Electrophysiological Responses To Noxious Stimuli in the Anaesthetised Child

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Doctor of Philosophy in Clinical Neurosciences
Trinity Term, 2015

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Declaration

I, Ravi Poorun, confirm that the work within this thesis, entitled 'Electrophysiological Responses to Noxious Stimuli in the Anaesthetised Child', is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Ravi Poorun
July 2015

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Dedication

To my parents.
For their love & support.

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Abstract

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In the UK, more than 235,000 children admitted to hospital each year receive an operation or investigation under general anaesthesia. It is not known whether nociceptive stimulation evokes a change in cortical brain activity in the anaesthetised child. The aim of this thesis is to determine whether noxious stimulation administered to anaesthetised children results in a measurable change in brain activity and whether this evoked activity is altered in children who have been born prematurely and experienced a high level of pain in early life.

Changing patterns of neuronal activity evoked by noxious and non-noxious stimuli were 'time locked' to electrophysiological recordings by means of a novel high-speed camera and an event detection interface developed during this thesis. Changes in band power were examined pre- and post-stimuli and across the different stimulus modalities.

In all children, background EEG activity was dominated by delta (<3 Hz) and alpha (8-12 Hz) band frequencies, consistent with previously reported anaesthetic literature. Clinical and experimental noxious stimulation, and tactile stimulation evoked a significant increase in delta activity ($p<0.05$) with no changes in average heart rate or ipsilateral EMG activity observed between pre- and post-stimulus. The application of local anaesthetic to the stimulation site diminished the evoked increase in delta activity. The response to noxious stimulation in the children born prematurely was not significantly different from the age-matched control group ($p>0.05$) but they had striking differences in their background EEG activity. Prematurely born children had significantly lower alpha and beta band activity.

The electrophysiological recordings we have obtained show that it is possible to measure evoked brain activity following a variety of noxious and non-noxious stimuli to investigate how the paediatric human brain processes sensory information under anaesthesia. The EEG measures were more sensitive to nociception than changes in autonomic activity and reflex withdrawal activity. Noxious stimulation caused a significant increase in delta activity, representing an increase in cortical synchronisation. While the children who were born prematurely did not respond differently to the noxious stimulation they had dramatically different background activity, which could have clinical relevance when using brain-derived patterns of EEG activity to help establish anaesthetic depth.

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Abbreviations

ACC	Anterior Cingulate Cortex
AEP	Auditory Evoked Potential
AS	Active Sleep
BIS	Bispectral Index
BOLD	Blood Oxygen Level Dependent
BPD	Bronchopulmonary Dysplasia
bpm	Beats Per Minute
CNS	Central Nervous System
dHCP	The Developing Human Connectome Project
DRG	Dorsal Root Ganglion
DTI	Diffusion Tensor Imaging
ED	Effective Dose
ECG	Electrocardiography
EEG	Electroencephalography
EMG	Electromyography
ERP	Event Related Potential
ELBW	Extremely-Low Birth Weight
ET	End Tidal Concentration
FDA	US Food and Drug Administration
FFT	Fast Fourier Transformation
FI	Inspired Fractional Concentration
FMRI	Functional Magnetic Resonance Imaging
fps	Frames Per Second
HD	High Definition
HFIP	Hexafluoroisopropanol
HTM	High-Threshold Mechanoreceptor
I-AMH	Type I A-fibre Mechano-Heat nociceptors
IASP	International Association for the Study of Pain
IES	Intraepidermal Electrical Stimulation
Ih	Hyperpolarisation Activated Current
ISI	Interstimulus Interval
IV	Intravenous
LC	Locus Coeruleus
LEP	Laser-Evoked Potential
LMA	Laryngeal Mask Airway
LOC	Loss Of Consciousness
LTM	Low-Threshold Mechanoreceptor
MAC	Minimum Alveolar Concentration
MLAEP	Mid-Latency Auditory Evoked Potential
MRI	Magnetic Resonance Imaging
N_2O	Nitrous Oxide
NICU	Neonatal Intensive Care Unit
NIRS	Near Infrared Spectroscopy
NREM	Non-Rapid Eye Movement
NS	Nociceptive Specific
P	Postnatal Day
PAG	Periaqueductal Grey
PBn	parabrachial Nucleus
PC	Principal Component
PCA	Principal Component Analysis
PEP	Pinprick Evoked Potentials
PET	Positron Emission Topography
QS	Quiet Sleep
QST	Quantitative Sensory Testing
REM	Rapid Eye Movement
RMS	Root Mean Squared
ROI	Region Of Interest
ROP	Retinopathy of Prematurity
RSN	Resting State Network
RSV	Respiratory Syncytial Virus
RVM	Rostroventral Medulla
SEP	Sensory Evoked Potential
SSEP	Somatosensory-Evoked Potential
SI	Primary Somatosensory Cortex
SII	Secondary Somatosensory Cortex
STT	Spinothalamic Tract
SWS	Slow Wave Sleep
TC	Thalamocortical
TENS	Transcutaneous Electrical Nerve Stimulation
TRP	Transient Receptor Potential
TTL	Transistor-Transistor Logic
VEP	Visual Evoked Potential
VLBW	Very-Low Birth Weight
VPL	Ventroposteriolateral Nucleus
WDR	Wide Dynamic Range

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Chapter 1

Introduction

Chapter 1

Introduction

1.1 The Question of Paediatric Pain Under Anaesthesia

Our environment, our emotions, our memories, our physiology, and our coexisting pathology influence the interpretation of nociceptive inputs. Pain is a conscious experience, with the resulting feelings and elicited behaviour of an individual not linearly correlated to the level of noxious input[1]. That it may further be modified to a particular situation provides evidence of just how personal the experience of pain can be.

Pain is always subjective.

The International Association for the Study of Pain (IASP) defines pain as *'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'*, with nociception being *'the neural process of encoding noxious stimuli'*[2]. By these definitions, nociception is neither necessary nor sufficient for the perception of pain. Although pain can often have a physical cause, pain perception can occur in the absence of a nociceptive stimulus. Nociceptive stimulation can evoke autonomic and behavioural responses, such as increased blood pressure or motor withdrawal reflex activity, but this may not necessarily lead to the perception of pain [2].

For example, noxious pinprick stimulation can be described as a sharp sensation, but not necessarily considered to be painful.

The way that we, as humans, convey our pain is most often communicated by language, and the self-report of our suffering forms the basis behind the management of pain. Our ability to communicate verbally with each other puts adults and older children at a distinct advantage to those with impaired consciousness or the inability to speak. This challenge can make the reliable and quantitative measurement of the pain experience extremely difficult.

This thesis has stemmed from the anecdotal evidence witnessed by paediatric anaesthetists during the induction of general anaesthesia (Greek - an-, *'without'*; aisthesis, *'sensation'*). Through personal communication, anaesthetists have reported that children appear to be more sensitive to an acutely noxious stimulus while under volatile anaesthesia if they were born prematurely.

With increased understanding of the plasticity behind normal somatosensory and pain processing, the potential of long-term consequences to early pain exposure has emerged as a key question. If responses observed clinically are quantified by alterations in sensory processing, then healthcare practitioners must be alerted to the possibility of a modified response to future pain and precautions taken. Perhaps more so when a child is under anaesthesia and unable to communicate.

There is limited evidence in humans for the long-term consequences of early pain experience and any findings are complicated by comorbidity, environmental and social

factors obtained during childhood, adolescence and adulthood. No human studies have been able to provide conclusive evidence of long-term sequelae, but there is evidence that early sensory experience can influence the development of central nociceptive wiring and the potential for an increased risk of pain disorders later in life.

Not knowing what, if anything, the anaesthetised patient is experiencing remains a puzzle to both adult and paediatric clinicians and researchers. However, with anaesthesia believed to cause an insensibility to pain, it remains unknown whether clinically noxious events evoke a change in the brain activity of children under general anaesthesia.

1.1.1 CHILDREN IN PAIN

Whether a child was experiencing pain and the decision to manage it was once decided upon by the personal beliefs of a physician and not scientific evidence. There was however, a distinct understanding that children were not simply 'little adults' and could not be treated as if they were. The fourth and fifth century BCE writings of Hippocrates described fundamental differences between adults and children, giving varying quantities of herbal remedies and alternative routes of administration[3].

Early medical writings provide little information, but the primary symptoms of childhood pain and distress appear to have been regarded as crying, restlessness, and sleepiness[4]. As is commonplace today, determining whether a child was in pain relied upon observed changes in general behaviour:

'The child groans in its sleep, rolls about, gnashes its teeth, tends to lie prone, cries out suddenly, or falls silent, is seized with convulsions, sometimes becomes somnolent, the face becomes emaciated and loses its colour; the child gets cold and answers questions with difficulty; sometimes throws itself about with outstretched hands, working itself into perspiration.'

~ Aurelianus (fifth century CE)[3]

Crying however was seen as the fundamental indicator of a child in pain, with some physicians believing that children only cried when in distress. The American physicians Starr (in 1895) and Holt (in 1897 & 1908) went so far as to describe different aspects of a cry and associating them with the severity of pain in specific illnesses. Headache was associated with a hydrencephalic cry - a sudden, loud, and paroxysmal shriek; and the acute pain of earache and colic accompanied by a sharp and piercing cry[3]. Acute pain was noted to cause leg withdrawal, exhaustion, sleep, and distinct changes of facial expression that was tentatively used to identify the source of pain.

Surgery has been performed on children since ancient times, with circumcision and trepanation widespread[3]. As surgical methods became more refined, the variety of surgeries in children increased to include procedures to repair cleft lips, inguinal hernias, tongue ties, as well as tonsillectomies[3]. Surgical procedures were extremely painful for children and they suffered terribly during this period[5]. Despite primitive analgesics and potions being available for pain relief during surgery, the physical restraint of children for procedures was common, and often difficult too for the physician to witness[3].

The advent of anaesthesia and the breakthrough that surgery could be performed painlessly was vital for all future developments, and the early modern history of childhood pain relief starts with the beginnings of anaesthesia.

1.1.2 THE DAWN OF PAEDIATRIC ANAESTHESIA

'A protracted and sanguine battle between surgeon and anaesthetist with the poor unfortunate baby as the battlefield'

~ P Ayre[6]

Pain had never prevented surgery from taking place, but until the advent of anaesthesia, operations were almost unbearable and the postoperative trauma that ensued often dangerous. Surgeons attempted to deaden the pain of surgery with the limited forms of pain relief that were available. This included the use of alcohol, nightshades, opium, and willow bark, and blunting pain by compressing nerves, exsanguination, or freezing limbs[7]. Friday, October 16th 1846 was the turning point in the history of anaesthesia when the successful demonstration of ether vapour made the idea of painless surgery an accepted possibility[7]. On what was to become known as *'Ether Day'*, William TG Morton administered ether and the surgeon, John Collins Warren, excised a salivary gland tumour from Gilbert Abbott. The patient's tumour was removed without any discomfort and to an astonished audience Warren declared, *'Gentlemen, this is no humbug!'*. The experience of pain was to be transformed during the era of general anaesthesia, however, its story had begun more than 50 years earlier and children were involved from its earliest clinical applications.

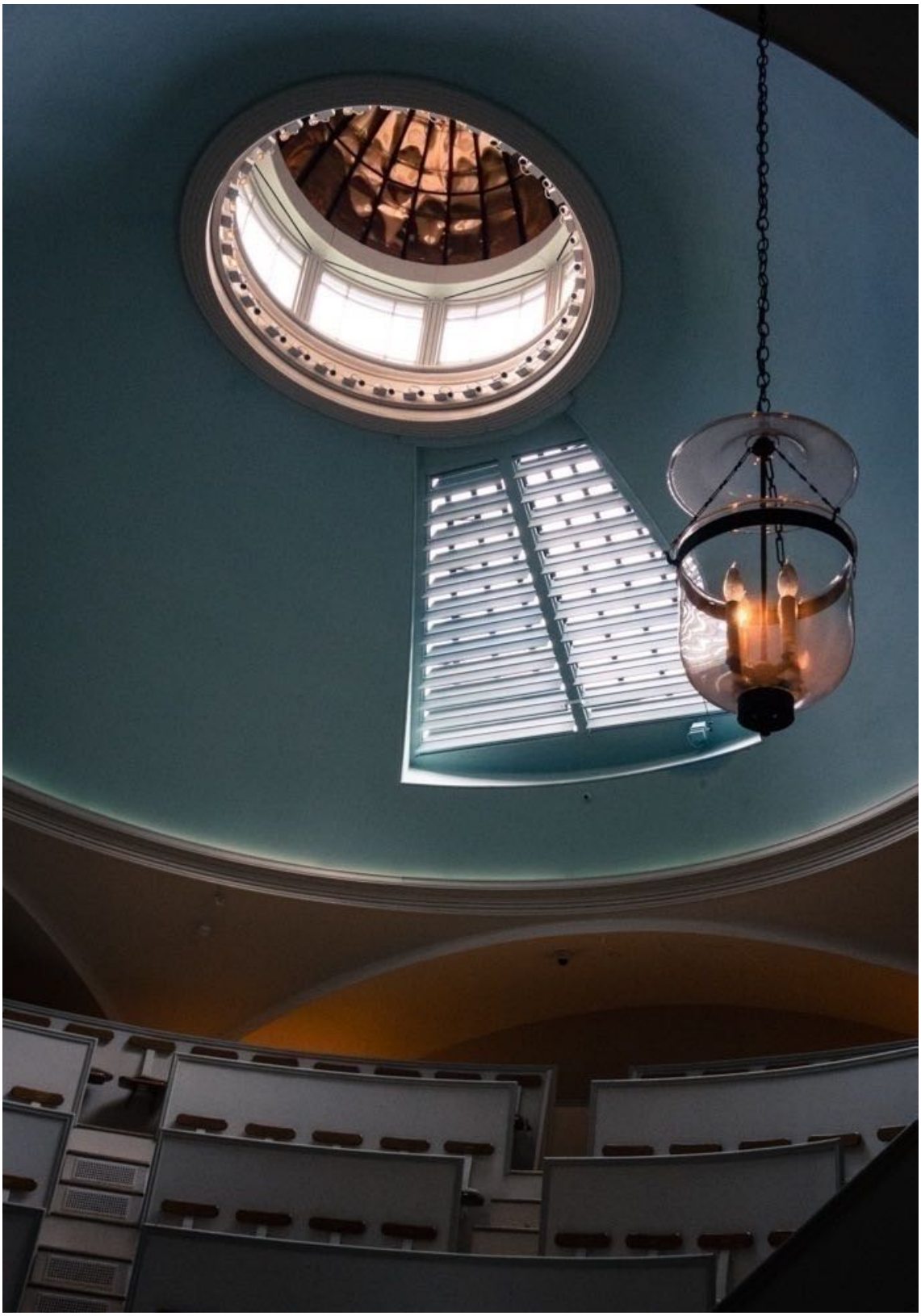

Figure 1-1: 'Inside The Ether Dome' by Ravi Poorun. The operating theatre where Morton's demonstration took place became known as 'The Ether Dome' in its honour. This photo was taken on my visit to Massachusetts General Hospital on the 27th July 2013.

Four years prior to Morton's demonstration, Crawford W Long had already begun to perform operations under ether anaesthesia. The first was the extraction of a neck cyst, but Long's third patient happened to be a child.

'My third experiment in etherization was made on the third of July, 1842, and was on a negro boy, the property of Mrs. S. Hemphill, who resides nine miles from Jefferson. The boy had a disease of a toe, which rendered its amputation necessary, and the operation was performed without the boy evincing the least sign of pain.'

~ CW Long (1849)[8]

In the same publication he tells of another boy he had anaesthetised. This time however, he describes being able to test the anaesthetic power of ether. What followed is quite possibly the first ever documented anaesthetic experiment performed on a child. Two fingers were amputated from a boy, one finger with, and the other without the influence of ether. The boy is said to have *'suffered from one operation, and was insensible during the other'*[8].

'I, Green L. Thompson, certify that I was present and witnessed Dr. C. W. Long, in the year 1845, cut off two fingers of a negro boy Isam, the property of my father in law, Ralph Daily, Sen. Before cutting off one of the fingers, Dr. C. W. Long gave the boy Isam, Sulphuric Ether to inhale from a towel or cloth, and while under the influence of the ether, cut off the finger without the boy showing the least sign of suffering pain. I have also heard the boy speak of the operation since and he always asserted that he did not feel the least pain when the operation was performed. The other finger was cut off without the boy being under the effects of the ether and the operation was painful.'

~ GL Thompson[8]

Long did not publish his work until three years after Morton's demonstration and what followed was the controversy over who had in fact discovered *'anaesthesia'*; this involved other claimants as well as the use of nitrous oxide. Long put the delay down to the fact that surgical operations were uncommon in his rural practice and his wanting to test the power of ether first. His publication contained many witness accounts used to support his claims, such as the one by GL Thompson, above.

Despite being a potent anaesthetic, ether's slow speed of onset made it relatively safe to be administered by novices. However, it was not without its drawbacks. The vapour is acrid, irritant, and unpleasant to breathe, eliciting coughing and vomiting in patients. Morton himself noted that young patients were at greater risk of complications and likely to have nausea and vomiting as a result of its use[9]. Anaesthesia soon became accepted for surgical interventions but the drawbacks of ether were not, and the search for a successful replacement did not take long.

When ether anaesthesia was demonstrated in Britain, James Young Simpson, Professor of Surgery in Edinburgh, saw the potential of utilising anaesthesia to alleviate the pain of childbirth. He began experimenting with other substances and accounts report that at a dinner party he had organised, Simpson and several of his guests were found unconscious around the dining table after having passed around one of these chemicals. This chemical was chloroform. An early case report by Simpson in 1847 describes the induction, peri-, and postoperative period of chloroform in which he anaesthetises *'a boy, four or five years old, with necrosis of one of the bones of the forearm'*[10].

Chloroform dominated anaesthesia for more than 60 years and was partly aided in its popularity because of the birth of Prince Leopold on the 7th April 1853. Queen Victoria had received chloroform during childbirth from the physician John Snow and recorded in her journal that *'the effect was soothing, quieting, and delightful beyond measure'*. Snow was a pioneer in anaesthesia and his use of chloroform extended to more than a hundred children:

'Chloroform may be given with propriety to patients of all ages. I have exhibited it to several infants aged from ten days to three weeks, and to one patient nearly ninety. I have notes of the cases of 145 infants under a year old, to whom I have administered this agent. A great number of them were operated on for hare-lip by Mr. Fergusson, who performs this operation at the earliest period of life, if the children are healthy. Chloroform acts very favourably on infants and children.'

~ J Snow[11]

However, in unskilled hands, the narrow therapeutic window of chloroform could lead to fatal hypotension, respiratory depression, and cardiac arrest (paving the way for specialists in anaesthesia). As reports of more and more deaths from chloroform use became more widespread, the death of 15-year-old Hannah Greener in 1848, who was undergoing the removal of an ingrowing toenail, came to attention when the jury at the inquest of her death came to the unanimous opinion *'that Hannah Greener died from congestion of the lungs, produced by chloroform, and that no blame could be attached to Dr. Meggison [the physician administering chloroform] or his assistant'*[12].

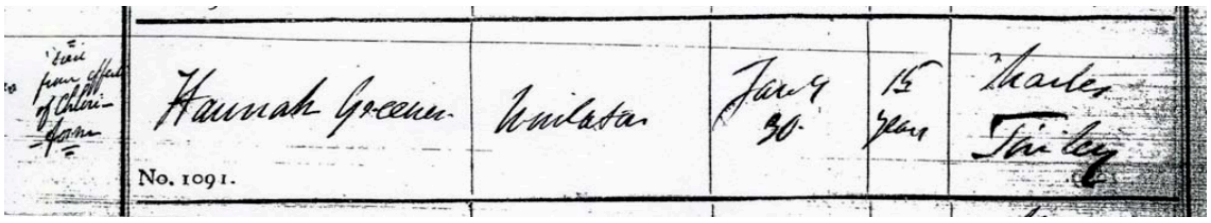

Figure 1-2: Burial Record of Hannah Greener - 30th January 1848. The notation in the margin to the left of the record states: 'Died from the effects of Chloroform'[12]

Simpson and Snow fell on different sides of the inquest's verdict. Simpson, advocating for chloroform, blamed the resuscitation methods for Hannah's death which caused her to asphyxiate, while Snow believed that the method of chloroform administration was to blame for her death[12]. It is likely that Hannah's death is more complicated than just the anaesthetic, technique, or resuscitation alone, however, from Hannah's burial record (Figure 1-2), we can see that the first ever recorded death due to anaesthesia was a paediatric patient, who died on Friday, 30th January 1848.

1.1.3 LONG-TERM ISSUES OF SURVIVING THE NEONATAL INTENSIVE CARE UNIT (NICU)

More than one in ten children around the world will be born premature[13], their birth occurring before 37 weeks gestation. However, significant advances made in neonatal medical care have improved the survival of preterm infants[14-16] that management strategies in the neonatal care unit (NICU) are being directed towards minimising the long-term outcomes of prematurity. The increased discharge rates from NICU[17] have put survivors at risk of complications due to their immaturity and long-term sequelae from their time in intensive care. Their medical needs differ from healthy term infants and there is a high rate of recurrent hospitalisations, neurodevelopmental impairment, and chronic health issues.

The risk of hospitalisation increases with decreasing gestational age[18-20], with readmission rates between 10–20%[21-24]. During their first year of life, ex-preterm infants are twice as likely to be readmitted into hospital than term-born infants[25]. Common causes include respiratory, feeding, and gastrointestinal problems such as respiratory syncytial virus (RSV) infection, bronchiolitis, reflux, gastroenteritis, and surgical conditions[18,24,26,27].

Additional risk factors include significant neonatal morbidity and extreme prematurity, with a greater number of morbidities increasing readmission rates. During the first three years of life, rehospitalisation is highest in infants later diagnosed with seizures and obstructive airway disease, and this number decreases with increased postnatal age[28]. With an average of 3.9 admissions to hospital, 58% of children requiring mechanical ventilation at birth are readmitted, 19% of whom needing intensive care[29]. Forty-five per cent of extremely premature (<28 weeks gestational age) neonates are readmitted to hospital by 18 – 22 months[27], the highest rates of rehospitalisation of any NICU survivor[24,30].

1.1.3.1 Respiratory Disease

It is common for ex-preterm children to have long-term respiratory disease beyond their NICU discharge. Most common is bronchopulmonary dysplasia (BPD), but ex-preterms are also at risk for reactive airway disease[26] and respiratory tract infections[31,32].

BPD is a respiratory disease where neonates at 36 weeks require supplementary oxygen and have persistent findings on chest X-rays[33]. Often during the first year of life, infants with BPD will have abnormal lung function and may require supplemental oxygen and medications at home. BPD can also lead to other respiratory morbidities and infections, growth failure, and cardiac issues (cor pulmonale and pulmonary hypertension)[34]. For the first two years, BPD patients have a high rate of rehospitalisation[35,36] but problems improve after two[37].

1.1.3.2 Ophthalmological Conditions

There is an increased risk of long-term ophthalmologic conditions in infants born with very low birth weight (VLBW) and extremely low birth weight (ELBW). This includes retinopathy of prematurity (ROP), amblyopia, anisometropia, myopia, and strabismus[38]. ROP is a common developmental vascular proliferative retinal disorder in preterm infants and is the most common cause of blindness in children. It presents from 32 weeks gestation and it is important that retinal screening occurs as early treatment drastically reduces morbidity and potential blindness from the condition.

Strabismus is more common in premature infants, with higher risk factors being lower birth weight and gestational age[39], and usually requires surgical correction. Anisometropia occurs more often in ex-preterm infants and because the eyes are unable to focus

independently a large refractive error can occur between the two eyes[40,41]. Refractive errors are more common at four and-a-half years old, with nearly 20% of ex-preterm children being diagnoses as myopic. Anisometropia and myopia require correction with contact lenses or glasses. Strabismus, anisometropia, and myopia in premature infants can lead to amblyopia where the lack of use of one eye before several years can lead to reduced vision that can become permanent between the years of six and ten[38,42,43].

1.1.3.3 Neurodevelopmental Problems

Compared to term-born children, ex-preterm infants have on-going health issues and are at increased risk of neurodevelopment disability with VLBW and ELBW infants at greatest risk of all[17]. These outcomes include impaired cognitive skills, fine and gross motor deficits, cerebral palsy - seen in approximately 8% of VLBW infants, and sensory impairment, with a lower gestational age constituting a greater risk[44].

There is a higher incidence of anxiety, depression, and behavioural problems including attention deficit hyperactivity disorder[45], particularly in female survivors who are more likely to form internalising behaviours[46]. Neonates born <1500 g have impaired cognition, neurosensory, behavioural and psychological problems putting them at greater risk of poor academic performance[17].

Despite the increased risk of neurodevelopmental disability, the majority will be functionally normal by adulthood and comparable to those born at term[47].

1.1.3.4 Surgical Issues

Prior to NICU discharge, some neonates will have undergone surgery for cardiac and gastrointestinal conditions. However, once discharged, hernias - both umbilical and inguinal - are more common in ex-preterm infants.

Up to 75% of infants weighing between 1000 –1500 g at birth will have an umbilical hernia[34]. Surgery is required if there are signs of an incarcerated bowel but most spontaneously resolve by two years old. Surgery is considered if the hernia persists at four years old, is greater than 2 cm in size or is associated with abdominal pain[48]. Inguinal hernias are more common in ex-preterm children with incidences increasing with lower birth weight[49], most common in boys[50], infants weighing less than 1000 g[51], and the prolonged use of mechanical ventilation[50]. Inguinal hernias should be referred to paediatric surgery, particularly as incarceration is more frequent in ex-preterms[52].

1.2 The Developmental Neurobiology of Nociception

Responses to noxious stimuli are functional at birth and arise from neural activity at varying levels of the CNS. Noxious chemical, mechanical and thermal stimuli are detected by nociceptors in the periphery where they are transduced and transmitted to the spinal cord. In the spinal cord, motor reflexes protect the body from further immediate damage, whilst excitatory and inhibitory spinal systems can alter afferent nociceptive inputs as well as undergoing long-term changes that may be the cause of altered sensory processing later in life.

Responses to noxious stimuli develop at different rates and results in specific age-related changes in nociceptive signalling. It is important that these responses are functional in order to promote protective behaviour to tissue injury and alert caregivers. Processing of nociceptive activity in the spinal cord and brainstem produce reflexive movements, autonomic, and metabolic responses in order to maintain homeostatic control of the body. These responses, along with facial expression and crying, form the basis of paediatric pain scores. Nevertheless, these surrogate measures, which in clinical practice are used in an attempt to quantify infant pain, may not necessarily relate to the true pain experience.

1.2.1 THE DEVELOPMENT OF PERIPHERAL NOCICEPTIVE PATHWAYS

Predominantly small diameter A∂-fibres and C-fibres are the neurones found in the skin, joints, muscles, vessels, fascia, and viscera that transmit nociceptive information. They are first-order afferents with free nerve endings that respond to noxious chemical, mechanical and thermal stimuli. They transduce and transmit this information to the dorsal horn of the spinal cord. A∂-fibres are myelinated with a diameter of 2.0-6.0 μm and a conduction velocity of 6-25 m/s[53], and the two classes of A∂-fibres respond to intense mechanical stimuli but differ in their response to intense heat[54]. These fibres transmit information that is well localised and are responsible for the acute sensation of pain that triggers the spinal withdrawal reflex. The conduction of C-fibres is slower (~1.0 m/s) due to their smaller diameter (0.4-1.2 μm) and lack of myelin[53]. They are responsible for the longer lasting dull-ache that is less well localised.

Nociceptors have their cell bodies in the dorsal root ganglion (DRG) where transcription and protein synthesis take place. From the DRG the central branch of

primary afferent axons are organised into dorsal root bundles which project to the superficial layers of the dorsal horn of the spinal cord[55]. Here, they synapse onto second-order neurones, which ascend to the thalamus via the contralateral spinothalamic tract. At the thalamus, second-order neurones synapse once more, onto third-order neurones, travelling to various cortical areas but most importantly to the primary somatosensory cortex[56].

Before reaching the thalamus, second-order neurones form collateral branches that leave the spinothalamic tract and travel to the midbrain and medulla[54]. Here, they synapse onto the periaqueductal grey (PAG) and rostroventral medulla (RVM), and along with the locus coeruleus are involved in the descending regulation of second-order neurones[1]

1.2.1.1 Peripheral Nociceptors

At birth, peripheral nociceptors respond to a range of chemical, mechanical, and thermal stimuli[57,58], and even though their responses develop and mature during childhood, nociceptors are able to respond to noxious stimuli and tissue damage from birth[59].

Chemical and thermal stimuli activate two families of peripheral receptors. The large transient receptor potential (TRP) ion channel family and the smaller purinergic receptor family comprising of the P2X inotropic channels and P2Y G-protein coupled receptors. TRP channels respond and transduce a variety of stimuli[60], with those expressed within skin and on sensory neurones responding to specific temperature ranges[61]. TRPV1 is activated by noxious heat (>43 °C) as well as capsaicin. Their mRNA is expressed in DRG cells before birth, and actively respond to capsaicin[62]. TRPA1 is activated by noxious cold, formalin, horseradish, and mustard oil[61]. Recordings from DRG cells have found that they

respond to mustard oil early in development[57,58], but their ability to activate the spinal reflex within the first week of rodent life is poor[63,64]. P2X3 receptors are found at the peripheral terminals of sensory neurones and respond to ATP released from endothelial cells, Merkel cells, and sympathetic neurones. At birth, P2X3 is expressed on many sensory neurones, but this becomes limited to the IB4+ve nociceptors during development[65].

Pressure sensitive mechanoreceptors and classified by their thresholds. Low threshold mechanoreceptors (LTMs) respond to innocuous pressure and are predominantly found on the nerve endings of Aß fibres. High threshold mechanoreceptors (HTMs) are activated by noxious pressure levels and are found on A∂ and polymodal C-fibres. Additional mechanical stimuli, such as touch and vibration, are detected by hair follicles, Meissner corpuscles, and Pacinian corpuscles[66] with glabrous skin more innervated and containing more HTMs than hairy skin[67]. Mechanoreceptors encode features of mechanical stimuli by their firing frequency. Not only are nociceptive stimuli transmitted in the small myelinated A∂ and unmyelinated C-fibres, they are mediated by slow and ultra-slow currents within them[66].

Touch, hearing, proprioception, and to some extent nociception, are thought to be mediated through mechanosensitive channels that are directly gated by mechanical forces[68]. These channels transduce mechanical stimuli into electrical activity and act as the force transducer of mechanoreceptors. The activity of these channels have been identified in most organisms, but their identity is uncertain in mammals[68]. In 2010, two piezo proteins, piezo1 and piezo2, were identified as components of mechanically activated non-selective cation channels[69]. In situ hybridisation has found that the protein, piezo2 is

expressed in somatosensory neurones of the DRG and trigeminal ganglion[69,70]. Within DRG neurones, piezo2 encodes rapidly adapting currents, activated by mechanical stimuli[69], and in mice, piezo2 can be found in the same location as markers for both nociceptive and non-nociceptive DRG neurones[69]. Piezo2 may in fact contribute to mechanical hyperalgesia and allodynia, as it is thought that piezo2 currents may be upregulated by bradykinin[71] and a selective Epac-1 agonist[72].

The transmission of action potentials along nociceptors is via voltage-gated sodium channels (Nav) expressed along their axons. Nav are either tetrodotoxin resistant (TTX-R) or sensitive (TTX-S) and both can be identified from birth in rodents[73]. Nav are expressed on all axons but the TTX-R Nav 1.8 and 1.9, and TTX-S Nav 1.3 and 1.7 are found specifically on nociceptors, with Nav 1.3 expressed at higher levels during embryological development.

1.2.1.2 Peripheral Sensitisation

The response to a nociceptive stimulus can change with prolonged activation, and sensitisation of nociceptive pathways can lead to hyperalgesia (increased response to noxious stimuli), or allodynia (pain in response to innocuous stimuli). Within an area of tissue damage, the increased activation of peripheral nociceptors can alter the threshold of nociceptive receptors leading to primary hyperalgesia. Secondary hyperalgesia occurs when an area surrounding the site of tissue damage becomes increasingly sensitive through centrally mediated mechanisms. Sensitisation is initially advantageous by encouraging protective behaviour of an injured area, but prolonged sensitisation can contribute to chronic pain states.

In early life, repeated peripheral stimuli can also cause sensitisation or hyperalgesia when older. However, normal physiological development requires activity to strengthen synaptic connections and because the activation of nociceptors is rare early in life, the activity from LTMs influences nociceptive pathway development[74]. Abnormal neonatal conditions can affect the activity within developing nociceptive circuits and if these arise during critical periods of development, impairments in normal function can occur. With excessive activity as a result of early life pain and injury, future sensitivities can be altered which are not seen when the same injury is received when older[75]. By reducing afferent activity, long-term decreases in sensory thresholds can be seen by altering synaptic function, spinal cord organisation, and increasing neuronal apoptosis[76-79].

Peripheral inflammation is a consequence of tissue damage and the inflammatory reaction occurs rapidly in order to repair damaged tissue. The area of inflammation becomes red (rubor) and hot (calor) due to increased blood flow, swollen (tumor) from vascular permeability, functionally compromised, and painful (dolor) by the activation and sensitisation of peripheral nociceptors. Pain occurs in order to protect the area from further harm by altering the organism's behaviour. Chemical (inflammatory) mediators are released from damaged cells, some of which directly activate nociceptors, others that exert their effects on the local area to release further chemical mediators from immune cells and attract leukocytes to the inflammation site. Inflammatory hyperalgesia is present early in development with responses to thermal stimuli accentuated when inflammatory mediators are applied to embryonic chick neurones innervating skin[80].

1.2.1.3 Spinal Reflexes

The ability to localise a noxious stimulus and produce reflex motor movements is an important protective mechanism to withdraw from potential injury. Spinal reflexes should not be interpreted as pain awareness or perception, but indicate that peripheral nociceptive mechanisms have developed sufficiently to protect the individual or organism from harm. During early life, spinal reflex responses are diffuse and have low thresholds. The lower thresholds in neonates and infants show a clear response between the intensity of a stimulus and the magnitude of response[81], a magnitude that decreases with age in rodents[82]. A noxious stimulus on the foot of a human infant will cause an entire body movement that over development will become localised to movement in an individual leg and subsequently foot. Following repeated heel lancing[83], mechanical stimuli[84], and surgery[85] in neonates and infants sensitisation of reflex responses occurs.

1.2.2 THE DEVELOPMENT OF CENTRAL NOCICEPTIVE PATHWAYS

The dorsal horn of the spinal cord and the trigeminal nuclei in the brainstem receive sensory afferent input from deep tissues, joints, muscles, skin, and viscera. Here, nociceptive input from the periphery is integrated into central sensory pathways and transmitted to higher centres by multimodal pathways and 'labelled lines'. Among the majority of interneurones found within the dorsal horn, approximately 5% are projection neurones with their long axons projecting up the spinal cord to higher CNS areas[86]. In the dorsal horn, a single wide dynamic range (WDR) neurone can receive synapses from many afferents resulting in a convergence of spatial information and modality, a larger and more overlapping receptive field than primary afferents, and multimodal ascending pathways. Furthermore, a small number of dorsal horn neurones are nociceptive specific

(NS) and only receive input from peripheral nociceptive afferents transmitting a 'labelled line' of solely nociceptive information to higher centres. The number of WDR and NS neurones that respond to noxious input changes postnatally and in the dorsal horn of the neonatal rat, their numbers are low and become more numerous during the first week of life[87].

1.2.2.1 Ascending Nociceptive Pathways

Projection neurones have their cell bodies in the lamina I, VI and V of the dorsal horn. Their long axons project in the contralateral spinothalamic tract (STT) that also transmit tactile and temperature information. Thalamocortical neuronal networks are essential for the perception of pain but only ten per cent of STT axons terminate in the thalamic ventrobasal complex with the majority projecting to the brainstem reticular formation - the periaqueductal grey (PAG), parabrachial nucleus (PBn), locus coeruleus (LC), and hypothalamus[86,88]. Projections directly to the thalamus mediate the sensory discriminative aspect of pain, while projections to the PBn and PAG mediate emotional and autonomic aspects of the pain experience through their connections to the hypothalamus and amygdala.

Thalamic output subsequently divides into lateral and medial streams. The lateral stream originates from the ventroposteriolateral nucleus (VPL) and is responsible for the sensory aspect of pain by projecting to the primary somatosensory cortex (SI)[89]. The medial stream projects to the secondary somatosensory (SII) and anterior cingulate cortices from the medial dorsal thalamic nuclei. This may contribute an important component to the emotional aspect of pain[89].

Unlike auditory or visual information, there is no specific 'pain cortex' and noxious stimulation activates multiple brain areas. Coordinated activity in the cingulate, frontal, insula, parietal, and somatosensory cortices result in a multifaceted perception of pain[1,90,91].

1.2.2.2 Ascending Spinal Projection Development

STT neurones develop before birth forming synaptic connections to thalamic cells from an early stage[92]. However, their functional maturation to the thalamus is slower. By day three in foetal and infant rats, the neural activity marker, fos, is expressed in the ventral lateral medulla where descending pathways originate[93], but are only labelled by day 14 in subcortical sensory and motor areas[93]. This ascending pattern of development is likely to underlie the development of pain perception[94].

It is challenging to study the development of STT ascending projections in man but we can infer their maturation through measurement of cortical activity in preterm infants[95]. Even when cerebral injury is present, changes in facial expression to noxious stimulation can be observed in extremely preterm infants suggesting that connections to the brainstem are present before birth[96]. In healthy one to four month old babies, diffusion tensor MRI imaging (DTI) has demonstrated that the spinothalamic and corticospinal tracts are among the most mature nerve bundles, with the internal capsule and cingulum among the most immature[97].

1.2.2.3 Thalamocortical Development

Thalamocortical afferent development has been anatomically documented in humans[98]. The transient subplate zone[99] is one of the main signs of cortical immaturity[100] and is significant for the normal development of the human telencephalon, which subsequently

develops into the cerebral cortex and basal ganglia[101]. From the subplate grows the thalamocortical and corticocortical connections[101], with its neurones penetrating the cortical plate between 24 and 26 week of gestation. These neurones contribute to the first evoked potentials recorded from 24 weeks[102]. Standard MRI techniques allow for the assessment of brain development in infants where a highly sensitive assessment of grey and white matter, and the differentiation of myelination in white matter can be visualised. In vivo, the transient subplate zone can be seen as an area of hypointensity on magnetic resonance images[103], but after 32 weeks of gestation, the transient subplate zone becomes less visible[100]. FMRI studies in preterm infants have shown blood oxygen level dependent (BOLD) activity in SI following tactile information, suggesting that thalamocortical connections are functioning from 2.5 weeks but that intra- and interhemispheric connections are not present until 34 weeks gestaion[104].

1.2.3 THE DEVELOPMENTAL SWITCH IN DESCENDING MODULATION

How we perceive a noxious stimulus is greatly influenced by its context and our past experiences, our mood, level of distraction, and expectation of the pain. Emotions and memories achieve this is by controlling the well-defined descending and endogenous pain modulatory pathways within the CNS, acting primarily on the dorsal horn of the spinal cord by inhibiting the transmission of noxious information.

Electrical stimulation of the grey matter that surrounds the cerebral aqueduct of the III ventricle (periaqueductal grey; PAG) and IV ventricle can induce analgesia. Top-down control of nociceptive input is integrated at the PAG arriving from multiple higher centres including the limbic system, amygdala, hypothalamus and frontal lobe[1,105]. From the PAG,

the processing of noxious information in the dorsal horn is controlled via projections to the rostroventral medulla (RVM) and dorsolateral pontine tegmentum within the brainstem. The RVM is the main output nucleus for brainstem descending control where adult pain sensitivity and excitability within the spinal cord can be either inhibited or enhanced[106].

In rodents, the maturation of the adult biphasic descending inhibition and facilitation from the RVM does not occur until P28 (P; Postnatal Day), relatively late in development[107,108]. Experimental lesioning and electrical stimulation during the first weeks of life show that facilitation initially dominates and after P21, the RVM undergoes a gradual switch where inhibitory control of spinal nociceptive circuits develops, dominating during P25 and P35, and reaching adult levels by P40[107]. This delayed maturation of RVM inhibitory pathways over this developmental period may explain why the extensive PAG projections do not produce analgesia until P21[109].

Highly expressed within all levels of the descending pathway are endogenous opioid peptides and their receptors. In addition to their presence within the dorsal horn of the spinal cord, opioid agonists activate descending pain-modulating pathways in the PAG and RVM. Additionally, the μ-opioid receptor that mediates this activation, facilitates the developmental switch within the RVM as μ-opioid receptor agonists injected into the RVM will initiate the switch from descending facilitation to inhibition during the critical periadolescent period[108]. By blocking opioid dependent activity specifically between P21-P28, the RVM descending inhibitory development is prevented, but is accelerated when morphine is chronically administered over P7-P14[108].

The understanding of how supra-spinal descending pain control mechanisms develop and how it may be manipulated by exogenous factors during critical developmental periods can aid us in understanding how a child may cope with pain experiences as an adult.

1.3 Methods of Assessing Pain in the Paediatric Population

'Pain is whatever the experiencing person says it is, existing whenever he says it does'
~McCaffery & Pasero, 1999[110]

The self-report of pain forms the basis behind its management. Newborn infants (Latin – infans, *'voiceless'*) are at a distinct disadvantage, for they cannot tell us of any pain they may experience. This, and the immaturity of the neonatal central and peripheral nervous systems had once underpinned the rationale that it was acceptable to refrain from providing analgesia for procedures that would most certainly be painful to adults[111].

It is now accepted that human infants process noxious stimuli, and that this information is transmitted to the brain[112]. However, the reliable subjective measurement of pain is impossible in this vulnerable population, and traditionally we have relied on objective measurements of physiological (heart rate, respiratory rate, and blood pressure) and behavioural (facial expression, movement patterns, crying, and consolability) responses to noxious stimulation[113].

1.3.1 CLINICAL PAIN ASSESSMENT IN CHILDREN

The assessment of pain in children is challenging and often confusing. The effective management of paediatric pain requires frequent measurement in order to adjust and maintain adequate analgesia. However, assessing pain in a population that may not be able to affectively describe their experience can lead to the underestimation of pain. This has led to a plethora of assessment methods that attempt to assess childhood pain.

The most accurate and desirable assessment of pain is ideally the personal self-report of one's pain. As with adults, older children and adolescents are able to understand and use a visual analogue scale (VAS) or similar. However, in younger children or those with impaired cognition or consciousness, this is not a suitable method. Toddlers to some degree are able to report their pain and a number of assessment methods have been created to help young children in communicating their pain intensity. The FACES scale[114] uses images of faces depicting varying degrees of pain from which a child can choose the image that most accurately represents their experience.

In those that cannot self-report, observations of pain related behaviour could be used. Behavioural tools predominantly use facial expression, crying, posture, and movement as the most reliable measurements of pain. Pain related behaviour can be modified by affective, developmental, environmental, and social factors, therefore, it is important that the correct scale is used, appropriate for the child's age and circumstance. A well used example is the FLACC score ([F]ace, [L]egs, [A]ctivity, [C]ry, and [C]onsolability) validated to be used in neonates, infants and children from two months to seven years[115]. An observer scores each of the five categories from 0 to 2 and a final pain score is made from 0 and 10. Repeating the score later can give an assessment of pain over time.

It was hoped that the measurement of physiological parameters would lead to a more accurate method of assessing and quantifying pain. However, this has not been the case as used alone, physiological measures - e.g. blood pressure, heart rate, plasma cortisol and catecholamine's - lack the sensitivity and specificity to pain.

The decision on which particular assessment method to use will usually be pragmatic one and current standards utilise a multi-dimensional approach to pain assessment. Methods utilising several of the above methods can improve pain assessment and at least 35 clinical pain assessment tools exist specifically designed for neonates[116], the best known and most validated being the premature infant pain profile (PIPP)[117]. However, it is important to remember that these proxy measures are not necessarily mediated by higher brain centres, but can be elicited from the brainstem or lower[77,118]. Therefore, a complete representation of an infant's experience of pain cannot be determined by surrogate measures alone, and with cortical involvement a requirement for the pain experience[1], physiological, behavioural and neurobiological tools must be used in conjunction with each other[119]. Such methods can be complicated and time consuming so their use in clinical practice may be limited.

1.3.2 NEUROPHYSIOLOGICAL MEASURES

Neuroimaging techniques provide us with a non-invasive understanding of the functional and regional anatomy of the human brain. The development of electroencephalography (EEG), functional magnetic resonance imaging (FMRI), near-infrared spectroscopy (NIRS), and positron emission topography (PET), has enabled us to examine the brain without interfering with normal function. Figure 1-3 shows various

imaging modalities used to image the central mechanisms of pain processing and which aspects of brain activity they measure.

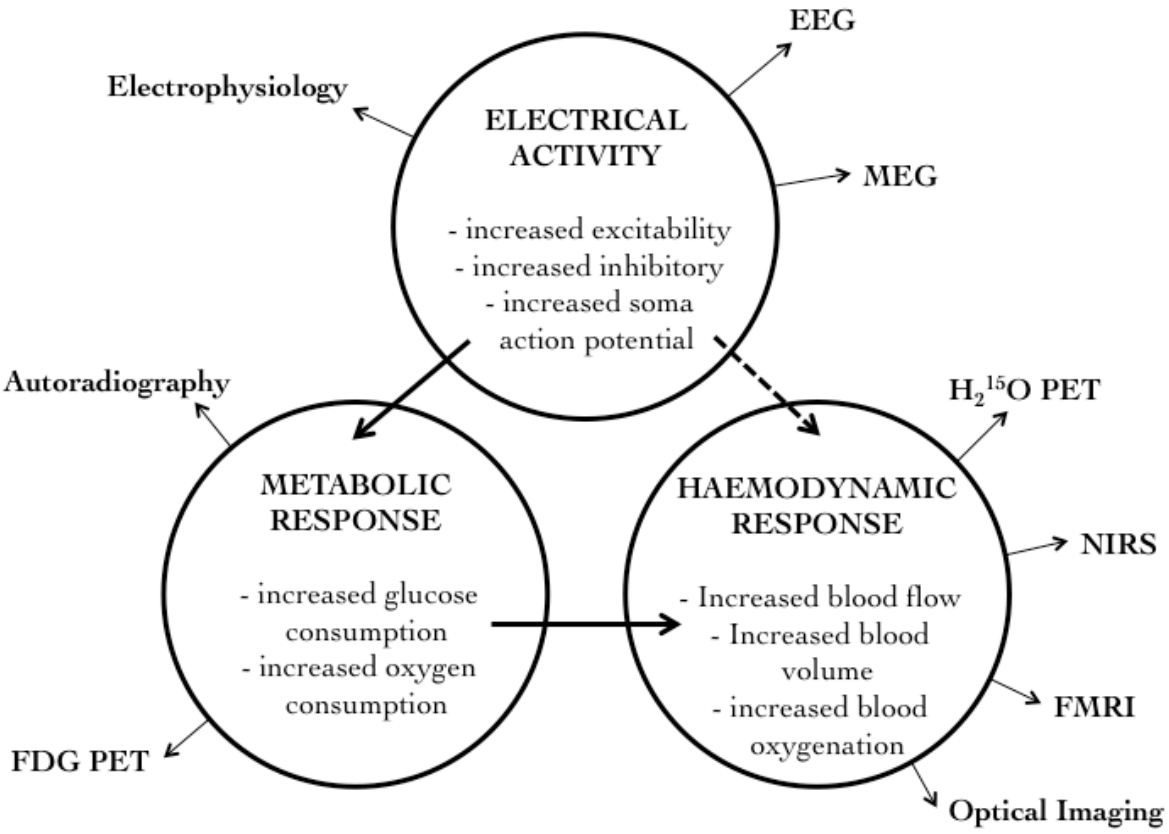

Figure 1-3: A schematic diagram displaying the neurophysiological correlates of neural activity and techniques used to detect that particular signal. EEG, electroencephalography; MEG, magnetoencephalography; FDG PET, flurodeoxyglucose positron emission tomography; $H_2{}^{15}O$-PET, water based positron emission tomography; NIRS, near infrared spectroscopy; FMRI, functional magnetic resonance imaging. Adapted from Tracey, 2008[120].

The use of neurophysiological and neuroimaging techniques has been extensively used in the study of adult pain, but these techniques have had relatively limited use in the evaluation of responses to nociceptive stimuli in neonates and infants. In the adult brain, numerous structures are involved in the experience of pain, including the brainstem, thalamus, and a rich variety of cortical areas, which includes the somatosensory cortex, cingulate cortex, insula cortex and amygdala[1]. However, the extent to which these structures are involved in infant pain is unknown, and any measure of CNS activity

following a noxious input in non-verbal patients is ultimately a surrogate measure of the pain experience.

Changes in haemodynamic brain activity have been recorded in response to nociceptive stimuli[121]. As the perception of pain requires the involvement of higher cortical brain structures, the measurement of evoked changes brain activity in adults have led to a better understanding of the brain structures that are involved in the perception of pain. In addition, nociceptive-evoked potentials have provided an electrophysiological representation of the changes in brain activity evoked by noxious stimulation[122]. These techniques often measure changes in brain activity in response to experimental stimuli, such as laser stimulation, however such techniques are not suitable for the study of pain in children. Acute pain in child is most often studied when essential medical procedures are performed. Clinically required painful procedures such as cannulation, vaccination or venepuncture are difficult, if not impossible to perform within a FMRI scanner and can only be carried out when there is clinical need for the procedure to be performed.

Both EEG and NIRS are ideal for performing studies in a clinical setting because of the equipment's portability and non-invasiveness. EEG has been used extensively in neonatal studies of procedural pain[95,123], and novel methods have been developed to 'time-lock' noxious events to the EEG[124].

The direct measurement of evoked brain activity in response to nociceptive stimuli in preterm and term born infants may help validate existing measures of nociception and must be considered alongside the existing body of literature that characterises the clearly observable neonatal behavioural and physiological responses to noxious stimulation.

Additionally, the development of more robust clinical tools for measuring infant pain may be aided by such techniques, and it would be ideal to develop a specific neural biomarker that is a direct correlate of pain experience.

1.3.2.1 Electromyography

The electrical activity of skeletal muscle can be measured using electromyography (EMG). The flexion withdrawal reflex[125] has been well documented and studied to understand the spinal cord processing of nociceptive stimuli[126] and analgesic sensitivity[127]. In neonates, both term and preterm, the flexion withdrawal reflex can be elicited by mechanical or electrical stimulation[81,84,128]. Evoking the reflex occurs at much lower thresholds than in adults and non-painful events can also produce reflexes[77].

During development there is reorganisation within the spinal cord and a decrease in its excitability[129,130]. This sees a threshold increase of the flexion withdrawal reflex[83,84]. Preterm infants less than 35 weeks postconceptional age show sensitisation and a reduced threshold to innocuous stimuli[84] but to the same innocuous stimuli, older infants display habituation. Habituation can also be seen in adults with intact or divided spinal cords[131,132] suggesting that it is activity within the spinal cord responsible for this.

Measuring the flexion withdrawal reflex using EMG cannot tell us about the subjective experience or perception of pain but is useful in understanding the development of spinal nociceptive processing.

1.3.2.2 Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) uses spectroscopic methods to measure the concentration of oxygenated and deoxygenated haemoglobin in blood and tissue. The absorption of near-infrared light is dependent on the amount of oxygenated and deoxygenated haemoglobin present in the tissue[133]. Optode pairs are placed on the surface of the scalp and local changes to the emitted and absorbed light can quantify changes in tissue oxygenation. Haemodynamic activity is a proxy of the functioning brain and assumes that the increase in tissue oxygenation is due to a change in regional cerebral blood flow, in response to an increase in underlying neural activity[134].

Cortical haemodynamic activity in response to noxious events - heel lance, venepuncture, and endotracheal tube suctioning and repositioning - has been recorded in infants[112,135,136], and widely used in other neonatal research[137]. From 25 weeks of gestation, an increase in haemoglobin concentration can be observed in the somatosensory cortex. This increase is dependent on the infant's age, but can also be influenced by other clinical features, including sleep state. This increase is not seen following innocuous stimulation on the surface of the skin, despite the flexion withdrawal reflex being elicited, suggesting specificity of the observed changes to noxious input.

Further studies have demonstrated a good correlation between the magnitude of the haemodynamic changes recorded in the contralateral somatosensory cortex and the change in neonatal facial expression following noxious stimulation[138]. The number of prior painful procedures and location of the stimulus have additionally been shown to alter the size of the haemodynamic response[112,139,140]. All these studies lead to the conclusion that infants exposed to noxious stimuli during the neonatal developmental period, process noxious

information at the level of the cortex, even though some infants do not manifest an observable behavioural response[141].

NIRS is prone to movement artefacts that can interfere with recording quality and because of this, its use at the bedside remains challenging. However, these studies provide evidence that the response to noxious stimulation is not purely reflexive in the neonate, but the information is transmitted to the infant cortex. As with EEG, NIRS cannot provide us with the knowledge of an infant's perception of pain, but rather, tells us that activity in cortical regions of the brain occurs and is necessary, for the infant 's perception of pain.

1.3.2.3 Electroencephalography & Evoked Potentials

The EEG signal represents the summation of synchronised excitatory and inhibitory postsynaptic potentials from populations of cortical neurones. Through scalp electrodes, the direct measurement of temporal changes in the field potential generated spontaneously or evoked by the local activity in neurones can be recorded.

Event-related potentials (ERPs) arise following the brain's response to receiving external stimuli and are changes in EEG activity associated with a specific stimulus. Underlying neuronal processing causes the synchronised firing of neurones to produce characteristic peaks and troughs on the EEG. Sensory evoked potentials (SEPs) are generated by visual, auditory, and somatosensory stimuli, often referred to as somatosensory-evoked potentials (SSEPs). SSEPs are clinically used to assess the function and integrity of the somatosensory system and pathways.

Pain-related SSEPs were first identified in the 1960s[142] and have traditionally been recorded using transcutaneous electrical stimulation (TENS) applied to peripheral nerves. Ideally, an experimental nociceptive stimulus should selectively activate nociceptors, be highly controllable, reproducible, and safe[122]. The stimulus must be short enough with a high enough intensity to cause sufficiently synchronised firing of afferent nerve fibres, facilitating the recording of time-locked brain responses. However, electrical stimulation is limited in the study of pain because it lacks selectivity, exerting its effects on a wide range of peripheral afferent fibres. The development of intraepidermal electrical stimulation (IES)[143] as a basic research and clinical tool has vastly improved the potential use of electrical stimulation to study nociception. The needle of IES electrodes protrude 0.2 mm and by pressing the electrode gently on the skin, the needle tip is inserted into the epidermis and superficial layer of the dermis where nociceptive fibres terminiate[144]. This method selectively activates Aδ nociceptors with no concomitant activation of deeper Aß mechanoreceptors. C fibre nociceptors have a very high electrical threshold, and the use of IES to selectively target them without activating deeper structures is more difficult[143].

Aδ and C fibre nociceptors do however; have differing thresholds to thermal stimuli. C fibre nociceptors are activated at 40°C, and Aδ fibre nociceptors at 46°C. This makes it possible to selectively activate them by laser beams[143]. Lasers generate radiant heat that can selectively excite free nerve endings in the superficial layers of the skin. The brain response following laser stimulation has been shown to produce highly synchronised firing of afferent fibres[145] producing laser-evoked potentials (LEP). Due to their high-synchronicity, LEPs can be observed in single trials.

Mechanical stimulation excites both non-nociceptive Aß fibres and nociceptive A∂ fibres. However, recent use of PinPrick stimuli have demonstrated that brain evoked potentials can be elicited following activation of type I A-fibre mechano-heat (I-AMH) nociceptors[146]. PinPrick stimuli are flat-tipped probes (diameter 0.25 mm) and in healthy subjects are able to produce pinprick-evoked potentials (PEP) at the vertex position on EEG recordings[146]. The increase in amplitude following sensitisation by capsaicin suggests that PEP may be useful in assessing experimental secondary hyperalgesia.

ERPs, such as those described above, are usually sensitive to the novelty of a stimulus. Differences in modality, location, intensity, and timing of the stimulus will cause a larger ERP than subsequent identical stimuli. Strong habituation has been described to repetition of LEPs[147], but novelty is not the only factor known to influence the amplitude and morphology of LEPs. For example, the magnitude of evoked response is also determined by the saliency of the stimulus. In an experiment involving a train of three consecutive laser stimuli, Ronga *et al.* observed a change in nociceptive ERP when the third stimulus differed in intensity from the previous two identical stimuli[148]. The study found that the third ERP was significantly larger than the first ERP following an increase in the intensity of the third stimulus[148].

Nociceptive-evoked potentials have also been successfully recorded following heel lancing in neonates[149] where time-locking hardware can be attached to the lance itself. Older children are not subject to heel lancing and must therefore be studied using other clinically required acutely painful procedures, such as vaccination and cannulation.

ERPs can be used during the intraoperative period to monitor neural pathways under general anaesthesia and potentially prevent injury to the nervous system. To a varying degree all anaesthetics, including sevoflurane, influence evoked potentials by increasing latency and reducing the amplitude of the ERP in a dose-dependent manner[150]. There are three types of SEPs used clinically, SSEPs, visual evoked potentials (VEP), and auditory evoked potentials (AEP). With volatile anaesthetics, the early spinal and subcortical waves are affected less than the later cortical potentials[151]. SSEPs and VEPs are therefore more affected than AEPs, which can be recorded from the brainstem. It should be noted however, that the concomitant use of N_2O should be avoided if recording cortical SEPs due to its profound depressant effect on SSEPs and VEPs when combined with a volatile anaesthetic[152]. SSEPs are most often recorded through electrical stimulation of the median nerve and, similar to adults[153], studies in children show an increase in the latency to the N20 component and a reduction of mean cortical amplitude of the N20-P45 component under maintenance anaesthesia of 2% sevoflurane[154].

Due to the resilience of AEPs under anaesthesia they have been identified as a potential marker for anaesthetic depth with peaks in the mid-latency auditory evoked potential (MLAEP) particularly sensitive to sevoflurane[155]. In children aged 2-10 years, MLAEPs are sensitive to volatile anaesthetics[156], with latencies to MLAEPs increasing with increasing age in response to sevoflurane in a dose dependent manner[157].

The majority of EEG studies investigating cerebral processing of noxious stimulation have analysed evoked potentials. However, these studies can only study cerebral signals that are implicitly phase-locked to the onset of a stimulus. Spectral analysis of the EEG has been useful in investigating long-lasting pain states[158]. By applying a Fourier transform

to the EEG signal, observations of oscillatory changes to the EEG signal can be made. Oscillatory responses may be important in central pain processing in both physiological and pathological pain conditions[159]. Oscillations in the EEG can be spontaneous, evoked, or induced, where evoked or induced oscillations relate to a stimulus. Spontaneous oscillations form background activity, and while evoked oscillations are phase-locked to a stimulus, induced oscillations are not. Pain-related oscillations can be influenced by internal and external factors with an increase in delta power highly correlated with stimulus intensity, perceived pain, and analgesia[160,161]. Reductions in alpha power can be observed following an increase in delta activity, and evoked by either A∂ or C fibre activity[162,163]. The relevance of alpha band reduction is unknown, but it is thought that the presence of alpha oscillations is the result of a relaxed wakeful brain and their suppression is also observed during sensory stimulation or cognitive tasks[159]. Reductions in the beta band activity following laser and electrical stimulation have also been recorded[164,165]. Beta band oscillations have been linked to sensory motor processing and may be involved in the coupling between the perception of pain and behavioural responses[159]. An increase in fast oscillations in the gamma band frequency can also be observed in the primary and secondary somatosensory cortices following noxious stimuli[165,166] and may be relevant in the central processing of nociceptive input[165]. The interplay between these networks of cortical oscillations are known to be altered in pathological conditions and may be a contributing factor to the formation of chronic pain states[159].

1.3.2.4 Magnetic Resonance Imaging

MRI has revolutionised the way we approach pain research. Being non-invasive and non-ionising, MRI is the ideal technique to investigate the anatomical (e.g. structure and

maturation) and functional (e.g. activity and connectivity) networks of the brain involved in the CNS processing of sensory stimulation during early human development.

Functional MRI (FMRI) is a non-invasive technique that assesses cortical activation. The most commonly used contrast method is blood oxygen level-dependent (BOLD) imaging. BOLD measures the magnetic change in haemoglobin between the oxygenated and deoxygenated states that accompany neuronal activity[167,168], but it is important to be aware that BOLD measures dynamic changes in blood flow and is not directly linked to neural activity. In newborns, most FMRI studies have focused on the visual system, and have demonstrated that visual activation can be obtained in sedated infants[169]. The ability to view abnormal decreases in visual activity[170], asymmetric, or absent activations[171] makes FMRI a feasible tool to view the visual cortex in disease[172]. This could be extrapolated to other neonatal conditions, possibly including the assessment of pain. The non-invasive nature of FMRI allows for repeated scanning in children and longitudinal studies investigating the development of neural networks can be achieved.

In the MRI scanner, neonates and infants are asleep, either naturally or with sedation, and this is one of the major limitations of FMRI - due to the lack of cooperation, tasks involving active participation cannot be performed[169]. However, even asleep, infants show distinct brain activations to cognitive[173] and sensory[174] passive tasks. The Developing Human Connectome Project (dHCP), a joint project led by three UK universities, aims to create a connectivity map of the developing human brain between 20 - 44 weeks post-conception age (www.developingconnectome.org). Linking together MRI, behaviour and genetic information, the project has begun by optimising neonatal FMRI protocols necessary to understand the physiology and pathophysiology of the developing brain[175].

A more recent discovery is the fluctuation in BOLD FMRI response to intrinsic neuronal activity[176]. Collecting FMRI data during periods of quiet rest (i.e. without any task) have revealed strong anatomical and functional connections forming a resting-state network (RSN) in the absence of external stimuli[177]. Resting-states in infants born preterm or at term have found five consistent RSN cortical 'hubs': the primary visual cortex; bilateral sensorimotor areas; bilateral auditory cortex; a network including the precuneus area, lateral parietal cortex, and the cerebellum; as well as an anterior network that incorporated the medial and dorsolateral prefrontal cortex[178]. With resting-state networks being identified in healthy infants there is a growing interest in evaluating network alterations in infants with brain injury[172].

In neonates, there is great potential for the various MRI techniques to elucidate the higher CNS processing of noxious stimuli. RSNs in healthy neonates do not entirely correspond to those commonly found in adults[179]. Assuming that the cortical hubs denote areas involved in tasks requiring high information processing[180] some reorganisation of RSNs must occur between birth and adulthood. It is proposed that cortical hubs in infants may be dominated by processes involving reflexive behaviour, and those in adults centred toward complex, adaptive behaviour[179].

While MRI has been extensively used in adult pain studies, and specific network disruptions have been described in several adult pathologies including chronic back pain[181], its application in children has only recently begun and to date has not been used to advance our understanding of infant pain. In adults, pain studies utilising FMRI have involved healthy volunteers or patients. Despite the variability in individual experience, a central 'pain matrix' has been described with some areas of the brain showing consistent

patterns of activation following noxious stimulation[182]. These brain areas include, but are not limited to, the thalamus, SI, SII, insula, forebrain, and anterior cingulate cortex (ACC)[1] and involves areas traditionally involved in modulating the cognitive[183] and emotional[184] aspects of pain perception.

To date, there is only one MRI study investigating the processing of noxious stimuli in children[185]. Cerebral pain responses in children and adolescents aged 11 to16 years were compared in children with experience of a NICU born preterm or at term, and term born children without NICU experience. Preterm but not term NICU children displayed an exaggerated noxious-specific response compared with controls when stimulated with moderately painful heat stimuli. Brain regions, which classically form part of the adult pain matrix, were activated during noxious stimulation but were not observed during non-painful warmth stimulation. Further, ex-preterm children displayed an increased sensitisation to painful stimuli with- out habituation. This would suggest that in the long term, previous experience of NICU might cause neurodevelopmental changes and cause an alteration in some aspects of nociceptive processing.

A recent imaging development may begin to disclose the reorganisation of cortical hubs. Diffusion tensor imaging (DTI) makes it possible to visualise the connections between different brain regions by measuring changes in the diffusion of water in white matter tracts[186]. DTI can identify differences between normal and abnormal tracts, and when combined with FMRI studies can help understand the structural connectivity between different brain regions[187]. With resting-state functional connectivity being based, but not exclusively, on direct anatomical connections[188], the assessment of the functional and structural relationships in the infant brain may begin to be investigated.

1.4 The Long-Term Consequences of Early-Life Pain

Only three countries have seen a fall in their preterm birth rates over the past 20 years[189]. Sadly this has not been the case in the UK, and with a 20% increase in the overall birth rate, 12% of all babies born in the UK will require special care with a further 2.5% needing neonatal intensive care[190]. As part of their essential medical treatment, preterm newborns receive multiple invasive procedures to compensate for their physiological immaturity in order to aid their survival to term. During the first two-weeks of admission to the NICU, newborns receive an average of 14 painful procedures a day (e.g. venepuncture, arterial or venous access, and artificial ventilation)[191]. In the most ill of babies however, this number can increase dramatically, with 50 painful or stressful procedures being commonplace each day[192].

On average infants receiving neonatal intensive care will remain in hospital for 56 days[190] and the repeated exposure to noxious procedures during this period is of particular concern. Furthermore, surgery may be required to treat complications of prematurity or correct congenital abnormalities. During this vulnerable time, an infant would normally be developing in the safety of an intrauterine environment, protected from excessive sensory stimuli and stressful events[193].

'If all that sugar and sucking does is reduce the external expression of pain then we are only treating ourselves, the carers, rather than the children'

~ Rogers (2000)[194]

Up to 80% of NICU admitted infants are not given specific analgesia for potentially painful procedures[192]. With a number of human studies indicating to long-term developmental consequences of early pain exposure[195], it is important to minimise these by

the effective management of early pain. Despite this, physicians remain hesitant in administering analgesia and there is little information on effective pain management strategies in neonates.

Only 48% of parents recall the use of pain-relieving drugs in their children[190] and despite 90% of medical staff believing that neonates experience pain as severely as adults, many procedures are performed without analgesia or comfort measures[196]. Opioids are the most frequently administered analgesic used in NICU[197], but with the risk of respiratory depression, hypotension, muscle rigidity and the potential increase of necrotising enterocolitis[191] they are rarely given for non-surgical invasive procedures. Topical anaesthetic cream is more effective against venepuncture than a heel lance[198] and the administration of sucrose reduces clinical pain scores after venepuncture but not following intramuscular injections or heel lancing[199].

The most frequently reported comfort methods used during clinical painful procedures include non-nutritive sucking, swaddling and cuddling [200]. Administration of sucrose prior to a painful procedure is also regularly used[201]. However, the term analgesia is not used to describe these interventions and they should not be considered as an adequate substitute for pharmacological analgesia[202].

There are very few studies looking at the effects of prematurity and early-life experience on adult chronic pain. To date only three exist and have provided mixed results. One cross-sectional study saw a 120% and 60% increased risk of chronic pain in young adults born with low birth weight and NICU admission respectively, though not significantly increased[203]. However, the risk of low back pain was shown to be lower in

young adults born at a low birth weight[204]. Neither of these studies takes into account the potential environmental, health, or social confounders that may play an important role. When adjusted for sex, social class at birth, age, or birth weight, the 1958 birth cohort study saw a non-significant 26% increase in risk of chronic widespread pain in those born prematurely or very low birth weight[205].

1.4.1 EFFECTS OF NEONATAL PROCEDURAL PAIN

'Repeated pain experiences in neonates may induce activity-induced changes in the functioning of pain pathways that persist well beyond infancy'

~Hermann et al. (2006)[206]

In neonates, the immediate effects of acute procedural pain can last for hours or days. This results in a greater behavioural response to both noxious and innocuous stimuli due to local peripheral changes at the site of injury[83,207,208]. Additionally, abdominal reflex studies show mechanical hypersensitivity in infants following abdominal surgery[85,209]. Beyond the neonatal period, developmental consequences following tissue injury also exist.

Neonates having spent at least 40 days hospitalised in NICU display greater nociceptive specific brain activity to noxious stimulation when they reach term age than healthy term-born infants[123] and circumcision performed during the first five days of life causes an increased behavioural response to routine vaccination at 4-6 months of age in circumcised boys than those uncircumcised[210]. A sustained hypersensitivity to innocuous mechanical stimulation is seen during the first year of life of premature infants[211], whilst at four and a half years, previous extremely low birth weight infants are likely to experience more unexplained stomach aches, headaches and other complaints compared to age-

matched term-born children[212]. Longitudinal studies of 9-12 year-olds found evidence of altered sensory processing in ex-NICU infants[213], and ex-premature 12-18 year-olds having an increased number of tender points with lower tenderness thresholds[214].

However, finger-lancing for blood collection in aged- and developmentally-matched premature and full-term infants have evoked the same behavioural responses[215], with parental reports of 18 month-old toddlers suggesting that ex-premature infants have a decreased reactivity to pain[216]. Reinforcing these findings, a study of 9-14 year olds found that children who had been admitted to NICU as an infant displayed elevated heat pain thresholds but a greater perceptual sensitisation - the gradual increase of a subjective pain score during prolonged noxious stimulation[206].

'Neonatal care and surgery in [extremely preterm] children are associated with persistent, modality-specific changes in sensory processing'

~Walker et al. (2009)[217]

While changes in local sensitivity at the site of tissue damage is due to alterations in peripheral nociceptive processing, it is proposed that global changes to thermal sensitivity is driven by centrally-mediated alterations in nociceptive pathways [217]. Prematurely-born children who have undergone major surgery in the first three months of life display localised decreases in thermal and mechanical sensitivity at the site of injury, as well as generalised decreased thermal sensitivity at a different body site (i.e. the thenar eminence). This suggests that both peripheral and centrally mediated long-term changes can occur following neonatal intensive care and surgery [217].

Long-term effects appear to be different if produced by acute procedural or deep somatic and visceral pain. Deep somatic and visceral can lead to sensitisation of pain

responses, but acute procedural pain may lead to a reduced responsiveness to subsequent noxious stimuli[218]. A dramatic illustration of the differences seen between injury severities is shown in children aged 9-16 years who suffered moderate or severe burns during infancy. Those moderately burned has a lower mechanical pain threshold, whereas those severely burned had an elevated thermal pain threshold[219].

Prematurity, length of NICU stay, and early surgery all have long-term consequences on subsequent nociceptive processing and pain perception. Invasive procedures may initially decrease nociceptive thresholds, but subsequent re-injury may produce an increased hypersensitivity that can only be explained by central nociceptive alterations.

1.4.2 EVIDENCE FROM ANIMAL STUDIES

'The postnatal period is a time of considerable synaptic growth and reorganisation in the dorsal horn of the spinal cord & the developing nociceptive system responds differently to injury when compared to the mature adult system'

~Fitzgerald & Howard, 2003[220]

Data from human studies show that early life experience can cause changes in future sensory and nociceptive processing. However, identifying the cellular mechanisms responsible is impossible in humans and animal models are used. The first 7-10 days of life in a rat is equivalent to a human infant of 24-40 weeks gestational age[59,221].

At birth, the rat spinal cord is still immature and throughout the first three weeks of life, it develops into its adult form. In the neonatal rat, the different penetrance rate of neuronal afferents from the dorsal root ganglion means that nociceptive reflexes are

mediated by myelinated A-beta and A-∂ rather than unmyelinated C-fibres - during embryogenesis, myelinated afferents enter the spinal cord earlier[222,223]. A-beta terminals are larger than in adulthood and extend into spinal laminae that will not be innervated in later life[76,78], this contributes to the increased receptive field of dorsal horn neurones and overlapping A and C fibre signalling[87]. This overlap causes reflex withdrawal to normally innocuous stimuli while noxious stimuli produce disorganised reflex responses[74]; these disorganised responses are also seen in young humans[209].

Nociception and the experience of pain are modulated by many mechanisms and there is a large descending modulatory influence on the spinal cord. The brainstem plays a major role and the spinal cord is modulated by signals from the PAG and RVM descending in the dorsolateral funiculus. These signals are either excitatory or inhibitory and act on nociceptive neurones in the dorsal horn to either increase or decrease nociceptive signals. The development of descending modulation is discussed in detail in Section 1.2.3.

The developmental immaturity of the nociceptive system during the first three weeks of life suggests that excitatory spinal processing predominates, causing an enhanced response to both noxious and non-noxious stimuli alike. This excitability and lack of any descending control may be important in allowing for plasticity in the nociceptive system following early pain and injury.

1.4.2.1 Long-Term Changes in Animals

'Evidence that early experience influences pain in later life suggests a specific developmental plasticity in the nociceptive system'

~Beggs et al. (2012)[224]

Just as human infants, animals are unable to report their pain. Alterations in their CNS also cause long-term changes to nociceptive responses and inflammation caused by repeated pin prick leaves rat pups hyperalgesic to thermal stimuli lasting several weeks[225]. Neonatal inflammation of seven days results in enhanced spinal neuronal activity and increased primary afferent innervation of the adult dorsal horn[226].

Global hyperalgesia in adult rats can be the result of pain experience as pups and is mediated by alterations in descending modulation[227]. Incisions of skin and underlying muscle cause the release of nerve growth factors evoking the hyperinnervation of the skin. Hypersensitivity to noxious stimuli is then seen in later life[228] and is consistent with human studies finding hypersensitivity following neonatal injury and surgery[85,210,229].

1.4.3 CRITICAL PERIODS OF NOCICEPTIVE DEVELOPMENT

As with all other sensations, the development of nociceptive pathways is modulated by sensory inputs during early childhood. There are critical periods during normal development where changes in neuronal activity can dramatically influence development. Such critical periods have already been defined for auditory and visual modalities along with the stress response[230]. More recently, critical periods for pain have begun to be seen[75,231]. Stimulation during this critical period will cause long-lasting developmental changes, but the same stimulation outside of this will cause no long-lasting effect[218].

The nociceptive critical period of a rat is during the first three weeks of life, and long-lasting nociceptive consequences are displayed in premature human infants between 24 and 38 weeks' gestation. It is thought that the first two weeks of a rat's life equates to the

last trimester of human gestation; this would suggest that the nociceptive critical period of a rat is similar to that of a human. Developmental mechanisms that mediate nociceptive processing in a rat are also likely to be similar to that of a human where any interference of the development and maturation of the spinal cord or descending modulatory pathways may have lasting effects.

1.4.4 MICROGLIAL REACTIVITY

'Glial activation seems to be a common underlying mechanism that leads to pathological pain in a number of pain syndromes with widely different aetiologies'

~Milligan & Watkins (2009)[232]

Increasingly, microglial reactivity has been implicated in the initiation of chronic pain in animals[233] through a positive feedback loop transforming acute to chronic pain[233,234]. Evidence of altered microglial reactivity can also be seen in chronic pain patients[235,236] and may play a major role in the 10-50% of postoperative patients who develop persistent pain[237]. Neonatal injury is associated with persistent enhancement of neuroimmune responses to noxious stimuli, and adult pain responses appear to be 'primed' by tissue injury during the neonatal period[224]. This suggests that the long-term impact of early life experience may predict enhanced painful responses to future injury.

1.5 General Anaesthesia

'All anaesthetic drugs are poisons. Remember that in achieving a state of anaesthesia you intend to poison someone, but not kill them - so give as little as possible'

~ 'Anaesthetics for Medical Students', 1980 in 'Anaesthesia: A Very Short Introduction'[238]

Muscle relaxation, narcosis (hypnosis/unconsciousness), and analgesia - the 'triad' of anaesthesia coined in 1950[239] is often represented on a triangle, and is still taught to medical students today. Yet for all its simplicity, the triad model is out of date and is insufficient to explain the complex pharmacokinetics and pharmacodynamics of anaesthetic agents, the distinct processes involved in consciousness, amnesia, and the modulation of other higher cortical functions.

At high enough doses, a general anaesthetic drug can induce all three points of the triad and by causing unconsciousness; the general anaesthetic is arguably the most important. However, as inferred by the quotation above, general anaesthetics are not without their problems and by adding an analgesic to prevent pain and a muscle relaxant to prevent reflexive movement, you can reduce the dose of anaesthetic required. Each component effects the other and a balanced anaesthetic technique where the three doses are minimised is ideally practiced[240].

During surgery, the dose of an anaesthetic agent is continually adjusted to maintain an adequate depth of anaesthesia in response to the many types stimuli that occur. This is important because inadequate anaesthetic dosing can lead to awareness during surgery, while excessive dosing may cause unwanted side effects.

1.5.1 CONSEQUENCES OF INADEQUATE & EXCESSIVE ANAESTHESIA

Reducing the anaesthetic dose by the addition of an analgesic and muscle relaxant has additional benefit of reducing reflexive movement and sympathetic activation, however, it can also lead to the increased possibility of becoming aware of intraoperative events[241], often reported postoperatively[242]. Intraoperative awareness is thought to be underreported due to the dose dependent nature of CNS depression caused by general anaesthetics[243] - at sub-anaesthetic doses, memory and recall are two of the first modalities to be suppressed[244].

Intraoperative awareness where the recollection of being awake at a time when consciousness is not intended[245]) is rare, and occurs in 0.1-0.7% of adults following anaesthesia[246]. This can increase to 1.0% in high risk patients where the use of anaesthesia is lessened[245]. In children, the recollection of real events can be found in 0.7% of patients[247,248] but more common is the recollection of dreams[249,250], where 10.4% of 5-12 year olds recall pleasant dreams unrelated to their hospital experience[251]. Dreaming during anaesthesia appears to be a common experience in children, mostly in younger children and those that experience intraoperative awareness[251]. Those children who recall intraoperative pain appear to be less psychologically affected than adults[252] where the memory of pain can unfortunately cause post traumatic stress disorder[253,254].

In excess, anaesthetic drugs cause cardiorespiratory depression and can have effects on other major organ functions. The immature physiology and pharmacokinetics of a child puts them at greater risk of these adverse effects and potential drug toxicity. Neonates experience the greatest cardiovascular depression[255,256] while infants reach anaesthetic equilibrium faster due to reduced tissue solubility[257]. The lungs of infants are also affected

and with increasing anaesthesia, respiratory rate slows and a reduction of functional residual capacity and tidal volume are seen[258]. Furthermore, postoperative apnoea is commonplace in preterm neonates[259].

1.5.2 VOLATILE ANAESTHETIC AGENTS

Anaesthetics have come a long way from the days of ether and chloroform, and in the 1930s, the development of CFC (chlorofluorocarbon) chemistry led to the discovery of alternative anaesthetic derivatives. In 1951, Charles Suckling created halothane. This potent anaesthetic dominated anaesthesia for more than 20 years and was derived from chloroform. Fluorinated ethers form the basis of all other modern volatile anaesthetics - similarly to halothane, adding fluorine to ether makes for a more stable and less flammable molecule.

Volatile anaesthetics are colourless liquids. Their low boiling point allows them to evaporate and be vaporised easily within anaesthetic machines. This allows them to be inhaled through the lungs into the alveolar capillaries and circulation. The aim is to achieve CNS drug concentrations to produce anaesthesia without any detrimental effects to other organs. Through inhalation, a volatile anaesthetic bypasses the venous phase seen with intravenously administered anaesthetics accounting for dilution of the anaesthetic agent. However, there is a delay from the time of first inhalation to sufficient alveolar concentration to induce anaesthesia. Within ten minutes, the end-tidal concentration of sevoflurane reaches a plateau that is close to a constant inspired concentration of sevoflurane. The small differences seen between inspired and expired concentrations are due to the distribution, metabolism, and non-pulmonary excretion of sevoflurane. The

speed of onset of volatile anaesthetics depends on the individual drug's solubility in water - the less soluble, the quicker the onset. Desflurane has the fastest onset, as it is the least water soluble, followed by nitrous oxide, sevoflurane, and isoflurane. Therefore, measurement of the end-tidal concentration of anaesthetic from the patient's breath at equilibrium is approximately the concentration of anaesthetic within the brain.

1.5.2.1 Minimum Alveolar Concentration (MAC)

How potent a volatile agent is expressed as the minimal alveolar concentration (MAC). The concentration of an anaesthetic agent which, at equilibrium, prevents reflexive movement to a standard surgical skin incision in 50% of subjects[260]. The MAC acts as a guide for the concentration of anaesthetic required to produce anaesthesia and is useful for comparing the potencies of individual anaesthetics, as a 1.0 MAC of any anaesthetic should cause similar behaviour across patients. The less potent an anaesthetic is, the greater the concentration required to reach 1 MAC. In pharmacology, MAC is a form of ED_{50} (ED, effective dose) with the ED_{95} preventing reflexive response in 95% of subjects. Volatile anaesthetics are very potent and the difference in concentration between ED_{50} and ED_{95} small[261].

The MAC value calculated and displayed on anaesthetic monitors is calculated from standard MAC values from adults and various factors can affect MAC readings, most importantly, age. Due to the scarcity of infants and children to study, MAC values have had to be estimated from small samples using an up-and-down method[262]. An individual concentration of anaesthetic is administered to a child and it is noted whether they respond to surgical stimulation or not. If one responds, the subsequent child will be given a higher dose or vice versa. Through this method, the ED_{50} can be estimated when equal numbers

of children move or not to stimulation[263], but this concentration changes with age. The MAC of halothane and isoflurane increases from birth and peaks at around six months of age, before declining over childhood and adulthood[264,265]. Whereas the MAC of sevoflurane decreases with increasing age from birth[266]. The MAC of sevoflurane in neonates (1 - 6 months) and infants (6 - 12 months) is similar at 3.3% and 3.2% respectively, whilst the MAC in children (1 - 12 years) plateaus at approximately 2.5%[266].

1.5.2.2 Sevoflurane

Of all modern anaesthetics, sevoflurane is the drug of choice in the paediatric population primarily due to its differences from others. Chemically similar to enflurane and isoflurane, sevoflurane is less volatile, however, being less blood soluble allows for a more rapid induction and recovery. Sevoflurane is less pungent too, while the unpleasant smell of desflurane or isoflurane can make them unsuitable for inhalation induction, the fruitier smell of sevoflurane makes it more pleasant. Anaesthesia is produced without analgesia or the potentially harmful epileptogenic spikes seen with desflurane and isoflurane.

Sevoflurane lowers blood pressure by vasodilatation, reducing systemic vascular resistance[267], and lowers heart rate by depressing cardiac contractility[268]. The lower heart rate reduces the oxygen requirement of myocardium, potentially improving the relationship between supply and demand.

As with all anaesthetics, sevoflurane reduces tidal volume and respiratory rate but is a relatively low respiratory irritant allowing for smoother induction. The hypoxic drive is reduced due to a lowered sensitivity to carbon dioxide and muscle tone is reduced within bronchial smooth muscle, increasing anatomical dead space.

Approximately 95-98% of the sevoflurane uptake is then eliminated through the lungs, with 2-5% being metabolised by cytochrome P_{450} to produce hexafluoroisopropanol (HFIP) and inorganic fluoride ions[269]. A plasma fluoride ion concentration of 50 µmol/L is quoted as the threshold for renal toxicity and after prolonged exposure, sevoflurane metabolism can produce levels above this threshold[269]. This has led to the US Food and Drug Administration (FDA) recommendation that sevoflurane be used with caution in patients with renal disease. At temperatures encountered clinically, the potentially hepatotoxic HFIP is conjugated rapidly in Compound A. Compound A is known to be nephrotoxic in rats[270], however there have been no signs of nephrotoxicity in adults or children[271-273].

1.5.3 MECHANISMS OF ACTION OF ANAESTHETIC DRUGS

Many agents can produce general anaesthesia and how they achieve this can be described by their molecular action and their effect on specific parts of the CNS.

1.5.3.1 Molecular Targets for General Anaesthetics

Anaesthetic agents are likely to have a myriad of cellular effects with individual agents being subtly different from one another. However, in 1984 it was found that anaesthetics interact with proteins[274] and that isomers of anaesthetic drugs, such as isoflurane[275], have

different potencies that cannot be explained by a non-selective mechanism. Many anaesthetic sensitive molecular targets have since been identified and the distribution of these receptors within the CNS implies that discrete neural pathways are involved in anaesthetic induced loss of consciousness (LOC).

The predominant molecular basis behind the action of general anaesthetics is through the $GABA_A$ receptor protein[276]. $GABA_A$ receptors are found throughout the CNS and their pentameric structure forms a chloride ion channel. The five subunits provide several potential binding sites for general anaesthetic agents along with benzodiazepines and anticonvulsants. A prominent example of discrete binding sites is with a N265M point mutation in the beta-3 subunit of transgenic mice[277]. This rendered the rodents insensitive to etomidate and propofol but they remained sensitive to alphaxalone, halothane and enflurane. With the identification of specific binding sites for volatile agents, nearly all anaesthetic agents have been found to potentiate postsynaptic $GABA_A$ inhibitory currents.

By inhibiting excitatory systems of the CNS, general anaesthetics have a further effect on synaptic transmission. AMPA receptors form the fast component of excitatory transmission but have little anaesthetic sensitivity[278]. However, the NMDA receptor plays an important role, as its blockade by nitrous oxide, xenon, and ketamine, produces analgesia. Found pre-, extra-, and postsynaptically, NMDA receptors have crucial roles in excitatory synaptic transmission, and plasticity through their uniquely slow activation and deactivation[279]. Most anaesthetics have some inhibitory effect at the NMDA receptor, but isoflurane and xenon compete with glycine at its specific site on the NMDA receptor[280].

Furthermore, volatile anaesthetics have been shown to target the two-pore domain K+ channels (K2P)[281,282]. K2P channels, which are similar to extrasynaptic GABA receptors, was originally identified in snails[283], where a potassium leak conductance was observed in the presence of anaesthetics. Similar observations were later identified in the K2P channels of mammals [284]. Halothane is known to activate many of the K2P receptor subtypes but the sensitivity of K2P receptors to other volatile anaesthetics is variable[285].

1.5.3.2 Central Targets for General Anaesthetics

There is currently no complete understanding for the mechanism for anaesthetic induced LOC. However, investigations of the thalamus, cortex, and hypothalamus suggest both a direct and indirect action of general anaesthetics.

Most anaesthetics produce delta oscillations (1-4 Hz) and spindles (waxing and waning 7-14 Hz events) that are generated within the thalamocortical (TC) system[286]. These features are also found in natural sleep and have lead to the belief that anaesthetic LOC and sleep share a common neurobiology. Studies into TC oscillations have predominantly been performed under steady state deep anaesthesia[287,288], and more recently begun to address how these oscillations relate to the onset of LOC[289-292]. Within the central medial thalamus, pharmacological activation is known to reverse the effects of sevoflurane[293,294], and the direct effect of etomidate and gaboxadol on extrasynaptic $GABA_A$ receptors enhance inhibition within the thalamus[295]. The expression of K2P channels on thalamic relay neurones suggests a further direct action of volatile anaesthetics in the thalamus[296].

During propofol and sevoflurane induction, the cortex reaches an electrophysiological state of anaesthesia ten minutes before the subthalamic nucleus[290]. This provides some argument against the thalamus as the sole site of action for general anaesthetics due to their major depressant effect within the cortex. The cortex also contains many of the molecular targets for anaesthetics and it is reasonable to suggest a direct action, particularly as ablation of the primary sensory ventromedial basal nucleus does not alter cortical arousal from anaesthesia in rats[297]. A direct thalamic action of propofol is unlikely because the hyperpolarisation activated current (Ih), responsible for spindle and delta oscillations[298] is inhibited by propofol[299].

In the hypothalamus, arousal and sleep active nuclei inhibit each other in the sleep regulating *'flip-flop'* switch[300]. Hypothalamic arousal nuclei from the ascending reticular activation system terminates in the cortex, passing through the thalamus and excitatory cholinergic input is provided directly to the thalamus from nuclei within the pons and hypothalamus[301]. Indirect inhibition of GABAergic reticular neurones causes an increased cortical arousal[302] with GABAergic agents administered to these nuclei resulting in EEG similarities with sleep[303,304]. Neurones within the adrenergic LC are sensitive to halothane[305] but are excited by sevoflurane and this is thought to be the source of the observed paradoxical excitation with sevoflurane[306].

In 1993, two studies discovered that it was the spinal cord and not the brain that mediated the immobility effects of volatile anaesthetics[307,308]. GABA is the most important inhibitory neurotransmitter within the CNS, however, often colocalised with and homologous to GABA receptors, are receptors for the inhibitory neurotransmitter glycine. Glycine receptors play a significant role in the lower brainstem and spinal cord where they

could potentiate the action of volatile anaesthetics[309-311]. The loss of response to a nociceptive stimulus appears to be determined by volatile anaesthetic action on the spinal cord[312]. The plausibility of glycine receptors as a target is emphasised by the finding that the MAC of isoflurane is not altered in the decerebrate rat[308]. Furthermore, volatile agents potentiate the inhibitory Cl^- currents activated by low but not high glycine concentrations indicating an increase in agonist affinity[313], while mutations in glycine receptors can ablate the effect of volatile anaesthetics[314]. Volatile anaesthetics have since been shown to disrupt motor output in the ventral horn of the spinal cord by specifically reducing interneuronal central pattern generator activity[315].

Anaesthetics have different receptor affinities and different sites of action, yet they all lead to the same state of loss of consciousness. It is likely that this is because of common mechanisms that modulate brain areas far from their receptor sites through global brain networks. Neurophysiological and neuroimaging techniques have started to identify brain networks involved in general anaesthesia.

It was initially thought that this common anaesthetic mechanism was due to a global depression in brain function. Positron-emission tomography (PET) studies have shown that most hypnotic anaesthetics cause a reduction in cerebral blood flow and cerebral metabolism[316], and EEG studies have shown that following a brief excitation, most anaesthetic agents depress EEG activity[317]. Ketamine is the exception to both these cases[318]. PET, FMRI, and EEG studies have shown that general anaesthetics act on specific brain regions. Propofol and volatile agents reduce activity in the cuneus/precuneus, parietal associative cortices, posterior cingulate cortex, prefrontal cortices, reticular formation, and thalamus in a dose dependent manner[319,320].

It has been suggested that anaesthetics trigger a switch in consciousness by altering thalamocortical connectivity. It is proposed that this switch could occur in the thalamus, where enhancement of GABA transmission, and the inhibition of glutamate and cholinergic transmission could depress thalamocortical, corticothalamic, and reticulothalamic loops, and therefore interfere with subsequent information transfer to higher brain structures[321]. The second possible site where this switch could occur is in the precuneus, which is known to play an important role in conscious processing[322]. However, recent FMRI evidence suggests that under propofol anaesthesia, the precuneus along with the posterior parietal and prefrontal cortices form an alternative cortical network that continues to respond to sensory stimuli[323]. It is therefore too simplistic to consider that there is a single brain region responsible for switching the brain into an unconsciousness state.

Anaesthetics that enhance inhibitory neurotransmission are likely to affect the cortex first and disrupt higher-level processing networks. Task-induced activation imaging studies show that anaesthetics modify connectivity in the default mode network, executive control network, and emotional and memory networks[318]. When anaesthetic agents induce a loss of consciousness, changes occur in brain networks where slow oscillations (0.1-1 Hz) appear across the cortex. The appearance of slow waves can disrupt functional connectivity between cortical areas, and may influence the central processing of stimuli under anaesthesia[323-325].

Feedforward projections from posterior to anterior cortical areas represent the transmission of incoming sensory information, whilst feedback projections transfer this information back from anterior to posterior cortical areas, which are important in the interpretation of sensory information[318]. Multichannel EEG measurements have seen feedback connectivity diminished with propofol and sevoflurane[326], but feedforward connectivity persisting[327], suggesting that primary sensory systems are intact under general anaesthesia.

1.6 The Electroencephalogram

Using a galvanometer, brain derived electrical activity was first measured in 1875 by Caton on rabbits and monkeys. A small fluctuating current was noted in the active animal that increased in amplitude during sleep and disappeared after death. However, it was more than half a century later that this important discovery was recognised and Berger began his galvanometer experiments on humans[328]. He demonstrated the alpha (8-12 Hz) rhythm in quiet conscious subjects and its blockade by higher frequency beta oscillations and eye blinks. Modern EEG recording devices in clinical practice are frequently used for localising focal brain disorders such as epilepsy or stroke, investigating sleep disorders and monitoring anaesthetic depth. Surface electrodes placed on the scalp records the voltage between two electrodes and provide a non-invasive technique for measuring electrical activity thought to derive from the pyramidal cells of the cerebral cortex.

It was originally thought that EEG rhythms were due to action potentials travelling along interconnected neurones. The frequency of the oscillations was proposed to be determined by the time taken for a series of action potentials to complete a loop of these

neurones - the circus movement theory. Later an alternative theory proposed that EEG oscillations were derived from the non-propagating potentials of cortical neurones[329]. It is now accepted that the EEG is formed by the summation of inhibitory and excitatory postsynaptic potentials in the cortex and that the deflections seen on the recording represent a change in voltage and polarity measured against a reference electrode over time. A degree of synchrony between the postsynaptic potentials of thousands of cells must occur to produce the oscillations in the delta (1-4 Hz) and theta (5-12 Hz) frequencies. Typically, the amplitude of the EEG is less than 100 μV and most commonly between 10-50 μV.

1.6.1 SLEEP

The EEG can be categorised in terms of its frequency. These are delta (<4 Hz), theta (4-7 Hz), alpha (8-13 Hz), beta (14-30 Hz), and gamma (>30 Hz). In the awake state, the EEG is disorganised, containing rapid oscillations of low amplitude. On eyes closing the EEG becomes more organised and alpha oscillation (8-13 Hz) are commonly seen. Once asleep, the EEG becomes more synchronised with slower oscillations and greater amplitude.

There are two distinct types of sleep and are categorised from the EEG. Rapid Eye Movement (REM) and Non-Rapid Eye Movement (NREM) sleep and a sleep cycle between these two states occur every 90 to 100 minutes. The stages of sleep are described in Table 1-1. Due to the lack of any clear distinction between NREM stages 3 and 4, both consisting of a large proportion of delta activity, stage 4 was merged into stage 3 (N3) in 2008[330]. These new sleep stage classifications are the terms used in this thesis.

Old Sleep Stage Classification	New Sleep Stage Classification	% Time Asleep	Frequency (Hz)	Amplitude (μV)	EEG Wave Type
Awake		N/A	>12	<30	Beta
Relaxed (Eyes-Closed)		N/A	8-12	<50	Alpha
NREM Stage 1	N1	5%	4-8	50-100	Theta
NREM Stage 2	N2	45%	4-8	50-150	Theta, spindles, K-complexes
NREM Stage 3	N3 Delta or SWS	12%	2-4	100-150	Delta & Theta
NREM Stage 4		13%	0.5-2	100-200	Delta & Theta
REM	REM	25%	>12	<30	Beta

Table 1-1: Stages of sleep. Taken from 'Sleep: A Very Short Introduction'[331].

N1 is also known as drowsy sleep and is characterised by the presence of theta waves. In this stage random limb movements and twitches of muscles occur. N2 contains sleep spindles and K-complexes (high-amplitude low-frequency events). During N2, conscious awareness of external events disappears and muscle activity diminishes. N3 or slow-wave sleep (SWS) is dominated by the presence of delta waves. It is here that a quarter of sleep time takes place and the amount of time spent in SWS is correlated to the amount of prior lack of sleep. As its name implies, REM sleep involves eye movements and EEG patterns are almost identical to those of an awake individual.

1.6.1.1 Paediatric Sleep

The EEG continues to develop with age[332]. By three years, theta oscillations have appeared, and by six, alpha oscillations are seen. This is slower than in adults but rises to 10 Hz between seven to eight years. The sleep pattern in children under six months is quite different than that of older children and adults. Rather than NREM and REM

stages, sleep is divided into quiet (QS) and active (AS) sleep[333]. Infants will also sleep more and throughout the day than adults. Normal NREM and REM pattern of sleep begin to develop from three months, and after six months, the individual stages of NREM sleep can be identified on the EEG.

1.6.2 ANAESTHESIA

As discussed in Section 1.5.3, the majority of general anaesthetics have effects on GABAergic systems. On the EEG, frequency changes are seen that are similar to that of sleep with the addition of burst suppression when under deep anaesthesia. The effects of anaesthesia has been described as biphasic describing an initial speeding of the EEG before slowing[334]. At subanaesthetic doses, rapid oscillations between 8-20 Hz are seen on the EEG representing the paradoxical excitation seen before anaesthetic doses are achieved. Once here, there is an increase of amplitude and a slowing of the EEG. Delta and theta waves become more prominent while alpha and beta waves diminish. Under very deep anaesthesia, most anaesthetics will inhibit EEG activity completely leading to an almost isoelectric EEG with short busts of slow frequency activity (burst suppression) that will disappear with continued anaesthesia administrations[335]. This characteristic slowing is reversed during recovery as anaesthesia is washed out.

In children aged two to 12 years a biphasic EEG response similar to that seen with adults has been seen with halothane and sevoflurane[336]. Following induction, 14-30 Hz oscillations are noted with sevoflurane causing spiky oscillations. As anaesthesia progressed, an increase in amplitude and delta oscillations appeared. Sevoflurane has been linked to the presence of seizure and spiking activity on the EEG[337].

1.6.3 THE EEG POWER SPECTRUM UNDER ANAESTHESIA

The EEG signal can be deconstructed into its constituent frequencies, their phase and amplitudes. Using the Fourier transformation, a power spectrum can be generated and the relative or absolute power of each individual frequency bands calculated.

1.6.3.1 Child Power Spectrum

Power within the EEG spectrum increases with age[338] and in children older than two years, an increase in low frequency power is seen during the induction of anaesthesia[336], similar to that found in adults. The effects of anaesthetics is proportional to its potency and in infants, EEG power at occipital electrodes is associated with arterial halothane concentration[339]. During anaesthesia, neonates and young infants have low power EEG signals mostly in the slow frequencies[339] and with sevoflurane, children up to ten years old have higher power in frontal electrodes than parietal[338], and frontal greater than occipital in infants[340].

1.7 Aims

In the UK, more than 235,000[341] children admitted to hospital each year receive an operation or investigation under general anaesthesia. The insertion of an intravenous cannula as part of normal clinical practice provides a useful nociceptive stimulus, creating an opportunity to measure electrophysiological responses using electroencephalography and electromyography. The aim of this DPhil project is not only to investigate, record and categorise any changes, but also to compare children with prior painful experiences and investigate the effect on pain sensitivity under anaesthesia.

1.8 Outline of Thesis

This thesis comprises of three interconnected studies. Beginning with the development and validation of clinically acceptable methods, the subsequent studies tackle measuring noxious stimulation in anaesthetised children and the potential long-term consequences of prematurity in anaesthetised children. Below is a brief overview of each study, described in the subsequent chapters.

Study One (*Chapter Three*): *Multi-Modal Pain Measurements in Anaesthetised Children*

This study describes the development of methods used to record motor, physiological and brain evoked activity in anaesthetised children. An integrative approach to studying nociceptive responses has been tested in neonates and has being translated into an anaesthetised paediatric population. A novel video-EEG time-locking technique has been developed and tested in this study and provides the possibility that sensitivity to noxious stimuli during anaesthesia could be investigated in other clinical populations.

Study Two (*Chapter Four*): *Noxious Stimulation in Anaesthetised Children*

Term-born children requiring an operation or investigation under general anaesthesia were recruited into this observational study describing altered patterns of EEG activity following experimental and clinically essential noxious stimuli. The novel techniques from the previous study have been used to demonstrate an increase in the EEG delta activity that is more sensitive than physiological and autonomic indicators of nociception. The size of the delta response was graded with stimulus intensity and the application of topical local anaesthetic to the site of stimulation inhibited the observed increase in delta activity.

Study Three (*Chapter Five*): *Anaesthesia & The Ex-Preterm Child*

The final study compares term-born children receiving a general anaesthetic with aged-matched prematurely born children. While under the influence of sevoflurane anaesthesia, ex-preterm children, born at less than 37 weeks gestational age, responded similarly to the noxious cannulation as term-born children. However, striking differences were seen in their background EEG activity under anaesthesia, with significantly lower levels of alpha and beta power observed.

Chapter 2

General Methods

Chapter 2

General Methods

2.1 Chapter Overview

This chapter covers the general methods used throughout this thesis. While the specific development of novel methods will be discussed in the following chapter (see Chapter 3 - Multi-Modal Pain Measurements in Anaesthetised Children), here, the ethical and practical consideration of pursuing clinical research in a paediatric population will be considered. The recruitment and exclusion criteria of study participants will be outlined, and the acquisition and collection of data from participating children will be discussed.

2.2 Clinical Research in the Paediatric Population

'Primum non nocere'
~ Hooker[342]

From James Young Simpson's dinner party discovery of chloroform in 1847 and Karl Koller's infamous experiment utilising cocaine as a local anaesthetic in 1884, self-experimentation has had a long history in anaesthesia[343]. The first documented case in medicine was by the 17th Century physician, Sanctorius of Padua, whose self-experimentation led to the discovery of metabolism[344]. However, whilst a few researchers

have advocated the use of self-experiments to gain knowledge and solve problems[345], conventional research studies are usually conducted on other people or animals. Human subject research has been through many atrocities in the past and several ethical codes have since been written, culminating in the Declaration of Helsinki. This states that *'Medical progress is based on research that ultimately must include studies involving human subjects'*, and that *'While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects'*[346].

Best clinical practice then, is based on the reliable evidence from scientific research. However, conducting clinical research in children and infants provides us with a specific set of ethical and practical challenges. With many historical accounts of demonstrations (Figure 2.1) and research studies performed on hospitalised children, often without their parent's permission[347], informed consent is the cornerstone to both clinical practice and scientific research.

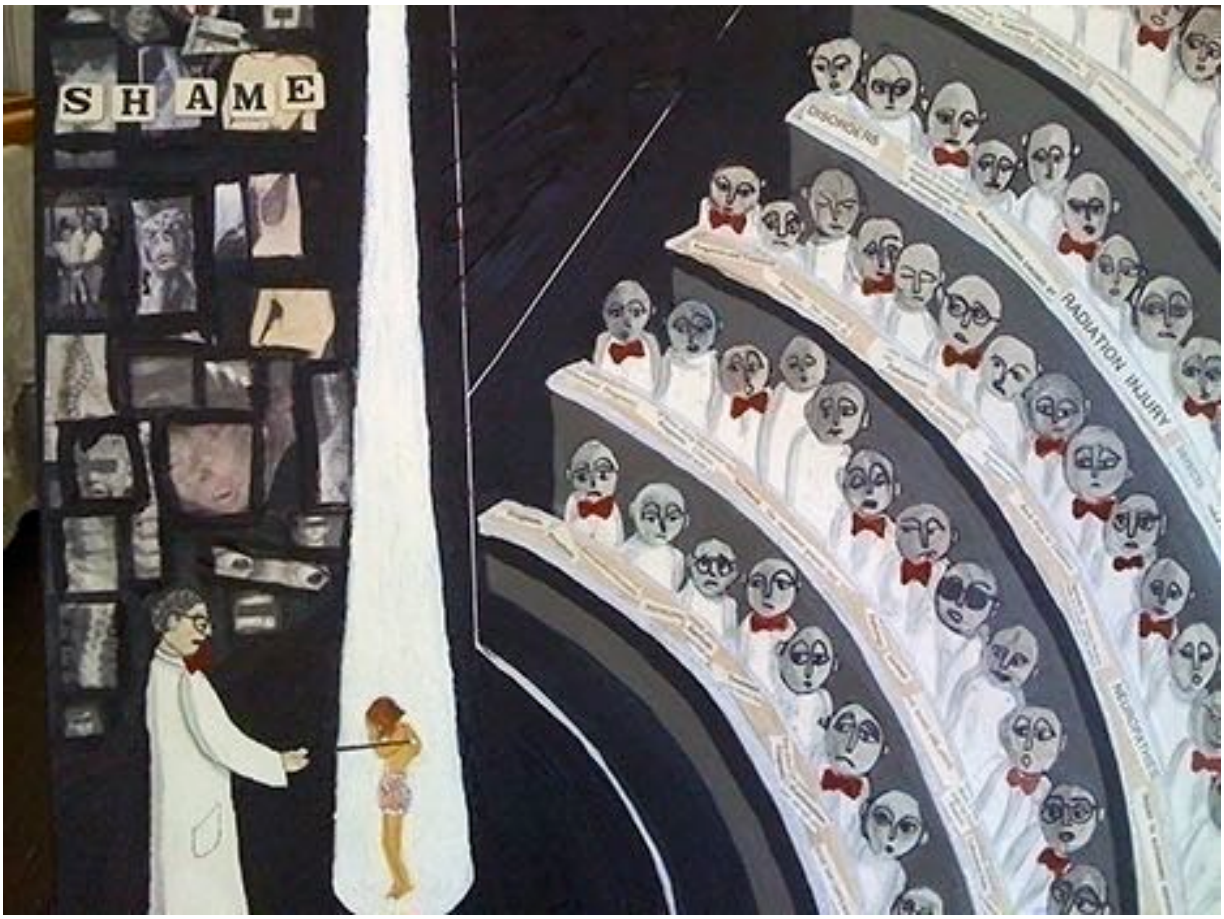

Figure 2-1: 'Shame' by Evelyn Berde

In order to give consent, a person must have the capacity to understand, retain, and consider the advantages and consequences of a situation, from adequate information and without coercion. The Mental Capacity Act (2005) also stipulates that a person must be able to communicate their decision. Risk may be acceptable in adults, who can evaluate their situation and provide fully informed consent, but this is unreasonable in young children; particularly those preverbal, who cannot understand the risk/benefit involved. Children and infants are therefore a vulnerable population, and the Declaration of Helsinki requires that *'All vulnerable groups and individuals should receive specifically considered protection'*[346].

Legally, to participate in research studies, informed consent is required by the parent or legal guardian of a child. However, studies should not only be presented to adult caregivers and the presumption of a child's comprehension should be applied to paediatric research with the assent being obtained[348]. A school-age threshold of 5-7 years has been proposed for obtaining assent due to the considerable capacity of children during this time[348].

'Primum non nocere' - 'Above all, do no harm'.

Studying pain in children can and does gain much media attention and becomes of great interest to the general public and scientists alike. With ethical concerns around the use of experimental pain procedures, the study of pain in children is often reliant on clinically essential noxious procedures when children require them.

2.2.1 ETHICAL APPROVAL

Children studied for this thesis were recruited from the Children's Hospital or Neonatal Unit at the John Radcliffe Hospital, part of the Oxford University Hospitals NHS Trust, Oxford UK. A favourable ethical opinion was granted by the Oxford Research Ethics Committee 'C' of the National Research Ethics Service with the study ID: 12/SC/0019 (for anaesthetic studies), and study ID: 12/SC/0447 (for neonatal studies).

2.2.2 NEONATAL SELECTION CRITERIA

All infants for neonatal studies were recruited from the postnatal wards of the John Radcliffe Hospital, Oxford. Healthy, term infants (≥37 weeks gestation) within the first seven days of birth undergoing antibiotic treatment were eligible to take part. Infants with hypoxic-ischemic encephalopathy, or any neurological problems were not eligible for inclusion.

2.2.3 CHILD SELECTION CRITERIA

Anaesthetic studies for this project were undertaken on children receiving an elective operation or investigation under general anaesthesia requiring a gas induction. Prior to recruitment, all children were assessed by a consultant paediatric anaesthetist as to their suitability for participation in the study. Both male and female children aged between 0-12 years inclusive, and requiring a general anaesthetic were eligible for inclusion in the study. Participants were excluded from the study if it was necessary for them to receive an intravenous (IV) anaesthetic induction requiring prior placement of an IV cannula, or the use of premedication before induction. Children who required emergency care or were not clinically stable were not approached for inclusion in the study.

2.2.4 CHILD RECRUITMENT

Throughout the duration of this thesis, 83 children were recruited between July 2012 and March 2014. Prior to the start of each study, informed (Appendix II - Patient Information Leaflet) written parental (or legal guardian) consent was obtained for each child (Appendix III - Consent Form), and where appropriate, written assent from the

child (Appendix IV - Assent Forms). The study conformed to the standards set by the Declaration of Helsinki and Good Clinical Practice guidelines.

2.2.5 DATA PROTECTION

On entering the study, each participant was allocated an anonymous study number and any identifying data restricted to the written consent and assent forms, and held in accordance with the Data Protection Act (1998). These forms and paper files were stored in a locked filling cabinet in a secure room at the John Radcliffe Hospital site. Participant's demographic data, study sheet, and clinical history were transferred to an electronic database and only accessible to approved research personnel by secure password.

2.3 Data Acquisition & Signal Processing

2.3.1 SINGLE EVENT RECORDINGS

Using a cannulation and heel lance as our clinical noxious stimuli presents a major challenge to the study. This only provides a single opportunity to record any clinically noxious evoked activity. Problems during the experimental protocol, or issues with equipment malfunction will result in a failed recording attempt and renders all preparatory work redundant for that subject. Preparatory work includes the identification of potential participants, letters sent home to the parents of children eligible for inclusion, clinical assessment and liaison with the healthcare team, recruitment, equipment preparation, electrode placement, and the recording of subject demographics. As a conservative

estimate, each study requires approximately five hours before an EEG is recorded so minimising the number of lost events is crucial.

2.3.2 EXPERIMENTAL RECORDING TECHNIQUES

Using the international 10-20 EEG electrode placement system[349], surface electrodes (Ambu Neuroline disposable Ag/AgCl cup electrodes) were placed on the scalp. Eight recording electrodes were positioned over the somatosensory cortex and midsaggital line depending on whether subjects were part of neonatal or anaesthetic studies (Figure 2-2). The primary difference in recording electrodes was the inclusion of Oz in neonatal studies. This was because the neonates were recruited as part of a larger study where other sensory stimuli, including auditory and visual stimuli, were presented to the subjects. Inclusion of Oz meant it was possible to record visual evoked potentials in this population. Differences in reference electrodes were used because of facial movement seen in neonates. Grimacing and other changes in facial expression have been known to cause movement artefacts in neonatal EEGs and so the reference electrode was positioned further back, at Fz. Movement artefacts due to facial grimacing were not present in the anesthetised children so the activity was referenced to FPz in this population.

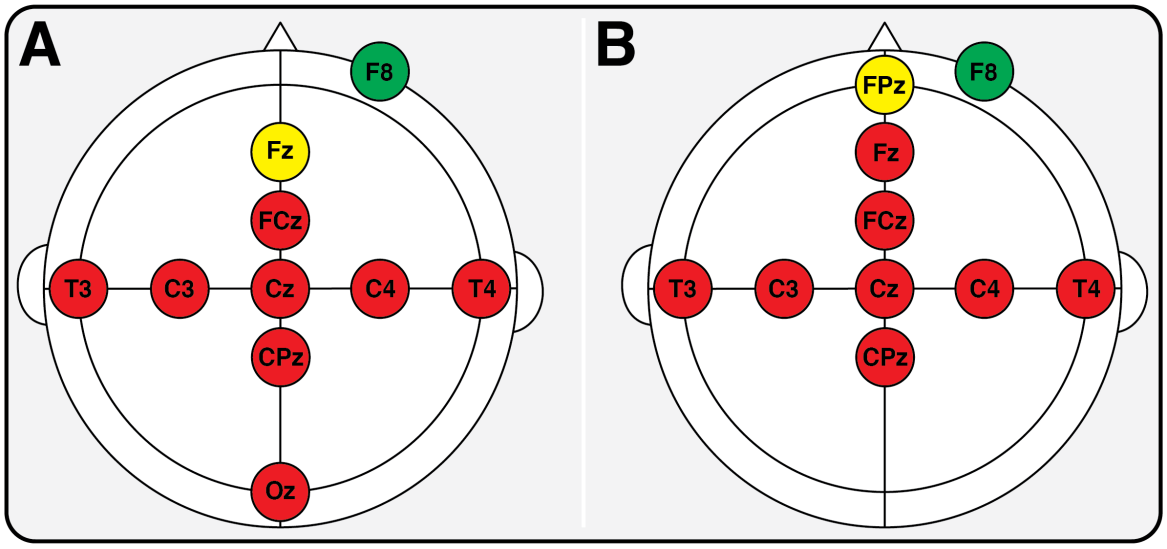

Figure 2-2: Electrode Positions. A schematic diagram of electrode positions for (A) Neonatal studies and (B) Anaesthetic studies. Following the international 10-20 EEG electrode placement system[349], eight recording electrodes were placed at Cz, CPz, C3, C4, Fz, FCz, Oz, T3, and T4 (Red Circles). Neonatal studies and Anaesthetic studies were referenced to Fz and Fpz respectively (Yellow Circles) with a ground electrode placed at F8 on the forehead (Green Circles).

To reduce electrode/skin impedance, each electrode site was prepared by rubbing the skin with an abrasive EEG preparation gel (NuPrep gel, D.O. Weaver & Co.). The gel was applied with a cotton wool bud in order to abrade the smallest area possible. Conductive EEG paste (Elefix EEG paste, Nihon Kohden) was used to optimise contact with the electrodes and improve conductance. If required, the electrodes were secured to the scalp using adhesive tape (Micropore, 3M) and/or an elasticated netting (Surgifix, Colorline). Once the recording electrodes were in place, leads were tied together to minimise external interference. Electrode impedance was assessed to be acceptable when less-than 5 kΩ was achieved, using the inbuilt impedance check within the acquisition software. Re-abrading the skin and reapplying the electrode corrected high impedances. Electrode application took approximately ten minutes, and the electrodes were disposed after each study.

Reflex withdrawal activity was recorded using bipolar surface electromyography (EMG) electrodes (Ambu Neuroline 700 solid gel surface electrodes) positioned on the biceps femoris/brachii muscles of both the stimulated and unstimulated limbs. The surface of the skin was rubbed with an EEG preparation gel to reduce electrode/skin impedance, then pairs of electrodes placed over the muscle belly. If required, electrodes were secured in place with tape, and all leads tied together in order to minimise external electrical interference. EMG activity is recorded from DC - 500 Hz and sampled at 2 kHz using the EEG recording system, with a 50 Hz notch filter applied when required. The single use electrodes were disposed of after each study.

An electrocardiography (ECG) electrode (Ambu Neuroline 700 solid gel surface electrodes) was placed on the left clavicle and referenced to the relevant EEG reference electrode.

Electrophysiological activity was acquired with the SynAmps RT 64-channel headbox and amplifiers (Neuroscan, Compumedics), with a bandwidth from DC-400 Hz and a sampling rate of 2 kHz used to acquire the data, and subsequently digitised at 24-bit resolution. A custom built PC using CURRYscan7 neuroimaging suite (Neuroscan, Compumedics) was used to record the cortical brain activity. All equipment conformed to the electrical safety standard for medical devices, IEC 60601-1.

2.3.3 SIGNAL PROCESSING

Due to time constraints of performing the study in a clinical setting, signal processing on the acquired data was performed post acquisition in CURRYscan7 neuroimaging suite (Neuroscan, Compumedics). Where automatic event marking was unavailable, event marking of clinically and experimentally noxious stimuli were performed post acquisition. This involved the viewing of video files played simultaneously with the EEG to mark the precise point of skin touch of the IV cannula and PinPrick stimulator (see Chapter 3).

The EEG trace was segmented into epochs of 5 seconds before and 5 seconds after the stimulus event mark. Each epoch was baseline corrected (to the mean across the whole epoch) to account for a DC shift, a 50 Hz notch filter and low pass filter at 70 Hz. The power spectrum before and after the stimulus was calculated in MATLAB (version R2013a, MathWorks, Natick, MA, USA) using the fast Fourier transform. Zero-padding was applied to the data to ensure that the data length reached the next power of 2. The power in the delta (0-3 Hz), theta (3-8 Hz), alpha (8-12 Hz), and beta (12-30 Hz) frequency bands was defined as the total power within these respective frequency ranges. Power was calculated for each EEG channel separately. To ensure that the delta activity was not driven by a DC shift, we divided the delta power into 2 ranges: infra-slow-delta (0-0.5 Hz) and high-delta (0.5-3 Hz).

EMG and ECG activity was also segmented into 10 second epochs of 5 seconds before and after each stimulus. For the EMG, a high pass filter at 10 Hz, a low pass filter at 500 Hz, and a 50 Hz notch filter were applied to the EMG signals, and they were baseline corrected to the pre-stimulus mean. The root mean square (RMS) of the signal was

calculated in 250 ms windows, and the average RMS value was calculated pre- and post-stimulus[350,351].

The ECG signal was high-pass filtered at 12 Hz (as the ECG lead was referenced to a head electrode, FPz, low frequencies associated with EEG signals were therefore removed) and low pass filtered at 40 Hz. Heart rate was determined from the R wave to R wave (RR) intervals of the ECG. The R waves were determined as the peak in the signal above a threshold three times the standard deviation and the RR intervals were calculated. Where spurious RR intervals (outside a range of mean +/- SD) were detected, the RR intervals were manually checked and corrected using the raw ECG signal. The average RR interval was calculated pre- and post-stimulus.

A summary of the sequence of processing for the pilot studies and main studies are shown in the flowchart in Figure 2-3. The data used for each study is shown in Appendix V.

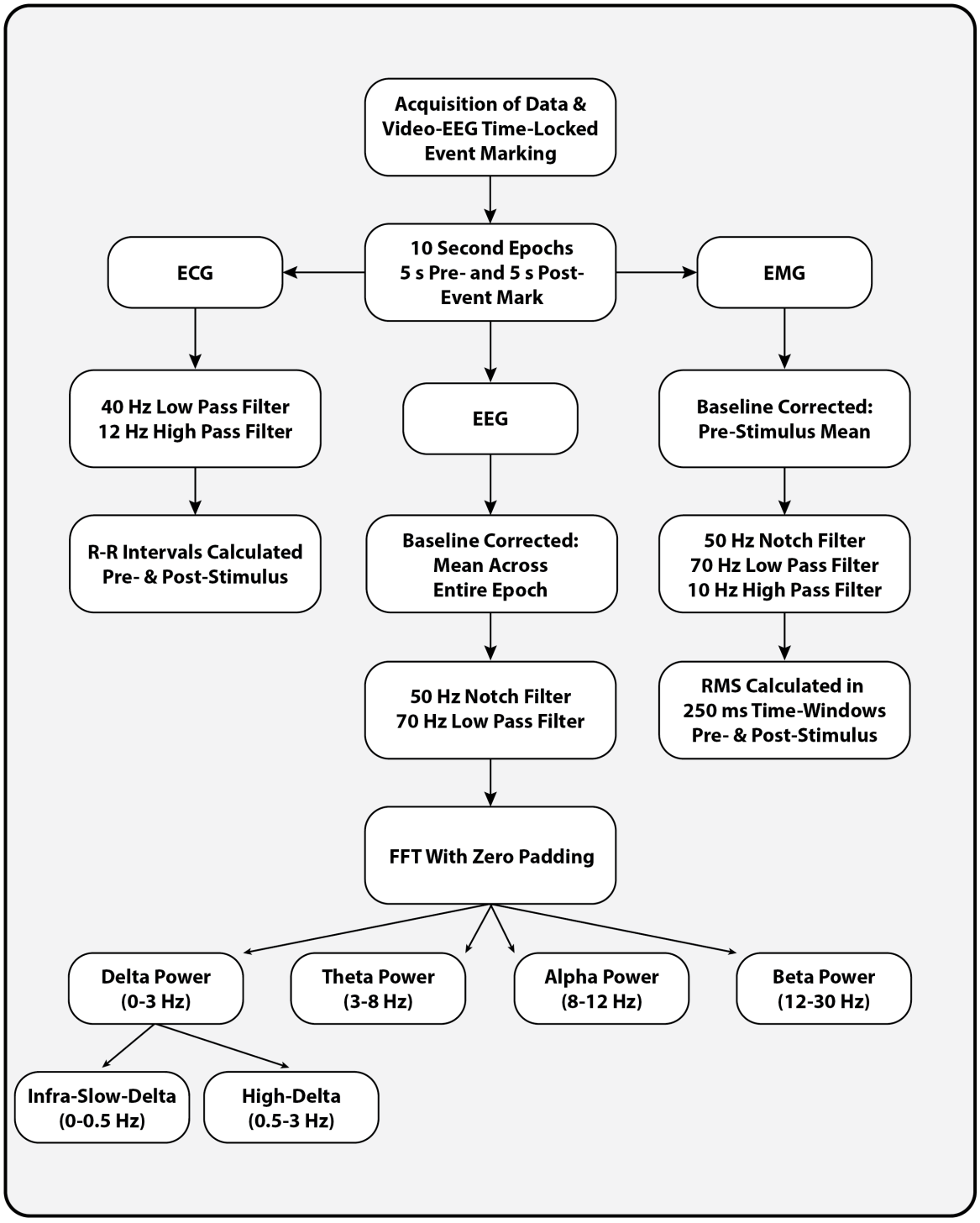

Figure 2-3: Sequence of Signal Processing. Flowchart of signal processing steps used for data in this thesis. ECG, Electrocardiography; EEG, Electroencephalography; EMG, Electromyography; R-R, R wave to R wave; RMS, Root Mean Square; FFT, Fast Fourier Transform.

Chapter 3

Multi-Modal Pain Measurements In Anaesthetised Children

Chapter 3

Multi-Modal Pain Measurements in Anaesthetised Children

3.1 Chapter Overview

The self-report of pain forms the basis of accurate pain assessment and management strategies but the lack of consciousness makes self-reporting impossible when anaesthetised. The prevention of pain remains one of the cornerstones of modern anaesthesia and in non-verbal populations, such as infants, comatosed or anaesthetised patients, observational measures of behavioural and autonomic activity can be used to infer levels of pain, forming the basis of pain scales. Anaesthesia however, can suppress both autonomic and motor responses following noxious stimulation, but it is unknown whether the abolition of these measures equates to the provision of adequate analgesia[352].

Nociception is the neural process of encoding noxious stimulation and the behavioural and autonomic responses observed are known to be indirect measures of pain, mediated by subcortical structures. Pain is a multi-factorial experience and for a stimulation to be perceived as painful, processing within the cerebral cortex is required[1]. In adults, the level of pain reported verbally is directly related to the intensity of a noxious stimulation and can be measured as an increase in the level of evoked cortical activity[353,354]. It is plausible

then, that in non-verbal subjects, directly measuring cortical activity may provide the best insight into whether an individual is in pain, and, in the case of the anaesthetised patient, whether the anaesthetic provision provided is anti-nociceptive.

This chapter describes the development of a reliable, non-invasive and clinically acceptable method to record motor, autonomic, and brain evoked activity in the anaesthetised child. Techniques pioneered in the neonate[123,149] have been further developed and made applicable to a wider range of subjects, and to both clinical and experimental noxious stimuli. This has provided a novel integrative approach to studying nociceptive responses in the anaesthetised paediatric population.

In order not to interfere with clinical procedures and experimental stimuli, a video-EEG time-locking method was established and validated in neonates before being translated into an anaesthetised population. In adult volunteers, it is possible to perform pain experiments in both the laboratory and clinical setting using a variety of electrical, laser, mechanical and thermal noxious stimuli, however, with research in the paediatric population, additional ethical values must be considered (see Section 2.2). While it is possible to use some of these techniques in the paediatric population, looking at more intensive noxious stimulation can only be done when the procedures are clinically necessary.

In this respect, the insertion of an intravenous cannula during part of routine anaesthetic practice provides us with a unique opportunity to study an acutely noxious stimulus in the anaesthetised child. During the cannulation, brain activity can be recorded using scalp EEG; flexion reflex withdrawal activity recorded using EMG; and autonomic

responses (including heart and respiratory rates) using ECG, pulse oximetry and respiratory monitoring. By synchronising these measurements with clinically and experimentally noxious and innocuous stimuli, a fully integrated method to measure evoked activity in the anaesthetised and non-anaesthetised paediatric population has been developed.

3.2 Materials & Methods

3.2.1 PHYSIOLOGICAL RECORDING TECHNIQUES

3.2.1.1 Neuronal Brain and Reflex Withdrawal Activity

Brain activity was recorded using electroencephalography (EEG) as described in Section 2.3. Eight EEG recording electrodes were positioned on the scalp according to the modified international 10-20 System[349]. In neonates, these were placed at Cz, CPz, C3, C4, Fpz, Oz, T3 and T4 with reference and ground electrodes placed at Fz and F8 respectively (Figure 2-2(A)). In the anaesthetised children, electrodes were placed at Cz, CPz, C3, C4, Fz, FCz, T3 and T4 with FPz used as the reference and a ground electrode placed at F8 on the forehead (Figure 2-2(B)). The differences in montages explained in Section 2.3.2.

Reflex withdrawal activity was recorded using bipolar surface electromyography (EMG) electrodes positioned on the biceps femoris/brachii muscles of both the stimulated and unstimulated limbs.

3.2.1.2 Behavioural Activity

Behavioural activity of neonates was monitored by high-definition (HD) video using a digital camera (Cybershot RX100, Sony) mounted on an articulated support arm with centre lever to lock all 3 movements (Magic Arm, Manfrotto), allowing for the optimal placement of the camera with minimal disruption of the surrounding environment. An LED was positioned near the head of the infant in the camera field of view and was manually flashed to indicate the onset of stimulus for analysis of facial expression. Videos were recorded as 1080p HD video MP4 files that could be analysed off-line, post study.

3.2.1.3 Physiological Activity Monitoring

Physiological activity was measured by recording oxygen saturation (SpO_2), respiratory rate, and heart rate. SpO_2 was recorded by a pulse oximeter (Nellcor N600x, Covidien). A movement transducer - sensitivity, 100 µV/mm; frequency range, 0.3 – 70 Hz - was placed on the abdomen of the infant or child to record respiration through a bipolar channel on the EEG headbox (Pico Movement Sensor, Unimed Electrodes). Heart rate was recorded using a single ECG electrode (Ambu Neuroline 700 solid gel surface electrodes) was placed on the left clavicle and referenced to FPz through the EEG headbox.

3.2.2 CLINICAL & EXPERIMENTAL STIMULI, AND EVENT DETECTION

Neurophysiological, behavioural, and physiological recordings were acquired concurrently and synchronised to clinical and experimental stimuli by means of an event-detection interface[124] and video recording. It was important that the methods of event

detection did not interfere with the child's clinical requirements, and complies with the infection control protocols of the hospital.

3.2.2.1 Noxious Heel Lance

In newborn infants, the collection of diagnostic blood specimens by skin puncture is often achieved by heel lancing. In the infant's heel, the major blood vessels are located 0.35–1.6 mm beneath the skin surface between the dermal and subcutaneous layers of the skin. The lancet used for the heel lance was the BD Quikheel Preemie Lancet (BD, Becton Dickinson UK Limited). When the trigger on the superior surface of lancet is depressed, a thin surgical blade is released, automatically and permanently retracted, creating a skin incision of 0.85 mm deep by 1.75 mm wide. When the blade is released, a characteristic sound and vibration is caused and this is detected by means of an accelerometer (K-shear type, Kistler Instruments Ltd.) attached to the lancet casing. The accelerometer output is fed through our event detection interface with a precision of 33 µs and an accuracy of 523 µs, generating a transistor-transistor logic (TTL) pulse and creating an event mark on the Neuroscan SynampsRT through its external digital input[124] marking the experimental recordings. All heel lances were performed when clinical need required them to be and not purely for the study.

3.2.2.2 Noxious Cannulation

During intravenous (IV) cannulation, a plastic tube is inserted into a peripheral vein to allow access to the patients circulation[355]. This allows administration of blood products, fluids, and drugs, and as part of every anaesthetic procedure, an IV cannula is required. The superficial veins of the skins are the usual site of access and these vessels are found within the dermis, beneath the epidermis, of the skin. The cannula used was a 22G BD

Venflon Peripheral IV catheter (BD, Becton Dickinson UK Limited) and pain experienced during cannulation originates from nerve endings within the skin, not the blood vessels themselves. During the cannulation procedure, a metal stylet (needle) within the plastic tube of the cannula is used to pierce the skin and blood vessel allowing for the insertion of the overlying cannula. Unlike the heel lance, a cannula does not lend itself to having an accelerometer or other device attached, allowing for synching with electrophysiological recordings, and so a novel video-EEG method was developed (see Section 3.3.1).

3.2.2.3 Noxious Punctate

Noxious experimental punctate stimuli are performed using a 'PinPrick' stimulator (PinPrick, MRC Systems) calibrated to a force of 128 or 512 mN. They are used during quantitative sensory testing (QST) of pain thresholds and allow for a reproducible measurement of cutaneous nociceptor activation. The calibrated force for the PinPrick stimulators are exerted through their 0.25 mm tip when held vertical to the stimulus site. PinPrick stimulators predominantly activate Aδ-nociceptive fibres[356-358] but the fibres activated are dependent on the stimulation force. As force increases, greater numbers of activated Aδ-nociceptive fibres are joined by C-fibres, however, the 0.25 mm tip diameter could also activate Aß-fibres so PinPrick stimuli may not be nociceptor specific[359].

3.2.2.4 Innocuous Tactile

Innocuous experimental tactile stimulation is performed with an adapted Buck (reflex) hammer. The adaptation involves an impedance head (Bruel & Kjaer, Type 8001, Denmark) placed between the handle and the rubber tip of the hammer[124]. A built in force transducer is calibrated to measure the force applied when tapping and is used to

determine the time of tactile stimulation with a precision and accuracy of 624 µs and 256 µs respectively[124]. The innocuous tactile stimuli were performed at the same site as other experimental stimuli, the heel or dorsum of the hand.

3.2.3 FUNCTIONAL EVALUATION

3.2.3.1 Development of a Video-EEG Technique

Cannulation and PinPrick stimuli do not lend themselves easily to being time-locked to EEG recordings. To tackle this, I developed a method to time-lock the stimuli to the EEG recordings using a fast frame rate video camera. Standard acquisition software only allows for simultaneous EEG and video recordings to be made with a standard IP camera running at 30 fps. This camera set-up only allows frames to be captured with an accuracy of 40 ms and a precision of 80 ms. However; this precision would not provide the temporal resolution to capture the exact moment that a cannula tip would pierce the skin.

My solution was to incorporate high-speed video recordings directly into the EEG acquisition software. I used the FireflyMV USB camera (Point Grey, Canada) that has the ability to produce high frame rate video files. The development and clinical validation of this system is described in this chapter.

3.2.3.2 Clinical Validation in Awake Neonates

The noxious heel lance and innocuous tactile stimuli, have previously been used in neonates have been shown to produce an event related potential maximal at the vertex[123,149]. It is unknown whether experimental noxious stimuli using the PinPrick stimulators evoke a similar pattern of activity in the neonate. I performed clinical tests in

awake neonates using the novel video-EEG time-locking methods that I developed to investigate whether experimental noxious stimuli can evoke nociceptive specific brain activity.

Twelve newborn infants were included in the study. The experimental cohort was divided into two groups. Six infants required a heel lance to monitor antibiotic levels or inflammatory markers and formed the first group. In the second group, six healthy infants received PinPrick stimuli. At the time of study, recruited infants were greater than 36 weeks gestation, awake, and studied within the first few days of life (mean postnatal age = 6 days). Data from infants in group 1 was obtained during a single heel lance procedure, with group 2 infants having had ten PinPrick stimuli (calibrated force = 128 mN) applied to their left heel with an inter stimulus interval of 10 seconds.

3.2.3.3 Experimental Development in Anaesthetised Children

Anaesthetic studies for this project were undertaken on children receiving an elective operation or investigation under general anaesthesia that required a gaseous anaesthetic induction. Prior to recruitment, all children were assessed by a consultant paediatric anaesthetist as to their suitability for participation in the study. Both male and female children aged between 0-12 years inclusive, and requiring a general anaesthetic were eligible for inclusion in the study. Participants were excluded from the study if it was necessary for them to receive an IV anaesthetic induction that required the prior placement of an IV cannula, or the use of premedication before induction. Children who required emergency care or were not clinically stable were not approached for recruitment into the study.

In order to investigate changes in electrophysiological responses to noxious stimulation, I chose to test the insertion of an intravenous cannula during anaesthesia. The insertion of a cannula formed part of the routine anaesthetic practice and provided me with a useful nociceptive stimulus, creating an opportunity to measure electrophysiological responses using EEG and EMG. However, before any studies or recruitment of children could begin, I observed several paediatric anaesthetists to establish what the standard methods of anaesthetic induction were being used so that I could optimise the methodology of my experimental protocol.

I also wanted to confirm that I could place EEG electrodes without interfering with clinical practice. Establishing the optimal time to place the electrodes was important because I did not want to interfere with patient care in the vulnerable situation where an anaesthetist is holding a mask over the patient's face, immediately post induction. Based on my observations I developed a protocol with the recommendation of anaesthetists, involving the insertion of a laryngeal mask airway (LMA) prior to IV access. This would allow time to place electrodes on the child as well as stabilise anaesthetic levels.

Through my observations, it became clear that Nitrous Oxide (N_2O) was used as an adjunct to volatile anaesthetic agents. This is commonplace, and with N_2O having analgesic properties, in order to standardise my experimental protocol, N_2O was turned off following LMA insertion. The finalised anaesthetic protocol is described in Section 4.2.2, Figure 4-2.

3.3 Results

3.3.1 DEVELOPMENT OF A HIGH-SPEED VIDEO-EEG TECHNIQUE

The FireflyMV is a small USB 2.0 digital camera, which, at approximately £200, is an affordable alternative to clinical video EEG systems. As with most cameras, at its highest resolution (640 x 480 pixels, Figure 3-1(A)), the FireflyMV is able to run at a frame rate of 30 fps (frames per second). However, customisation of the video formats and modes can allow for faster frame rates. This can be achieved by selecting a specific region of interest (ROI) within the image, and by aggregating the camera pixel values to sub-sample the image using a process known as pixel-binning. Additionally, the maximum frame rate allowed by the camera is not only dependent on the resolution but also by the bandwidth required to transmit data. Changing video modes and removing colour information can reduce bandwidth.

Selecting a specific ROI allows the camera to send images at faster frame rates by skipping the rows on the image sensor until the desired area of interest is reached (See Figure 3-1). ROI does not effect the processing of colour information so the image obtained is smaller with an increased frame rate of 193 fps (Figure 3-1(B)).

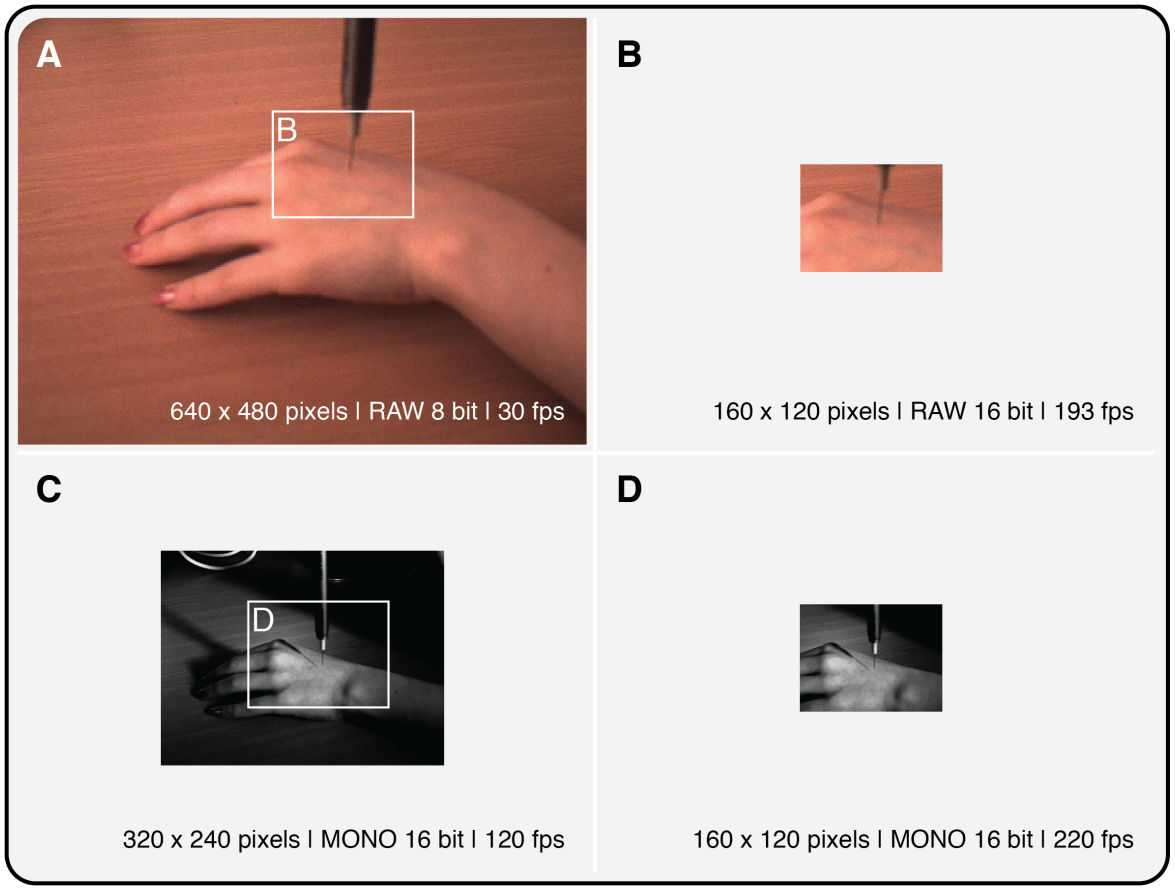

Figure 3-1: Maximising Frame Rate. (A) Original colour image at 30 fps with the maximum possible resolution. (B) The effect of selecting a specific region of interest resulting in a lower resolution image but a higher frame rate of 193 fps. (C) The effect of pixel-binning, colour data is lost but an image of half the original resolution achieves a higher frame rate of 120 fps. (D) Using a combination of pixel-binning and selecting a specific area of interest, the highest possible frame rate of 220 fps is achieved.

Pixel-binning involves combining groups of pixels into one single pixel. With standard binning, the mean analogue charge of two or more pixel values is calculated before formulating a final, single pixel value. During subsampling, the mean digital values from two or more pixels formulate a single pixel value. This occurs automatically and is implemented in groups of two pixels (2x) either horizontally or vertically. A combination of both horizontal and vertical (2x2) binning and subsampling results in an image that is half the size of the original in height and width and results in a faster frame rate of 120 fps due to the decreased bandwidth requirement (Figure 3-1(C)). With the Firefly model used

in our studies, colour information is lost resulting in a black and white image, but with a subtle increase of image intensity (contrast).

A combination of both selecting a ROI and pixel-binning results in a monochrome image, a further increase in frame rate to 220 fps, and a final image resolution of 160 x 120 pixels (Figure 3-1(D)).

CURRYscan7 neuroimaging suite (Neuroscan, Compumedics) is able to record and directly link video and electrophysiological recordings through Microsoft's DirectShow with compatible cameras. CURRYscan7 is a 32-bit application and the FireflyMV source and decoder filters must also be 32-bit to run correctly. Unfortunately, most modern Windows operating systems are 64-bit and as a result, 32-bit filters must be installed manually for an image to appear within CURRYscan7.

Once the appearance and recording of video files was achieved, the 'time-locking' of clinical and experimental stimuli to electrophysiological recordings could be performed. Capturing video recordings at 220 fps allowed for a precision and accuracy of 9 ms and 4.5 ms respectively. The events were marked on the electrophysiological recording post acquisition on review. The 'event' was defined as the point where the stimulus (experimental noxious or cannulation) first made contact with the surface of the skin (Figure 3-2). A video example demonstrating the first point of contact can be viewed at the following web address: https://youtu.be/Cq_CzPJRlzc. The point of first contact was used because it was consistent throughout all stimuli. However, it must be noted that by using this point, there is likely to be bias in time-locking during the PinPrick stimulation because non-nociceptive afferent fibres will be activated prior to the nociceptive afferent fibres. Due to the diffuse nature of the experimental tactile stimulus, even high-speed video

recordings were unable to capture the exact onset of stimulus and so an event detection interface was used[124].

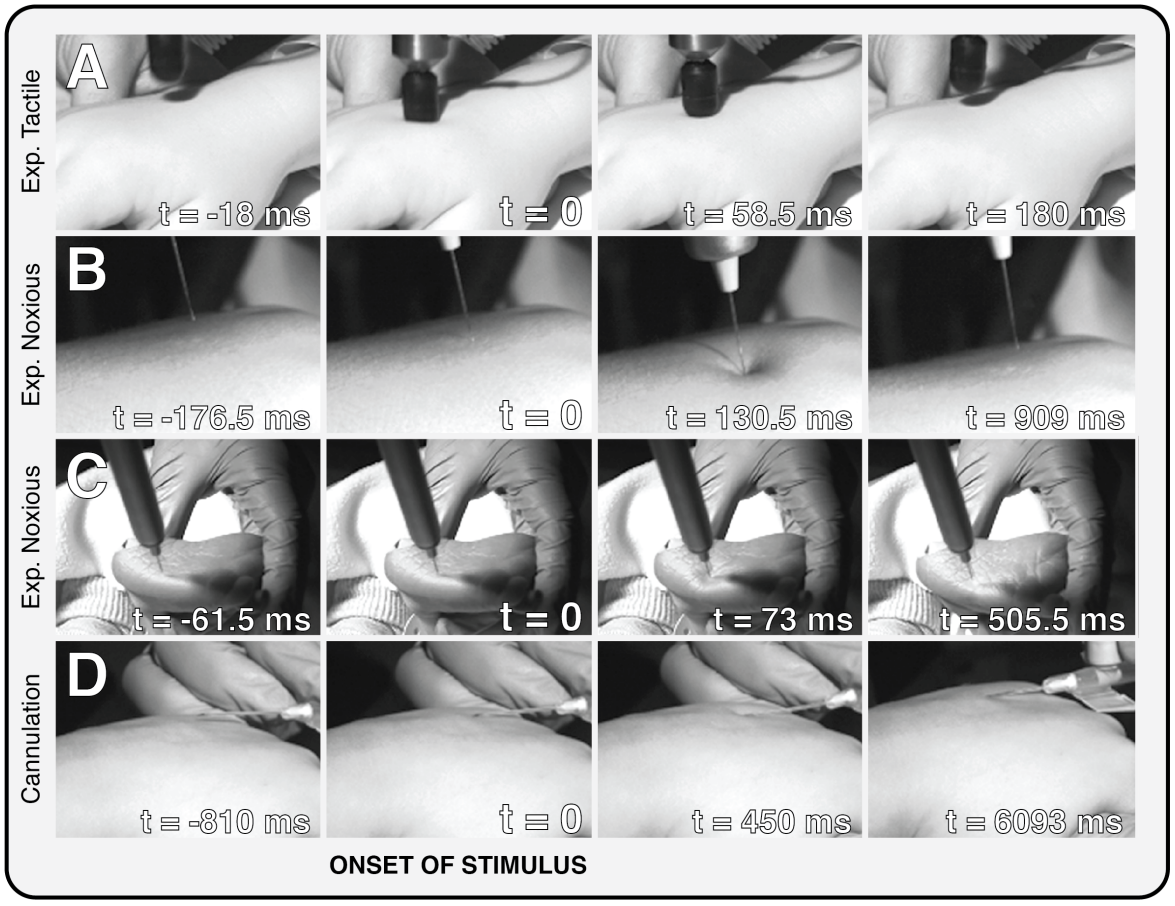

Figure 3-2: EEG Time-Locking. Example stills showing the video recordings. Experimental tactile stimuli (row A) were time-locked to recordings through an event-detection interface. Experimental noxious stimuli (rows B (in anaesthetised children) & C (in neonates)) and cannulation (row D) were time-locked to the EEG recording using high-speed video recordings. 't=0' refers to the time when the stimuli first came into contact with the skin. The times shown in milliseconds (ms) in the frames refer to these particular samples.

3.3.2 CLINICAL VALIDATION IN AWAKE NEONATES

Simultaneous and synchronised recordings of autonomic, behavioural, and CNS responses following a heel lance (Figure 3-3(A)) and experimental noxious (Figure 3-3(B)) stimuli were successfully recorded in a group of awake term neonates. Consistent

with previous literature[149], evoked potentials and reflex withdrawal were seen following the clinically required heel lance in this population.

We wished to test whether experimental noxious stimuli could evoke a similar response to clinically required procedures. We did this using a 128 mN PinPrick stimuli, time-locked by video recordings as demonstrated in Section 3.3.1. On looking at high speed video-recordings there were two options for the point of stimulation of the PinPricks - the first point at which the pin head touches the skin (Figure 3-4(A)), or the point at which the barrel drops (Figure 3-4(B)). When the barrel drops, the full force of the PipPrick is applied to the pin and this point has been used previously in adults as the point of PinPrick stimulation[146]. In neonates, the point of barrel depression was found to evoke a nociceptive-specific response most similar to that of a heel lance (Figure 3-3).

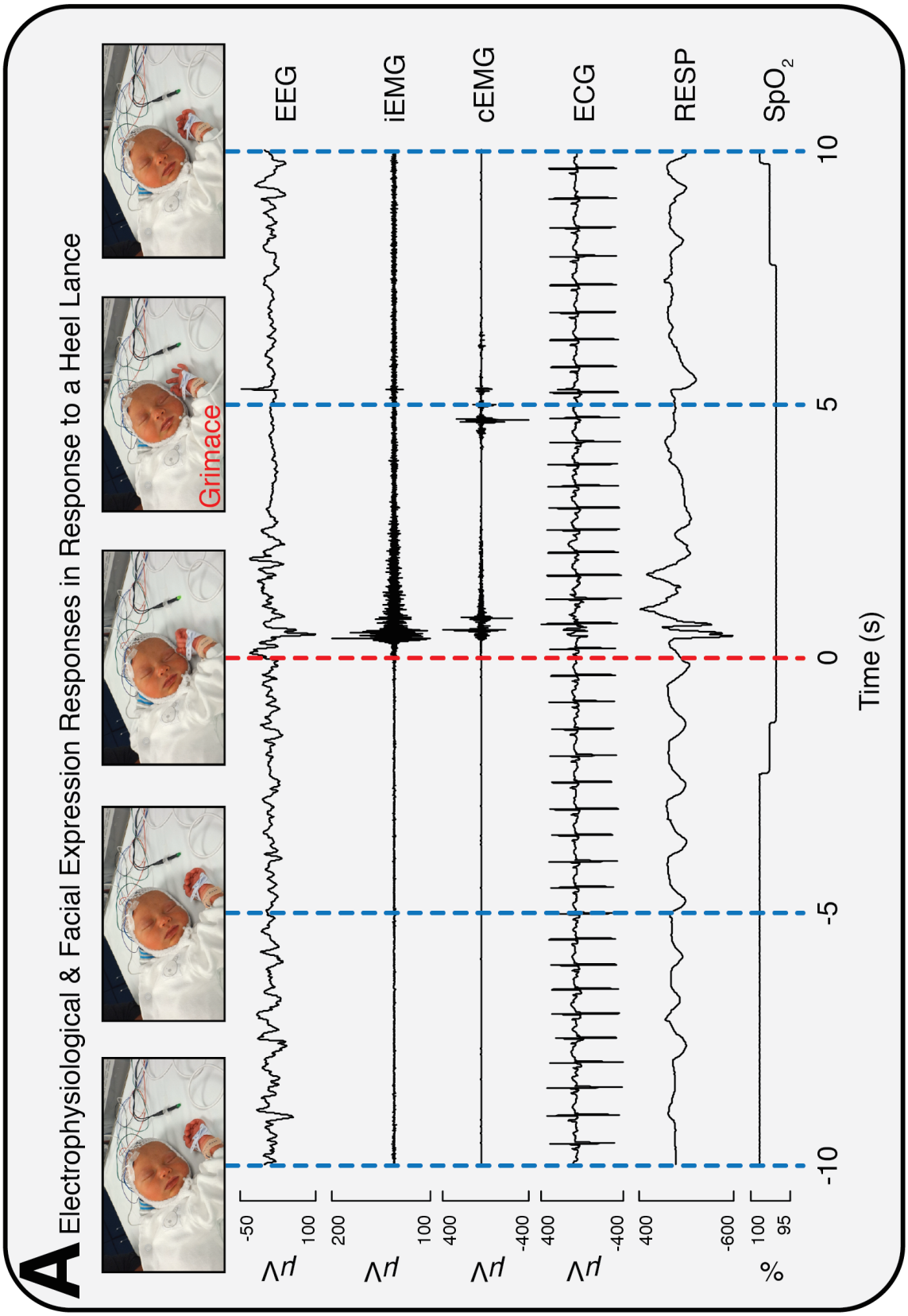

Figure 3-3(A): Simultaneous Recordings. See legend on p114.

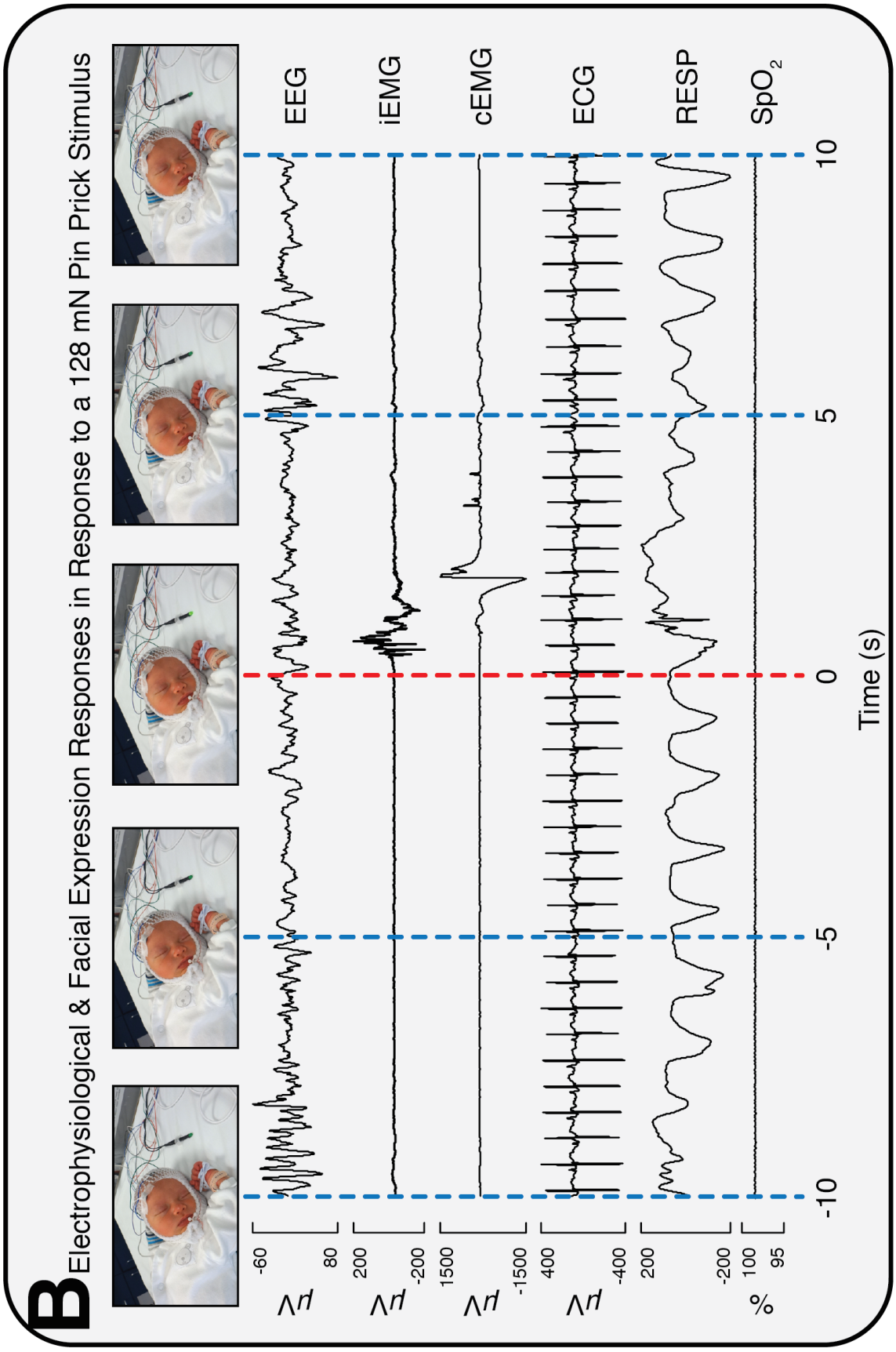

Figure 3-3(B): Simultaneous Recordings. See legend on p114.

Figure 3-3: Simultaneous Recordings. Simultaneous electrophysiological recordings to noxious stimuli in the awake neonate. Simultaneous recordings of video (showing behavioural responses and video-time locking of experimental noxious stimuli), EEG (showing cortical brain activity at Cz) referenced to Fz, contra- and ipsilateral EMG, ECG, and respiration in response to (A) a heel lance, and (B) experimental noxious stimulus (128 mN PinPrick stimulus). Consistent with previous literature[149], evoked potentials, reflex withdrawal, and changes in facial expression were seen following the clinically required heel lance. These changes were not seen following a PinPrick stimulus. iEMG, ipsilateral EMG; cEMG, contralateral EMG.

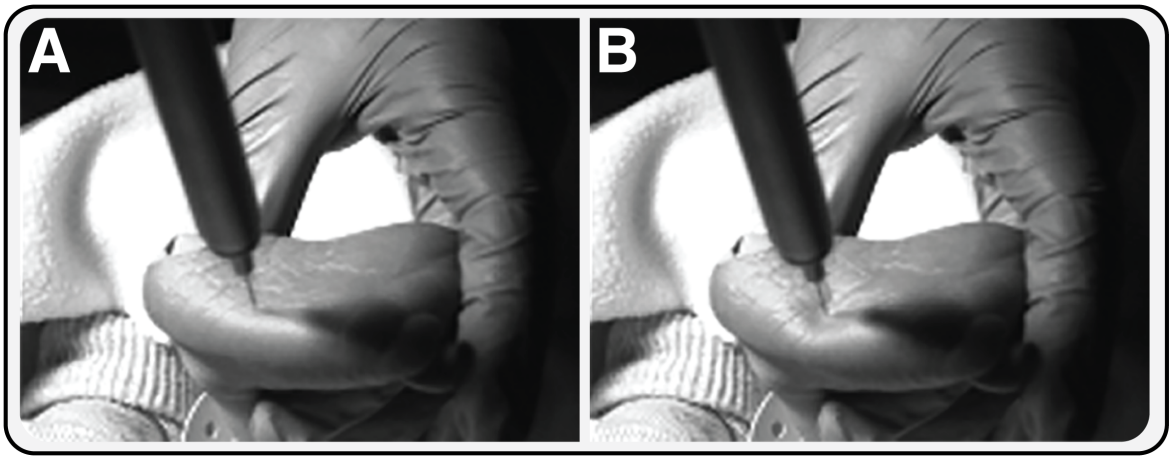

Figure 3-4: Selection of Onset of Stimulus. Possible onsets of stimulation to experimental noxious stimulation in neonates. (A) the point at which the pin first touches the skin, and (B) the point of barrel depression.

The average response from a 6 subjects to a heel lance and experimental noxious stimuli at the Cz (vertex) electrode are shown in Figure 3-5. Figure 3-5(A) shows the average response to 6 heel lance trials, and Figure 3-5(B) shows the average response to experimental noxious stimuli from 60 trials (10 PinPricks per subject). Nociceptive-specific EEG responses have been discussed in detail in Section 1.3.2.3 with a heel lance has been previously shown to evoke a nociceptive-specific component between 400 - 750 ms[123]. Consistent with this data, it appears that the average traces from both the heel lance and experimental noxious stimuli evoked a similar positive deflection in this region. The remarkable similarity of the morphology of the waveforms following clinical and

experimental stimuli is apparent. Visual inspection of the activity (Figure 3-5) show that the amplitude of the potential is smaller following the PinPrick simulation compared to the heel lance. There was no significant difference found between the latency to the maximum positive deflection evoked by the heel lance and experimental noxious stimuli (p=>0.05, Student's t-test) when compared on a trial-by-trial basis.

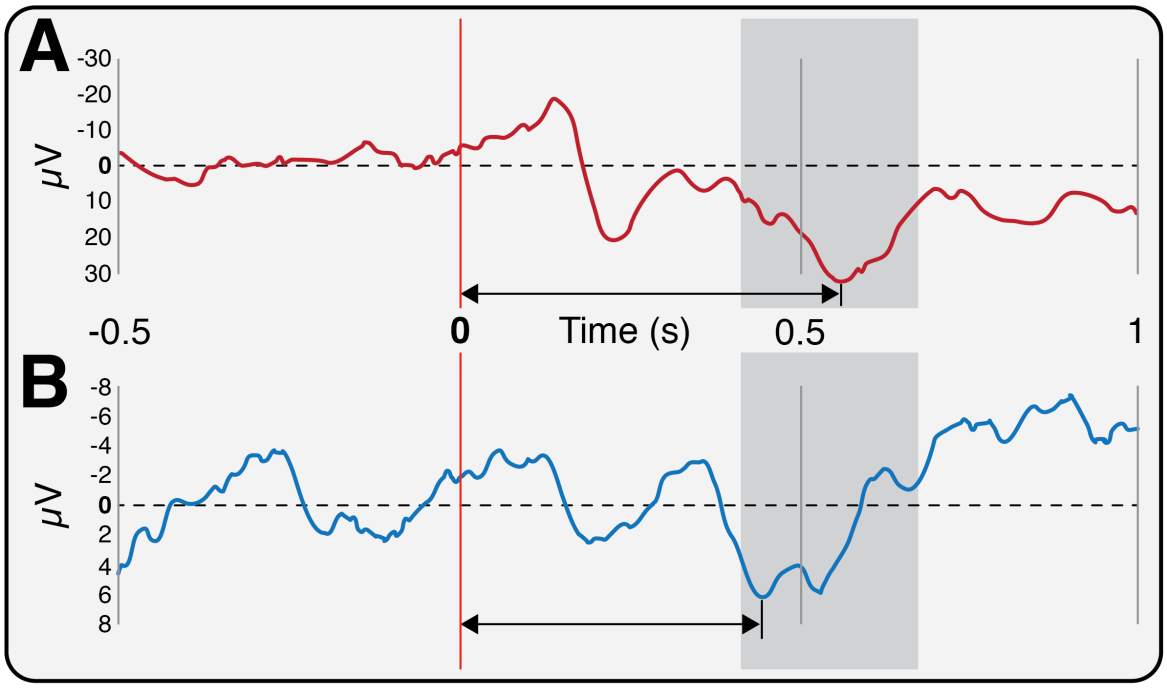

Figure 3-5: Heel Lance & PinPrick Averages. Average EEG response to noxious stimuli in neonates at the Cz (vertex) electrode. (A) Heel lance (n=6 trials), (B) experimental noxious stimuli (N=60 trials). The nociceptive specific component was defined as the latency (arrows) to the first positive deflection within a time window of 400 - 750 ms post-stimulus (grey shaded boxes)[123].

3.3.3 EXPERIMENTAL DEVELOPMENT IN ANAESTHETISED CHILDREN

Using our time-locked video EEG, we were able to record and measure EEG responses to cannulation under sevoflurane anaesthesia in children. This was achieved through post acquisition review of video files and manually event-marking the onset of cannulation, chosen as the point of first skin touch. Figure 3-6(A) shows example traces at

all electrode sites of the activity evoked following cannulation, in a single anaesthetised child. Figure 3-6(B) shows the average trace at Cz from all anaesthetised subjects and includes a magnified version of the data epoched from -0.5 to 2 seconds. This is compared to Figure 3-6(C), which highlights data in a published study that shows the activity evoked by inoculation in an awake 2-month-old child[360]. Figure 3-6(C) clearly shows the tactile and nociceptive specific response at approximately 500 and 700 ms respectively, while these evoked responses are not seen under anaesthesia (Figure 3-6(B)).

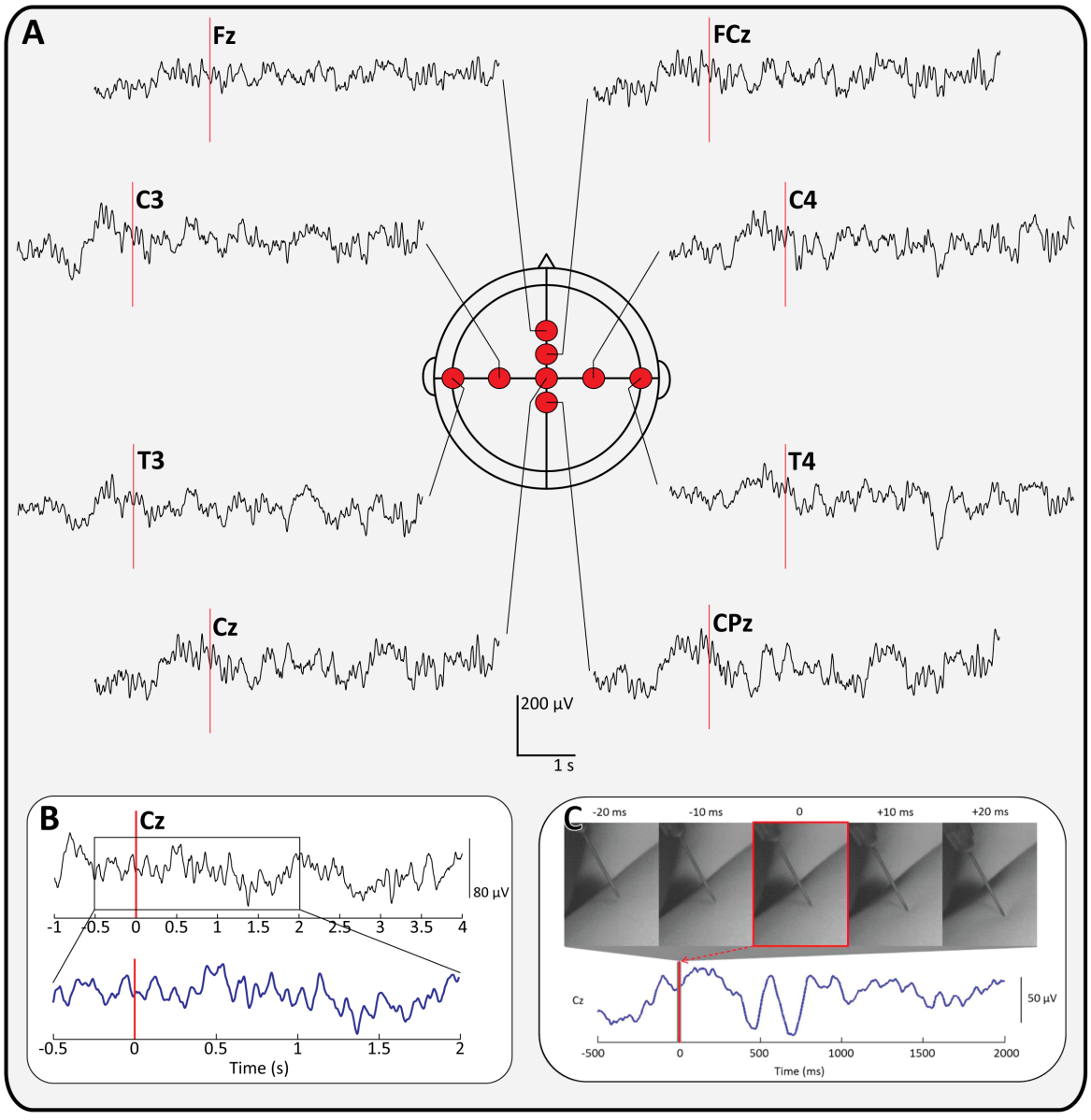

Figure 3-6: Cannulation Recordings. See legend on p117.

Figure 3-6: Cannulation Recordings. (A) Raw EEG recordings in a single child in eight recording electrodes are shown. Each four-second epoch consists of one-second pre-stimulus and three-seconds post stimulus with the onset of cannulation (T0) identified by the red vertical line. Stimulus onset was manually event marked post acquisition using our high-speed video-EEG method. (B) Average trace at Cz electrode from all anaesthetised subjects and zoomed into a -0.5 to 2 second epoch, showing the absence of any visually-observed evoked potentials. (C) A comparison trace from an inoculation event in an awake 2-month-old child showing tactile and nociceptive specific response at approximately 500 and 700 ms respectively[360].

I conducted a pilot study in 12 children (age range: 19 -143 months) examining the EEG response to cannulation at Cz and CPz electrodes. Vertex evoked potentials that would be expected in the awake child are not observed when anaesthetised. Initial analysis demonstrated there was an increase in delta power from baseline at both electrode sites (20.0 ± 13.4 % at Cz and 13.5 ± 12.0% at CPz; mean ± standard error of the mean, Figure 3-7). Based on these results I hypothesised that noxious stimulation in children receiving sevoflurane anaesthesia evokes an increase in EEG delta activity at Cz and CPz.

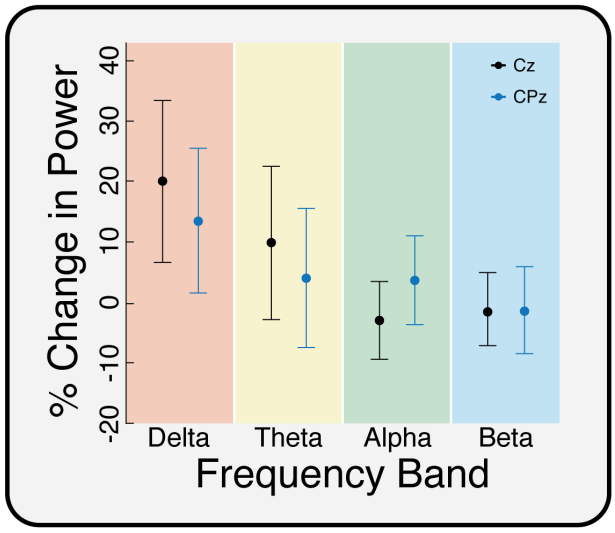

Figure 3-7: Change in Delta Power From Cannulation. This figure shows the percentage change in power from baseline at Cz (black) and CPz (blue) electrodes in response to cannulation across the delta (0-3 Hz), theta (3-8 Hz), alpha (8-12 Hz), and beta (12-30 Hz) frequency bands.

Of the 12 children studied in the pilot, three were born prematurely. Analysis of EMG activity showed that one of the children (age at study: 26 months, gestational age at birth: 26 weeks) had a reflex withdrawal response on the ipsilateral arm to cannulation (Figure 3-8). This demonstrates that under this level of anaesthesia, the spinal reflex withdrawal is possible to be seen. The anecdotal evidence that premature-born children are more responsive under anaesthesia and our EMG finding, leads me to the hypothesis that prematurely born children are more reactive to noxious stimulation while under anaesthesia.

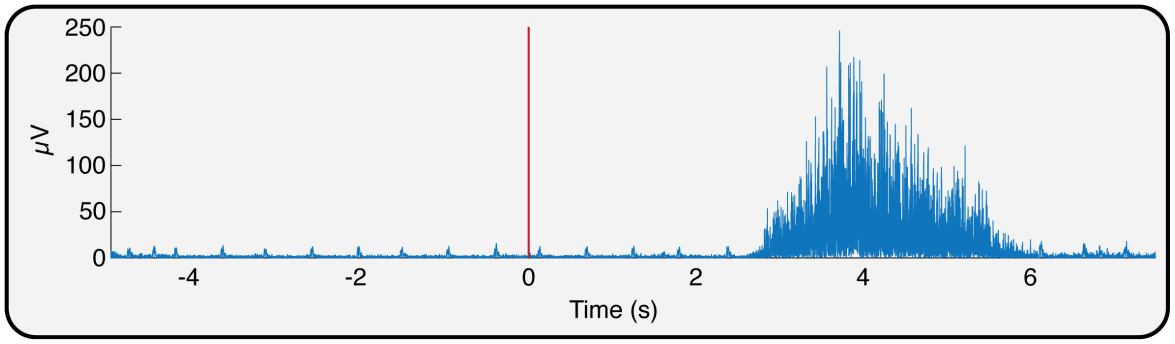

Figure 3-8: Example EMG Response to Cannulation. One premature-born subject in the pilot study showed a clear EMG response to cannulation (red line of onset), with a rise in EMG activity lasting for approximately 3 seconds. A visible hand/arm movement was coupled with this response.

3.4 Discussion

In this study, we have shown the development and use of a novel video-EEG technique to study event-locked physiological responses to clinically essential and experimental noxious and innocuous stimulation in human infants and anaesthetised children. The integrated system allows for the simultaneous monitoring of autonomic, behavioural, brain, and reflex withdrawal activity in response to sensory mechanical stimuli.

The main advantage of this methodology is the use of non-invasive and ethical techniques to study the cortical processing of pain in human infants and children under anaesthesia. Furthermore, experimental procedures do not interfere with clinical protocols and provides the opportunity for evoked cortical responses to more complex clinical procedures, such as cannulation, to be investigated in children as well as other potential patient groups. The precision of 9 ms of the new 'time-locking' interface means it is possible to get a much more detailed understanding of how different types of experimental and clinical stimulation affect cortical brain function in an anaesthetised population. This methodology may provide a more sensitive way of investigating whether current analgesic strategies used by anaesthetists are adequately antinociceptive. Video time-locked EEG is often used clinically to study epileptic brain activity in response to subtle behavioural manifestations. Most clinical EEG systems can only synchronise the EEG to the video recording at conventional frame rates, that is, with a precision of ~60 ms, the ability to time-lock EEG to a video recording with a precision of 9 ms, using simple and easily available technology, may be useful in other clinical and experimental settings.

Initial analysis of the pilot anaesthetic study conducted in 12 children, demonstrated that vertex-evoked potentials, as seen in neonatal studies, were not observed in response to cannulation. This led to the investigation of stimulus induced changes, where an increase in delta power was observed at both Cz and CPz electrodes. This study led me to hypothesise that noxious stimulation in children receiving sevoflurane anaesthesia would evoke an increase in EEG delta activity at the Cz and CPz electrode sites.

In summary, the methods described in this chapter have build upon similar methods used in other clinical investigations examining nociceptive brain activity in premature and newborn infants[123,149]. The methods were further developed to use a high-frame-rate video camera so that the timing of more complex clinical events, such as a cannulation, could be established. The feasibility of using this approach to event-mark the EEG recording in the anaesthetic room has been confirmed, where it is hypothesised that an increase in EEG delta activity is observed in response to noxious stimuli.

Chapter 4

Noxious Stimulation in Anaesthetised Children

Chapter 4

Noxious Stimulation in Anaesthetised Children

4.1 Chapter Overview

In the UK, more than 235,000 children admitted to hospital each year undergo an operation or investigation with general anaesthesia[341]. The prevention of pain is one of the primary goals of anaesthetic practice, and a variety of different compounds are used to independently control anaesthesia, analgesia, amnesia, muscle relaxation, and the reduction or elimination of autonomic reflexes - described as balanced anaesthesia[240]. The suppression of autonomic and motor responses in both adults and children limits the use of objective clinical assessment tools for pain in the anaesthetic setting, and it is not known whether the abolition of physiological and reflexive measures relates directly to the provision of adequate analgesia[352].

Recording measures of neurophysiological brain activity in response to noxious stimulation has the potential to provide a more complete understanding of how nociceptive information is being processed in the anaesthetised child. In adults, noxious stimuli administered during anaesthesia alters EEG activity[361-370]. In the majority of studies, an increase of delta activity is reported[361,363,367-369]. Additionally, this increase has been seen in animals[366,371]. However, a smaller number of adult studies report the opposite finding, a

reduction of delta activity[363,365,367,370]. These contradictory observations in adults have been attributed to different doses and types of anaesthesia used in these studies, as well as the different surgical procedures investigated[361,367,370,372]. In anaesthetised subjects, delta activity is the dominant pattern of brain activity and how this is modulated by external events, such as nociceptive stimulation, is important in understanding how the brain processes information when patients are anaesthetised.

Little is known about how the anaesthetised paediatric brain responds to nociceptive events and to date, cortical responses to noxious stimuli have not been investigated. During childhood and adolescence the brain continually undergoes development and it cannot be assumed that when anaesthetised, children will respond to noxious stimuli in the same way as adults. In the first few years of life there is a rapid increase in myelination[373] and an increase in synaptic density, followed by a period of synaptic pruning until mid-adolescence[374]. These developmental changes are reflected in changes in EEG frequency[375] and synchrony[376], and grey and white matter volume[373]. Directly recording neurophysiological measures of brain activation in response to noxious stimulation has the potential to provide a more complete understanding of how nociceptive information is being processed in the anaesthetised child. A first step toward this goal is to characterise the electrophysiological brain response evoked by a controlled nociceptive stimulus in children who are receiving a fixed dose of a single anaesthetic agent.

Due to its wide therapeutic index and fast speed of induction, sevoflurane is the preferred agent for volatile anaesthetic induction in the paediatric population. This chapter describes the innovative study where my novel video-EEG time-locking technique (see section 3.3.1) has been used to establish whether tactile (innocuous) and noxious stimuli

evoke changes in the electrophysiological brain activity of children receiving sevoflurane monoanaesthesia.

Following the pilot study in anaesthetised children (see section 3.3.3), I hypothesised that under sevoflurane monoanaesthesia, noxious stimuli would evoke a transient increase in cortical synchrony characterised by an increase in EEG delta activity, primarily at Cz and CPz electrodes. The aim of this study was to test whether this evoked rise of delta activity was observed at other electrode sites, and whether the increase would occur following clinical cannulation, and experimental innocuous and noxious stimuli. In addition, I examined whether this increase in delta activity was blocked by the application of a topical local anaesthetic agent.

4.2 Materials & Methods

4.2.1 PARTICIPATING CHILDREN

Thirty-nine children were studied at the John Radcliffe Children's Hospital, Oxford between July 2012 and February 2014 (see recruitment flowchart in Figure 4-1).

Children were studied between 1-12 years, and this age range was chosen due to an end-tidal concentration of 2.5% equating to 1 MAC of sevoflurane[266]. Children were included in the study if they were to undergo gaseous induction of anaesthesia. Examples of procedures that children underwent include MRI scans, squint and hernia repairs, and orchidopexy. A full demographic list including procedures can be found in Appendix V. Children were not eligible if premedication was received before anaesthetic induction or if

they required an IV anaesthetic induction. Participants requiring emergency care, having central nervous system disease or developmental delay were also excluded.

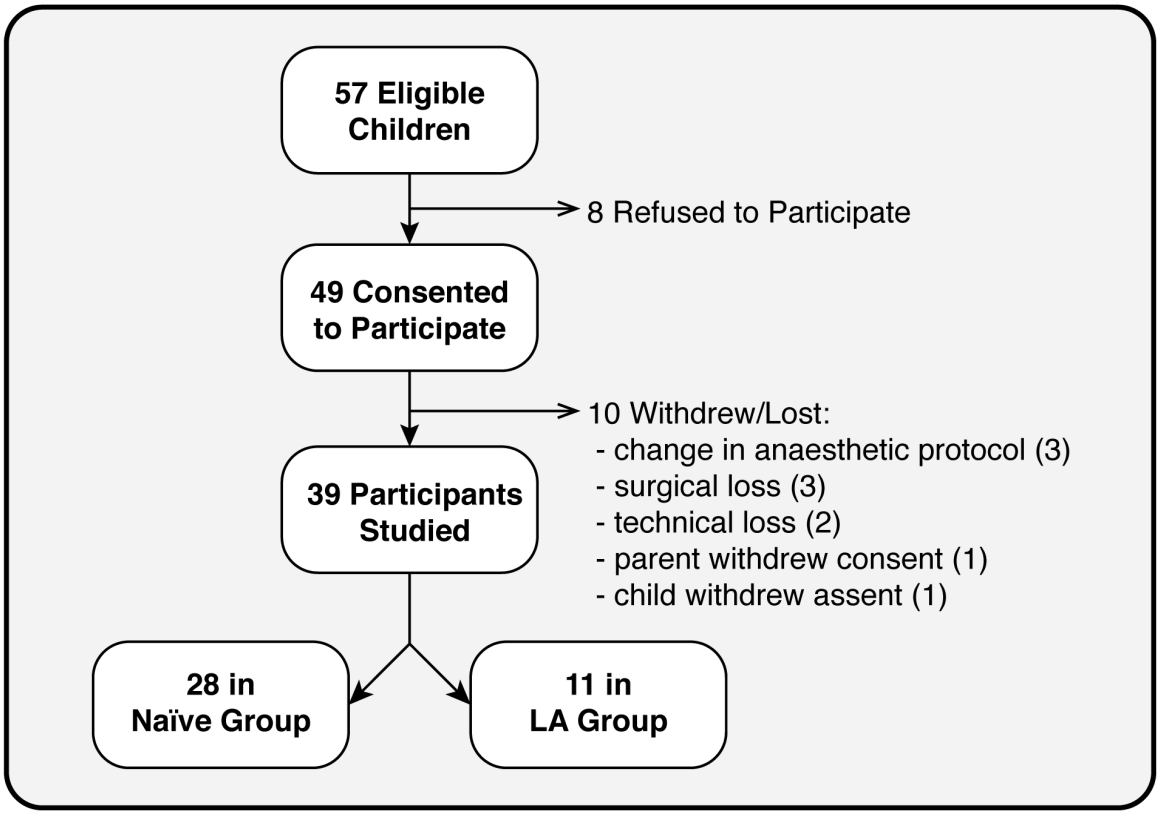

Figure 4-1: Recruitment Flowchart. Thirty-nine children participated in the study forming this chapter. They were divided into two experimental groups: (i) the naïve group (n = 28, age range: 14-153 months) and (ii) the local anaesthetic (LA) group (n=11, age range: 25-83 months) who had topical local anaesthetic cream applied to the stimulus site.

Children were assigned into two study groups depending on whether topical local anaesthetic gel had been administered prior to anaesthetic induction. Group 1 were the naïve group (n = 28, age range: 14-153 months) and Group 2 were the local anaesthetic (LA) group (n=11, age range: 25-83 months) who had topical local anaesthetic gel applied to the stimulus site.

4.2.2 EXPERIMENTAL PROCEDURES

Gaseous induction of anaesthesia was performed following routine anaesthetic practice using sevoflurane (Baxter, UK), oxygen, and nitrous oxide (N_2O). A LMA was inserted once the child was settled and an end-tidal sevoflurane concentration of 2.5% was achieved by reducing the inspired sevoflurane and turning off N_2O. The experimental study protocol is shown in Figure 4-2. The children were maintained under sevoflurane monoanaesthesia. The time between induction of anaesthesia and experimental start was at least 10 minutes. This was due to the placement of head and body electrodes, and by this point, N_2O concentration was confirmed to be less than 5% and the end-tidal sevoflurane concentration at 2.5%. Data acquisition did not commence until these levels were reached and the anaesthetist was happy for me to continue with the study.

Topical local anaesthetic (tetracaine 4% w/w – Ametop Gel, Smith and Nephew Healthcare, UK) was administered to children by nursing staff on the day care unit. If nursing staff were not informed by the anaesthetists that children were going to receive a gaseous induction, and then local anaesthetic was applied in case IV anaesthetic induction was required. The gel was administered to children on the dorsum of both their hands at least 30 minutes before being taken to theatre. Once in the anaesthetic room, theatre staff removed the gel and this subset of children (n=11) formed our LA group - Group 2.

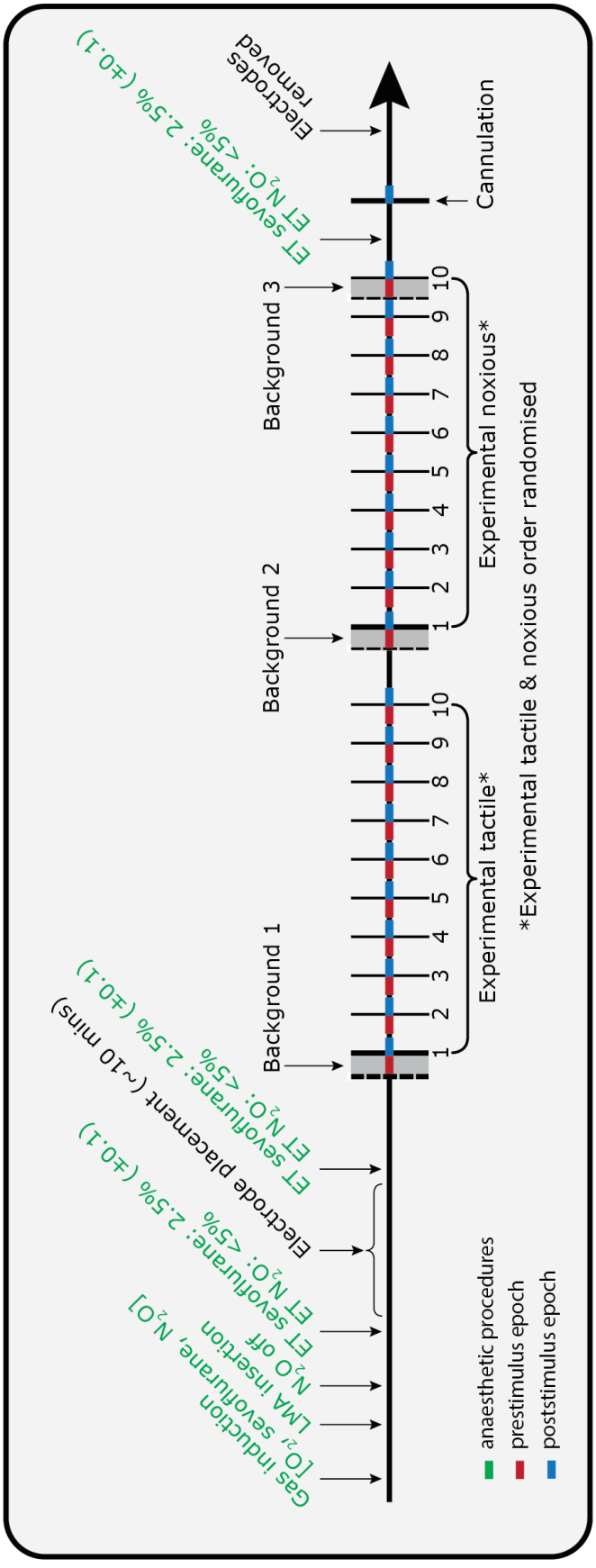

Figure 4-2: Final Anaesthetic & Experimental Protocol. See legend on p128.

Figure 4-2: Final Anaesthetic & Experimental Protocol. Anaesthetic induction was performed following routine practice with sevoflurane and nitrous oxide. A laryngeal mask airway (LMA) was then inserted and the end-tidal (ET) concentration of sevoflurane was set to 2.5% and nitrous oxide <5%. Following electrode placement, the experimental protocol was carried out, with two blocks of experimental stimuli followed by cannulation. The 5 second period before (red) and after (blue) each stimulus were analysed and 3 background periods (grey shaded boxes) were taken throughout the recording.

Immediately following anaesthetic induction and go-ahead by the supervising anaesthetist, eight EEG recording electrodes were placed on the scalp, with reference and ground electrodes positioned at FPz and the forehead respectively (see Section 2.3.2). Bipolar EMG was placed on the belly of both biceps brachii muscles, and a single ECG electrode on the left clavicle referenced to FPz (see Section 2.3.2).

In this study, three stimuli were used - experimental tactile, experimental noxious, and a clinically required cannulation (see Section 3.2.2). A train of ten time-locked experimental tactile stimuli was presented to each participant with an inter-stimulus interval (ISI) of 11.2±2.5 seconds (mean±standard deviation). Ten experimental noxious stimuli, calibrated to a force of 512 mN were presented to each participant with an ISI of 11.2±1.8 seconds (mean±standard deviation). The experimental stimuli were always performed in two blocks before the routine cannulation, but the order that they were presented was randomised between subjects (see Figure 4-2).

All stimuli were performed on the dorsum of the same hand, which was selected by the anaesthetist. Due to the arrangement of anaesthetic rooms, there was a slight bias for stimuli to be performed on the left hand (73% of studies). To avoid possible sensitisation, small changes were made to the stimulation site between experimental stimuli.

Experimental noxious stimuli and cannulation were 'time-locked' using our novel high speed video-EEG system (see Section 3.3.1), directly linking video and electrophysiological recordings. Events were marked on the electrophysiological recording after the experiment, and were defined as the point where the stimulus (experimental noxious or cannula) first made contact with the surface of the skin (see Figure 3-2). A schematic of the anaesthetic room setup is shown in Figure 4-3 where a minimum of four personnel (excluding the patient) would be present during the experiment - the anaesthetist, anaesthetic assistant, and two researchers. During the induction of anaesthesia, researchers remained outside of the anaesthetic room and would enter once the child's parent and accompanying nurse had left. At this time, the anaesthetist and anaesthetic assistant would be with the patient as researchers set up equipment and prepared for electrode placement. When ready to begin, one researcher would perform the experimental stimuli on the chosen hand, while the other worked on the EEG acquisition computer and camera placement.

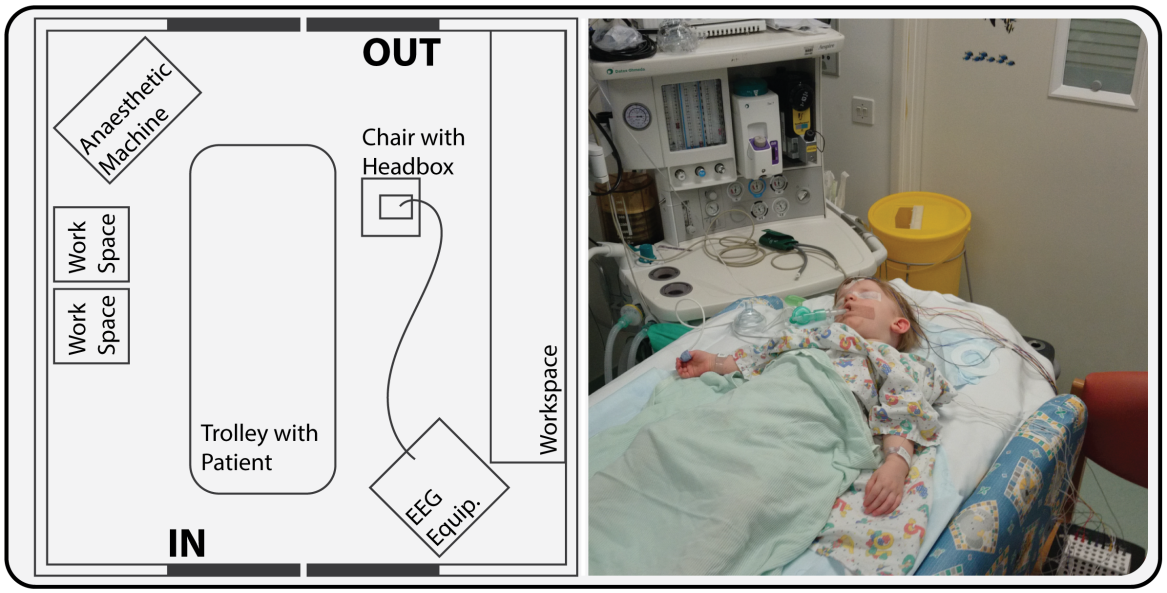

Figure 4-3: Anaesthetic Room Setup. See legend on p130.

Figure 4-3: Anaesthetic Room Setup. On the left is a schematic diagram of where equipment is positioned within the anaesthetic room. As a minimum, excluding the patient, there would be 4 other persons in the room - the anaesthetist, anaesthetic assistant, and two researchers. The high-speed camera was movable and would be placed in the optimal view point of the hand to be experimented on; this was connected to the EEG equipment via a USB cable. On the right is a photograph taken just after electrode placement on a research subject. Note: not to scale.

4.2.3 ELECTROPHYSIOLOGICAL ANALYSIS

For each individual stimulus, the raw EEG was split into 10 second epochs - 5 seconds before and after the stimulus event mark. Due to an obscured view of the stimulus not allowing for the precise onset of the stimulus to be determined, 10 experimental noxious stimuli were excluded from analysis.

A minimum of eight experimental stimuli in each stimulus block was suitable for analysis. Cannulation was included in the analysis for all subjects. Each epoch underwent the sequence of signal processing as described previously in Section 2.3.3.

It is possible that background EEG activity was changing throughout the recording as a result of change in anaesthetic depth or drift in the EEG signal. To determine whether there was a change, three 5 second background epochs of EEG were defined (see Figure 4-2). The first epoch was prior to any stimuli; the second prior to the second train of experimental stimuli, and the third was prior to the last experimental stimulus. Due to artefacts caused by movement and repositioning of the upper limb in preparation for the cannulation, the background period immediately prior to cannulation could not be used for analysis. Therefore, the change in response to cannulation was calculated from the first epoch of background activity, prior to any stimuli. This change in activity evoked by cannulation was compared to the change in activity between background periods 1 and 2.

EMG and ECG activity was exported as ten second epochs - five seconds before and five seconds after the stimulus and processed as described previously in Section 2.3.4.

4.2.4 STATISTICAL ANALYSIS

Statistical analysis was carried out using custom scripts written in R (The R Project for Statistical Computing). Determined from Q-Q plots, the majority of the data was non-parametric. To examine the differences in band power in all four of the frequency bands across the three background periods the data was normalised by taking the logarithm and a three-way MANOVA (multivariate analysis of variance) was performed; EEG channel, subject age, and background period as factors, and the four frequency bands as variables. The change in delta power between cannulation and background EEG periods was assessed using a permutation test; with subject age and EEG channel as additional factors, for both the naïve and LA groups. A permutation test was also used to consider the change between pre-stimulus baseline data, experimental tactile and experimental noxious stimuli, with age, EEG channel and stimulus number as additional factors. A change in power that was different from zero was assessed using a one-sample median test with Bonferroni correction for multiple comparisons. All permutation tests were carried out using the R package 'lmPerm' with iterations terminated when the estimated standard error of the estimated p-value was less than 0.001. For EMG and ECG analysis the average RMS and heart rate were compared using a Wilcoxon signed-rank test.

4.3 Results

4.3.1 DELTA ACTIVITY IS THE DOMINANT EEG PATTERN IN ANAESTHETISED CHILDREN

Figure 4-4 (A) shows the power spectrum of background EEG activity prior to any stimulation, this is averaged across all electrodes and all subjects in the naïve group (n=28). The majority of activity occurs in the delta band (62.6±2.8%, mean ± standard error of the mean) and this result is consistent with previous reports in anaesthetised individuals[377] and my pilot study. The percentage of power in theta, alpha, and beta frequency bands were 18.9±1.5%, 14.3±2.1% and 4.2±0.5% respectively (Figure 4-4 (B)).

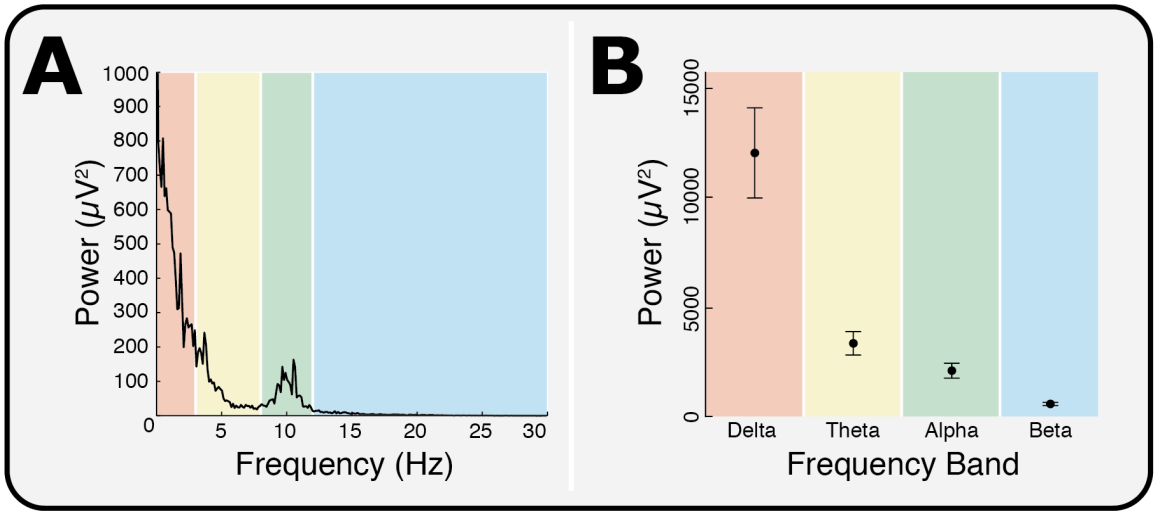

Figure 4-4: Power-Spectra For Background EEG. (A) average power spectrum of background EEG across all channels (naïve group; n=28). The spectrum was divided into four frequency bands: delta (0-3 Hz, orange), theta (3-8 Hz, yellow), alpha (8-12 Hz, green), and beta (12-30 Hz, blue). (B) comparison of the power in the background of each frequency band.

Background EEG activity was stable across the whole recording period and no significant differences in power (in any frequency band) occurred across the three background epochs (Figure 4-2) (p=0.42; three-way MANOVA).

4.3.2 DELTA ACTIVITY IS MAXIMAL ACROSS CENTRAL ELECTRODES

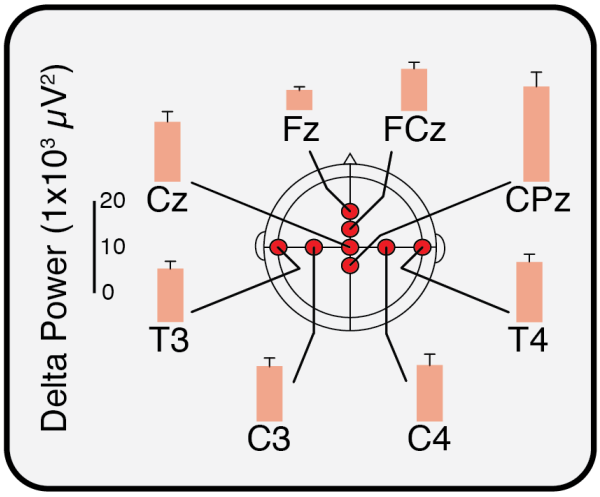

Figure 4-5: Delta Activity Across Electrodes. The delta power in the background EEG at each of the EEG channels. Error bars indicate standard error of the mean.

The highest level of delta power in the background EEG was recorded at CPz, and there was a significant difference in the background EEG between channels (p<0.001, three-way MANOVA). However, there was not a significant difference across the subject age range (p=0.11). The central electrodes (CPz & Cz) accounted for the majority of the delta activity (Figure 4-5) and because of the significant effect of channel on background activity, subsequent analysis focused on the delta activity at these two central electrodes. This is consistent with the approach taken in previous studies involving anaesthetised adults[367,378].

4.3.3 DELTA ACTIVITY IS INCREASED FOLLOWING CLINICALLY NOXIOUS STIMULI

In the naïve group, the evoked change in delta power following cannulation, at Cz and CPz, was significantly greater than the change in delta power across the background periods (Figure 4-6 (A), p=0.042, permutation test; with no effect of age or channel). There

was an average increase in delta power of 34.2% ± 8.3 % following cannulation compared with background activity. Figure 4-7 shows the change in delta power at CPz evoked by the cannulation for each individual subject, and the mean±standard deviation of the change across background periods. Thirty-six per cent (10/28) of the subjects showed an increase in delta activity, above the level of background variability, in response to cannulation. In comparison, only 18% (5/28) of the subjects showed an increase in delta activity, above the level of background variability, in response to tactile stimulation. This increase in delta power in response to cannulation was blocked by the application of a topical local anaesthetic to the stimulation site (Figure 4-6 (B)). The application of local anaesthetic gel (Group 2 - LA group) reduced the mean evoked change in delta activity by 95.7% (mean change in delta power post-cannulation compared with background EEG in the naïve group = 5598.0 ± 2538.2 μV^2, and in the LA group = 241.1 ± 3727.4 μV^2, ± standard error of the mean) and was not significantly different from background activity (p=0.30, permutation test).

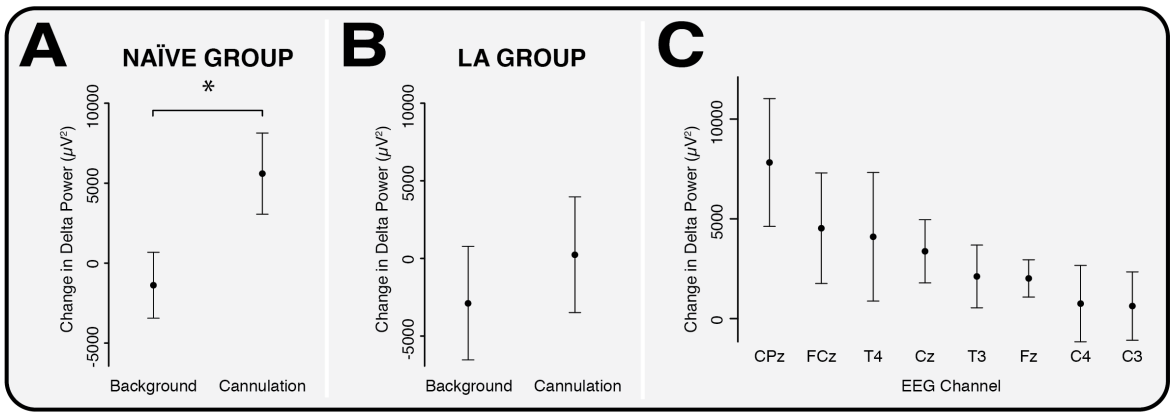

Figure 4-6: Change in Delta Band Power in Response to Cannulation.
See legend on p135.

Figure 4-6: Change in Delta Band Power in Response to Cannulation. The change in delta band power between cannulation and background activity, compared with the change between two background periods at Cz and CPz in (A) the naïve group, and (B) the LA group. Cannulation evoked a significant increase in delta power in the naïve group (*, $p<0.05$), which is not seen in the LA group. (C) Change in delta power between cannulation and background activity at each of the EEG channels (ordered according to mean response). Error bars indicate standard error of the mean.

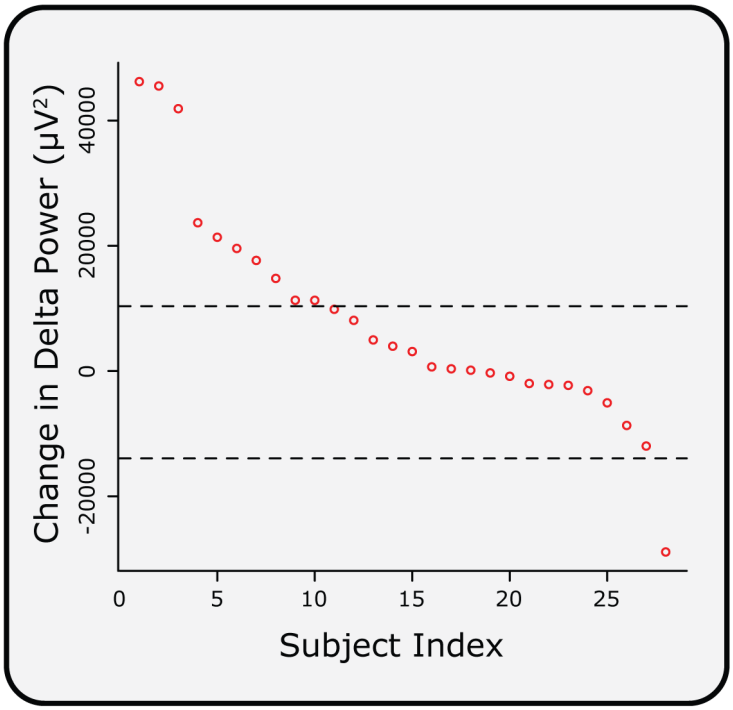

Figure 4-7: Change in Delta Power in Individual Subjects in Response to Cannulation. Individual subjects were ranked according to change in delta power between cannulation and background. Dashed lines indicate the mean ± standard deviation of the change in delta power between the background periods.

The evoked change in delta power following cannulation was not associated with other autonomic or behavioural measures. No change was observed in the average heart rate between the background ECG (107.8 ± 3.7 bpm) and post-cannulation (106.3 ± 3.6 bpm) (beats per minute, mean ± standard error of the mean, p=0.09, Wilcoxon signed-rank test). No movement was observed when looking at the change in the ipsilateral and contralateral

EMG activity between background and cannulation (p=0.26 and 0.55 respectively; Wilcoxon signed-rank test).

Figure 4-6(C) shows the change in delta power post cannulation compared with background EEG across all recording electrodes. While the largest response was observed at CPz, all electrodes showed an increase in delta power. In order to exclude the possibility that DC artefacts, such as potential changes across the skin, were responsible for the increase in delta activity, power was also examined in the infra-slow-delta band (0-0.5 Hz) and high-delta band (0.5-5 Hz) separately (Figure 4-8). In response to cannulation, there was a significant increase in the high-delta band (p=0.031) and a non-significant increase (p=0.13) in the infra-slow-delta band. This suggests that the majority of the evoked change in delta activity was caused by an increase in the high-delta band.

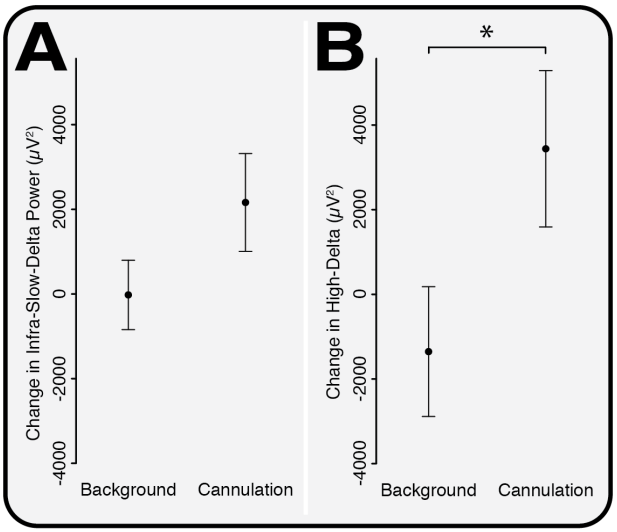

Figure 4-8: Change in Infra-Slow-Delta & High-Delta Activity in Response to Cannulation. To determine whiter the delta power response to cannulation was related to infra-slow or higher frequency delta activity, the delta band was split into infra-slow-delta (0-0.5 Hz) and high-delta (0.5-3 Hz) components. (A) Infra-slow-delta increased with cannulation compared with background periods, but this increase was not significant (p=0.13). (B) High-delta significantly increased in response to cannulation (p=0.031). Error bars indicate standard error of the mean.

4.3.4 DELTA ACTIVITY IS INCREASED BY EXPERIMENTAL NOXIOUS AND INNOCUOUS TACTILE STIMULI, AND IT IS GRADED WITH THE INTENSITY OF THE STIMULUS

The experimental noxious stimuli (9±1 per subject) applied to the dorsum of the hand caused an average increase in delta activity of 620.4±193.4 μV^2, which was significantly different from zero (Figure 4-9, p=0.0099, one-sample median test with Bonferroni correction for multiple comparisons). The experimental tactile stimuli (9±1 per subject) caused a lower but significant increase in delta power of 291.4±186.5 μV^2 (p=0.012) than that evoked by experimental noxious stimuli. Comparison of the innocuous and noxious experimental stimulation, Figure 4-9, with the response to cannulation, Figure 4-6(A), shows a graded increase in delta power with the intensity of the stimulus.

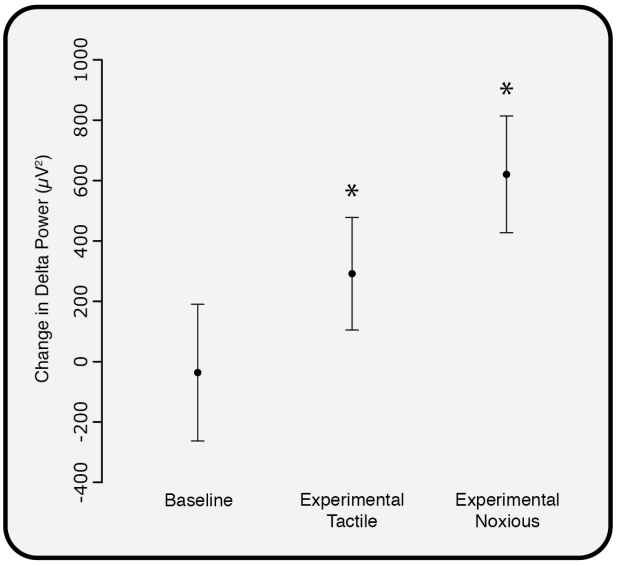

Figure 4-9: Change in Delta Power in Response to Experimental Noxious & Tactile Stimuli. The change in delta band power (post-stimulus minus pre-stimulus) for all experimental tactile and noxious stimulation (*, p<0.05, significant difference from zero) compared with the change between sequential pre-stimulus (baseline) periods. Error bars indicate standard error of the mean.

The average change across subjects evoked by each individual stimulus is shown in Figure 4-10. There was no significant difference in the change in delta power with

progressive stimulus number for either the noxious or tactile stimuli (ρ=0.40, permutation test). This indicates that habituation or sensitisation to the stimulus is unlikely to have occurred at this intensity or inter-stimulus level.

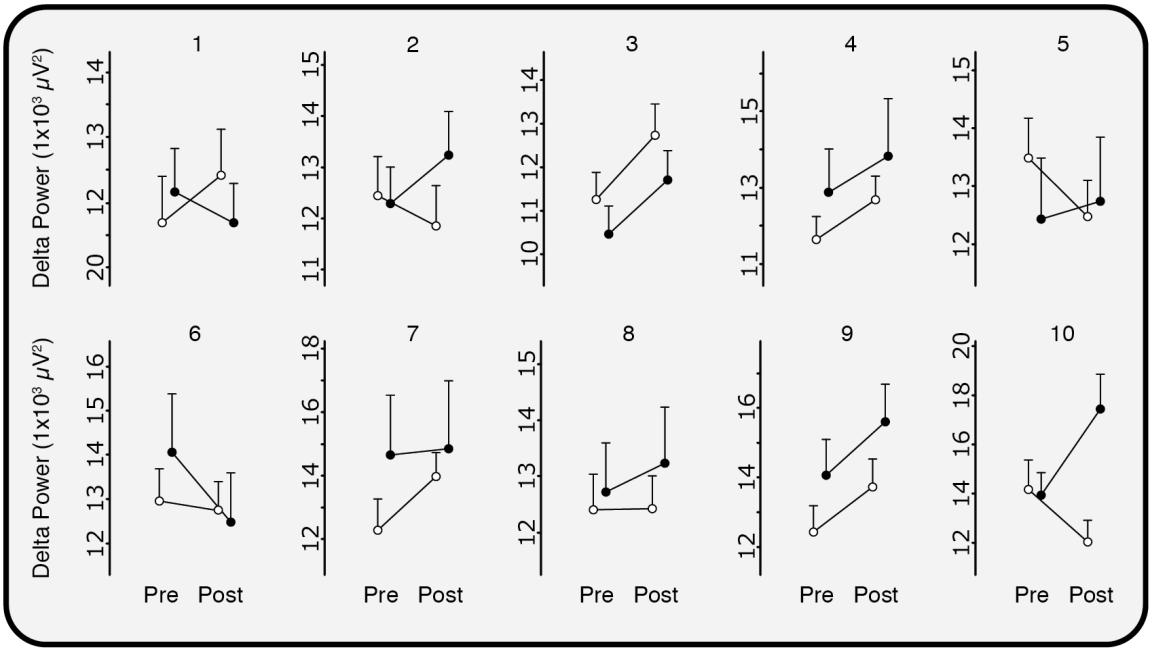

Figure 4-10: Change in Delta Power for Each Experimental Stimulus. Pre-stimulus baseline compared with post-stimulus delta band power for the experimental tactile (open circles) and experimental noxious (solid circles) stimuli according to stimulus number (1-10). Error bars indicate the standard error of the mean.

4.4 Discussion

Described in this chapter is the observation of altered patterns of EEG activity following experimental and clinically-essential noxious stimuli in anaesthetised children. Using my novel and innovative techniques (see Chapter 3), I have demonstrated that acute noxious events evoke a significant increase in the EEG delta activity of children receiving sevoflurane monoanaesthesia. This increase in activity is diminished by the application of topical local anaesthetic to the surface of the skin, and cannot be attributed to movement

or autonomic responses, as no concomitant changes in either heart rate or spinal reflex withdrawal were recorded in the same time period.

During the experiment, background EEG activity remained constant throughout, implying that any changes in response to noxious stimuli could not be attributed to a drift in anaesthetic depth during the experiment. There was also no effect of age across the age range (1-12 years), suggesting that the observed stimulus-evoked increase in delta activity is already developed by one year of age. The increase in delta activity was graded by the intensity of the stimulus with noxious stimuli evoking a greater response than an experimental tactile stimulus, but the skin piercing cannulation evoking a greater increase compared with to both experimental noxious and experimental tactile stimuli.

The mechanisms of general anaesthetic action is not fully understood but nearly all commonly used volatile agents, including sevoflurane[316], are thought to enhance GABAergic inhibition by potentiating GABA-induced Cl^- currents or, in high concentrations, by directly activating $GABA_A$ receptors[316]. The EEG in an awake subject is predominantly characterised by higher frequency components and has low levels of delta activity compared to anaesthetised patients or when subjects are asleep. When anaesthetised[323], the EEG recordings of adults results in an increase of delta activity compared to when awake. In this study, the administration of sevoflurane monoanaesthesia also resulted in a high level of synchronous firing, with delta activity accounting for 62.6% of the total signal power.

It is thought that large networks of cortical neurones synchronously firing generate this increase of delta activity. Under anaesthesia, the absence of ascending excitatory input

from arousal nuclei to the thalamus[316,379,380] causes thalamic neurones to default into their rhythmic burst-firing mode. Thalamic neurones are extensively connected to the cortex, and the rhythmic and synchronous firing of large populations of cortical neurones give rise to delta oscillations[316].

In this study, I have shown that in anaesthetised children, noxious and innocuous events lead to a further increase in delta activity. The mechanism underlying this phenomenon remains unknown. It may be plausible that when nociceptive activity arrives at the thalamus of an anaesthetised patient, more thalamic neurones are recruited into the corticothalamic loop, thereby intensifying the cortical oscillations. On an EEG recording, this would be observed as an increase in activity in the delta frequency band[316], consistent with observations in this study.

An increase in delta power to noxious stimulation has been reported in anaesthetised adults, where a range of clinical events, including surgical incision and laryngoscopy, have been shown to cause an increase in delta activity[361,363,367,368]. The opposite effect (i.e. a decrease in delta activity) has also been reported in some studies, with the differences in results attributed to the different surgical stimuli, anaesthetic agents, and dose[361,367,370,372]. In this study, cannulation evoked an increase in delta activity above the expected level of background variability in 36% of the children. The individual differences between subjects cannot be attributed to age, different surgical techniques, or anaesthetic agents or dose. It is possible however, that the differences reflect the children's individual variability in terms of their sensitivity to the anaesthetic agent or sensory processing. However, there is a complex interaction between the effects of anaesthesia and noxious stimuli on the generation of delta activity. Children who did not show an increase in delta activity may

have received a level of anaesthesia that was sufficient to suppress the response. Given recent evidence that suggests that changes in slow wave activity can be used as an individualised biomarker to identify the point when patients transition from awareness to unresponsiveness[323], the importance of investigating the factors that drives this response is clear.

In this study, the level of noxious stimulation that participants received was very small compared to the level of stimulation they are likely to receive during surgery. Nevertheless, despite being relatively small, a change in delta power could still be observed. With my results suggesting that the evoked delta response is graded with the intensity of the stimulus, it would be important to use these techniques to investigate a more intense surgical stimulus, such as the first surgical incision, and a broader range of anaesthetic and analgesic strategies currently administered to children. The observation that the increase in delta activity observed in this study has also been observed in adults is also of interest. Despite the continuous brain development that occurs throughout childhood and adolescence[373-375,381], it appears that the mechanisms involved in the generation this evoked response is already present from 12 months of age. However, a more extensive developmental study would need to be conducted to establish how this activity is modulated by age.

The clinical utility of these measures and techniques cannot yet be assessed. However, what this study does is provide mechanistic insight into how the brain in anaesthetised children responds to noxious events. This work may lead to direct improvements in patient care as our new methodology provides a more sensitive way of investigating whether current protocols used by paediatric anaesthetists are anti-nociceptive.

Assessing whether an anaesthetised patient is receiving adequate analgesia will always be problematic and in the absence of self-report, it is impossible to know what, if anything, a person is feeling. We can infer from changes in heart rate or spinal reflex withdrawal activity a patient's level of pain, but these are surrogate measures with limited validity. In anaesthesia, where multiple drugs are used, care must be given to what measures are chosen because the lack of a motor response is likely to be due to the use of muscle relaxants and not an adequate level of anaesthesia or analgesia. The pupillary dilation reflex has recently emerged as a possible alternative measure because clinical doses of sevoflurane do not inhibit the reflex when autonomic and motor activity is inhibited[352,382]. Cortical activity is fundamental to the perception of pain[1], and so the changes in cortical synchronisation following noxious stimulation observed in this study may provide the most specific measure to consider when assessing if anaesthetic techniques are anti-nociceptive. This is particularly interesting in light of our observation that the application of a topical local anaesthetic diminished the evoked increase in cortical synchronisation caused by cannulation.

The simultaneous recording of multiple behavioural and physiological responses, including cortical activity, is likely to give the best insight into nociceptive processing and allow for further investigations[383]. The novel techniques presented in this study provide a methodology whereby standardised levels of anaesthesia and experimental stimulation can be investigated, which will provide a platform in future studies to investigate what drives the inter-subject variability, and thus determine the clinical usefulness for individual patients.

Chapter 5

Anaesthesia & The Ex-Preterm Child

Chapter 5

Anaesthesia & The Ex-Preterm Child

5.1 Chapter Overview

With more than one in ten children around the world being born prematurely[13], survivors of neonatal care have the potential of being increasingly at risk of complications due to their immaturity and possible long-term sequelae from their time in intensive care. Their medical needs differ from healthy term infants and premature-born children have an increased incidence of neurophysiological deficits, such as cerebral palsy[17], are more likely to have respiratory complications, such as bronchopulmonary dysplasia[384], and have a greater likelihood of strabismus[385], retinal detachment[386], cryptorchidism[387], and short bowel syndrome[388]. Readmission rates into hospital are between 10–20%[21-24] and during their first year of life, premature-born infants are twice as likely to be readmitted into hospital than term-born infants[25]. This increases the chance that a premature-born child will require general anaesthesia for surgical and non-surgical interventions or investigations (e.g. A MRI scan).

To avoid the dangers of under or over dosing, the optimal titration of an anaesthetic dose is paramount to achieve effective analgesia, unconsciousness, and immobility[389-391]. With the uncertainty behind the neurotoxic effects of general anaesthesia in infants[392], it is

important to consider the effects of anaesthesia on the premature-born child who may undergo multiple operations during critical periods of brain development, and who may have structural and functional neurological abnormalities[393-399] that could alter the effect of anaesthetics.

Children born too soon are exposed to a high incidence of pain during neonatal care and may experience altered sensitivities to subsequent pain in later life (see Section 1.4). Because the prevention of pain is one of the cornerstones of anaesthetic practice, it is possible that premature-born children may be more sensitive to noxious stimuli and the effects of anaesthesia. Peters *et al.* reported an increase in subsequent perioperative analgesic requirements and greater postoperative pain in children who had undergone surgery during neonatal life[229]. Evoked patterns of nociceptive activity under anaesthesia are not similar to those seen in awake children, however, in the previous chapter; an increase in delta activity in response to noxious stimulation was recorded (see Chapter 4). This measurement provides a unique method of assessing whether premature-born children have altered responses to nociceptive stimuli under anaesthesia compared to term-born children.

In awake, premature-born children, structural and functional neurological changes have been noted and may result in selective cognitive deficits that have been directly related to the number of painful procedures during neonatal life[394-398,400]. Frequency band power is altered in school-age children born prematurely, with a reduction of alpha power and a slower alpha oscillating frequency than term-born children[394]. Additionally, those school-aged children born at less than 28 weeks gestation displayed a slowing of alpha-band frequency related to the amount of pain exposure during the neonatal period[396].

During the early twenties, young adults who were less than 1000 grams at birth exhibited lower alpha and beta power and higher delta and theta power in background EEG recordings[401]. It is plausible that premature-born children may also exhibit different patterns of background EEG activity compared with term-born children while anaesthetised.

Clinically, anaesthetists utilise autonomic and behavioural responses to titrate the levels of anaesthesia and provide appropriate anaesthesia. However, autonomic and behavioural responses alone are not sufficient to assess analgesia in the anaesthetised patient as these responses may be of brainstem or spinal cord in origin rather than cortical, a requirement for the experience of pain. More recently, electrophysiological measures of brain activity have been used to monitor anaesthetic depth[323,402-406] and identify changes in brain activity in response to nociceptive stimuli[361,363,367-369]. Differences in the patterns of background brain activity between anaesthetised patient groups could be clinically relevant in order to develop and optimise electrophysiological measures of anaesthetic depth.

This chapter aims to determine whether anaesthetised premature-born children display different patterns of background brain activity compared to aged-matched term-born children and to establish whether their electrophysiological responses to noxious stimuli are altered. The first hypothesis is that in response to noxious stimulation, premature-born children display a significantly increased delta activity response. Secondly, that premature-born children would have a reduction in alpha and beta power in their background EEG under anaesthesia.

5.2 Materials & Methods

5.2.1 PARTICIPATING CHILDREN

Forty-five children were studied at the John Radcliffe Children's Hospital, Oxford between July 2012 and February 2014 (see recruitment flowchart in Figure 5-1).

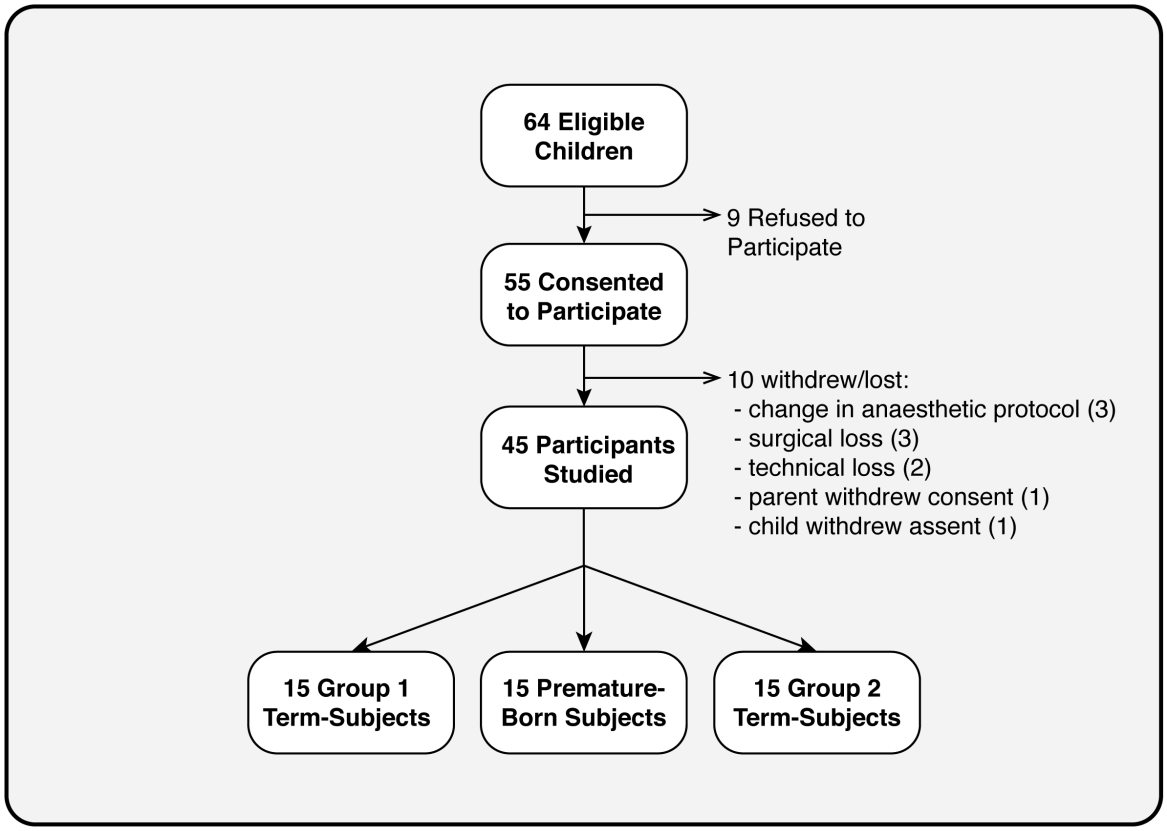

Figure 5-1: Recruitment Flowchart. Forty-five children participated in the study forming this chapter. They were divided into three experimental groups: (i) the first term-born group (n = 15, age range: 18-153 months), (ii) the premature-born group (n=15, age range: 15-150 months), and the second term-born group (n = 15).

Children were studied between 1 – 12 years receiving an elective operation or investigation (MRI) under general anaesthesia. Thirty children were recruited as part of the main study, with 15 premature-born children (mean gestational age at birth: 29.2 weeks, range 23 - 34 weeks, mean age at study: 5.2 years, range 15 - 150 months). As the sample size is relatively small, and there is a higher prevalence of term born children, I

wanted to test whether the mean band power and standard errors reported in the term-born children could be reproduced in an independent sample. Therefore, a separate cohort of 15 aged-matched term-born children (mean age at study: 4.4 years, range 14 - 100 months) was recruited for secondary comparison. This independent group were taken from the term-born group of neurologically normal children included in Chapter 4 and were selected based on how closely their age matched the original group of term-born children (see Subject Demographics in Appendix V).

Term-born children were excluded from the study if they had central nervous system disease or developmental delay. As prematurity leads to a number of developmental problems, premature born children were not excluded on these grounds. Instead, the study was designed to investigate the full range of premature-born children seen in the clinical setting (see Subject Demographics in Appendix V). However, given this approach, a number of premature born children had neurological impairment. To determine whether neurological impairment influenced the observations, in part of the analysis, premature-born children were split into two groups according to whether they had been clinically diagnosed with a neurological impairment. This was determined from medical notes and included any child where neurological abnormality was identified (see Subject Demographics in Appendix V).

5.2.2 EXPERIMENTAL PROCEDURES

Anaesthetic procedures used are previously described in Chapter 4. Gaseous induction of anaesthesia was performed using sevoflurane, oxygen, and nitrous oxide (N_2O) and once the child was stable, a LMA was inserted and an end-tidal sevoflurane concentration

of 2.5% was achieved by reducing the inspired sevoflurane and turning off N_2O (see Figure 4-2).

Immediately following anaesthetic induction, eight EEG recording electrodes were placed on the scalp, with reference and ground electrodes positioned at FPz and F8 respectively (see Section 2.3.2). Bipolar EMG was placed on the belly of both biceps brachii muscles, and a single ECG electrode on the left clavicle referenced to FPz (see Section 2.3.2).

Clinically-required cannulations were time-locked to electrophysiological recordings by means of a high-speed video camera at the time of acquisition (see Section 3.3.1). Cannulations performed on the dorsum of the hand (in 73% on the left hand - 80% in the term-born group and 67% in the premature-born group), and the exact point of cannulation, defined as the point where the cannula first made contact with the surface of the skin, was then marked on the electrophysiological recording after the experiment (see Figure 3-2).

5.2.3 ELECTROPHYSIOLOGICAL ANALYSIS

For each individual stimulus, the raw EEG was split into 10 second epochs - 5 seconds before and after the cannulation event mark, and each epoch was signal processed by the methods described in Section 2.3.3.

It is possible that due to a change in anaesthetic depth or drift in EEG signal that the background EEG activity may change during the recording. To determine whether there

is a change, three background periods were defined (see Figure 4-2). Due to artefacts caused by movement and repositioning of the upper limb for cannulation, the background period immediately prior to cannulation could not be used for analysis. The change from background activity evoked by cannulation was compared to the change in activity between two background periods.

EMG and ECG activity were exported as ten second epochs - five seconds before and five seconds after the stimulus and processed as described previously in Section 2.3.4.

5.2.4 STATISTICAL ANALYSIS

Statistical analysis was carried out using R (The R Project for Statistical Computing). Assessment of QQ plots showed that delta, theta, alpha and beta power, as well as change in delta power, heart rate and EMG, were all non-normally distributed. The four band powers were normalised prior to statistical analysis by taking the logarithm. The change in delta power in response to cannulation from background levels (with both the premature-born and term-born groups pooled together, i.e. without age-matching) was compared with the change between two background periods using a permutation test with subject age and EEG channel as additional factors. A permutation test was used because the data was not normally distributed and the sample size was relatively small. In addition the permutation test allowed for additional factors (i.e. subject age and EEG channel) to be considered. The change in delta power post-cannulation was then compared between the two aged-matched groups using a permutation test, with subject group (premature-born or term-born) and EEG channel as within subject factors. For each frequency band, the difference between background EEG was compared between the age-matched subject

groups, using a three-way ANOVA (with subject group, background period and EEG channel all within subject factors). Holm's method was used to correct for multiple comparisons across the four frequency band powers. In addition, a three-way ANOVA (channels considered as a within subject factor) was performed across the three subject groups (term-born group 1; term-born group 2; premature-born group) to check that significant differences identified in the previous analysis were still present when the data was considered in a single analysis.

Changes in heart-rate and in EMG between background and cannulation were assessed using a Wilcoxon-signed rank test.

5.3 Results

5.3.1 NOXIOUS CANNULATION EVOKES A SIGNIFICANT INCREASE IN DELTA POWER

In both term-born and premature-born children, the clinically required noxious cannulation evokes a significant increase in delta power (p=0.032, permutation test, with no effect of channel or age; Figure 5-2).

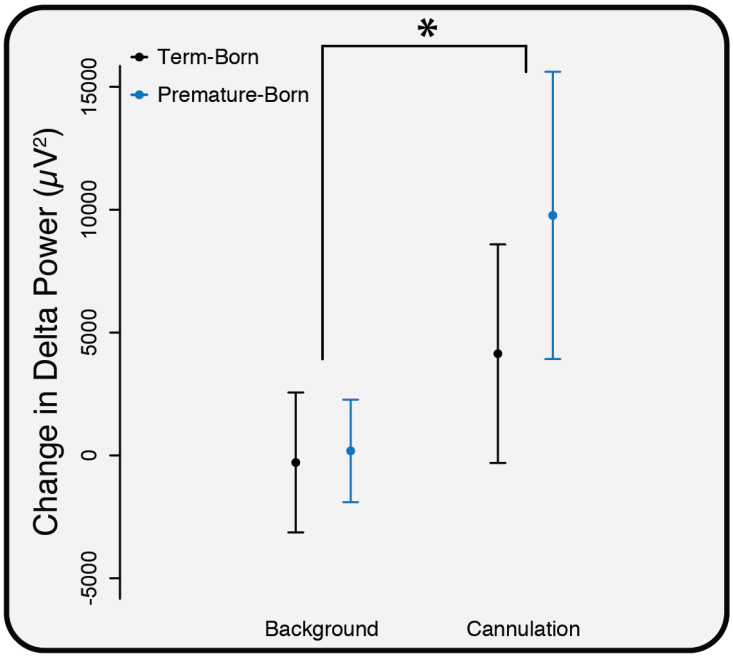

Figure 5-2: The Change in Delta Power in Response to Cannulation. The change in delta band power from cannulation to background activity ('cannulation') is compared with the change in background activity between two background periods ('background'). There were no significant differences in the change between the premature-born (blue) and term-born (black) groups, though a significant increase was observed between background and cannulation (*: $p<0.05$). Error bars indicate standard error of the mean.

This is consistent with results found in Chapter 4. The increase in delta activity was not significantly different between the term-born and premature-born groups ($p=0.44$, permutation test). The average background delta power in the term-born children was 1.6 ± 0.2 x10^4 μV^2 (mean ± standard error of the mean (SEM)) and in the premature-born children was 1.4 ± 0.2 x10^4 μV^2. The average delta power following cannulation was 2.0 ± 0.3 x10^4 μV^2 in the term-born children and 2.5 ± 0.4 x10^4 μV^2 in the premature-born children. There was no affect of age on the change in delta power (Figure 5-3 (A)). When both groups of children were combined, there was also no affect of age on background alpha or beta power ($p=0.60$ and $p=0.70$ respectively, Figure 5-3 (B & C)).

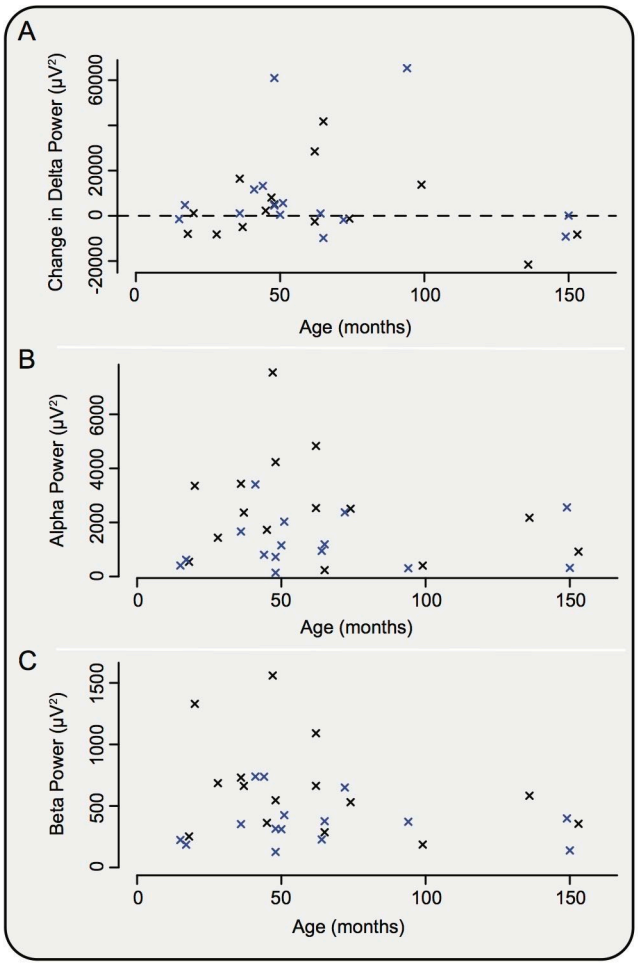

Figure 5-3: Relationship Between Patterns of Brain Activity With Age. (A) The change in delta power in response to cannulation, (B) background alpha power, and (C) background beta power, plotted against age at study for term-born (black) and premature-born (blue) children. Each point represents the average EEG activity across channels for each child.

5.3.2 NOXIOUS CANNULATION DID NOT ALTER HEART RATE OR MOTOR ACTIVITY

Noxious cannulation did not alter the average heart rate in either the premature-born children or term-born children (p=0.14 & p=0.33 respectively, Wilcoxon signed-rank test). In background recordings, the average heart rate in term-born children was 106.5 ± 4.8 bpm (mean ± standard error of the mean, beats per minute). In premature-born children this was 108.2 ± 5.0 bpm. In both groups the average heart rate following noxious cannulation changed by less than 2 bpm.

Across all background periods, there was no change in ipsilateral or contralateral EMG activity in response to cannulation in the premature-born children (p=0.39 and p=0.30 respectively, Wilcoxon signed-rank test) or the term-born children (p=0.25 and p=0.23 respectively).

5.3.3 BACKGROUND ALPHA & BETA POWER IS SIGNIFICANTLY LOWER IN PREMATURE-BORN THAN AGE-MATCHED TERM-BORN ANAESTHETISED CHILDREN

To test whether being born prematurely altered the background EEG activity under anaesthesia the delta, theta, alpha and beta bands, power was calculated during three background periods in both the term-born and premature-born children. The average background power spectrum for the premature-born and term-born children is shown in Figure 5-4.

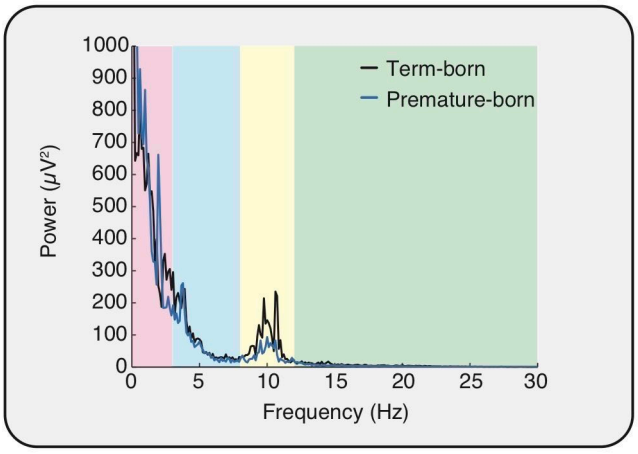

Figure 5-4: Average Power Spectra in the Background EEG. Power spectra, averaged across all channels and all children, in the background period for the term-born (black) and premature-born (blue) children. For the analysis, the power was calculated in the delta (0-3 Hz, red), theta (3-8 Hz, blue), alpha (8-12 Hz, yellow), and beta (12-30 Hz, green) bands.

The background delta and theta activity was not significantly different between the two groups of children (Figure 5-5 (C & D), p=0.66 and p=0.28 respectively; three-way ANOVA). Consistent with Chapter 4, delta activity accounted for the majority of power in both term-born and premature-born groups (63.4 ± 2.0 % in the term-born group and 70.1 ± 2.3 % in the premature-born group). However, both the background alpha and beta band power were significantly lower in the premature-born children compared with the term-born children (Figure 5-5 (A & B), p=0.048 and p=0.048 respectively, p-values corrected for multiple comparisons across the four frequency bands. NB. Error bars in figure are standard error of the mean. Uncorrected p values: alpha; 0.013 and beta; 0.012).

The decrease in alpha and beta power in the premature-born children was observed across all three background periods (see Figure 5-5 (A & B)) and both the alpha and beta power remained stable across these periods of background EEG activity (p=0.36 and p=0.16 respectively; three-way ANOVA). Compared to term-born children, alpha and beta power in the premature-born children was lower across all EEG channels. However, there was a significant effect of channel with the greatest difference in band power between term-born and premature-born children being observed at FCz, Fz, and Cz (p<0.001; three-way ANOVA, Figure 5-6).

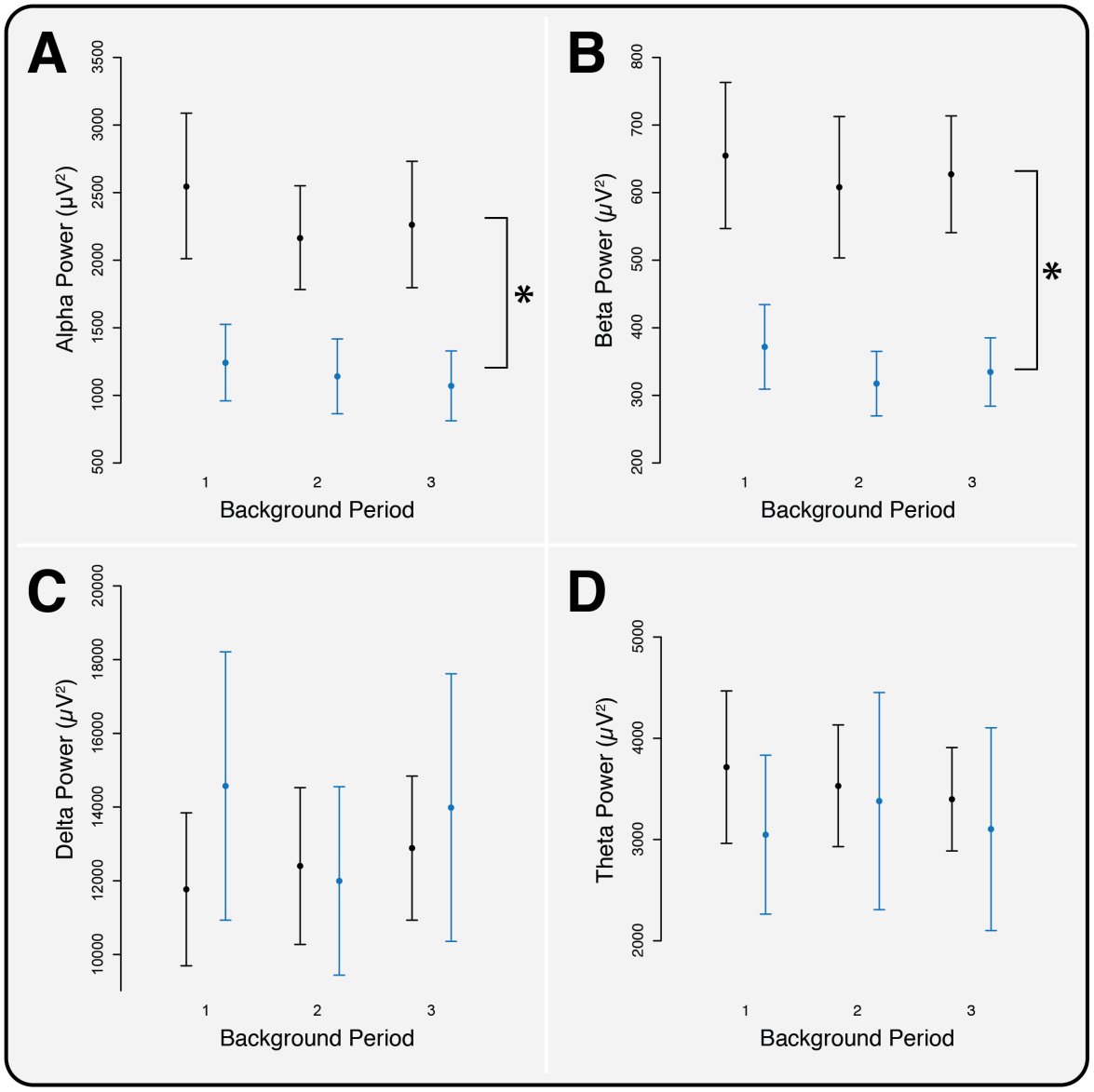

Figure 5-5: Background Power Between Term-Born & Premature-Born Children. Band power across three background periods are compared between term-born (black) and premature-born (blue) children in (A) alpha band, (B) beta band, (C) delta band, and (D) theta band. (*: $P<0.05$ indicates significant differences between the two groups across all background periods, p-values were corrected for multiple comparisons using Holm's method, across the four frequency bands). Error bars indicate standard error of the mean.

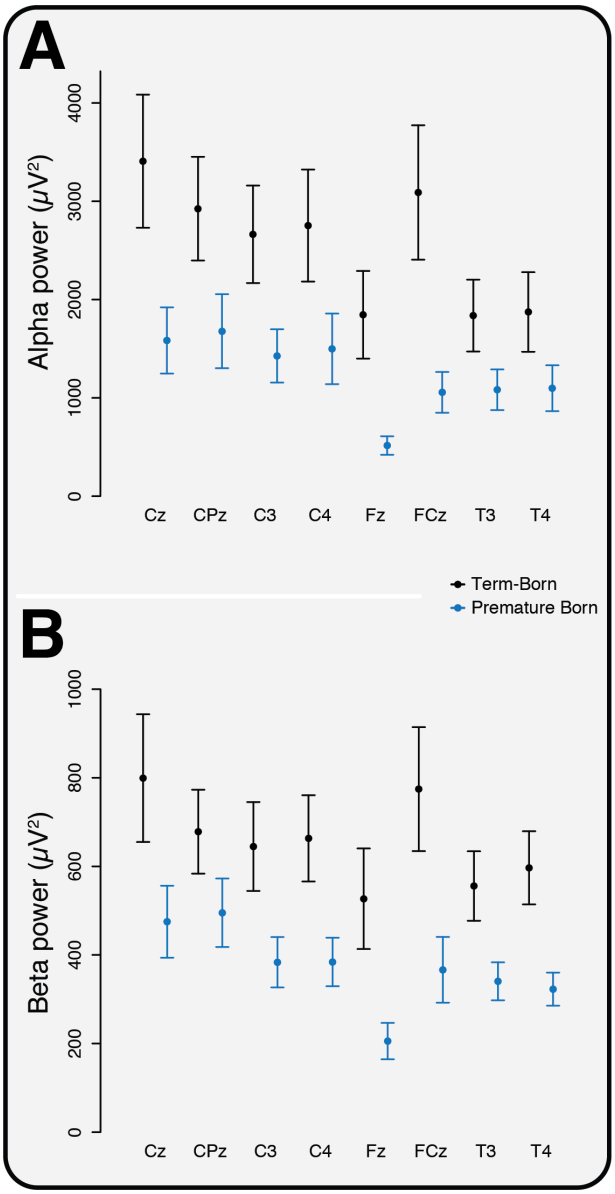

Figure 5-6: Comparison of Alpha & Beta Band Power With EEG Electrode. (A) Alpha and (B) beta power in the first background period for each EEG electrode, compared between term-born (black) and premature-born (blue) children. Error bars indicate standard error of the mean.

Due to my relatively small sample size of the premature-born group (n=15), I validated this observation by comparing the alpha and beta band power of my term-born group to an independent sample of age-matched term-born children (n=15). Figure 5-7 shows that across all three backgrounds, alpha and beta power was consistent between both groups of term-born children (p>0.05 in both cases). The premature-born group also had a

significantly lower alpha and beta power compared with the second group of term-born children (p=0.042 and p=0.008 respectively). This analysis indicates that the observed decrease of alpha and beta power in premature-born children relates to the group's prematurity rather than subject variability. While, without having powered to guard against false negatives, no strong conclusion can be gained from the lack of differences between the two term groups, it nevertheless increases the evidence to suggest that the observed differences between the term-born and premature-born children are related to the groups prematurity rather than individual subject variation. In addition, the premature-born group had significantly lower alpha power (p=0.009; three-way ANOVA; post hoc comparison p<0.05) and significantly lower beta power (p=0.003; three-way ANOVA; post hoc comparison p<0.05) compared with both groups of term-born children. There was no significant difference in the alpha power (post hoc comparison; p=0.74) or beta power (post hoc comparison; p=0.1) between the independent groups of term-born children.

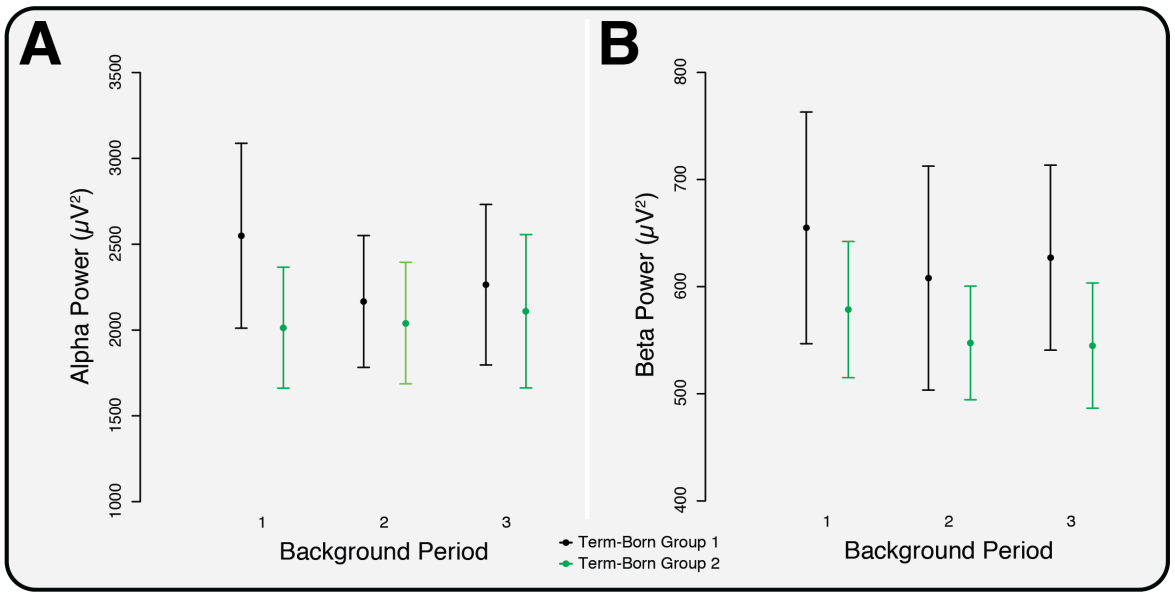

Figure 5-7: Comparison of Band Power Between Two Independent Groups of Term-Born Children. (A) alpha and (B) beta power across three background periods in the main group of term-born children (black) compared with the second independent group of term-born children (green). There was no significant difference between the two groups. Error bars indicate standard error of the mean.

5.3.4 BACKGROUND EEG & BRAIN PATHOLOGY

As discussed in Section 1.1.3, prematurity can lead to numerous developmental sequelae during childhood, of which, neurological issues are of particular concern. This chapter considered a clinically representative population of premature-born children with a wide range of pathologies (see Appendix V). It is possible that the observed differences between the term-born and premature-born children are driven by subjects with neurological impairment rather than a direct result of being born prematurely. To address this issue, the premature-born children were split into two groups according to whether or not they had neurological impairment identified in their medical records. Premature-born children with neurological impairments (n=6) had lower average levels of background alpha and beta power (764.3 ± 230.0 μV^2 and 233.2 ± 55.2 μV^2 respectively, mean ± standard error of the mean) compared to the premature-born children without

neurological impairment (n=9, average alpha: 1408 ± 373.1 μV^2, average beta: 413.2 ± 66.0 μV^2). However, the average alpha and beta power in the premature-born subjects without neurological impairment was still lower than the term-born children (Figure 5-8). The reduction of alpha and beta power is therefore not only a feature of prematurity, but additionally related to the level of neurological impairment.

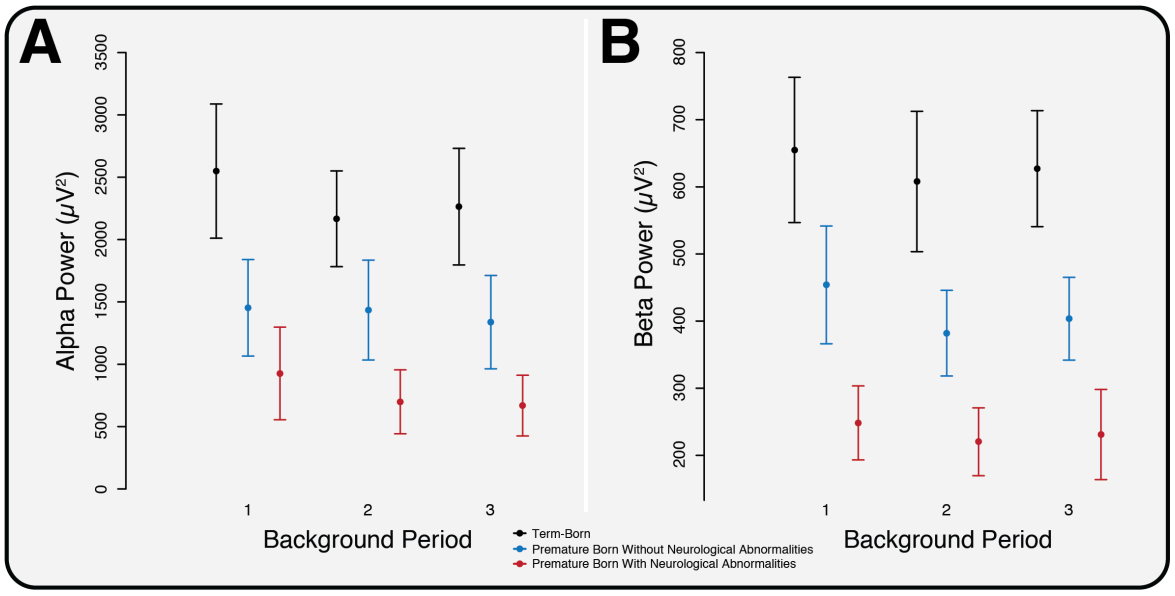

Figure 5-8: Comparison of Children With & Without Neurological Impairment. (A) alpha and (B) beta power across the three background periods in the term-born children (black), compared with the premature-born children without neurological impairment (blue), and the premature-born children with neurological impairment (red). Error bars indicate standard error of the mean.

5.4 Discussion

In this chapter, the differences in electrical activity of the brains of premature-born and term-born children have been investigated using EEG under sevoflurane monoanaesthesia. The first hypothesis tested was that premature-born children display an increased response to noxious stimulation compared to term-born children. The second

was that premature-born children would have an altered background brain activity under anaesthesia when compared to term-born controls.

Consistent with the results from Chapter 4, clinical noxious cannulation evoked a significant increase in delta activity that could be observed in both premature-born and term-born children. However, contrary to my hypothesis, the activity evoked by cannulation was not significantly different between the two groups of children. This suggests that nociceptive processing in anaesthetised children is not significantly altered by age at birth. In this study, significant differences were observed in the background EEG activity between premature-born and term-born children. Across all three background periods there was a reduction in alpha and beta power in premature-born children. This cannot be attributed to differences in the level of given anaesthetic as sevoflurane was kept at a consistent end-tidal concentration of 2.5%.

Previous research in awake premature-born children has seen a reduction in alpha power[394], and an increase in the gamma/alpha ratio[396] compared with term-born children. A decrease in alpha and beta power, with relative increase in delta and theta power, has also been shown in premature-born young adults when compared to their term-born peers[401]. These spectral changes have been associated with the increased likelihood for premature-born infants to experience later cognitive deficits[396,397].

Alpha and beta power are reduced in pathologies, including schizophrenia[407], attention-deficit hyperactivity disorder[408], Parkinson's disease[409], and Alzheimer's disease, in which EEG spectral measure correlate with scores of cognitive deterioration[410]. As thalamocortical interactions are thought to underlie alpha rhythm generation, it has been

suggested that the changes in spectral power in these pathologies relate to alterations in thalamocortical connectivity[411]. Even without overt neurological sequelae, premature-born children may have structural and functional white matter alterations[393,399]. Thus, it has been suggested that changes in thalamocortical circuits also leads to alterations in spectral power in awake premature-born children[394]. While a limitation of this study is that EEG activity in the children was not recorded before induction of anaesthesia, we demonstrate that these spectral differences are seen under anaesthesia. As thalamocortical feedback loops are also thought to drive alpha activity under anaesthesia[412], it seems likely that alterations in thalamocortical circuits in premature-born children also give rise to the reduction in alpha power observed in premature-born children under anaesthesia

In recent years there has been a drive towards the use of EEG monitoring in the clinical setting to measure depth of anaesthesia[323,404,406]. The aim of these monitors is to aid the anaesthetist in allowing the patient to be sufficiently anaesthetised and reducing adverse anaesthetic events, such as neurotoxicity[389-391], by minimising the anaesthetic dose required and tailoring anaesthetic care for the individual[404]. BIS (Bispectral Index, Aspect Medical Systems), Entropy and Narcotrend monitors are three examples of monitors that use forehead electrodes to process multiple EEG factors in order to obtain a score of 0-100 (coma to alert states respectively). How each monitor derives their score is different but all use the power from spectral measures in various frequency bands. Additionally, BIS measures the phase synchronisation between different frequencies to produce a beta ratio[413]. The 'depth scores' decrease with decreasing levels of consciousness[414] and increasing doses of general anaesthetic agents[415]. A BIS score between 40-60 is termed 'surgical anaesthesia' where an absence of explicit recall is noted and as such, a lower dose of anaesthetic can be used allowing for a quicker recovery from anaesthesia[416].

In children as well as adults, the most common monitor in use is BIS. There is much controversy over the use of such monitors in adults, and the validity of BIS and similar measures is even more problematic in children[402,403,405]. BIS scores are similar to that in adults but appear to be less reliable in infants[417-419]. At the same MAC of sevoflurane, infants show a higher BIS value than older children[420], with the highest BIS values in the youngest infants[421]. At present, it is too early to make clinical recommendations regarding endpoints for titration of anaesthetic depth in premature-born children based on the results shown in this chapter. However, given the background EEG differences observed, it is worth noting that depth of anaesthesia monitors may have altered values in premature-born children compared with their term-born counterparts. Indeed, elderly subjects with cognitive impairment, who have lower alpha and beta power compared with controls, also have lower BIS values while awake[422,423], and it has been suggested that under anaesthesia, maintenance of these subjects at standard BIS values may not be appropriate[422].

Electrophysiological measures used to assess anaesthetic depth have been reported to be lower in children with intellectual impairments while both awake and under anaesthesia[424,425]. Children with cerebral palsy require less propofol during induction to reach a steady state BIS value compared with controls[426], and have lower BIS values after premedication and at some anaesthetic levels[427]. It therefore seems likely that premature-born children with neurological pathology will have lower BIS values. Indeed, in this study, the subjects with the lowest alpha and beta powers were those with neurological impairment.

Prematurity clearly influences the brain activity of the child and this study has shown that this may have an impact on their anaesthetic requirements. I have taken a pragmatic approach to the recruitment of premature-born children, as all children born at a gestational age of less than 37 weeks (receiving a gas induction) were eligible to take part in the study. The children in the premature-born group were born at a wide range of gestations from 23 - 34 weeks, and 6 out of 15 had neurological impairment. Using this approach I aimed to study the full spectrum of premature-born children seen in the clinical setting. While the greatest reduction in alpha and beta band power (compared with term-born children) was observed in the premature-born children with neurological impairment, a reduction in power was also seen in the premature-born children without neurological impairment. Though I did not perform neuropsychological assessments of the children, premature-born children are at a higher risk of cognitive impairment, compared with term-born children, even without overt pathologies[428-431]. Future studies should therefore investigate background EEG measures under anaesthesia compared with neuropsychological indices. I did not find a relationship between age and background alpha and beta power, or the magnitude of the evoked delta response to cannulation. While the age range was relatively wide (1-12 years), 57% of the participants were between 3-6 years. Given the relatively small sample size, a future study is needed to assess any age related changes.

In the previous chapter, I demonstrated that there was an increase in delta activity in response to noxious cannulation. Here, I have confirmed this increase and shown that there was no significant difference between premature-born and term-born children. Additionally there was no significant increase in EMG activity or heart rate following

cannulation between the two groups, indicating that the resultant increase in delta activity was not due to movement or changes in autonomic activity.

Previous studies have demonstrated altered pain responses following early life pain experience, both in infants[123,207,210,432,433] and in older children[185,206,213]. Early life surgery also increases pain sensitivity later in life, with subsequent surgery leading to an increase in perioperative analgesic requirements[229]. However, pain is a subjective experience, influenced by many factors such as memories, social context, attention, and mood, as well as changes in the peripheral and central nociceptive pathways[1]. Premature-born children have a heightened level of pain catastrophising, which may contribute to altered pain responses[213]. In this study, contrary to my hypothesis, no difference in the response to cannulation was observed between the premature-born and term-born children. It is therefore plausible that the altered pain sensitivity seen in awake individuals is predominantly related to the conscious processing of the stimulus. Hohmeister and colleagues showed that while heat pain threshold was different between children with neonatal intensive care experience compared with those without, in both groups the pain threshold increased when the child's mother was present - possibly because the child was less anxious or more distracted[213]. Moreover, the cortical response to cannulation observed here is different from what we would expect to see in an awake individual[146,149], indicating a difference between conscious and unconscious processing of noxious stimuli.

Due to gaps in medical records, the precise number of painful procedures experienced during early life is not known. While it is likely that the premature-born children experienced a number of painful procedures as part of their essential medical care in the neonatal period, children in the term-born group may have also experienced painful

procedures in early life that have not been accounted for in this study. Moreover, cannulation is a relatively small noxious stimulus. As increase in delta power has been observed in response to surgical incision in anaesthetised adults[361,363,367-369]. In a future study it would be interesting to investigate responses to surgical incision, to determine whether premature-born children exhibit differences in nociceptive processing at greater stimulus intensities.

In conclusion, I have demonstrated that premature-born children have decreased alpha and beta power compared with term-born controls while under sevoflurane anaesthesia, and that both groups show an increase in delta power in response to cannulation. The results suggest that, when the same level of anaesthetic is given, premature-born infants reach a different depth of anaesthesia due to altered thalamocortical connectivity.

Chapter 6

Conclusion

Chapter 6

Conclusion

6.1 Chapter Overview

With hundreds of thousands of children undergoing general anaesthesia each year, the opportunity to investigate the cortical processing of nociception is an important one. The lack of any neurophysiological signature of analgesia and the development of such a measure using non-invasive techniques may be of considerable interest to the paediatric anaesthetist. The ability to further tailor an anaesthetic technique to the individual child is essential in order to minimise adverse and undesirable effects of too much or too little anaesthesia.

In this thesis, I have aimed to determine whether noxious stimulation administered to anaesthetised children results in a measurable change in brain activity and on the basis of anecdotal evidence, whether any evoked activity is altered in children who have been born prematurely and experienced a high level of pain during early life.

6.2 A Novel Method of Time-Locking Clinical & Experimental Noxious Stimuli

Conducting EEG studies in children, in a busy operating theatre setting was bound to be challenging, and in this study I have had to make several practical decisions to study such a population. Most difficult perhaps was in how to record the various clinical and experimental stimuli. The insertion of an intravenous cannula as part of routine anaesthetic practice provided me with a useful nociceptive stimulus, creating the opportunity to measure electrophysiological responses using EEG, EMG, and change in heart rate. However, a cannula does not lend itself to being time-locked to electrophysiological recordings with ease. I overcame this problem by synchronising the stimulus to EEG recordings with video. These systems are commercially available and in use clinically, however, the video speed of these systems is insufficient to capture the subtleties of a cannula (or PinPrick stimulus) touching and piercing the skin. My new method to time-lock experimental stimulations improves on current systems not only by capturing video at a faster frame rate, but by being more compact and portable, allowing cameras to get much closer to the stimulus than ever before. Here, I have recorded cannulations, but the possibility of other more complex clinical stimulations, such as vaccinations, venepuncture and others, could be recorded with ease.

An additional benefit to using high-speed video-EEG is in relation to the clinical investigation of epileptic brain activity. Conventional frame rate systems can only synchronise EEG to video recordings with a precision of 60 ms, but the ability to time-lock EEG with a precision of 9 ms may be useful in relating subtle behavioural manifestations to epileptogenic brain activity.

In addition to the investigation of clinical stimuli and benefits to clinical EEG investigations, using this technique, I have also demonstrated the effectiveness of recording experimental noxious stimuli in both neonates as well as anaesthetised children. Unlike the clinical cannulations or heel lances, the opportunity to record evoked potentials or cerebral changes to experimental stimuli provides a repeatable measure that can be used to measure the efficacy of potential pharmacological agents in these vulnerable populations.

6.3 Do Anaesthetised Children Process Noxious Stimuli At the Cortical Level?

The first aim of my thesis was to address the question of whether noxious stimulation performed in anaesthetised children evokes a stimulus-related change in brain activity, which can be recorded using electrophysiological techniques. The studies presented here demonstrate that children between one and 12 years can respond to nociceptive stimuli under sevoflurane anaesthesia, with a significant increase in delta band power following cannulation. This increased response was inhibited by the application of topical local anaesthetic, and supports the view that the evoked response is stimulus-related because peripheral transmission of nociceptive information is inhibited. Changes in delta power were also observed following tactile stimulation. The magnitude of change in delta power was graded with stimulus intensity. Greatest changes in delta power were observed following noxious cannulation and smaller magnitude changes were evoked by non tissue damaging noxious PinPrick stimulation. The evoked changes were not nociceptive specific as delta power also increased following tactile stimulation, albeit with a smaller magnitude.

This study was predominantly observational, with children from many medical histories and ages being included. It could be argued that a myriad of factors could influence the observed results. Standardising the concentration of sevoflurane was a strength of this study. However, it is appreciated that sevoflurane concentration may drift over the course of the study. By measuring the background anaesthetic levels at various points along the experiment, we were able to ensure that there were no differences in the background EEG activity throughout the recording.

This study has demonstrated that the application of noxious stimulation to the anaesthetised child does evoke changes in brain activity. This innovative technique provides the possibility that sensitivity to noxious stimuli during anaesthesia could be investigated in other clinical populations.

6.4 Do Prematurely-Born Children Have An Altered Response to Nociceptive Stimulation Under Anaesthesia?

My second aim was to determine whether children born prematurely have an altered response to noxious stimulation while under anaesthesia, compared to age-matched term-born controls. My results have led me to the conclude that at the same level of anaesthesia, premature-born infants achieve a greater depth of anaesthesia, possibly due to altered thalamocortical connectivity. This has been demonstrated through premature-born children displaying a decreased alpha and beta power compared with term-born controls. Surprisingly, I have demonstrated an increase in delta power in response to cannulation in both premature-born and term-born children. However, if the ex-preterm child is indeed at a greater depth of anaesthesia, the true response to noxious stimulation may be masked,

or indeed be very different due to structural and functional changes in their neuroanatomy.

6.5 Limitations

This thesis began by aiming to look at the differences between term-born and premature-born children under anaesthesia. This was because anaesthetists had communicated that prematurely-born children appeared to be more sensitive to an acutely noxious stimulus while under volatile anaesthesia compared to term-born children. I hypothesised that it may be possible to observe evoked potentials following noxious stimulation in anaesthetised children. To test this hypothesis, I characterised the response to noxious stimulation in anaesthetised children across all age groups. From my pilot studies, it became obvious that evoked potentials were not seen, but rather a general change in delta activity could be observed. The increase in delta power was not nociceptive specific but was also evoked in response to experimental non-noxious tactile stimulation. Furthermore, the pilot studies indicated that this increase was seen in both term-born and premature-born children.

During the study, I aimed to achieve a steady inspired concentration of sevoflurane. In a pragmatic approach, I believed that using a steady end-tidal concentration of sevoflurane would be the most appropriate way to standardise anaesthetic dose across a wide range of subjects. However, it must be noted that end-tidal concentrations do not necessarily match blood anaesthetic concentration and there is a chance that there is a difference in the pharmacokinetics of sevoflurane between term-born and premature-born children. Additionally, by not using anaesthetic 'depth' as my end point, there is a distinct possibility

that at the identical end-tidal concentration of sevoflurane, premature-born children may be more or less deeply anaesthetised than term-born children.

There was in fact no difference in the response to noxious stimulation between term-born and premature-born children when this was fully tested in the main study. This may be because volatile anaesthetics could have a greater effect on nociceptive processing at the level of the spinal cord compared to the brain. The sevoflurane concentration of 2.5% was chosen as it related to 1 MAC across the age range studied. However, it might be possible to observe differences between premature and term born children at higher or lower concentrations of anaesthesia, which were not tested as part of this study.

A further limitation was that the effect size we saw was very small. This is likely to be due to the fact that the stimuli used was very mild and of relatively low intensity compared to surgical stimuli. This puts into question the clinical significance of my finding, as a cannulation is not as salient as a surgical event. Due to the small effect size, my powering and sample sizes would need to be very large to see any difference between the term-born and premature-born children at the stimulus intensity tested in this study. Unfortunately, due to my experimental setup and ethical approval, it was not possible to record activity to more noxious stimuli, such as surgical events. Indeed, this would also require that premedication or analgesics be given to the child, which would alter the experimental design, which was highly controlled in this experimental study.

Another concern is that participants were not randomised into a local anaesthetic group. This decision was made based on nursing decisions and the welfare of the child at the time. This meant that within the realms of my ethical approval, the recruitment of

children with and without local anaesthetic was opportunistic. In order to address this in future studies, a randomised controlled trial could be implemented.

My original hypothesis that premature-born and term-born children would respond differently to noxious stimulation while under general anaesthesia yielded a negative result. However, interesting differences in the background activity in term-born and premature-born anaesthetised children were observed. However, as differences in background brain activity have been observed in awake premature-born children compared to term-born children, a clear limitation to this observation is the lack of background data acquired prior to the induction of anaesthesia. Consequently, it was not possible to establish whether the differences observed in this study where caused by the administration of the anaesthetic or if they where present prior to induction.

It was interesting to observe that there was no age related changes in my results. This is a little surprising because the EEG is known to change dramatically over childhood, and my result may be because of the small sample sizes I was able to recruit for the study. I began recruitment with the aim to study the effects of differing degrees of prematurity and experience of early life surgery, however, after six months of recruitment there were fewer premature-born children than I had anticipated and I was limited to recruiting children in specific surgical lists, due to the complexity of some of the cases. This meant that I needed to pool together all my previous subgroups to form a mixed premature-born group that covered a wide age range. This also led to issues whereby I included children with and without neurological impairment in my analysis. I addressed this complexity by splitting my analysis according to known neurological impairments; however, it remains difficult to

know the extent to which the results are influenced by different clinical sub-populations, which were grouped in my analysis.

6.6 Further Work

This exciting project has shown that when under anaesthesia, children respond to noxious stimuli with an increase in their delta band activity of the EEG. Furthermore, premature-born children display a notably different level of background EEG activity under anaesthesia than their term-born counterparts. The great strength of this project has been the development of a robust technique to time-lock and record electrophysiological activity in the anaesthetised child. However, there have been several limitations to the study that I would like to address in the future.

Of particular note are the small sample sizes of children. Despite recruitment rates being high, identifying suitable premature-born children was a challenge, which led to the pooling of premature-born children from a variety of gestational ages at birth. Beyond the time constraints of a DPhil, I would like to use the Oxfordshire database of all neonatal patients to be able to follow up on premature-born children. This could allow for the identification of these children much earlier and allow them to be contacted prior to their arrival to hospital for any operation or investigation under general anaesthesia.

The choice of using a cannulation was in part a pragmatic one. The practical constraints related to the use of an anaesthetic room and work flow within UK operating theatres meant that it would be impractical to initially study the effects of other clinically noxious stimuli in such a controlled manner. The placement on an intravenous cannula is

however; performed thousands of times each day without the use of anaesthesia and such a mild stimulus is not predictive to responses that could be seen to major surgical stimuli. In the future I would like the opportunity to investigate the magnitude of EEG responses to major surgical interventions, such as the first surgical incision.

Furthermore, through studying the demographics of children included, certain operations, such as orchidopexy or hernia repair, are frequently encountered. It would be of particular interest to group studies according to surgical procedures and compare the effects of minimal and more invasive interventions. However, as it was possible to study responses to mild clinical and experimental stimulation, which were graded with stimulus intensity, it suggests that it would be of interest to study more intense surgical stimulation. Having had the opportunity to study children with topical anaesthetic provided me with a highly desirable additional control and it would be beneficial to incorporate such a group into future studies. To achieve this, it would require a more formal randomised controlled drug trial to be conducted in the future.

Due to a cannulation being a minor noxious stimulus, its clinical importance to the anaesthetist is unknown. With further work, I would like to consider whether this increase in delta power is sustained beyond the initial few seconds. It would also be of interest whether the effect could be observed in the same patient minutes later and potentially to record changes in brain activity post surgery. Additionally, it would be useful to elucidate the effect of differing doses of anaesthetic. This is of particular interest because increases in delta activity can be observed with deepening anaesthesia.

Perhaps the most important addition to this study would be to record background EEG activity in premature-born and term-born children prior to the induction of anaesthesia. This would enable us to determine if the altered brain activity seen under anaesthesia occurs specifically as a consequence of the premature child being under anaesthesia or whether these observations are also present as a result of being born too soon, regardless of the administration of the anaesthesia. If known differences in background brain activity in premature-born children persist under general anaesthesia, this could have clinical relevance when using brain-derived patterns of EEG activity to help establish anaesthetic depth.

The discovery that premature-born children with concomitant neurological impairment display the greatest reduction in alpha and beta band powers is particularly interesting. In performing further work, this population should be identified in advance of the study so that the degree of their neurological impairments can be assessed along with their degree of prematurity.

Many, if not all, of the limitations (Section 6.5) and further work of this study can be covered by a change in the logistical and experimental design. Keeping the strong technique developed here, I believe that being able to identify early on suitable premature-born children and children with neurological impairment would aid in their recruitment and increase subject numbers. A more inclusive study, which would take into account all stages of the pre-, peri- and post-operative period, would be of great benefit. Assessing background EEG activity in awake children prior to the induction of anaesthesia would allow for any differences in background activity to be quantified and to see if these persist under anaesthesia.

6.7 Concluding Remarks

In conclusion, this thesis describes a novel method that can be used to further our understanding and mechanic insight into how the developing nervous system processes noxious information. My innovative method has improved upon current clinically available systems to study nociception in a challenging and highly understudied clinical population. The better understanding of childhood pain processing under anaesthesia may allow us to estimate the dose response or effectiveness of analgesics through clinical trials, and with the use of monitors to assess depth of anaesthesia, the knowledge that children with early-life painful experiences are different is an important consideration to take note.

Appendix I

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Appendix II

Patient Information Leaflet

NUFFIELD DEPARTMENT OF CLINICAL NEUROSCIENCES

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UNIVERSITY OF OXFORD

INFORMATION SHEET FOR PARENTS/PARTICIPANTS

Dear parent,

You and your child are invited to take part in a research study. Before you decide, we would like you to understand why the research is being done and what it will involve for you and your child. Please take time to read the following information carefully. Ask us if there is anything that is not clear to you or if you would like more information.

1. Study title

LONG-TERM CONSEQUENCES OF NEONATAL INTENSIVE CARE AND EARLY LIFE SURGERY ON FUTURE PAIN SENSITIVITY

2. What is the purpose of the study?
If a child is born early they are cared for in a neonatal intensive care unit (NICU). Better treatments and technologies have allowed babies as young as 24 weeks gestation to survive. In NICU, infants are subject to many clinical procedures, which for some babies can last months but are essential for their care and survival. Additionally, surgery may be required to treat complications or to correct congenital abnormalities.

Unlike older children or adults, babies are unable to tell us whether or not they are hurting and it is difficult to assess the amount of pain they feel and to be sure they are receiving the right pain medication. Evidence from clinical practice suggests that this pain exposure so early in life alters an infant's long-term sensitivity to pain when older.

The purpose of this study is to understand the extent to which ex-premature infants and those requiring early surgery have long-term consequences as a result of their previous experience.

3. Why has my child been chosen?
Your child will be coming to the hospital for an elective day case procedure involving general anaesthesia. The doctors involved in your child's anaesthetic care have identified your child as eligible for inclusion into the study. We hope to recruit children into the study who were either born prematurely or at term and those children who required surgery within the first year of life. In this study a control group will include children who were born healthy and at full term.

4. Does my child have to take part?
No. It is up to you to decide whether or not your child will take part in the study. If you agree

that your child can take part you will be asked to sign a consent form. If you decide you do not wish your child to take part in the study, we fully understand and assure you that this will not affect your child's treatment in any way.

5. What will happen to my child if he/she takes part?
The purpose of this study is to understand the extent to which children respond when they experience touch or pain after they are asleep under anaesthetic. As part of every anaesthetic it is necessary to place an intravenous cannula ("drip") into the patient. This can be done when your child is asleep under anaesthetic. We would like to see how your child responds to insertion of the cannulae by monitoring their brain activity ("brain waves") and behaviour whilst they are asleep under anaesthetic. At the same time we will measure other physiological factors such as muscle activity, heart rate and oxygenation, and your child's response to gentle touch using a specially designed touch device.

Children recruited into this study will have their required anaesthetic performed by means of anaesthetic gas. The study will not interfere with your child's clinical care and no painful procedures will be carried out solely for research purposes.

Your child will be involved in the study only on the day and at the time of his/her elective day case procedure. The collection of the required data will take approximately 10 minutes whilst your child is under general anaesthetic, this will mean that your child will be under anaesthetic for ten minutes longer than usual clinical care.

We will collect basic clinical information about your child's delivery, condition at birth and their progress and medical treatment since birth, and if they were in the neonatal unit. For example, their gestational age at birth and their age on the day of the study, their need for support with breathing or any medication they are/were receiving.

Measuring brain activity
The technique used to monitor the way in which children respond to pain and other sensations is Electroencephalography (EEG). This is where electrodes are gently placed on the child's head to measure brain waves. EEG is routinely used on the neonatal unit, on children's wards and clinics. Following brain recordings from the cannulation, a specially designed touch device will be used to test touch stimulation and will be time-locked to the monitoring equipment. This touch device is similar to an eraser on the end of a writing pencil and is used to time your child's brain recordings to the touch.

Measuring muscle activity
To monitor your child's muscle activity we will measure their reflex movements using Electromyography (EMG). For this, two electrodes will be gently placed on the muscle just above the site of cannulation. These are the same electrodes used for EEG recordings and will be placed on the skin.

All the recording systems are portable and studies can be performed in the anaesthetic room prior to you child's required clinical procedure

Videoing your child
We will video your child during the study. After the study, we will assess facial expression

changes in response to the cannulation. We will then calculate a pain score. Pain scores are a widely used means of measuring pain. Recorded images by video and/or photograph will not be for public use.

6. What are the known risks of the study?
Obtaining video footage of your child is non-invasive and does not present any risk to your child. EEG and EMG have been used on the neonatal unit, children's wards and clinics without any adverse effects.

7. What are the benefits of taking part?
There are no direct benefits for your child, for taking part in this study. This study is designed to gather information that will help to develop better ways to care for premature babies, infants and children in the future.

8. Expenses and payment
We are grateful for your child's participation in this study, however, we are unable to pay you or your child for taking part in the study.

9. What will happen if I don't want my child to carry on with the study?
You are free to withdraw your child from the study at any time without having to give a reason. If your child becomes distressed and/or clinically unwell before, and/or during the research, the research procedures will stop. The clinically required anaesthetic procedure will go ahead subject to a review by a suitably qualified clinician. All information regarding their medical records will be treated as strictly confidential.

10. What if something goes wrong?
The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor.

If you or your child are concerned or 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 Dr Rebeccah Slater, Tel: 01865 234 537, Email: rslater@fmrib.ox.ac.uk , or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572 224 or the head of CTRG, email heather.house@admin.ox.ac.uk .

11. Will my child's taking part in this study be kept confidential?
All information and videos that are collected about your child will be kept anonymised and strictly confidential. Each child will be allocated a study number so that all information is anonymised.

All documents will be stored securely and will only be accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so. Paperwork e.g. Consent forms, test occasion, demographic, drugs taken at time of testing and medical information, will be stored under lock and key, accessed only by the research team within restricted/secured areas of the John Radcliffe Hospital. These will remain anonymous and confidential. All information, videos and results will be kept strictly confidential.

The named Consultants involved in this study will have access to the participants personal data. They will allow the research personnel to access this information under their supervision.

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.

12. What will happen to the results of the study?
The results from all children will be analysed individually and then combined to provide information about this cohort of children. There will be no identifying information about any child, when the data is presented in any forum. The findings from this study will be published in order to disseminate this information to all professionals who care for children and preterm babies/infants.

The John Radcliffe Hospital is the organisation that will collect, store, handle and process the data. The principal investigator for this project is Dr Rebeccah Slater.

13. Who is funding the research?
This study is being funded by The Wellcome Trust.

14. Who has reviewed this research?
All research in the NHS is looked at by an independent group of people called a Research Ethics Committee. This study has been reviewed and given favourable opinion by a Research Ethics Committee, Oxfordshire REC C, Reference: 12/SC/0019.

15. Further information and contact details
In addition to this study's potential to change future clinical practice for children in pain, this study also forms part of a PhD project where Dr Rebeccah Slater will supervise the student, Ravi Poorun.

<u>The study investigators are:</u>

Dr Rebeccah Slater	Wellcome Trust Career Development Fellow	University of Oxford
Ravi Poorun	Wellcome Trust MBPhD Student	University of Oxford
Dr Tariq Ali	Consultant Paediatric Intensivist	Oxford University Hospitals
Dr Richard Rogers	Consultant Paediatric Anaesthetist	Oxford University Hospitals

You can contact the investigators by post, email, telephone or fax:

Nuffield Department of Clinical Neurosciences
Level 6, West Wing
John Radcliffe Hospital
Headington. Oxford.
OX3 9DU

Email: rslater@fmrib.ox.ac.uk

Telephone: 01865 234 537

Fax: 01865222717

Thank you for reading this information leaflet.

Appendix III

Consent Form

NUFFIELD DEPARTMENT OF CLINICAL NEUROSCIENCES

Level 6, West Wing, John Radcliffe Hospital, Oxford. OX3 9DU
Tel: +44(0) 1865 234 537 | Fax: +44(0)1865 222 717
rslater@fmrib.ox.ac.uk

Version number: **3.0** | Date: **17/12/12**

UNIVERSITY OF OXFORD

CONSENT FORM

Title of Project:

LONG-TERM CONSEQUENCES OF NEONATAL INTENSIVE CARE AND EARLY LIFE SURGERY ON FUTURE PAIN SENSITIVITY

Name of Researcher: **Dr Rebeccah Slater** Please Initial Box

1. I confirm that I have read, understand and received a personal copy of the information sheet dated 17/12/12 (Version 3.0) for the above study. I have had the opportunity to consider the information, 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 him/her at any time without giving any reason, without my child's medical care or legal rights being affected. ☐

3. I understand that of any of my child's medical notes and relevant data collected during the study may be looked at by responsible individuals from the University of Oxford, Oxford University Hospitals NHS Trust or from regulatory authorities, for the purpose of audit and monitoring, where it is relevant to my child taking part in this research. I give permission for these individuals to have access to my child's records. ☐

4. I agree to the use of video-taping and photographs for study method analysis only. Use of this material for any other purpose will be consented for separately from this project and will be optional. ☐

5. I agree to the use of anonymized quotes in reports and publications. ☐

6. I agree for my child to take part in the above study. ☐

Please Turn Over

Name of Child: _____

Name of Parent/Guardian: _____

Date: _____ Signature: _____

Name of Person Taking Consent: _____

Date: _____ Signature: _____

Appendix IV

Assent Forms

INFORMATION SHEET FOR CHILDREN AGED 5 & UNDER

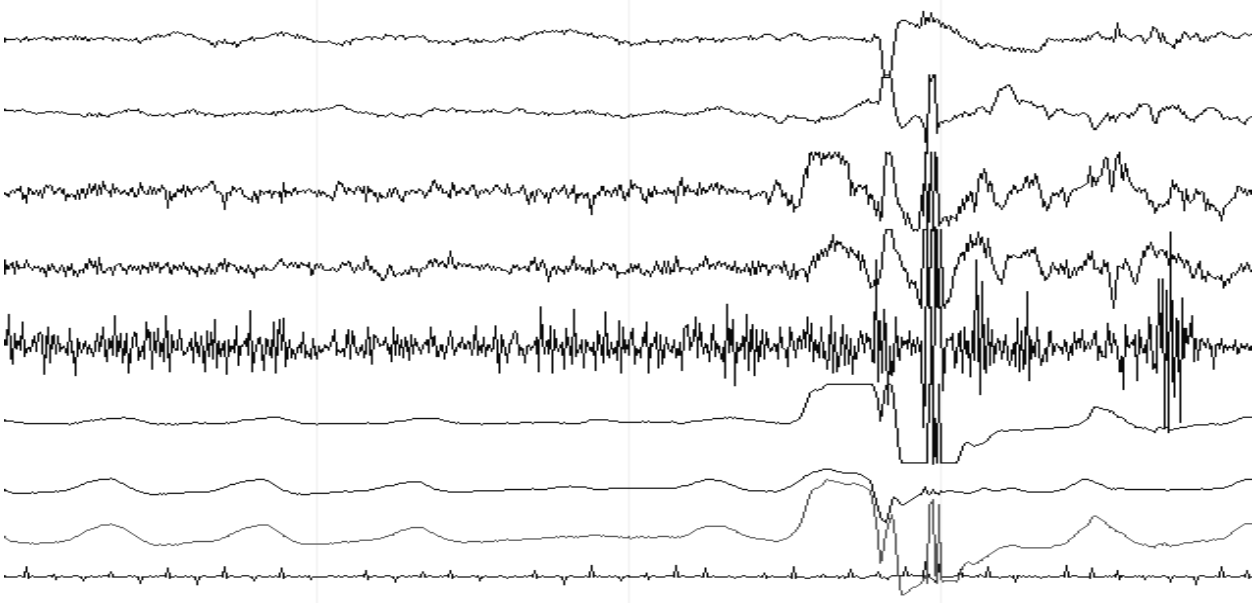
Nuffield Department of Clinical Neurosciences
Level 6, West Wing. John Radcliffe Hospital. Oxford. OX3 9DU
Tel: +44(0)1865 234 537 | fax: +44(0)1865 222 717
rslater@fmrib.ox.ac.uk

Version Number: 3.0 | Date: 17/02/12

TO BE SHOWN/READ TO THE CHILD BY THEIR PARENTS/GUARDIANS

LONG-TERM CONSEQUENCES OF NEONATAL INTENSIVE CARE AND EARLY LIFE SURGERY ON FUTURE PAIN SENSITIVITY

We are trying to help children who are in pain after operations. To do this we want to record your brain waves whilst you are asleep.



Why are we doing this research?

We are doing this because we want to see if babies born too early hurt more when they are older.

Why are you asking me to join?

You have been asked to join in because you are already coming into the hospital and will be asleep with an anaesthetic. We will be asking other children your age to join in too.

Do I have to join?

No, you don't. It is up to you. No one will mind if you don't join and no one will ask you why. It will not make any difference to the way that doctors and nurses will look after you.

What will happen to me if I join?

If you decide to join, and once the doctor makes you fall asleep, the researchers will put little discs on your head and arm to record your brain waves. We will use some gel to stick them to you and will take it all off before you wake up. The doctor will need to put a "drip" into your arm when you are asleep. When the doctor does this we will record your brain waves. All of this will take about 10 minutes and you will be asleep. Once you wake up everything will be finished and your Mum or Dad will be with you.

Will anything I have make me ill?

No. When we are recording your brain waves you will be asleep and it will not make you ill.

Is there anything else that I should be worried about?

No. If you are worried about anything you can talk to your Mum or Dad and to the researcher and your doctor and nurse.

What if something goes wrong?

There is not really anything that could go wrong. You may wake up with a little bit of gel in your hair but this will come out with a wash. If something does happen, you or your Mum or Dad can talk to the researcher or doctor in charge.

Will anyone know that I'm joining in?

Only your Mum or Dad, your doctor and nurse, and the researchers. All the things you give to the researchers will have a number on instead of your name so they will not know who you are.

What will happen to my brain wave recordings?

The researchers will look at your brain waves and put them together with brain waves from other children in the study. All the brain waves together will tell the researchers how the brain responds when children in the study are asleep.

Thank you for reading this. Have a talk with your Mum or Dad and let them know if you want to join in our research and if you do, look at the form on the next pages. Thank you.

INFORMATION SHEET FOR CHILDREN AGED 6-12

Nuffield Department of Clinical Neurosciences
Level 6, West Wing. John Radcliffe Hospital. Oxford. OX3 9DU
Tel: +44(0)1865 234 537 | Fax: +44(0)1865 222 717
rslater@fmrib.ox.ac.uk

Version Number: 3.0 | Date: 17/02/2012

LONG-TERM CONSEQUENCES OF NEONATAL INTENSIVE CARE AND EARLY LIFE SURGERY ON FUTURE PAIN SENSITIVITY

We are trying to help the treatment of other children who are in pain after operations. To do this we want to record your brain waves whilst you are asleep with the anaesthetic.

Why are we doing this research?

When a baby is born too early they are looked after in baby intensive care. Unlike older children, babies can't tell us if it hurts when the doctors and nurses are helping them. We want to know if the pain they might have when born too early affects them when they are older.

Why are you asking me to join?

You have been asked to join in because you are already coming into the hospital and will be asleep with the anaesthetic. We will be asking other children your age to join in too.

Do I have to join?

No, you don't. It is up to you. You can decide to join in and then change your mind later if you want to. No one will mind if you don't join and no one will ask you why. If you decide not to join in now, or if you change your mind later, it will not make any difference to the way that doctors and nurses will look after you.

What will happen to me if I join?

If you decide to join, and once the doctor makes you fall asleep, the researchers will put little discs on your head and arm to record your brain waves. We will use some gel to stick them to you and will take it all off before you wake up. The doctor will need to put a "drip" into your arm when you are asleep. When the doctor does this we will record your brain waves. All of this will take about 10 minutes and you will be asleep. Once you wake up everything will be finished and your Mum or Dad will be with you.

Will anything I have make me ill?

No. When we are recording your brain waves you will be asleep and it will not make you ill.

Is there anything else that I should be worried about?

No. If you are worried about anything you can talk to your Mum or Dad and to the researcher and your doctor and nurse.

What if something goes wrong?

There is not really anything that could go wrong. You may wake up with a little bit of gel in your hair but this will come out with a wash. If something does happen, you or your Mum or Dad can talk to the researcher or doctor in charge.

Will anyone know that I'm joining in?

Only your Mum or Dad, your doctor and nurse, and the researchers. All the things you give to the researchers will have a number on instead of your name so they will not know who you are.

What will happen to my brain wave recordings?

The researchers will look at your brain waves and put them together with brain waves from other children in the study. All the brain waves together will tell the researchers how the brain responds when children in the study are asleep.

Thank you for reading this. Have a talk with your Mum or Dad and let them know if you want to join in our research and if you do, look at the form on the next pages.

Thank you.

UNIVERSITY OF OXFORD

ASSENT FORM

Title of Project:

LONG-TERM CONSEQUENCES OF NEONATAL INTENSIVE CARE AND EARLY LIFE SURGERY ON FUTURE PAIN SENSITIVITY

Name of Researcher: **Dr Rebeccah Slater**

For Child Participants

Please circle *'Yes'* or *'No'* (or if unable, parent/guardian on their behalf):

Have you had read to you the information about this project?	Yes / No
Has somebody told you about this project?	Yes / No
Do you know what this project is about?	Yes / No
Do you understand the answers to your questions?	Yes / No
Do you know that it's OK to stop taking part at any time?	Yes / No
Are you happy to be videotaped?	Yes / No
Are you happy to take part?	Yes / No

Please Turn Over

If any answers are *'No'* or you don't want to take part, don't sign your name.

If you do want to take part, you can write your name below:

Your Name:

.....

Date:

.....

The researcher or doctor who explained this project to you needs to sign too:

Print Name:

.....

Sign:

.....

Date:

.....

Thank you for your help.

Appendix V

Anaesthetic Subject Demographics

CHAPTER 3: PILOT STUDY SUBJECT DEMOGRAPHICS

Subject	Sex	Age (months)	Term/Preterm	Stim. Site	Laterality	Procedure
01	F	49	Term	Foot	L	MRI Investigation Under General Anaesthesia
02	F	59	Term	Foot	L	MRI Investigation Under General Anaesthesia
03	M	71	Term	Hand	L	MRI Investigation Under General Anaesthesia
04	M	65	Preterm	Foot	L	MRI Investigation Under General Anaesthesia
05	F	143	Term	Hand	L	Open Ileal Biopsy
06	M	92	Term	Hand	L	Osteotomy
07	M	20	Term	Hand	L	MRI Investigation Under General Anaesthesia
08	F	19	Preterm	Hand	L	MRI Investigation Under General Anaesthesia
09	F	26	Preterm	Hand	L	Botox Injections
10	F	78	Term	Hand	L	Flexor Tenotomy
11	F	18	Term	Hand	R	Arthrogram & Reduction
12	M	134	Term	Hand	R	Gastrocnemius Lengthening

CHAPTER 4: NAÏVE GROUP SUBJECT DEMOGRAPHICS

Subject	Sex	Age (months)	Term/Preterm	Stim. Site	Laterality	Procedure
16	F	28	Term	Hand	L	CT Investigation Under General Anaesthesia
17	F	45	Term	Hand	L	MRI Investigation Under General Anaesthesia
18	M	99	Term	Hand	L	Examination Under Anaesthesia
19	M	18	Term	Hand	L	Orchidopexy
20	F	20	Term	Hand	L	Neck Mass Incision
21	M	62	Term	Hand	L	Orchidopexy
22	F	36	Term	Hand	L	MRI Investigation Under General Anaesthesia
23	F	37	Term	Hand	R	Evacuation and Biopsy Under General Anaesthesia
25	F	65	Term	Hand	L	Examination Under Anaesthesia
27	M	41	Preterm	Hand	L	Squint Repair
29	F	48	Preterm	Hand	L	Clitoroplasty
30	M	94	Preterm	Hand	R	Arthrogram
31	M	47	Term	Hand	L	Circumcision
32	F	100	Term	Hand	L	Cystoscopy
33	M	83	Term	Hand	R	Orchidopexy
34	M	74	Term	Hand	L	Hernia Repair
35	F	62	Term	Hand	L	Hernia Repair
36	M	51	Preterm	Hand	L	Tonscillectomy & Adenoidectomy
37	F	36	Preterm	Hand	L	Examination Under Anaesthesia
38	F	50	Preterm	Hand	R	Squint Repair
45	F	17	Preterm	Hand	L	MRI Investigation Under General Anaesthesia
48	M	136	Term	Hand	R	Osteotomy
50	M	48	Term	Hand	L	Hernia Repair
51	M	72	Preterm	Hand	L	Orchidopexy
52	M	62	Term	Hand	R	Arthrogram
53	F	153	Term	Hand	R	Osteotomy
55	F	14	Term	Hand	L	Cystoscopy
56	F	44	Preterm	Hand	R	Botox Injections

CHAPTER 4: LOCAL ANAESTHETIC GROUP SUBJECT DEMOGRAPHICS

Subject	Sex	Age (months)	Term/Preterm	Stim. Site	Laterality	Procedure
13	F	39	Term	Hand	L	Squint Repair
14	M	83	Term	Hand	L	Squint Repair
15	F	42	Term	Hand	L	Grommit Insertion
24	M	33	Term	Hand	L	Orchidopexy
26	M	25	Term	Hand	L	Cyctoscopy
39	M	32	Term	Hand	R	Trigger Thumb Release
40	F	41	Term	Hand	R	Arthogram
41	M	40	Term	Hand	L	Trigger Thumb Release
42	M	79	Term	Hand	R	Anterior Tibial Transfer
43	M	35	Term	Hand	R	Orchidopexy
44	M	78	Term	Hand	L	Umbilicoplasty

CHAPTER 5: TERM-BORN GROUP 1 SUBJECT DEMOGRAPHICS

Subject	Sex	Age (months)	Term/Preterm	Stim. Site	Laterality	Procedure
16	F	28	Term	Hand	L	CT Investigation Under General Anaesthesia
17	F	45	Term	Hand	L	MRI Investigation Under General Anaesthesia
18	M	99	Term	Hand	L	Examination Under Anaesthesia
19	M	18	Term	Hand	L	Orchidopexy
20	F	20	Term	Hand	L	Neck Mass Incision
21	M	62	Term	Hand	L	bilateral orchidoplexy & preputial adhesiolysis
22	F	36	Term	Hand	L	MRI Investigation Under General Anaesthesia
23	F	37	Term	Hand	R	Evacuation and Biopsy Under General Anaesthesia
25	F	65	Term	Hand	L	Examination Under Anaesthesia

31	M	47	Term	Hand	L	Circumcision
34	M	74	Term	Hand	L	Hernia Repair
35	F	62	Term	Hand	L	Hernia Repair
48	M	136	Term	Hand	R	Osteotomy
50	M	48	Term	Hand	L	Hernia Repair
53	F	153	Term	Hand	R	Osteotomy

CHAPTER 5: TERM-BORN GROUP 2 SUBJECT DEMOGRAPHICS

Subject	Sex	Age (months)	Term/Preterm	Stim. Site	Laterality	Procedure
13	F	39	Term	Hand	L	Squint Repair
14	M	83	Term	Hand	L	Squint Repair
15	F	42	Term	Hand	L	Grommit Insertion
24	M	33	Term	Hand	L	Orchidopexy
26	M	25	Term	Hand	L	Cystoscopy
32	F	100	Term	Hand	L	Cystoscopy
33	M	83	Term	Hand	R	Orchidopexy
39	M	32	Term	Hand	R	Trigger Thumb Release
40	F	41	Term	Hand	R	Arthogram
41	M	40	Term	Hand	L	Trigger Thumb Release
42	M	79	Term	Hand	R	Anterior Tibial Transfer
43	M	35	Term	Hand	R	Orchidopexy
44	M	78	Term	Hand	L	Umbilicoplasty
52	M	62	Term	Hand	R	Arthogram
55	F	14	Term	Hand	L	Cystoscopy

CHAPTER 5: PREMATURE-BORN GROUP SUBJECT DEMOGRAPHICS

Subject	Sex	Age (months)	Term/Preterm	Stim. Site	Laterality	Procedure	Neurological Impairment
27	M	41	Preterm	Hand	L	Squint Repair	
28	**F**	**150**	**Preterm**	**Hand**	**R**	**Osteotomy**	**Cerebral Palsy**
29	F	48	Preterm	Hand	L	Clitoroplasty	
30	M	94	Preterm	Hand	R	Examination Under Anaesthesia	
36	M	51	Preterm	Hand	L	Tonscillectomy & Adenoidectomy	
37	F	36	Preterm	Hand	L	incision & curettage	
38	F	50	Preterm	Hand	R	Squint Repair	
45	F	17	Preterm	Hand	L	MRI Investigation Under General Anaesthesia	
46	**M**	**65**	**Preterm**	**Hand**	**L**	**MRI Investigation Under General Anaesthesia**	**Spastic Dysplasia**
47	**M**	**64**	**Preterm**	**Hand**	**L**	**MRI Investigation Under General Anaesthesia**	**Hydrocephalus**
49	**F**	**48**	**Preterm**	**Hand**	**L**	**MRI Investigation Under General Anaesthesia**	**Bilateral Ventriculomegaly**
51	M	72	Preterm	Hand	L	Orchidopexy	
56	F	44	Preterm	Hand	R	Botox Injections	
53	**M**	**149**	**Preterm**	**Hand**	**R**	**Multilevel Orthopaedic Surgery**	**Left Hemiplegia**
54	**M**	**15**	**Preterm**	**Hand**	**L**	**Squint Repair**	**Grade 4 IVH**