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Cortical Pain Processing in the Infant Brain

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DECLARATION

I, Rebeccah Louise Elizabeth Ann Slater, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Rebeccah Slater
2007

ABSTRACT

Premature infants are exposed to multiple invasive procedures as part of their essential medical care. It is not known, however, if nociceptive information is processed by the cortex at this age. The fundamental question to be addressed by this thesis is whether premature infants display cortical responses to noxious stimulation.

This thesis describes a series of studies where the question of cortical pain processing is addressed by directly measuring cortical responses to noxious stimulation using near-infrared spectroscopy (NIRS) and electroencephalography (EEG). The NIRS results show that, following an acute noxious event, the contralateral somatosensory cortex is functionally activated in infants from 25 weeks postmenstrual age (PMA). Awake infants have a larger cortical response than asleep infants and, in the awake group, the size of the response increases with PMA.

The magnitude of the haemodynamic response correlates with pain scores calculated using the premature infant pain profile (PIPP), although infants who do not display a change in facial expression can still process noxious stimuli at the cortical level. Latency to response is longest in the youngest infants using either the haemodynamic response or change in facial expression as an output measure.

The underlying pain-related neuronal activity in the cortex has been investigated using EEG. Nociceptive-specific event related potentials have been observed in infants from 31-42 weeks PMA, with a recognisable N-P complex visible in the contralateral somatosensory cortex in 82% of studies. Noxious stimulation can evoke specific patterns of neural activity within the cortex of preterm and term infants that can be observed on a single-trial basis.

The studies represent the first measurements of cortical activation in the immature preterm cortex following a noxious event. The fact that noxious information is transmitted to higher levels of the central nervous system highlights the importance of developing a systematic approach to reduce pain and improve analgesic strategies in this vulnerable population.

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CHAPTER 1: INTRODUCTION

1.1 Pain Processing in the Premature Infant

1.1.1 An overview of neonatal pain

To treat infant pain successfully and improve developmental outcome, we must understand the neurobiological mechanisms involved in processing noxious information (Fitzgerald, 2005). As infants are unable to report pain verbally, our understanding of pain processing in this vulnerable group has relied heavily upon measures of nociception, such as reflex responses to noxious stimulation and behavioural and physiological responses (Andrews and Fitzgerald, 1999; Craig et al., 1993). The distinction between nociception and pain is fundamental to our understanding of pain processing. While nociception can involve the activity of central pathways at the level of primary sensory neurones and spinal and brainstem circuits, pain perception has an emotional and affective component that requires higher-level cortical processing (Treede et al., 1999). Nociception refers only to the biological signal processing of the noxious input and is independent of conscious perception. Pain has been defined by The International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. An additional note is included with the definition, which states that the inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment.

Studies in neonates have used a number of indirect methods to demonstrate that noxious stimulation can activate sensory pathways, resulting in a wide range of physiological, biochemical, and behavioural responses (McNair et al., 2004; Franck et al., 2000). Other more direct neurophysiological methods use nociceptive spinal flexion reflex responses to study the postnatal development of the nociceptive responses to single and repetitive noxious stimulation (Andrews and Fitzgerald, 2002; Andrews et al., 2002; Andrews and Fitzgerald, 1999; Andrews and Fitzgerald, 1994). Despite these observations, which show that infants are able to mount a strong and organised response to noxious stimulation, the question remains as to how much pain human infants actually experience. The anatomical and functional immaturity of the nervous system, especially at levels higher than the spinal cord, means that the conscious perception of pain may not be achievable until the neural circuitry is developed sufficiently (Fitzgerald, 2005; Lee et al., 2005). It is feasible that the strong behavioural and physiological responses observed in premature infants may be entirely mediated at the spinal or brainstem level with little or no

cortical involvement. This would imply that these infants do not experience true pain. A recent study demonstrated that infants below 32 weeks postconceptional age (PCA) with parenchymal brain injury (often associated with white matter damage) had the same behavioural and physiological pain responses as normal age-matched controls (Oberlander et al., 2002). This suggests that in infants below 32 weeks PCA, these responses may largely be mediated subcortically. If the neonatal cortex had an important role in mediating these responses, it would be anticipated that severe brain injury would affect these pain scores. It is therefore possible that nociceptive afferent input either does not reach the cortex in preterm human infants before 32 weeks, or that the cortical circuits cannot be activated before this time.

The study by Oberlander and colleagues refers to infant maturity in terms of postconceptional age (PCA), which represents the number of weeks since conception or post fertilisation. Clinicians often use gestational age or postmenstrual age (PMA), which represents the number of weeks since the first day of the woman's last menstrual period. PMA or gestational age is therefore approximately two weeks greater than the PCA or developmental age. In this thesis, results are reported in terms of PMA. When other work is cited, however, infant maturity is described using the terminology present in the original article.

1.1.2 The emergence of pain assessment methods for neonates

For many years, it was thought that neonates do not feel pain. The theory was based on the premise that infants have an immature central nervous system (CNS) and are thus incapable of pain experience (Agarwal et al., 1985). Early studies concluded that neonatal responses to noxious events did not involve the infant cortex, and that perception and localisation of pain was therefore not possible (Agarwal et al., 1985). The neonate's inability to remember pain was considered indicative that infants do not process pain in the same way as adults (Agarwal et al., 1985) and it was speculated that a high pain threshold may be essential to protect the infant during childbirth (Bondy, 1980). In the late 1980's direct methods emerged to assess neonatal responses to noxious stimulation and as a result these assertions were questioned. A review written in 1986, concluded that it can no longer be assumed that neonates do not feel pain and that such assumptions need to be proven (Owens, 1986). The methods that emerged to assess neonatal pain are discussed in the ensuing paragraphs.

Early suggestions that infants feel pain came from studies conducted in 1984, which demonstrated that an increase in heart rate and crying could be observed in newborn infants following a heel lance (Owens and Todt, 1984). The authors concluded that infants were thus

capable of feeling pain and that infant pain can be reliably measured in a clinical setting. Since this time, changes in physiological activity in response to both innocuous and noxious events have been extensively studied (Stevens and Johnston, 1994; Craig et al., 1993; Porter, 1989). Some studies have shown, however, that an increase in heart rate can occur following both a heel lance and a control procedure, so the usefulness of heart rate, as an indicator of neonatal pain, has been questioned (Craig et al., 1993). It was concluded that change in heart rate may reflect a generalised state of physiological stress as opposed to a specific response to a noxious stimulus. Further difficulties associated with the use of heart rate as an index of neonatal pain were demonstrated in a study in preterm infants aged 26-31 weeks PCA (Johnston et al., 1995). The authors demonstrated that although a greater maximum heart rate was observed in response to a heel lance compared with a control event, the response was the least robust in the younger infants (Johnston et al., 1995).

Studies of cry, as an index of neonatal pain, have shown that intensity and duration of cry are the most useful parameters, as they both increase with increasing pain intensity (Owens and Todt, 1984). Nevertheless, since crying is the primary method by which infants express their basic needs, it is difficult to know whether crying occurs as a direct result of pain, or due to other factors, such as hunger or excessive heat or cold. The use of cry as an index of neonatal pain requires complex analysis and, as it can be pharmacologically and mechanically suppressed in the intensive care environment, there are considerable difficulties associated with the interpretation. A further method which has been used to assess neonatal responses to noxious stimulation is emotional sweating (Gladman and Chiswick, 1990; Harpin and Rutter, 1982). Sweating can be assessed by measuring either palmar water loss (Harpin and Rutter, 1982) or plantar skin conductance (Gladman and Chiswick, 1990). A study of 124 infants found that infants undergoing a heel lance, who were greater than 37 weeks PCA, had a raised level of palmar water loss, which returned to baseline when the infants settled (Harpin and Rutter, 1982). Palmar sweating and plantar skin conductance have not been observed in infants less than 36 weeks PCA, which means their use for pain assessment in preterm infants in the intensive care environment is limited (Gladman and Chiswick, 1990; Harpin and Rutter, 1982).

Other measures of the physiological component of pain include changes in respiration rate, transcutaneous partial pressure of oxygen ($TcpO_2$) and carbon dioxide ($TcpO_2$), blood pressure and endorphin levels. Respiratory rate has been used to monitor the autonomic response to noxious procedures (Craig et al., 1993; Brown, 1987; Rawlings et al., 1980). Where both accelerated breathing (Brown, 1987; Rawlings et al., 1980) and breath-holding (Craig et al.,

1993) have been reported, however, the natural physiological variability in respiratory rate renders it unusable as a sole indicator of neonatal pain (Porter, 1989). Attempts to use changes in transcutaneous partial pressure of oxygen ($TcpO_2$) and carbon dioxide ($TcpCO_2$) as neonatal pain indicators (Porter, 1989; Rawlings et al., 1980) have also yielded conflicting results. Marked decreases in $TcpO_2$ have been reported following circumcision (Rawlings et al., 1980), however, when the same measures were used by Craig et al. (1993) they reported no distinction between the response to a heel lance or sham procedure. Changes in oxygen saturation appear to be more reliably reported; a number of studies have shown a reduction in oxygen saturation following a heel lance and other invasive procedures (Stevens and Johnston, 1994; Craig et al., 1993). Once again, this cannot be used as a sole measure of neonatal pain because there are many situations where oxygen saturation in a preterm infant may fluctuate.

An alternative approach used in neonatal pain assessment is to consider the behavioural responses following noxious procedures. Specific facial features were shown to occur following painful procedures and methods of analysing facial responses to pain, such as the maximum discriminative facial movement coding system (MAX) and the facial action coding system (FACS) were developed (McGrath and Unruh, 1987). These systems were eventually revised to create the neonatal facial coding system (NFCS) (Grunau and Craig, 1987). Grunau and Craig (1987) studied the facial response in 140 healthy infants following a heel lance and found that facial activity can be used as a reliable measure of pain processing. They demonstrated that the response was dependent on sleep state and questioned the dogma that infant responses to noxious stimulation only consisted of uncoordinated whole body movements. Since this time, facial expression has repeatedly been used to assess neonatal pain and is now an important component of current clinical pain assessment tools (Grunau et al., 1998; Stevens et al., 1996; Craig et al., 1994; Stevens and Johnston, 1994; Craig et al., 1993). This is discussed in more detail in Section 1.1.3. Body movements, such as leg extension, finger splay, and hand to mouth movements, have also been used in neonatal pain assessment (Craig et al., 1993; Porter, 1989; Franck, 1986); however, as the responses are less specific than facial responses, body movements are considered a less effective measure of neonatal pain (Craig et al., 1993; Porter, 1989). In addition, there is the disadvantage that body movements are often mechanically or pharmacologically suppressed, and can therefore be difficult to investigate under normal clinical conditions (Porter, 1989). A further tool that has been used to study responses to noxious stimulation in neonates is the spinal flexion reflex response. It has been used successfully in the neonatal intensive care environment, and has yielded useful information on human somatosensory development (Andrews and Fitzgerald, 2000; Andrews and Fitzgerald, 1999;

Andrews and Fitzgerald, 1994). In both preterm and full-term human neonates, this reflex can be evoked with low intensity mechanical stimulation and has a lower threshold than the nociceptive flexion reflex in adults (Andrews and Fitzgerald, 2000; Andrews and Fitzgerald, 1999; Andrews and Fitzgerald, 1994).

In 1987, studies by Anand and Aynsley-Green provided the first objective evidence that neonates mount a substantial stress response during surgery. The study demonstrated that infants who received fentanyl during surgery had improved postoperative outcome (Anand et al., 1987). The importance of providing adequate analgesia during and after surgical procedures was highlighted, which further challenged the belief that infants were incapable of pain perception. Despite this pioneering work, conducted over 20 years ago, pain-control in neonates remains sporadic and suboptimal. The problem that underlies neonatal pain research is that we do not know whether these measures provide an objective measure of pain processing. While it is clear that neonatal pain assessment is dependent on the level of the nervous system from which the pain response emanates, there is a lack of evidence regarding whether the neonatal cortex is involved in pain responses measured using these behavioural and physiological indicators. If these behavioural and physiological responses to nociception are mediated subcortically, they may not reflect true pain experience.

1.1.3 Current clinical pain assessment tools

Based on the measures discussed in Section 1.1.2, a number of pain assessment tools have been designed to quantify pain responses in premature infants. The most commonly used scales for preterm infants are the premature infant pain profile (PIPP), neonatal infant pain score (NIPS), neonatal facial coding system (NFCS), CRIES and COMFORT (van Dijk et al., 2000; Stevens et al., 1996; Krechel and Bildner, 1995; Craig et al., 1994; Lawrence et al., 1993; Ambuel et al., 1992; Grunau and Craig, 1987). The NFCS was the first pain assessment tool to describe a set of 10 facial features that can be observed in infants following a noxious procedure (Grunau and Craig, 1987). The scoring system calculates a pain intensity score based on whether 10 specific facial actions are either present or absent during specified phases of a noxious procedure. These facial actions include, eye squeeze, brow bulge, nasolabial furrow, lip purse, open lips, vertical mouth stretch, horizontal mouth, taut tongue, tongue protrusion and chin quiver (Grunau and Craig, 1987). The NFCS was initially developed and validated by videoing infants during painful and non-painful procedures. This meant that the video footage could be intensively analysed in a frame-by-frame approach. The feasibility and validity of using the NFCS at the bedside in real time has also been shown (Grunau et al., 1998).

The best validated pain scale for premature infants is the PIPP, which has been specifically developed to assess neonatal pain (Stevens et al., 1996). It uses three facial actions from the NFCS and is based on the behavioural observations that an infant experiencing acute pain has eyes that are forcefully closed, a brow that is lowered and furrowed, and nasal roots that are broadened and bulged with a deepened nasolabial furrow (Grunau and Craig, 1987). The seven other facial actions were eliminated because they were not considered to be specific or sensitive indicators of pain (Stevens et al., 1996). In addition, the PIPP also scores change in heart rate and oxygen saturation, and includes a weighting factor for gestational age and behavioural state. Each indicator is used to calculate a composite numerical score that attempts to quantify pain experience. An example score sheet is given in Figure 1.1. Weighting factors were introduced into the PIPP to increase the score for the infants who are considered least able to mount a response following a noxious procedure. In a clinical setting, this may be a useful way to ensure that pain processing in the youngest and sickest infants is not under treated, but as a research tool, a weighting factor that arbitrarily increases the pain score is less useful and potentially misleading.

Infant Study Number: _____
Date/time: _____
Event: _____

Process	Indicator	0	1	2	3
Chart	Gestational age	36 weeks and more	32–35 weeks, 6 days	28–31 weeks, 6 days	less than 28 weeks
Observe infant 15 s	Behavioral state	active/awake eyes open facial movements	quiet/awake eyes open no facial movements	active/sleep eyes closed facial movements	quiet/sleep eyes closed no facial movements
Observe baseline Heart rate __ Oxygen saturation __					
Observe infant 30 s	Heart rate Max __	0–4 beats/min increase	5–14 beats/min increase	15–24 beats/min increase	25 beats/min or more increase
	Oxygen saturation Min __	0–2.4% decrease	2.5–4.9% decrease	5.0–7.4% decrease	7.5% or more decrease
	Brow bulge	None 0–9% of time	Minimum 10–39% of time	Moderate 40–69% of time	Maximum 70% of time or more
	Eye squeeze	None 0–9% of time	Minimum 10–39% of time	Moderate 40–69% of time	Maximum 70% of time or more
	Nasolabial furrow	None	Minimum	Moderate	Maximum

Figure 1.1 Premature infant pain profile

An example score sheet used to calculate the PIPP score (Stevens et al., 1996).

1.1.4 Latency between noxious stimulation and PIPP facial expression indicators

One of the difficulties associated with the use of neonatal pain assessment tools is that change in facial expression has been reported to be of limited use in very preterm infants. It has been consistently shown that the most premature infants have the least robust responses (Johnston et al., 1995; Craig et al., 1993). Facial pain expressions may be weak or absent before 28-32 weeks and it is widely acknowledged that babies less than 27 weeks are challenging to score (Ballantyne et al., 1999). Some studies suggest that the youngest premature infants are able to demonstrate little or no facial response to pain (Johnston et al., 1999). Stevens et al. (1996) found that the total facial pain coding score was lower for babies less than 28 weeks, and accounted for this observation by introducing a weighting factor to the PIPP score. The reason why younger infants have lower facial pain scores remains unclear. It may be that the very immature central nervous system (CNS) is unable to mount a co-ordinated contraction of the facial muscles involved in the behavioural response, thus preventing a fully developed facial response to pain. An alternative explanation may be that the scoring system used in these studies does not evaluate pain behaviour for the appropriate time-period required to observe the full manifestation of the response. A time window of only 10 seconds after the noxious event has been used to assess the pain response in some studies (Ogawa et al., 2005). According to the PIPP, a facial expression score is calculated by determining the percentage of time, during a 30-second time window, that an infant demonstrates three different facial expressions following an acute noxious event. If the youngest infants have a longer latency to response, it is possible that the low scores reflect the delay in response rather than an infant's inability to mount a response. In the youngest premature infants, particular care must be taken when interpreting clinical pain scores that use change in facial expression as an indicator of pain processing.

1.1.5 Noxious procedures in premature infants

Advances in neonatal intensive care have led to the survival of very premature infants. The UK has the highest rate of low birthweight babies in Western Europe: 12% of babies need some level of special care at birth (~ 80,000 per annum) and 2.5% need neonatal intensive care (~ 17,000 per annum) (Green, 2005). To maintain their wellbeing and compensate for their physiological instability, multiple invasive procedures are performed as part of their essential medical care. Estimates suggest that on average an infant receiving intensive care will undergo 14 painful procedures per day (Simons et al., 2003). A meta-analysis of 5 studies conducted between 1995 and 2003 showed that 603 infants in intensive care, aged between 23 and 42 weeks gestational age, underwent a total of 38,426 invasive procedures while in intensive care (D'Apolito, 2006).

Repeated exposure to noxious procedures is a particular concern in the preterm neonatal population because it occurs at a time when the infant should be developing in a protective uterine environment where sensory input would be limited (Anand, 2000). Furthermore, there is evidence that exposure to painful stimuli in the neonatal period leads to long-term changes in pain processing (Peters et al., 2005; Grunau et al., 2001a; Anand, 2000; Porter et al., 1999; Taddio et al., 1997).

There is a lack of information about effective pain management strategies in neonates. Analgesia is provided in less than 35% of procedures that health-care professionals consider to be painful (Simons et al., 2003) and in the UK over 80% of neonatal units still do not have any pain assessment protocol in place (Redshaw and Hamilton, 2005). A recent report, commissioned by the premature baby charity BLISS, found that less than 60% of units regularly use analgesia for pain relief. The report stated that the most regularly used methods for comforting babies during painful procedures are gentle touch, talking to the infant, and swaddling (Green, 2005). Only 48% of parents reported that infants were given pain-relieving drugs to make them more comfortable (Green, 2005), and a survey of medical staff showed that, while 90% believed that infants feel pain as severely as adults, most procedures are performed without analgesia or comfort measures (Porter et al., 1997). Clearly, pain in adults and older children would not be managed in this way.

In humans, normal gestation lasts between 37 and 40 weeks. During this time, the foetus is dependent on the maternal host to provide support for all its respiratory and nutritive needs. If an infant is born before this time then its survival may be threatened and it may be necessary for life support to be provided by an intensive care unit. There are approximately 220 units offering neonatal intensive care in the UK and the number of infants requiring specialist care is rising. Of the 42,500 infants that the Department of Health estimates are born prematurely, approximately 300 are born at less than 24 weeks and 2880 are born at less than 28 weeks. The increase in the number of infants being born prematurely is partly due to improved obstetric care and improved fertility treatment. Better obstetric care means that infants are identified as having problems earlier during pregnancy. Consequently, these infants are likely to be delivered earlier to prevent the problems developing. More women are having babies later in life and as a result there has been an increase in the number of obstetric complications, and number of premature births. Improved fertility treatment has led to an increase in the number of multiple gestation pregnancies, which is a known risk factor for premature birth. It is also significant that England has the highest rate of teenage pregnancy in Europe; teenage mothers have a higher risk of

delivering a premature or low birth weight baby. The increase in the number of teenage pregnancies has contributed to the increase in number of premature births (Radshaw and Hamilton, 2005). Increased survival of younger infants means that the duration of stay has also increased, particularly for the extremely low birth weight infants. A national survey of neonatal unit organisation and policy found that the duration of stay ranged from 4-140 days with a mean of 56 days (Green, 2005).

1.1.6 Long term consequences of early pain exposure in human neonates

A number of studies in humans have suggested that exposure to pain in the neonatal period can lead to long term developmental consequences (Grunau et al., 2006). While animal models have shown that long-term changes in pain processing are evident following early pain exposure (Ren et al., 2004; Torsney and Fitzgerald, 2003; Bhutta et al., 2001; Ruda et al., 2000; Anand et al., 1999; Reynolds and Fitzgerald, 1995), the effect of early pain experience in man has been difficult to assess and has produced conflicting results. In 1995, The Lancet published a frequently cited study which showed that exposure to pain in the neonatal period had long-term developmental consequences. The study showed that boys circumcised at birth had an increased response to routine vaccination at 4-6 months of age compared to uncircumcised boys (Taddio et al., 1995). A subsequent follow-up study, which provided further evidence to support this finding, demonstrated that boys treated with lidocaine-prilocaine cream (EMLA) during circumcision had lower pain responses when they were vaccinated 4-6 months later than those treated with a placebo; an uncircumcised control-group demonstrated the lowest pain responses (Taddio et al., 1997). Further studies suggested that children aged 4.5 years, who were formerly extremely low birth weight (ELBW) infants, are more likely to experience unexplained stomach aches, headaches and other complaints as compared with age-matched term-born controls (Grunau et al., 1994b).

Other studies appear inconsistent with these findings. Premature infants and full-term infants, age-matched in terms of developmental age, have been shown to demonstrate the same behavioural responses to finger-lancing, undertaken for the purpose of blood collection (Oberlander et al., 2000) and parents of 18 month old toddlers report that premature infants have decreased reactivity to pain compared to term-born aged-matched controls (Grunau et al., 1994a). In agreement with these findings, a study of children who were 9-14 years of age, which compared the responses of children who had received neonatal intensive care with age-matched controls, found that the ex-neonatal intensive care unit (ex-NICU) groups exhibited elevated heat pain thresholds compared to controls. Despite the fact that the ex-NICU children were

hyposensitive to thermal pain, it was also demonstrated that the ex-NICU children had greater perceptual sensitisation (Hermann et al., 2006). Greater perceptual sensitisation refers to the gradual increase in subjective pain intensity during prolonged noxious stimulation.

Peters and colleagues demonstrated that infants who had undergone surgery in the first three months of life only showed higher sensitivity to subsequent surgery if it was performed in the same dermatome (Peters et al., 2005). The evidence in humans for the long-term consequences of early pain experience is limited, and interpretations of these findings are complicated by confounding factors, including comorbidity, and social and family factors. A more recent study, using quantitative sensory testing (QST), showed that children who experienced tissue injury in early infancy during thoracic heart surgery had altered responses to mechanical and thermal stimulation in later life (Schmelzle-Lubiecki, 2005). At 10 years of age, infants who had undergone surgery during the neonatal period showed a decreased sensitivity to tactile stimulation at the site of the scar compared to the unaffected contralateral site. It was also shown that infants were significantly less sensitive to touch at a different site compared to an age-matched control group, suggesting non-somatotopic changes have occurred that affect sensory processing. The study concluded that the children who had undergone surgery during infancy had a baseline hyposensitivity to touch. While QST has been useful is assessing pain processing in older children and adults it is clearly an inappropriate and unsuitable tool for the neonatal population because it requires active attention and feedback from the subjects being tested. These studies highlight the importance of developing an effective strategy to manage pain while infants are in the intensive care environment.

1.1.7 Nociception and pain perception

Nociception is the process whereby primary sensory neurones detect pain-producing stimuli. The activation of nociceptors is usually caused by stimuli capable of causing tissue damage, such as noxious heat, intense pressure, or irritant chemicals, but not by innocuous stimuli such as warming or light touch (Julius and Basbaum, 2001). The nociceptors are first-order afferents with free nerve endings. The basic task of nociceptors is the transduction and transmission of noxious information to the spinal cord. The afferent fibres are classified in terms of structure, diameter and conduction velocity. Noxious potentials are propagated by C and Aδ fibres. C fibres are unmyelinated, with a diameter between 0.4-1.2mm and conduction velocity of 0.5-2.0m/s, whereas Aδ fibres are myelinated, more rapidly conducting fibres, with a conduction velocity between 12-30m/s and diameter range from 2.0-6.0mm. When a noxious event is

detected, the primary afferents have different patterns of propagation. There are two classes of Aδ fibres, which propagate modally specific information, with graded intensity and short latency. Both respond to intense mechanical stimuli but can be distinguished by their response to intense heat (Julius and Basbaum, 2001). They are responsible for the induction of the first acute pain sensation and trigger the reflex withdrawal response. In contrast, the propagation of action potentials in C fibres is much slower. The C fibres are responsible for the occurrence of the longer lasting dull pain sensation. Other classes of first order afferent fibres, known as Aα and Aβ fibres, which are large myelinated fibres with conduction velocities ranging between 30-100m/s, mainly propagate non-noxious stimuli. In humans, it has been suggested that the first cutaneous sensory receptors develop between 7.5-10.5 weeks gestation (Humphrey, 1978) and the first primary afferent fibres synapse with neurones in the spinal cord at 14 weeks. C fibres, which terminate in the more superficial parts of the spinal dorsal horn, enter the spinal cord at 19 weeks gestation (Konstantinidou et al., 1995).

The primary afferents, which arrive at the spinal cord, are organised into bundles of dorsal roots that form synapses with second order neurones distributed along the dorsal horn of the spinal cord. Intrinsic neurones of the dorsal horn promote the interaction of afferent and efferent nociceptive stimuli and are responsible for the transfer to supraspinal structures. There are a number of different types of second order neurones present in the spinal cord; these include projection neurones, which directly transmit information to supraspinal centres; intersegmental propriospinal neurones, which integrate several spinal levels; and interneurones, which can be further divided into interlaminar and intersegmental types. For a full review of these pathways refer to (Almeida et al., 2004). The sensory receptors and spinal cord synapses, required to experience nociception, develop earlier than the afferent pathways and thalamocortical pathways, which are essential for the conscious perception of pain (see Table 1.1).

Anatomical characteristic	Description	Gestational age (weeks)
Peripheral cutaneous sensory receptors	Perioral cutaneous sensory receptors	7.5
	Palmar cutaneous sensory receptors	10-10.5
	Abdominal cutaneous sensory receptors	15
Spinal Cord	Neurones for nociception in dorsal root ganglion	19
Thalamic afferents	Thalamic afferents reach subplate zone	20-22
	Thalamic afferents reach cortical plate	23-24

Table 1.1 Anatomical development of human nociceptive pathways in humans
Table adapted from Lee et al. (2005).

Noxious stimulation can evoke reflex movements at the level of the spinal cord or brainstem without involving higher centres. In the spinal cord, the afferent fibres make synaptic connections with spinal cord interneurones that in turn synapse onto motor neurones. The classic nociceptive flexion reflex involves activation of flexor motorneurones by nociceptors afferents, which triggers muscle contractions, causing limb flexion away from a stimulus. At birth, strong spinal nociceptive reflexes can be evoked by mechanical stimulation, which are exaggerated compared to the adult (Fitzgerald et al., 1988), with lower thresholds and larger prolonged muscle contractions. The excitability of the mechanical neonatal withdrawal reflex is attributable to underlying changes in dorsal horn sensory processing and is likely to be due to absence of fine-tuning of sensory and nociceptive neuronal circuitry in the newborn. In the human neonate, as in the cat (Sherrington, 1910) and rat (Woolf and Swett, 1984), there is a graded distribution of thresholds within the receptive field, with an area of maximum sensitivity on the plantar surface of the foot, and a decrease in sensitivity progressing up the leg towards the knee (Andrews and Fitzgerald, 1994). A study of 50 neonates, with PCA between 27.5 and 42.5 weeks, showed that infants exhibit a withdrawal reflex following innocuous stimulation. The activation threshold is lowest in the youngest infants and gradually increases with PCA (Andrews and Fitzgerald, 1994). One suggestion is that the high level of excitability in the spinal cord of youngest infants may in part be due to the late development of interneuronal pathways, including those involved in descending inhibition from the brainstem (Fitzgerald and Koltzenburg, 1986). Table 1.1 provides a brief overview of the anatomical development of the nociceptive pathways in humans.

In contrast to nociception, which simply signals noxious stimuli to the central nervous system, pain perception requires that the stimulus be recognised as unpleasant. The fundamental difference between physically processing a nociceptive stimulus and perceiving a nociceptive stimulus as painful depends on the level of the nervous system at which the response is processed. True pain experience requires that we consciously perceive the input at higher levels of the nervous system. It is a prerequisite for the supraspinal processing of pain that peripheral sensory receptor afferents, in the skin or other organs, form synaptic connections with spinal cord neurones. Following integration in the dorsal horn, sensory and nociceptive information must be conducted to supraspinal centres, via ascending tracts, to targets in the brain stem, mid-brain, hypothalamus, thalamus and amygdala. Here, further central processing takes place and projections are established with the somatosensory cortex and other structures. Functional imaging studies in adults have provided a picture of a 'pain matrix' in the brain, subdivided into a medial and lateral system, based on the projection sites of the lateral and medial thalamic structures to the cortex. The somatosensory cortices, SI and SII, in the lateral system are thought to have a discriminatory role in detecting pain localisation and intensity, whereas the medial system involving the anterior cingulate cortex and the insular cortex are thought to mediate the cognitive-evaluative component of pain (Apkarian et al., 2005; Brooks and Tracey, 2005).

The thalamus is essential for the integration of cortical and subcortical information for both motor and sensory function. It represents the main relay structure for information to be transmitted to the cortex and receives descending nerve impulses from the cortex. The developmental age at which thalamic fibres, involved in the transmission of noxious information, reach the cortex is inferred from histological studies of other thalamocortical circuits. Studies in human foetuses that report on the visual and auditory thalamic projections conclude that thalamic projections reach the visual cortex at 21-25 weeks developmental age and that thalamic afferents reach the auditory cortical plate at 24-26 weeks developmental age (Kostovic and Rakic, 1984; Krmpotic-Nemanic et al., 1983). Further studies have shown that afferents from the thalamic region reach the prefrontal cortex by 27 weeks, and conclude that thalamic fibres enter the cortex between 26-28 weeks developmental age (Mrzljak et al., 1992). It has also been shown that thalamocortical fibres are not present in the cortical plate by 22 weeks (Kostovic and Rakic, 1990). Thalamocortical fibres are thought to emerge somewhere between 21-28 weeks developmental age. Once the critical thalamocortical connections are in place, there is the potential for noxious information to be transmitted to the cortex; however, confirmation of the anatomical presence of these fibres does not mean that these pathways are functionally active or that the cortex can produce an organised response to noxious stimulation.

A more detailed discussion of the functional responses of these pathways, assessed using somatosensory evoked potentials in neonates, is given in Section 1.2.12.

Nociceptive stimuli do not only activate sensory and motor pathways. They will also stimulate the autonomic nervous system and endocrine responses. It has been shown in both adults and premature infants that exposure to hostile conditions initiates a response, which alters behaviour, autonomic function and the secretion of multiple hormones (Carrasco and Van De Kar, 2003). Neuroendocrine responses, such as increases in the concentration of cortisol, b-endorphin and noradrenaline have been used as indicators of stress following painful procedures in neonates. However, they are not specific responses to pain and cannot be used as surrogate measures of pain perception because they can be mediated by the autonomic nervous system and hypothalamic-pituitary-adrenal axis, which does not require conscious cortical processing (Carrasco and Van De Kar, 2003). As discussed in the previous section, vital signs, such as heart rate, respiration, transcutaneous oxygen and carbon dioxide levels have also been used to assess neonatal pain (Lindh et al., 1999; Johnston et al., 1995; Craig et al., 1993). As some studies have shown that using these measures infants respond to painful and non-painful procedures in the same way, care must be taken when interpreting results based on these measures alone (Craig et al., 1993).

1.2 Brain Imaging

1.2.1 Investigating the human brain

A number of different approaches have been used to investigate the function and regional anatomy of the human brain. Some examples are summarised in Table 1.2. Studies of the living brain have been advanced by the development of technology, such as positron emission topography (PET), functional magnetic resonance imaging (fMRI) and near-infrared spectroscopy (NIRS), which have enabled the brain to be examined in a manner that does not interfere with normal function.

Brain Imaging Technique	Energy Source	Spatial resolution (mm)	Temporal Resolution (s)	Constraints	Output Measured
FMRI	Radio waves	4-5	4-10	Immobilisation, Loud, cooperation	Relative cerebral blood flow
EEG/MEG	Intrinsic electricity	10	0.001	Artifact, lack of unique localisation	Electrophysiology
Nuclear (PET/SPECT)	Radiation	5-10	60-1000	Radiation limits, Immobilisation	Physiology, neurochemistry, absolute values
MR spectroscopy	Radio waves	10	10-100	Immobilisation, Loud	Relative chemical concentrations
Near-infrared spectroscopy	Near-infrared light	5	0.05	Immobilisation, Surface>depth, limited field of view	Relative Haemoglobin concentrations
Transcranial magnetic/electric stimulation	Magnetic/electric fields	10	0.01	Risk of seizures, Immobilisation, loud	Electrophysiology, conduction times
Structural MRI	Radio waves	1	N/A	Immobilisation, loud	Structure, vasculature, white matter
Post mortem	N/A	0.0001	N/A	Post mortem	Microarchitecture, Chemoarchitecture
Single or multi-unit electrophysiology	Intrinsic electricity	0.01-1	0.01	Invasive, direct access to brain	Electrophysiology

Table 1.2 Summary of brain imaging techniques
Table adapted from Apkarian et al. (2005).

1.2.2 Brain imaging – a haemodynamic approach

Numerous studies, in particular those using PET and fMRI, have demonstrated that cortical activity is associated with an increase in cerebral blood flow (CBF) and cerebral blood volume. fMRI, using blood oxygen level-dependent (BOLD) contrast imaging, is the most widely used technique for generating images or maps of human brain activity. It uses haemoglobin as an endogenous contrast agent and relies on the fact that oxygenated and deoxygenated haemoglobin have different magnetic properties. The physiological changes measured using these techniques arise from changes in cerebral blood flow, produced as a secondary consequence of increased neuronal activity (Logothesis et al., 2001). Logothesis and colleagues simultaneously measured neuronal electrical activity and the haemodynamic response following visual stimulation in primates, to assess whether the fMRI signal occurred due to changes in neuronal activity or due to a vascular change. The study demonstrated that the BOLD response in primates directly

reflected an increase in neuronal activity. The BOLD response correlated with local field potentials (LFP), which represent the synchronised synaptic inputs of a given neuronal population. The study also reported that multi-unit spiking (MUA), which reflects the spiking activity of neurones situated very near to the electrode tip, did not correlate as well as the LFPs. Other studies have also reported a proportional relationship between average neuronal firing and the fMRI signal (Heeger et al., 2000).

When a region of the brain becomes functionally active, there is an increase in local neuronal activity. This drives an increase in regional CBF, which increases the supply of oxygenated haemoglobin; this creates the increase in the BOLD signal. The BOLD signal is also dependent on concomitant changes in the deoxygenated haemoglobin concentration, which occur because of oxygen extraction. As the signal is based on the ratio of oxygenated to deoxygenated haemoglobin, it is not possible to determine whether BOLD increases arise due to an increase in oxygenated haemoglobin, caused by an increase in regional CBF, or due to an increase in deoxygenated haemoglobin caused by increased oxygen metabolism. This has implications for the interpretation of fMRI studies which only report BOLD increases, because other active areas of the brain may be ignored (Nair, 2005).

Although fMRI and PET are both based on the basic principle that images of blood flow change can be constructed based on a comparison with a baseline condition, there are key differences in the physiological mechanisms responsible for the detected changes. Images obtained using PET rely on tissues absorbing a positron-emitting tracer, hence PET is dependent only on blood flow and is independent on the ratio of oxygenated to deoxygenated haemoglobin. The PET scanner detects gamma rays that are emitted when a positron, emitted by the tracer, collides with an electron and is annihilated. fMRI has a number of advantages over PET. fMRI has a faster image acquisition time, hence images can be acquired in a few seconds whereas PET scans are acquired over a few minutes. fMRI scans also have much higher spatial resolution and, importantly, in fMRI studies it is not necessary to inject radioactive tracers into the subjects. The use of PET and fMRI has increased our understanding of the function of the brain from a haemodynamic perspective. These techniques have been used to study pain processing above the level of the spinal cord and have provided an insight into how cortical and sub-cortical areas are involved in different aspects of pain processing.

1.2.3 Pain processing above the level of the spinal cord

The first suggestion that painful stimulation causes increased blood flow in specific cortical areas came from studies conducted in the 1970s. Investigators used the ^{133}Xenon clearance technique to demonstrate that moderate pain gives a global increase in CBF, which was predominantly observed in the frontal regions (Lassen et al., 1978). The observation that noxious stimulation can activate specific cortical and subcortical regions was later confirmed using PET. Functional activation following acute noxious heat stimulation was observed in human subjects in numerous brain regions. Jones and colleagues (1991) showed activation in the contralateral anterior cingulate cortex (ACC), thalamus and lenticular nucleus, and Talbot et al. (1991) showed activation in the primary and secondary somatosensory cortices, as well as the contralateral ACC. More recently, studies have used fMRI to confirm that multiple cortical areas are activated following noxious stimulation. A recent meta-analysis, which reviewed the brain areas activated in normal subjects following a noxious stimulus, identified six brain regions as being consistently activated across different pain studies using variety of imaging techniques (Apkarian et al., 2005). The areas consistently activated were the ACC, the primary and secondary somatosensory cortex (SI and SII), the insular cortex (IC), the thalamus (Th) and the prefrontal cortex (PFC). More recently, the focus of pain-neuroimaging studies has moved away from simply identifying functionally active brain regions. Recent studies have concentrated on understanding the specific roles that the different cortical and sub-cortical areas perform during pain perception and the analysis of more complex drives evoked by painful stimuli, such as reward, fear, anxiety and decision-making.

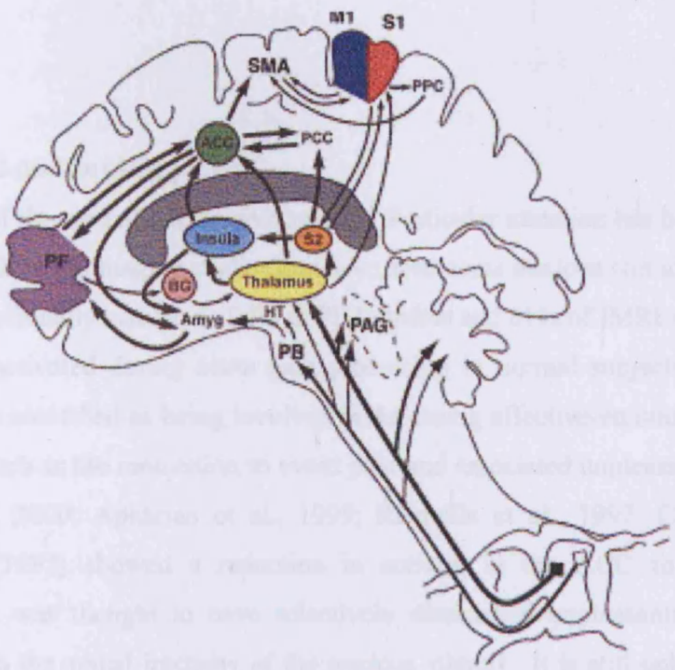


Figure 1.2 Cortical and subcortical regions involved in pain perception

Schematic of brain regions involved in pain perception taken from Apkarian et al., (2006), modified from Price (2000) to include additional areas and connections. S1, primary somatosensory cortex; S2, secondary somatosensory cortex; IC, insular cortex; ACC, anterior cingulate; PFC, prefrontal cortex; BG, basal ganglia; CB, cerebellum; PCC, posterior cingulate; PMC, premotor cortex; BS, brainstem; Amyg, amygdala; Hippo, hippocampus; PAG, periaqueductal gray; VT, ventral tegmentum; M1, primary motor cortex; PPC, posterior parietal cortex; PMC, premotor cortex; PVG, periventricular gray; SMA, supplementary motor area; Hyp, hypothalamus.

Primary and secondary somatosensory cortices

SI and SII have been consistently identified as being involved in the sensory processing of pain. Numerous studies have confirmed activity in SI contralateral to the stimulated site. The sensory-discriminative features of pain are considered to include detection, stimulus localisation, intensity discrimination and quality discrimination (Treede et al., 1999). It is a key feature of the nociceptive system, which enables subjects to locate the source of pain and have the capacity to encode different intensities of noxious stimulation. Within SI the somatotopic arrangement is consistent with the tactile homunculus for hand and foot (Ploner et al., 1999; Tarkka and Treede, 1993). Pain intensity coding has been demonstrated for nociceptive neurones in SI using both haemodynamic (Bornhovd et al., 2002) and electrophysiological (Iannetti et al., 2005b; Arendt-Nielsen, 1994) correlates of pain. Using painful laser stimulation, Iannetti and colleagues showed that the amplitude of nociceptive specific components, driven by the operculoinsular and

the primary somatosensory cortex, correlate with the perceived intensity of the pain (Iannetti et al., 2005b).

Anterior cingulate cortex (ACC)

The ACC is part of the medial nociceptive system. Particular attention has been focused on the ACC as virtually all brain imaging studies that have used acute noxious stimuli provide evidence to suggest it is functionally activated. 94% of PET studies and 81% of fMRI studies have shown that the ACC is activated during acute pain processing in normal subjects (Apkarian et al., 2005). It has been identified as being involved in the strong affective-emotional components of pain processing, such as the motivation to avoid pain and associated unpleasantness and anxiety (Sawamoto et al., 2000; Apkarian et al., 1999; Rainville et al., 1997; Craig et al., 1996). Rainville et al. (1997) showed a reduction in activity in the ACC following hyponotic suggestion, which was thought to have selectively diminished unpleasantness, despite there being no change in the actual intensity of the noxious stimuli. It is still unknown whether the ACC has a nociceptive-specific area or whether activation is caused by a non-specific effect, possibly due to the affective processing and autonomic responses. Many other imaging studies that have used non-noxious stimulation also show activation of the ACC (Raz et al., 2005; Killgore and Yurgelun-Todd, 2005; Najib et al., 2004). This suggests that the ACC may have a more general role in terms of affective-emotional processing, which is not specific to noxious stimulation.

Insular cortex (IC)

The insular cortex takes up an intermediate position between the lateral and medial system. It receives its major input from the lateral system but projects to the limbic system. The affective-emotional components of pain are also thought to be processed by the insular cortex. In contrast to the ACC, the insular cortex has been shown to encode the intensity and laterality of painful stimuli (Brooks et al., 2002; Coghill et al., 1999). Craig et al. (2000) used PET to show that contralateral activity in the dorsal margin of the IC correlated with graded cooling stimuli. The results suggest that the intensity of heat pain is encoded in the IC and that this site is involved in discriminating between noxious and innocuous temperature (Craig et al., 2000).

Prefrontal cortex (PFC)

The PFC is the most anterior portion of the frontal lobes. The PFC is thought to be related to the cognitive-evaluative processing of pain, such as the memory of pain and pain-related attention processing. As activation does not increase with stimulus intensity, intensity coding is not a task of the prefrontal cortex (Coghill et al., 1999). The prefrontal cortical regions are activated in a number of imaging studies but activation is not as common as the other cortical areas that have been described (Apkarian et al., 2005). The prefrontal cortex receives input from the ACC, but there is no evidence that it receives direct thalamocortical nociceptive input.

The studies that have been discussed outline a number of brain regions consistently activated following noxious stimulation. There are also considerable differences that occur across different experimental paradigms. In fact, even when considering the same individual in the same experimental conditions there is considerable variation in the subjective processing of pain. Many of the observed differences in pain imaging studies can be accounted for by the disparity between technical procedures. It is also likely that there is considerable variation in pain processing when comparing responses between different individuals.

1.2.4 Brain imaging and the preterm infant

Our understanding of human pain processing in the adult brain has increased considerably over the last 15 years (Apkarian et al., 2005). Studies of the haemodynamic correlates of pain have led to a better understanding of pain processing in adults at supraspinal levels, and analysis of pain-evoked potentials has provided an electrophysiological representation of pain processing (Kakigi et al., 2005). However, the imaging techniques and experimental paradigms adopted for the study of adult pain processing are not appropriate for the study of infants in intensive care. In the neonatal population, there are clear technical difficulties associated with the use of imaging techniques such as fMRI and PET. Although many successful fMRI studies have been conducted in neonates, it would be extremely difficult to perform a clinically required noxious procedure while an infant was in an fMRI scanner. Many infants cannot easily be moved from the intensive care environment and, in this vulnerable population, it would be unethical to use the radioactive tracers required for PET studies. Further experimental difficulties arise because studies can only be undertaken when there is a clinical requirement for a painful procedure to be carried out; it is not possible to use repetitive painful stimuli at predetermined intervals to assess the development of pain processing throughout early development. Hence, many of the experimental paradigms used in adults cannot be applied to studies in infants. For these reasons,

alternative brain imaging techniques and experimental approaches need to be adopted to investigate pain processing in the neonatal population.

The studies presented in the following chapters use near-infrared spectroscopy (NIRS) to assess the haemodynamic correlates of pain and electroencephalography (EEG) to assess the underlying cortical electrical activity. NIRS and EEG are ideal for the study of neonates because the equipment is portable and non-invasive. NIRS measures regional changes in the concentration of natural chromophores such as oxygenated and deoxygenated haemoglobin. A further benefit of this technique over other optical imaging methods is that studies can be done at the bedside, while the infant remains in intensive care. Many studies have confirmed the cerebral origin of such optical haemodynamic recordings from the surface of the head and the feasibility of using NIRS to detect neural activity (Gratton et al., 2005). This technique is widely used in neonatal research and has been successfully applied in infants to measure the haemodynamic correlates of neural activity during motor, auditory, olfactory and visual tasks (Zaramella et al., 2001; Isobe et al., 2001; Bartocci et al., 2000; Sakatani et al., 1999; Meek et al., 1998). It has also successfully been applied to detect evoked responses in infants, from neonates to 12-month-olds during visual, memory, and language tasks (Taga et al., 2003; Pena et al., 2003; Baird et al., 2002). NIRS and EEG are discussed in more detail in the following sections.

1.2.5 Near-infrared spectroscopy

NIRS is a technique used to measure blood and tissue oxygenation. The method is based on spectroscopic measurements of oxygenated and deoxygenated haemoglobin concentration. The technique depends on the transparency of biological tissue to near-infrared light and uses the fact that the absorption of near-infrared light is dependent on the amount of oxygenated and deoxygenated haemoglobin present within the tissue (Owen-Reece et al., 1999). A source and detector are placed on the surface of the head and the change in intensity between the emitted and absorbed light is used to measure changes in tissue oxygenation. Furthermore, the absolute haemodynamic parameters such as blood flow (Edwards et al., 1988) and cerebral blood volume (Wyatt et al., 1990a) can be calculated. In recent years, NIRS has been used to study functional activation of various areas of the brain. As with other techniques that use a haemodynamic approach to assess the functional activation of the brain, it is based on the assumption that increased tissue oxygenation represents an increase in regional cerebral blood flow, which is in turn associated with an increase in underlying neural activity (Logothetis et al., 2001).

Light in the visible region is strongly attenuated and cannot penetrate more than 1cm of tissue, whereas near-infrared light can still be detected after it has traversed through 8cm of tissue (Elwell, 1995; Wyatt et al., 1986). There are a number of compounds that absorb near-infrared light. Of particular interest are oxygenated and deoxygenated haemoglobin because measurements of these compounds can provide information on the tissue oxygenation status. Changes in the amount of absorption or scattering will result in changes in the light reaching the detector. The amount of light that a compound absorbs is dependent on two major features; the wavelength of light and the properties of the tissue being penetrated. This wavelength-dependent absorption is known as the absorption spectrum of the compound. The loss in light intensity can be described using the Beer-Lambert Law, which states that for an absorbing compound in a non-absorbing medium, the attenuation (A) is proportional to the concentration of the compound in the solution (c), the specific extinction coefficient of the absorbing compound (α) and the optical path length (d), which is the distance between points where the light enters and leaves the medium. The product of αc is known as the absorption coefficient (μ_a). I_0 and I are the light intensities incident on the medium and transmitted through the medium respectively.

$$A = \log\left[\frac{I_0}{I}\right] = \alpha c d$$

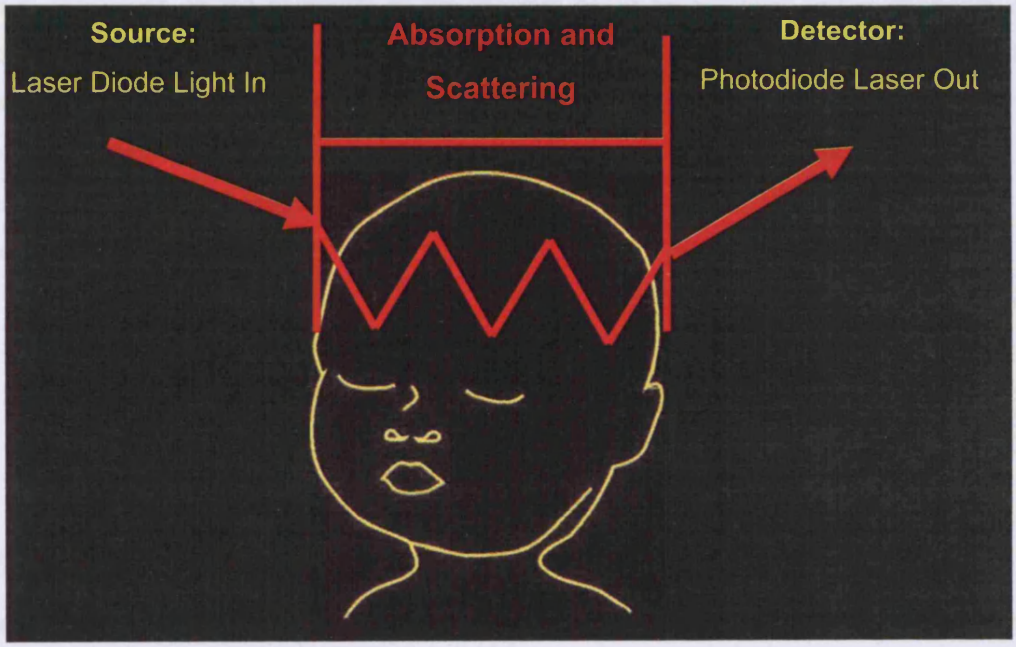


Figure 1.3 Schematic diagram showing the effect of scattering

The total optical path length is greater than the geometrical displacement of the source and detector.

The consequences of scattering also need to be considered. Scattering has the effect of increasing observed tissue attenuation at a given point. The amount of scattering is dependent

on the size of the scattering particle, the wavelength of light, and the refractive indices of the scattering medium (Elwell, 1995). In a highly scattering medium, the mean distance travelled by each photon is far greater than the geometrical displacement (see Figure 1.3 for a schematic representation). In this case, the modified Beer-Lambert law can be used to describe the loss in light intensity. The true path length, known as the differential path length (β) can be determined by multiplying the geometrical displacement by a scaling factor, which is dependent on the biological tissue being penetrated and the relative proportion of the different mediums. For example, the scaling factor accounts for the proportion of soft tissue to muscle to bone. The scaling factor (B), known as the differential path length factor, has been shown to be age-dependent and in infants is estimated to be 4.39 +/- 0.28 (Wyatt et al., 1990b).

The modified Beer Lambert Law, as written below accounts for the losses due to scattering. G is the additive term that accounts for scattering losses.

$$A = \log\left[\frac{I_0}{I}\right] = \alpha c d(B + G)$$

As G is dependent on the geometry of the medium being investigated and is unknown, the modified Beer-Lambert law does not calculate an absolute concentration of the compound, but instead uses the *change* in attenuation to calculate the change in the concentration of the compound. Since absolute concentrations are unknown, all measurements are expressed as absolute concentration changes from an arbitrary zero, which is determined at the start of the measurement period.

$$\Delta A = \Delta c \alpha d B$$

1.2.6 Physiological interpretation of the near-infrared response

As described previously NIRS is a brain imaging method that relies on a vascular response to define cerebral activation. Functional studies are based on the premise that changes in haemoglobin content and oxygenation are related to regional cerebral blood flow (rCBF). It is reasonable to suppose that an increase in haemodynamic activity correlates with an increased level of cortical electrical activity (Logothetis and Wandell, 2004). In adults, functional

responses typically observed in activated cortical areas consist of an increase in oxygenated haemoglobin and a decrease in deoxygenated haemoglobin, resulting in an overall increase in total haemoglobin (Villinger et al., 2002). This has been observed using NIRS, where increased blood volume, increased oxygenated haemoglobin and decreased deoxygenated haemoglobin concentrations were observed in the visual cortex during visual stimulation (Meek et al., 1995). These findings are supported by fMRI studies which have consistently shown an increase in the BOLD signal in the visual cortex during visual stimulation tasks (Ogawa et al., 1993; Schneider et al., 1993).

Despite the apparent similarities in these findings, care must be taken when comparing results using NIRS to fMRI. If NIRS studies only report increases in oxygenated haemoglobin, which is most often the parameter of choice, and the increases are not coupled with a decrease in deoxygenated haemoglobin, then had these studies been conducted using fMRI the reported activation may not have been observed. While increases in oxygenated haemoglobin are consistent with our understanding of activation in terms of fMRI, the decrease in deoxygenated haemoglobin also corresponds to an increase in BOLD contrast (Mehagnoul-Schipper et al., 2002). Areas of the brain that have a metabolic demand for oxygen higher than the rate of supply may cause the BOLD signal to be negated or result in an overall decrease in the BOLD signal.

In contrast to findings in adults, there is evidence in neonates that an increase in oxygenated haemoglobin is not coupled to a decrease in deoxygenated haemoglobin. This has been shown using NIRS and confirmed by observations using fMRI, which have shown that the BOLD effect is inverted in this population (Sakatani et al., 1999). Using NIRS to study cerebral blood oxygenation changes during auditory stimulation in newborn infants, Sakatani (1999) reported parallel increases in the deoxygenated and oxygenated haemoglobin concentrations in 60% of subjects. In fMRI studies, BOLD decreases have also been reported in neonates despite an apparent increase in blood flow to the activated cortex (Anderson et al., 2001). These observations are illustrated by two studies, which measured signal changes in the auditory cortex of unsedated neonates during auditory stimulation. In the first study NIRS was used to demonstrate that the concentrations of both oxygenated and deoxygenated haemoglobin (measured with respect to the volume of cortex under the detectors) increased in two thirds of the infants (Zaramella et al., 2001). Notably, if fMRI had been used to investigate the haemodynamic response then an increase in the observed BOLD signal, caused by the rise in oxygenated haemoglobin, would have been attenuated by the increase in the deoxygenated

haemoglobin. In agreement with these findings the study using fMRI showed that BOLD decreases were recorded in the auditory cortex in two-thirds of the infants (Anderson et al., 2001).

During sensory stimulation in the neonate, an increase in both oxygenated and deoxygenated haemoglobin has been observed in the visual cortex during visual stimulation (Meek et al., 1998); in the motor cortex during passive motor movements of the arms or legs (Isobe et al., 2001; Hintz et al., 2001; Benaron et al., 2000), over the temporal cortex during auditory stimulation (Zaramella et al., 2001), and over the anterior orbito-frontal gyri of the frontal lobes during the presentation of different smells (Bartocci et al., 2000). The findings indicate that in the human infant, the cerebral metabolic rate of oxygen consumption may exceed the increase in oxygen supply provided by the increase in regional CBF. The metabolic ratio that appears to exist in adults may not hold for neonates. It is probable that the inversion of the BOLD signal occurs because oxygen metabolism exceeds the supply. Whether the vascular response in infants differs from adults and the age range at which this differs remains controversial (Martin and Marcar, 2001). Due to the uncertainty in the direction of the deoxygenated haemoglobin response in neonates, when using functional NIRS it is particularly important to ensure that the detected changes occur as a direct response to the stimulation. It is essential to ensure that the observed changes do not occur due to other factors such as movement artefacts.

1.2.7 Neurophysiological basis of the electroencephlogram (EEG)

EEG can be used to assess the underlying cortical electrical activity in infants. An EEG recording measures the fluctuating field potentials as a function of time. The deflections in an EEG trace, measured against an inactive electrode, represent a change in voltage and polarity. When multiple recording electrodes are used, it gives a representation of the spatial distribution of the field over the scalp. A normal adult EEG recording will consist of activity that occurs over a range of frequencies. The slow activity (0.3-7.0Hz) and fast activity (greater than 30Hz) are sparsely represented with the majority of activity occurring in a frequency range between 8-30Hz. The amplitude range for a cortical EEG signal most commonly lies between 10-50μV. A nerve cell body has a resting potential of -60 to -70mV, which is subject to fluctuations chiefly elicited by synaptic activity. If an action potential travels along a nerve fibre that ends in an excitatory synapse, an excitatory postsynaptic potential (EPSP) occurs in the following neuron. Two action potentials travelling along the same fibre within a short time interval results in the summation of the EPSP in the postsynaptic neurone. If a threshold membrane potential is reached an action potential will be produced in the second order neurone. If an action potential

travels along a fibre ending in an inhibitory synapse then hyperpolarisation will result, representing an inhibitory postsynaptic potential (IPSP). The generation of an EPSP results in the net flow of cations across the synaptic membrane and consequently gives rise to a depolarisation. The potential gradient causes cations to move along the nerve cell membrane through the extracellular space in the direction of the subsynaptic region. These ion fluxes in the extracellular space are of paramount importance in the generation of field potentials and are the basis of an EEG recording. The primary transmembranous currents generate secondary ionic currents along cell membranes in the intracellular and extracellular space. The portion of these currents that flow through the extracellular space is directly responsible for the generation of the field potentials. Figure 1.4 shows the basic mechanism underlying the generation of field potentials and the resultant EEG recording.

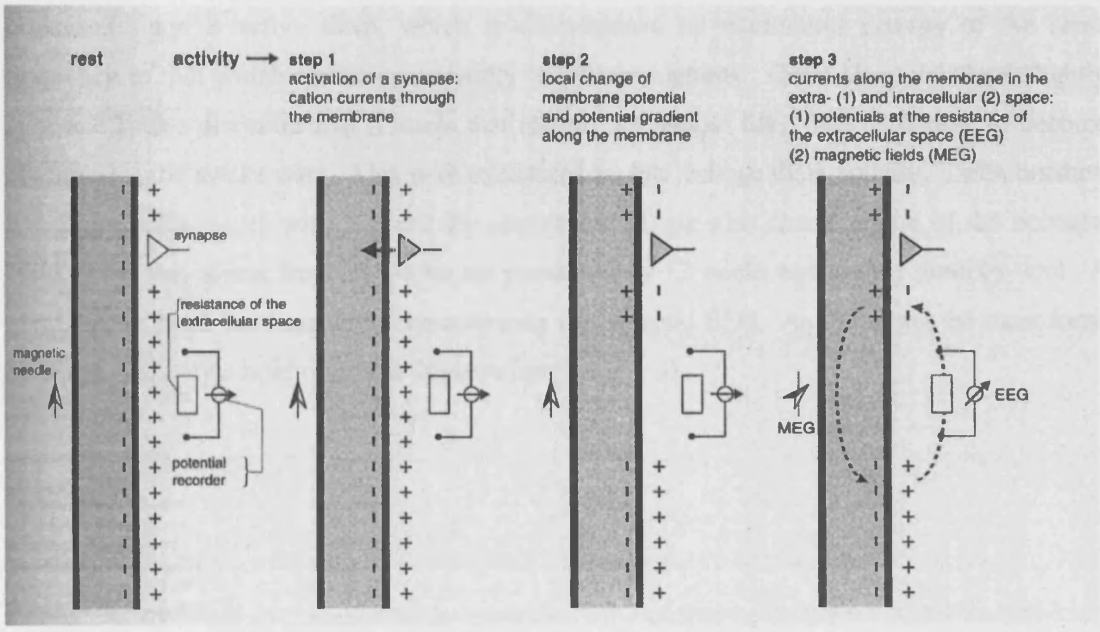


Figure 1.4 Underlying mechanisms generating an EEG and MEG signal

Basic mechanisms underlying the generation of potentials (electroencephalogram; EEG) and magnetic fields (magnetoencephlogram; MEG) in the extracellular space of the CNS. It is based on the principle that an extended neuronal process is locally depolarised by the activation of an excitatory synapse. Diagram from Niedermeyer and da Silva (2005).

1.2.8 Neonatal EEG

As EEG activity rapidly changes throughout early development, the correct interpretation of a neonatal EEG is dependent on knowledge of the post menstrual age (PMA). The earliest EEG activity has been recorded in infants from 22-23 weeks PMA. The characteristic feature of the neonatal EEG is that the activity is not continuous but consists of small bursts of activity against

a quiescent background. The quiescent periods, often referred to as tracé discontinué, vary enormously in duration. In the youngest infants bursts of activity can be separated by up to 8 minutes (Hughes, 1994). The periods of quiescence decrease in time as the infants approach term and are thought to cease from about 32 weeks. If term infants demonstrate periods of quiescence, this is considered to suggest neurophysiological immaturity and is indicative of poor outcome at 3 years old. After the tracé discontinué the earliest distinctive EEG pattern that can be observed is sharp theta activity on the occipitals of prematures (STOP). The activity is 5-6Hz, is maximal in the occipital regions and can be either unilateral or bilateral. STOP activity is most prominent in the youngest premature infants and has normally disappeared by term. The next distinctive pattern, which is maximal between 29-31 weeks, is the premature temporal theta (PTθ) activity. Like STOP it is theta activity, but in contrast, it is maximal in the temporal areas and is normally bilateral. Different sleep stages can be distinguished by 32 weeks. The first organised stage is active sleep, which is characterised by continuous activity of the same frequency to that which occurs sporadically in younger infants. Quiet sleep develops slightly later and shows discontinuous features that remain throughout life. The final state to become organised is the awake state. This is characterised by low voltage theta activity. Delta brushes, which are delta waves with fast activity superimposed, are also characteristic of the neonatal EEG. They can appear from 28 weeks, are prominent by 32 weeks and usually gone by term. A great deal of work has been done characterising the neonatal EEG. As this is not the main focus of this thesis, only a brief overview is given (see Table 1.3).

	27-27 weeks	28-31 weeks	32-35 weeks	36-41weeks
Continuity	Discontinuous, long flat stretches	Discontinuous	Continuous in waking state and REM sleep. Discontinuous in non-REM sleep	Continuous except for tracè alternant in non-REM sleep
Low voltage	Long flat stretches	Flat stretches, mainly asynchronous	Low-voltage record suspect of serious cerebral pathology	Very low-voltage records are due to severe cerebral pathology, prognosis ominous
Interhemispheric synchrony	Short bursts in synchrony	Mostly asynchronous activity	Partly synchronous	Minor asynchronies present
Occipital theta (STOP)	Prominent	Decreasing	Decreasing	Absent
Temporal theta (PTθ)	Present and increasing	Prominent	Decreasing and disappearing	Disappearing or absent
Alpha rhythm	None	None	None	None
Differentiation of awake and asleep state	Undifferentiated	Undifferentiated	Waking distinguished from sleep early in period, then differentiation of non-REM and REM sleep	Good
Slow activity (awake)	Slow bursts high voltage (state of vigilance undifferentiated)	Very slow activity predominant	Slow (delta) occipital maximum	Slow (delta), mostly of moderate voltage
Fast activity (awake)	Very little beta activity	Frequent ripples or brushes around 16/sec	Frequent ripples or brushes (16-20 Hz)	Decreasing ripples, sparse fast activity
Tracé alternant	None	None	Present in non-REM (quiet sleep)	Present in non-REM (quiet sleep)
Spindles	None	None (ripples present)	None (ripples present)	None (but scanty ripples)
REM sleep	Undifferentiated	Undifferentiated	Continuous slow activity; REM present	Continuous slow activity (more REM than non-REM sleep

Table 1.3 Summary of EEG maturation

Table adapted from Niedermeyer and da Silva (2005).

1.2.9 Principles of event related potentials

EEG is a technique used to investigate human brain function in health and disease. Further specialised techniques use EEG to assess underlying sensory and cognitive processing associated with specific types of stimulation. Changes in EEG activity, associated with a specific sensory stimulation, are generally referred to as event related potentials (ERPs). ERPs arise following the brain's reception and response to an external stimulus. Characteristic peaks and troughs, caused by the synchronised bursting of neurones, correspond to the underlying neuronal processing. The functional significance of the ERP characteristics involves the simultaneous consideration of its components, such as polarity (positive or negative), timing (latency), and scalp distribution (topography). The amplitude and latency of successive peaks in the waveform can be used to determine the time course of information processing in the brain, whereas the distribution of voltage over the scalp can be used to estimate the neuroanatomical loci of these processes. A subset of ERPs, known as sensory evoked potentials, includes those generated by visual, auditory and somatosensory stimuli. Potentials evoked by somatosensory stimuli, referred to as somatosensory evoked potentials (SEPs), are discussed in the next section.

It is difficult to observe ERP activity in a raw EEG trace because few stimuli generate clear-cut changes in the EEG signal that can be observed above the background activity. The difficulty in detecting ERPs is that the activity can be masked by the ongoing EEG activity. Conventionally, ERPs are detected using averaging techniques so that the signal of interest is amplified and observed over and above background activity. The basic assumption is that the signal of interest occurs at a fixed latency, time-locked to the stimulus, which means that the ongoing EEG activity can be treated as noise. This simple model is an approximation as it does not take into account that sensory stimuli can cause a reorganisation of the EEG signal (Sayers et al., 1974) or, for example, that some stimuli, such as visual stimulation, can reduce the amplitude of the ongoing EEG activity (Vijn et al., 1991).

1.2.10 Somatosensory evoked potentials

SEPs are routinely used to provide information for clinical assessment about the function of the somatosensory system and the integrity of somatosensory pathways. The most common method used to generate SEPs is transcutaneous electrical stimulation. Electrical stimuli depolarise the nerve fibres by generating a potential difference across the nerve fibre membrane, therefore bypassing the encoding of natural stimuli by the receptors. This has advantages over other physiological stimuli because it is easy to control and produces potentials that are easily measured. A comparison between electrical stimulation and mechanical stimulation (Ogawa et

al., 2005) has shown that waveforms produced by the former are similar but of greater amplitude than those produced by the latter (Pratt and Starr, 1981). The difficulty with mechanical stimulation is that a reliable delivery method, essential to record time-locked neural events, is hard to achieve. Designing a mechanical device that produces a quick and well-defined impact can be problematic. Although some mechanical stimulation systems have been designed, such as the use of short-duration air puffs, these have not been routinely used clinically because only a small population of fibres are excited. Electrical stimulation non-selectively activates peripheral afferent fibres, and has the disadvantage that it produces a variety of sensations, such as buzzing and ringing. It is also an unnatural stimulus that is not experienced in our normal environment. Cortical SEPs generated by electrically stimulating the median nerve, and recorded in the parietal region contralateral to the stimulation site, peak at around 18-35ms. In adults, up to 500 averages are used to generate the SEPs. Figure 1.5 shows an example SEP with the negative (N) and positive (P) components identified. The EEG traces presented in this thesis have the negative potentials represented by a downward deflection.

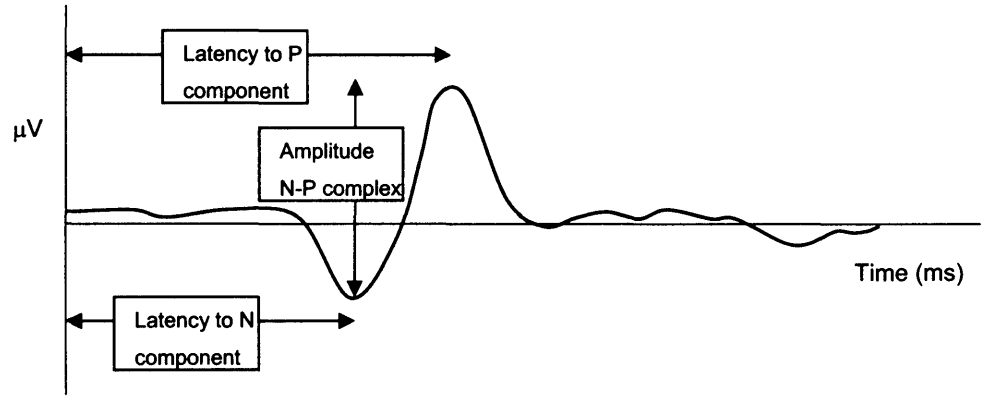


Figure 1.5 Schematic of a somatosensory evoked potential
Schematic of an SEP with the latency and amplitude of the N-P complex labelled.

1.2.11 Background to pain-evoked somatosensory potentials

A particular challenge for clinical neurophysiologists has been to use EEG to record pain-related somatosensory evoked potentials (SEPs). The excellent temporal resolution provided by EEG means that neural activity in the cerebral cortex can be recorded when afferent input reaches the brain. SEPs have conventionally been recorded using electrical stimulation applied to the peripheral nerves. It provides an excellent way of assessing the integrity of the nervous system because activity can be recorded from the peripheral nerves to the spinal cord to the thalamus and finally the cortex. Its use is limited, however, in terms of assessing pain processing because

electrical stimulation non-selectively activates a wide range of the peripheral afferent fibres, which triggers a various sensations.

Evoked potentials following a painful stimulus were first identified in the 1960s (Spreng and Ichioka, 1964). Since then a wide range of stimulation protocols have been used in the attempt to measure pain-related SEPs. Examples include, painful impact, where small solid bullets were fired at the upper arm or trunk of participants (Kohlloffel et al., 1991) and dental pulp stimulation (Hari et al., 1983). Each method has its advantages and disadvantages; however, the ideal stimulus would be one that generates evoked potentials that are nociceptive-specific, reproducible, highly controllable and safe (Kakigi et al., 2005). In addition, the stimulus must be short enough to cause a volley of afferent nerve fibre activity that is sufficiently synchronised to allow the recordings of time-locked brain responses. In 1975 the first EEG signals following laser stimulation were recorded (Mor and Carmon, 1975). These signals are now referred to as laser evoked potentials (LEPs). As lasers rapidly heat the skin, afferent volleys are highly synchronised, which allows time-locked events to be observed on a single trial basis. Laser stimulators selectively activate thermosensitive Aδ and C fibres, and as there is no contact with the skin, mechanosensitive afferent fibres are not activated. A number of different laser emission sources have been developed, of which the CO_2 laser is most often used (Arendt-Nielsen and Chen, 2003). Laser stimulation evokes a characteristic double pain sensation; which includes a sharp pricking sensation followed by a longer lasting dull pain. Neurophysiological studies have shown that these different perceptual responses are respectively related to the activation of Aδ and C primary afferents (Plaghki and Mouraux, 2003). The earliest cortical potentials activated by laser stimulation of the hand have a dipolar distribution, which peaks at about 160ms. The negative component is generated by the contralateral temporal region and the positive component is generated by the midfrontal region. The latency of the N-P complex is compatible with input transmission occurring via Aδ fibres (Kunde and Treede, 1993). A later potential, which peaks between 200-350ms, is maximum at the vertex (Cz). This N-P vertex potential represents the largest amplitude response following laser stimulation and is the easiest to record. This vertex potential is thought to reflect a late cognitive response driven by the cortical processing of the noxious stimulation.

1.2.12 Somatosensory processing in the preterm infant brain

Somatosensory evoked potentials (SEPs) have been used in neonates to assess the functional integrity and maturity of the neonatal central nervous system. Studies have shown that the

immature brain is able to respond to external stimuli, and careful characterisation of these responses provides an excellent way of looking at age-dependent changes in the developing neonate. In most studies an electrical stimulus has been used to activate the median or tibial nerve (Pike et al., 1997; Taylor et al., 1996) but other methods, such as tactile stimulation of the fingers, have also been used (Pihko et al., 2004). Cortical SEPs have been reported to occur from the 25th week of gestation (Taylor et al., 1996). Such reports confirm that at this early stage of development the somatosensory pathways are able to conduct peripheral impulses to cortical structures. These studies have consistently shown a decrease in peak latency with increased gestational age (Smit et al., 1999; Taylor et al., 1996; Klimach and Cooke, 1988). This decrease in latency is attributed to the immature myelination of the sensory pathways, the immature functioning of the synapses and the smaller diameter of the axons in the younger infants (Pihko and Lauronen, 2004). The latency to response is dominated by two developmental features that have the opposite consequences. Increased myelination causes a progressive increase in the conduction velocity and synchronisation of the potentials, whereas increased body length causes a decrease in latency and a decrease in synchronisation.

While there is good evidence to suggest that SEPs can be reliably recorded from 30 weeks PCA, the evidence is less good in infants below 28 weeks. A handful of studies have looked at responses in extremely premature infants and to date the results have been variable. One study, which looked at SEPs in infants below 32 weeks, found that they were only able to record reliable SEPs in 24 out of 59 infants (Smit et al., 2000). In contrast, by term the same investigators could get reliable EP recordings in 58 out of 65 studies performed (Smit et al., 2000). The first study to record EPs in infants below 28 weeks was conducted by Klimach and Cooke in 1988. They recorded EPs from 3 infants below 28 weeks and although they reported an overall success rate of 90% across the whole population (26-41 weeks PCA) they stated that most unsuccessful studies were conducted in infants below 28 weeks PCA (Klimach and Cooke, 1988).

It is possible that the poor success rate in this population is due to the extreme technical difficulty in undertaking these studies. It is equally plausible that while sensory pathways may be anatomically intact at this age, they may not have formed mature synaptic connections and therefore cannot be functionally activated. Findings by Pike and colleagues dispute this (Pike et al., 1997). They looked at posterior tibial SEPs in 67 preterm infants, aged between 27-37 weeks gestation, and were able to obtain reliable recordings from all the infants in their study. They argue that poor success rates in recordings is not due to variable rates of myelination and

neurogenesis but a consequence of the practical and technical difficulties involved in undertaking these studies in the challenging NICU environment. In a recent review, Vanhatalo et al. suggest that methods for recording and analysing SEPs in preterm babies are suboptimal and that the unique properties of the preterm SEP call for novel analytical methods (Vanhatalo and Lauronen, 2006). The review highlights the point that previous studies have empirically shown that less averaging gives more clearly defined waveforms. This reflects the natural variability in the waveforms between trials, and challenges the validity of using conventional grand averaging approaches.

CHAPTER 2: GENERAL METHODS

2.1 Neonatal Research

2.1.1 General overview

This thesis describes a set of studies where the question of cortical pain processing in very young infants is addressed by directly measuring cortical responses to noxious stimulation using near-infrared spectroscopy (NIRS) and electroencephalography (EEG). To date, neither EEG nor NIRS have been used to investigate the onset or development of the cortical response to pain in infants. By combining these techniques, we hypothesised that it might be possible to assess whether the nociceptive pathways conduct impulses to the cortex and at what stage during development the cortex is able to produce a response. Because the exact relationship between the haemodynamic response and underlying electrical activity in neonates is both unclear and conceivably different from that in adults, it is advantageous to measure responses using both recording modalities simultaneously. In addition, behavioural and physiological responses to pain are monitored and scored using a clinical pain assessment tool. A series of experiments are described which set out to test whether the immature cortex is functionally active in response to noxious stimulation; how the cortical responses change throughout early development; and whether other factors modulate the cortical processing of pain. The overall aim of these studies is to develop a systematic approach to measure pain in the neonatal population.

2.1.2 Neonatal intensive care

The site of the current study, University College London Hospital (UCLH) is a level 3 unit, which means it provides intensive care for babies needing respiratory support; for babies weighing less than 1,000 grams and/or less than 28 weeks gestation receiving nasal continuous airway pressure; for babies with severe respiratory disease or for those who require major surgery. UCLH is attached to a foetal medicine department, which means a high number of the infants are identified antenatally as having problems. UCLH has approximately 3000-4000 deliveries per annum of which up to 400-500 are admitted to the intensive care unit. In specialist London units, babies born at less than 23 weeks have a 42 per cent chance of survival. Those born at 25 weeks have an 80 per cent chance of survival and those born at 28 weeks have a 90 per cent chance of survival (Green, 2005).

2.1.3 Research challenges in a neonatal unit

A number of specific challenges need to be addressed when research is undertaken on infants receiving intensive care. As with all studies involving humans, the first requirement is to obtain ethical approval from the institutional ethics board. The aim of the board is to review research proposals to ensure that the research complies with recognised ethical standards. The purpose is to protect the dignity, rights, safety and well-being of all research participants. This is particularly important when studying neonates because they cannot personally give informed consent. Consent is given on their behalf by their parents or legal guardians.

Research studies should ideally be conducted without having to move the infant, which means studies are conducted at the bedside. The equipment needs to be designed such that it is suitable for use in an intensive care environment. Medical and nursing staff must have access to the infants at all times, so it needs to be compact and portable. It is essential that equipment and research personnel are kept to the absolute minimum so as not to hinder the medical care provided for the infants. It is also important that the equipment is compatible with the medical equipment in use. This has the advantage that the ongoing physiological monitoring of the infants can be recorded and downloaded for research purposes. To prevent the spread of infection it must be possible to clean the equipment completely and to dispose of parts that come into contact with the infants. Cross infection can be reduced if infants colonised with bacteria are identified. Although all infants are handled using universal precautions, it may be safer to avoid studying infants colonised with known bacteria, such as MRSA. This is of particular importance when doing research involving premature infants because the immune system is immature and they have a high risk of developing infections.

A good relationship needs to be established with the nursing and medical staff. Even if ethical approval has been obtained, it is still essential to gain approval from the staff caring for the infant at the time of the study. If clinical staff are concerned about how a research study is being conducted then they are within their rights to prevent the study from taking place. Staff are working in a stressful and difficult environment so every attempt should be made not to interfere with medical or nursing practices. The priority of a neonatal unit is to provide clinical care to the infants and research undertaken on this population should in no way compromise the infants' well-being.

Written informed consent is obtained from parents before any study commences. A sensitive approach needs to be adopted when requesting consent from parents. It is not appropriate to

approach a parent if they have recently received bad news about the wellbeing of their child. Parents need to be kept informed about the research and, where possible, should be invited to keep copies of video footage, photographs or other research data that has been recorded from their children. If photographs or video footage is to be used for any other purposes, such as publication, further consent needs to be sought. It is a requirement of the ethics board that the informed consent is maintained but it is important to remember that parents have the option to withdraw their infants from the study at any time. Studies will only be successful if a good relationship is maintained with parents. This is particularly important in the case of longitudinal studies. Patient and parent confidentiality must be protected. Data is anonymised by allocating a unique study number for each infant and test occasion. Data files are password protected and paper records are stored in a locked filing cabinet.

Studies presented in this thesis involve research undertaken when a blood sample is clinically required. As the needs of the infants are constantly reviewed based on clinical assessment it is essential to check infants medical records regularly for changes in their condition and the intended treatment. When considering the optimum time to do a study numerous factors need to be considered, such as the working hours of the pathology laboratory, whether parents wish to be present, and why the blood samples are required. It is also important to establish whether other clinical procedures are also being performed at the same time. If an infant is undergoing other procedures, such as an eye examination for retinopathy of prematurity (ROP), then it may not be appropriate to study the child on that occasion. Infants are limited in their ability to tolerate excessive handling and the well-being of the child needs to be considered at all times. By liaising with the clinical staff, it is possible to ascertain whether an infant is stable enough to tolerate the extra handling. It is important to be prepared to abort a study at any time when these conditions are not met. As all the blood samples are clinically required, it may still be necessary to perform the medical procedure even if the research cannot be undertaken.

2.1.4 Single event recordings

One of the major challenges of this study is that the stimulus cannot be administered unless a heel lance is required for clinical purposes. This means that once the equipment has been set up there is only one opportunity to record the evoked activity. On some occasions, it is necessary to perform two heel lances in order to get the required quantity of blood; however, it is optimal for the infant if only one heel lance is performed. Any methodological or technical problems resulting in a failed recording attempt means all the preparatory work is redundant. The preparatory work for this type of study includes recruitment, clinical assessment of the infant,

preparation of equipment, application of electrodes, optodes and other recording equipment, liaising with parents and doctors to find out when studies can be conducted, recording demographic details and other data required by the ethics board. It is estimated that each study takes in excess of six hours to set up so it is crucial that the number of aborted attempts are minimised. By checking that all the equipment is working correctly before starting each study the number of technical difficulties that arise can be limited. Due to the clinical instability of the infants, there are many reasons why studies may be aborted on clinical grounds. By ensuring the infants are handled in the best possible way when preparing for a study means they will be more settled and less likely to become unstable as a result of the extra handling.

2.1.5 Study design and ethical permission

Studies were undertaken on inpatients in the neonatal intensive care unit (NICU) and the special care baby unit (SCBU) at the Elizabeth Garrett Anderson and Obstetric Hospital, which is part of University College London Hospital (UCLH). Ethical approval was obtained from the UCLH ethics committee and informed written parental consent was obtained for each infant prior to the commencement of each study.

2.1.6 Infant recruitment

Infants were recruited between March 2004 and June 2006. A copy of the parent information sheet and the parental consent form are provided in the appendix (Appendices 1.1 and 1.2). 138 parents were approached and invited to enrol their baby or babies into the study. Of these parents, 21 did not give an answer as their babies were either transferred to another hospital or discharged before a decision was made. Parents were given at least 24 hours before they were asked if they had made a decision. 71 babies were successfully recruited to the study, which gave a recruitment success rate of 61%. Overall, infants were studied on 204 test occasions using either NIRS, EEG, video recording or a combination of these techniques. The stimulus in each study was either a clinically required heel lance or non-noxious tactile stimulation.

2.1.7 Selection criteria

Medical charts were reviewed for pertinent medical history and, unless specifically stated, all infants included in the study had normal appearances on cranial ultrasound scans. At the time of the study, infants were assessed as clinically stable. Infants were not receiving analgesics or sedatives at the time of the study unless specifically stated. Infant handling was kept to a

minimum; hence, infants were studied in a variety of positions and sleep states. Postmenstrual age (PMA) was determined from cranial ultrasound scans taken at 19-20 weeks gestation or from the maternal report of the last menstrual period.

2.1.8 Heel lance protocol

The heel lance was performed when the heart rate and oxygen saturation were stable. Following heel lance the foot was not squeezed for a period of at least 30 seconds to ensure that the evoked response occurred only as a result of the initial stimulus and can be timed from a single discrete event. When the blood was free-flowing the foot was not squeezed, however, as the blood samples are needed for clinical purposes, if blood flow was poor and it was necessary to squeeze the foot the study was excluded from further analysis. It was often possible to collect blood samples without squeezing the foot.

2.1.9 Collaborators

A number of collaborators were involved in aspects of the work presented in this thesis. Anne Cantarella (BNurs) is a neonatal research nurse who was employed by UCLH to assist with the study. She was involved in obtaining parental consent prior to each study and performed the heel lances when required. She was present when each study was undertaken and was responsible for ensuring the infants wellbeing. In addition, she did some of the behavioural scoring presented in Chapter 4. Alan Worley (MSc, MIEEE) is a clinical scientist employed by Great Ormond Street Children's Hospital. He was involved in the design, development and integration of the multi-modality physiological monitoring systems. In addition, he built the trigger system described in Chapter 5. Dr Martin King (PhD) provided statistical assistance with the mixed-model regression.

CHAPTER 3: RESULTS PART 1

Cortical haemodynamic responses to noxious stimulation in preterm infants

3.1 Introduction

3.1.1 Chapter overview

In this chapter, we attempted to measure cortical pain activity in very young infants by direct measurement of the haemodynamic response to noxious stimulation using near-infrared spectroscopy (NIRS). Although previous studies have shown that painful procedures in preterm and term infants evoke a range of behavioural and physiological responses, such as change in facial expression, crying, and change in heart rate and oxygen saturation, the purpose of the studies presented in this chapter was to establish whether noxious stimulation also evokes cortical activity. The intention was to establish whether a fully integrated pain response may be achievable in the immature infant brain. When this research began, NIRS had not been used to investigate the onset or development of the cortical responses to noxious stimulation. We hypothesised that using this technique, it would be possible to establish whether the immature cortex is functionally activated in response to noxious stimulation; the developmental profile of the response; and whether the response can be modulated depending on the physiological state of the infant.

3.2 Materials and Methods

3.2.1 Participating infants

Eighteen infants aged between 25 and 45 weeks postmenstrual age (PMA) were tested on 31 occasions during clinically required routine heel lancing. Table 3.1 provides details of the individual clinical characteristics of each infant and gives the PMA at the time of each study. Infants included in the study were tested on one or more occasions (see Table 3.1). Two infants were receiving morphine at the time of heel lance. In a separate study, the cortical response to non-painful mechanical stimulation of the foot using von Frey hairs was studied in 11 infants, aged 26 to 35 weeks PMA.

Neonatal characteristics of each infant included in the study

Baby no.	PMA at birth (completed weeks)	Birth weight (g)	Days on mechanical ventilator	PMA at time of study (completed weeks)	Diagnosis
1	24	560	8	25	extreme prematurity
2	24	547	36	25,27	extreme prematurity, RDS
3	24	639	15	26,32,34,39,42	extreme prematurity, CLD, GOR
4	24	613	42	28,32,35,43	extreme prematurity, CLD, GOR
5	24	768	54	34,36	extreme prematurity, PDA, CLD, GOR
6	27	1108	1	29,30	Preterm
7	27	1014	1	36	Preterm
8	28	858	3	35,45	preterm, RDS, CLD, PDA
9	29	1482	1	29	Preterm
10	29	1106	1	34	Preterm
11	30	683	1	37	preterm, hyperbilirubinemia, IUGR, CLD
12	32	1324	0	34	Preterm
13	33	1408	1	38	Preterm
14	33	2118	0	34	preterm, RDS
15	32	1798	0	33	preterm, RDS
16	34	2350	3	35,40	preterm, NEC
17	34	1340	0	37,37	Preterm
18	37	2100	0	39	IUGR

Table 3.1 Neonatal characteristics for each infant included in the study

PMA: postmenstrual age, NEC: necrotising enterocolitis, IUGR: intra-uterine growth restriction, RDS: respiratory distress syndrome, CLD: chronic lung disease, GOR: gastro-oesophageal reflux, PDA: patent ductus arteriosus (ligated).

3.2.2 Infant demographics

Table 3.2 details the demographic characteristics of the infants.

Demographic characterisation of the neonatal population	
Mean gestational age at birth (weeks), n=18	28.2 (4.3); range, 24.0-37.3
Mean PMA at time of study (weeks), n=31	35.0 (5.2); range, 25.7-45.6
Mean postnatal age at time of study (days), n=31	7.8 (38.5); range, 5-34
Mean birth weight (g), n=18	1212.0 (574.3); range, 547-2350
Mean weight at time of study (g), n=31	1914.9 (952.9); range, 520-4184
No. of multiple gestation infants, n=18	7
No. of infants with chronic lung disease, n=31	7
Mean no. of days on mechanical ventilator, n=18	9.4 (16.7); range, 0-54
Mean no. of test occasions, n=18	1.7 (1.1); range, 1-5
Mean no. of days between consecutive studies, n=13	27.3 (20.7); range, 1-69

Table 3.2 Demographic characterisation of the neonatal population
Standard deviations are in parentheses.

3.2.3 Assessment of sleep state

Sleep state was assessed using the behavioural state indicator in the premature infant pain profile (Stevens et al., 1996). Behavioural state was determined by observing the infant for 15 seconds prior to the heel lance and selecting one of four categories from the PIPP that best describe the infant's state. The four behavioural state categories are active/awake (eyes open with facial movements), quiet/awake (eyes open with no facial movements), active/sleep (eyes closed with facial movements) or quiet/sleep (eyes closed with no facial movements). Infants in the first two categories were classified as awake and those in the second two categories were classified as asleep.

3.2.4 Near-infrared spectroscopy

Cortical activity is associated with increased localised cerebral blood flow. In this study, a double channel near-infrared spectrophotometer (NIRO-200, Hamamatsu Photonics) was used to measure regional changes in oxygenated and deoxygenated haemoglobin concentration. This is a non-invasive technique which has been widely used in neonatal research to measure functional activation of the cortex (Kusaka et al., 2004; Sakatani et al., 1999). The light emitters and

detectors (optodes) were positioned symmetrically on either side of the head over the somatosensory cortex, using the international 10-20 EEG placement system to identify key landmarks (Jasper, 1958). The optodes were attached to the head using double-sided adhesive tape. To help prevent displacement of the optodes, and to prevent ambient light from affecting the recording, the optodes were positioned under a snugly fitted hat. Figure 3.1 shows a photograph of an infant with the optodes placed over the somatosensory cortex. Optodes were placed on the head at least 15 minutes prior to recording and real-time changes in cerebral haemodynamic activity were monitored. The measured absorption changes at each wavelength were converted to the relative concentration changes. The changes were expressed in μmol/L, and the optical path length was calculated by multiplying the inter-optode distance by the neonatal differential path length factor (DPF). DPF has been calculated to be 4.39 for neonates (Wyatt et al., 1990b). A sampling frequency of either 2Hz or 6Hz was selected. Changes in oxyhaemoglobin [HbO_2] and deoxyhaemoglobin [HHb] were measured. Total haemoglobin [HbT] concentration change was calculated [HbT] = [HbO_2] + [HHb].

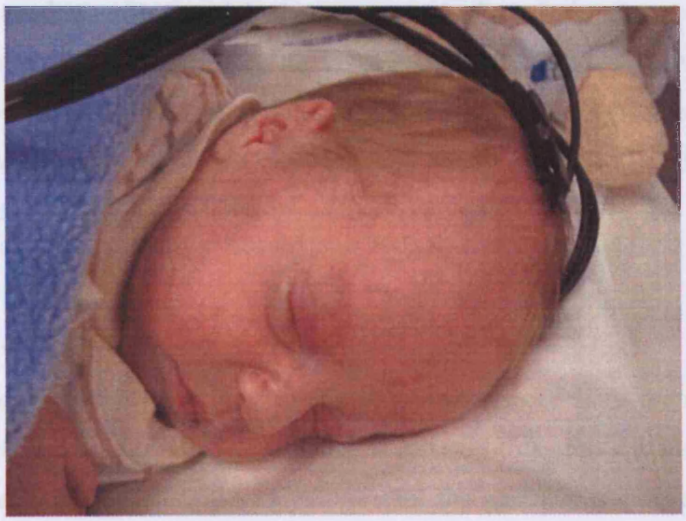


Figure 3.1 Optode placement

Photograph of premature infant (33+4 weeks PMA) with optodes positioned over the somatosensory cortex.

3.2.5 Analysis of NIRS data

Baseline activity was recorded for 20 seconds prior to the heel lance. The maximum change in [HbT] was defined as the maximum change from the mean pre-stimulus recording in the 20-second period post heel lance. Calculating the maximum change in haemoglobin concentration from a prestimulus baseline has been used in other NIRS studies (Kusaka et al., 2004; Isobe et

al., 2001). In five studies, only unilateral data was obtained due to loss of contact of an optode on one side while the other optode remained in good contact. Data analysis was undertaken by a blinded observer using a prewritten MATLAB script (MATLAB version 7.0). If more than one heel lance was required on a given test occasion only the response to the first heel lance was included in the analysis.

3.2.6 Tactile stimulation

In a second series of measurements, the selectivity of the cortical response was investigated using non-noxious stimulation. von Frey hairs were used to apply a known calibrated force to the plantar surface of the foot using techniques described by Andrews and Fitzgerald (1994). The force applied to the foot was increased using graded von Frey hairs. Each von Frey hair was applied to the plantar surface of the foot five times in each infant at intervals greater than 60 seconds. The reflex withdrawal threshold was defined as the stimulus at which a distinct movement of the foot away from the same von Frey hair occurred in at least three out of five occasions. The maximum force applied was one von Frey hair above reflex withdrawal threshold. The range of force applied by the von Frey hairs (0.096-25.81g) was similar to that previously reported (Andrews and Fitzgerald, 1999). For each hair, a haemodynamic response was defined as more than 10% of the post-stimulus response lying two standard deviations outside the pre-stimulus baseline in three out of five responses.

3.2.7 Statistical analyses

The maximum change in [HbT], defined as the maximum change from the mean pre-stimulus recording in the 20 second period post stimulus, is referred to as the stimulus response and is used in all the statistical analyses. To assess whether the stimulus response was significantly different from the pre-stimulus baseline within an individual subject, the maximum post-stimulus value was compared with the pre-stimulus sample mean (two-sample t-test). To establish whether there was a significant difference between the cortical response in the awake and asleep infants restricted maximum likelihood mixed-model regression was used (Hand and Crowder, 1996). The regression analysis provides an estimate of the mean stimulus response in the awake and asleep states as a function of age; it takes into consideration the non-independence among observations made in infants where more than one measurement was acquired. The analysis was performed using SAS proc mixed (SAS Version 9.1).

A linear dependence on age was adopted, and an (age*state) interaction term allowed for a potential difference in age dependence between the awake and asleep states. The likelihood ratio test was used to test whether the response to heel lance was different in the ipsilateral and contralateral cortices. A number of calculations, including sensitivity analysis and the usual regression diagnostics were performed to ensure that the regression results are robust. The results were satisfactory. The inclusion of infants receiving morphine did not substantially change the statistical assessment; however, these infants were excluded from all the statistical analysis used to calculate regression coefficients.

3.3 Results

3.3.1 Noxious stimulation produces a cortical response in preterm infants from 25 weeks

Figure 3.2 shows a typical somatosensory haemodynamic response to noxious stimulation of the heel during routine blood sampling in a preterm infant. An increase in the total haemoglobin concentration over the contralateral somatosensory cortex following heel lance was observed in all but one infant. The mean increase in [HbT] in the contralateral somatosensory cortex, calculated from the whole sample of infants, was 7.74μmol/L (SE: 1.10, N=30), while a mean decrease in [HbT] of -0.10μmol/L (SE: 1.10, N=26) was recorded on the ipsilateral side. While the magnitude of the ipsilateral response was variable and sometimes positive, it was always smaller than the contralateral response (see Figure 3.3).

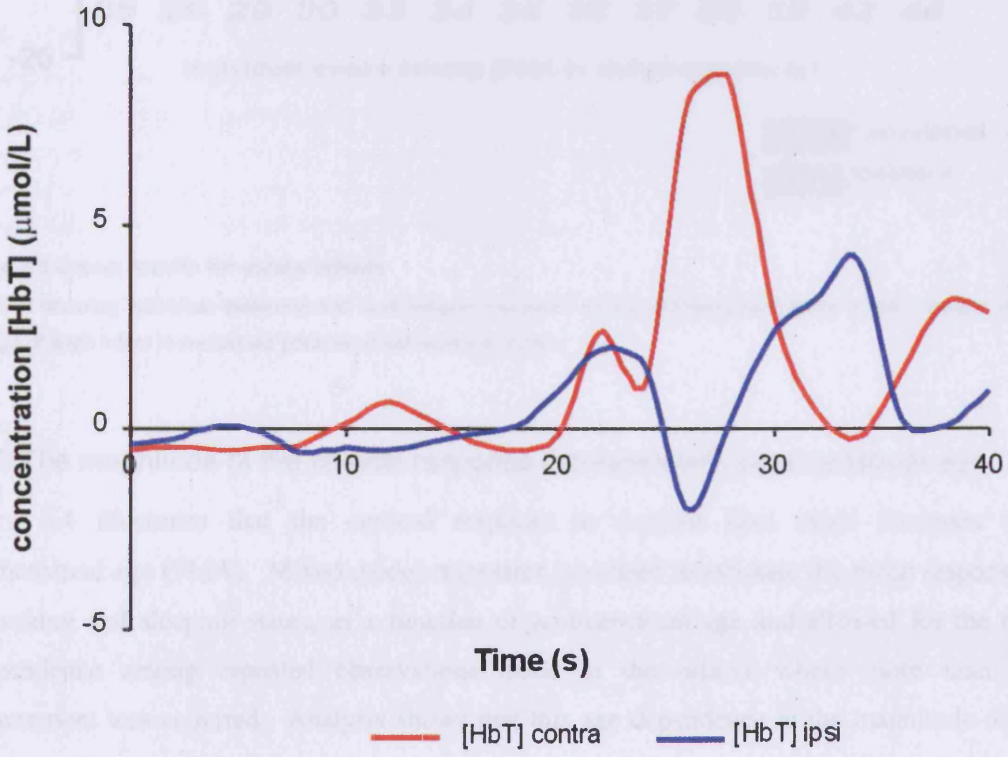


Figure 3.2 Haemodynamic response

Sample trace in a single infant (29+5 weeks PMA) demonstrating the evoked change in [HbT] in the contralateral and ipsilateral somatosensory cortex following a painful stimuli given at t=20 seconds. Sampling frequency: 2Hz.

The likelihood ratio test, used to compare the responses in the ipsilateral and contralateral cortices, showed a highly significant difference between the two sides. (The likelihood ratio: 34.6 with 4 degrees of freedom; where 0.05 level chi-sq critical value = 9.49). This confirms that the cortical response to heel lance was a localised effect that did not occur due to a global haemodynamic change.

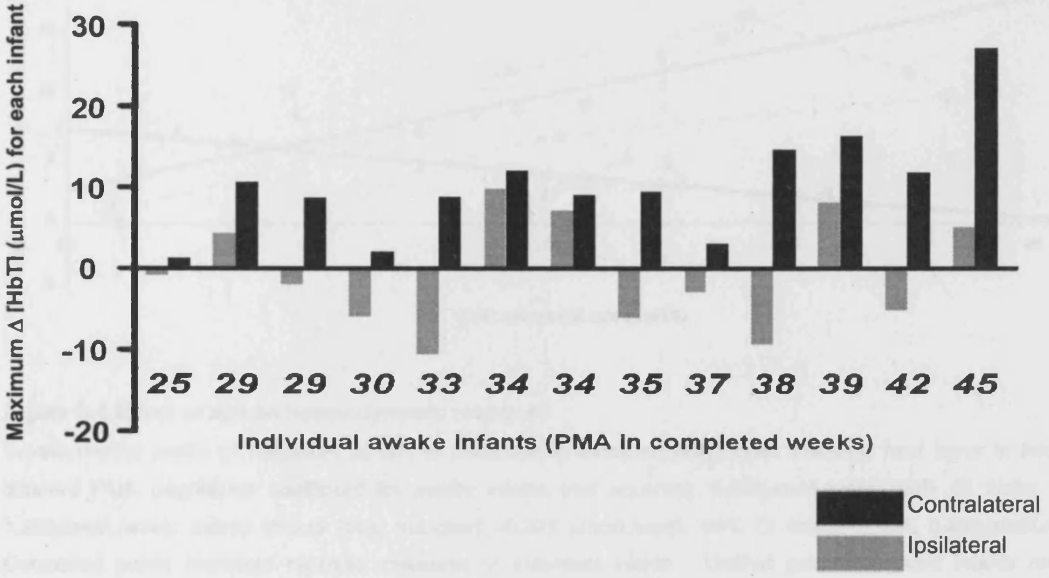


Figure 3.3 Group results for awake infants

Bar chart showing individual ipsilateral and contralateral maximum Δ[HbT] following heel lance in each awake infant. The age of each infant in completed postmenstrual weeks is shown.

3.3.2 The magnitude of the cortical response increases with postmenstrual age

Figure 3.4 illustrates that the cortical response to noxious heel lance increases with postmenstrual age (PMA). Mixed-model regression was used to estimate the mean response in the waking and sleeping states, as a function of postmenstrual age and allowed for the non-independence among repeated observations made in the infants where more than one measurement was acquired. Analysis shows that this age dependence in the magnitude of the response only occurs in awake infants (PMA regression coefficient: 0.698μmol/L/week, 95% CI limits: 0.132, 1.265μmol/L/week). Age dependence was not observed in the sleeping state (PMA regression coefficient in the asleep state: -0.291μmol/L/week, 95% CI limits: -1.004, 0.423μmol/L/week). Furthermore, as illustrated in Figure 3.3 there was no age dependence in the ipsilateral responses. It should be noted that although the youngest infants displayed the smallest responses, even the very youngest infant (25+5 weeks PMA) demonstrated a clear and

statistically significant response above the pre-stimulus baseline ($p<0.005$, two-sample t-test). Figure 3.5 shows the cortical response to heel lance in the youngest infant in the sample.

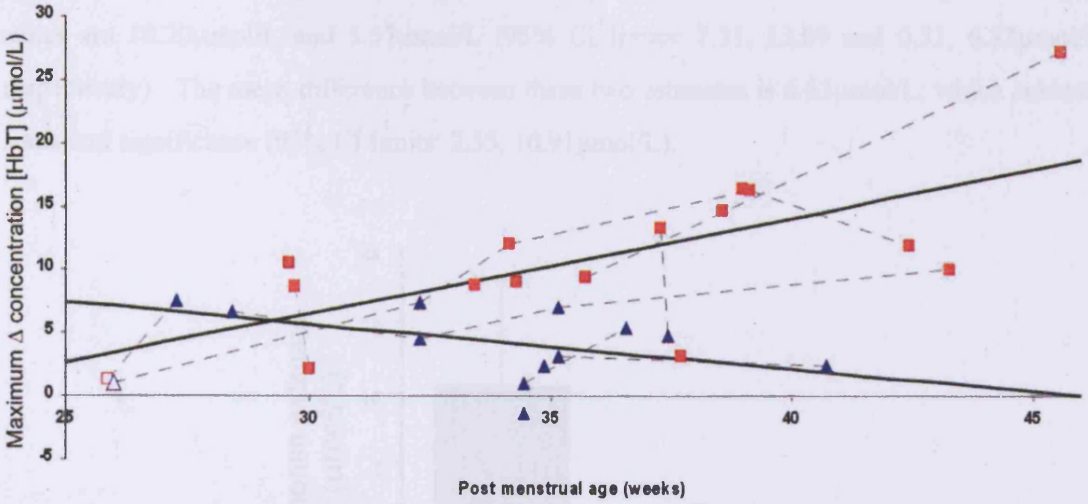


Figure 3.4 Effect of age on haemodynamic response

Developmental profile of maximum Δ[HbT] in contralateral somatosensory cortex following heel lance in infants at different PMA (regression coefficient for awake infants (red squares): 0.698μmol/L/week, 95% CI limits: 0.132, 1.265μmol/L/week; asleep infants (blue triangles): -0.291 μmol/L/week, 95% CI limits: -1.004, 0.423μmol/L/week). Connected points represent repeated measures in individual infants. Unfilled points represent infants receiving morphine; these infants are excluded from the regression analysis.

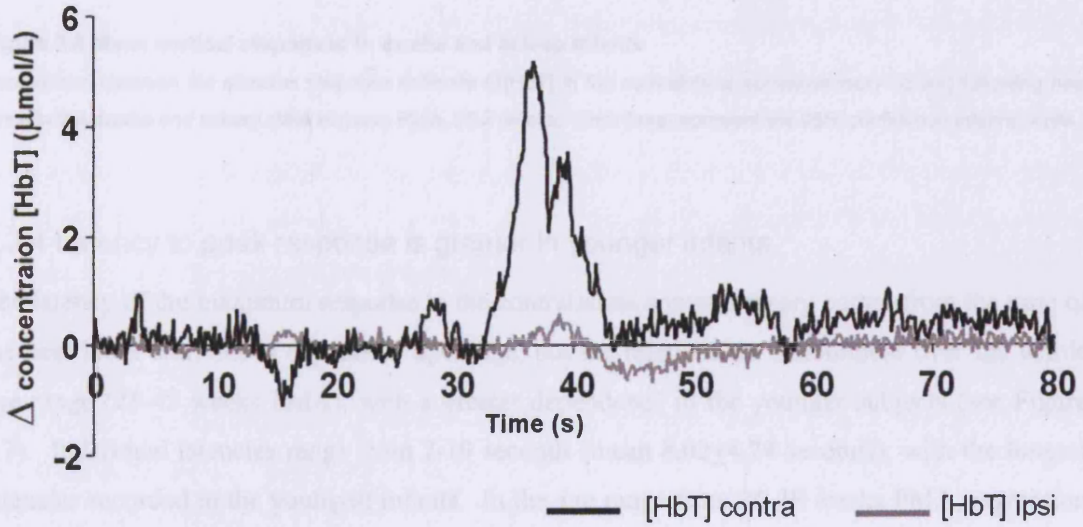


Figure 3.5 Haemodynamic response in the youngest infant

Sample trace in the youngest infant in our sample (25+5 weeks PMA) demonstrating the evoked change in [HbT] in the contralateral and ipsilateral somatosensory cortex following a painful stimuli given at t=20 seconds. Sampling frequency: 6Hz.

3.3.3 Awake infants have larger cortical responses than sleeping infants

The magnitude of the response was dependent upon sleep state (Figure 3.6). At the mean PMA (35.2 weeks) the least-squares mean stimulus response estimates in the waking and sleeping states are 10.20μmol/L and 3.57μmol/L (95% CI limits: 7.31, 13.09 and 0.31, 6.83μmol/L, respectively). The mean difference between these two estimates is 6.63μmol/L, which achieves statistical significance (95% CI limits: 2.35, 10.91μmol/L).

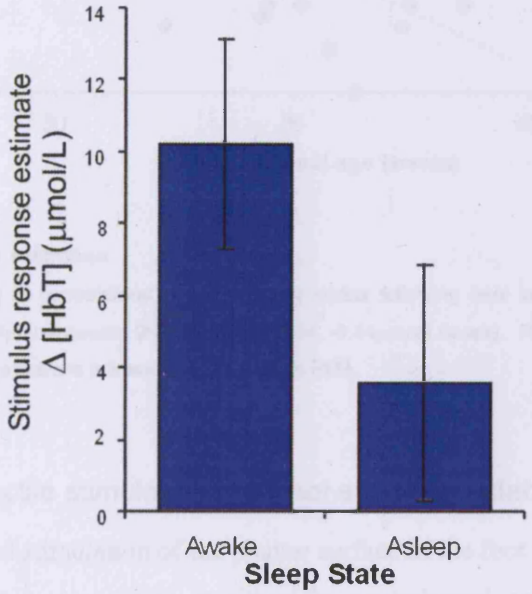


Figure 3.6 Mean cortical responses in awake and asleep infants

Comparison between the stimulus response estimate (Δ[HbT] in the contralateral somatosensory cortex) following heel lance in the awake and asleep state at mean PMA, 35.2 weeks. Error bars represent the 95% confidence interval limits.

3.3.4 Latency to peak response is greater in younger infants

The latency of the maximum response in the contralateral somatosensory cortex from the time of the heel lance stimulus is dependent upon age, but the relationship is nonlinear over the whole age range (25-45 weeks PMA), with a greater dependence in the younger subjects (see Figure 3.7). Individual latencies range from 2-19 seconds (mean 8.02±4.74 seconds), with the longest latencies recorded in the youngest infants. In the age range from 25-38 weeks PMA, regression analysis, used to estimate the time to peak response as a function of PMA, demonstrates a clear increase in latency with age (regression coefficient = -0.99μmol/L/week, 95% CI limits: -1.54, -0.44μmol/L/week).

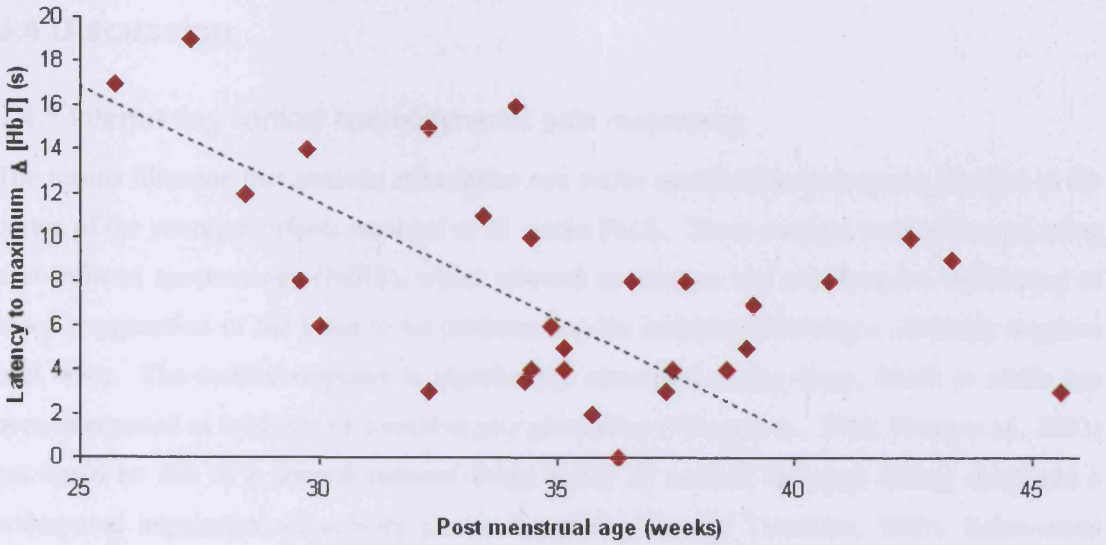


Figure 3.7 Latency to peak response

Latency to maximum Δ[HbT] in contralateral somatosensory cortex following heel lance in infants at different PMA (regression coefficient = -0.99μmol/L/week, 95% CI limits: -1.54, -0.44μmol/L/week). Regression line represents infants aged 25-38 weeks PMA where there is a linear dependency on PMA.

3.3.5 Non-noxious tactile stimulation does not evoke detectable cortical responses

Non-noxious mechanical stimulation of the plantar surface of the foot did not elicit a measurable change in the haemodynamic activity in either the contralateral or ipsilateral somatosensory cortex (N=11, 26-35 weeks PMA). In each case, a stimulus intensity was used that produced a visible foot withdrawal, demonstrating that the sensory input was sufficient to produce a reflex motor response. Despite this, no measurable cortical haemodynamic response was recorded. It is probable that the non-noxious von Frey hair stimulation evokes activity in the somatosensory cortex that NIRS is not sensitive enough to detect. Hence, these results do not mean that non-noxious sensory input cannot activate the somatosensory cortex, but rather a single non-noxious stimulus, strong enough to evoke reflex movement of the foot, does not evoke a cortical response equivalent to that recorded following noxious stimulation. Furthermore, the reflex withdrawal is not responsible for the activation of the somatosensory cortex observed following noxious stimulation.

3.4 Discussion

3.4.1 Interpreting cortical haemodynamic pain responses

The results illustrate that noxious stimulation can evoke specific haemodynamic changes in the cortex of the youngest infants recorded at 25 weeks PMA. These changes were measured using near-infrared spectroscopy (NIRS), which allowed continuous and non-invasive monitoring of blood oxygenation of the brain to be performed at the bedside, following a clinically required heel lance. The cortical response is significantly attenuated during sleep, which in adults has been interpreted as evidence of cognitive pain processing (Wang et al., 2004; Wang et al., 2003) but could be due to a general reduced firing ability of cortical neurones during sleep and a widespread impairment of sensory processing (Rosanova and Timofeev, 2005). Behavioural states are less differentiated in preterm infants and a large proportion of their sleep is classified as indeterminate, that is neither active nor quiet sleep (Lehtonen and Martin, 2004), which complicates interpretation of the data on the basis of cognitive processing. The smaller cortical responses to noxious stimulation in younger infants are likely to reflect lower energy requirements due to less neuronal activity (Kostovic and Judas, 2002). The linear relationship between subjective pain intensity and regional cerebral blood flow in the contralateral somatosensory cortex observed in adults (Bornhovd et al., 2002) may also be reflected in neonates. Our results suggest that infants undergoing intensive care process painful experiences at the cortical level and that the activity associated with this process increases with postmenstrual age. A true confirmation of this requires electrophysiological measures such as recording of somatosensory evoked potentials (see Chapter 5).

3.4.2 The development of spinal and supraspinal pain processing in human infants

In contrast to the cortical responses reported here, spinally mediated reflex limb withdrawal from a noxious stimulus is greater both in magnitude and duration in the youngest infants and decreases with age (Andrews and Fitzgerald, 1999; Andrews and Fitzgerald, 1994; Fitzgerald et al., 1988). The data highlight the dissociation between spinal reflex responses and cortical nociceptive processing in human infants, and illustrate that the strong reflex responses in preterm neonates do not necessarily mean more perceived pain. Reflexes may have a protective function for an organism that is less able, through cortical immaturity, to perceive and organise a more directed response. Reflex thresholds are also lower in infants than in adults and can be evoked by von Frey hair mechanical stimulation, as observed here. The postnatal development of cutaneous flexion reflexes in rat pups and preterm human infants show remarkable parallels

(Fitzgerald and Jennings, 1999; Fitzgerald et al., 1988), and it is likely that this reflex excitability is due to an altered balance of local and descending excitatory and inhibitory processing in the developing dorsal horn (Fitzgerald, 2005). Here it is shown that von Frey hair mechanical stimulation of the foot, sufficient to evoke a flexion reflex, did not elicit a measurable haemodynamic response in the somatosensory cortex, again highlighting the differences between spinal and cortical pain pathways at this stage.

3.4.3 The onset and maturation of cortical pain processing

Much has been written about when exactly a human preterm infant or foetus may begin to process pain (Lee et al., 2005). In principle, with more data it would be possible to establish whether there is a well-defined age of onset in the cortical pain response. The age of onset, however, may precede the limits of viability (23-24 weeks PMA). Even if it were possible to look at the neonatal responses to noxious stimulation in this age group, it cannot be assumed that cortical responses seen in neonates are directly translatable to the foetus in utero due to the unique features of the uterine environment (Mellor et al., 2005).

The long latencies of the cortical responses in the youngest infants is likely to be due to the low conduction velocities and slow synaptic responses in the nociceptive circuitry, which is consistent with the long latency reflex responses observed at the same age (Andrews and Fitzgerald, 1999). The long latencies observed in the youngest infants reflect the lack of myelination and decreased synaptic strength, which means the immature nervous system is slow to mount a directed coordinated response following external stimulation. Other studies in neonates that have measured haemoglobin oxygen saturation in the somatosensory area following passive knee movement, have also reported latencies consistent with these findings (Isobe et al., 2001). Isobe and colleagues reported the mean time corresponding to the maximum changes in [HbO_2] and [HHb] to be 11.9 ± 3.3s and 19.1 ± 6.7s respectively (gestational age, 24-35 weeks). The mean latency to the maximum response has also been reported following visual stimulation and is a similar duration (Kusaka et al., 2004). Despite the long latencies, the cortical responses following noxious stimulation were well defined with a clear onset. The responses were even evident in the two extremely premature infants that were receiving morphine at the time of study. This is interesting in light of recent reports that show that morphine has no effect on behavioural and physiological pain scores in infants following heel lance (Carbajal et al., 2005), and requires further investigation.

3.4.4 Cortical pain processing in human infants

The results from this chapter were published in 2006 in the Journal of Neuroscience providing, for the first time, a direct measure of cortical activation in response to noxious stimulation in the preterm infant (Slater et al., 2006b). Since this study was completed, further evidence has confirmed that the somatosensory cortex in premature infants is activated during noxious stimulation (Bartocci et al., 2006b). In agreement with the results presented in this chapter, Bartocci et al. conclude that noxious stimulation causes functional activation of the somatosensory cortex and that premature infants are able to process pain selectively in the cerebral cortex. In their paper, they present a study where one optode is positioned over the somatosensory cortex and one optode is positioned over the occipital cortex. Following noxious stimulation, they found an increase in the oxyhaemoglobin concentration [Hb] in the somatosensory region but detected no change in the occipital region.

There are, however, a number of differences in the findings in the Bartocci study and the results presented in this chapter. While here we show an increase in [HbT] in the contralateral somatosensory cortex and a mean decrease in [HbT] in the ipsilateral somatosensory cortex, Bartocci et al., (2006) report bilateral increases in [Hb] in both cortices. The apparent discrepancies may be accounted for by the different methodological approaches adopted in each study. The noxious stimulus described in this chapter was a brief discrete event that occurred when the heel was lanced. The foot was not squeezed for a period of 30 seconds following the heel lance, which ensured that the measured cortical response was a direct result of the acute stimulus. By contrast, the stimulus used in the Bartocci study was considerably more variable. It lasted between 35 and 60 seconds, while a needle was being inserted into a vein (Bartocci et al., 2006b). The long duration of this stimulus may lead to bilateral activation of the somatosensory cortex, whereas the acute noxious event used here causes selective activation of the contralateral somatosensory cortex. Furthermore, while the output measure in our study was the peak haemodynamic response that occurred within 20 seconds of the stimulus, in the Bartocci study they report the average response that occurred over a 60-second period. It remains plausible that early contralateral activation is followed by a bilateral or ipsilateral response. This is supported by evidence in adults, where transient suppression of the BOLD response has been reported to occur in the ipsilateral somatosensory cortex during tactile stimulation (Hlushchuk and Hari, 2006). The peak negative BOLD response occurs within 10 seconds and the average duration of the deactivation is 18 seconds.

The response to tactile stimulation was reported in both studies (Slater et al., 2006b; Bartocci et al., 2006b). In this chapter, it has been shown that carefully controlled tactile stimulation using graded von Frey hairs does not produce a measurable cortical response. By contrast, Bartocci et al. report bilateral activation in response to the infant having their skin disinfected with an alcohol-soaked pad. This variable, long-duration stimulus, which also has a cooling effect, may activate specific cortical areas that are not activated when a more selective approach is adopted using von Frey hairs. The importance of the tactile stimulation study presented in this chapter was that a cortical response was not detected by near-infrared spectroscopy, despite the presence of a spinal reflex withdrawal of the foot. This confirmed that movement of the foot was not responsible for the haemodynamic activity recorded following the heel lance. Bartocci et al. conclude that both tactile and painful stimulation activate somatosensory cortical areas. Progress in pain research is dependent upon precise definitions and characterisations of the conditions of the stimuli used, and in this respect term and preterm infants are no different from adults (Slater et al., 2006a). It is important that reported cortical activations are a direct response to the noxious stimulation and are distinguished from a diffuse barrage of sensory stimulation. The poorly defined stimulus used in the Bartocci study could potentially lead to misinterpretations of the precise cause of the evoked activity. To be confident that the response is due to the noxious element of the stimulus it is required that the stimulus is well defined. In this way, a defined activation time can be determined from the stimulus-locked event.

Anand et al. argue that because the postnatal age of the infants in our study range from 5 to 134 days the results may be confounded by the fact that infants have experienced variable degrees of previous pain exposure (Anand et al., 2006). Many studies have shown that neonatal responses to acute pain are dependent on exposure to previous painful events (Grunau et al., 2005; Grunau et al., 2001b; Johnston et al., 1995). It is plausible that the larger haemodynamic responses observed in infants who have a greater PMA may represent maturational changes as well as a degree of previous pain experience. Since there is likely to be a correlation between age in postnatal days and number of painful experiences, it follows that if previous pain exposure were the most significant factor, than the magnitude of the haemodynamic response would show a better degree of correlation with postnatal age than postmenstrual age. In this study, however, the correlation between postnatal age and the maximum change in contralateral [HbT] was much weaker than the correlation with PMA, which is not consistent with suggestions made by Anand et al. (see Appendix 5 for details). Hence, it is more likely that the varying degree of exposure to painful procedures, which has not been accounted for in this model, may partly explain the large scatter observed in the results. Another reason why the scatter is wide may be due the difficulty

in locating the key landmarks on the skull. Premature infants, and in particular those who were born extremely prematurely, often have unusual shaped heads due to positional moulding, which can make it more difficult to ensure that the optodes are located in the correct position. This means that identifying the somatosensory region can be more difficult.

In conclusion, neonates are able to mount a specific and lateralised cortical response to noxious stimuli from 25 weeks PMA. The results highlight the clinical need for a systematic approach to reduce the occurrence of acute pain and to improve the analgesic treatment of repetitive pain in neonates. Analysis of the underlying neural activity will elucidate the extent of this cortical processing, but this study clearly demonstrates that noxious information is transmitted to the immature preterm cortex and therefore has the potential to influence higher levels of the developing central nervous system.

CHAPTER 4: RESULTS PART 2

Clinical Pain Assessment

4.1 Introduction

4.1.1 Understanding the infant response to noxious procedures

It is accepted that pain assessment in non-verbal populations is a challenging clinical problem (Stevens, 2006). As we do not have a direct measure of pain, it is difficult to assess its existence and severity. In the previous chapter it was shown that noxious stimulation does activate cortical areas in the premature infant brain (Slater et al., 2006b; Bartocci et al., 2000). From 25 weeks PMA infants are able to mount a localised haemodynamic response in the contralateral somatosensory cortex following noxious heel lance (Slater et al., 2006b). These results suggest that infants do have the required neuronal connections to experience the affective components of pain and are not simply displaying reflex responses to nociception. Despite these findings, it remains unclear whether pain scores, calculated using current pain assessment tools, are a true reflection of higher-level pain processing. As infants cannot report subjective pain intensity verbally, it is useful to establish whether clinical pain scores, calculated on the infant's behalf, correlate with the magnitude of cortical activation that occurs following a noxious procedure (Slater et al., 2006b).

The rationale for undertaking this study is partly based on the fact that we cannot know whether noxious stimulation is actually causing pain in the neonatal population (Bowsher, 2006). It is only possible to measure responses to nociception, which in some cases, are assumed to be surrogate measures of pain processing (Bartocci et al., 2006a). True pain experience must involve supraspinal processing, so it is possible that measures of the cortical haemodynamic response may provide a better measure of central nervous system pain processing as compared with either behavioural or physiological measures. Although pain scores used in neonatal pain assessment cannot provide an objective measure of the intensity of perceived pain, they do provide a reliable measure of the behavioural and physiological responses that manifest following a noxious procedure (Stevens et al., 1996). In adults, it has been shown that the magnitude of the regional haemodynamic response in the contralateral somatosensory cortex correlates with perceived pain intensity (Bornhovd et al., 2002). If this holds true in the neonatal period, then it would be beneficial to know whether current neonatal pain assessment measures

correlate with activity in the somatosensory cortex, an area of the brain known to be involved in nociceptive processing in preterm infants (Slater et al., 2006b; Bartocci et al., 2006b).
The behavioural, physiological and cortical responses to noxious stimulation in a neonate can be measured directly (Slater et al., 2006b; Stevens et al., 1996). In this study the measures include change in heart rate and oxygen saturation, driven by the autonomic nervous system; change in facial expression, driven by motor neurone activity which results in a coordinated set of facial muscle contractions; and cortical haemodynamic activity in the contralateral somatosensory cortex. As these responses are driven by a common noxious stimulus in the periphery, albeit activating multiple ascending pathways, it is possible that there will be a degree of correlation between these measures. However, it is also possible that cortical activation can occur independently of autonomic and motor responses or vice versa. The behavioural and physiological responses to noxious stimulation will be assessed using the validated PIPP scoring system (Stevens et al., 1996) and the haemodynamic response will be assessed using NIRS, as described in Chapter 3. The study provides the first objective evidence of whether cortical processing following acute noxious stimulation correlates with the observed responses that are used clinically to assess neonatal pain.

In addition, the latency to change in facial expression following a noxious procedure will be investigated. It was shown in Chapter 3 that the latency between the noxious stimulation and peak haemodynamic response in the contralateral somatosensory cortex is greater in younger infants. It is possible that the latency to change in facial activity will mirror this trend. By carefully analysing an infant's facial response, it is possible to measure the time to onset of change in facial activity after a well-defined noxious stimulus. Studies presented in this chapter are designed to establish whether latency to change in facial expression is a function of maturity. It is hypothesised that latency to facial expression change will be longer in younger infants.

4.2 Materials and Methods

4.2.1 Participating infants

36 infants aged between 25 and 43 weeks postmenstrual age (PMA) were studied on 94 test occasions. Clinical pain assessment scores were calculated using the PIPP when heel lances were performed to provide blood samples for routine clinical assessment. Within this group, a subset of 12 infants was studied on 33 test occasions using near-infrared spectroscopy to assess the haemodynamic response to the noxious stimulus simultaneously (PMA range: 25-43 weeks). Table 4.1 details the demographic characteristics of the infant population. Infants with congenital abnormalities were excluded from the study. One infant included in the study had an intraventricular haemorrhage (IVH). The results from this infant have been highlighted throughout. Table 4.2 details the demographic characteristics of the subset of infants also studied using NIRS.

Demographic characterisation of the neonatal population	
Mean gestational age at birth (weeks), n=36	30.5 (4.3); range, 22.8-37.4
Mean PMA at time of study (weeks), n=94	34.5 (3.3); range, 25.7-43.3
Mean postnatal age at time of study (days), n=94	36.9 (30.2); range, 2-134
Mean birth weight (g), n=36	1440.7 (696.9); range, 533-3330
Mean weight at time of study (g), n=94	1777.6 (691.2); range, 532-4184
No. of multiple gestation infants, n=36	12
No. of infants with chronic lung disease, n=36	7
Mean no. of days on mechanical ventilator, n=36	7.2 (13.3); range, 0-54
Mean no. of test occasions, n=36	3.0 (2.5); range, 1-11
Mean no. of days between consecutive studies, n=94	6.2 (11.6); range, 1-69

Table 4.1 Demographic characterisation of the neonatal population
Standard deviations are given in parentheses.

Demographic characterisation of the neonatal population	
Mean gestational age at birth (weeks), n=12	28.6 (4.4); range, 24.0.8-34.6
Mean PMA at time of study (weeks), n=33	34.7 (3.7); range, 25.7-43.3
Mean postnatal age at time of study (days), n=33	45.1 (34.2); range, 5-134
Mean birth weight (g), n=12	1079.1 (750.8); range, 547-2350
Mean weight at time of study (g), n=33	1702.7 (747.9); range, 532-4184
No. of multiple gestation infants, n=12	6
No. of infants with chronic lung disease, n=12	1
Mean no. of days on mechanical ventilator, n=12	10.6 (17.6); range, 0-54
Mean no. of test occasions, n=12	2.8 (1.9); range, 1-7
Mean no. of days between consecutive studies, n=33	8.6 (14.2); range, 0-57

Table 4.2 Demographic characterisation (combined NIRS studies)
Standard deviations are given in parentheses.

4.2.2 Near-infrared spectroscopy

The NIRS techniques have been described in detail in chapter 3 (Section 3.2.4). As with all the NIRS studies the haemodynamic response is defined as the maximum change in [HbT] from a prestimulus baseline, in the 20-second period following the heel lance.

4.2.3 Heel lance protocol

The heel lance was performed as described in the general methods in Chapter 2 (Section 2.1.4). If the foot was squeezed before a change in facial expression was observed, the study was excluded from subsequent analysis.

4.2.4 Premature infant pain profile

Infant pain was measured using the PIPP. The Infants were video-recorded for at least 15 seconds prior to the heel lance and for at least 30 seconds after the heel lance (Sony DCR-DVD201E camcorder). This is the minimum requirement to calculate the PIPP score. In most cases, infants were videoed for at least 5 minutes. The time of the heel lance stimulus was marked on the video footage by an LED that flashed when the spring-loaded blade was released

from the heel lancet (Tenderfoot). The behavioural data was analysed from videotapes and the physiological data was downloaded to a computerised data collection system. Postmenstrual age (PMA) was determined as described in the general methods in Section 2.1.6.

The video footage was analysed after the heel lance procedure to establish the behavioural state score and facial expression score. Behavioural state was assessed by observing the 15-second period prior to the heel lance and categorising the infant into one of four groups. Categorisation depended on whether facial movement could be observed in the video footage and on whether the infant's eyes were open or closed (refer to Section 3.2.3 for a description of the different behavioural categories in the PIPP). The facial expression component of the PIPP was analysed by watching the video footage for 30 seconds immediately following the heel lance. The presence of each facial expression indicator (nasolabial furrow, eye squeeze and brow bulge) was assessed individually. The observer focused on one facial expression and used a stopwatch to calculate the duration over which the facial expression could be observed in the 30-second time window. This was repeated for each indicator. The video footage was repeatedly watched until the coder was satisfied that the duration of each expression had been correctly recorded. A score between 0 and 3, assigned for each of the three facial expressions, was dependent on the percentage of time that the facial expression was observed in the 30 second period after the heel lance (see Figure 1.1 for a copy of the score sheet). 10% of the sessions were randomly scored by an independent assessor. The intrarater reliability and interrater reliability were determined. The second coder was blinded to whether or not the infant was undergoing a heel lance. In 12 cases it was not possible to video record the infants. In these studies, the infants were scored in real-time at the bedside. A full description of the PIPP score can be found in the paper that describes its initial development and validation (Stevens et al., 1996).

Infants were monitored for changes in heart rate and oxygen saturation on a beat-to-beat basis using a Nellcor 200 transcutaneous monitor. The physiological readings of heart rate and blood saturation, required for the PIPP assessment, were downloaded to an external PC. Data analysis software was used to calculate the mean heart rate and oxygen saturation in the 15 seconds prior to the heel lance. This was recorded on the PIPP record sheet (see Figure 1.1). The software also determined the maximum heart rate and the minimum oxygen saturation in the 30 second period immediately following the heel lance. These values were used to calculate the percentage increase or decrease from the prestimulus baseline for heart rate and oxygen saturation respectively. A score between 0 and 3 was also assigned based on the gestational age of the infant (refer to Figure 1.1 for the exact categories). For each heel lance, all the different aspects

of the PIPP were recorded and a total composite score was calculated. The total score ranged between 0 and 21.

4.2.5 Comparison between pain scores and haemodynamic activity

The degree of correlation between the PIPP score and the magnitude of the haemodynamic response was assessed. As the magnitude of the haemodynamic response is dependent on PMA and sleep state (Slater et al., 2006b) the relationship between the unweighted PIPP score (i.e. the total PIPP score excluding weighting factors for gestational age and sleep state) and the haemodynamic response was also established. In addition, the relationship between the facial expression score and the magnitude of the cortical activity was assessed. The sum of the three PIPP facial expression indicators (i.e. eye squeeze, brow bulge and nasolabial furrow) is referred to throughout this chapter as the facial expression score. The percentage of infants who had a facial expression score of zero was calculated. The individual cortical responses (i.e. the maximum Δ[HbT] in the contralateral somatosensory cortex) for each of these infants is presented. The relationship between the physiological responses in the PIPP score (i.e. the score from the change in heart rate and oxygen saturation categories) and magnitude of the cortical response was assessed.

4.2.6 Latency to facial response

The latency to facial response was defined as the time between the heel lance stimulus and the first occasion when any of the three PIPP facial indicators were observed. The latency to facial response was plotted against PMA and regression analysis was used to estimate the facial expression score as a function of PMA. Infants classified as 'facial non-responders' were excluded from the latency analysis, as they did not show any observable change in facial expression.

4.2.7 Statistical Analysis

The NIRS data was analysed in the same way as described in chapter 1 (see Section 3.2.7). To establish the degree of correlation between the PIPP facial expression score and the cortical response restricted maximum likelihood mixed-model regression was used (Hand and Crowder, 1996). The model effectively treats the problem as a simple regression problem because the effect of the repeated measures in the same infant was not significant.

4.3 Results

4.3.1 Facial expression change

Figure 4.1a and 4.1b shows a photograph of an infant before and after a heel lance. After the heel lance the infant's eyes are forcefully closed, the brow is lowered and furrowed, and the nasal roots are broadened and bulged with a deepened nasolabial furrow. It is these changes in facial expression that are observed by health care professionals when attempting to assess or treat pain and it is these facial expressions that are assessed by the PIPP.

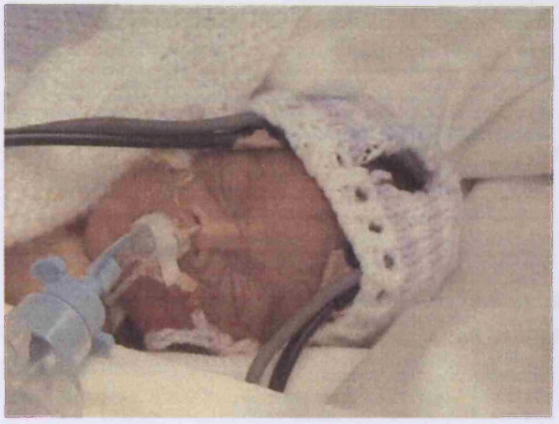


Figure 4.1a

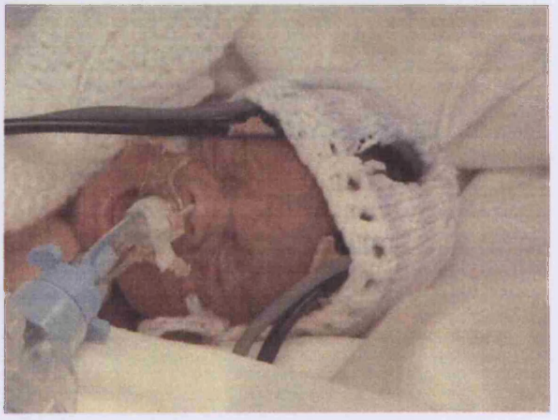


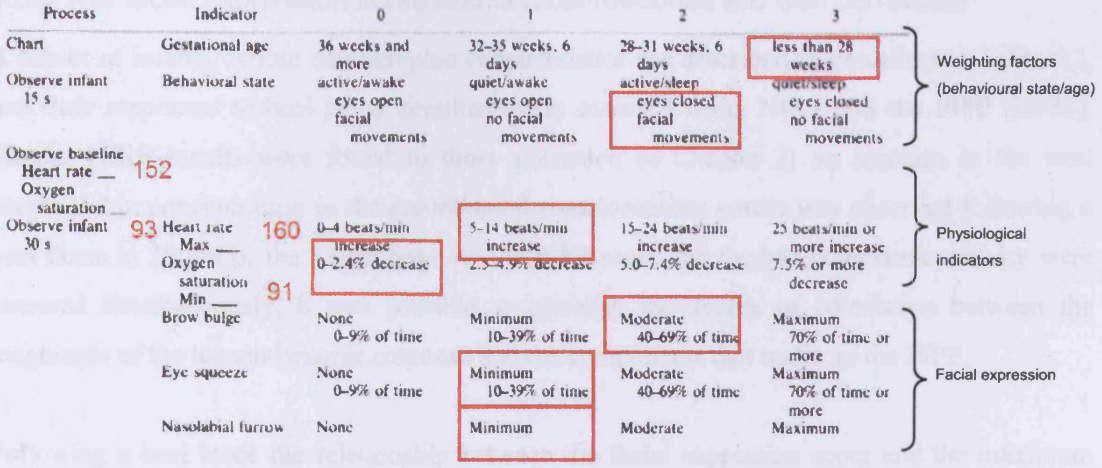
Figure 4.1b

Figure 4.1 Photograph of infant before and after a heel lance

Photograph of infant (27+3 weeks PMA) showing characteristics features before (Figure 4.1a) and after a heel lance (Figure 4.1b). NIRS optodes are placed over somatosensory cortex during the heel lance procedure. Brow bulge, eye squeeze and nasolabial furrow can be seen in Figure 4.1b.

4.3.2 Assessment of the components of the premature infant pain profile

The PIPP score, the unweighted PIPP score (which is the complete PIPP excluding the weighting factors for age and sleep state), and the facial expression score alone was calculated for each infant. For clarity, Figure 4.2 shows a breakdown of how the different scores were calculated for the infant in the photograph in Figure 4.1.



Total PIPP score (sum of all indicators) = 10

Unweighted PIPP score (exclude gestational age and behavioural state indicators) = 5

Facial expression score (brow bulge + eye squeeze + nasolabial furrow indicators) = 4

Figure 4.2 Example calculation of the PIPP, unweighted PIPP and facial expression score

Individual components that make up the PIPP score, unweighted PIPP score, and facial expression score for the infant in Figure 4.1.

The mean PIPP score, the unweighted PIPP score, and the facial expression score for the whole population are given in Table 4.3. By analysing the facial expression components of the PIPP score (i.e. brow bulge, eye squeeze and nasolabial furrow), it was established that 43% of studies were classified as 'facial non-responders'. On each test occasion an infant was defined as a 'facial non-responder' if they received a score of zero in all of the facial expression categories following the heel lance procedure. This occurred in 41 out of 94 test occasions. This group of infants is of particular clinical interest as they are at risk of having their pain under-treated, and are therefore considered as a subset in more detail in Section 4.3.6.

Analysis of behavioural and physiological responses using the PIPP score	
Mean PIPP score, n= 88	**7.35 (0.46)**
Mean unweighted PIPP score, n=88	**4.34 (0.52)**
Mean facial expression score, n=94	**2.42 (0.37)**

Table 4.3 Group analysis - behavioural and physiological responses to heel lance

SEMs are given in parentheses.

4.3.3 The facial expression score and cortical response are well correlated

A subset of infants, whose demographic characteristics are described in described in Table 4.2, had their responses to heel lance simultaneously assessed using NIRS and the PIPP (N=33). Similar NIRS results were found to those presented in Chapter 2; an increase in the total haemoglobin concentration in the contralateral somatosensory cortex was observed following a heel lance in 30 out of the 33 studies. As the PIPP score and the haemodynamic activity were assessed simultaneously, it was possible to establish the degree of correlation between the magnitude of the haemodynamic response and the components that make up the PIPP.

Following a heel lance the relationship between the facial expression score and the maximum change in the haemodynamic response in the contralateral somatosensory cortex was established. This is illustrated in Figure 4.3. The highest facial expression scores were observed in the infants with the highest level of cortical activity. Regression analysis, used to estimate the facial expression score as a function of the cortical response gives a regression coefficient = 0.773μmol/L, 95% CI limits: 0.45482, 1.091μmol/L, demonstrating a high degree of correlation between the cortical response and the facial expression score.

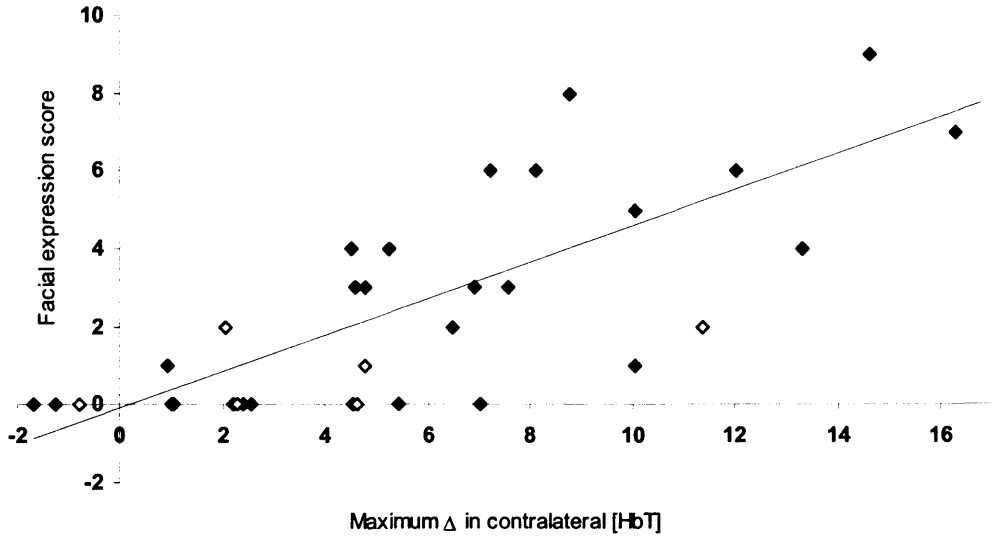


Figure 4.3 Relationship between facial expression score and haemodynamic response

Relationship between the facial expression score and the maximum Δ [HbT] in the contralateral somatosensory cortex. The open diamonds represent an infant with intraventricular haemorrhage (IVH).

4.3.4 The haemodynamic and autonomic responses are weakly correlated

The maximum increase in heart rate and the maximum decrease in oxygen saturation are the two indicators in the PIPP score that assess the physiological response to noxious stimulation. Figure 4.4 shows that the maximum change in [HbT] in the contralateral somatosensory cortex, weakly correlates with the physiological score calculated from the sum of the two physiological indicators (regression coefficient = 0.45μmol/L, 95% CI limits: 0.05, 1.92μmol/L). This demonstrates that the physiological and autonomic changes do not always parallel the cortical response following noxious stimulation.

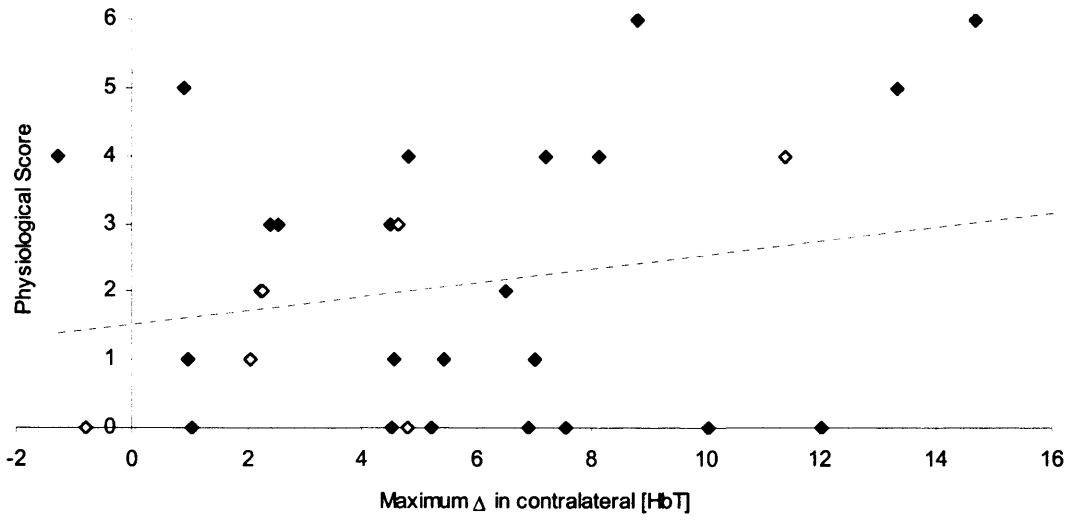


Figure 4.4 Relationship between the physiological and haemodynamic activity

Relationship between maximum Δ[HbT] and the physiological response following noxious stimulation. The open diamonds represent an infant with intraventricular haemorrhage (IVH).

4.3.5 PIPP weighting factors do not improve the correlation with the cortical response

Figure 4.5 shows the relationship between the total PIPP score and the maximum change in [HbT] in the contralateral somatosensory cortex. Although the haemodynamic response results from the functional activation of the somatosensory cortex, and the PIPP score uses physiological and behavioural indicators to assess the pain response, there is a strong correlation between the measures (r = 0.716μmol/L, 95% CI limits: 0.318, 1.114μmol/L).

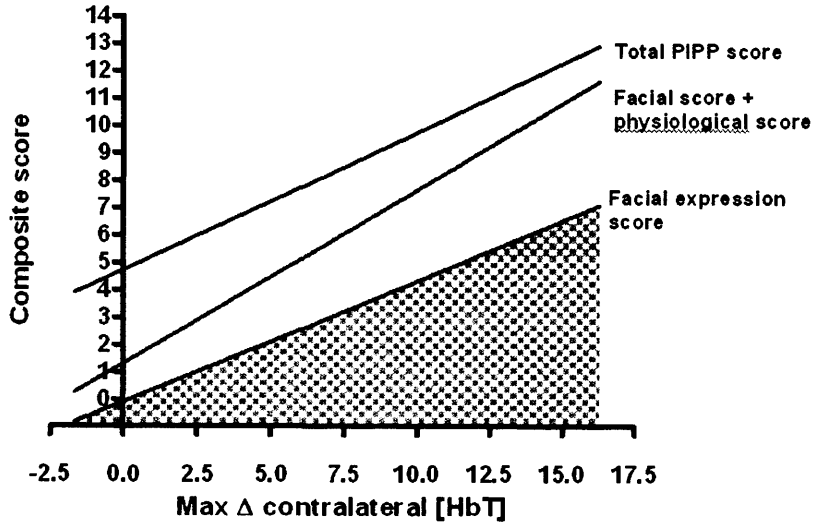


Figure 4.5 Assessment of the components which contribute towards the PIPP score

Relationship between maximum Δ[HbT] and individual components of the PIPP score. The lines represent the regression lines for different components of the PIPP score. The filled areas represent the various contributions of the different components of the PIPP score.

In addition, Figure 4.5 also shows the correlation between the haemodynamic response and the facial expression score, and the correlation between the haemodynamic response and the unweighted PIPP score. Figure 4.5 demonstrates how the addition of the different components of the PIPP influences the degree of correlation with the haemodynamic cortical response. Regression analysis shows that the regression values for the total PIPP score (r = 0.716μmol/L, 95% CI limits: 0.318, 1.114μmol/L) and total facial expression score (r = 0.773μmol/L, 95% CI limits: 0.454, 1.091μmol/L) are similar, which implies that the weighting factors, for sleep state and gestational age, combined with the physiological score does not improve the correlation with the cortical response. When the physiological components are added to the facial expression score, the degree of correlation is improved (r = 0.870μmol/L, 95% CI limits: 0.149, 1.888μmol/L), however, when the weighting factors are also added this improvement is lost. This arises because the youngest sleeping infants tend to have the smallest haemodynamic responses and it is these infants whose scores are weighted most heavily.

4.3.6 'Facial non-responders' still mount a cortical response to noxious stimulation

On 33 test occasions, 12 infants had their PIPP score and haemodynamic response simultaneously measured in response to a heel lance. According to the PIPP score, infants did

not display a change in facial expression on 13 test occasions and therefore had a facial expression score of zero; however, most of these infants demonstrated a robust haemodynamic response. Figure 4.6 shows the maximum change in [HbT] in the contralateral somatosensory cortex in the 13 individual infants who were classified as 'facial non-responders'. The maximum change from the pre-stimulus baseline ranged from -1.68 to 7.00μmol/L (see Figure 4.6). Although there is a good overall correlation between the cortical activity and the facial expression (see Figure 4.5), it cannot be assumed that if an individual infant does not display a facial response that there is no underlying cortical activity. It is possible to mount a cortical response to a noxious event without displaying a change in facial activity, therefore an infant classified as a 'facial non-responder' may still process the noxious information at the cortical level. The infants who did not display a change in facial expression were not different in terms of their clinical assessment. In fact, as infants were studied on a number of test occasions some of the infants included in Figure 4.6 displayed changes in facial expression on other test occasions; therefore the lack of facial response observed in these infants does not arise because the infants are intrinsically unable to mount a coordinated facial response.

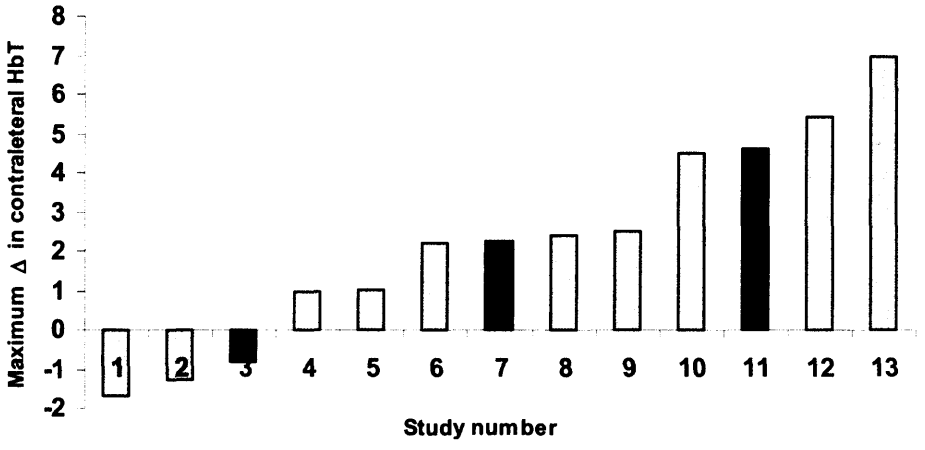


Figure 4.6 Cortical responses for infants classified as 'facial non-responders'

Bar chart showing contralateral maximum Δ [HbT] in the somatosensory cortex following heel lance in individuals classified as facial non-responders. The infant with intraventricular haemorrhage (IVH) is identified in black.

4.3.7 Latency to first facial response

The latency to the first facial expression response following a heel lance is plotted as a function of PMA in Figure 4.7. In the age range, from 25-43 weeks PMA, regression analysis used to estimate the latency to change in facial expression as a function of PMA, demonstrates that the younger infants have a longer latency to response than the older infants (regression coefficient =

-0.70 μmol/L/week, 95% CI limits: -1.08, -0.31μmol/L/week). The latency ranged from 1-21 seconds; however, all five infants under the age of 31 weeks PMA had a latency to facial expression response greater than 10 seconds (see Figure 4.7). This range is a similar order of magnitude to the results presented in chapter 3, where the latency to the peak haemodynamic response was also observed to be longer in the younger infants (see Section 3.3.4).

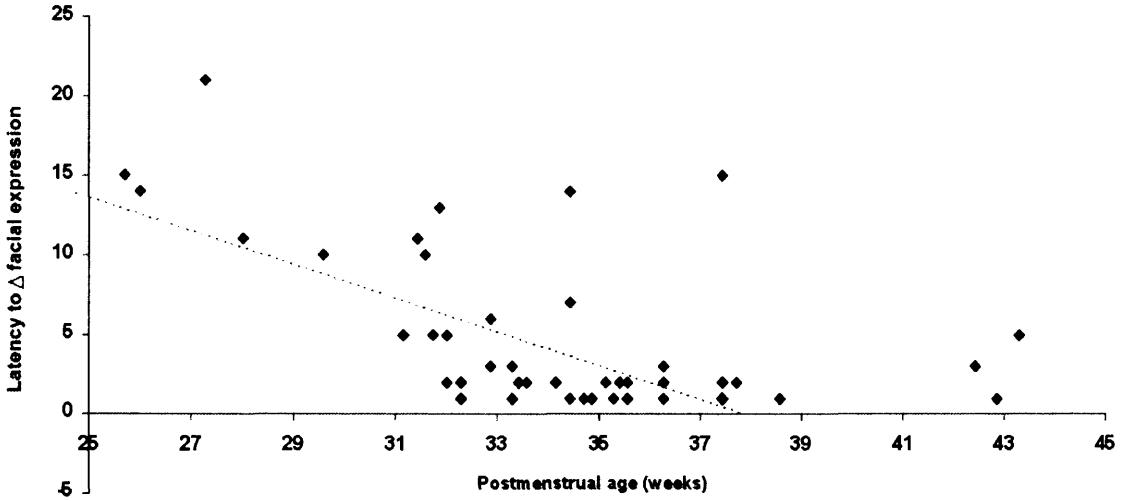


Figure 4.7 Latency to facial expression change

Latency to change in facial expression plotted against PMA. (Regression coefficients = -0.70μmol/L/week, 95% CI limits: -1.08, -0.31μmol/L/week). Regression line represents infants aged 25-38 weeks PMA where there is a linear dependency on PMA.

4.4 Discussion

4.4.1 A comparison of cortical responses and clinical behavioural assessment

The PIPP is the most commonly used quantitative method for assessing neonatal pain. In the present study, heel lancing produced PIPP scores similar to those previously reported (Stevens et al., 1996). Concurrent measures of the behavioural, physiological and cortical responses to noxious stimulation allowed us to break down the PIPP score and examine the relationship between the cortical haemodynamic response and the individual components that contribute to the PIPP. This provided the opportunity to see how various neonatal pain assessment measures, which include change in facial expression and physiological activity, correlate with the cortical haemodynamic activity.

Regression analysis demonstrates that following a noxious event, change in facial expression is the component of the PIPP that correlates best with the level of cortical activity in the contralateral somatosensory cortex. A weaker correlation was observed between the physiological response and the cortical haemodynamic response. Previous studies have also shown that following noxious stimulation change in facial expression is the most specific and consistent response (Grunau et al., 1998; Grunau and Craig, 1987) and it has also been reported that the behavioural and physiological indicators of pain are not highly correlated with one another (Johnston et al., 1995). The correlations that have been observed are likely to have arisen partly because of the shared underlying neural mechanisms. For example, nociceptive pathways originating from the spinal cord dorsal horn directly activate brain structures, such as the medullary and midbrain reticular formation, and deep layers of the superior colliculus that are involved in motor organisation, as well as activation of the autonomic nervous system. Multiple ascending pathways project to several brainstem and cortical regions and individual neurones often project in more than one of these pathways (Price, 2000). The spinothalamic pathway, which relays nociceptive information to the somatosensory region via nuclei of the thalamus, converges on the same limbic and subcortical structures accessed by other ascending spinal pathways involved in the behavioural and autonomic responses. This suggests that the physiological responses controlled by the sympathetic nervous system, which functions for the most part at a subconscious level and involves a limited amount of cognition, may not be the most suitable surrogate measure of higher-level pain processing.

The arbitrary weighting factors included in the PIPP may not improve how well the PIPP score represents perceived pain intensity. The weighting factors result in an increased pain score for the younger infants and those who are asleep. If, as has been shown in adults, activity in the somatosensory cortex in infants correlates with perceived pain intensity then it is possible that the reduced level of cortical activation observed in these infants arises because the level of pain perception is in fact lower. The fact that infants also demonstrate an attenuated facial response when they are asleep, may occur because they actually perceive less pain. Weighting factors were initially introduced to ensure that pain in the youngest infants was not underestimated. While this may be useful in a clinical setting, arbitrarily increasing the pain score in these infants does not tell us anything about how these infants actually respond to noxious stimulation nor does it provide a better measure of pain processing.

4.4.2 Infants classified as 'facial non-responders' display cortical activity

The premature infant pain profile results in a high percentage of preterm infants being classified as 'facial non-responders' but it is not clear that these infants are truly pain free. Clear facial grimacing, universally recognised as an expression of pain (Craig et al., 1993), was observed following heel lancing; however, in 43% of studies a change in facial expression was not observed. 'Facial non-responders' refer to infants who achieved a zero facial expression score according to the PIPP. Infants who do not mount a behavioural or physiological response to noxious stimulation are of particular clinical interest as these infants are at risk of having their pain underestimated. While noxious stimulation generally evokes parallel cortical and behavioural responses in infants, pain may be processed at the cortical level without producing detectable behavioural or physiological changes. The ability for an infant to display a change in facial expression in response to a noxious stimulus requires that motor neurone activity is sufficiently coordinated to produce a visible set of facial muscle contractions. This relies on a complex set of interactions at the spinal or brainstem level. Our study has demonstrated that if an infant does not demonstrate a change in facial expression, it cannot be assumed that the stimulus does not reach the neonatal cortex. It is possible that while the noxious information is being transmitted to the higher-levels of the central nervous system, behavioural observations do not reflect this process. As a result, pain assessment based on behavioural tools alone should be interpreted with caution. Infants who do not display a change in facial expression may still process pain at higher levels of the cortex.

The physiological and behavioural measures assessed using the PIPP rely on spinal, brainstem and autonomic responses. Responses that rely on sensorimotor reflex circuitry, which is

developmentally regulated, may not respond in a manner that is directly proportional to the transmitted noxious sensory information. Furthermore, this input-output relationship may alter with age, further distorting the true nociceptive processing that is being assessed. For example, it has been shown that non-noxious stimulation in preterm infants can produce reflex limb withdrawal, while the same stimulus fails to produce any haemodynamic cortical response (Slater et al., 2006b). It has also been suggested that the facial activity evoked by heel lance is blunted in the youngest infants (Johnston et al., 1993) while the magnitude and spread of motor activity in the limbs is greater than that in older infants (Andrews and Fitzgerald, 2000; Andrews and Fitzgerald, 1999). These factors explain the complex relationship between the different measures in the PIPP and the haemodynamic response. The relationship between spinal, brainstem and cortical responses to noxious stimulation could be tested by measuring the flexion reflex electromyography (EMG), changes in facial expression and cortical responses, in response to both noxious and non-noxious events. It would be of interest to test whether the EMG summary-parameters change with age and stimulus type along the same trajectories as the summary-parameters used to characterise the cortically evoked response. It would also be beneficial to establish whether reflex EMG responses can occur independently of a cortical response and vice versa. Results from studies like these could inform us about the relationship between spinal reflexes and facial action coding relative to the cortical responses to noxious stimulation.

The percentage of infants classified as 'facial non-responders' in this study is high compared with previous reports. Another study that used the PIPP to assess pain responses following heel lancing found that 20% of infants did not demonstrate a facial or physiological response (Johnston et al., 1999). A more detailed study of change in facial expression, which used the full neonatal facial coding system (NFCS), found that during a heel lance 22% of infants had no change in eye squeeze, 27% had no change in brow bulge, and 35% did not demonstrate nasolabial furrow (Grunau et al., 1998). The high percentage of 'facial non responders' in this study can also be explained by a number of factors. Previous studies have reported that squeezing the foot is more stressful (Lindh et al., 1999) and painful (Grunau et al., 1998) than the actual heel lance. In these studies, the foot was not squeezed during the recording period so it is possible that the overall heel lance procedure was less painful. In addition, to reduce the number of studies affected by movement artefact a number of steps were taken to ensure that the babies were settled. Positioning techniques, which included side lying and swaddling were used and particular care was taken to expose only the foot that was to be lanced. As a result, this allowed the babies to maintain a stable state; often an asleep infant would not wake up as a result

of the procedure. The high percentage of infants that did not demonstrate a behavioural response to the heel lance may have arisen due to the developmentally-sensitive approach used when handling the infants (Als, 1998). 80% of infants in this study were classified as asleep. Previous work has shown that infants who are asleep have a smaller cortical response following a noxious procedure (Slater et al., 2006b). Lower pain responses have also been observed using behavioural indicators (Johnston et al., 1999). It was to account for this observation that a weighting factor was included in the PIPP to increase the score for sleeping infants. The intention was to prevent sleeping infants from having their pain underestimated.

4.4.3 Latency to change in facial expression is greater in younger infants

The youngest infants have the longest latency to change in facial expression. In this study the youngest infant did not display a facial expression response until 21 seconds after the initial stimulus. According to the PIPP the infant would not have shown a response for at least 60% of the observation period. As a result, there is a risk that pain in extremely preterm infants is being underestimated and under treated. Previous studies, which have used a limited post-stimulus response period, may have come to the erroneous conclusion that the youngest infants display little or no response following noxious stimulation. These findings were predicted from the NIRS results, where latency to peak response was also longest in the youngest infants (Slater et al., 2006b). The long latencies are attributed to low conduction velocities and slow synaptic responses in the nociceptive circuitry. As described in Section 4.4.1, shared ascending pathways transmit information to the cortex and to nuclei that initiate the motor response. It is therefore not surprising that if responses are observed to be slower in one system then they are also likely to be slower in others. This study has shown that it takes longer for the youngest infants to produce a coordinated facial response to nociception. These data provide further evidence to suggest that pain scores calculated for the most premature infants should be used cautiously.

CHAPTER 5: RESULTS PART 3

Nociceptive-Specific Event Related Potentials in the Preterm Infant

5.1 Introduction

5.1.1 Chapter overview

In Chapter 3 it was shown, using near-infrared spectroscopy, that cortical haemodynamic responses to heel lance can be observed in infants from 25 weeks PMA (Slater et al., 2006b). The rationale for measuring the haemodynamic response was based on the assumption that the haemodynamic activity is driven by underlying cortical electrical events that result in an increase in energy demand and subsequent increase in blood flow (Logothetis and Wandell, 2004). A more direct measure of neuronal activity can be achieved from the study of somatosensory evoked potentials. The aim of this chapter is to establish whether noxious stimulation can evoke electrical activity in somatosensory cortical neurones that can be measured and characterised. In this study, we recorded electroencephalography (EEG) activity following noxious stimulation in premature infants, and analysed the traces for nociceptive-specific responses. It was postulated that the neonatal EEG would be characterised by periods of quiescence and that because a noxious event would have a large impact on the nervous system it may be possible, on a single trial basis, to observe activity evoked by a noxious event over and above the background EEG. As the hypothesis is based on findings from a single noxious event, it is essential to establish whether repeated stimulation in the same infant generates reproducible responses and whether comparable responses can be observed between infants. In addition, careful consideration needs to be given to how the heel lance will be time-locked to the EEG recording. The aim was to produce the first ever, direct recordings of cortical electrical activity in human preterm infants following noxious sensory input.

5.2 Materials and Methods

5.2.1 Participating infants

Studies were successfully performed on 33 occasions in 21 infants aged 28-42 weeks postmenstrual age (PMA) when heel lances were performed for routine blood tests. Eleven studies were rejected due to movement artefact. Table 5.1 details the demographic characteristics of the infants in this study.

Demographic characterisation of the neonatal population (EEG study)	
Mean gestational age at birth (weeks), n=21	30.9 (4.3); range, 24.1-40.1
Mean PMA at time of study (weeks), n=33	35.4 (3.3); range, 28.0-42.9
Mean postnatal age at time of study (days), n=33	33.9 (32.6); range, 2-128
Mean birth weight (g), n=21	1580.0 (783.2); range, 533-3330
Mean weight at time of study (g), n=21	2041.4 (787.2); range, 899-4184
No. of multiple gestation infants, n=33	4
No. of infants with chronic lung disease, n=21	3
Mean no. of days on mechanical ventilator, n=21	6.43 (13.59); range, 0-51
Mean no. of test occasions, n=21	1.6 (0.81); range, 1-4
Mean no. of days between consecutive studies, n=21	4.2 (10.0); range, 0-34

Table 5.1 Demographic characterisation of the neonatal population (EEG study)
Standard deviations are given in parentheses.

5.2.2 Design and development of time-locked trigger system

To undertake these studies, it was necessary to use a novel approach to time lock the heel lance to the EEG recording. Only by time locking the stimulus to the recording would it be possible to observe activity associated with the noxious event. The equipment had to be designed in such a manner that it did not interfere with the clinical requirements of the infant and it complied with the protocols currently in use on the unit. The heel lance device normally used for obtaining blood samples is a sterile disposable automatic incision device (Tenderfoot, International Technidyne Corporation). The lancet houses a spring-loaded surgical steel blade that is released when a small switch on the surface of the device is depressed. When the device is activated, the blade swings in an arc motion so that for a short time the blade protrudes from the device such that it can make an incision in the superficial layers of skin. After the blade is released, it automatically and permanently retracts. The smallest lancet (Tenderfoot Preemie) produces a standardised wound with a depth of 0.85mm and a length of 1.75mm.

A number of designs to time lock the release of the blade to the EEG recording equipment were considered. One example was to attach a small microphone to the surface of the lancet. The release of the spring-loaded blade is accompanied by an auditory cue that could be detected by a microphone. Due to the noisy nature of the neonatal environment, the likelihood that the system would not detect the auditory cue or would trigger in response to other sounds was too high; this design was not pursued. An alternative approach was to attach an accelerometer (K-shear accel., Kistler Instruments Ltd) to the superior surface of the heel lancet using Blu-Tack. An accelerometer can detect the vibration generated by the release of the blade and the output can be sent to a recording device. The system could be designed such that it only triggered once a certain threshold of activity was detected. If the received signal does not match a stored electrical template then the system will not trigger the EEG recording to start. This design was eventually used and an engineer at Great Ormond Street Children's Hospital built an in-house system suitable for clinical use (Worley et al., 2006). A block diagram of the trigger circuit is given in Appendix 6 (Figure 5.16).

5.2.3 Electrodes and skin preparation

Surface electrodes (Ambu Neuroline disposable cup electrodes) were placed on the scalp using the international 10-20 EEG electrode placement system (Jasper, 1958). Four recording electrodes were positioned over the somatosensory cortex; recording electrodes were located at Cz, CPz, CP3 and CP4. Reference and ground were placed at Fz and on the forehead respectively. If access was difficult, the reference electrode was placed at POz. Electrode positions are shown in Figure 5.1. To reduce the electrode/skin impedance each electrode site was prepared by rubbing the skin with an abrasive EEG prepping gel (NuPrep gel D.O.Weaver and Co). 'Nu-prep' is the skin preparation gel in use at Great Ormond Street Hospital and has been shown to be safe for use with neonates. The gel was applied with a cotton wool bud in order to abrade the smallest area possible. Conductive EEG paste (Elefix Nihon Kohden, Corp) was used to optimise contact with the electrodes and improve conductance. The electrodes were secured to the scalp using adhesive tape (mefix Mölnlycke Health Care AB). Once the recording electrodes were in place, leads were coiled and tied together to minimise electrical interference. Electrode impedance was assessed to be acceptable by visually inspecting the EEG traces. High impedances were corrected by re-abrasing the skin and re-applying the electrode or by completely replacing the electrode. Electrode application took approximately 30 minutes. Electrodes were disposed of after each study.

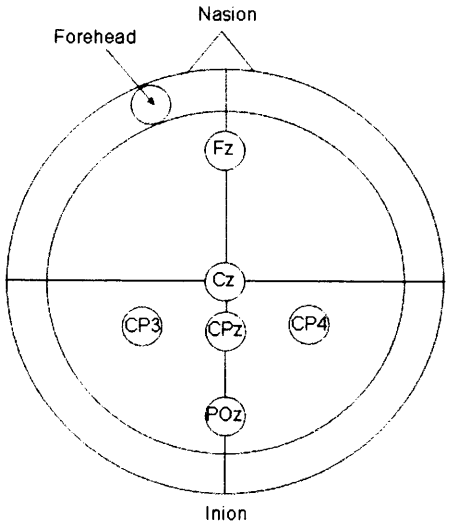


Figure 5.1 Electrode positions

Schematic diagram of electrode positions. Recording electrodes are placed at Cz, CPz, CP3 and CP4. They are referenced to either Fz or POz depending on access to the infant.

5.2.4 EEG recording

EEG activity was recorded using a 4-channel data acquisition unit (Cambridge Electronic Design, micro1401) and an isolated pre-amplifier (Cambridge Electronic Design, 1902). The bandwidth was 0.5-30Hz and the sampling frequency was set at 2 KHz. Data acquisition software (Signal, version 1.82, Cambridge Electronic Design Ltd) was used to record the activity. The release of the blade from the heel lancet (Tenderfoot) electrically triggered the EEG recording and evoked activity was recorded for a 10 second period. A calibration signal was applied at the start of each recording to ensure that the amplitude of the signal was correctly scaled. All equipment conformed to the electrical safety standard for medical devices, IEC-601-1.

In a subset of studies, a tri-axial accelerometer (K-shear accel., Kistler Instruments Ltd) was placed on the infant's head in order to detect movement artefact. In these studies, the recording electrode at position CPz was not used as it was necessary to use one of the recording channels to record the accelerometer output.

5.2.5 Heel lance protocol and event trigger

The heel lance was performed using the methods described in Chapter 2 (Section 2.2.6). In addition, an accelerometer, connected to a trigger circuit, was attached to the superior surface of the heel lancet using Blu-Tack (K-shear accel., Kistler Instruments Ltd). Prior to the heel lance, the lancet was placed on the heel and the system was manually triggered to create a control EEG trace. The aim of the control procedure was to deliver a stimulus where the tactile component was identical to the heel lance but the noxious component not received. Results from all heel lances were included in the analysis. If more than one heel lance was required on the same test occasion both heel lances were included in the analysis.

5.2.6 Combined haemodynamic and electrical monitoring

In two studies, EEG recordings were combined with simultaneous NIRS recordings. The NIRS measurements were performed as described in chapter 3 (Section 3.2.4).

5.2.7 Premature infant pain profile

Infants were videoed throughout the study in order to examine behavioural responses. The PIPP was used to assess infant pain as described in Chapter 4 (Section 4.2.4).

5.2.8 Data analysis

The EEG data was imported into a data analysis package (Clampfit 8.1, Axon Instruments, Inc.). The data was filtered using a 50Hz notch filter to reduce electrical interference and then baseline corrected. The traces were visually inspected and clearly identifiable waveforms above the background EEG were manually characterised. The latency to the negative (N) and positive (P) components were identified independently by two observers. As this was part of an exploratory study, the observers were not blinded to the nature of the recordings. Traces from different infants were compared to see if similar features between the trials could have arisen as a direct result of the noxious event. When waveforms of interest were identified, analysis software was used to determine the latency and amplitude of each of the components of interest (Clampfit 8.1, Axon Instruments, Inc.). Results from each observer were compared. Trials were placed in one of five categories. Classification was dependent on the quality of the EEG recording and whether a waveform of interest could be identified (see Table 5.2). Trials in Categories 3, 4 and 5 were excluded from further analysis. Through an iterative process a set of parameters was established which characterised the components of interest. All the analysis was initially

performed on the contralateral somatosensory cortex (CP3 or CP4). In the trials where a waveform of interest was identified in the contralateral somatosensory cortex, further analysis was then undertaken on the other three channels. The additional channels were analysed using normal averaging techniques.

Classification of EEG traces following heel lance	
Good EEG trace with identifiable waveform	
Good EEG trace with no identifiable waveform	e.g. waveforms cannot be visually identified
Movement artefact	e.g. identified using the accelerometer
Quality of EEG too poor to analyse	e.g. due to electrical interference
Technical failure	e.g. due to trigger failure

Table 5.2 Classification of EEG traces following heel lance

5.2.9 Characterisation of waveforms

The mean response across the cohort of infants was found using normal averaging techniques and a time-shifted averaging approach (Purves and Boyd, 1993). Individual trials following heel lance stimulation were manually assessed and the observed activity was time-shifted so that peaks were aligned to correct for latency jitter. The heel lance and control responses were analysed using the same methods. The latency to the peak N and P response was determined and the amplitude of the components was calculated for each trial.

5.3 Results

5.3.1 Integrated time-locked physiological monitoring

A trigger system was successfully designed to enable a number of physiological responses to be time locked to a noxious event. Each time the system was triggered, an event marker was sent to the pulse oximeter data file, an LED flashed, which was recorded on the video footage and the EEG recording was initiated. The system was also capable of sending an event marker to the NIRS data file. A block diagram of the system is given in Figure 5.2. The main advantage of this trigger system is that the heel lance protocols used in the neonatal unit were not changed when a study was undertaken. Nursing staff can perform heel lances following routine procedures.

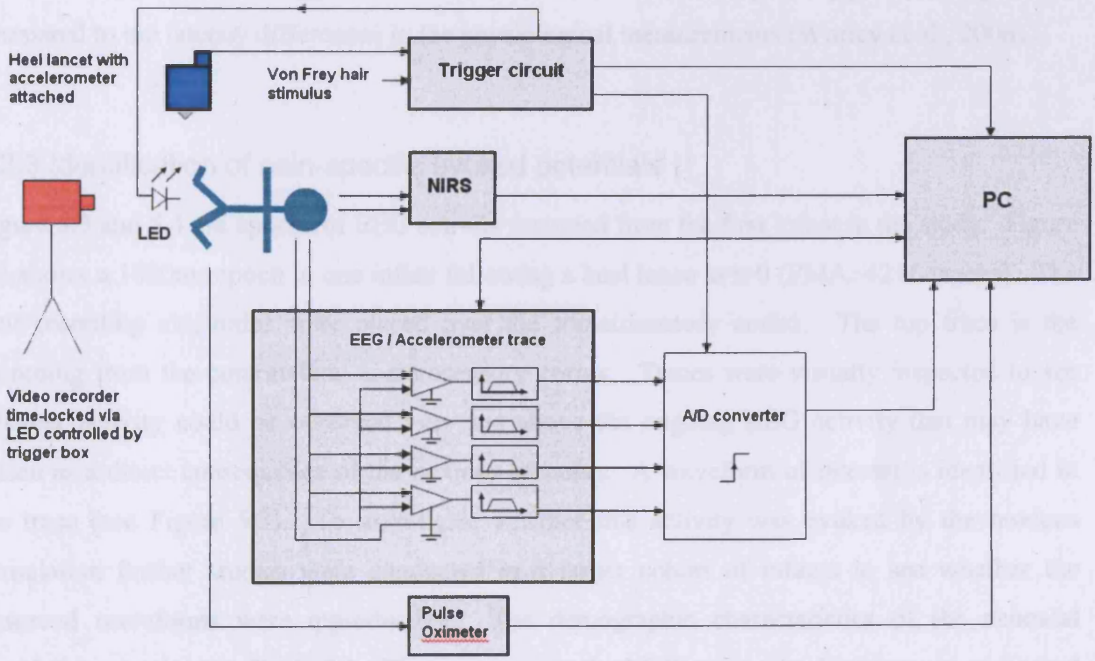


Figure 5.2 System Diagram

Block diagram of recording system.

The system was successfully designed to prevent the trigger from firing following spurious signals, for example, when the heel lancet is handled or when the security tag is released. To date the trigger system has been 100% successful. During all the recording sessions, it has never falsely fired following a spurious event and on the few occasions where the heel lance has not triggered, it was identified that the accelerometer was incorrectly attached to the lancet. Care has to be taken to ensure that the Blu-Tack remains adhesive until the time when the heel lance

is performed. Adhesion can be impaired if the lancet becomes too warm, i.e. when left in an incubator for a long time, or if it is contaminated by solvents used to clean the apparatus.

5.3.2 Variability in trigger timing

The heel lancets used in this study are routinely used on the Neonatal Unit to enable blood samples to be collected for clinical assessment (Tenderfoot, International Technidyne Corporation). Using an infrared optical measuring device, the stimulus duration, which is the duration in which the blade protrudes from the heel lancet, was determined to be $124\pm4.8\mu s$ (N=10). In addition, the time taken from the midpoint of the stimulus duration to the event trigger was calculated to be $104\pm113\mu s$ (N=10) (Worley et al., 2006). These measurements were established to verify that the latency variation between the observed waveforms was not caused by the timing jitter in the trigger circuit. The variation in the latency to trigger was insignificant compared to the latency differences in the physiological measurements (Worley et al., 2006).

5.3.3 Identification of pain-specific evoked potentials

Figure 5.3 and 5.4 are epochs of EEG activity recorded from the first infant in the study. Figure 5.3 shows a 1000ms epoch in one infant following a heel lance at t=0 (PMA: 42+6 weeks). The four recording electrodes were placed over the somatosensory cortex. The top trace is the recording from the contralateral somatosensory cortex. Traces were visually inspected to see whether activity could be observed over and above the ongoing EEG activity that may have arisen as a direct consequence of the noxious stimulus. A waveform of interest is identified in the trace (see Figure 5.3). To investigate whether this activity was evoked by the noxious stimulation further studies were conducted in a larger cohort of infants to see whether the observed waveforms were reproducible. The demographic characteristics of the neonatal population are given in Table 5.1. The responses evoked following a heel lance were compared to responses following a control procedure. In total, 61 attempts were made to record the resultant EEG activity following a clinically required heel lance. After accounting for exclusions due to technical failure and movement artifact, there were 33 successful trials. A summary of the trials that were undertaken is given in Table 5.3.

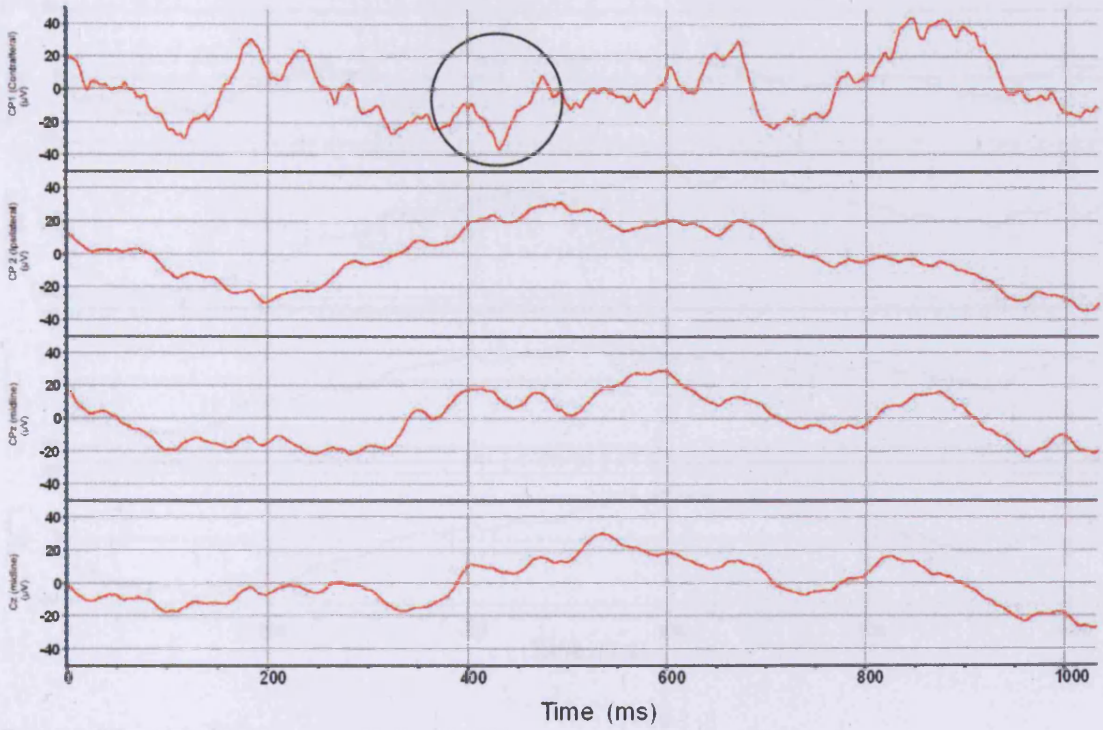


Figure 5.3 Example EEG trace

Four recording electrodes positioned at CP1, CP2, CPz and Cz in a single infant (PMA = 42+6 weeks). Heel lance given at t=0ms. The top trace represents the activity in the contralateral somatosensory cortex (CP1). The activity that has been circled identifies the waveform of interest.

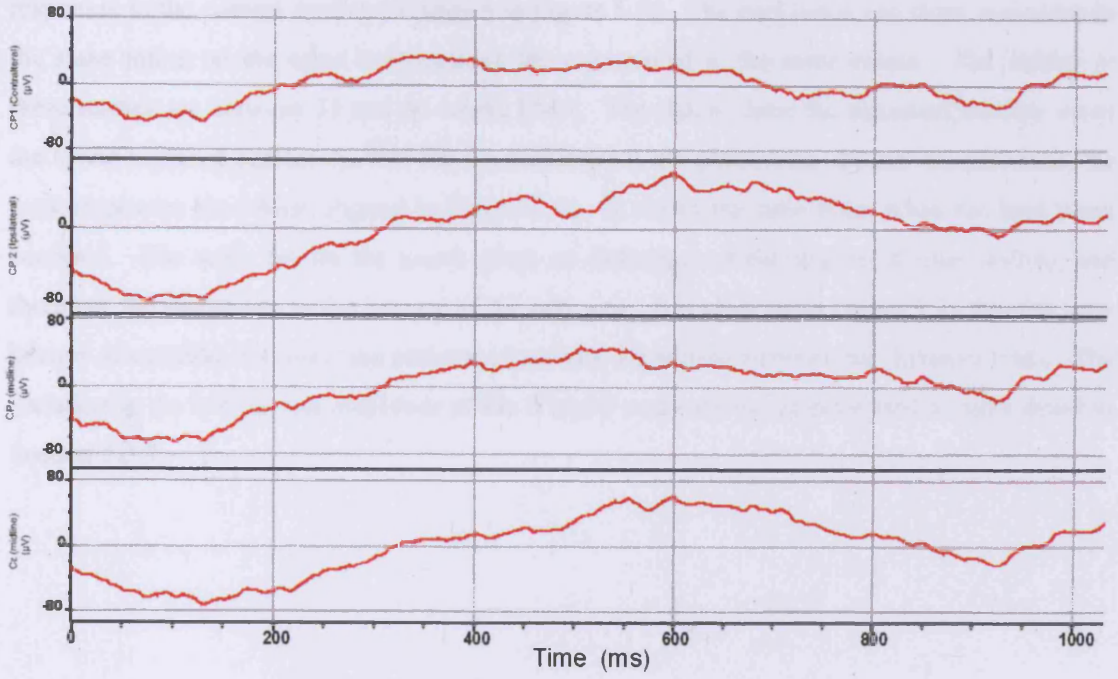


Figure 5.4 Example EEG trace - control response

Four recording electrodes positioned at CP1, CP2, CPz and Cz in a single infant (PMA = 42+6 weeks). The response is recorded when the heel lancet is placed against the surface of the foot but not triggered.

5.3.4 Reproducibility of the evoked response

In 27 out of the 33 studies comparable negative/positive waveforms were observed in response to a single acute noxious event in the contralateral somatosensory cortex (CP3 or CP4 depending on which foot was heel lanced). The N-P complex was visible in 82% of the studies (see Table 5.3). The latency and amplitude of the N and P components for each individual trial are presented in Appendix 7 (Table 5.4) and each of the raw traces is shown in Appendix 8 (Figure 5.17).

Classification of single-trial EEG traces		
Technical failure	20%	(12/61)
Quality of EEG too poor to analyse	8%	(4/49)
Movement artefact	27%	(12/45)
Good EEG trace no identifiable waveform	18%	(6/33)
Good EEG trace with identifiable waveform	82%	(27/33)

Table 5.3 Classification of single-trial EEG traces

The responses to five different heel lance trials are shown in Figure 5.5a and the associated responses in the control studies are shown in Figure 5.5b. The heel lance and sham responses in the same infant on the same test occasion are represented in the same colour. The infants in these studies are between 33 and 36 weeks PMA. The shams show the electrical activity when the lancet is placed against the foot but the heel lance is not performed. To aid identification, the peak responses have been aligned in Figure 5.5a. X marks the time point when the heel lance occurred. The scale bar on the x-axis gives an indication of the degree of time shifting and therefore the variability in the latency of the response. It is clear from Figure 5.5a that the peak latency is variable; however, the patterns of activity are similar between the different trials. The variation in the latency and amplitude of the N and P components are presented in more detail in Section 5.3.7.

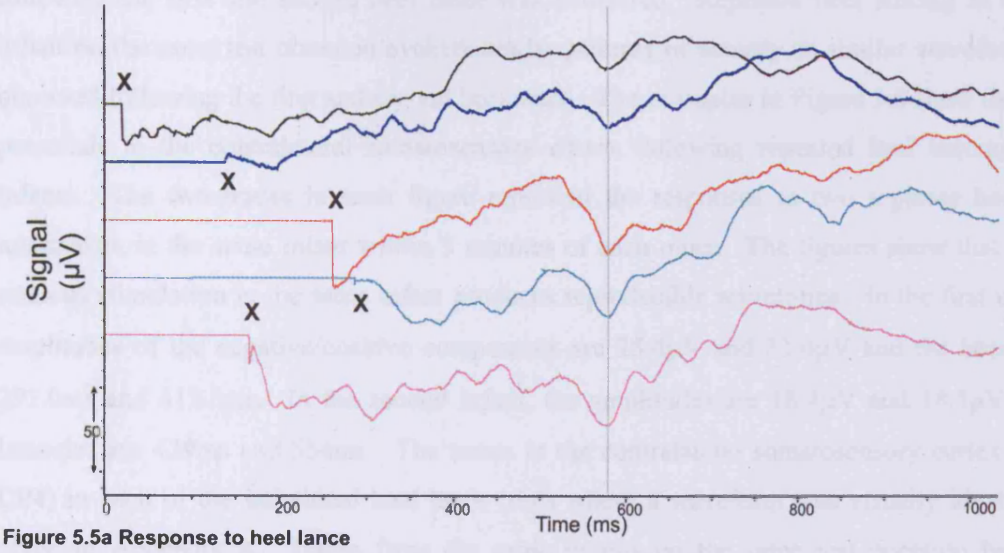


Figure 5.5a Response to heel lance

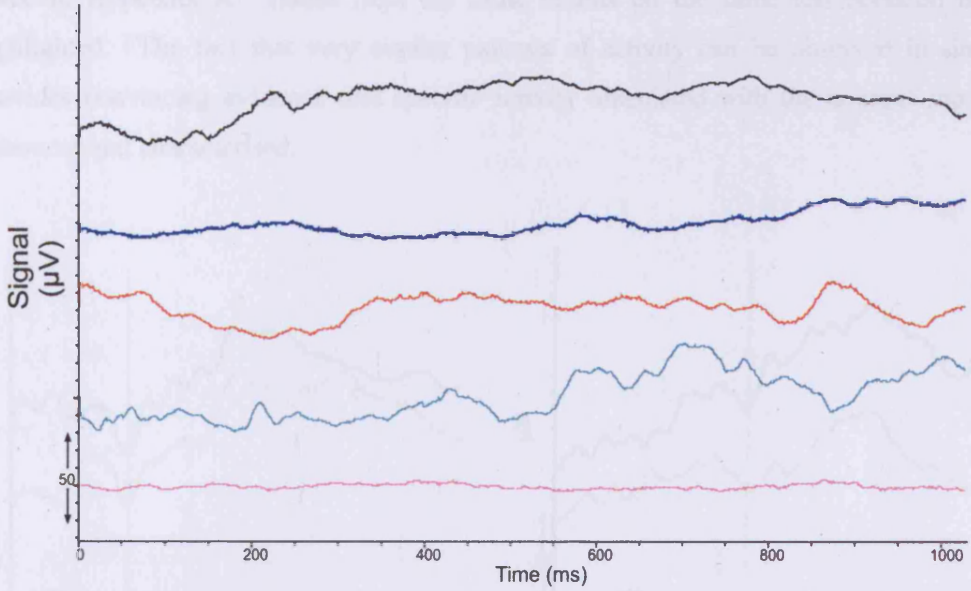


Figure 5.5b Response to control study

Figure 5.5 EEG response following heel lance in five studies

Response to heel lance (Figure 5.5a) and associated control response (Figure 5.5b) in five individual heel lance trials. The matching colours in each figure represent trials undertaken in the same infant on the same test occasion. In Figure 5.5a the peak responses are aligned to aid identification. X marks the point where the heel lance occurs. (Bandwidth 0.5-30Hz. Sampling rate 2000Hz).

5.3.5 Repeated stimulation in the same infant generates reproducible waveforms

Further evidence to support the hypothesis that these potentials are specifically generated in response to the noxious stimulation comes from repeated studies conducted in the same infant. In four cases, in order to get sufficient blood for the required blood sample, two heel lances were

performed in the same infant on the same test occasion. The morphology of the waveforms following the first and second heel lance was compared. Repeated heel lancing in the same infant on the same test occasion evoked similar patterns of activity as similar waveforms were observed following the first and second heel lance. The examples in Figure 5.6 show the evoked potentials in the contralateral somatosensory cortex following repeated heel lancing in two infants. The two traces in each figure represent the responses to two separate heel lances undertaken in the same infant within 5 minutes of each other. The figures show that repeated noxious stimulation in the same infant produces reproducible waveforms. In the first infant the amplitudes of the negative/positive components are 25.4μV and 31.0μV and the latencies are 291.0ms and 412.5ms. In the second infant, the amplitudes are 18.4μV and 18.5μV and the latencies are 439ms and 554ms. The traces in the contralateral somatosensory cortex (CP3 or CP4) in each of the individual heel lance trials where a waveform was visually identified are given in Appendix 8. Traces from the same infants on the same test occasion have been highlighted. The fact that very similar patterns of activity can be observed in single trials provides convincing evidence that specific activity associated with the noxious input can be measured and characterised.

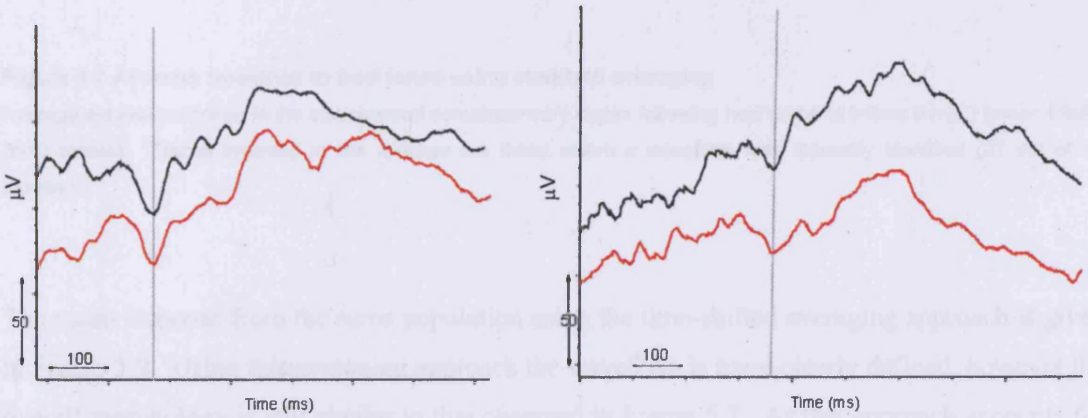


Figure 5.6 Responses to heel lance in the same infant

Evoked potentials in the contralateral somatosensory cortex following heel lance in two infants. The two traces in each figure represent the responses to two separate heel lances undertaken in the same infant within 5 minutes of each other (heel lance 1 is black, heel lance 2 is red). The figure shows that repeated noxious stimulation in the same infant produces reproducible waveforms. (Bandwidth of 0.5-30Hz. Sampling rate 2000Hz.)

5.3.6 Classification of waveforms

Figure 5.7 shows the mean response following a heel lance in the contralateral somatosensory cortex from the 27 single trials where a waveform was visually identified (age range: 31–42

weeks PMA). The activity seen in Figure 5.7 is a direct average and time-shifted averaging techniques have not been used in this analysis. Figure 5.8 shows the mean control response, calculated using the same standard averaging techniques, when the heel lancet was placed against the foot but not triggered. It is clear from Figure 5.7 that following the heel lance there is activity in the EEG trace that occurs as a direct result of the noxious stimulus, which is not present in the control data presented in Figure 5.8.

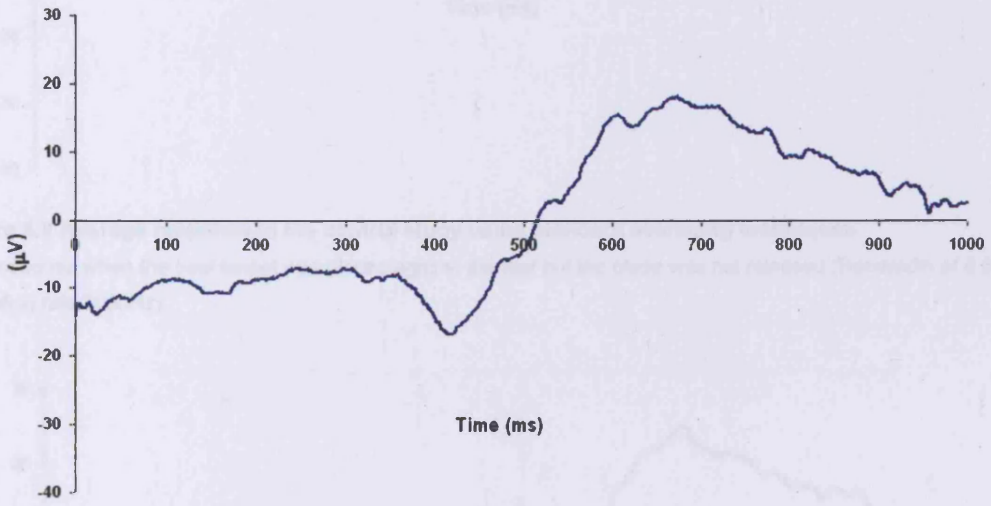


Figure 5.7 Average response to heel lance using standard averaging

Average evoked response in the contralateral somatosensory cortex following heel lance at t=0ms (N=27) (mean PMA= 35+3 weeks). Traces included in the average are those where a waveform was manually identified (27 out of 33 studies).

The mean response from the same population using the time-shifted averaging approach is given in Figure 5.9. Using this averaging approach the waveform is more clearly defined, however the overall morphology is still similar to that observed in Figure 5.7. As this approach accounts for the latency variation between trials, it produces an average waveform that is a better representation of the waveforms in the individual trials.

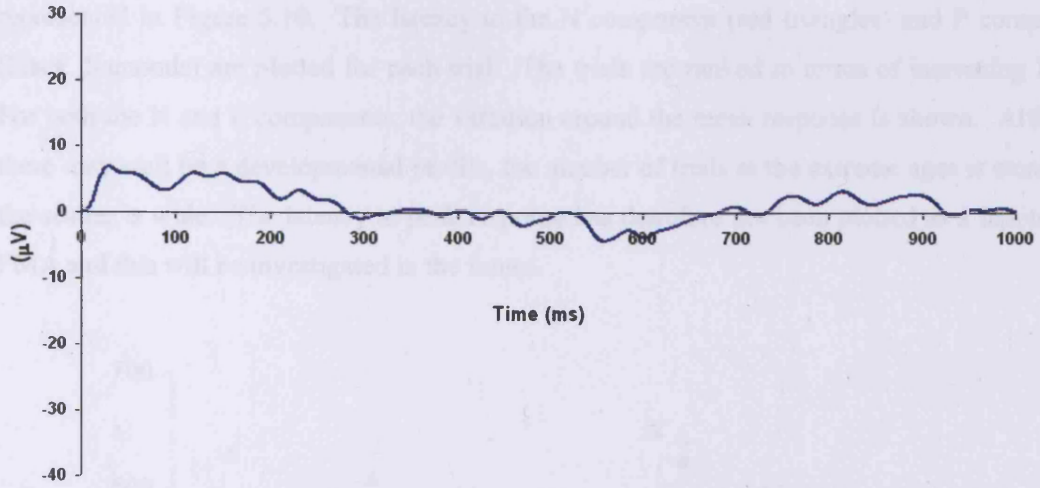


Figure 5.8 Average response in the control study using standard averaging techniques

The response when the heel lancet was placed against the foot but the blade was not released (Bandwidth of 0.5-50Hz. Sampling rate 2000Hz).

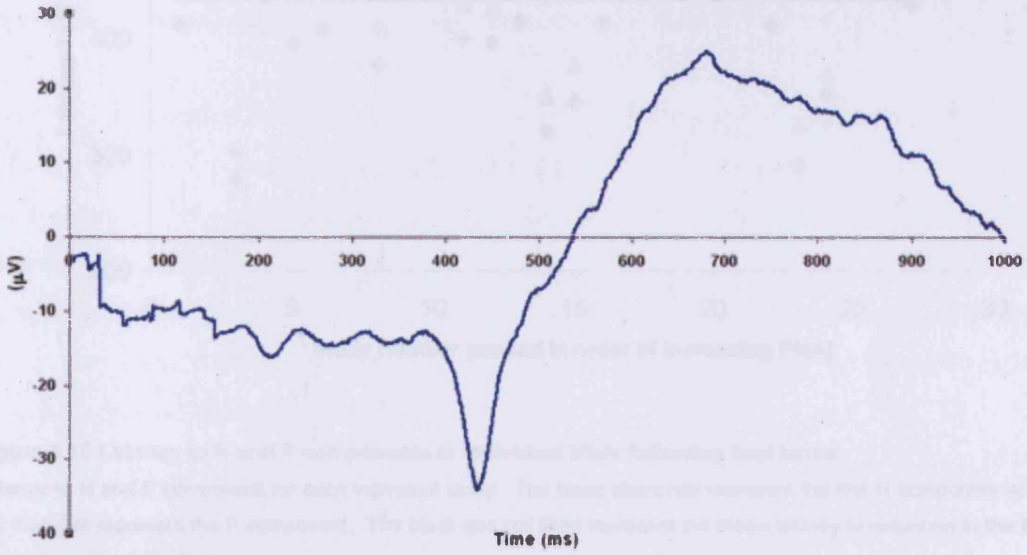


Figure 5.9 Average response using time-shifted averaging

Average evoked response in the contralateral somatosensory cortex following heel lance at t=0ms (N=27) (mean PMA= 35+3 weeks). The trace is averaged around the peak negative component in each single trial. The discontinuities in the trace between 0-200ms arise when time-shifted traces are appended to the average (Bandwidth of 0.5-50Hz. Sampling rate 2000Hz).

5.3.7 Variability in pain-specific evoked potentials

There is considerable variation in the latency to response in the individual trials. The latency of the N component ranges from 279-625ms and the latency of the P component ranges from 306-

646ms (N=27, PMA range 31-42 weeks). The degree of latency scatter between trials is represented in Figure 5.10. The latency to the N component (red triangles) and P component (black diamonds) are plotted for each trial. The trials are ranked in terms of increasing PMA. For both the N and P components, the variation around the mean response is shown. Although there may well be a developmental profile, the number of trials at the extreme ages is small and the scatter is wide. The latency to peak response has therefore not been plotted as a function of PMA and this will be investigated in the future.

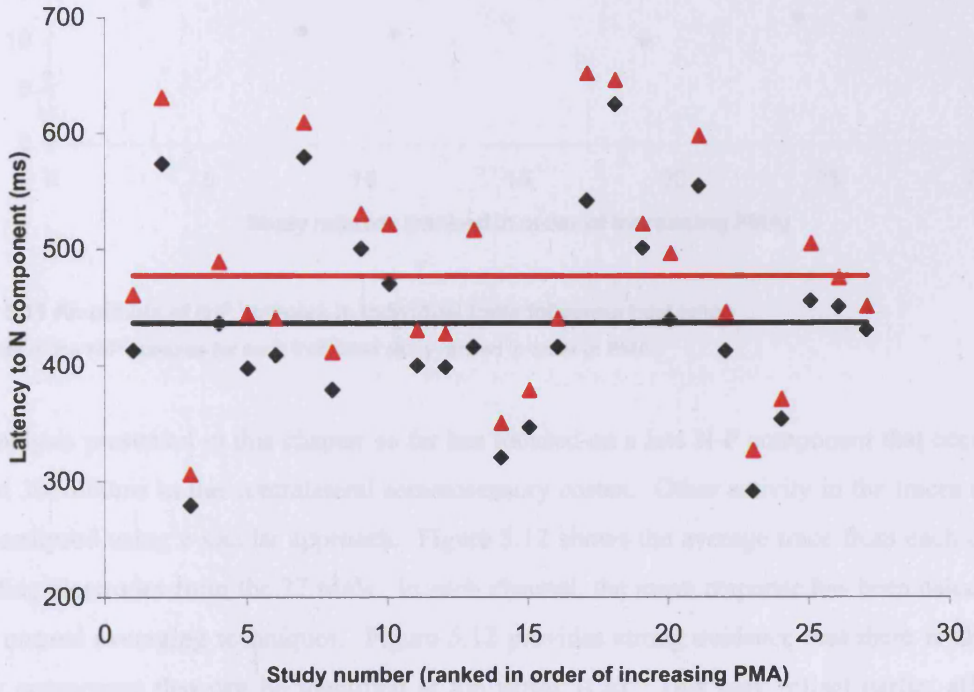


Figure 5.10 Latency to N and P components in individual trials following heel lance

Latency to N and P component for each individual study. The black diamonds represent the first N component and the red triangles represent the P component. The black and red lines represent the mean latency to response to the N and P components respectively.

Figure 5.11 shows the amplitude of the N-P complex plotted for each individual trial. The amplitude of the N-P complex ranges from 10-48μV, with a mean amplitude of 24.4μV. In this figure, the trials have also been ranked in terms of PMA; however, a more detailed analysis of the relationship between amplitude and PMA has not yet been undertaken. Further analysis of this data may enable the factors that contribute to the variation in the size of response, such as sleep state, PMA and previous exposure to painful procedures to be determined.

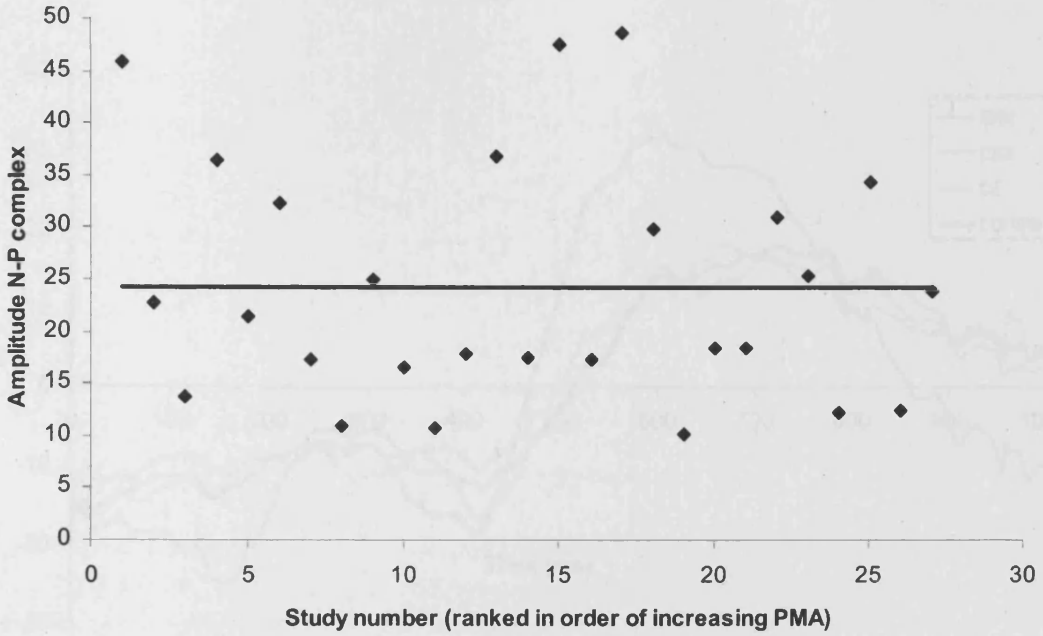


Figure 5.11 Amplitude of N-P complex in individual trails following heel lance

Amplitude of the N-P complex for each individual study ranked in order of PMA.

The analysis presented in this chapter so far has focused on a late N-P component that occurs at around 300-600ms in the contralateral somatosensory cortex. Other activity in the traces needs to be analysed using a similar approach. Figure 5.12 shows the average trace from each of the recording electrodes from the 27 trials. In each channel, the mean response has been calculated using normal averaging techniques. Figure 5.12 provides strong evidence that there is also an earlier component that can be identified at the vertex (Cz). This may reflect earlier afferent input and needs to be investigated further.

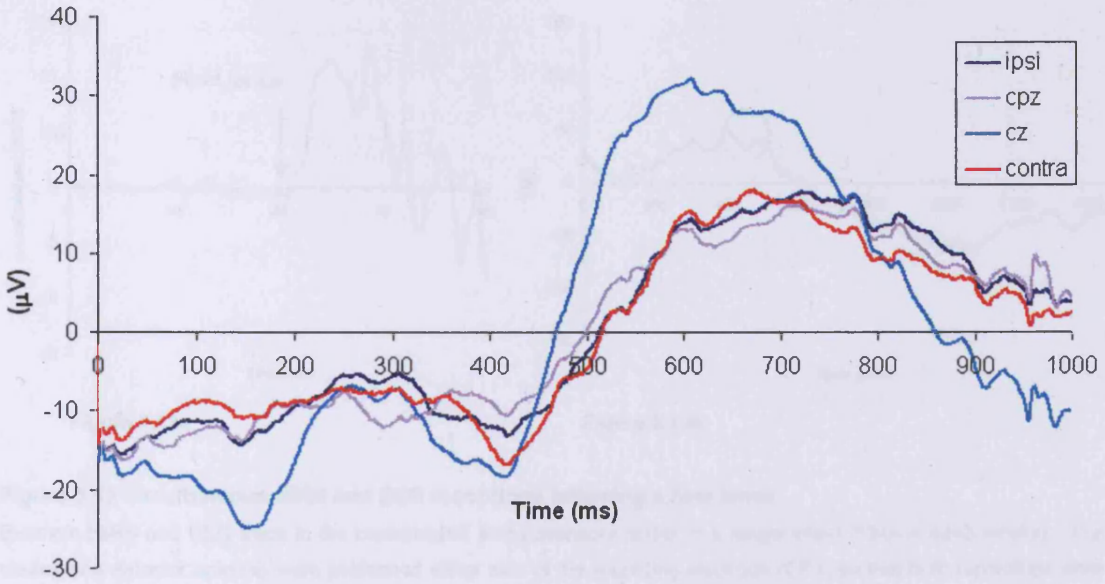


Figure 5.12 Average response from four recording electrodes following a heel lance

Average evoked response in four recording electrodes placed over the somatosensory cortex following heel lance at t=0ms (N=27) (mean PMA= 35+3 weeks). Trials included in the average are those where a waveform was manually identified in the contralateral somatosensory cortex (CP3 or CP4).

5.3.8 Combined NIRS and EEG recordings

Figure 5.13 shows the haemodynamic and electrical response to a heel lance in a single infant on one test occasion when both NIRS and EEG activity were simultaneously measured. To date combined NIRS and EEG recordings have been successfully recorded in two infants. The preliminary data demonstrates that it is technically feasible to record the time-locked haemodynamic and electrical activity following a heel lance in preterm infants (Slater et al., 2006c).

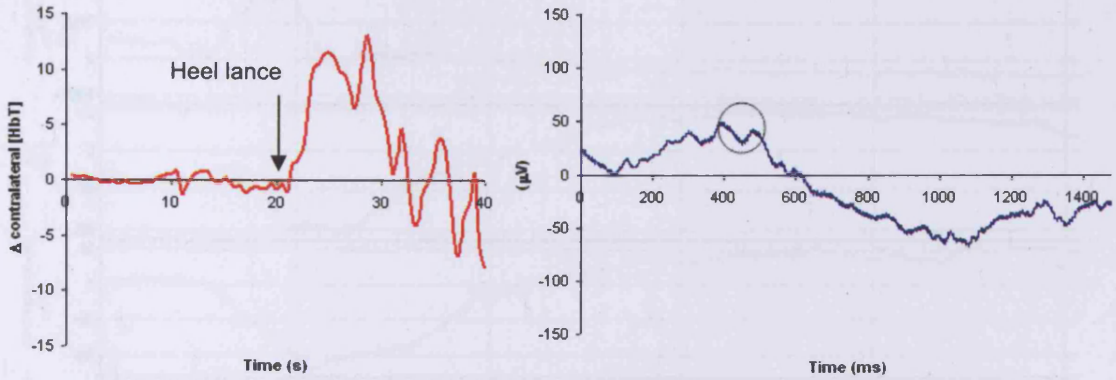


Figure 5.13a

Figure 5.13b

Figure 5.13 Simultaneous NIRS and EEG recordings following a heel lance

Example NIRS and EEG trace in the contralateral somatosensory cortex in a single infant (PMA = 42+3 weeks). The source and detector optodes were positioned either side of the recording electrode (CP1) so that both recordings were made from the same area of the brain. Figure 5.13a shows the NIRS recording (heel lance occurred at t=20 s) and Figure 5.13b shows the evoked EEG activity (heel lance occurs at t=0ms). The waveform of interest has been circled in Figure 5.13b.

5.3.9 Control for movement artefact

To ensure that the observed activity did not occur because of movement artefact, a subset of studies was undertaken where an accelerometer was placed on the forehead of each infant. The accelerometer trace could be compared to the recorded EEG activity. When movement artefact was visible in the accelerometer trace the study was excluded from further analysis. An example trace is given in Figure 5.14. The top trace shows the movement artefact recorded by the accelerometer. Movement can clearly be observed in the trace between 100 and 300ms. The resultant activity in the other channels can also be observed.

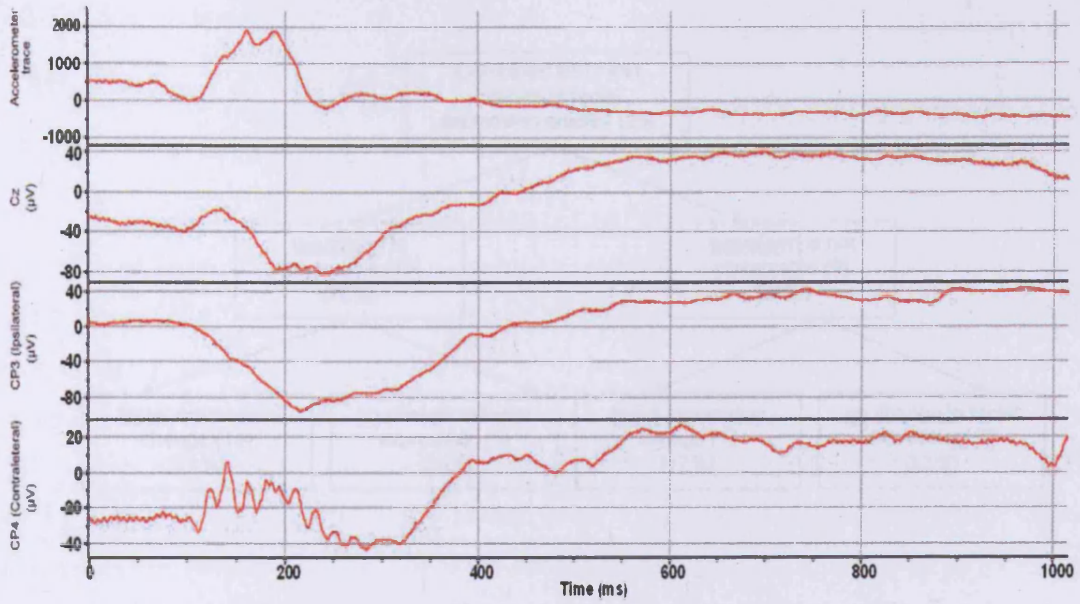


Figure 5.14 The effect of movement artefact

An accelerometer trace and the EEG traces from three recording electrodes positioned at Cz, CP3 and CP4 in a single infant. Heel lance given at t=0ms. The top trace represents the head movement recorded by an accelerometer.

5.3.10 Correlation of EEG responses and behavioural responses

The average PIPP score across all trials was 7.6 $\pm$ 4.6 (see chapter 4 for further details regarding how this was calculated). The range of the PIPP score in individual trials was 1-17. Although the data is preliminary, it is of interest to note that of the 6 infants where a waveform was not identified in the contralateral somatosensory cortex, 5 also did not display a change in facial expression. By comparison, of the 19 studies where a waveform was identified, only 5 infants did not demonstrate a change in facial expression. This is represented in the tree diagram in Figure 5.15. The number of infants in this analysis is lower than the number of infants in the total population because the facial expression score was not determined for every infant when an EEG study was performed.

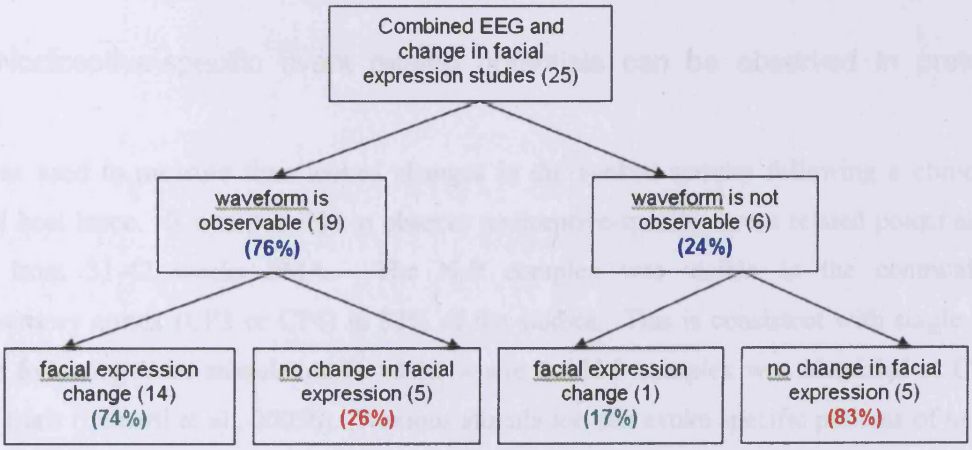


Figure 5.15 Facial expression change and the presence of an observable waveform

Tree diagram to show the relationship between change in facial expression and the presence of an observable waveform in the contralateral somatosensory cortex.

5.4 Discussion

5.4.1 Nociceptive-specific event related potentials can be observed in preterm infants

EEG was used to measure time locked changes in the evoked activity following a clinically required heel lance. It was possible to observe nociceptive-specific event related potentials in infants from 31-42 weeks PMA. The N-P complex was visible in the contralateral somatosensory cortex (CP3 or CP4) in 82% of the studies. This is consistent with single-trial analysis following laser stimulation in adults where the N-P complex was identified at Cz in 79% of trials (Iannetti et al., 2005b). Noxious stimulation can evoke specific patterns of neural activity within the cortex of preterm and term infants. Individual traces from different infants show similar patterns of activity, and when the stimulus is repeated in the same infant on the same test occasion, identical waveforms were produced in some cases. The study provides further evidence that infants undergoing intensive care process painful experiences at the cortical level. The study suggests that from 31 weeks the sensory pathways are sufficiently developed to transmit noxious information to the preterm infant cortex.

The observed activity has striking similarities to that observed in adults following laser stimulation (Iannetti et al., 2005b). In the infants where a waveform was identified the latency of the N component ranges from 279-625ms and the latency of the P component ranges from 306-646ms. Although there is considerable variability in the latency of the response it is in keeping with that seen in adults following laser stimulation (Iannetti et al., 2005b). In a single adult subject, Iannetti and his co-authors showed that the variability in the latency to the peak response was 214-250ms. In an earlier study by Purves and Boyd (1993), a latency variation of +/-60ms was observed in LEP trials performed in different adults. The latency variation between trials observed in these studies is greater in infants than it is in adults. Given that the heel lance stimulus is less easily controlled than LEPs, is undertaken on infants of different developmental ages and in different behavioural states, and that the stimulus cannot be repeated in the same infant for the purpose of the study, it seems acceptable that there is more variation between trials. In addition, as the neuroanatomical pathways are less developed in the neonatal population it is likely that this will also result in more latency variation between individual trials.

The latency of these components is longer in infants compared to adults. This is consistent with other studies in premature infants where electrical stimulation has been used to evoke SEPs

(Smit et al., 1999; Taylor et al., 1996; Klimach and Cooke, 1988). As described in the previous chapters, the long latency is thought to reflect the immaturity of the nervous system (Andrews and Fitzgerald, 1999). Infants in the neonatal unit range from 22 to 45 weeks PMA. Based on the SEP studies in infants (Smit et al., 1999; Taylor et al., 1996; Klimach and Cooke, 1988) and the work presented in Chapter 3 (Section 3.3.4) and Chapter 4 (Section 4.3.6) of this thesis, it is hypothesised that the latency to response will be age dependent over this age range. To confirm whether the developmental trends are similar to those observed in the haemodynamic activity it would be necessary to study more infants at the extreme ages. This is particularly important because of the large latency variation between trials. The developmental profile could easily be masked by the latency jitter if too few infants were included in the analysis.

5.4.2 Identification and analysis of evoked potentials

The standard technique used to observe stimulus-evoked activity is to average the response across multiple trials. For example, the SEP studies that have been conducted in neonates, described in detail in Section 1.2.12, use repeated electrical stimulation to increase the signal to noise ratio. Using this method the signal of interest can be observed over and above the ongoing background EEG activity. This type of analysis is based on the assumption that the evoked activity is time-locked to the stimulus and that the ongoing EEG activity can be treated as noise. There are a number of reasons why using this approach alone was not ideal in this study. For ethical reasons the noxious stimuli cannot be repeated in the same subject to improve the signal to noise ratio. Therefore, an average response would have to be taken from a single infant at various stages during development or from a cohort of different infants who were the same developmental age. Both these approaches are potentially problematic because of the wide latency jitter that has been observed between trials. Even if the stimulus is repeated on the same infant on the same test occasion, long-latency evoked potentials, generated by a nociceptive input, will exhibit marked latency jitter between trials (Iannetti et al., 2005b). This is attributed to the slow conduction velocity of the primary sensory neurones and the intrinsic variation in latency that is associated with cognitive processing.

Despite the latency variation between trials, it was still possible to use these normal averaging techniques to see evidence of the time-locked activity. The waveforms of interest were not completely masked by the averaging process even if some aliasing does occur. As the latency to response varies considerably between trials, it is possible that standard averaging techniques could distort the waveform and cause a significant reduction in peak amplitude. An approximation of average latency may also be misleading. In this study comparable activity

could be observed between trials, hence it was possible to characterise the responses using a time-shifted averaging approach, similar to that used for the analysis of LEPs (Iannetti et al., 2005b; Purves and Boyd, 1993). Time-shifted averaging was first used to analyse LEPs in 1993. Individual trials following laser stimulation were manually assessed and the observed activity was time-shifted so that peaks were aligned to correct for latency jitter (Purves and Boyd, 1993). This method enabled the maximum amount of biological information to be disclosed from single-trial responses. Using this technique, it was possible to compare directly the evoked response in each trial and produce an average response that was a better representation of the individual waveforms. In February 2006 a paper published in Clinical Neurophysiology outlined an automated method to identify the amplitude and latency of LEPs on a single-trial basis using a time-shifted averaging approach (Mayhew et al., 2006). The paper described a method that used multiple linear regression to determine the amplitude and latency of N2 and P2 components following laser stimulation. The authors demonstrated that estimates calculated using the automated approach correlated with corresponding estimates determined manually by a human expert. The automated technique can be used to determine latency and amplitude components from evoked potentials generated from a range of sensory stimuli (Mayhew et al., 2006). The authors suggest that it may be possible to implement this technique to measure the latency and amplitude of evoked potentials generated by a range of other sensory modalities. If the algorithms used in the automated analysis could be used to assess the neonatal response to a heel lance, it would strengthen the case that nociceptive-specific activity is being observed and remove some of bias created when traces are manually analysed.

The range in amplitude of the N-P complex was also wide (10-48μV). In the Iannetti study the variation in amplitude, in a single adult was reported to be 22.9-49.5μV (Iannetti et al., 2005b). Although the amplitude range is similar in both studies, the underlying reasons for these observations may be different. In the neonatal study, the variation in amplitude may be due to factors such as, sleep state, age at time of study or previous pain exposure. The influence of each of these factors can be considered when a larger data set is available. The lower amplitude calculated using standard averaging techniques is explained by the wide latency jitter between trials. As the time-shifted averaging approach is less affected by latency variation, it produces a response that better represents the waveforms produced in the single trials. It is possible that the amplitude of the observed response correlates with perceived pain intensity. If the total number of activated neurones is critical for encoding noxious events and stronger inputs activate more neurones synchronously, then this is likely to result in an increase in the observed amplitude, which could potentially reflect the level of perceived pain. It is also possible that pain

perception may correlate with the latency to the response, but given the intrinsic wide variation in the latency of these components, it is probable that a subtle latency distribution would be masked. In addition, it is important to note that pain perception cannot be directly measured in this population. It would, however, be possible to compare the outcome measures discussed in this chapter with other surrogate measures of nociception, such as change in facial expression and autonomic responses.

Evoked activity observed following noxious stimulation in the neonates might be analogous to LEPs in adults. Despite the salient differences in the latency and topography of the evoked responses, there are clear similarities. It will be beneficial to look at the power spectrum of the neonatal evoked potentials to establish whether it is similar to that reported in LEP studies. In addition, it would also be useful to compare the power spectrum of the neonatal evoked potentials to that of the long slow transient potentials that have also been observed in the neonatal period (Vanhatalo et al., 2005). These spontaneous slow activity transients are characterised by a large voltage deflection, with an amplitude of up to 800μV and a maximum rate of 8/min. This would provide further evidence to suggest that the potentials, which arise following nociceptive input in the neonate, do not represent spontaneous transient responses that only occurs during this developmental period. Vanahtalo et al., (2005) suggest that the spontaneous slow activity transients, confined to the neonatal period, terminate in parallel with the maturation of functional GABAergic inhibition. In this thesis, the small number of studies conducted in infants that are post term suggest that the activity is still present at this age. This provides further evidence that the potentials are specific responses to the noxious stimulation, which do not represent transient developmental activity.

Numerous LEP studies in adults have confirmed that the largest scalp signal is a late negative-positive complex (N2-P2) with maximal amplitude at Cz. The bilateral operculoinsular and the contralateral somatosensory areas has been shown to generate the N2 component (Ohara et al., 2004; Frot et al., 1999; Tarkka and Treede, 1993) and the cingulate gyrus has been shown to generate the P2 component (Iannetti et al., 2003; Tarkka and Treede, 1993). An earlier negative component has also been characterised in the adult studies, as a lateralised response originating from the operculoinsular cortex (Vogel et al., 2003; Tarkka and Treede, 1993). In the neonatal studies, two distinct responses have also been observed. The first occurs at approximately 150ms and is maximum at the vertex (Cz) and the second occurs at approximately 450ms and is maximum in the contralateral somatosensory cortex. The difference in the location of the maximum cortical responses may be due to differences in the type of stimulation, or perhaps,

due to differences in how the immature nervous system processes pain at the cortical level. Most studies that have used LEP have activated the dorsal surface of the hand. It is plausible that activation of the heel may have a different topographic representation. In order to establish the source generators of the potentials in the neonatal studies, and to compare these studies directly with LEP studies, more electrodes need to be placed on the surface of the head. Even if technical difficulties means that it is not possible to achieve full head coverage, it is particularly important to have additional electrodes placed over the temporal regions.

The majority of the analysis conducted so far has focused on the late cortical component that is most well defined in the contralateral somatosensory cortex. In the initial analysis, the late negative component was the most easily identified potential. This may be due to the separate source generators that are thought to drive the N and P components. The earlier activity may represent the initial afferent input. It is expected that latency jitter between trials will not be as wide in the earlier components; however, the amplitude of these components may be smaller and more difficult to observe in the single trial analysis. It would be particularly useful to analyse results in subsets of infants who are the same gestational age in order to control for developmental changes in the observed waveform. Studies of this type will enable us to establish whether a developmental profile can be observed. In addition, further work needs to be done to assess the relationship between changes in facial expression and the presence or absence of the described waveforms.

5.4.3 Limitations of the study

One of the major limitations of this study is that the EEG activity was assessed using only four recording electrodes. As this was an exploratory study, it was decided that the available electrodes would be placed over the somatosensory region of the cortex to give the best possible chance of recording the evoked activity. This meant that full coverage of the head was not possible and prevented activity in other areas of the brain from being investigated. The limited topography meant that no electrodes were placed over indifferent areas of the skull. Future studies need to be performed using a high-resolution EEG/EP recording system with more recording electrodes. It would then be possible to look at the voltage distribution over the scalp and confirm whether the observed waveforms are specifically located in the somatosensory region of the cortex. With the appropriate computational analysis, the neuroanatomical loci of these processes could be identified. More recording channels would also facilitate the time-locked recording of other measures. For example, an accelerometer could be included in all studies to detect movement artefact without the disadvantage of losing a recording electrode.

These studies provide pilot data on which further work can be based. The studies were designed to establish whether it is possible to record EEG activity in neonates that occurs as a direct result of a noxious event. Based on the findings, future experiments can be hypothesis driven and designed to answer specific questions about the cortical processing of pain during early development. Further studies and other stimulation paradigms are discussed in more detail in Chapter 6. The data strongly suggest that it may be possible to observe nociceptive-specific event related potentials in premature infants following a single noxious event. These responses need to be carefully characterised to optimise the recording parameters and to determine the underlying neurological processes that are driving this activity.

5.4.4 Simultaneous recording of haemodynamic and electrical cortical processing

It is technically feasible to use EEG and NIRS to measure pain-related activity in the cortex of premature infants. Simultaneous collection of EEG and NIRS data on a single-trial basis allows paired comparisons to be made between the two measures. By using both these techniques on the same test occasion, a further insight into how the premature infant brain processes pain at the higher cortical levels can be achieved. It is valuable to undertake these measures simultaneously because the techniques measure different aspects of cortical processing. While it is clear that both EEG and NIRS are measures of stimulus-evoked activity, it is important to remember that the aspect of the neural activity being captured by each technique is completely different (Villringer, 1997). EEG measures time-locked activity over a short time-interval whereas NIRS integrates both time-locked and non-time-locked activity and detects longer lasting changes in haemoglobin concentration. It appears that the latencies and amplitudes of waveforms are not affected by the combined recordings; although more combined studies need to be undertaken to confirm this. In adults studies, it has been shown that simultaneous use of fMRI and EEG does not affect the quality of the recordings (Iannetti et al., 2005a).

The experimental set-up enables a number of different recording modalities to be used to investigate the response to noxious stimulation in premature and term infants. Measuring the time-locked haemodynamic, electrical, physiological and behavioural responses to an acutely painful procedure provides an excellent experimental paradigm to further our understanding of the evoked activity that occurs during this early stage of development. This integrative approach provides an opportunity to assess the effect of pain on the immature nervous system. Using EEG to measure the electrically evoked activity has demonstrated that reproducible waveforms can be observed as a specific consequence of activation of nociceptors (Slater et al., 2006d). Further work will establish how the electrical evoked activity is related to the haemodynamic

activity and confirm whether there is an observable change in amplitude or latency with increasing PMA.

CHAPTER 6: CONCLUSION

6.1.1 Do premature and term infants process noxious stimuli at the cortical level?

One of the aims of this thesis was to address the question of whether premature infants can mount a cortical response following noxious stimulation. The studies presented in this thesis demonstrate that infants, from 25 weeks PMA, can mount a cortical haemodynamic response in the contralateral somatosensory cortex following a heel lance, which can be recorded using near-infrared spectroscopy (Slater et al., 2006b). By comparing the responses to non-noxious tactile stimulation, the specificity of the response to the noxious event has been confirmed. The magnitude of the response increases with postmenstrual age and is modulated by sleep state. Infants who are awake display a greater cortical response than those who are asleep. These studies confirm that noxious procedures performed in infants can activate cortical structures.

The premature infant pain profile (PIPP) scoring system was used to assess how the behavioural and physiological responses correlated with cortical response to noxious stimulation. There is a strong correlation between the magnitude of the haemodynamic response and change in facial expression, whereas, there is a weaker correlation with change in heart rate and oxygen saturation. Despite the strong correlation observed between the facial expression change and the haemodynamic activity, care needs to be taken when interpreting these findings. In some studies, infants demonstrated a clear cortical response following a heel lance but did not display any change in facial expression. Although these infants did not display a behavioural response that could be observed by an onlooker, they were still processing the noxious stimuli at the cortical level. As behavioural responses to noxious stimulation are routinely used to assess pain in premature infants, it is useful that the limitations of using facial expression as the sole pain scoring system have been identified.

The underlying cortical electrical activity has also been considered. EEG recordings following a heel lance were analysed and the existence of clearly identifiable nociceptive-specific event related potentials was recognised. Noxious stimulation can evoke specific patterns of neural activity within the cortex of preterm and term infants. While the EEG data demonstrates that it is possible to record nociceptive-specific brain responses following a single noxious event, these responses need to be carefully characterised.

6.1.2 Further work

The summary measures used to characterise the haemodynamic and electrical activity are latency to peak response, onset latency, response duration and peak amplitude. In order to characterise the responses more carefully, further analysis of this data should include the within-subject and between-subject variability for each of these measures. Careful characterisation of the cortical electrical responses, which have been observed following noxious events, is essential if these measures are to be used as a measure of higher-level pain processing. This is particularly important for the evoked potential data because it may be possible to use this measure to test whether cortical responses to noxious and non-noxious stimuli are affected by a variety of analgesic regimes. Simultaneous studies using NIRS and EEG could assess the relationship between the haemodynamic and electrical activity evoked by both noxious and non-noxious somatosensory stimuli. It may be possible to consider the temporal relationship between the two responses using appropriate modelling methods (King et al., 2003). Characterisation of the responses would be aided if procedures other than a clinically required heel lance could be performed to activate Aδ and C fibres. One possibility is that a transcutaneous electrical stimulator, referred to as a prickle stimulator, could be used to give a time-locked prickle sensation, which is known to activate these fibres (Garnsworthy et al., 1988). If a stimulator of this type could be used, it would be easier to perform longitudinal studies, because the experimental protocol would not be dependent on the clinical needs of the infants.

Further work also needs to be carried out using non-noxious mechanical stimulation to distinguish between the tactile and the noxious component of the response. In principle, this could be tested using standard von Frey hair stimulation; however, in pilot studies and in studies presented in Chapter 2, it was not possible to record a measurable response to von Frey hair stimulation using either EEG or NIRS. This may be because the required number of stimuli, essential to increase the signal to noise ratio, may be unrealistic in this population. This requires further investigation; however, other non-noxious stimulation could also be used. One possible method to activate Aβ fibres would be to place a small vibrating disc in contact with the dorsal surface of the foot. The advantage of studies using non-noxious stimulation is that it may be possible to measure a graded response to varying stimulus strengths. Experiments of this kind would confirm that patterns of neural activity in the human infant somatosensory cortex can be measured using neurophysiological methods, and would provide a clearer picture of the specificity, repeatability and reliability of the responses.

The cortical haemodynamic response to heel lancing increases with increasing postmenstrual age (Slater et al., 2006b). There was no doubt, however, that the two youngest participants in the study, aged 25 weeks PMA, showed a clear cortical haemodynamic response. It would be beneficial to use EEG to measure the nociceptive evoked neural activity in infants of this gestational age because it is in this population that there is least data. To date, the youngest infant in the EEG study was 28 weeks; a recognisable waveform was not observed in this infant but it is not known at this stage whether this is representative of the whole population of this age. Characterising these responses during this early stage of development would further our understanding of the neurobiology of nociceptive processing at this age.

Several studies have shown that reflex responses and biobehavioural markers of nociception increase with repeated heel lancing and other invasive procedures. This suggests that hyperalgesia may be occurring in these infants. It would be of interest to test whether sensitisation from repeated heel lances leads to an increase in evoked cortical responses to both noxious and non-noxious procedures. One method that could be used would be to compare the responses of newborn infants, born between 36-40 weeks gestation, with age-matched premature infants who have already undergone weeks of intensive care. It would be possible to establish whether the mean responses, using each outcome measure, differ between the two groups. The results would confirm whether cortical responses in young infants are sensitised by repeated noxious stimulation, as suggested by other studies (Grunau et al., 2005).

Numerous clinical trials have been undertaken in the neonatal population to investigate how analgesic strategies and nursing interventions can alter 'pain responses' (Boyle et al., 2006; Hall et al., 2005; Gal et al., 2005; Anand et al., 2004). The trials involving the effect of analgesics tend to have complex designs and rely heavily on pain scores as the sole outcome measure (Carbajal et al., 2005). While such trials face substantial challenges in terms of ethical considerations and confounding comorbid conditions, the real problem lies in the inadequate measures of the pain itself. It is not certain whether the outcome measures used in these studies are reliable measures of nociceptive processing. One of the goals in establishing a reliable measure of the cortical response to nociception is to test whether cortical responses, to both noxious and non-noxious stimuli, are significantly affected by current analgesic regimes. Numerous trials that have investigated the effectiveness of sucrose, show statistical differences in pain measures between infants randomised to receive or not to receive sucrose (Boyle et al., 2006; Gal et al., 2005). As a result, sucrose is increasingly being used in neonatal intensive care units (NICU) to treat procedural pain. Despite behavioural evidence that sucrose relieves pain in

young infants (Stevens et al., 2004), it remains controversial, particularly among clinicians who are unsure of its effectiveness, and reluctant to link a sweet taste with a noxious procedure. It would be possible to test the effects of sucrose on the cortical response to noxious stimulation by undertaking a clinical trial, where the endpoint could be the summary measures of the observed nociceptive-evoked potential. Infants could be randomised to receive either water or sucrose, and it could be established whether there is a significant difference in the response in the sucrose and non-sucrose groups.

It may also be possible to measure directly the effect of morphine on the cortical response to nociception. Morphine is the most commonly used analgesic and sedative medication in the NICU. Many ventilated babies are routinely prescribed morphine infusions and, as they become tolerant, the dose often needs to be increased. Morphine infusions are prescribed after surgery or after having suffered a pathological fracture or an extravasation injury. In addition, bolus doses of morphine may be prescribed prior to single painful procedures. The effects of morphine on cortical evoked potentials and haemodynamic activity in response to a heel lance could be tested. It would be possible to analyse whether there is a significant difference between the magnitude of response in infants receiving morphine compared to those who are not. It is important to bear in mind that in the early stages it would not be possible to assign infants at random to a morphine and non-morphine group so we could not definitively attribute differences in the group mean outcome to an analgesic effect. Nevertheless, after taking account of treatment group allocation, studies of this type could provide essential pilot data for future trials where it may be possible to randomised babies to higher or lower doses of morphine.

Most avenues that have been discussed regarding the future areas of research have involved using the nociceptive-specific evoked potential as a quantifiable outcome measure to assess the different aspects of the neurobiology of pain processing. It has not been suggested that NIRS is used, for example, to assess the efficacy of sucrose or morphine. This is because the reproducibility between trials appears to be less consistent. The morphology of the haemodynamic responses is more variable and it is more difficult to establish the area of maximum response. Ensuring that optodes are located in the same place between trials is more difficult as compared with locating the correct EEG positions. For these reasons, an analysis of the summary measures of the evoked potential would be more appropriate to answer this kind of question. It would be possible to refine the NIRS measurements if the responses were measured with better anatomical precision. This could be achieved using 2-dimensional mapping systems

(Isobe et al., 2001). The peak response in the somatosensory cortex could be measured directly, which would greatly reduce the between-trial variability.

6.1.3 Concluding remarks

In conclusion, this study describes a number of methods that can be used to further our understanding of pain processing in the neonatal population. By measuring cortical activity following noxious events in preterm infants, it may be possible to further our understanding of pain processing during early development. A better understanding of infant pain processing will pave the way for the development of a systematic approach to reduce pain and improve analgesic strategies in this vulnerable population.

APPENDIX

Appendix 1 Information sheet for parents

University College London Hospitals NHS
NHS Foundation Trust

Neonatal Unit

Information Sheet for Parents

Version 3 April 2005

Babies in hospital are regularly exposed to painful stimuli as part of their routine medical treatment. However, infants are unable to say whether or not it hurts. Therefore other ways need to be used to assess the amount of pain being felt by newborn babies. It is useful to know how pain is being processed because the long term effect of being exposed to pain when you are young is not known. It is possible that babies who experience pain during early development may have an altered response to pain when they are older. This study may offer an insight into the best way to manage pain relief in very young infants.

In this study we would like to use three techniques to assess the way in which babies respond to pain and other sensations. We will only study your baby's response to pain while they are having their routine blood tests. We will not do any extra blood tests for the purpose of the study. Non painful sensations, such as light touch with nylon hairs, vibration or very low level electrical nerve stimulation may also be used.

The first technique will look at the baby's behaviour and will assess how facial expression changes when infants are exposed to pain. Sometimes it would be useful for us to video your baby's face and calculate a pain score after the study is over. We do not plan to keep these video recordings.

The other techniques will monitor brain activity. This will be done using EEG, whereby electrodes are gently placed on the infant's scalp to measure brain waves. EEG is routinely used in the intensive care unit. The third technique is called near-infrared spectroscopy. It has been developed so that sensors placed on the head can detect changes in blood flow and oxygen levels resulting from brain activity.

All the imaging systems are portable and studies can be performed at the cotside. We have used these techniques at the neonatal unit for almost 20 years without any adverse effects.

These tests will be carried out in the neonatal unit while your baby is comfortable in their cot or while being held. If your baby starts to cry we will stop the study. The study will not interfere with the routine clinical care. As we are interested in how your baby's response to pain changes as they grow, we may study your baby on a number of occasions during their stay in hospital.

All proposals for research involving newborn babies are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the joint University College London and UCL Hospitals committees on the ethics of human research. There is no pressure to agree to the study and you are free to withdraw at any time without giving a reason.

Thank you for taking the time to read this sheet.

	Rebeccah Slater	
Consultant Neonatologist	Research Fellow	Research Nurse

Appendix 2 Parent consent form

University College London Hospitals NHS
NHS Foundation Trust

Neonatal Unit

Pain Processing in Neonates

Consent Form
Version 2 March 2004

1. Have you read the information sheet about this study? YES/NO

2. Have you had an opportunity to ask questions about and discuss this study? YES/NO

3. Have you received satisfactory answers to all your questions? YES/NO

4. Have you received enough information about this study? YES/NO

5. Who have you spoken to about this study? .....

6. Do you understand that you are free to withdraw from this study

at any time?	YES/NO
without giving a reason for withdrawing?	YES/NO
without affecting your future medical care?	YES/NO

7. Do you agree to take part in this study?
YES/NO

Signed..... Date.....

Name (block letters) .....

Consent obtained by.....

UCL HOSPITALS

UCL Hospitals is an NHS Trust incorporating the Eastman Dental Hospital, Elizabeth Garrett Anderson Hospital, Hospital for Tropical Diseases, The Middlesex Hospital, National Hospital for Neurology & Neurosurgery and University College Hospital.

Appendix 3 Record of infant data

DATA SHEET

THE DEVELOPMENT OF PAIN PROCESSING IN NEWBRON INFANTS

Study Number	
Date of Birth	
Gestation at Birth	
Type of Delivery	
Sex	
Ethnicity	
Significant Medical/Surgical History	
Cranial Ultrasound Results MRI Yes / No	
Additional Information	
Antenatal Steroids	
Number of days on ventilator Date:	
Date Recruited to Study	

Version 3 / October 2005

Appendix 4 Test occasion record sheet

TEST OCCASION RECORD

THE DEVELOPMENT OF PAIN PROCESSING IN NEWBORN INFANTS

Study Number	
Test Occasion Number	
Date of Study	
Nursery	
Age in Days	
Postmenstrual Age	
Current Weight	
Current Mode of Ventilation	
Number of days on ventilator	
Medication	
Position	
Last Feed	
Significant Prior Handling	
Time commenced:	Time finished:

Study Number:

Cranial Ultrasound Results MRI Yes / No				
Baseline observations before commencing study	Heart Rate Respiratory Rate		Oxygen Saturation	Sleep State Blood pressure
Imaging Study	Spectroscopy		EEG	Video
Stimulus	Von Frey	Electrical	Heel lance	Vibration
Stimulus application site	Foot		Arm	
	Right		Left	
Name of person who did heel lance				

Spectroscopy	NIRO				TOPOGRAPHY	
Optode position – channel 1	Right		Left		Area of cortex	
Optode position – channel 2	Right		Left		Area of cortex	
EEG Lead Placement Electrode Position	1	2	3	4	Ground	Reference
Frame no.						

Appendix 5 Relationship between postnatal age and haemodynamic response

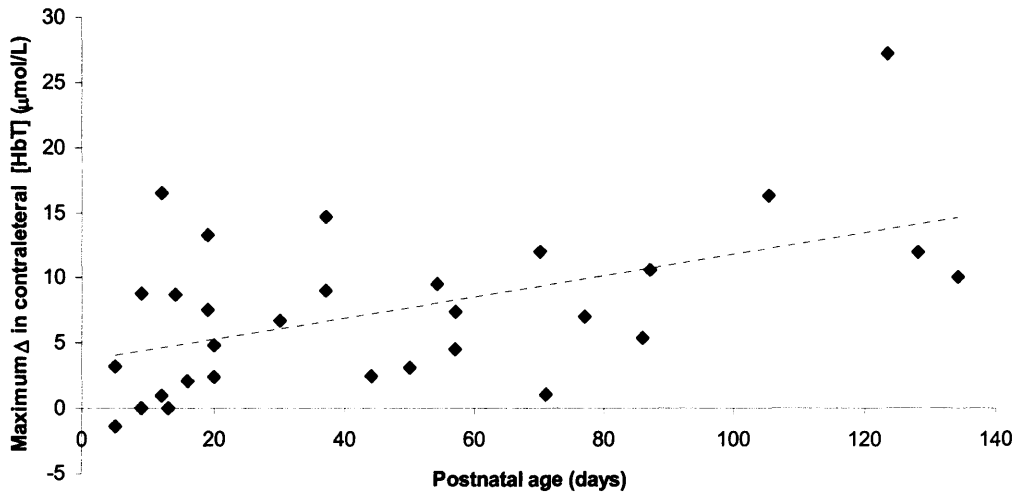


Figure 3.8 Effect of age on haemodynamic response

Developmental profile of maximum Δ[HbT] in contralateral somatosensory cortex following heel lance in infants at different postnatal ages (regression coefficient: 0.073μmol/L/week, 95% CI limits: 0.018, 0.128μmol/L/week). Infants receiving morphine were excluded from the regression analysis.

Appendix 6 Block diagram of trigger circuit

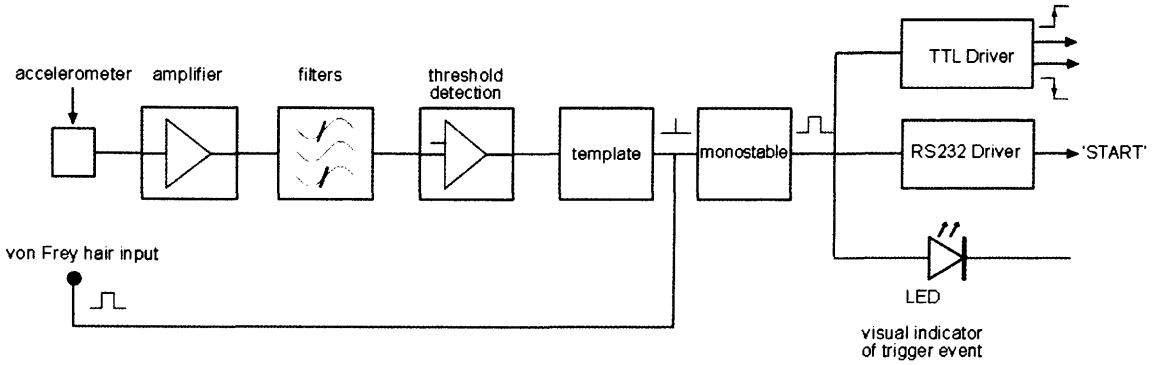


Figure 5.16 Block diagram of trigger circuit

Appendix 7 Analysis of waveform in individual trial

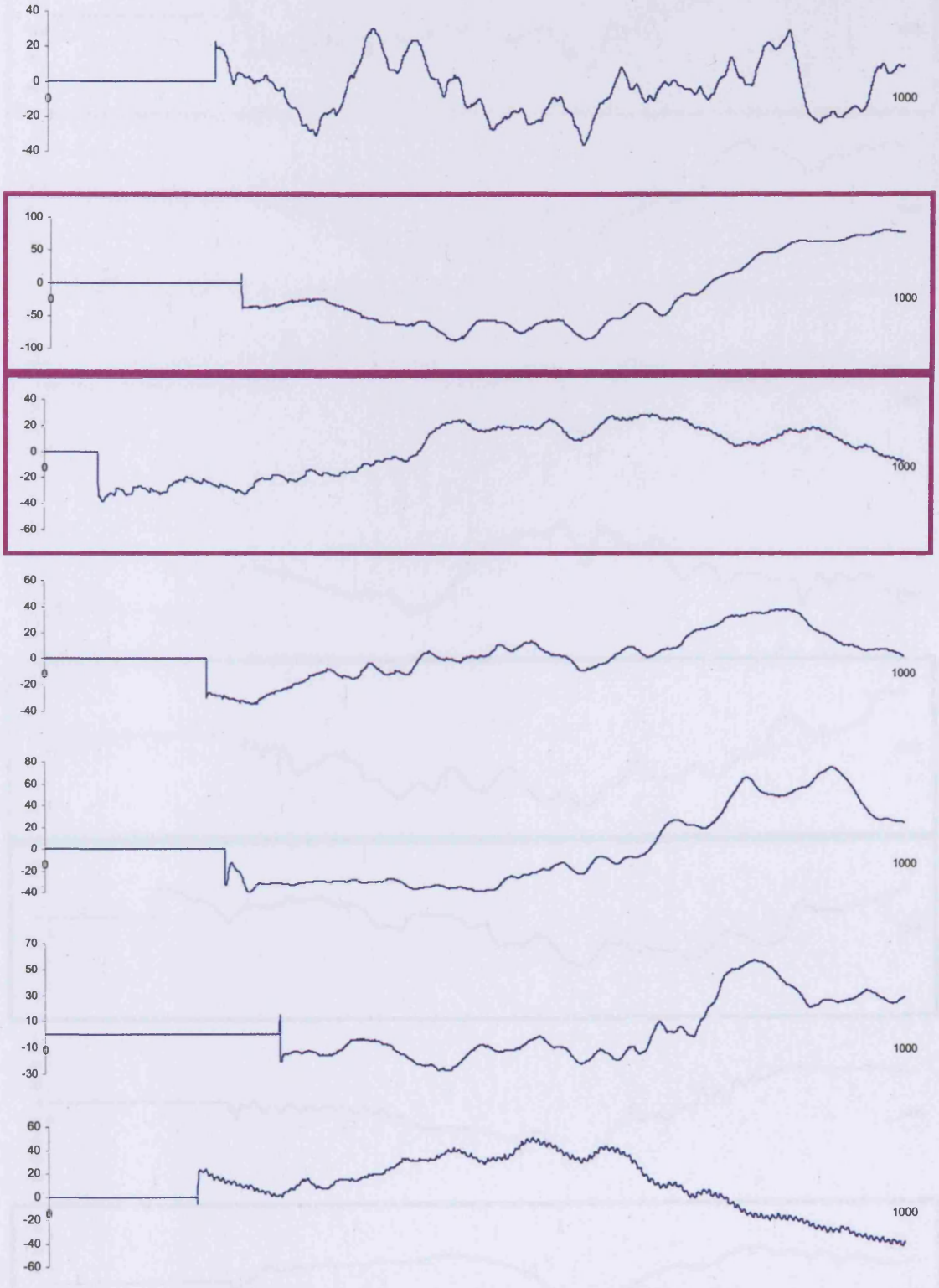
Detailed analysis of N-P complex

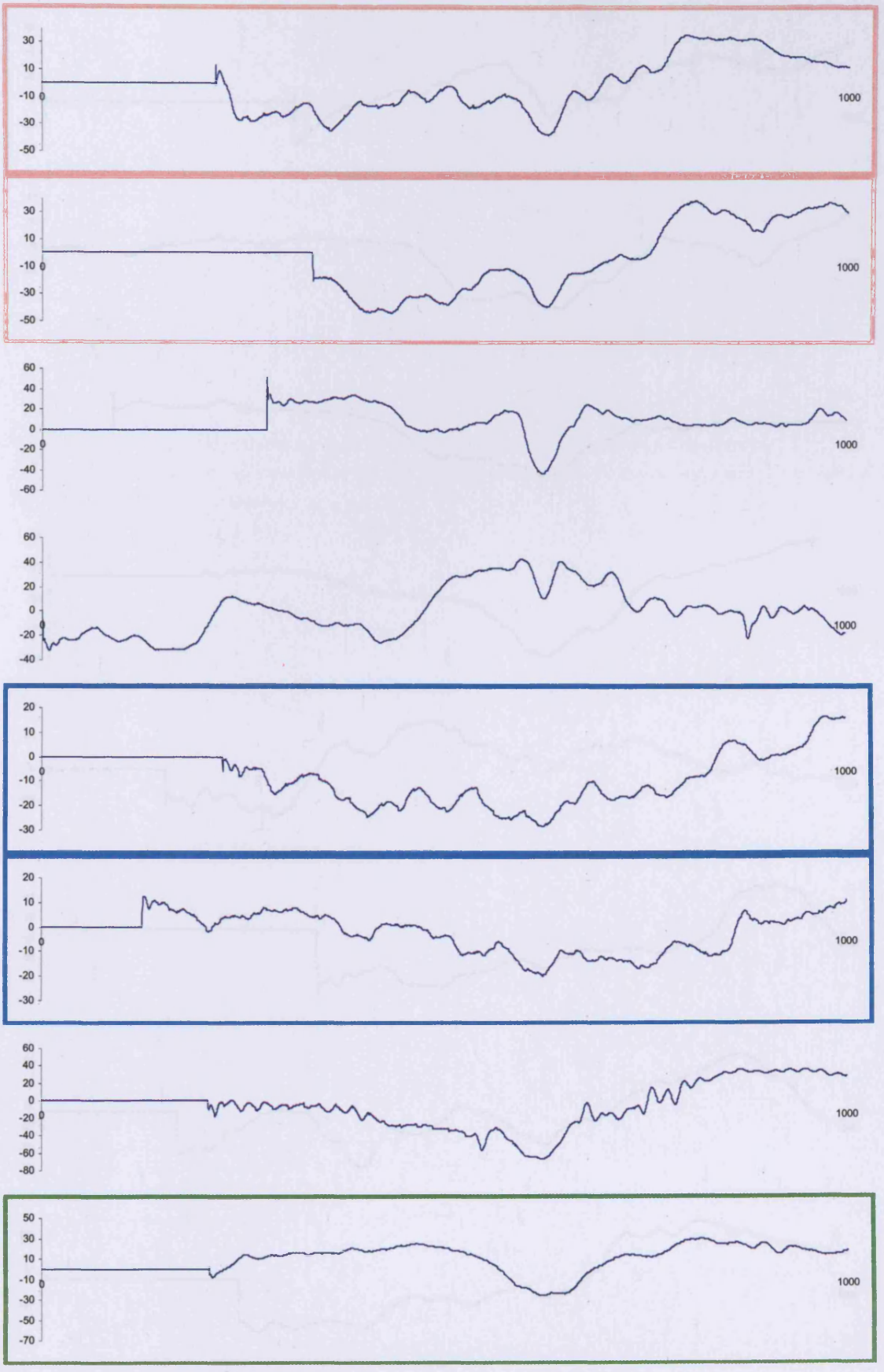
Study Number	Sex	PMA (weeks)	Foot lanced	Latency to N1 (ms)	Latency to P1 (ms)	Amplitude (μV)	Duration (ms)
1	Female	42+6	Right	430.5	451.0	23.8	20.5
2	Female	42+3	Left	450.5	475.5	12.5	25.0
3	Male	35+4	Left	416.0	517.0	36.9	101
4	Male	35+4	Left	320.5	351.0	17.4	30.5
5	Male	33+3	Right	409.0	440.5	32.4	31.5
6	Male	33+3	Left	579.5	609.5	17.2	30.0
7	Female	35+4	Left	346.5	379.0	47.6	32.5
8	Female	36+2	Right	439.5	496.5	18.5	57.0
9	Female	36+2	Right	554.5	598.0	18.4	43.5
10	Female	36+2	Right	412.5	446.0	31.0	33.5
11	Female	36+2	Right	291.0	327.0	25.4	36.0
12	Male	35+4	Left	416.0	440.5	17.3	24.5
13	Female	37+5	Left	353.5	371.0	12.3	17.5
14	Female	31+1	Left	413.0	460.5	46.0	47.5
15	Female	36+0	Left	501.0	522.0	10.1	21.0
16	Male	31+1	Left	573.5	630.5	22.7	57.0
17	Male	35+4	Left	542.0	652.0	48.6	110
18	Female	31+3	Right	279.0	306.0	13.6	27.0
19	Female	31+6	Left	436.5	489.0	36.5	52.5
20	Female	41+1	Right	455.5	505.0	34.5	49.5
21	Female	35+2	Left	470.5	521.5	16.5	51.0
22	Male	35+4	right	625.0	646.0	30.0	21.0
23	Female	35+2	Left	397.5	444.5	21.3	47.0
24	Female	33+2	Right	379.0	411.5	10.9	32.5
25	Female	34+6	Right	500.5	530.5	25.1	30.0
26	Female	34+6	Right	398.5	427.5	17.9	29.0
27	Male	35+2	right	400.0	430.5	10.8	30.5
28	Female	42+6	Right	-	-	-	-
29	Male	37+6	Right	-	-	-	-
30	Female	34+3	Left	-	-	-	-
31	Female	31+6	Right	-	-	-	-
32	Female	28+0	left	-	-	-	-
33	Female	30+2	Right	-	-	-	-
			Mean	**436.7(86.5)**	**477.0(93.6)**	**24.3(11.5)**	**40.3(22.1)**

Table 5.4 Analysis of N-P complex in individual trials

Studies highlighted in the same colour were undertaken on the same infant on same test occasion. Standard deviation given in parentheses.

Appendix 8 Traces from individual heel lance trials

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1000
60
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-60
-80
0
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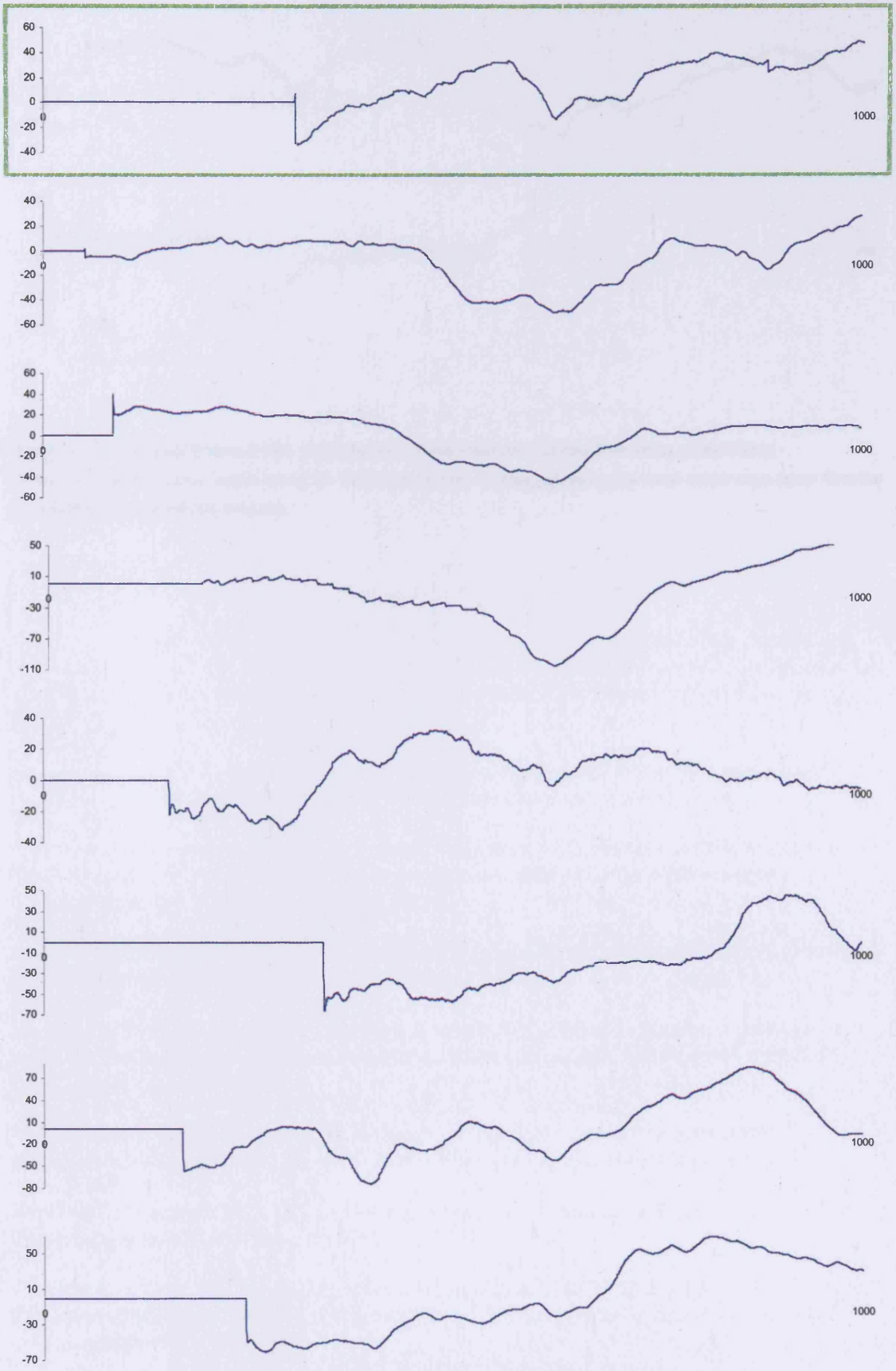
50
30
10
-10
-30
-50
-70
0
1000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-10
-30
-50
-70
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40
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-50
-80
0
1000
50
10
-30
-70
0
1000

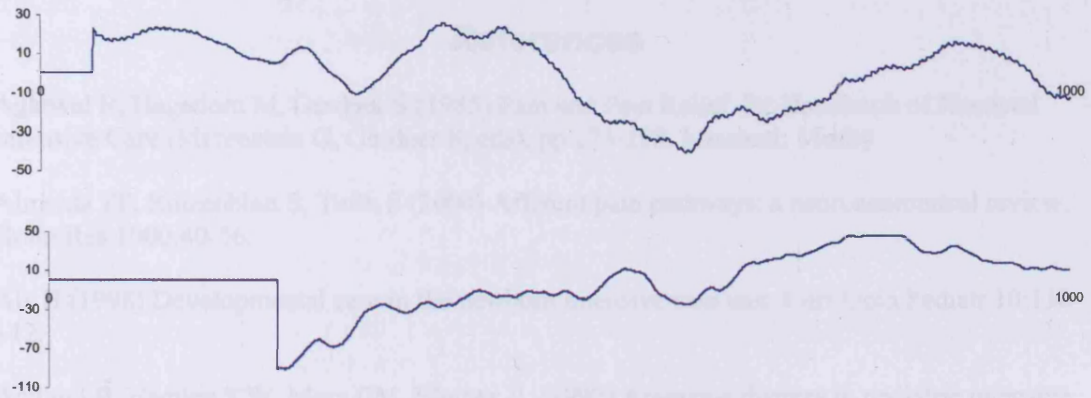

Figure 5.17 Individual traces in the contralateral somatosensory cortex following a heel lance

In each trial, the heel lance occurs where the EEG trace begins. Traces outlined in the same colour were taken from the same infant on the same test occasion

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