

ASSESSMENT OF PAIN AND ITS TREATMENT IN NEONATES

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A thesis submitted in fulfilment of the requirements for the
degree of MPhil, Imperial College London.

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ABSTRACT

Babies on the neonatal unit are often exposed to a number of noxious stimuli. Procedures such as venepuncture, insertion of cannulae, heel prick and endotracheal ventilation can occur on a daily basis, and the majority of these babies do not receive any pain relief treatment. Current methods of assessing pain in neonates, comprising behavioural cues and physiological markers, can be difficult to relate directly to pain perception, especially in preterm infants, as they can also be indicative of other states such as stress, anxiety and hunger. Accurate recognition of pain in a neonate could enable more appropriate use of analgesics, thereby reducing morbidity and limiting potential side-effects.

We investigated 48 babies using techniques that may evaluate the function of nociceptor-specific nerve fibres and central pain pathways, in comparison with currently used behavioural and physiological indicators. These techniques included “objective” physiological responses to procedural pain, such as skin axon-reflex vasodilatation (flare) responses, and novel contact cerebral evoked potentials in response to warm (non-painful) stimuli.

We recorded physiological responses (i.e. sweat rate, changes in blood flow, sympathetic skin response) during clinically required heel prick procedures in babies on the neonatal unit. Palmar sweat rate was higher in babies ≥ 36 weeks, and significantly correlated with gestational age. We did not see any relationship between the painful heel prick procedure and palmar sweat response. We were unable to demonstrate any increase in the sympathetic skin response waveforms post-heel

prick procedure in the one baby we studied. Changes in local skin blood flow around the heel prick (ipsi-lateral limb) and in the opposite foot (contralateral limb) were very variable, although many babies showed an increase in the blood flow post-heel prick. We also performed a behavioural analysis (using PIPP) during heel prick procedures in babies whilst simultaneously recording their palmar sweat levels. We could not demonstrate any definite association between the palmar sweat response to pain and the PIPP scores.

The feasibility of recording warm temperature induced cerebral evoked potentials in neonates was investigated, using scalp EEG electrodes, in response to a cutaneous stimulus with a thermode destination temperature 37°C. However, the EEG waveforms were obscured by ocular artefact, and thus warm temperature evoked potentials could not be demonstrated in neonates, unlike adults.

In conclusion, current methods of behavioural and physiological assessment of pain in neonates have an important role, but are not specific and unlikely to be helpful in monitoring the effects of new analgesics. Warm stimuli evoked cerebral potentials are technically difficult to record in neonates, and heat pain stimuli are unjustified, but other techniques such as functional MRI may provide a useful measure and deserve study. The assessment and treatment of pain in neonates remains a major unmet clinical need.

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ACKNOWLEDGEMENTS

This thesis and the work within it would not have been accomplished without the support of a number of people, who have all contributed in some way to my work over the last one year.

My greatest thanks go to my supervisor, Professor Praveen Anand (Professor of Clinical Neurology, Imperial College London, UK) for allowing me to undertake such an interesting and varied project within his department. He has expanded my understanding of neuroscience immeasurably with his wealth of knowledge on the subject.

I am also extremely grateful to my supervisors at St Mary's Hospital. Very special thanks to Dr Sunit Godambe, who has supported and encouraged me and provided me with some wonderful opportunities throughout the whole of my project. I am also very thankful to Dr Peter Chow and Dr Peter Misra for their insight, advice and assistance throughout my project.

A special thanks to Ms Mary Tighe, for sharing her expertise in EEG, in helping out with the study and analysis of the evoked potential traces. I would like to thank Dr Les Huson, Medical Statistician, Clinical Research Unit, Imperial College London, who has reviewed and helped with the statistical analyses.

I would also like to thank all my colleagues at the Peripheral Neuropathy Unit especially Dr Ravikiran Shenoy and Dr Yiangos Yiangou. They have made my stay

so enjoyable and rewarding, and have tirelessly shared their knowledge and expertise.

I would also like to thank the staff at the Winnicott Baby Unit for their help and support throughout this project without which this wouldn't not have been possible.

I would also like to thank Stuart Rawling at the Winnicott Baby Unit for his technical and IT assistance.

This project was supported by the Winnicott Foundation, UK. I would like to thank them for their financial support.

Most importantly, this project would not have been possible without all the babies and their parents who have given up their precious time to participate in the individual studies. To them I express my deepest gratitude, and I hope that the data presented in this thesis may go some way towards benefiting babies in the future.

Finally I would like to thank my family who have provided never failing support in all manner of ways throughout my project, and have always inspired me.

DECLARATION

The work presented herein was carried out at the Winnicott Baby Unit, 3rd Floor, Clarence Memorial Wing, St Mary's Hospital, Praed Street, Paddington, London W2 1NY and between January 2011 and September 2011 under the supervision of Professor Praveen Anand.

Within the Winnicott Baby Unit I have participated in all aspects of the study including consenting and patient testing including measurement of sweat rate, skin blood flow and sympathetic skin response. I also performed the audio/ video recording for behavioural analysis.

I helped conduct recording of contact warm evoked potentials, with assistance from Mary Tighe (Brain Products, UK) and the team from the Peripheral Neuropathy Unit, Hammersmith Hospital.

The study was approved by the Research Ethics Committee (REC Reg. No: 10/H0718/34) and the Imperial College R&D (R&D No: JROSM0075).

Informed written parental consent was obtained for each infant.

The study conformed to the standards set by the Declaration of Helsinki.

ABBREVIATIONS

AGA	Appropriate for Gestational Age
BAEP	Brain Auditory Evoked Potentials
CHEPS	Contact Heat Evoked Potential Stimulator
CRIES	Crying, Requires Increased FiO ₂ , Increased vital signs, Expression & Sleeplessness
EEG	Electroencephalogram
EP	Evoked Potential
ERP	Event Related Potential
HPA	Hypothalamo-Pituitary-Adrenal Axis
IVH	Intraventricular Haemorrhage
LGA	Large for Gestational Age
NIDCAP	Neonatal Individualised Developmental Care and Assessment Program
NIPS	Neonatal Infant Pain Scale
NIRS	Near Infrared Spectroscopy
PIPP	Premature Infant Pain Profile
PU	Perfusion Units
PVL	Periventricular Leucomalacia
SCR	Skin Conductance Response
SGA	Small for Gestational Age
SSEP	Somatosensory Evoked Potential
SSR	Sympathetic Skin Response
VEP	Visual Evoked Potentials

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CHAPTER 1

INTRODUCTION

1.1 PAIN

1.1.1 DEFINITION

International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Loeser and Treede 2008). As per this definition, pain is said to be subjective and best assessed by self-report which is not possible in neonates. Nociception is defined as "the neural processes of encoding and processing noxious stimuli" (Loeser and Treede 2008). The term nociception was coined by Charles Scott Sherrington in 1906.

1.1.2 PATHOPHYSIOLOGY OF PAIN

Sensation of pain is elicited by a stimulus that causes or can potentially cause tissue damage. Nociceptors are free nerve endings that have their cell bodies in the dorsal root ganglia. After transduction of the first noxious stimuli into action potentials in the afferent neuron, a series of changes occur in components of the pain pathway that alter the way these components respond to subsequent stimuli. Both increased and decreased sensitivity to painful stimuli can occur. The term 'hyperalgesia' is used to describe a condition when these changes result in an increased sensitivity to painful stimuli. The term 'analgesia' is used to describe selective suppression of pain without effects on consciousness or other sensations. Pain differs significantly from other somatosensory modalities in that it can be altered by past experiences, suggestion, emotions and the simultaneous activation of other sensory modalities. Thus the level of pain experienced is not solely a physical property of the stimulus (Eric P. Widmaier 2006).

There are two major somatosensory pathways, the anterolateral pathway and the dorsal column pathway.

The ascending anterolateral pathway (spinothalamic tract) is responsible for transmission of pain and temperature to the brain. It makes its first synapse between the sensory receptor neuron and a second neuron located in the gray matter (dorsal column) of spinal cord (Eric P. Widmaier 2006). Two types of nociceptor fibres have been identified: the myelinated A δ fibres carry signals faster (2.5–35 m/s) than the unmyelinated C fibres (0.5–2.0 m/s). Pain evoked by the A δ fibres is described as sharp, acute, pricking pain and is felt first. This is followed by a duller poorly localised pain, often described as burning, aching or throbbing pain that is carried by the C fibres (Craig 2003). The axons of these neurons travel up the spinal cord to the brain and cross the midline through the anterior white commissure, passing upwards in the contralateral anterolateral columns. The second order neuron projects through the anterolateral column of the cord to the thalamus, where it synapses on cortically projecting neurons. As the pathways cross from the side where the afferent neurons enter the central nervous system to the opposite side in the spinal cord, the sensory pathways from somatic receptors on the left side of the body go to the somatosensory cortex of the right cerebral hemisphere. Pain-related activity in the thalamus relays to the insular cortex and anterior cingulate cortex, and pain that is distinctly located also activates the primary and secondary somatosensory cortices (Romanelli and Esposito 2004). In the somatosensory cortex, the endings of the axons of the specific somatic pathways are grouped according to the peripheral location of the receptors that give input to the pathways (Eric P. Widmaier 2006).

When the signals are sent to the reticular formation and thalamus, the sensation of pain enters consciousness in a dull poorly localized manner. From the thalamus, the signal can travel to the somatosensory cortex in the cerebrum, when the pain is experienced as localized and having more specific qualities. Nociception can also cause generalized autonomic responses before or without reaching consciousness to cause pallor, diaphoresis, tachycardia, hypertension, light headedness, nausea and fainting (Feinstein, Langton et al. 1954).

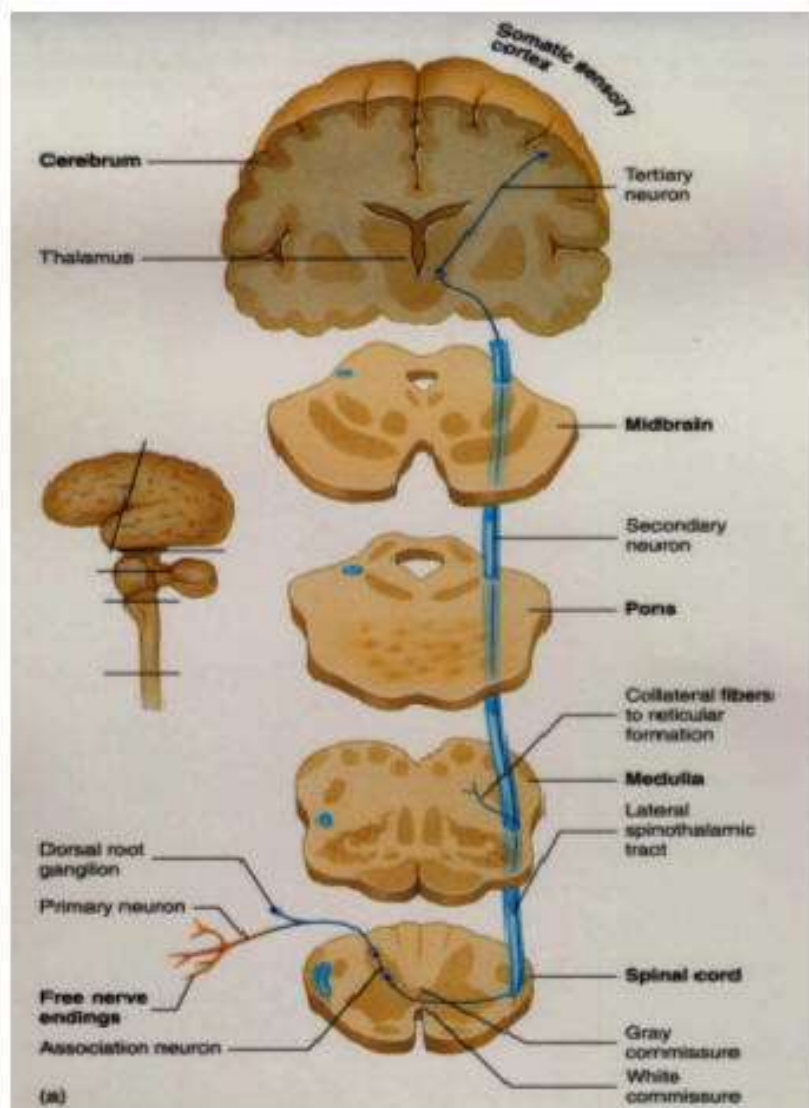


Image 1.1: The Pain Pathway (adapted from <http://grants.hhp.uh.edu/clayne/6397/Unit5.htm>)

In the central nervous system, monoamines, catecholamines and neuropeptides are all thought to act as neurotransmitters or neuromodulators and have been implicated in the pain pathways (Ruda et al. 1987). The nociceptors are activated by mechanical, thermal or chemical stimulation. The arachidonic acid pathway produces the inflammatory mediators involved in the pain pathway, namely the prostaglandins, leukotrienes and thromboxanes. The prostaglandins and leukotrienes sensitize the nociceptors to other inflammatory mediators (like bradykinin and histamine) which interact with substance P (a neuropeptide released from the peripheral neurons) to stimulate the nerve endings. Substance P also stimulates mast cells to release more histamine as well as more prostaglandins and bradykinins, further stimulating the nociceptors. Substance P is also known to contribute to swelling and redness in the area of pain (Yudcovitch LB, adapted from

<http://www.pacificu.edu/optometry/ce/courses/22746/ocularpainpg1.cfm>).

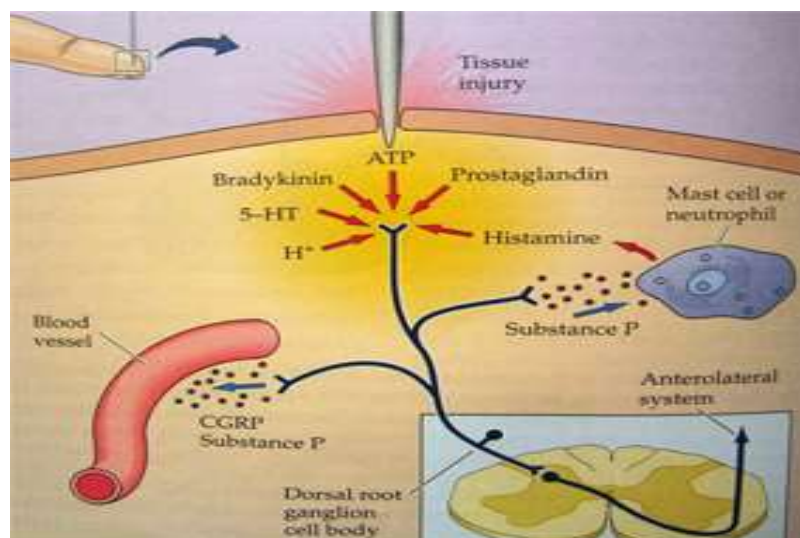


Image 1.2: Physiological pathway of pain (adapted from
[http://instruct1.cit.cornell.edu/courses/psych396/student2006/](http://instruct1.cit.cornell.edu/courses/psych396/student2006/the_biology_of_pain_mac_version/receptor2.jpg)
[the_biology_of_pain_mac_version/receptor2.jpg](http://instruct1.cit.cornell.edu/courses/psych396/student2006/the_biology_of_pain_mac_version/receptor2.jpg))

1.1.3 PAIN PATHWAYS IN THE NEWBORNS

Development of neural pathways has been studied in human infants using histological studies. It was previously thought that neonates did not perceive any noxious stimuli as unpleasant, and even if they felt pain they probably did not remember it in the long term. Contrary to this belief, we now know that the pathways responsible for conducting pain signalling up to the brain have developed by 20-24 weeks of gestation and the pathways that inhibit incoming pain impulses do not mature until the last trimester of pregnancy. The first cutaneous sensory receptors have been found between 7.5 and 10.5 weeks gestational age. The primary nociceptive afferents synapse with the spinal cord neurons at around 19 weeks. Even though the thalamic projections to the cortical plate occur at 22 weeks, the true connections to the cortex are thought to develop between 21 and 28 weeks gestational age (Anand and Hickey 1987; Anand and Carr 1989; Fitzgerald 1991).

In foetal life, presence of large receptive fields in the sensory neurones in the spinal cord along with diffuse central connections are likely to lead to poorer discrimination between noxious and non-noxious events and poorer spatial localisation (Fitzgerald 1991; Lloyd-Thomas and Fitzgerald 1996). Also, the descending inhibitory system of afferent neurons does not develop until after birth, resulting in large receptive fields and prolonged responses to stimuli during neonatal period (Anand and Carr 1989; Schechter NL 1993; Andrews and Fitzgerald 1994). This may increase the pain sensitivity of infants born prematurely by lowering the threshold of pain perception and make them more susceptible to pain (Craig, Whitfield et al. 1993; Johnston, Stevens et al. 1995; Giannakouloupoulos, Teixeira et al. 1999; Franck, Greenberg et

al. 2000; Smith, Gitau et al. 2000; Vanhatalo and van Nieuwenhuizen 2000; Gunnar and Quevedo 2007).

Having known the chronological order of the appearance of these pathways, mere anatomical presence of these does not necessarily mean that they are functionally active as well. As the somatosensory system continues to evolve and grow even after birth, it is still unclear whether neonates have the ability to discriminate between sensory and other type of stimuli, as do adults.

1.1.4 PAIN IN NEONATES – THE PROBLEM

Unrecognised and under-treated pain remains a major issue of concern in Neonatal Intensive Care Units. With advances in neonatal and obstetric care, the survival of extremely preterm neonates has increased over the past two decades. As a result, the number of painful procedures that neonates undergo has also multiplied. During hospitalisation in a Neonatal Unit, babies may undergo between 2 and 14 invasive, potentially painful procedures each day and up to 100 in first 14 days of hospitalisation, for which less than one in three receive an analgesic therapy (Johnston, Collinge et al. 1997; Simons, van Dijk et al. 2003; Carbajal, Rousset et al. 2008). Babies undergo a number of different procedures like heel prick, intubation, endotracheal suctioning, intravenous cannulation, arterial lines placement etc, the most common being endotracheal suctioning and heel prick sampling (Barker and Rutter 1995; Johnston, Collinge et al. 1997; Porter FL 1998; Benis MM 2001; Simons, van Dijk et al. 2003). Most of these procedures are done in the first 24-48 hours of admission.

1.1.5 PAIN IN NEONATES – WHY BOTHER?

As well as ethical consideration of having babies suffer pain, there is evidence to support adequate analgesia in reducing long term morbidity. Pain can adversely affect both short term and long term outcomes of babies (Porter, Grunau et al. 1999; Anand 2000). Repeated exposure to painful stimuli may alter babies' physiological responses, hormone levels and stimulate HPA - axis in a group of population whose nervous system is still developing (Grunau, Holsti et al. 2005; Grunau, Haley et al. 2007). These changes may result in tissue injury and increase the risk of IVH and PVL (Hall, Kronsberg et al. 2005). These painful experiences could adversely affect the neuro-developmental outcomes (Grunau, Holsti et al. 2006). Studies have shown that following a stimulus such as peripheral tissue injury, there is a reduction in the threshold of the nociceptive specific neurones in addition to the increase in the excitability of the central nervous system (Woolf 1983). An increased response to pain was shown following a higher frequency of painful procedures in low birth weight neonates at 32 weeks as compared to controls (Grunau, Oberlander et al. 2001). These may result in prolonged states of hyperalgesia and cutaneous hypersensitivity (Fitzgerald and Walker 2009). These studies have shown that infants exposed to multiple painful procedures earlier in life showed greater pain response than babies who were exposed to fewer painful stimuli. There is also evidence showing that untreated procedural pain results in adverse long term cognitive, behavioural and emotional consequences (Grunau 2002; Klein, Gaspardo et al. 2009). Helleurd and Storm (2002), in their study showed that non-painful sensory stimulation of infants (especially preterm and early newborns) can produce equal or higher levels of physiological stress activation than painful stimulation and that

repeated nociceptive stimulation probably sensitises the infants to pain (Hellerud and Storm 2002).

We, therefore, have physiological, moral and ethical reasons for prevention, assessment and treatment of pain in neonates.

1.1.6 ASSESSMENT OF PAIN IN NEONATES

Recognition and treatment of pain can still be termed a 'gray zone' in Neonatal Intensive Care Units. In spite of advances in all other aspects of neonatal care, huge knowledge gaps still exist in the area of assessment and management of pain.

Unlike adults, babies cannot communicate verbally and rely on their caregivers for the assessment and treatment of their pain. Current methods of assessing neonatal pain include both behavioural cues (like facial expressions, crying and body movements) and physiological markers (autonomic responses such as heart rate, respiratory rate and oxygen saturation). Based on these parameters, more than 40 different pain scores are currently in use all around the world (Ranger, Johnston et al. 2007). However, these parameters are very non-specific and can sometimes be difficult to attribute directly to pain, as they can also be indicative of other states such as agitation, stress, anxiety and hunger and could be altered by babies' neurological state and use of medication. At present less than 20% of the neonatal units in the UK have a pain assessment protocol (Redshaw M 2005).

In the last two decades, numerous studies have been done looking at pain transmission in neonates and its management. These include:

1. Behavioural responses (Grunau and Craig 1987; Johnston, Stevens et al. 1995; Grunau, Oberlander et al. 1998; Slater, Fitzgerald et al. 2007).
2. Physiological responses:
 - Skin blood flow (McCulloch, Ji et al. 1995; Kunzek, Quinn et al. 1997).
 - Sweat rate (Verbov and Baxter 1974; Harpin and Rutter 1982a; Harpin and Rutter 1982b).
 - Skin conductance (Roeggen, Storm et al. ; Storm 2000; Hellerud and Storm 2002; Hernes, Morkrid et al. 2002; Storm, Myre et al. 2002; Harrison, Boyce et al. 2006).
3. Changes in total haemoglobin concentration in cerebral cortex (NIRS) (Baird, Kagan et al. 2002; Pena, Maki et al. 2003; Taga, Asakawa et al. 2003; Slater, Cantarella et al. 2006).
4. Evoked potentials to noxious heel prick stimuli (Slater, Cantarella et al. 2006; Slater, Fitzgerald et al. 2007; Slater, Fabrizi et al. 2010; Slater, Worley et al. 2010).

However, none of the studies have provided us with any robust objective measures of neonatal pain. Hence, there is a need for a robust pain assessment scale in neonates that is easy to use, is easily reproducible and takes into account baby's clinical / neurological condition, use of medication and baby's gestational age.

Hormonal responses to pain have also been measured but these need laboratory assessment and cannot be done bedside (Franck and Miaskowski 1997). Previous studies have shown foetal stress responses to invasive procedures from at least 23 weeks gestation (Giannakouloupoulos, Sepulveda et al. 1994). Increased concentrations of plasma catecholamines and corticosteroids and beta-endorphin levels in the CSF of neonates following painful procedures have been shown in several studies, but the techniques to obtain these samples are invasive and painful (Anand and Hickey 1987; Marshall 1989). Lagercrantz et al, in a combined group of term and preterm neonates, showed that there was a greater increase in the levels of catecholamines following routine nursing procedures like changing nappies etc than following heel prick procedures (Lagercrantz, Nilsson et al. 1986).

These physiological responses and behavioural scores may not reliably reflect the underlying degree of pain in a clinically unstable baby who is intubated and is on sedatives and paralysing agents. Even though these studies have shown behavioural, physiological and biochemical responses to pain, whether or not premature neonates process pain at cortical level remained unanswered. As we know that cortex is important in pain perception, it is possible that the above physiological and behavioural responses could be just reflex responses mediated at the lower levels and that premature babies do not perceive any noxious stimulus as being painful. Thus, these responses could be measures of nociception rather than perception of pain (Slater, Fitzgerald et al. 2007). Studies have shown that spinally mediated reflex limb withdrawal from a noxious stimulus is greater in magnitude and duration in the youngest infants and decreases with age, in contrast to the cortical

responses (Fitzgerald, Shaw et al. 1988; Andrews and Fitzgerald 1994; Andrews and Fitzgerald 1999).

1.1.7 TREATMENT OF PAIN IN NEONATES

Both pharmacological (such as morphine, midazolam etc.) and non-pharmacological (oral sucrose, pacifiers, developmental care etc) means of pain control are currently in use in neonatal units. Even though pain relief is provided after surgery, daily routine procedures are still being done without any form of pain control measures being taken into consideration (Franck 1987; Tohill and McMorrow 1990; Bauchner, May et al. 1992; McLaughlin, Hull et al. 1993; Fernandez and Rees 1994; Johnston, Collinge et al. 1997). There does not seem to be any consensus amongst clinicians with regards to the best means of managing pain in neonates. Anxiety still exists with regard to the use of drugs like opiates in neonates, arising mainly from fear about their side-effects, and uncertainty about long-term effects on the neuro-developmental outcomes. More recently, attention is being paid to the individualised developmental care programs (NIDCAP) to reduce the risk of complications such as IVH in this vulnerable group of patients with unstable physiological conditions (Als, Duffy et al. 1996; Symington and Pinelli 2000).

1.2 HYPOTHESIS

We hypothesised that sweat rate, skin blood flow and cerebral evoked potentials are objective correlates of procedural pain in neonates.

1.3 AIMS AND OBJECTIVES

1.3.1 PRIMARY OBJECTIVES

(1) To systematically investigate the relevance of three physiological observations to procedural pain (heel prick) in neonates:

- (a) Sweat rate.
- (b) Skin blood flow.
- (c) Sympathetic Skin Response.

(2) To record warm evoked potentials to assess temperature and pain pathway maturation in neonates.

1.3.2 SECONDARY OBJECTIVES

(3) To investigate whether behavioural measures of pain (using PIPP) correlate with the results from above objective measures of pain.

(4) To investigate whether there are any effects of prematurity and maturation on the physiological or evoked potential data.

(5) To investigate whether drug and non-drug pain relief treatments given as part of routine clinical care have any effect on the above results.

The ability to monitor pain and the effects of its treatment objectively will advance pain assessment procedures in neonatal units and enable new, safer drug and non-drug treatments to be investigated in neonates with confidence.

1.4 INCLUSION AND EXCLUSION CRITERIA

1.4.1 INCLUSION CRITERIA

- All babies born at 24-44 weeks postmenstrual age admitted to the Winnicott Baby Unit at St Mary's Hospital, London.
- Who had a parent / guardian who were able and capable of giving informed consent on their behalf.
- Were free from any known neurological conditions / abnormalities that may occlude or compromise the results of the study i.e.
 - Antenatally or postnatally detected congenital anomalies
 - Intracranial abnormalities detected on antenatal scans
 - Intraventricular haemorrhage of any grade
 - Babies with neonatal encephalopathy

1.4.2 EXCLUSION CRITERIA

- Babies who did not have a parent / guardian who was able and capable of giving informed consent on their behalf.
- Babies who had any known neurological conditions / abnormalities that may occlude or compromise the results of the study (as elaborated in inclusion criteria).

CHAPTER 2

USE OF SWEAT RATE FOR THE ASSESSMENT OF PAIN IN NEONATES

2.1 INTRODUCTION

2.1.1 SWEAT RATE IN NEONATAL PAIN RESEARCH

Palmar sweat rate has been investigated as a marker of stress and pain in neonates by several previous investigators. Sweating from the palm of the hand and sole of the foot is a measure of the electro dermal activity and reflects the emotional state of a person, also called 'Emotional Sweating or Mental Sweating'. Sweating from these areas is independent of factors such as ambient room temperature and humidity, unlike rest of the body (thermal sweating), and has been shown to increase at times of stress, fear, anxiety etc.

Palmar and plantar sweat glands are well developed from about 28 weeks with their innervation by the sympathetic nervous system being present much earlier, at about 18 weeks gestational age (Nessmann and Baverel 1972). Whenever the sympathetic nervous system is activated, as in a stress response, there is a release of acetylcholine from the post-ganglionic nerve terminals which activates muscarinic receptors. This causes secretion from the palmar and the plantar sweat glands, and hence sweating. In both thermal and emotional sweating, the cholinergic fibres of sympathetic nervous system are involved. However, emotional sweating is controlled by a higher centre in premotor cortex (anterior cingulate cortex) and the thermal sweating is controlled by the hypothalamus (Tranel and Damasio 1994).

Palmar Sweat Index was used in early paediatric research but the results were highly variable and difficult to interpret (Gedaly-Duff 1989). Initial studies in babies

failed to show the presence of emotional sweating or its correlation with the state of arousal and it was thought that palmar sweating did not occur until 2 months of age (Verbov and Baxter 1974). However, subsequent studies have demonstrated a clear relationship between the palmar sweating (as measured with evaporimeter) and the state of arousal in babies ≥ 36 -37 weeks gestation and also showed that by third week of life the levels in vigorously crying babies were comparable to anxious adults (Harpin and Rutter 1982a; Harpin and Rutter 1982b). These studies have also shown that the thermal sweating response is dramatically increased in postnatal life, and almost all babies sweat in response to high ambient temperature by the age of 2 weeks (Harpin and Rutter 1982a; Harpin and Rutter 1982b).

In our study we have measured the palmar sweat levels in response to the heel prick procedure, in order to investigate its relationship to procedural pain.

2.2 METHODS

2.2.1 SUBJECTS

A total of 18 recordings for the sweat test were made on 12 babies whose gestational ages varied from 28+5 to 39 weeks, and who were between 1 and 43 days old (Appendix A - Table 2.1, page 97).

2.2.2 SWEAT RATE TESTING

The palmar sweat levels were measured using an Evaporimeter EP1 (Servomed,

Sweden). This device works on the principle of measurement of water vapour pressure gradient close to the skin surface. This gradient is directly proportional to the rate of evaporation of water from the skin (trans-epidermal water Loss).

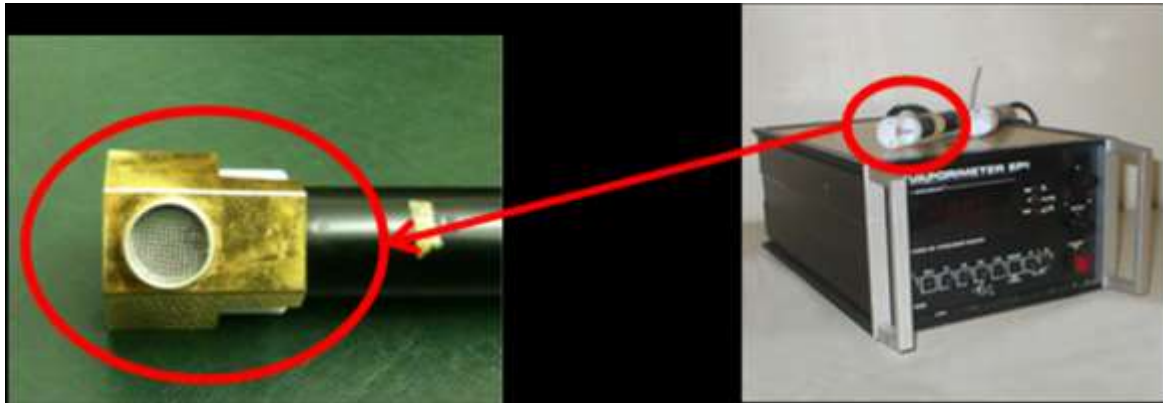


Image 2.1: Evaporimeter with the probe (highlighted in the red circle)

Babies were studied in a cot or in an unhumidified incubator. The clinically required heel prick was performed by the clinician on the unit using a standard Unistix 3 lancet. The readings were taken from the palm of the hands. The evaporimeter probe was gently placed on the skin surface until a steady reading of water loss was obtained. This water loss was measured in g/m^2 per hour. A baseline reading was obtained before the heel prick procedure till the reading on the evaporimeter stabilised. The monitoring was continued during the heel prick procedure and after the heel prick procedure till stable readings were obtained.

During this period of monitoring, babies' heart rate, respiratory rate and the oxygen saturations were also noted (Appendix A - Table 2.2, page 97). Where possible, a video recording of the babies was made for a later behavioural analysis. A note was

also made of any pain relief given to the babies. None of the babies studied received any other medication (other than oral sucrose) including inotropes or opiates.

The protocol was approved by the ethics committee and written consent was taken from parents before the study.

Details of babies' gestational age, postnatal age, birth weight, current weight and other clinical details were obtained from the Badger system (Electronic clinical (patient) record system). Gestational age was determined from antenatal ultrasound scans or from the maternal report of the last menstrual period (as obtained from the Badger).

2.2.3 ANALYSIS OF RESULTS

Results were analysed and graphs were created using GraphPad Prism version 3.02 for Windows (GraphPad Software, SanDiego, California USA).

2.2.4 RESULTS

The babies were divided into different groups and the analysis was done as described below. We could not study the effect of analgesics used as all babies received oral sucrose (as a part of unit protocol) before the heel prick procedures.

Babies who were born at different gestational ages (grouped as 24-29+6 weeks, 30-33+6 weeks, 34-36+6 weeks and ≥ 37 weeks) were further subdivided into various

postnatal ages (grouped as 1st week, 2nd week, 3rd week and >3 weeks) (Appendix A - Table 2.2, page 97). The sweat levels were measured as described above.

However, because the numbers in each of the subgroups above were small, we could not perform any statistical tests to analyse any differences amongst them (Details of the subgroup analysis are presented in Appendix B – page 104).

Hence without dividing them into subgroups based on their gestational and postnatal ages, we performed a linear regression analysis of the pre (baseline) and post heel prick sweat levels of all babies based on their corrected gestational ages at the time of testing. The corrected ages of babies ranged from 29 weeks to 39 weeks. Using the Spearman test, we looked for the correlation between the pre (baseline) / post heel prick sweat levels and the corrected gestational ages of the babies. We then used the Mann-Whitney test to compare the pre (baseline) sweat levels with the post heel prick sweat levels.

As seen in figure 2.5, the mean baseline (pre or resting) sweat levels clearly increased with gestational age ($r = 0.5438$, $p = 0.0197$). The post heel prick sweat levels also increased with the gestational age of babies ($r = 0.6794$, $p = 0.0019$).

When we compared the mean pre heel prick levels with the mean post heel prick levels at all ages (figure 2.6), there was no significant change ($p = 0.4964$).

We then used the ratio of post / pre heel prick values to analyse the results. Firstly a simple linear regression relating this ratio to gestational age gave results which are not statistically significant ($p = 0.565$). Secondly, a multiple regression analysis was

performed relating the ratio to gestational age, the pre-heel prick value, and the age of the baby. This shows a statistically significant relationship between the post / pre heel prick ratio and gestational age ($p = 0.0366$), after adjusting for pre-heel prick value ($p = 0.0449$) and the age ($p = 0.0858$). No other factors were statistically significant in this exploratory multiple regression. Hence, there is evidence that the change in sweat measurements (post as a ratio of pre) are related to gestational age but that the pre-heel prick results and perhaps the age of the baby are confounding factors in this relationship.

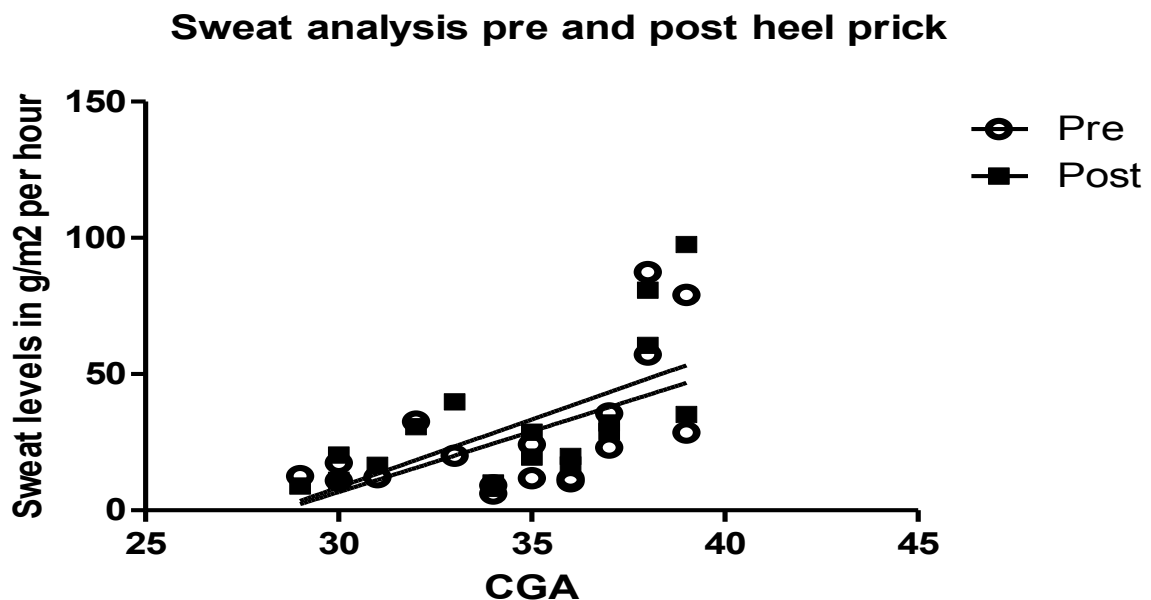
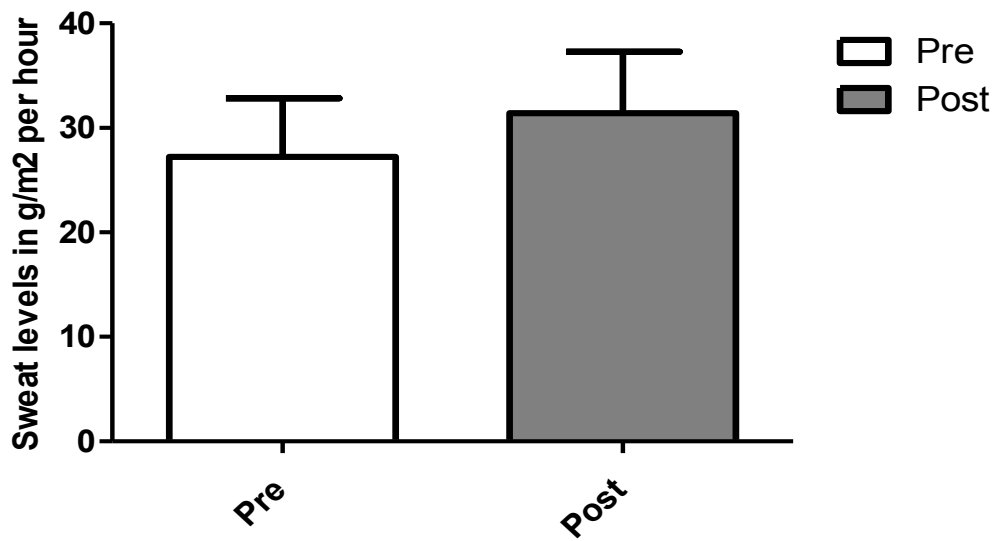


Figure 2.5: Linear regression analysis comparing pre and post heel prick sweat levels with corrected gestational ages (CGA)

Comparison of pre and post heel prick sweat levels



Comparison of pre and post heel prick sweat levels

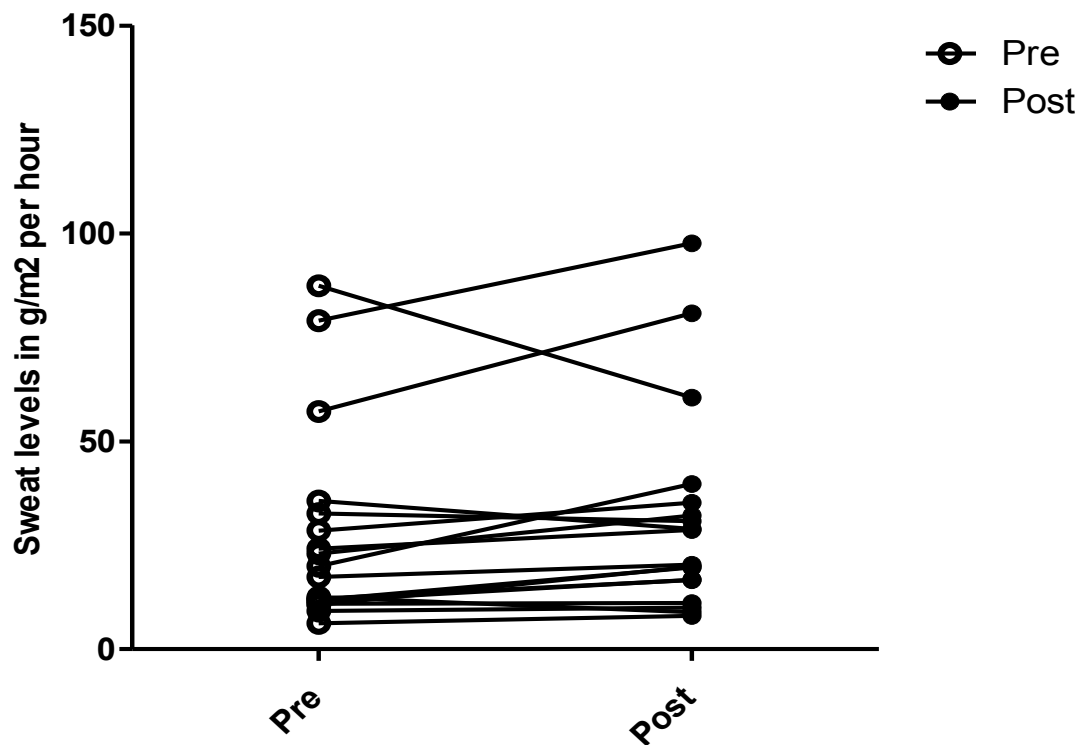


Figure 2.6: Comparing the pre heel prick with post heel prick sweat levels at all ages (Top – Bar chart showing mean $\pm$ SEM, Bottom – Before & After plot showing individual mean readings)

In order to find out if there were any differences in the mean baseline and post heel prick sweat levels in babies with different birth weights, the babies were divided into three major groups based on their birth weight and the mean pre and post heel prick results were analysed which did not show any statistical difference (Details of this sub group analysis presented in Appendix B – page 104).

2.3 DISCUSSION

Palmar sweat response has been investigated as a marker of pain in neonates previously. It seems unlikely that the sweating from palmar or plantar skin in neonates just reflects the trans-epidermal water loss, since the epidermis in these areas is thicker than elsewhere and the rates of water loss from these areas exceeds other sites in the absence of thermal sweating (Hammarlund, Nilsson et al. 1977; Rutter and Hull 1979; Harpin and Rutter 1982a; Harpin and Rutter 1982b).

We performed a linear regression analysis of the sweat results based on corrected ages (29-39 weeks). The mean baseline (pre or resting) sweat levels clearly increased with gestational age ($p = 0.0197$). The post heel prick sweat levels also increased with the gestational age of babies ($p = 0.0019$). When comparing the mean pre heel prick levels with the mean post heel prick levels at all ages, there was no significant change ($p = 0.4964$). A simple linear regression relating post / pre heel prick sweat ratio to gestational age gave results which are not statistically significant ($p = 0.565$). A multiple regression analysis relating this ratio to gestational age, the pre-heel prick value, and the age of the baby showed a statistically significant relationship between the post / pre heel prick ratio and gestational age ($p = 0.0366$),

after adjusting for pre-heel prick value ($p = 0.0449$) and the age ($p = 0.0858$). No other factors were statistically significant in this exploratory multiple regression. So our study has shown some evidence that the change in sweat measurements (post as a ratio of pre) are related to gestational age but that the pre-test results and perhaps the age of the baby are confounding factors in this relationship.

We also performed sub group analysis of sweat levels based on babies' gestational ages after dividing them into four major groups as described in the earlier sections of the thesis. In our study, babies ≥ 36 -37 weeks show a definite palmar emotional sweating response (mean baseline and post heel prick levels higher than at earlier gestations) which varies greatly with their state of arousal – being lowest when asleep and highest when crying. Our study did not show a striking increase in sweat levels post heel prick as in the previous studies (Harpin and Rutter 1982a; Harpin and Rutter 1982b). However, we could not perform any statistical tests in this group because of low numbers ($n = 4$ in week 1 and 0 in week 2, 3 and 4). Babies born at 34-36+6 weeks showed only slight but definite increases in the palmar sweat loss in the first and second week of life. We could not perform any statistical tests in this group either because of low numbers ($n = 3$ in week 1, $n = 1$ in week 2, $n = 0$ in week 3 and $n = 0$ in week 4). Babies born at 30-33+6 weeks also showed minimal variations in the palmar water loss till the third week of life with the variation slightly more pronounced after 3 weeks of life ($p = 0.7$). Babies born at 24-29+6 weeks showed little variation in the palmar sweat loss in the first 3 weeks of life and the results were not statistically significant ($p = 1.0$).

Subgroup analysis of sweat levels based on babies' birth weights did not show any difference in the three groups ($p = 0.4$).

The major limitation of our study was a very small sample size. Dividing babies into various gestational age groups and then further into different postnatal age groups further reduced the numbers in each group thereby limiting the ability to perform statistical analysis of the results. This problem was however overcome by performing a linear regression analysis of results based on corrected gestational ages. Whether the administration of sucrose to the babies could have altered our results would have been possible to ascertain by doing a subgroup analysis comparing babies who received sucrose with babies who did not receive any sucrose. This was not possible in our study since all babies received oral sucrose prior to heel prick as a part of unit protocol.

Our study agrees with the previous studies (Harpin and Rutter 1982a; Harpin and Rutter 1982b) in that the palmar sweat response reflects the emotional state and correlates with the state of arousal, but this response may not be very specific to pain. As palmar/ plantar sweat loss reflects the sympathetic response to stress, it may not be a very reliable tool in assessing pain in neonates. However, since the sample size was small in our study, further studies need to be done with a larger sample size in order to be able to draw conclusions with confidence.

CHAPTER 3

BEHAVIOURAL ANALYSIS OF PAIN USING PREMATURE INFANT PAIN PROFILE AND ITS CORRELATION WITH PALMAR SWEAT RATE

3.1 INTRODUCTION

3.1.1 BEHAVIOURAL ANALYSIS OF NEONATAL PAIN

Over the years, behavioural responses to pain in neonates have been extensively studied. These responses to pain include crying, movements of limbs, facial expressions and changes in sleeping/ waking patterns. Based on various physiological and behavioural indicators, more than 40 different pain assessment scores are in use worldwide (Ranger, Johnston et al. 2007), many using similar criteria although no 'gold standard' exists. The most commonly used pain assessment tools such as the PIPP (Premature Infant Pain Profile), NIPS (Neonatal Infant Pain Scale), CRIES (Crying, Requires Increased FiO₂, Increased vital signs, Expression & Sleeplessness) are intended primarily for evaluating pain experience during acute procedures such as heel sticks and are often difficult to score accurately in the clinical setting. Scores may be invalidated by the babies' neurological state and drugs like sedatives and paralysing agents. Some scales are adjusted to take into account that behavioural and physiological responses of infants differ depending on gestational age. Also, not all scales in use are appropriate for both term and preterm babies. There are fewer scales for evaluating ongoing pain, the most commonly cited being the French EDIN (Échelle Douleur Inconfort Nouveau-Né, neonatal pain and discomfort scale) (Debillon, Zupan et al. 2001) and N-PASS (Neonatal Pain, Agitation and Sedation Scale) (Hummel, Puchalski et al. 2008). Hence, there is a need for a pain assessment method that is easy to use, gives quick results, and is valid and reliable and is more objective.

3.1.2 PREMATURE INFANT PAIN PROFILE

The reason we selected the Premature Infant Pain Profile (PIPP) for our study is that PIPP is one of the most frequently used scores and has been validated for use in premature neonates (Stevens, Johnston et al. 1996). It was developed at the Universities of Toronto and McGill in Canada. It is a 7 indicator composite pain measure (Appendix A – Table 3.1, page 100) that includes:

- (1) Gestational age
- (2) Behavioural state before painful stimulus
- (3) Change in heart rate during painful stimulus
- (4) Change in oxygen saturation during painful stimulus
- (5) Brow bulge during painful stimulus
- (6) Eye squeeze during painful stimulus
- (7) Nasolabial furrow during painful stimulus

Babies' gestational age is scored before the assessment. Baby is then observed for 15 seconds prior to the potentially painful event to score the behavioural state.

Babies' baseline heart rate and oxygen saturation are recorded. The baby is then observed for 30 seconds immediately following the painful event to score the facial and physiologic changes during this time. Premature infant pain profile is calculated as the sum of points for all 7 indicators. The minimum score is 0 and the maximum score 21; higher the score, greater the pain behaviour.

In our study we performed the behavioural analysis in response to a clinically required heel lance procedure in babies using PIPP score and compared the scores with the findings of the sweat analysis to see if there is any correlation.

3.2 METHODS

3.2.1 SUBJECTS

We performed behavioural analysis using the Premature Infant Pain Profile (PIPP) in response to the clinically required heel lance procedure. A total of 16 video recordings were done in 11 babies. However PIPP scoring could not be done in case of 9 recordings (6 babies) due to poor quality video (1 recording), no monitoring of heart rate and saturations as babies stable and in cots (6 recordings) and monitoring with Doppler assessments (2 recordings). Hence, we were able to perform PIPP analysis only in case of 7 recordings (6 babies) whose corrected gestational ages varied from 29+3 to 38+4 weeks, and who were between 1 and 43 days old (Appendix A - Table 3.2, page 101).

3.2.2 BEHAVIOURAL ANALYSIS

Babies were studied in a cot or in an unhumidified incubator. Sweat levels were obtained in response to heel prick procedure as above. Where possible, a video recording of the babies was made for a later behavioural analysis. During this period of monitoring, babies' heart rate, respiratory rate and the oxygen saturations were also noted. PIPP scoring was later on done by two neonatologists independently

(score 1 and 2) on the Winnicott Baby Unit who were not present at the time of the procedure/ recordings to avoid any possibility of bias. A mean of these scores was calculated for each baby separately and then compared with the changes in the sweat levels. For the purposes of this study, even the mean scores in decimals have been used. We also compared the scores given by the two neonatologists with each other to look for any inter-observer bias.

The protocol was approved by the ethics committee and written consent was taken from parents before the study.

Details of babies' gestational age, postnatal age, birth weight, current weight and other clinical details were obtained from the Badger system (Electronic clinical record system). Gestational age was determined from antenatal ultrasound scans or from the maternal report of the last menstrual period (as obtained from the Badger).

3.2.3 RESULTS

We performed linear regression analysis to compare pre (baseline) and post heel prick sweat levels with the mean PIPP scores (Figure 3.1). Using Spearman test ($n = 7$), this showed a negative correlation between mean PIPP scores and pre heel prick sweat levels ($r = -0.50$, $p = 0.26$) and post heel prick levels ($r = -0.82$, $p = 0.03^*$).

Table 3.2 (Appendix A, page 101) illustrates the correlation between the PIPP scores and the sweat levels. As seen, in case of recording 1, 2, and 3, the mean PIPP score was low which correlated very well with the mean sweat levels post heel prick that did not show any significant increase. However, in case of recording 4, 5, 6 and 7,

the mean PIPP scores were very low but there was a definite increase in the mean sweat levels post heel prick as compared to the baseline levels.

Comparison of PIPP scores with sweat levels

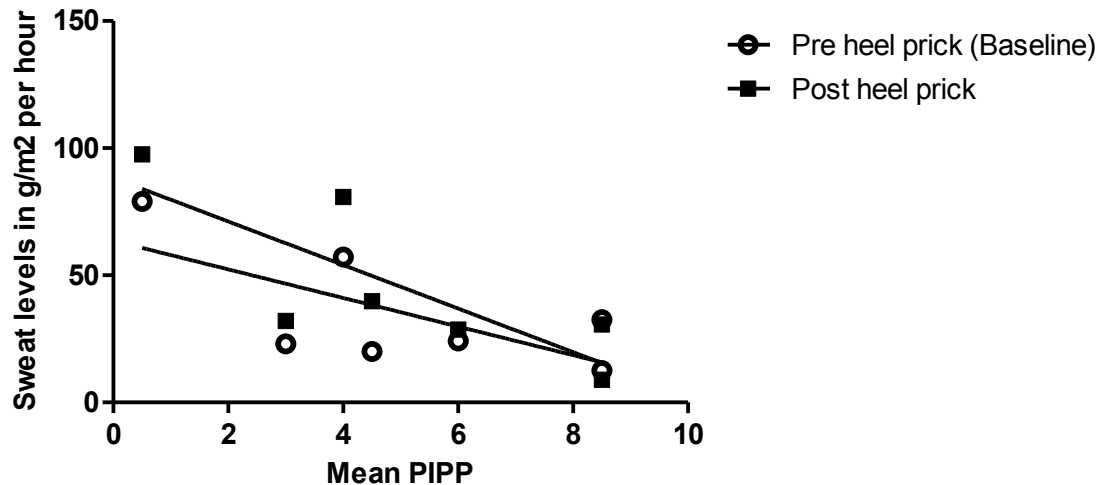


Figure 3.1: Linear regression analysis comparing mean pre and post heel prick sweat levels with mean PIPP scores

Interestingly, when we compared the two PIPP scores with each other (Table 3.2 - Appendix A, page 101), although they agreed with each other in most cases (recordings 1, 3, 4 and 6), there were marked differences in some (recordings 2, 5 and 7). As seen in figure 3.2, we compared the scores using Mann Whitney test ($n = 7$) that failed to show any significant correlation between the scores ($p = 0.27$).

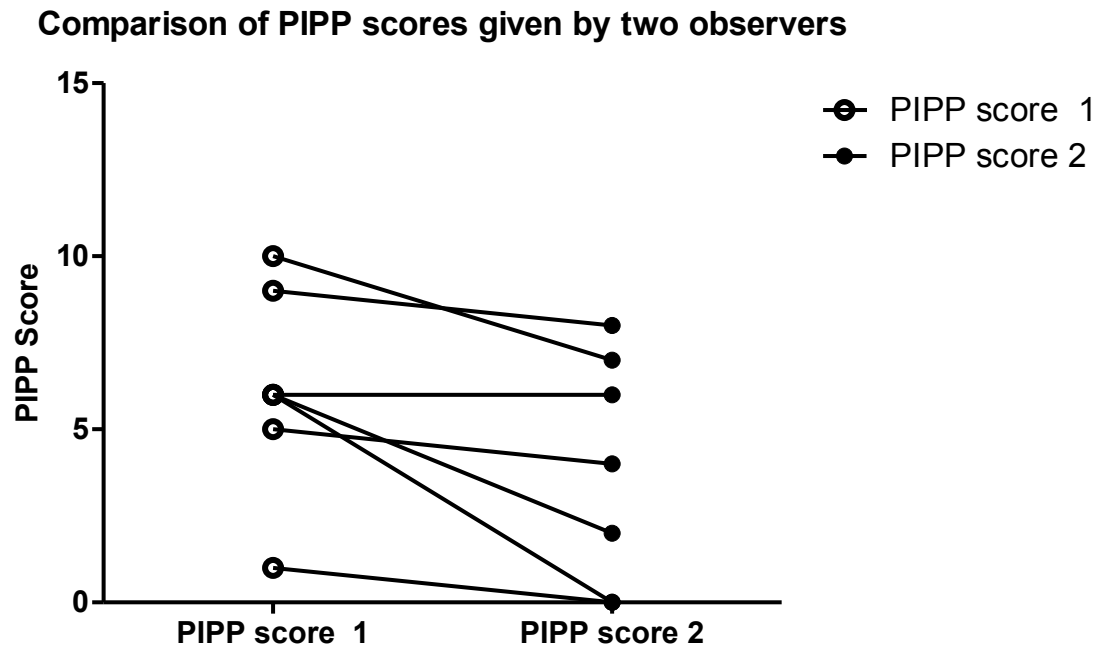


Figure 3.2: Comparison of PIPP scores given by the two observers (Before & After plot)

3.3 DISCUSSION

Amongst the behavioural responses, facial expression has been shown to be the most consistent and specific pain response (Grunau and Craig 1987; Grunau, Oberlander et al. 1998). However, there is a poor correlation with behavioural and physiological indicators of pain (Johnston, Stevens et al. 1995). This is supported by attenuation of the behavioural but not the physiological responses to venepuncture by oral glucose intervention suggesting that the latter may be related to stress rather than pain (Bauer, Ketteler et al. 2004). It has been suggested that it is possible that the nociceptive pathways originating from the spinal cord and conveying information to the thalamus and somatosensory cortex more reliably activate the brainstem

nuclei responsible for facial expression than the cardiovascular and respiratory control centres (Bauer, Ketteler et al. 2004). In their study, Slater et al showed that the infants demonstrated a clear cortical response following a heel prick lance, but did not show any changes in facial expression (Slater, Fitzgerald et al. 2007). The authors have postulated that the absence of facial activity may be due to immature motor circuitry failing to produce synchronised and coordinated muscle contraction or may indicate true absence of emotion. This means that if an infant does not demonstrate a change in facial expression, it cannot be assumed that the stimulus has not reached cortex (Slater, Fitzgerald et al. 2007).

In our study, we could not demonstrate a clear relationship between the behavioural responses (as assessed using PIPP score) and an objective marker of stress (palmar sweat levels). Linear regression analysis ($n = 7$), showed a negative correlation between mean PIPP scores and pre heel prick sweat levels ($p = 0.26$) and post heel prick levels ($p = 0.03^*$). Comparison of the PIPP scores with each other ($n = 7$) failed to show any significant correlation between the scores ($p = 0.27$).

The major limitation of our study again was a small sample size. In addition, some of the videos could not be assessed due to poor quality. This also highlights the technical difficulties in using a scoring system to assess pain in neonates in a clinical setting. One of the major challenges was trying to score facial responses in babies who were in an incubator, with various tubing attached and limited access / visibility. Besides, inter-observer variability was also noted in our PIPP scores, questioning their reliability. Whether the administration of sucrose to the babies could have altered our results would have been possible to ascertain by doing a subgroup

analysis comparing babies who received sucrose with babies who did not receive any sucrose. This was not possible in our study since all babies received oral sucrose prior to heel prick as a part of unit protocol.

CHAPTER 4

USE OF SYMPATHETIC SKIN RESPONSE FOR THE ASSESSMENT OF PAIN IN NEONATES

4.1 INTRODUCTION

4.1.1 SYMPATHETIC SKIN RESPONSE IN NEONATAL PAIN RESEARCH

Sympathetic Skin Response (SSR) is a way of assessing electrodermal activity, and reflects sympathetic cholinergic function which leads to a change in the skin resistance to electrical conduction. This principle is well used in adults in psychological research, and forms one component of 'Lie Detector' tests (Christie 1981; Bauer 1998).

The SSR can be recorded using the EMG machine. SSR is generally recorded with the active electrodes placed on the palm or sole. It has also been recorded from the forehead, axilla (Baba, Watahiki et al. 1988) and the genitalia (Ertekin, Ertekin et al. 1987). The stimulus can be an inspiratory gasp, a cough, a loud noise, an electrical stimulus, a skin stroke etc. SSR response curves normally consist of a negative and a positive phase (Baba, Watahiki et al. 1988; Arunodaya and Taly 1995). The negative or upward deflection (low amplitude) is followed by a positive or downward deflection (high amplitude). The SSR potential can, however, vary with ambient and baby's temperature, emotional state, skin potential level, arousing stimuli (surprise effect), habituation of response with repeated stimulations, and inter- and intra-individual variability (Vetrugno, Liguori et al. 2003). The source of the negative component of SSR is sweat gland itself, and depends directly on neuronal activation (Takagi and Nakayama 1959; Shaver, Brusilow et al. 1962). However, the source of the positive component is not fully established (Yokota, Takahashi et al. 1959; Shaver, Brusilow et al. 1962; Vetrugno, Liguori et al. 2003).

SSR has been studied in adults with excessive and defective sweating, peripheral arterial diseases (Argyriou, Tsolakis et al. 2006), carpal tunnel syndrome (Kiylioglu, Akyol et al. 2005; Bayrak, Tilki et al. 2007), COPD (Bir, Ozkurt et al. 2005), reflex sympathetic dystrophy (Bolel, Hizmetli et al. 2006), depression (Boettger, Greiner et al. 2010), post auricular region in Meniere's disease (Yildiz, Koybasi et al. 2007) and Parkinson's disease (Schestatsky, Ehlers et al. 2006). To our knowledge, SSR has never been studied in neonates.

SSR is simple, fast and readily obtainable on most electrophysiological equipment. However it has several limitations. The response readily habituates, may be difficult to reproduce consistently, there may be incomplete reactions to inappropriate stimuli and may be unpredictably absent even in normal subjects (Vetrugno, Liguori et al. 2003).

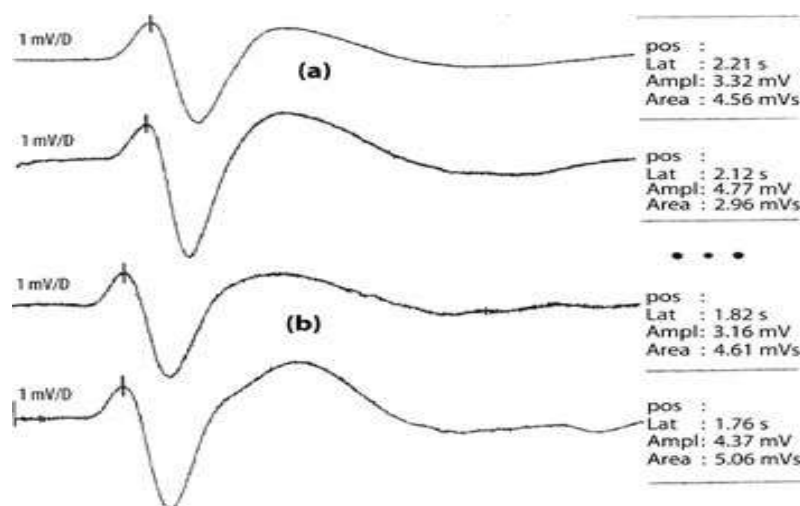


Figure 4: Typical SSR recordings taken from the right palm (upper trace) and left palm (lower trace) following stimulus of (a) left median nerve and (b) cervical cord (Chroni et al. 2006)

Sweating from the palmar and plantar sweat glands as measured by skin conductance activity (using different equipment from SSR) has been studied by several investigators. Skin conductance is measured in terms of a baseline activity and frequency and amplitude of the waves. A study by Storm et al (2000) has concluded that this spontaneous skin conductance reflects the stress response to heel stick in premature infants from at least 29 weeks gestation, and the behavioural state mirrored these changes (Storm 2000). More recent studies have shown SCRs to occur in infants from 25 weeks gestational age (Luis Pereira-da-Silva I M 2009; Slater, Cantarella et al. 2009).

In our study, we recorded the sympathetic skin response in a baby using a standard EMG machine in response to a clinically required heel prick.

4.2 METHODS

4.2.1 SUBJECTS

A total of 1 recording for the sympathetic skin response was made on 1 baby whose gestational age at birth was 31+2 weeks, with a corrected gestational age of 34+4 weeks and who was 23 days old. Baby's birth weight was 923 grams and the weight at the time of assessment was 1090 grams.

4.2.2 SYMPATHETIC SKIN RESPONSE TESTING

We measured the SSR using a Dantec Keypoint EMG machine using surface ECG electrodes with appropriate amplifier filter frequencies.

Baby was studied in an unhumidified closed incubator. The heel prick was performed using a standard Unistix 3 lancet.

Before the heel prick procedure was done, standard surface ECG leads were placed, one on the right palm, one on the dorsum of right hand and one on the right thigh. A baseline recording of the number of SSR waves per minute was done prior to the heel prick. A subsequent recording of the number of waves per minute was made for two successive minutes after the heel prick.

During this period of monitoring, baby's heart rate, respiratory rate and the oxygen saturations were also noted. A note was also made of any pain relief given to the baby. As per the unit protocol, this subject received oral sucrose prior to the heel prick procedure, and did not receive any other medication including inotropes or opiates.

The protocol was approved by the ethics committee and written consent was taken from parents before the study.

Details of babies' gestational age, postnatal age, birth weight, current weight and other clinical details were obtained from the Badger system (Electronic clinical record system). Gestational age was determined from antenatal ultrasound scans or from the maternal report of the last menstrual period (as obtained from the Badger).

4.2.3 RESULTS

Our results did not show any increase in the number of SSR waves per minute in response to the clinically required heel prick procedure. The baseline SSR rate was 5 SSRs / minute and the rates during the first and second minutes post heel prick were 2 SSRs / minute and 4 SSRs / minute respectively.

4.3 DISCUSSION

SSR has already been proposed as an indicator of sudomotor function and as an index of body's reaction to emotion and attention in adults (Shahani, Halperin et al. 1984; Knezevic and Bajada 1985). The feasibility of using SSR as an indicator of body's response to stress or pain in neonates remains to be established.

We were able to record SSR in only one baby and that was the major limiting factor in our study. Hence would need a much larger sample in order to interpret the results with confidence. On the basis of our finding and previous reports, we considered that study of more babies with SSR was unlikely to show correlations with pain scores.

CHAPTER 5

USE OF SKIN BLOOD FLOW FOR THE ASSESSMENT OF PAIN IN NEONATES

5.1 INTRODUCTION

5.1.1 DOPPLER SKIN BLOOD FLOW IN NEONATAL PAIN RESEARCH

Skin blood flow changes around a painful stimulus (like heel prick) is a function of nociceptive fibres; whereas changes in skin blood flow in other parts of body in response to heel prick or any painful stimulus reflects the sympathetic response to pain. These changes in skin blood flow in response to various stimuli have been studied previously by several investigators.

McCulloch et al (1995) measured blood flow changes in 15 infants using laser Doppler technique with a probe placed on the lateral abdominal wall. They found that skin blood flow increased significantly (27-134%) during lancet puncture of the heel, physical handling, standard suctioning and chest physiotherapy, and there were no changes during closed suctioning. Skin blood flow reduced significantly (35% by 20 minutes) after intravenous morphine (McCulloch, Ji et al. 1995). They postulated that substance P could be the link between pain and increased skin blood flow.

Substance P is a neurotransmitter known to be associated with nociception and vasodilatation (Piercey, Schroeder et al. 1981; Charnay, Paulin et al. 1983; Walmsley and Wiles 1990; Spigelman and Puil 1991). Studies have shown that both the release of substance P from primary sensory afferent nerve terminals and substance P induced vasodilatation are inhibited by opioid agonists in a naloxone-reversible manner (Jessell and Iversen 1977; Lembeck and Donnerer 1985; Khalil and Helme 1991).

A study done by Kunzek et al (1997) concluded that skin perfusion as measured using doppler fluximetry was unreliable in quantifying the sympathetic response to a noxious stimulus in preterm infants (Kunzek, Quinn et al. 1997). They studied skin blood flow changes in the heel contra lateral to the heel prick site. They looked at 16 preterm infants undergoing standard heel prick and found that although there was a significant reduction in skin blood flow following heel prick, this was variable and dependent on basal skin blood flow and there were also loss of data due to movement artefact. Kunzek and colleagues showed that preterm newborns react to noxious stimulus by reducing their skin blood flow as described in adults but the degree of reduction appeared much lower than adults (median % reduction 11% vs. 25-40% in adults). They concluded that these differences could be attributed to differences in anatomy between babies and adults i.e. capillary vasculature may be poorly developed in babies; incompletely developed arterio-venous anastomosis in the sole of foot (responsible for 90% of laser Doppler signal from the area of skin investigated) (Sherman 1963); and the degree of underlying basal vasoconstrictor tone.

In our study we used a laser Doppler technique to look for blood flow changes around the heel prick site and in the contra lateral foot in response to a clinically required heel lance.

5.2 METHODS

5.2.1 SUBJECTS

A total of 19 recordings for the skin blood flow in the peri-heel prick site (ipsi-lateral) were made on 15 babies whose gestational ages varied from 29+1 to 37 weeks, and who were between 2 and 26 days old (Appendix A - Table 5.1, page 102).

A total of 27 recordings for the skin blood flow in the foot opposite to the heel prick (contra-lateral) were made on 13 babies whose gestational ages varied from 24+4 to 41+4 weeks, and who were between 2 and 77 days old (Appendix A - Table 5.2, page 102).

5.2.2 SKIN BLOOD FLOW TESTING

We measured microvascular perfusion using PeriFlux 4001 Master multichannel laser Doppler system (Perimed, Stockholm, Sweden) which works on the principle of Doppler shift. The range for Doppler shift measurements for the instruments is 20 Hz to 20 kHz.



Image 5.1: Laser Doppler system (probe highlighted in red circle)

Babies were studied in an open cot or an unhumidified incubator. The heel prick was performed using a standard lancet Unistix 3 lancet.

The laser doppler probe (Standard 408 Probe) was placed in a plastic probe holder (PF104), and the holder was fixed using tensoplast adhesive plaster without pressure to the skin around the heel prick site in case of peri-heel prick recordings and in the middle of the heel of the opposite foot in case of contra lateral recordings. In case of readings taken around the heel prick site (peri-heel prick), the doppler probe was gently placed on the heel for a few minutes prior to the heel prick stimulus till a stable baseline reading was obtained. The probe was re-placed at the same site after the heel prick to look for the changes in blood flow.

In case of readings taken from the contra lateral limb, the probe was left on the contra lateral foot continuously throughout the procedure.

The mean skin blood flow in PU (red trace – measured readings) was recorded during the following observation periods:

1. Pre-heel prick (in both peri- and contra-lateral readings).
2. Post heel prick (during squeezing) at 1 min/ 3 min/ 5 min (only in case of contra-lateral limb recordings).
3. Post end of sampling/ squeezing at 1 min/ 3 min/ 5 min (both peri- and contra-lateral readings)

The data was recorded continuously as a trace on a laptop screen using the Perisoft software (Perisoft for windows, version 2.50). The mean readings of skin blood flow

were obtained using the same software. Spikes associated with limb movements were identified and rejected as artefacts.

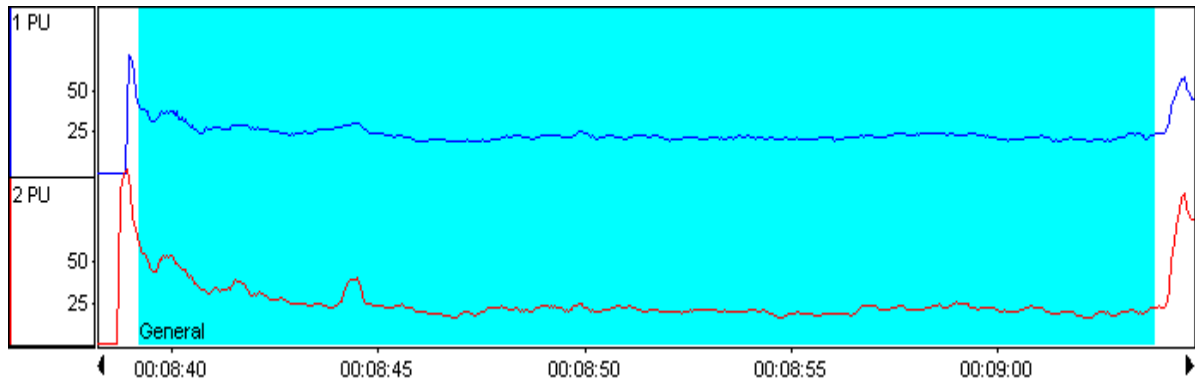


Figure 5.1a: Sample trace from a baby illustrating the baseline skin blood flow as measured in PU (red trace representing the output / measured readings)
X - axis: time in minutes and seconds, y - axis: skin blood flow

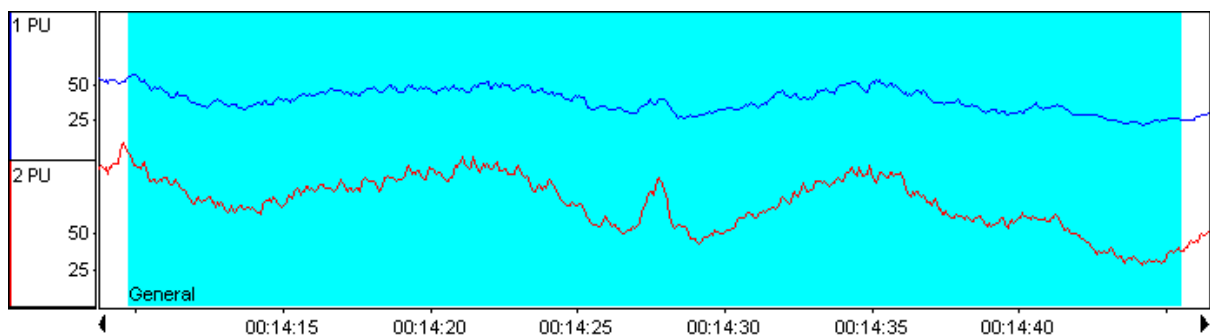


Figure 5.1b: Sample trace from the same baby illustrating the increase in skin blood flow post heel prick (red trace representing the output and measured readings)
X - axis: time in minutes and seconds, y - axis: skin blood flow

The protocol was approved by the ethics committee and written consent was taken from parents before the study.

During this period of monitoring, babies' heart rate, respiratory rate and the oxygen saturations were also noted (Appendix A - Table 5.3, page 103). A note was also made of any pain relief given to the babies. None of the babies studied received any other medication (other than oral sucrose) including inotropes or opiates.

Details of babies' gestational age, postnatal age, birth weight, current weight and other clinical details were obtained from the Badger system (Electronic clinical record system). Gestational age was determined from antenatal ultrasound scans or from the maternal report of the last menstrual period (as obtained from the Badger).

5.2.3 ANALYSIS OF RESULTS

Results were analysed and graphs were created using GraphPad Prism version 3.02 for Windows (GraphPad Software, SanDiego, California USA).

5.2.4 RESULTS

Mean skin blood flow changes pre-heel prick (baseline) and at various intervals post heel prick were calculated by taking into account the results obtained by using the Perisoft software (Perisoft for windows, version 2.50). Our results have shown a wide range of changes in the blood flow both around the heel prick site and in the contra lateral foot after the procedure. Even though the mean skin blood flow in the babies

post heel prick increased, the changes were varied with some babies showing a decrease and some an increase post heel prick (Figure 5.2, 5.3 and Appendix A - Table 5.4, 5.5, page 103-104). There was also loss of some data due to movement artefact. We could not study the effect of analgesics used as all babies received oral sucrose (as a part of unit protocol) before the heel prick procedures.

Table 5.4 (Appendix A, page 103) and Figure 5.2 illustrate the mean blood flow changes around the heel prick site. We used Mann Whitney test ($n = 22$) to compare the baseline skin blood flow around the heel prick site with changes at various intervals post heel prick. The analysis did not reveal the results to be statistically significant i.e.

Baseline vs. Post end sampling 1 min ($p = 0.75$),

Baseline vs. Post end sampling 3 min ($p = 0.13$),

Baseline vs. Post end sampling 5 min ($p = 0.87$).

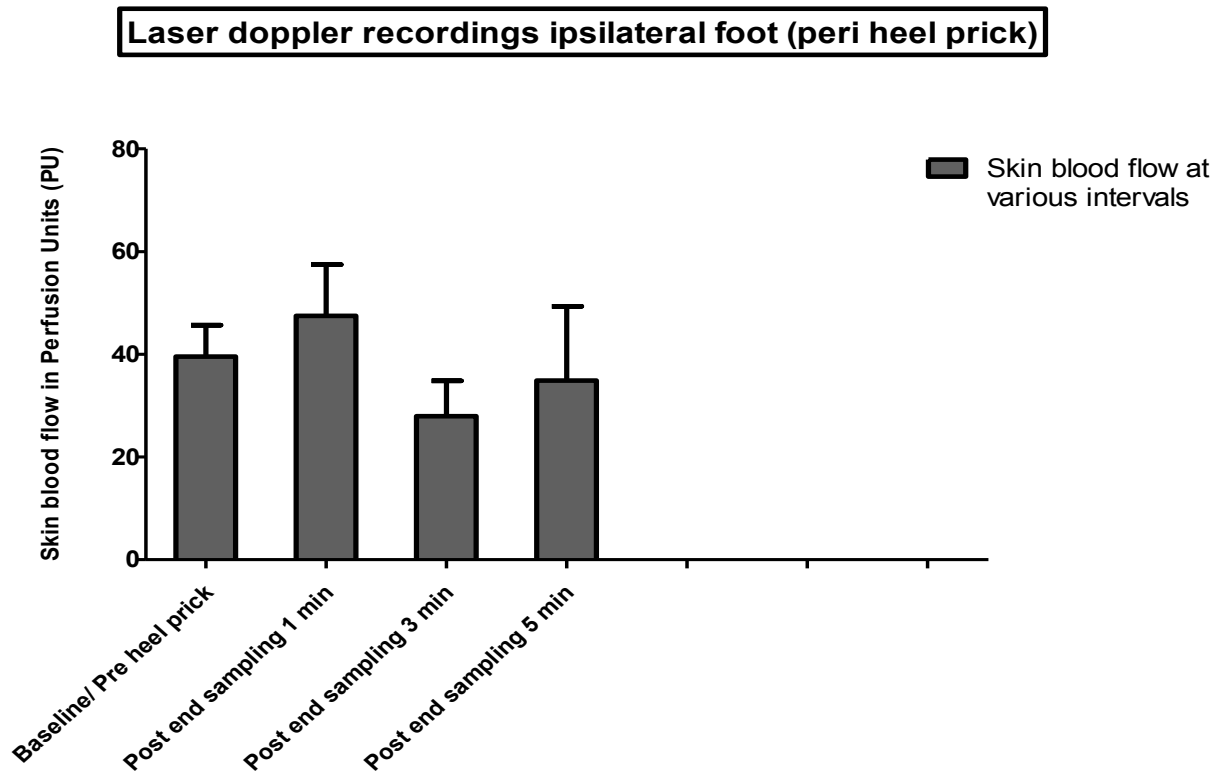


Figure 5.2: Mean blood flow changes in the peri-heel prick (ipsi-lateral foot) recordings following a heel prick (Bar chart showing mean $\pm$ SEM)

Table 5.5 (Appendix A, page 103) and Figure 5.3 illustrate the mean blood flow changes in the contra-lateral limb. We used Mann Whitney test ($n = 29$) to compare the baseline skin blood flow in the contralateral foot with changes at various intervals post heel prick. The analysis did not reveal the results to be statistically significant except post heel prick at 1 minute i.e.

Baseline vs. Post Heel Prick 1 min ($p = 0.03^*$),

Baseline vs. Post Heel Prick 3 min ($p = 0.07$),

Baseline vs. Post Heel Prick 5 min ($p = 0.640$,

Baseline vs. Post End Sampling 1 min ($p = 0.26$),

Baseline vs. Post End Sampling 3 min ($p = 0.86$),

Baseline vs. Post End Sampling 5 min ($p = 0.93$).

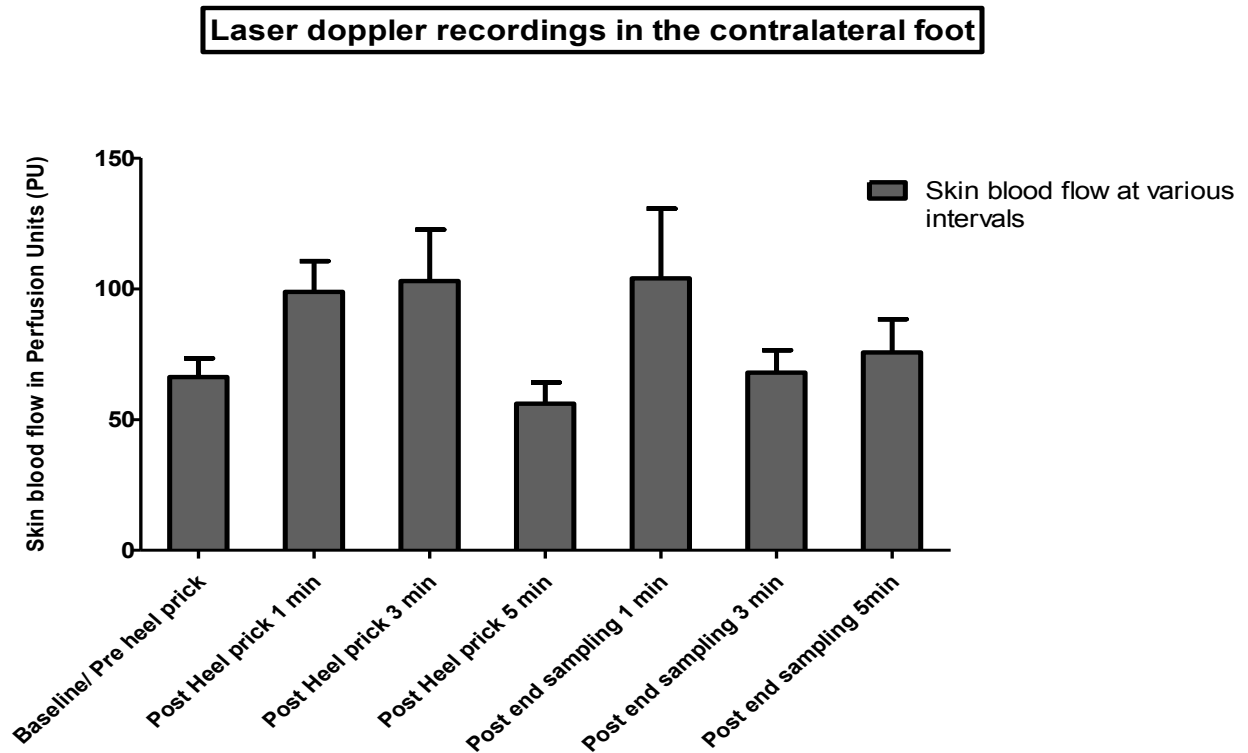


Figure 5.3: Mean blood flow changes in the contra-lateral foot following a heel prick (Bar chart showing mean $\pm$ SEM)

We performed a linear regression analysis of the pre (baseline) and post (peak levels) heel prick skin blood flow of all babies based on their corrected gestational ages at the time of testing.

In the ipsi-lateral (peri-heel prick) group, the corrected ages of babies ranged from 30 weeks to 38 weeks. Using the Spearman test, we looked for the correlation between the pre (baseline) / post (peak) heel prick skin blood flow and the corrected gestational ages of the babies. We used the Mann-Whitney test to compare the pre (baseline) skin blood flow with the post (peak) heel prick levels to look for any changes.

As seen in figure 5.4, there does not appear to be any correlation between either the pre (baseline) skin blood flow ($r -0.526$, $p 0.816$) or the post (peak levels) heel prick skin blood flow ($r 0.063$, $p 0.803$) with gestational age in the peri-heel prick group.

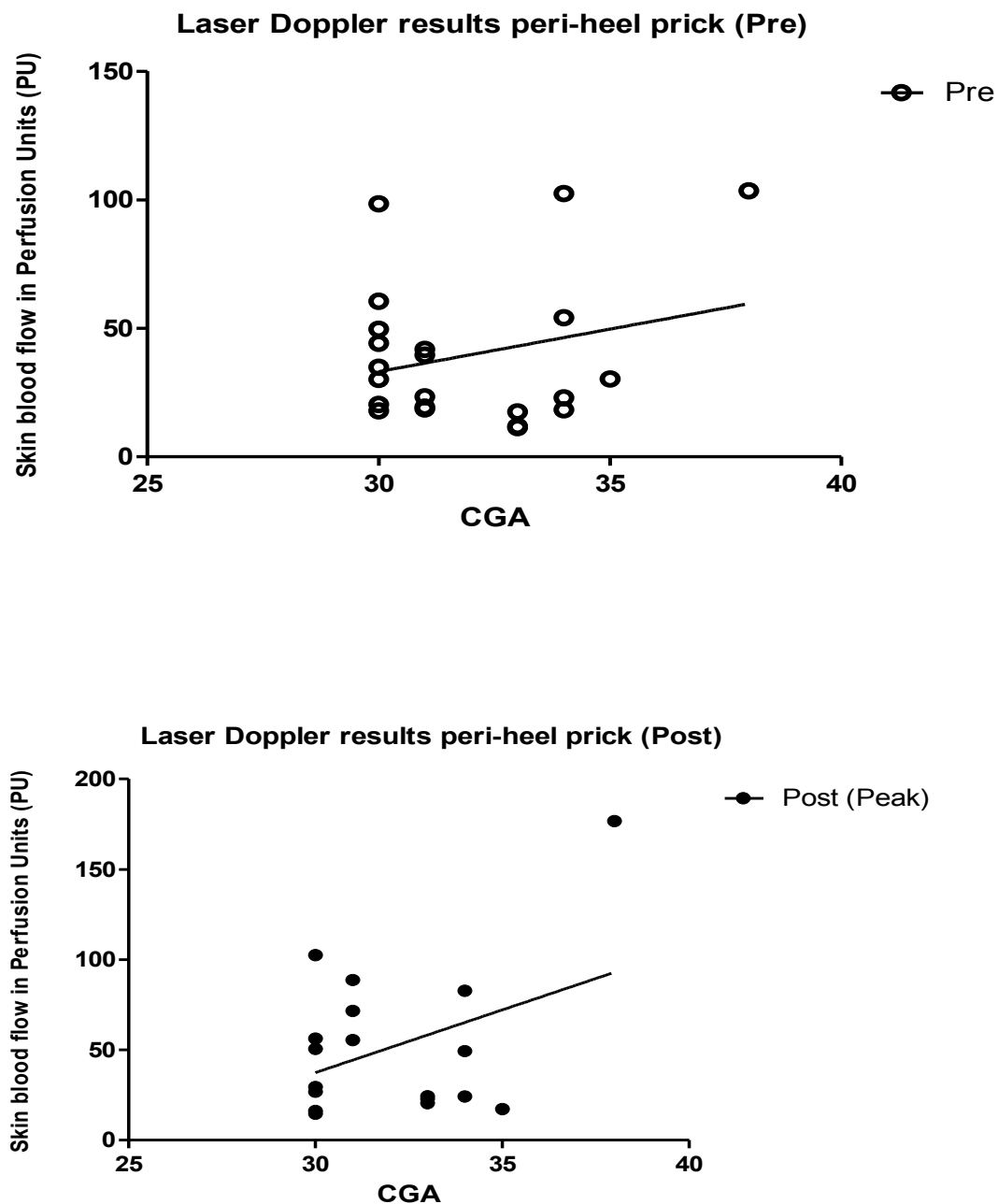


Figure 5.4: Linear regression analysis comparing pre and post heel prick skin blood flow with corrected gestational ages in ipsi-lateral group

Figure 5.5 illustrates that there was no significant increase in skin blood flow post heel prick (p 0.377).

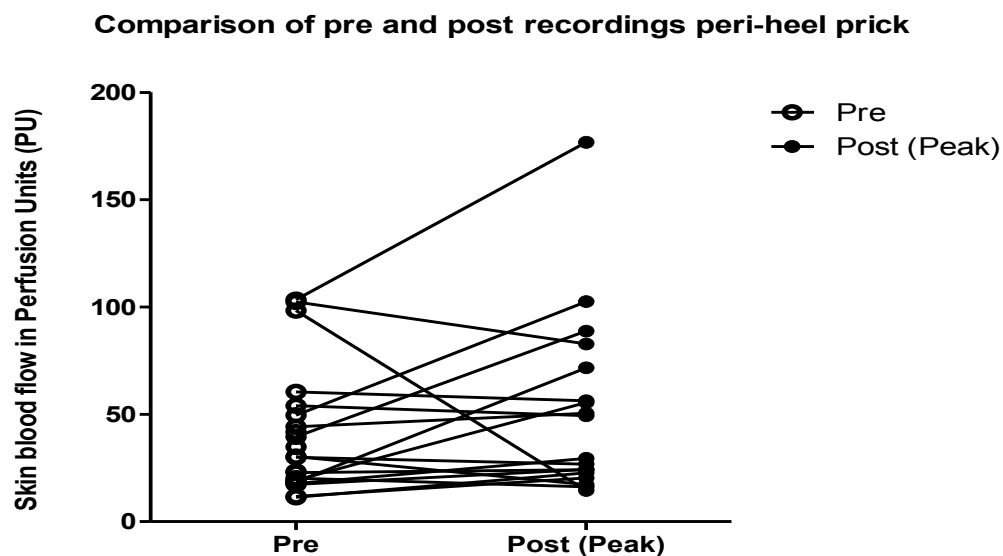
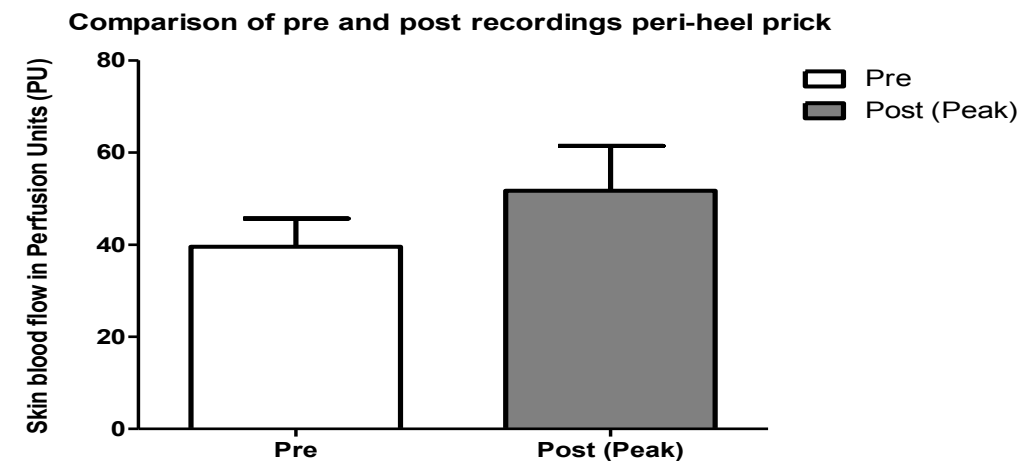


Figure 5.5: Comparing the pre heel prick with post heel prick skin blood flow at all ages in ipsi-lateral group (Top – Bar chart showing mean $\pm$ SEM, Bottom – Before & After plot showing individual mean readings)

In the contra-lateral foot group, the corrected ages of babies ranged from 30 weeks to 42 weeks. As seen in figure 5.6, there appears to be a trend for negative correlation between the pre (baseline) skin blood flow (r -0.318, p 0.099) and the post (peak levels) heel prick skin blood flow (r -0.345, p 0.084) with their gestational ages in the contra-lateral foot group.

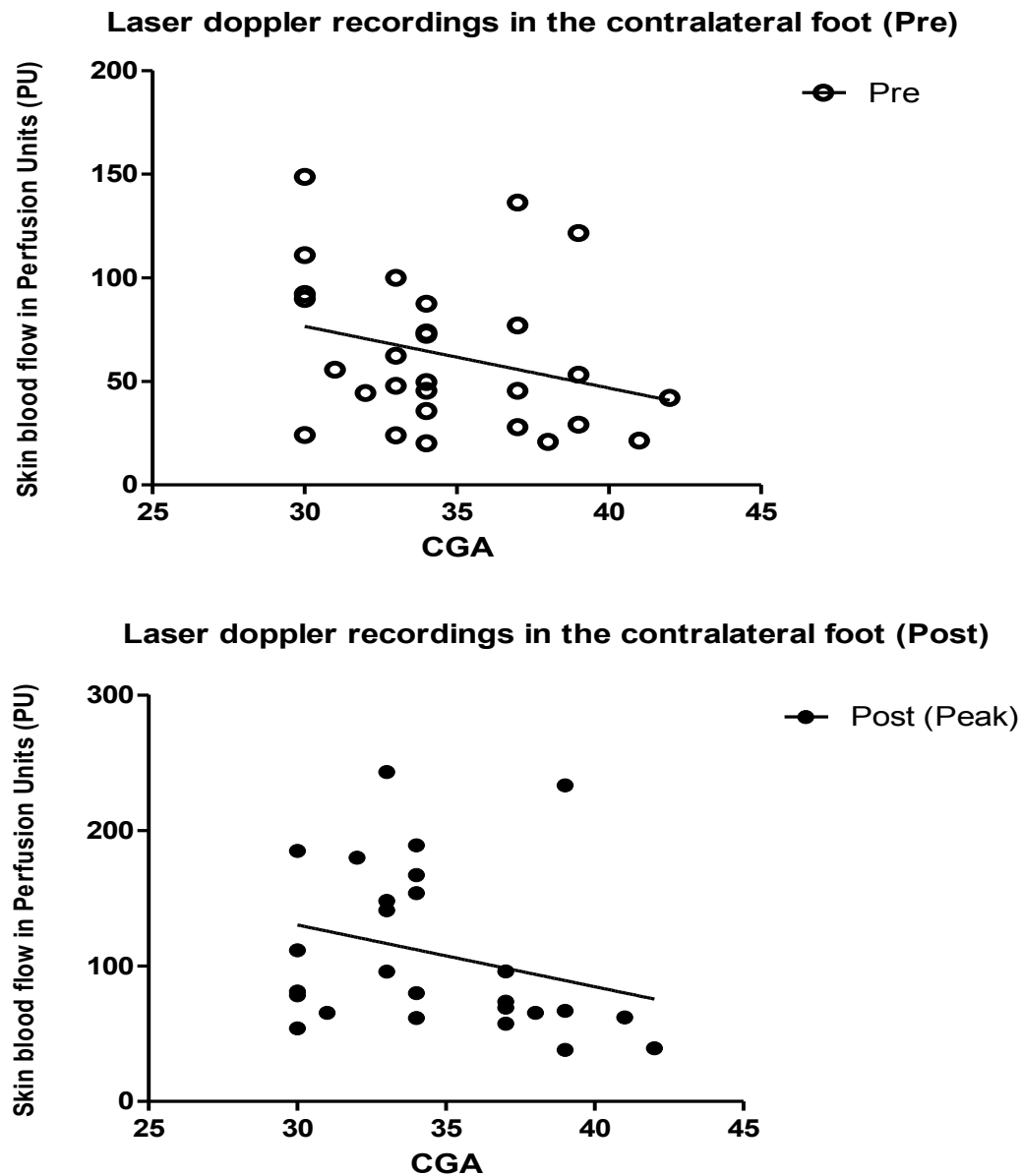


Figure 5.6: Linear regression analysis comparing pre / post heel prick skin blood flow with corrected gestational ages in contra-lateral group

Figure 5.7 illustrates that there was a significant increase in skin blood flow in the contra-lateral limb post heel prick as compared to the pre (baseline) ($p^{**} 0.0016$). However, this result needs to be interpreted with caution as seen the results could be skewed by a few readings (2 values were very high).

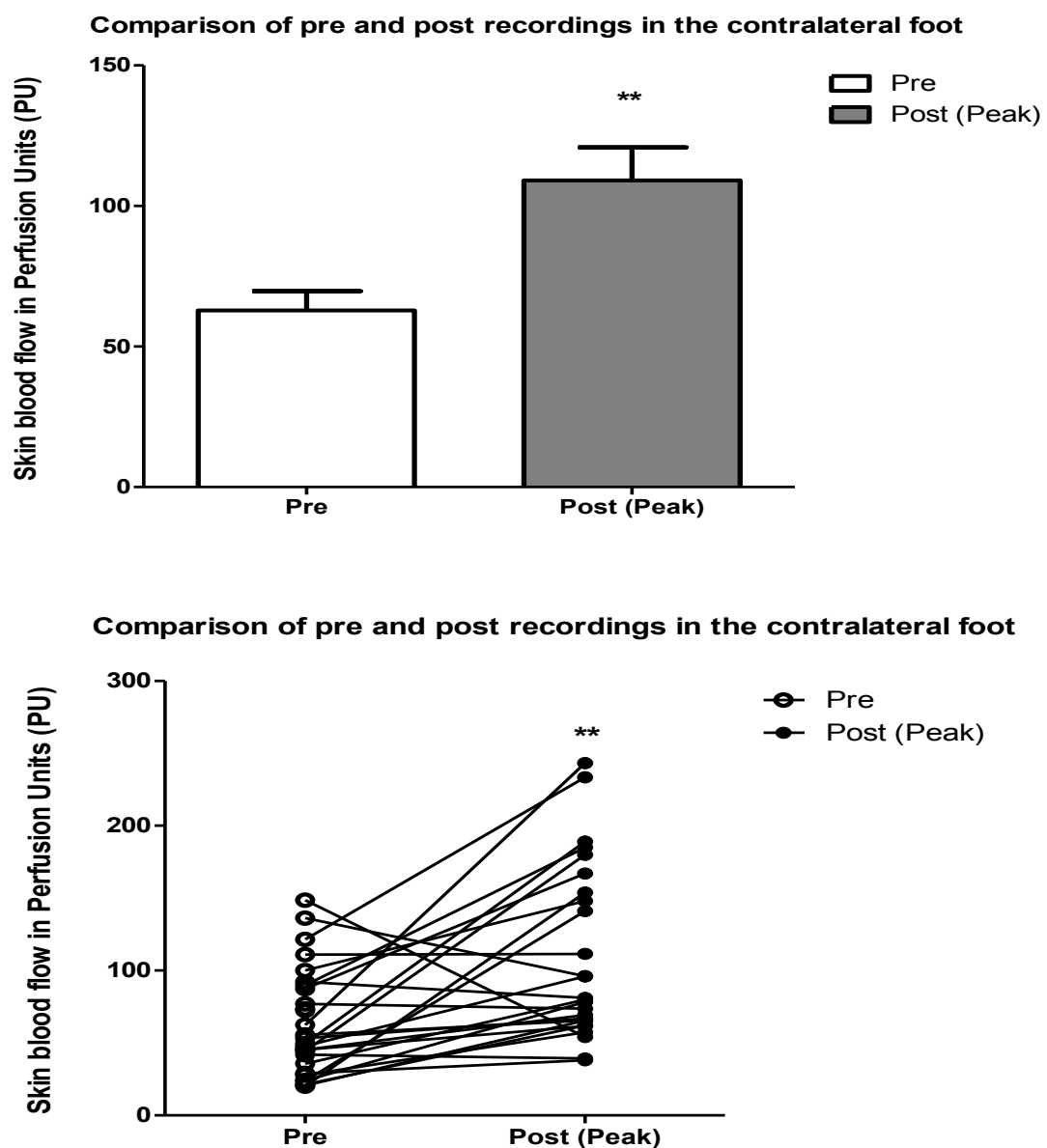


Figure 5.7: Comparing the pre heel prick with post heel prick skin blood flow at all ages in contra-lateral group (Top – Bar chart showing mean $\pm$ SEM, Bottom – Before & After plot showing individual mean readings)

We then used the ratio of post / pre heel prick values to analyse the results with linear regressions fitted to test the relationship with gestational age. In neither set of measurements was the relationship statistically significant ($p = 0.73$, ipsilateral data; $p = 0.67$ contralateral data). So, for the Doppler changes, there is no evidence of a relationship to gestational age, but perhaps this is confounded by other factors.

5.4 DISCUSSION

Changes in skin blood flow in response to various procedures have been studied in neonates by some investigators previously (McCulloch, Ji et al. 1995; Kunzek, Quinn et al. 1997).

In our study we used PeriFlux 4001 Master multichannel laser doppler system (Perimed, Stockholm, Sweden) that works on the principle of doppler shift. A beam of laser-generated monochromatic light is applied to the surface of tissue being studied via a fibre optic probe. Back-scattered light is picked up by sensitive photo detectors via a separate fibre in the probe. The returned signal is analyzed by the instrument giving a value of blood cell movement. The result presented as an arbitrary Perfusion Unit (PU) can be viewed on the instrument, on a chart recorder or using a computer with PeriSoft software. Since the PU is arbitrary, it cannot be given any physiological definition such as the actual number of cells flowing through a given volume of tissue during a given time period. Thus, perfux perfusion values must be compared relative to one another and are not absolute. The measuring depth in the skin means that only movements in micro vessels contribute to the value. In tissues other than skin, movement in large vessels passing near the

surface can influence the measurement. However, the thickness of large vessels is sufficient to exclude most of the laser light. The moving objects measured are mainly red blood cells but leucocytes and platelets also contribute. This technique has advantages of being non invasive or invasive depending on the tissue under study.

Our results have shown a wide range of changes in the blood flow both around the heel prick site ($n = 22$) and in the contra-lateral foot ($n = 29$) after the procedure and the results were not statistically significant. Linear regression analysis in the peri-heel prick group did not show any correlation between either the pre (baseline) skin blood flow ($p = 0.816$) or the post (peak levels) heel prick skin blood flow ($p = 0.803$) with gestational age. Also, there was no significant increase in skin blood flow post heel prick ($p = 0.377$). In the contra-lateral group our study showed a negative correlation between the pre (baseline) skin blood flow ($p = 0.099$) and the post (peak levels) heel prick skin blood flow ($p = 0.084$) with their gestational age. And there was a significant increase in skin blood flow in the contra-lateral limb post heel prick as compared to the pre (baseline) ($p = 0.0016^{**}$). However, this result needs to be interpreted with caution as seen the results could be skewed by a few readings. We then used the ratio of post / pre heel prick values to analyse the results with linear regressions fitted to test the relationship with gestational age. In neither set of measurements was the relationship statistically significant ($p = 0.73$, ipsilateral data; $p = 0.67$ contralateral data). So, for the Doppler changes, there is no evidence of a relationship to gestational age, but perhaps this is also confounded by other factors.

As with the previous analysis, the major limitation of our study was a small sample size. Also, skin blood flow changes are dependent on the baseline skin temperature,

room temperature, baby's cardiovascular status and post heel prick squeezing (in case of peri-heel prick recordings). They are also affected to a large extent by movements (movement artefacts). Hence, skin blood flow changes as measured with laser Doppler may not be useful to monitor response to pain as the results can be altered by many other factors. However, in order for the results to be interpreted with confidence, a much larger study group will have to be looked at. Whether the administration of sucrose to the babies could have altered our results would have been possible to ascertain by doing a subgroup analysis comparing babies who received sucrose with babies who did not receive any sucrose. This was not possible in our study since all babies received oral sucrose prior to heel prick as a part of unit protocol.

CHAPTER 6

USE OF THE NOVEL CONTACT HEAT EVOKED POTENTIAL STIMULATOR (CHEPS) FOR THE ASSESSMENT OF PAIN IN NEONATES

6.1 INTRODUCTION

6.1.1 EVOKED POTENTIALS IN PAIN RESEARCH

6.1.1.1 DEFINITION

EEG has been used to study pain since 1960s, especially the pain evoked potentials that offer an objective way of measuring pain. An evoked potential (EP) is an electrical potential recorded from the nervous system following presentation of a stimulus (Wikipedia ; Andrew S Blum 2007). EPs test and record how quickly and completely the nerve signals reach the brain from the site of the stimulus and hence can be used to examine the functional integrity of somatosensory pathways. An event-related potential (ERP) is a measured brain response that is directly the result of a thought or perception i.e. any stereotyped electrophysiological response to an internal or external stimulus as measured with EEG (Wikipedia ; Wikipedia ; Andrew S Blum 2007).

There are three kinds of evoked potentials in clinical use (Wikipedia ; Andrew S Blum 2007):

1. Brain auditory evoked potentials (BAEP): generated by specific brain regions in response to stimulation of the auditory pathways.
2. Visual evoked potentials (VEP): generated in the occipital cortex in response to visual stimulation.

3. Somatosensory evoked potentials (SSEP or SEP): generated by various portions of the ascending sensory pathways in response to stimulation of peripheral sensory nerves.

6.1.1.2 PHYSIOLOGIC BASIS OF EVOKED POTENTIALS

EP signals can be recorded from cerebral cortex, brain stem, spinal cord and peripheral nerves. EP surface electrodes record changes in extracellular voltage at the skin or scalp surface. When gray matter activity in the cortex or spinal cord is responsible for the main electrical generators of the recorded waveform, the evoked potential is termed a “near-field” potential and when the white matter tracts or subcortical structures are the main generators the evoked potential is called “Far-field” potential.

The components of an EP/ ERP are referred to by a letter indicating polarity of their voltage peak, followed by a number indicating either the latency to maximal amplitude after stimulation (measured in milliseconds) or the component's ordinal position in the waveform, e.g. a negative-going peak that is the first substantial peak in the waveform and often occurs about 100 milliseconds after a stimulus is presented is often called the N100 (indicating its latency) or N1 (indicating that it is the first peak and is negative); it is often followed by a positive peak usually called the P200 or P2.

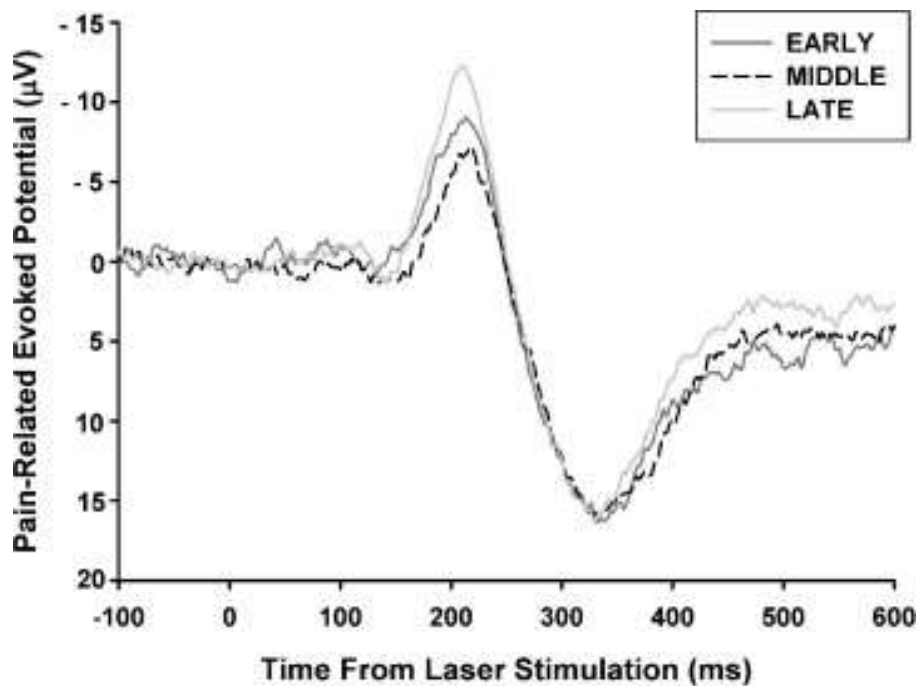


Figure 6.1: Normal Evoked Potential waveform (Edwards et al. 2011)

Evoked potential amplitudes tend to be low, ranging from less than a microvolt to several microvolts. Signal averaging can be done to resolve these low-amplitude potentials against the background of ongoing EEG, ECG, EMG and other biological signals and ambient noise.

Because of their ease of stimulation, reliability of recording sites, and ample existent normative data, median nerve and posterior tibial nerve responses are most commonly used for recording SSEPs.

6.1.1.3 EVOKED POTENTIALS IN NEONATAL PAIN

SSEPs have been used in neonates to assess the functional integrity and maturity of the neural pathways. There is good evidence of recording SSEPs from 30 weeks gestation but not so good in babies less than 28 weeks (Klimach and Cooke 1988;

Pike, Marlow et al. 1997; Smith, Gitau et al. 2000). Some studies have even reported cortical SSEPs from as early as 25th week (Taylor, Boor et al. 1996). These studies have shown a decrease in the peak latency with increased gestational age (Klimach and Cooke 1988; Taylor, Boor et al. 1996; Smith, Gitau et al. 2000). The possible reasons postulated for not being able to record SSEPs in babies born at <28 weeks are technical difficulties in this age group and / or functional immaturity of the nervous system in premature infants (Slater, Fitzgerald et al. 2007).

Most data on normative values for SSEPs have been obtained from adults. Median and posterior tibial nerve SSEPs can be recorded in infancy, although an incompletely developed nervous system renders markedly different recordings than those of adults. The validity of the median nerve SSEPs to predict neurodevelopmental outcomes especially in the preterm infants is not fully established (Mercuri, von Siebenthal et al. 1994; Majnemer and Rosenblatt 1996; Taylor, Boor et al. 1996; Ekert, Taylor et al. 1997).

Amplitudes in evoked potential studies are highly variable and of limited clinical use. Araki et al (1998) in their study concluded that the amplitudes of the cortical components of SSEP in children are greatly influenced by the stimulus rate (Araki, Takada et al. 1999). N2-P2 amplitude differences have been used in adult studies in the assessment of pain (Carmon, Dotan et al. 1978; Carmon, Friedman et al. 1980; Bromm and Scharein 1982; Stowell 1985; Bromm and Lorenz 1998).

A study done by Smit et al (2000) concluded that N1 latency reflects the functional integrity of the somatosensory pathway in the nervous system and can be affected

by conditions like intraventricular haemorrhages etc (Smith, Gitau et al. 2000). In their study, the observed N1 latency at term and at 6 months corrected age suggests that extra uterine maturation of the somatosensory pathway in infants born at less than 30 weeks' gestation is delayed by extra uterine life. However, Tombini et al (2008) concluded that extra uterine life does not affect maturation of somatosensory pathways in preterms without neurological deficit and hence SSEPs could be considered a useful tool for a non-invasive assessment of somatosensory pathways integrity in preterm infants (Tombini, Pasqualetti et al. 2009).

Slater et al (2009) in their study showed the presence of a distinct nociceptive-specific potential in newborn infants (Slater, Worley et al. 2010). The data show that noxious stimulation evokes distinct neuronal activity in the infant brain and that different somatosensory modalities can be discriminated and processed from an early age. The same group demonstrated that infants who are born prematurely and who have experienced at least 40 days of intensive or special care have increased brain neuronal responses to noxious stimuli compared to healthy newborns at the same postmenstrual age (Slater, Fabrizi et al. 2010). They showed that these noxious-evoked potentials are clearly distinguishable from shorter latency potentials evoked by non-noxious tactile sensory stimulation.

6.1.2 CONTACT HEAT EVOKED POTENTIAL STIMULATOR STUDIES

Heat evoked potentials have been used to study nociceptive pathways in adults for many years (Carmon, Mor et al. 1976; Bromm, Neitzel et al. 1983; Bromm and Treede 1984; Bromm and Treede 1987; Bromm and Treede 1987; Bromm and Treede 1991; Morley, Lau et al. 2005). Until recently most studies of thermally

evoked potentials were performed using laser stimulators that selectively excite A δ and C nerve fibres (Bromm, Neitzel et al. 1983; Bromm and Treede 1984). Contact heat pain evoked potentials were first reported by Harkins and colleagues (Harkins, Price et al. 2000; Itskovich, Fei et al. 2000).

Contact heat stimuli provide a more natural thermal activation than lasers, evoking both the sharp, pricking, first pain and slow burning second pain of natural thermal injury (Craig, Chen et al. 2000; Arendt-Nielsen and Chen 2003). In addition, long duration stimuli and large areas on stimulation can also be used (Arendt-Nielsen and Chen 2003). Contact heat evoked potentials were hard to elicit until recently because of the slow temperature rise and fall times of the equipment utilised. This problem has been overcome with the use of a newly developed device, the contact heat evoked stimulator (CHEPS), using which heat pulses can be delivered rapidly with adjustable peak temperatures; thus the differential warm/ heat thresholds of receptors expressed by A δ and C fibres can be stimulated.

CHEPS offers important advantages for studying evoked potentials. It is easy to use in a clinical situation with a dedicated software program to run the system. The superficial burns or erythema sometimes associated with lasers are avoided. The protective eyewear (required for lasers) is unnecessary. It can be used in areas unsuitable for other diagnostic tests (e.g. skin biopsy) such as the face or glabrous skin. Repetitive stimulation or “wind-up” is possible, offering an additional clinical tool for the assessment of small sensory nerve fibre function. CHEPS is also compatible with fMRI, increasing its range of application in the study of evoked potentials and brain activation.

The suitability of CHEPS has already been illustrated in assessing nociceptive pathways in adults, particularly neuropathic pain patients (Atherton, Facer et al. 2007). Evoked potentials to warm temperature stimulus have not been recorded in neonates by any other investigators, but there is a large amount of data from adults, including published data from the Peripheral Neuropathy Unit. Furthermore, the feasibility of recording contact heat evoked potentials in the MRI environment in adults has also been demonstrated (Craig, Chen et al. 2000; Ohara, Crone et al. 2004; Qiu, Noguchi et al. 2006; Granovsky, Granot et al. 2008; Roberts, Papadaki et al. 2008).

Instead of using high temperatures, we have used non-painful, warm (temperatures of 37°C) in our unblinded, pilot study.

6.2 METHODS

6.2.1 SUBJECTS

A total of 9 recordings of contact warm evoked potentials using CHEPS were made in 7 babies whose gestational ages varied from 29+1 to 39 weeks, and who were between 4 and 37 days old (Appendix A, Table 6.1, page 104).

6.2.2 CONTACT WARM EVOKED POTENTIALS RECORDING USING CHEPS

Babies were studied in a cot or in an unhumidified incubator. We performed contact warm stimulation using the contact heat evoked stimulator (CHEPS). CHEPS has

been developed and produced by Medoc (Ramat Yishai, Israel). It has a heat foil thermode (Minco Products, Inc., Minneapolis, MN) with an area of 572.5mm² covered with a 25µm layer of thermo-conductive plastic (Kapton®, thermal conductivity of 0.1 - 0.35 W/m/K at 23 °C). Two thermocouples are embedded 10µm within the conductive coating which contact the skin directly. CHEPS has an adjustable, maximum temperature rise time of 70°C/sec and cooling rate 40°C/sec, with a baseline temperature of 32°C. Heat pulses are delivered rapidly in less than 300 milliseconds with adjustable peak temperatures. Thus the differential warm/heat thresholds of receptors expressed by Aδ and C fibres can be stimulated.



Image 6.1: CHEPS machine with the thermode

We applied a set of twenty stimuli of 37°C over the lumbar spine area with an inter-stimulus interval of 7 seconds. The thermode was moved after each stimulus to

avoid habituation. A repeat set of recordings was then done with the same settings with the thermode placed on the cervico-thoracic spine area.

Electroencephalogram (EEG) was acquired from 6 electrodes (Fz, Cz, Pz, infra-orbital EOG, reference electrode Fpz and ground TP9) and reported from Cz and Fz. Electrodes were contacted with Nuprep electrode gel and impedance maintained below 5k Ω . A sampling rate of 500 Hz was applied for recording. Online low and high pass filters (0.15 Hz and 100 Hz respectively) were also applied to the EEG data recorded.

EEG data was analysed using dedicated software Vision Analyser Version 1.05.0002 (Brain Products GmbH, Munich, Germany). Using the software, the baseline EEG data was filtered (low pass 0.5305 Hz, high pass 40 Hz). It was then segmented around the trigger input from CHEPS, corrected for ocular blinks using the Gratton and Coles correction algorithm, averaged (10 segments), and re-referenced to an average reference.

During this period of monitoring, babies' heart rate, respiratory rate and the oxygen saturations were also noted. None of the babies studied received any analgesic medication including opiates or inotropes.

The protocol was approved by the ethics committee and written consent was taken from parents before the study.

Details of babies' gestational age, postnatal age, birth weight, current weight and other clinical details were obtained from the Badger system (Electronic clinical record system). Gestational age was determined from antenatal ultrasound scans or from the maternal report of the last menstrual period (as obtained from the Badger).

6.2.3 RESULTS

Similar waveforms were noted in all the leads which were obscured by eye movement artefact. No definitive negative (N2) and positive (P2) peaks were identifiable in any of the leads (Figure 6.2).

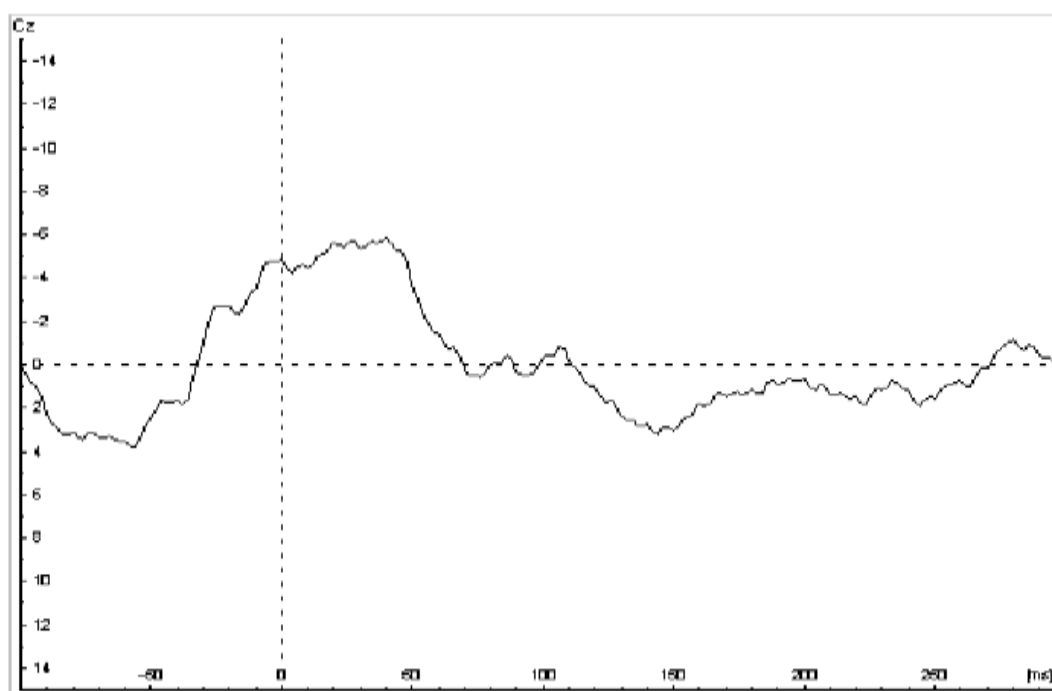


Figure 6.2 Sample warm evoked potential trace recorded from a baby and extracted from Cz (Note no identifiable N2 and P2)

Normally the latencies of heat evoked potentials are measured from the first definitive negative peak (N2), and the amplitude measured peak to peak (N2 to P2). However, it was not possible to measure the latencies or the amplitudes of the waveforms in any of our traces.

6.3 DISCUSSION

In adults, reliable and quantifiable heat evoked potentials have been produced with consistent peak latencies and amplitudes and with significant correlation to pain intensity scores (Chen, Xu et al. 2001; Le Pera, Valeriani et al. 2002). The suitability of CHEPS in adults has already been illustrated by several authors (Craig, Chen et al. 2000; Ohara, Crone et al. 2004; Qiu, Noguchi et al. 2006; Atherton, Facer et al. 2007; Granovsky, Granot et al. 2008; Roberts, Papadaki et al. 2008). However, this was not the case in our study. In our study we could not demonstrate any clear waveform patterns with definitive negative and positive peaks.

A study done by another group previously also indicated that responses in cortical activity recorded by EEG are not useful for clinical assessment of infants' responses to noxious stimuli (Norman, Rosen et al. 2008). In this study, painful stimuli induced changes in the EEG record which correlated with behavioural pain responses. However, these changes occurred mainly within the highest frequencies studied, were not consistently lateralized, and were more pronounced when the child was awake. Careful analysis suggested that a major part of these responses came from muscle activity rather than from cerebral cortex and were hence not reliable.

The reasons for not being able to demonstrate any definitive wave patterns could be multiple. Firstly, the temperature that we used in our study was only warm (37°C) instead of painful hot temperature as used by the previous investigators in adults. Any higher temperature could not be used in babies especially preterm because of ethical and clinical (fragile skin, risk of burns) reasons. Thus it is possible that this temperature may have not been enough to stimulate the heat thresholds of receptors expressed by Aδ and C fibres. Secondly, it is possible that the thermo receptors in newborn babies are not physiologically mature enough to recognise and transmit signals with a temperature stimulus of 37°C. Lastly, the responses seen in all the leads corresponded to the ocular responses (EOG). In other words, the waveforms identified probably represented ocular artefacts, illustrating the technical difficulties (lack of control of eye movements) in recording evoked potentials in babies.

The major limitation of our study again was a small sample size. Again, whether the administration of sucrose to the babies could have altered our results would have been possible to ascertain by doing a subgroup analysis comparing babies who received sucrose with babies who did not receive any sucrose. This was not possible in our study since all babies received oral sucrose prior to heel prick as a part of unit protocol.

CHAPTER 7

CONCLUSION

7.1 MAIN FINDINGS AND THEIR SIGNIFICANCE

We investigated 48 babies using techniques that may evaluate the function of nociceptor-specific nerve fibres and central pain pathways, in comparison with currently used behavioural and physiological indicators. These techniques included “objective” physiological responses to procedural pain, such as skin axon-reflex vasodilatation (flare) responses, and novel contact cerebral evoked potentials in response to warm (non-painful) stimuli.

Our study showed an increase in both mean baseline (pre or resting) and post heel prick sweat levels with the gestational age of babies. However, when comparing the mean pre heel prick levels with the mean post heel prick levels at all ages, there was no significant change. Our study has shown some evidence that the change in sweat measurements (post as a ratio of pre) are related to gestational age but that the pre-heel prick results and perhaps the age of the baby are confounding factors in this relationship. Our study has shown definite palmar emotional sweating response in babies ≥ 36 -37 weeks, which varied greatly with their state of arousal. The sweat levels were lowest with babies asleep and highest when crying. Previous studies (Harpin and Rutter 1982a; Harpin and Rutter 1982b) had shown a striking increase in sweat levels post heel prick, but our study failed to demonstrate this effect. Babies born ≤ 36 weeks showed only slight or no increase in palmar water loss post heel prick stimulus. The sweat levels did not vary significantly in the three groups based on birth weight as well. We conclude that the palmar sweat response reflects the emotional state and correlates with the state of arousal, but this response was not specific to pain. As palmar/ plantar sweat loss reflects the sympathetic response to

stress, it may not be a reliable tool in assessing pain in neonates. Moreover, the wide range of sweat levels in various age groups makes it difficult to use sweat levels to monitor stress / pain response.

In our study we could not demonstrate a clear relationship between the behavioural responses (as assessed using PIPP score) and an objective marker of stress (palmar sweat levels). Moreover, inter-observer variability was also noted in the PIPP scores. This demonstrates how difficult it can be to use a pain assessment score in a clinical setting (Neonatal Intensive Care Unit) especially if the babies are sick, in closed incubators, on ventilators or on various medications that could alter their neurological status.

Our study has shown a wide range of changes in the blood flow both around the heel prick site and in the contra lateral foot after the procedure. We could not demonstrate any evidence of a relationship between skin blood flow and gestational age, but perhaps this could be confounded by other factors. Skin blood flow can be altered by changes in baseline skin temperature, room temperature, baby's cardiovascular status and post heel prick squeezing (in case of peri-heel prick recordings) and is subject to movement artefacts. Hence, skin blood flow changes as measured with laser Doppler may not be useful to monitor response to pain.

We recorded SSR in only one baby and hence would need a much larger sample in order to interpret the results with confidence, but it appeared that further studies were unlikely to show correlations with procedural pain.

In our study the waveforms identified with CHEPS stimuli probably represented ocular artefacts, and we could not demonstrate any clear waveform patterns in response to a temperature stimulus of 37°C. The reasons for this could be the temperature of 37°C failing to sufficiently stimulate thermo receptors (A δ and C fibres), functional immaturity of these thermo receptors in newborns or the technical difficulties (eye movements) in recording evoked potentials in babies. Hence, future studies need to look at ways of controlling various parameters that may potentially produce artefacts and obscure the definitive waveforms.

One of our study aims was to investigate whether drug and non-drug pain relief treatments given as part of routine clinical care have any effect on the above results. However we could not study this effect as all babies received oral sucrose (as a part of unit protocol) before the heel prick procedures. Babies enrolled for our study did not receive any other analgesics. Since all babies received sucrose prior to the heel prick testing, any subgroup analysis (sucrose vs. no sucrose) could not be done. Also, whether the administration of sucrose to the babies could have altered our results would have been possible to ascertain by doing a subgroup analysis comparing sucrose vs. no sucrose groups that was not possible in our study.

The major limitation to our study was a very small sample size. This could be explained on the basis of three main reasons. Firstly, having approached 110 parents for the study, only 56 consented (about 50%). Out of those, we could perform our studies on 48 babies only as rest of the babies were either discharged or transferred to other units before we could attempt any studies. The term 'pain research' itself creates anxiety and concerns amongst parents whose stress levels

are already very high due to the admission of their baby to the unit, explaining the low recruitment rate in our study. One of the main challenges that neonatal and paediatric research faces is consent. Enrolment of children into studies requires informed consent of the parents. Previous studies have shown that the main barriers to the children's recruitment into trials may be factors like parental misconceptions about perceived benefits & risks of research, randomisation etc (Nabulsi, Khalil et al. ; Harth and Thong 1995; Stephenson and Walker 1996; Zupancic, Gillie et al. 1997; Langley, Halperin et al. 1998; Hayman, Taylor et al. 2001). Secondly, as we were performing different studies like sweat rate testing, evoked potentials etc at different periods of time, we could only study the babies whose parents had given consent at that particular time. So, perhaps focussing on only one study like evoked potentials for the duration of the project would have been a more sensible option. Finally, grouping of babies based on their gestational ages and further division based on postnatal ages reduced the sample sizes even further. Our pilot study has illustrated the difficulties one faces whilst doing studies in neonates. Factors like unpredictability of responses at different gestational and postnatal ages (related to the functional maturity of nervous system) and technical difficulties like lack of control over limb and body movements, ocular movements, state of arousal and electrical interference from the equipment used in intensive care settings make neonatal research challenging. Our study has also highlighted the difficulties that one encounters in a neonatal intensive care in using any pain assessment scores especially in babies who are in closed incubators, on respiratory support or with neurological impairment etc.

7.2 FUTURE DIRECTIONS

Bedside non-invasive techniques like EEG, Event related potentials and NIRS have shown promising results in their usefulness to detect cortical activation related to painful events like heel pricks. However, further studies looking at their utility, feasibility and clinical significance during differing painful situations, including ongoing pain, and within varying populations are required. Large samples coming from multiple centres will allow robust analyses of the range of painful conditions using various assessment techniques as well as individual variability.

The advances in techniques to assess pain in adults (like fMRI, Evoked potentials etc) have the potential to greatly advance the assessment of pain and its treatment in neonates. Since these tests do not rely on the co-operation of the subject; they are ideally suited for the assessment of neonates and “pre-verbal” children. These techniques can be adapted for use in neonates, and have the potential to advance the assessment of pain and its treatment in neonates and pre-verbal children. Simultaneous acquisition of fMRI images whilst recording evoked potentials to various painful procedures could be pivotal in our understanding of pain transmission in neonates.

Further studies with a large cohort of babies in both preterm and term neonates and in well as well as sick neonates could confirm some of the data presented in this thesis, and make a contribution to the understanding of mechanisms of pain in neonates, and contribute to the development of ways of assessing and managing pain in this group of vulnerable population in a better way. A major challenge would

be to find out the best approaches to allow a smooth transmission of research findings to the bedside.

APPENDIX A – TABLES

Table 2.1: Details of babies studied at for palmar sweat levels

Gestation (weeks)	Number of babies	Number of measurements	Postnatal age range in days	Birth weight (Kg)	
				Mean	Range
37 or more	4	4	2-6	3.34	2.16-4.49
34-36+6	3	4	1-13	1.78	1.45-2.0
30-33+6	1	3	14-28	1.41	1.41
24-29+6	4	7	2-43	1.02	0.8 - 1.13

Table 2.2: Babies' observations (range) during sweat rate recording

	Temperature (°C)	Heart Rate (per min)	Respiratory Rate (per min)	Oxygen saturation (%)
Range	36.5-37.3	106-185	17-93	79-100

Table 2.3: Palmar sweat levels in babies at various gestational ages

Gestation (weeks)	Post-natal age	Baseline (Pre heel prick)		Post heel prick	
		Palmar water loss (g/m ² per hour)		Palmar water loss (g/m ² per hour)	
		Mean	Mean	Mean	Range
≥ 37	1st week	63.01	68.53	68.53	5-9
	2nd week	-	-	-	
	3rd week	-	-	-	
	>3 weeks	-	-	-	
34-36+6	1st week	23.47	26.87	26.87	4-16
	2nd week	11.77	16.7	16.7	7
	3rd week	-	-	-	
	>3 weeks	-	-	-	
30-33+6	1st week	-	-	-	
	2nd week	6.22	8	8	16
	3rd week	9.2	10	10	18
	>3 weeks	10.7	19.8	19.8	11
24-29+6	1st week	11.7	10	10	5-17
	2nd week	14.8	18.45	18.45	6-18
	3rd week	-	-	-	-
	>3 weeks	25.6	33.6	33.6	6-9

Table 2.4: Palmar sweat levels in babies (baseline and post heel prick) in the three groups based on birth weight

Baby groups	Post natal ages	Sweat levels in g/m ² per hour			
		Baseline (Pre heel prick)		Post heel prick	
		Mean	Range	Mean	Range
AGA	1st week	37.74	8.5-109.6	29.4	7-97.2
	2nd week	9.38	4.4-15.6	12.41	7-18.7
	3rd week	9.21	6.4-10.1	9.97	7.6-11.9
	>3 weeks	20.22	3.1-38.6	28.8	13.7-48.5
SGA	1st week	30.16	5.5-97.6	36.28	13.2-100.2
	2nd week	14.38	6.3-24.9	18.53	11.7-24.4
	3rd week	-	-	-	-
	>3 weeks	-	-	-	-
LGA	1st week	28.54	15.2-33	35.18	33.9-36.1
	2nd week	-	-	-	-
	3rd week	-	-	-	-
	>3 weeks	-	-	-	-

Table 3.1 The Premature Infant Pain Profile, adapted from (Stevens, Johnston et al. 1996)

Indicator	Finding	Points
Gestational age	≥ 36 weeks	0
	32-35+6 weeks	1
	28-31+6 weeks	2
	< 28 weeks	3
Behavioural state	Active/ awake, eyes open, facial movements	0
	Quiet/ awake, eyes open, no facial movements	1
	Active/ sleep, eyes closed, facial movements	2
	Quiet/ sleep, eyes closed, no facial movements	3
Heart rate maximum	0-4 beats/ min increase	0
	5-14 beats/ min increase	1
	15-24 beats/ min increase	2
	≥25 beats/ min increase	3
Oxygen saturation minimum	0-2.4% decrease	0
	2.5-4.9% decrease	1
	5.0-7.4% decrease	2
	7.5% decrease or more	3
Brow bulge	None (≤ 9% of time)	0
	Minimum (10- 39% of time)	1
	Moderate (40- 69% of time)	2
	Maximum (≥70% of time)	3
Eye squeeze	None (≤ 9% of time)	0
	Minimum (10- 39% of time)	1
	Moderate (40- 69% of time)	2
	Maximum (≥70% of time)	3
Nasolabial furrow	None (≤ 9% of time)	0
	Minimum (10- 39% of time)	1
	Moderate (40- 69% of time)	2
	Maximum (≥70% of time)	3

Table 3.2 Illustrating babies' details with the PIPP scores and mean sweat levels

Recording	Baby's CGA	Day	PIPP Score 1	PIPP Score 2	Mean PIPP Score	Mean sweat in g/m ² per hour	
						Baseline	Post heel prick
1	29+3	5	9	8	8.5	12.5	8.9
2	31+4	35	10	7	8.5	32.6	30.74
3	34+4	35	6	6	6	24.2	28.7
4	32+5	43	5	4	4.5	20.01	39.75
5	37	1	6	0	3	23	32.12
6	38+4	2	1	0	0.5	79	97.6
7	38+2	2	6	2	4	57.13	80.81

Table 5.1: Details of babies studied for ipsi-lateral recordings

Gestation (weeks)	Number of babies	Number of measurements	Postnatal age range in days	Birth weight (Kg)	
				Mean	Range
≥37	1	1	8	-	2.97
34-36+6	1	1	3	-	1.64
30-33+6	5	5	5-19	1.36	1.16-1.5
24-29+6	8	12	2 - 26	1.16	0.86-1.34

Table 5.2: Details of babies studied for contra-lateral recordings

Gestation (weeks)	Number of babies	Number of measurements	Postnatal age range in days	Birth weight (Kg)	
				Mean	Range
≥37	4	4	2-3	3.04	1.77-3.94
34-36+6	-	-	-	-	-
30-33+6	3	4	6-48	1.17	0.92-1.66
24-29+6	6	19	2 - 77	1.02	0.67-1.46

Table 5.3: Babies' observations (range) during blood flow recording

Range	Temperature (°C)	Heart Rate (per min)	Mean Blood Pressure (mm Hg)	Respiratory Rate (per min)	Oxygen saturation (%)
Ipsi- lateral	36.5-37.1	131-158	38-39	31-76	90-100
Contra- lateral	36.2-37.3	119-165	36-43	28-77	88-100

Table 5.4: Skin blood flow changes in the ipsi-lateral foot

Skin blood flow measured in PU	Mean	Range
Pre heel prick	39.57	13.24-224.85
Post end sampling 1 min	47.5	7.54-231
Post end sampling 3 min	27.96	7.87-135.04
Post end sampling 5 min	34.9	33.08-72.69

Table 5.5: Skin blood flow changes in the contra-lateral foot

Skin blood flow measured in PU	Mean	Range
Pre heel prick	66.24	11.81-750.86
Post heel prick 1 min	98.8	8.76-999.97
Post heel prick 3 min	102.94	17.27-999.97
Post heel prick 5 min	56.04	17.52-189.03
Post end sampling 1 min	104.04	11.11-999.97
Post end sampling 3 min	67.95	7.14-333.62
Post end sampling 5 min	75.61	5.62-579.04

Table 6.1: Details of babies studied with CHEPS

Gestational Age	Corrected Gestational Age (on test date)	Age (in days)	Birth weight	Current weight (on test date)
29+1	30	6	1.3 Kg	1.3 Kg
31+2	36+3	36	0.92 Kg	1.22 Kg
29+4	31	10	1.46 Kg	1.46 Kg
29+4	32	17	1.46 Kg	1.6 Kg
32+3	33+5	9	1.57 Kg	1.57 Kg
39	39+4	4	3.16 Kg	3.16 Kg
29+2	33+4	30	1.2 Kg	1.54 Kg
29+2	34+4	37	1.2 Kg	1.54 Kg
31+2	34	19	1.63 Kg	1.87 Kg

APPENDIX B – ADDITIONAL ANALYSIS

PALMAR SWEAT RATE SUBGROUP ANALYSIS BASED ON GESTATIONAL AGE:

Babies born at 24-29+6 weeks showed little variation in the palmar sweat loss in the first 3 weeks of life (Using Mann Whitney test, $p = 1.0$ with $n = 2$ in week 1, $n = 2$ in week 2, $n = 0$ in week 3 and $n = 3$ in week 4). This variation became slightly more pronounced as babies approached a corrected gestational age of 36 weeks (Figure 2.1).

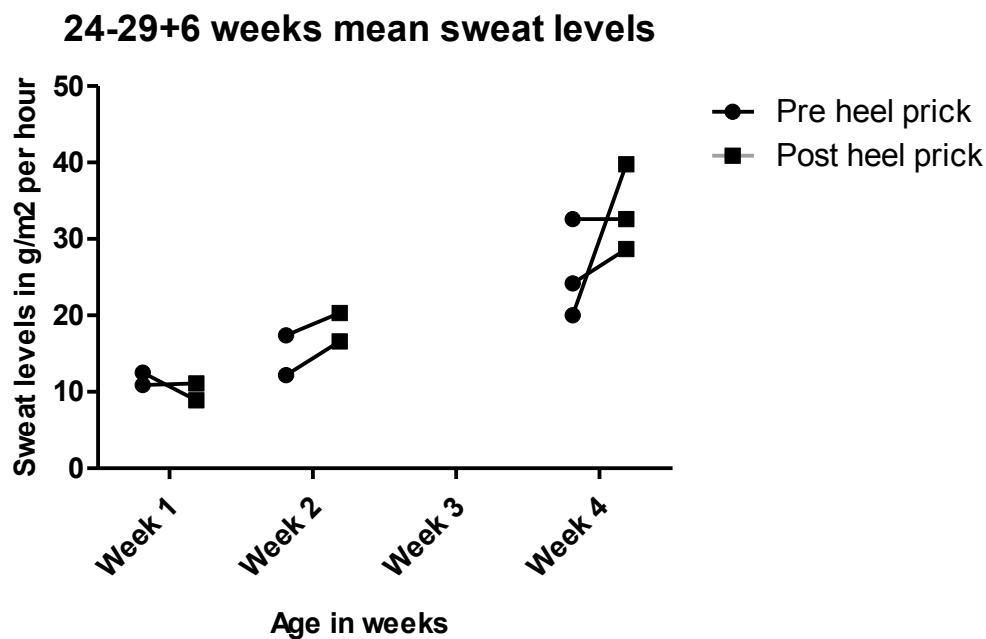


Figure 2.1 Mean sweat levels in g/m² per hour pre (baseline) and post heel prick in babies born at < 30 weeks (Before & After plot showing individual mean readings)

Babies born at 30-33+6 weeks also showed minimal variations in the palmar water loss till the third week of life with the variation slightly more pronounced after 3 weeks of life (Using Mann Whitney test, $p = 0.7$ with $n = 0$ in week 1, $n = 1$ in week 2, $n = 1$ in week 3 and $n = 1$ in week 4) (Figure 2.2).

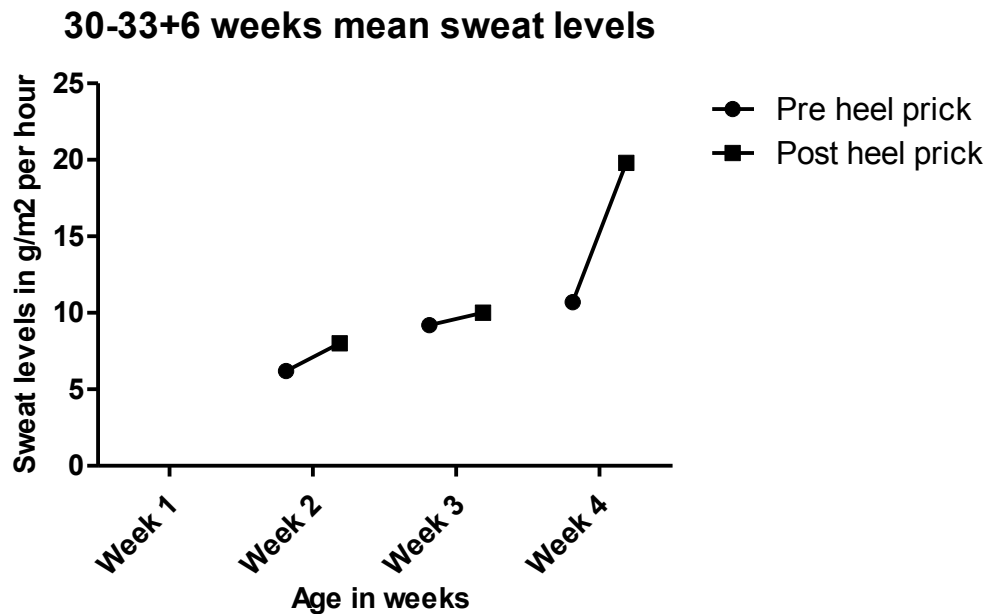


Figure 2.2: Mean sweat levels in g/m² per hour pre (baseline) and post heel prick in babies born at 30-33+6 weeks (Before & After plot showing individual mean readings)

Babies born at 34-36+6 weeks showed only slight but definite increases in the palmar sweat loss in the first and second week of life. However, we could not perform any statistical tests because of low numbers ($n = 3$ in week 1, $n = 1$ in week 2, $n = 0$ in week 3 and $n = 0$ in week 4) (Figure 2.3).

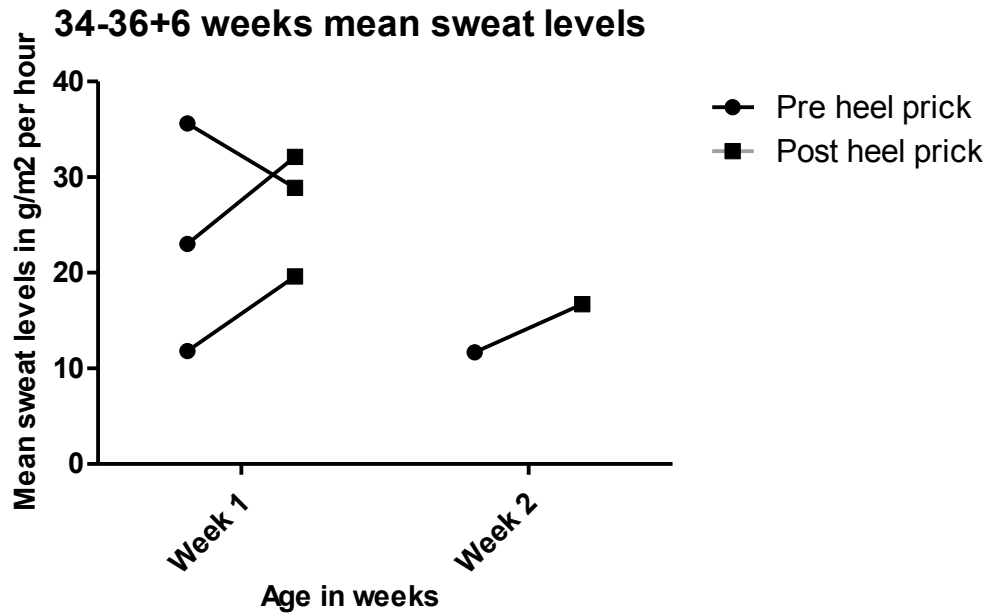


Figure 2.3: Mean sweat levels in g/m² per hour pre (baseline) and post heel prick in babies born at 34-33+6 weeks (Before & After plot showing individual mean readings)

Babies born at ≥ 37 weeks showed a definite palmar water loss with some increase post heel prick but the range was very wide (Figure 2.4). The increase in the palmar water loss post heel prick stimulus was not as striking as the previous studies (Harpin and Rutter 1982; Harpin and Rutter 1982). Moreover, sweat response in this age group correlated more with the state of arousal than with the heel prick stimulus. It was seen that the sweat rate was significantly higher in babies who were awake and distressed (crying as hungry) than babies who were asleep. This higher sweat rate in babies who were crying prior to heel prick did not change significantly after the heel prick and the levels came down after the babies settled down (Figure 2.4).

However, as with the previous group we could not perform any statistical tests because of low numbers (n = 4 in week 1, n = 0 in week 2, n = 0 in week 3 and n = 0 in week 4).

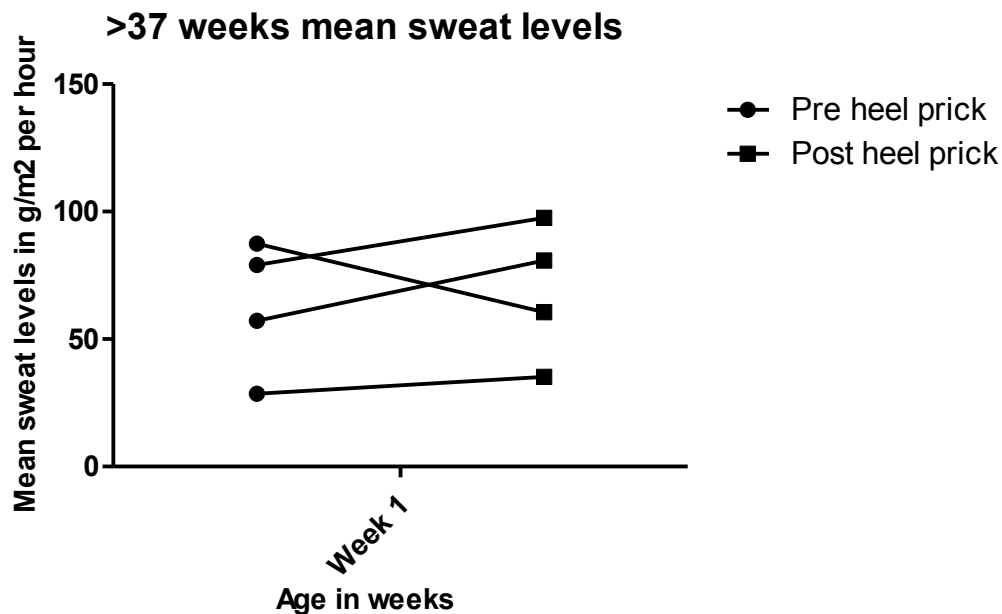


Figure 2.4: Mean sweat levels in g/m² per hour pre (baseline) and post heel prick in babies born at ≥ 37 weeks (Before & After plot showing individual mean readings)

Case study (Figure 2.7):

This is a continuous trace recording of the sweat levels pre heel prick (baseline) and post heel prick in a 2 day old baby who was born at 38 weeks. It was noted that the sweat levels started increasing with the onset of crying (which was independent of heel prick procedure) and started coming down as the baby stopped crying and settled down.

Continuous sweat recording in a term baby

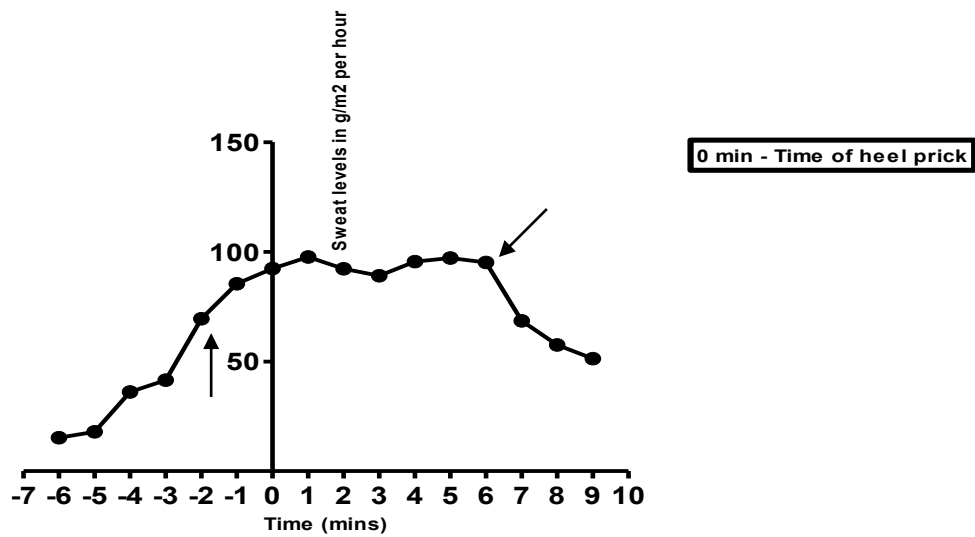


Figure 2.7: Sweat response recorded in a 2 day old baby born at 38 weeks

(Arrows marking the start and end of crying)

PALMAR SWEAT RATE SUBGROUP ANALYSIS BASED ON BIRTH WEIGHT

In order to find out if there were any differences in the mean baseline and post heel prick sweat levels in babies with different birth weights, the babies were divided into three major groups:

1. Appropriate for gestational age (AGA): Birth weight 10th-90th centile for gestational age
2. Small for gestational age (SGA): Birth weight < 10th centile for gestational age
3. Large for gestational age (LGA): Birth weight > 90th centile for gestational age

The mean sweat levels and the range for each weight group were calculated at different postnatal ages as before (grouped as 1st week, 2nd week, 3rd week and >3 weeks).

Our study did not show any difference in the mean sweat levels in the three groups. As seen in Table 2.4 (Appendix A, page 99), the mean sweat levels in the three groups at various postnatal ages were variable with no direct correlation with the heel prick procedure.

Figure 2.8 illustrates the mean sweat levels (mean of all readings at different postnatal ages) in babies in the three groups. Although the mean sweat levels post heel prick were higher than the levels before heel prick (baseline), there did not appear to be any statistically significant difference in mean sweat levels amongst the three groups i.e. AGA, SGA and LGA (Mann Whitney test, $p = 0.4$).

Mean sweat levels in babies in three groups based on birth weight

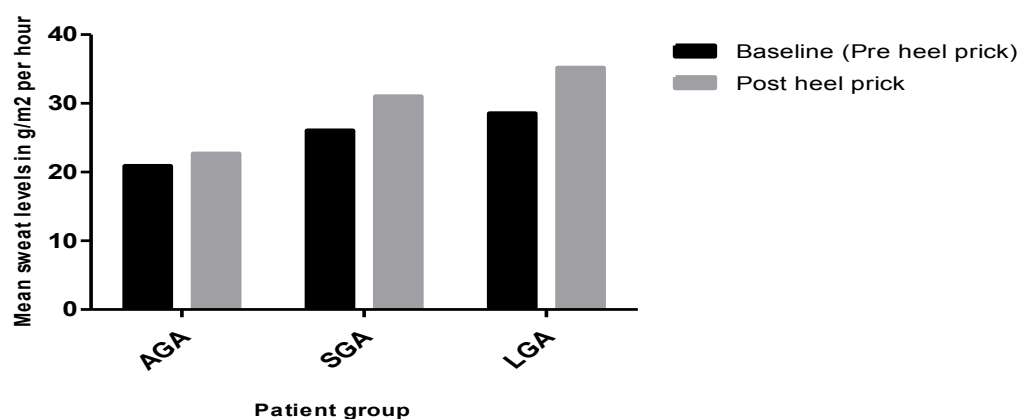


Figure 2.8: Mean baseline and peak sweat levels in AGA, SGA and LGA babies

REFERENCES

- Als, H., F. H. Duffy, et al. (1996). "Effectiveness of individualized neurodevelopmental care in the newborn intensive care unit (NICU)." Acta Paediatr Suppl **416**: 21-30.
- Anand, K. J. (2000). "Pain, plasticity, and premature birth: a prescription for permanent suffering?" Nat Med **6**(9): 971-3.
- Anand, K. J. and D. B. Carr (1989). "The neuroanatomy, neurophysiology, and neurochemistry of pain, stress, and analgesia in newborns and children." Pediatr Clin North Am **36**(4): 795-822.
- Anand, K. J. and P. R. Hickey (1987). "Pain and its effects in the human neonate and fetus." N Engl J Med **317**(21): 1321-9.
- Andrew S Blum, S. B. R. (2007). The Clinical Neurophysiology Primer, Humana Press Inc.
- Andrews, K. and M. Fitzgerald (1994). "The cutaneous withdrawal reflex in human neonates: sensitization, receptive fields, and the effects of contralateral stimulation." Pain **56**(1): 95-101.
- Andrews, K. and M. Fitzgerald (1999). "Cutaneous flexion reflex in human neonates: a quantitative study of threshold and stimulus-response characteristics after single and repeated stimuli." Dev Med Child Neurol **41**(10): 696-703.
- Araki, A., A. Takada, et al. (1999). "The effects of stimulus rates on the amplitude of median nerve somatosensory evoked potentials: the developmental change." Brain Dev **21**(2): 118-21.
- Arendt-Nielsen, L. and A. C. Chen (2003). "Lasers and other thermal stimulators for activation of skin nociceptors in humans." Neurophysiol Clin **33**(6): 259-68.
- Argyriou, A. A., I. Tsolakis, et al. (2006). "Sympathetic skin response in patients with peripheral arterial occlusive disease." Clin Neurophysiol **117**(2): 414-9.
- Arunodaya, G. R. and A. B. Taly (1995). "Sympathetic skin response: a decade later." J Neurol Sci **129**(2): 81-9.
- Atherton, D. D., P. Facer, et al. (2007). "Use of the novel Contact Heat Evoked Potential Stimulator (CHEPS) for the assessment of small fibre neuropathy: correlations with skin flare responses and intra-epidermal nerve fibre counts." BMC Neurol **7**: 21.
- Baba, M., Y. Watahiki, et al. (1988). "Sympathetic skin response in healthy man." Electromyogr Clin Neurophysiol **28**(5): 277-83.
- Baird, A. A., J. Kagan, et al. (2002). "Frontal lobe activation during object permanence: data from near-infrared spectroscopy." Neuroimage **16**(4): 1120-5.
- Barker, D. P. and N. Rutter (1995). "Exposure to invasive procedures in neonatal intensive care unit admissions." Arch Dis Child Fetal Neonatal Ed **72**(1): F47-8.
- Bauchner, H., A. May, et al. (1992). "Use of analgesic agents for invasive medical procedures in pediatric and neonatal intensive care units." J Pediatr **121**(4): 647-9.
- Bauer, K., J. Ketteler, et al. (2004). "Oral glucose before venepuncture relieves neonates of pain, but stress is still evidenced by increase in oxygen consumption, energy expenditure, and heart rate." Pediatr Res **55**(4): 695-700.

- Bauer, R. M. (1998). "Physiologic measures of emotion." J Clin Neurophysiol **15**(5): 388-96.
- Bayrak, A. O., H. E. Tilki, et al. (2007). "Sympathetic skin response and axon count in carpal tunnel syndrome." J Clin Neurophysiol **24**(1): 70-5.
- Benis MM, S. G. (2001). "Frequency of invasive procedures in very low birth weight (VLBW) infants in the neonatal intensive care unit (NICU)." Pediatric Res: 2001:49:392A.
- Bir, L. S., S. Ozkurt, et al. (2005). "Impaired sympathetic skin response in chronic obstructive pulmonary disease." Tohoku J Exp Med **207**(4): 243-8.
- Boettger, M. K., W. Greiner, et al. (2010) "Sympathetic skin response following painful electrical stimulation is increased in major depression." Pain **149**(1): 130-4.
- Bolel, K., S. Hizmetli, et al. (2006). "Sympathetic skin responses in reflex sympathetic dystrophy." Rheumatol Int **26**(9): 788-91.
- Bromm, B. and J. Lorenz (1998). "Neurophysiological evaluation of pain." Electroencephalogr Clin Neurophysiol **107**(4): 227-53.
- Bromm, B., H. Neitzel, et al. (1983). "Evoked cerebral potential correlates of C-fibre activity in man." Neurosci Lett **43**(1): 109-14.
- Bromm, B. and E. Scharein (1982). "Principal component analysis of pain-related cerebral potentials to mechanical and electrical stimulation in man." Electroencephalogr Clin Neurophysiol **53**(1): 94-103.
- Bromm, B. and R. D. Treede (1984). "Nerve fibre discharges, cerebral potentials and sensations induced by CO₂ laser stimulation." Hum Neurobiol **3**(1): 33-40.
- Bromm, B. and R. D. Treede (1987a). "Human cerebral potentials evoked by CO₂ laser stimuli causing pain." Exp Brain Res **67**(1): 153-62.
- Bromm, B. and R. D. Treede (1987b). "Pain related cerebral potentials: late and ultralate components." Int J Neurosci **33**(1-2): 15-23.
- Bromm, B. and R. D. Treede (1991). "Laser-evoked cerebral potentials in the assessment of cutaneous pain sensitivity in normal subjects and patients." Rev Neurol (Paris) **147**(10): 625-43.
- Carbajal, R., A. Rousset, et al. (2008). "Epidemiology and treatment of painful procedures in neonates in intensive care units." Jama **300**(1): 60-70.
- Carmon, A., Y. Dotan, et al. (1978). "Correlation of subjective pain experience with cerebral evoked responses to noxious thermal stimulations." Exp Brain Res **33**(3-4): 445-53.
- Carmon, A., Y. Friedman, et al. (1980). "Single trial analysis of evoked potentials to noxious thermal stimulation in man." Pain **8**(1): 21-32.
- Carmon, A., J. Mor, et al. (1976). "Evoked cerebral responses to noxious thermal stimuli in humans." Exp Brain Res **25**(1): 103-7.
- Charnay, Y., C. Paulin, et al. (1983). "Distribution of substance P-like immunoreactivity in the spinal cord and dorsal root ganglia of the human foetus and infant." Neuroscience **10**(1): 41-55.
- Chen, S. R., Z. Xu, et al. (2001). "Stereospecific effect of pregabalin on ectopic afferent discharges and neuropathic pain induced by sciatic nerve ligation in rats." Anesthesiology **95**(6): 1473-9.
- Christie, M. J. (1981). "Electrodermal activity in the 1980s: a review." J R Soc Med **74**(8): 616-22.
- Chroni, E., A. A. Argyriou, et al. (2006). "The effect of stimulation technique on sympathetic skin responses in healthy subjects." Clin Auton Res **16**:396-400.

- Craig, A. D. (2003). "Pain mechanisms: labeled lines versus convergence in central processing." Annu Rev Neurosci **26**: 1-30.
- Craig, A. D., K. Chen, et al. (2000). "Thermosensory activation of insular cortex." Nat Neurosci **3**(2): 184-90.
- Craig, K. D., M. F. Whitfield, et al. (1993). "Pain in the preterm neonate: behavioural and physiological indices." Pain **52**(3): 287-99.
- Debillon, T., V. Zupan, et al. (2001). "Development and initial validation of the EDIN scale, a new tool for assessing prolonged pain in preterm infants." Arch Dis Child Fetal Neonatal Ed **85**(1): F36-41.
- Edwards, L., K. Inui, Ring C, et al. (2008). "Pain-related evoked potentials are modulated across the cardiac cycle." Pain **137**(3): 488-94.
- Ekert, P. G., M. J. Taylor, et al. (1997). "Early somatosensory evoked potentials in preterm infants: their prognostic utility." Biol Neonate **71**(2): 83-91.
- Eric P. Widmaier, H. R., Kevin T. Strang (2006). Vander's Human Physiology: The Mechanisms of Body Function, Mcgraw-Hill.
- Ertekin, C., N. Ertekin, et al. (1987). "Skin potentials (SP) recorded from the extremities and genital regions in normal and impotent subjects." Acta Neurol Scand **76**(1): 28-36.
- Feinstein, B., J. N. Langton, et al. (1954). "Experiments on pain referred from deep somatic tissues." J Bone Joint Surg Am **36-A**(5): 981-97.
- Fernandez, C. V. and E. P. Rees (1994). "Pain management in Canadian level 3 neonatal intensive care units." Cmaj **150**(4): 499-504.
- Fitzgerald, M. (1991). "Development of pain mechanisms." Br Med Bull **47**(3): 667-75.
- Fitzgerald, M., A. Shaw, et al. (1988). "Postnatal development of the cutaneous flexor reflex: comparative study of preterm infants and newborn rat pups." Dev Med Child Neurol **30**(4): 520-6.
- Fitzgerald, M. and S. M. Walker (2009). "Infant pain management: a developmental neurobiological approach." Nat Clin Pract Neurol **5**(1): 35-50.
- Franck, L. S. (1987). "A national survey of the assessment and treatment of pain and agitation in the neonatal intensive care unit." J Obstet Gynecol Neonatal Nurs **16**(6): 387-93.
- Franck, L. S., C. S. Greenberg, et al. (2000). "Pain assessment in infants and children." Pediatr Clin North Am **47**(3): 487-512.
- Franck, L. S. and C. Miaskowski (1997). "Measurement of neonatal responses to painful stimuli: a research review." J Pain Symptom Manage **14**(6): 343-78.
- Gedaly-Duff, V. (1989). "Palmar sweat index use with children in pain research." J Pediatr Nurs **4**(1): 3-8.
- Giannakoulopoulos, X., W. Sepulveda, et al. (1994). "Fetal plasma cortisol and beta-endorphin response to intrauterine needling." Lancet **344**(8915): 77-81.
- Giannakoulopoulos, X., J. Teixeira, et al. (1999). "Human fetal and maternal noradrenaline responses to invasive procedures." Pediatr Res **45**(4 Pt 1): 494-9.
- Granovsky, Y., M. Granot, et al. (2008). "Objective correlate of subjective pain perception by contact heat-evoked potentials." J Pain **9**(1): 53-63.
- Grunau, R. (2002). "Early pain in preterm infants. A model of long-term effects." Clin Perinatol **29**(3): 373-94, vii-viii.
- Grunau, R. E., D. W. Haley, et al. (2007). "Altered basal cortisol levels at 3, 6, 8 and 18 months in infants born at extremely low gestational age." J Pediatr **150**(2): 151-6.

- Grunau, R. E., L. Holsti, et al. (2005). "Neonatal procedural pain exposure predicts lower cortisol and behavioral reactivity in preterm infants in the NICU." Pain **113**(3): 293-300.
- Grunau, R. E., L. Holsti, et al. (2006). "Long-term consequences of pain in human neonates." Semin Fetal Neonatal Med **11**(4): 268-75.
- Grunau, R. E., T. Oberlander, et al. (1998). "Bedside application of the Neonatal Facial Coding System in pain assessment of premature neonates." Pain **76**(3): 277-86.
- Grunau, R. E., T. F. Oberlander, et al. (2001). "Demographic and therapeutic determinants of pain reactivity in very low birth weight neonates at 32 Weeks' postconceptional Age." Pediatrics **107**(1): 105-12.
- Grunau, R. V. and K. D. Craig (1987). "Pain expression in neonates: facial action and cry." Pain **28**(3): 395-410.
- Gunnar, M. and K. Quevedo (2007). "The neurobiology of stress and development." Annu Rev Psychol **58**: 145-73.
- Hall, R. W., S. S. Kronsberg, et al. (2005). "Morphine, hypotension, and adverse outcomes among preterm neonates: who's to blame? Secondary results from the NEOPAIN trial." Pediatrics **115**(5): 1351-9.
- Hammarlund, K., G. E. Nilsson, et al. (1977). "Transepidermal water loss in newborn infants. I. Relation to ambient humidity and site of measurement and estimation of total transepidermal water loss." Acta Paediatr Scand **66**(5): 553-62.
- Harkins, S. W., D. D. Price, et al. (2000). "Somatosensory evoked potentials associated with thermal activation of type II Adelta mechanothermal nociceptive afferents." Int J Neurosci **104**(1-4): 93-111.
- Harpin, V. A. and N. Rutter (1982a). "Development of emotional sweating in the newborn infant." Arch Dis Child **57**(9): 691-5.
- Harpin, V. A. and N. Rutter (1982b). "Sweating in preterm babies." J Pediatr **100**(4): 614-9.
- Harrison, D., S. Boyce, et al. (2006). "Skin conductance as a measure of pain and stress in hospitalised infants." Early Hum Dev **82**(9): 603-8.
- Harth, S. C. and Y. H. Thong (1995). "Parental perceptions and attitudes about informed consent in clinical research involving children." Soc Sci Med **41**(12): 1647-51.
- Hayman, R. M., B. J. Taylor, et al. (2001). "Participation in research: informed consent, motivation and influence." J Paediatr Child Health **37**(1): 51-4.
- Hellerud, B. C. and H. Storm (2002). "Skin conductance and behaviour during sensory stimulation of preterm and term infants." Early Hum Dev **70**(1-2): 35-46.
- Hernes, K. G., L. Morkrid, et al. (2002). "Skin conductance changes during the first year of life in full-term infants." Pediatr Res **52**(6): 837-43.
- Hummel, P., M. Puchalski, et al. (2008). "Clinical reliability and validity of the N-PASS: neonatal pain, agitation and sedation scale with prolonged pain." J Perinatol **28**(1): 55-60.
- Itskovich, V. V., D. Y. Fei, et al. (2000). "Psychophysiological and psychophysical responses to experimental pain induced by two types of cutaneous thermal stimuli." Int J Neurosci **105**(1-4): 63-75.
- Jessell, T. M. and L. L. Iversen (1977). "Opiate analgesics inhibit substance P release from rat trigeminal nucleus." Nature **268**(5620): 549-51.

- Johnston, C. C., J. M. Collinge, et al. (1997). "A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units." Clin J Pain **13**(4): 308-12.
- Johnston, C. C., B. J. Stevens, et al. (1995). "Differential response to pain by very premature neonates." Pain **61**(3): 471-9.
- Khalil, Z. and R. D. Helme (1991). "Effects of serotonin and opioid agonists on tachyphylaxis to SP-induced vasodilatation." Ann N Y Acad Sci **632**: 418-21.
- Kiylioglu, N., A. Akyol, et al. (2005). "Sympathetic skin response in idiopathic and diabetic carpal tunnel syndrome." Clin Neurol Neurosurg **108**(1): 1-7.
- Klein, V. C., C. M. Gaspardo, et al. (2009). "Pain and distress reactivity and recovery as early predictors of temperament in toddlers born preterm." Early Hum Dev **85**(9): 569-76.
- Klimach, V. J. and R. W. Cooke (1988). "Short-latency cortical somatosensory evoked responses of preterm infants with ultrasound abnormality of the brain." Dev Med Child Neurol **30**(2): 215-21.
- Knezevic, W. and S. Bajada (1985). "Peripheral autonomic surface potential. A quantitative technique for recording sympathetic conduction in man." J Neurol Sci **67**(2): 239-51.
- Kunzek, S., M. W. Quinn, et al. (1997). "Does change in skin perfusion provide a good index to monitor the sympathetic response to a noxious stimulus in preterm newborns?" Early Hum Dev **49**(2): 81-9.
- Lagercrantz, H., E. Nilsson, et al. (1986). "Plasma catecholamines following nursing procedures in a neonatal ward." Early Hum Dev **14**(1): 61-5.
- Langley, J. M., S. A. Halperin, et al. (1998). "Parental willingness to enter a child in a controlled vaccine trial." Clin Invest Med **21**(1): 12-6.
- Le Pera, D., M. Valeriani, et al. (2002). "Contact heat evoked potentials to painful and non-painful stimuli: effect of attention towards stimulus properties." Brain Topogr **15**(2): 115-23.
- Lembeck, F. and J. Donnerer (1985). "Opioid control of the function of primary afferent substance P fibres." Eur J Pharmacol **114**(3): 241-6.
- Lloyd-Thomas, A. R. and M. Fitzgerald (1996). "Do fetuses feel pain? Reflex responses do not necessarily signify pain." Bmj **313**(7060): 797-8.
- Loeser, J. D. and R. D. Treede (2008). "The Kyoto protocol of IASP Basic Pain Terminology." Pain **137**(3): 473-7.
- Luis Pereira-da-Silva I M, S. G., Patricia Rodrigues, Daniel Virella, Micaela Serelha, Hanne Storm (2009). Effectiveness of skin conductance in assessing the nociceptive response from heel prick in neonates compared with the Neonatal Infant Pain Scale. 17th European Workshop on Neonatology. Lipson, Portugal.
- Majnemer, A. and B. Rosenblatt (1996). "Evoked potentials as predictors of outcome in neonatal intensive care unit survivors: review of the literature." Pediatr Neurol **14**(3): 189-95.
- Marshall, R. E. (1989). "Neonatal pain associated with caregiving procedures." Pediatr Clin North Am **36**(4): 885-903.
- McCulloch, K. M., S. A. Ji, et al. (1995). "Skin blood flow changes during routine nursery procedures." Early Hum Dev **41**(2): 147-56.
- McLaughlin, C. R., J. G. Hull, et al. (1993). "Neonatal pain: a comprehensive survey of attitudes and practices." J Pain Symptom Manage **8**(1): 7-16.
- Mercuri, E., K. von Siebenthal, et al. (1994). "Multimodality evoked responses in the neurological assessment of the newborn." Eur J Pediatr **153**(9): 622-31.

- Morley, C. J., R. Lau, et al. (2005). "What do parents think about enrolling their premature babies in several research studies?" Arch Dis Child Fetal Neonatal Ed **90**(3): F225-8.
- Nabulsi, M., Y. Khalil, et al. "Parental attitudes towards and perceptions of their children's participation in clinical research: a developing-country perspective." J Med Ethics **37**(7): 420-3.
- Nessmann, C. and F. Baverel (1972). "[Skin development in the human embryo and fetus. Histological study]." J Gynecol Obstet Biol Reprod (Paris) **1**(6): 527-50.
- Norman, E., I. Rosen, et al. (2008). "Electroencephalographic response to procedural pain in healthy term newborn infants." Pediatr Res **64**(4): 429-34.
- Ohara, S., N. E. Crone, et al. (2004). "Amplitudes of laser evoked potential recorded from primary somatosensory, parasympathetic and medial frontal cortex are graded with stimulus intensity." Pain **110**(1-2): 318-28.
- Pena, M., A. Maki, et al. (2003). "Sounds and silence: an optical topography study of language recognition at birth." Proc Natl Acad Sci U S A **100**(20): 11702-5.
- Piercey, M. F., L. A. Schroeder, et al. (1981). "Sensory and motor functions of spinal cord substance P." Science **214**(4527): 1361-3.
- Pike, A. A., N. Marlow, et al. (1997). "Posterior tibial somatosensory evoked potentials in very preterm infants." Early Hum Dev **47**(1): 71-84.
- Porter FL, A. K. (1998). "Epidemiology of pain in neonates." Res Clin Forums **20**: 9-18.
- Porter, F. L., R. E. Grunau, et al. (1999). "Long-term effects of pain in infants." J Dev Behav Pediatr **20**(4): 253-61.
- Qiu, Y., Y. Noguchi, et al. (2006). "Brain processing of the signals ascending through unmyelinated C fibers in humans: an event-related functional magnetic resonance imaging study." Cereb Cortex **16**(9): 1289-95.
- Ranger, M., C. C. Johnston, et al. (2007). "Current controversies regarding pain assessment in neonates." Semin Perinatol **31**(5): 283-8.
- Redshaw M, H. K. (2005). A survey of Current Neonatal Unit Organisation and Policy, National Perinatal Epidemiology Unit.
- Roberts, K., A. Papadaki, et al. (2008). "Contact heat evoked potentials using simultaneous EEG and fMRI and their correlation with evoked pain." BMC Anesthesiol **8**: 8.
- Roeggen, I., H. Storm, et al. "Skin conductance variability between and within hospitalised infants at rest." Early Hum Dev **87**(1): 37-42.
- Romanelli, P. and V. Esposito (2004). "The functional anatomy of neuropathic pain." Neurosurg Clin N Am **15**(3): 257-68.
- Ruda, M. A., G. J. Bennett, and R. Dubner. (1987). "Neurochemistry and neural circuitry in the dorsal horn. In: Emson, P.C., Rasser, M. N., Tohyama, M (eds). Progress in Brain Research. Amsterdam, Elsevier: pp 219-268.
- Rutter, N. and D. Hull (1979). "Water loss from the skin of term and preterm babies." Arch Dis Child **54**(11): 858-68.
- Schechter NL, B. C. (1993). Pain in infants, children, and adolescents, Baltimore, MD: Williams and Williams.
- Schestatsky, P., J. A. Ehlers, et al. (2006). "Evaluation of sympathetic skin response in Parkinson's disease." Parkinsonism Relat Disord **12**(8): 486-91.
- Shahani, B. T., J. J. Halperin, et al. (1984). "Sympathetic skin response--a method of assessing unmyelinated axon dysfunction in peripheral neuropathies." J Neurol Neurosurg Psychiatry **47**(5): 536-42.

- Shaver, B. A., Jr., S. W. Brusilow, et al. (1962). "Origin of the galvanic skin response." Proc Soc Exp Biol Med **110**: 559-64.
- Sherman, J. L., Jr. (1963). "Normal Arteriovenous Anastomoses." Medicine (Baltimore) **42**: 247-67.
- Simons, S. H., M. van Dijk, et al. (2003). "Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates." Arch Pediatr Adolesc Med **157**(11): 1058-64.
- Slater, R., A. Cantarella, et al. (2006). "Cortical pain responses in human infants." J Neurosci **26**(14): 3662-6.
- Slater, R., A. Cantarella, et al. (2009). "Latency to facial expression change following noxious stimulation in infants is dependent on postmenstrual age." Pain **146**(1-2): 177-82.
- Slater, R., L. Fabrizi, et al. (2010). "Premature infants display increased noxious-evoked neuronal activity in the brain compared to healthy age-matched term-born infants." Neuroimage **52**(2): 583-9.
- Slater, R., M. Fitzgerald, et al. (2007). "Can cortical responses following noxious stimulation inform us about pain processing in neonates?" Semin Perinatol **31**(5): 298-302.
- Slater, R., A. Worley, et al. (2010). "Evoked potentials generated by noxious stimulation in the human infant brain." Eur J Pain **14**(3): 321-6.
- Smith, R. P., R. Gitau, et al. (2000). "Pain and stress in the human fetus." Eur J Obstet Gynecol Reprod Biol **92**(1): 161-5.
- Spigelman, I. and E. Puil (1991). "Substance P actions on sensory neurons." Ann N Y Acad Sci **632**: 220-8.
- Stephenson, T. and D. A. Walker (1996). "Gaining patients' consent. Telling parents all relevant details might reduce recruitment of children to trials." Bmj **313**(7053): 362-3.
- Stevens, B., C. Johnston, et al. (1996). "Premature Infant Pain Profile: development and initial validation." Clin J Pain **12**(1): 13-22.
- Storm, H. (2000). "Skin conductance and the stress response from heel stick in preterm infants." Arch Dis Child Fetal Neonatal Ed **83**(2): F143-7.
- Storm, H., K. Myre, et al. (2002). "Skin conductance correlates with perioperative stress." Acta Anaesthesiol Scand **46**(7): 887-95.
- Stowell, H. (1985). "Human evoked potentials and C-fiber pain." Int J Neurosci **26**(3-4): 219-24.
- Symington, A. and J. Pinelli (2000). "Developmental care for promoting development and preventing morbidity in preterm infants." Cochrane Database Syst Rev(4): CD001814.
- Taga, G., K. Asakawa, et al. (2003). "Brain imaging in awake infants by near-infrared optical topography." Proc Natl Acad Sci U S A **100**(19): 10722-7.
- Takagi, K. and T. Nakayama (1959). "Peripheral effector mechanism of galvanic skin reflex." Jpn J Physiol **9**(1): 1-7.
- Taylor, M. J., R. Boor, et al. (1996). "Preterm maturation of the somatosensory evoked potential." Electroencephalogr Clin Neurophysiol **100**(5): 448-52.
- Tohill, J. and O. McMorrow (1990). "Pain relief in neonatal intensive care." Lancet **336**(8714): 569.
- Tombini, M., P. Pasqualetti, et al. (2009). "Extrauterine maturation of somatosensory pathways in preterm infants: a somatosensory evoked potential study." Clin Neurophysiol **120**(4): 783-9.

- Tranel, D. and H. Damasio (1994). "Neuroanatomical correlates of electrodermal skin conductance responses." Psychophysiology **31**(5): 427-38.
- Vanhatalo, S. and O. van Nieuwenhuizen (2000). "Fetal pain?" Brain Dev **22**(3): 145-50.
- Verbov, J. and J. Baxter (1974). "Onset of palmar sweating in newborn infants." Br J Dermatol **90**(3): 269-76.
- Vetrugno, R., R. Liguori, et al. (2003). "Sympathetic skin response: basic mechanisms and clinical applications." Clin Auton Res **13**(4): 256-70.
- Walmsley, D. and P. G. Wiles (1990). "Assessment of the neurogenic flare response as a measure of nociceptor C fibre function." J Med Eng Technol **14**(5): 194-6.
- Woolf, C. J. (1983). "Evidence for a central component of post-injury pain hypersensitivity." Nature **306**(5944): 686-8.
- Yildiz, S. K., S. Koybasi, et al. (2007). "Sympathetic skin responses from postauricular region in Meniere's disease." Clin Neurophysiol **118**(9): 1991-8.
- Yokota, T., T. Takahashi, et al. (1959). "Studies on the diphasic wave form of the galvanic skin reflex." Electroencephalogr Clin Neurophysiol **11**: 687-96.
- Yudcovitch, L.B. "The Use of Anesthetics, Steroids, Non-steroidals, and Central-Acting Analgesics in the Management of Ocular Pain." Available online at <http://www.pacificu.edu/optometry/ce/courses/22746/ocularpainpg1.cfm>
- Zupancic, J. A., P. Gillie, et al. (1997). "Determinants of parental authorization for involvement of newborn infants in clinical trials." Pediatrics **99**(1): E6.