Measuring analgesic efficacy in neonates using a brain-derived outcome measure: a multicentre randomised controlled trial on gentle parental touch



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Abstract

Hospitalised neonates undergo numerous painful medically-required procedures, yet providing adequate pain management remains challenging. In the absence of verbal expression, assessing the efficacy of pain-relieving interventions relies on surrogate measures such as vital signs and facial expressions. Given that pain is an experience processed in the brain, assessing pain in a multimodal manner including an objective, brain-derived outcome measure can provide valuable insights to inform clinical care teams and parents in their choice of pain-relieving intervention.

This thesis presents the Parental Touch trial (Petal), a multicentre, randomised, parallel-group interventional superiority trial investigating a non-pharmacological intervention involving parents, namely parental touch, provided to their babies either before (intervention group) or after (control group) a clinically-required blood test. Parents stroked their baby's skin at a speed that optimally activates C-tactile fibres, a group of nerve afferents which have been linked to pain relief in adults and neonates. In total, 112 neonates were randomised with a 1:1 allocation ratio to either arm and the effect of the intervention was assessed on multimodal outcome measures. The primary outcome measure was the magnitude of noxious-evoked brain activity in response to the heel lance measured with electroencephalography (EEG). Secondary outcome measures were Premature Infant Pain Profile-Revised (PIPP-R) score, development of tachycardia, and a parental anxiety score.

A full statistical analysis plan was developed and published online prior to unblinding the data. The intervention did not show a significant effect on any of the primary or secondary outcome measures. However, in the exploratory analysis of parental feelings while being involved in their baby's care, most parents reported positive feelings, such as feeling 'useful' or 'reassured', and 98% of parents reported being pleased to have taken part in the study and that they would consider taking part in future research studies.

Scientists, clinicians, and regulatory agencies such as EMA and FDA are striving to improve analgesic provision for neonates, which requires having objective and validated outcome measures to assess pain. The Petal trial methodology was developed following the relevant guidelines and provides a framework for performing randomised controlled trials in the neonatal population to assess pain-relieving

interventions on multimodal outcome measures focusing on an objective, brain-derived primary outcome measure, with the ultimate goal to advance how pain is assessed and managed in neonates and improve the well-being of neonates and their families.

Acknowledgements

This thesis is dedicated to the neonates and parents who participated in the trial, as well as to my family.

I extend my heartfelt thanks to my exceptional supervisors, Professor Rebeccah Slater and Dr Luke Baxter, for their wise insights, driving enthusiasm, and unwavering support throughout my DPhil. Working with you has been truly inspiring. A special thank you also goes to Dr Sean Fitzgibbon for the supervision during the first year of my DPhil.

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Thank you to my partner Oliver for the steadfast support through every step of the DPhil; despite the distance, you have always felt close. I also extend my gratitude to my family and parents for supporting me as I ventured into the unknown landscape of academia. This thesis is also dedicated to the memory of my father: I know you would have loved to be a part of this journey a little longer.

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List of Abbreviations

a.u. . . . . . Arbitrary unit
ACTTION . . . . . Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks
BOLD . . . . . Blood oxygenation level dependent
bpm . . . . . Beats per minute
CACE . . . . . Complier average causal effect
CI . . . . . Confidence interval
CONSORT . . . . . Consolidated Standards of Reporting Trials
COSMIN . . . . . COnsensus-based Standards for the selection of health Measurement INstruments
CT . . . . . C-tactile
ECG . . . . . Electrocardiogram
EEG . . . . . Electroencephalography
EMA . . . . . European Medicines Agency
EMG . . . . . Electromyography
EQUATOR . . . . Enhancing the QUAlity and Transparency Of health Research
ERP . . . . . Event-related potential
FDA . . . . . Food and Drug Administration
fMRI . . . . . Functional magnetic resonance imaging
FP . . . . . Fractional polynomials
GA . . . . . Gestational age
GCP . . . . . Good Clinical Practice
GLM . . . . . General linear model
HR . . . . . Heart rate
HRF . . . . . Haemodynamic response function
IASP . . . . . International Association for the Study of Pain
ICC . . . . . Intraclass correlation coefficient
ICH . . . . . International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
INC . . . . . International Neonatal Consortium
IQR . . . . . Interquartile range
ISRCTN . . . . . International Standard Randomised Controlled Trial Number

ITT ..... Intention-to-treat
MAR ..... Missing at random
MCAR ..... Missing completely at random
MEG ..... Magnetoencephalography
MNAR ..... Missing not at random
MRI ..... Magnetic resonance imaging
n-NRF ..... Noxious neurodynamic response function
NICU ..... Neonatal Intensive Care Unit
NIHR ..... National Institute for Health and Care Research
NIRS ..... Near-infrared spectroscopy
OLS ..... Ordinary least squares
OR ..... Odds ratio
PALM ..... Permutation Analysis of Linear Models
PCA ..... Principal component analysis
PedIMMPACT Pediatric Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials
Petal ..... Parental touch trial
PIPP ..... Premature Infant Pain Profile
PIPP-R ..... Premature Infant Pain Profile-Revised
PMA ..... Postmenstrual age
PMG ..... Project Management Group
PNA ..... Postnatal age
RCT ..... Randomised controlled trial
RoB ..... Risk-of-bias tool for randomized trials
SAP ..... Statistical analysis plan
SD ..... Standard deviation
SPIRIT ..... Standard Protocol Items: Recommendations for Interventional Trials
SSNAP ..... Supporting sick newborn and their parents
STAI ..... State Trait Anxiety Inventory
STAI-S ..... State Trait Anxiety Inventory-State
STAI-T ..... State Trait Anxiety Inventory-Trait
WHO ..... World Health Organization

Chapter 1

Publications, presentations and funding

1.1 Publications relevant to this thesis

The publications listed here form the core of the thesis. Although the primary publication serving as the foundation of each chapter is cited at its onset, elements from other publications listed here might be present elsewhere in the thesis. My personal contributions are described at the start of each chapter.

Chapter 3 is primarily based on two manuscripts which I co-authored. The protocol of the trial was published in July 2022 in *BMJ Open* under a CC BY 4.0 license with copyright retained by the authors [1].

- Cobo, M. M., Moultrie, F., Hauck, A. G. V., Crankshaw, D., Monk, V., Hartley, C., Evans Fry, R., Robinson, S., van der Vaart, M., Baxter, L., Adams, E., Poorun, R., Bhatt, A., & Slater, R. (2022). Multicentre, randomised controlled trial to investigate the effects of parental touch on relieving acute procedural pain in neonates (Petal). BMJ Open, 12(7), e061841-e061841. doi:10.1136/bmjopen-2022-061841

The statistical analysis plan (SAP) was published online on International Standard Randomised Controlled Trial Number (ISRCTN) on 31 March 2023, prior to unblinding the data on 4 April 2023. It was later submitted to Wellcome Open

Research as an article of which I am co-first author under a CC BY 4.0 license with copyright retained by the authors [2]. It is currently undergoing peer review.

- Baxter, L.*, Hauck, A. G. V.*, Bhatt, A., Cobo, M. M., Hartley, C., Marchant, S., Poorun, R., van der Vaart, M.‡ & Slater, R.‡ (2023) Statistical Analysis Plan for the Petal Trial: The Effects of Parental Touch on Relieving Acute Procedural Pain in Neonates [version 1; peer review: 2 approved with reservations] Wellcome Open Res, 8:402. doi:10.12688/ wellcomeopenres.19819.1

*equal contributions ‡ equal contributions

Chapter 4 is primarily based on the manuscript reporting the trial results published in *The Lancet Child & Adolescent Health* in 2024, of which I was co-first author. The article is published under a CC BY 4.0 license with copyright retained by the authors [3]. The methods are described in detail in Chapter 3. The results reflect those published with some minor additions, while the introduction and discussion of this chapter have undergone more conspicuous adaptation.

- Hauck, A. G. V.*, van der Vaart, M.*, Adams, E., Baxter, L., Bhatt, A., Crankshaw, D., Dhami, A., Evans Fry, R., Freire, M. B. O., Hartley, C., Mansfield, R. C., Marchant, S., Monk, V., Moultrie, F., Peck, M., Robinson, S., Yong, J., Poorun, R., Cobo, M. M.‡, & Slater, R.‡ (2024). Effect of Parental Touch on Relieving Acute Procedural Pain in Neonates and Parental Anxiety (Petal): A Multicentre, Randomised Controlled Trial in the UK. The Lancet Child & Adolescent Health. doi.org/10.1016/S2352-4642(23)00340-1.

*equal contributions ‡ equal contributions

Chapter 5 is primarily based on the following manuscript published in *PAIN* in 2024 [4], of which I am co-first author. The article is published under a CC BY 4.0 license with copyright retained by the authors [4]. The methods and results section reflect those published with some minor additions, while the introduction and discussion of this chapter have undergone more conspicuous adaptation.

- van der Vaart, M.*, Hauck, A. G. V.*, Mansfield, R., Adams, E., Bhatt, A., Cobo, M. M., Crankshaw, D., Dhami, A., Hartley, C., Monk, V., Evans Fry, R., Moultrie, F., Robinson, S., Yong, J., Poorun, R., Baxter, L.‡, & Slater, R.‡ (2024). Parental experience of neonatal pain research while participating in the Parental touch trial (Petal). Pain. doi:10.1097/j.pain.0000000000003177

*equal contributions ‡ equal contributions

1.2 Presentations and posters

- **5^{th} Congress of joint European Neonatal Societies (jENS), Italy, 2023**

  'Parental touch trial (Petal): a multicentre randomised controlled trial investigating parental touch as an intervention to relieve neonatal pain and reduce parental anxiety', 15-min presentation in parallel session

- **13^{th} International Newborn Brain Conference (INBBC), online/USA, 2022**

  'Parental touch trial (Petal) – study protocol: a multicentre randomised controlled trial on the effect of parental touch on pain relief in neonates', poster presentation

- **28^{th} European Workshop on Neonatology (EWON), Germany, 2021**

  'Parental Touch Trial (Petal): study protocol', plenary presentation

1.3 Funding and awards

- European Society for Paediatric Research: Young Investigator START-UP Grant, 2020

- University of Oxford, Dept. of Paediatrics (Oxford, United Kingdom): Departmental Scholarship, 2020

- Swiss Study Foundation (Zurich, Switzerland): scholarship, 2020-2021
- Department of Paediatrics (Oxford, United Kingdom): Paediatric Neuroimaging Award, 2020
- Janggen-Poehn Foundation (Sankt Gallen, Switzerland): scholarships, 2021-2024
- Lincoln College (Oxford, United Kingdom): Senior Scholarship, 2022
- Lincoln College (Oxford, United Kingdom): The Woldenberg Bursary, 2022

Chapter 2

Background

This chapter provides an overview of some of the seminal events that shaped the field of neonatal pain management, highlighting radical shifts in the understanding of neonatal pain. The burden of neonatal pain is still weighing on babies and their families, and a brief overview of its current prevalence and consequences will be described. Challenges on the path to improving pain assessment and management will be presented, in particular in relation to the rapidly developing human body and the changing repertoire of pain-related responses that can be used as proxy measures to assess pain in the youngest population. Following a description of indicators of neonatal pain, including brain-derived methods, an overview of pain management options will be provided. Interventions can be of environmental, pharmacological and non-pharmacological nature, and can be delivered in collaboration with parents. The introduction will conclude on ongoing challenges in establishing the effectiveness of pain-relieving interventions for neonates, and on the efforts by regulatory agencies to prompt an increase in clinical trials in the paediatric population including efforts targeted specifically at the neonatal population, which will hopefully contribute to advancing the knowledge required to provide each neonate with adequate analgesia and comfort.

2.1 Pain in early life: an historical perspective

While anaesthesia during surgical procedures was introduced in the mid-nineteenth century for both adult and paediatric patients, its use remained minimal in the

paediatric population until the 1980s [5, 6]. Interpreting past views and beliefs requires some caution, and efforts have been made to understand the roots of little analgesic provision to children during surgical procedures. In the nineteenth and twentieth century until the 1980s, one of the prevailing views was that the youngest children were less sensitive or even insensitive to pain [5, 7, 8]. Rodkey and Pillay Riddell attribute this theory primarily to four intertwined reasons: the view that the nervous system was not yet developed enough to perceive pain, behaviourism, which interpreted pain responses primarily as a reflex to a noxious stimulus, a Darwinian perspective portraying infants as 'lower beings', and a different understanding of scientific hypothesis testing relying on the lack of indisputable evidence that infants could feel pain [6]. While not everyone agreed with the theory that infants were less sensitive to pain, use of analgesics in children was complicated by side and adverse effects which appeared more often than in older children or adults [5]. The deficiencies of the monitoring equipment available even in the early 1970s and the daunting episodes of physiological instability observed in some patients during general anaesthesia hampered the implementation of anaesthetic regimens for infant surgeries [8]. Although efforts were made to anaesthetise even the youngest infants safely [8], the co-existence of views on reduced pain sensitivity in early life and concerns of complications related to the use of analgesics obstructed the implementation of anaesthesia and analgesia for surgical procedures in the youngest children [5].

Up until the 1980 neonates were often being administered little or no anaesthesia during surgeries: in light of the limitations described above, nitrous oxide and oxygen anaesthesia, in combination with a muscle relaxant, was considered as a light form of anaesthesia appropriate for infant surgery [5, 6]. In the second half of the twentieth century, the development of intensive care units and more advanced equipment allowed better monitoring of possible complications due to the procedure and anaesthesia [9], in parallel with changes in the understanding of pain perception in infants, and consequently in pain assessment and management [5]. Among the most notable contributions to pain research, Melzack and Wall

published the gate control theory in 1965, which described a role of the spinal cord and brain in modulating peripheral input [10]. While pain in neonates or infants is not specifically addressed, the gate control theory led to significant advances towards a more comprehensive approach to pain which emphasises the potential of sensory interventions to modulate noxious stimuli. Over fifty years later, it is among the most cited publications in the field of infant pain management [11].

A bibliometric analysis on publications related to pain management in infants identified the period between 1982 and 1992 as one of rapid progress [11]. Pivotal events occurred in the 1980s, which were set to shape infant pain management and research for decades to follow. Observations that premature neonates are more likely to develop respiratory complications from minor surgery with a potent anaesthetic (halothane) compared to term neonates underscored the importance of careful perioperative monitoring of premature neonates receiving general anaesthesia [12]. Owens reflected on conceptual and methodological issues on infant pain and presented arguments in favour of the infant's potential ability to experience pain, advocating that the burden of proof should be shifted to those who remain of the opinion that neonates cannot feel pain [13]. Furthermore, he suggested clinically-required procedures such as heel lances for metabolic tests in the first few days of life and immunisations as an opportunity to study pain in neonates without applying any additional noxious stimuli, and proposed a multi-faceted approach to assessing pain in neonates, including facial expression, body movements, crying, heart rate and endocrine activity [13]. To dispute the assumption that infants do not respond to noxious stimuli, which was one of the rationales underpinning the belief that infants could not perceive pain, Owens and Todt assessed heart rate changes and crying duration on the second postnatal day in response to a heel lance. They reported increases in heart rate and crying following the heel lance, which supported their dispute of the view that neonates are insensitive to noxious stimuli [14]. In the same years, Anand was collecting evidence about the effects of pain in early life as part of his PhD at the University of Oxford, when he conducted studies which are among the most cited in the field of infant pain management [11]. In 1985, he published

findings of a study in preterm and term neonates which showed that they can mount an endocrine and metabolic response to surgery [15]. This was followed two years later by a landmark publication on a randomised controlled trial in a population of premature neonates undergoing open surgical ligation of patent duct arteriosus comparing standard practice at the time, which consisted of nitrous oxide and the muscle relaxant tubocurarine, with or without fentanyl: fewer complications and decreased stress markers were observed in the intervention group receiving fentanyl [16]. In another seminal work, Anand and Hickey summarised evidence supporting the ability of neonates to perceive noxious stimuli [17]. According to a survey of anaesthesiologists in the United Kingdom and Republic of Ireland published in 1988, the majority of respondents thought that neonates were able to perceive pain, but they rarely prescribed opioids to neonates even for major surgery, possibly due to concerns of potential development of respiratory suppression, while higher rates of opioid prescription were reported for older children [18]. Similarly, while they more often provided local analgesia to older children, the survey respondents never (52%) or rarely (21%) prescribed local analgesia to neonates [18].

Some of the most important documents leading to practice changes did not originate from the scientific or medical community, but from parents. In 1985, Jeffrey Lawson was born premature and hospitalised in Washington (US). He required open heart surgery, which he underwent with a paralytic agent [6]. Similarly, Edward Harrison had undergone a shunt placement procedure for hydrocephalus a decade earlier under muscle relaxants. Their mothers' advocacy following Edward's procedure and Jeffrey's death merely a few weeks after the surgery were central in increasing the public's awareness on the medical practices of the time and initiating discussions on practice changes [19, 20]. The increased attention paid not only by the scientific and medical community but also by the public [7] to improving pain management in infants which followed parental advocacy and landmark publications on the topic is reflected by the launch of a special interest group on Pain in Childhood by the International Association for the Study of Pain (IASP) in 1987. Practice changes were introduced following these seminal papers. If the survey of

anaesthesiologists in the United Kingdom and Republic of Ireland had shown in 1988 that most thought neonates could feel pain, but only a small proportion prescribed analgesia to neonates even for major surgery [18], by 1995 the responses to a follow-up survey had largely changed: almost all respondents agreed that infants of any age could perceive pain, and the prescription of opioids for major surgery in neonates increased from 10% to 91% [21]. In 1995, the anaesthetists who did not provide opioids offered alternative forms of analgesia [21]. Even for the youngest neonates where opioids were avoided due to fears of respiratory depression, local anaesthetic techniques were provided in the majority of cases [21]. In the following years, guidance documents and consensus statements developed and endorsed by medical associations were established, which paved the way for consecutive guidelines. For example, the Fetus and Newborn Committee of the Canadian Paediatric Society and the Committee on Fetus and Newborn, Committee on Drugs, Section on Anesthesiology and Section on Surgery of the American Academy of Pediatrics released a joint statement in 2000 which not only encompassed pain management but also the prevention of pain and stress in the neonate [22]. Similarly, experts from several countries shared a consensus statement in 2001 listing strategies to prevent and manage pain in early life in order to facilitate the development of national and international pain management guidelines [23].

2.2 A revised definition of pain

Changes in the views on neonatal pain were reflected in the revision of the IASP definition of pain. The original definition finalised by the IASP in 1979 was ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage’ [24]. This definition specified that pain may or may not be linked to actual tissue damage, but used the word ‘described’, which was criticised as excluding non-verbal populations such as neonates [25]. However, the criticism was not unanimous [26], underscoring the complexity of providing a pain definition generally agreed upon. A revision of the taxonomy of pain-related terms led to a release of an updated definition of pain in 2020 (Box

2.1) [27]. Furthermore, bullet points to clarify important aspects of the terminology were presented alongside the definition (Box 2.1).

Box 2.1: IASP revised definition of pain (2020) [27]

Pain
'An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage.'

Notes

- 'Pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors.'
- 'Pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons.'
- 'Through their life experiences, individuals learn the concept of pain.'
- 'A person's report of an experience as pain should be respected.'
- 'Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being.'
- 'Verbal description is only one of several behaviors to express pain; inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain.'

The last bullet point is particularly relevant to neonates, as it underscores that pain experience is not excluded in this population, as well as in infants and non-human animals (Box 2.1), reflecting the deliberate effort by the IASP to include such populations in the new definition [27]. Furthermore, a distinction is made between pain and nociception, where the latter is defined as 'the neural process of encoding noxious stimuli' and does not necessarily lead to pain [28]. While a large body of literature has studied how pain can be conceptualised via various pain models [29], an exhaustive introduction to all pain models would go beyond the scope of this thesis. In the context of this thesis, the term 'pain' is generally used to describe the resulting state from being exposed to procedures which adults would typically report as painful. In the neonatal population, the

focus lies on pain responses due the absence of verbal report providing access to the personal nature of the pain experience.

Although the understanding of pain in early life and its management vastly improved over the past century, and scepticism towards pain sensitivity was largely dismissed, pain in early life remains an important issue [30, 31]. It is difficult to tackle this as long as the safety and efficacy of pain-relieving interventions are not established and widely accepted. The following sections will highlight the current prevalence of noxious clinically-required procedures in neonates, the still insufficient provisions of pain relief, and the consequences of being exposed to clinically-required painful procedures in early life, leading onto the approaches to assess analgesic efficacy and current pain management strategies.

2.3 The current burden of neonatal pain

While numbers vary depending on the context and healthcare system, data from the US indicates that approximately nine percent of all neonates are admitted to a Neonatal Intensive Care Unit (NICU). In the UK, one in seven babies is admitted to neonatal care [32], and a similar proportion (17%) requires special nursery care or NICU care in Australia [33].

Neonates worldwide often undergo medically-required painful procedures in a NICU setting. A large observational study in France, the *Epidemiology of Procedural Pain in Neonates (EPIPPAIN)* study, prospectively collected data on painful and stressful procedures, as well as analgesia, in the first 14 days since admission to tertiary neonatal units in the Paris region [34]. They counted a median of 10 (range 0–51) painful procedures per day, and a median of 75 (range 3–364) painful procedures in the first two weeks since admission. Data on the number of heel sticks per neonate in the same region was prospectively collected in 2016 in *EPIPPAIN 2*: each neonate had on average 16 (range 1–86, standard deviation (SD) 14.4) heel sticks in the first two weeks since admission [35], and 1,887 venepunctures were performed in a cohort of 589 neonates [36]. Regional variations in the type and number of painful procedures exist, but overall neonates in NICUs worldwide often undergo painful

procedures, as reported in a cross-sectional study from Kenya [37], in prospectively collected data from Canada [38], in a prospective study from South Korea [39], and based on prospective data gathered in Brazil [40]. In a review of epidemiological studies, Cruz and colleagues identified six studies focussing on the first two weeks since NICU admission: in total, 6,832 to 42,413 painful procedures were counted with each neonate having on average 7.5–17.3 invasive procedures per day [41].

Limitations of some of these studies include the lack of reporting of repeated attempts [41], despite the fact that it can constitute a significant additional burden. For example, the prospective study by Carbajal and colleagues showed that approximately 20% of painful procedures required at least two attempts, with some procedures such as intubation requiring in some cases even fifteen attempts [34]. A further aspect to consider when attempting to summarise the burden of neonatal pain is that most of the studies count the number of skin-breaking and other painful procedures, but do not further investigate how different procedures may vary in their contribution to neonatal pain [42]. Laudiano-Dray and colleagues reported estimates of how various procedures affect neonatal pain scores based on the literature [42]. The procedures described as most frequent in the review by Cruz and colleagues, namely heel lance, suctioning, venepuncture and insertion of peripheral venous catheter, tend to fall in the categories of moderate and severe intensity according to the ranking of Laudiano-Dray and colleagues [41, 42]. There is large inter-individual variability in the number of painful procedures recorded, with the highest number of painful procedures being recorded in the most vulnerable neonates, i.e. those born at lower gestational age (GA), smaller and sicker [34, 39, 40, 43]. According to the World Health Organization (WHO), more than one in ten babies (13.4 million, or one baby every two seconds) were born prematurely worldwide in 2020, defined as birth below 37 completed gestational weeks [44, 45]. Even healthy term-born neonates can undergo painful procedures such as routine metabolic blood tests in the first week of life or immunisations.

In contrast to the high number of painful procedures which neonates may undergo, pain management provision can be low and is generally inconsistent,

even within the same region [35, 37, 40, 41, 46], or for the same indication [47]. In the *EUROpean Pain Audit In Neonates (EUROPAIN)*, a prospective study assessing sedation and analgesia in NICUs across 18 countries, a wide variation in sedation and analgesia practices and provision rate (0–100 %) in tracheally ventilated neonates was observed among centres [46]. Exposure to pain without adequate analgesia in a critical period of rapid neurodevelopment, such as the neonatal period, can have short and long-lasting effects. The following section will briefly describe the maturation of nociceptive pathways, and the impact of early life pain on neurodevelopment.

2.4 The impact of pain in early life

2.4.1 The development of nociceptive circuits

Peripheral noxious (mechanical, thermal and chemical) stimuli can be conveyed to the dorsal horn in the spinal cord via afferent nerve fibres, such as myelinated Aδ and unmyelinated slow-conducting C-fibres [48–50]. The signal conducted by the faster, thinly myelinated Aδ fibres is often referred to as leading to a 'first' pain, as well as to the initiation of spinal reflexes such as limb withdrawal from a noxious stimulus, whereas the slower signal transmitted by C-fibres contributes to the following 'second pain' [51]. In the spinal cord, the incoming signal from the periphery is modulated [52] and conveyed to a large network of supraspinal areas involved in the processing of the nociceptive input [53]. No neocortical domain has been identified as pain-specific [54], and pain processing in the brain is currently conceptualised in terms of a pain network [55] and a dynamic pain connectome [56], which highlights the temporal fluctuations and dynamic aspect of the connectome. A particular model of the pain network called the neurologic pain signature uses specific brain regions to predict pain and includes the ventrolateral thalamus, anterior and dorsal posterior insula, the secondary somatosensory cortex, and the anterior cingulate cortex [57]. Descending pathways projecting to the spinal cord can modulate nociceptive processing [53]. The timing of development of the

dynamic pain connectome is not yet fully understood, and may be dependent on the maturation of brain areas which are part of the adult pain connectome [58].

At term birth, the brain is still undergoing significant maturation including formation of synapses and pruning, whereas its macroanatomical layout already resembles that of an adult [58–60]. Sensory receptors innervate the skin in the first trimester in humans, and sensory afferents Aδ, Aβ, and C- fibre start to innervate the dorsal horn at slightly different times early in gestation [61]. Receptive fields are relatively large at birth and decrease in size postnatally [62]. Furthermore, the balance of spinal cord excitation develops over time, with premature neonates having a hyperexcitable dorsal horn compared to term neonates [63], reflecting findings from studies in rats where limb withdrawal reflexes occurred at a lower mechanical threshold in the first postnatal weeks compared to later in life [62]. The limb withdrawal reflex not only occurs at higher threshold, but also becomes more localised to the ipsilateral leg with increasing gestational age [64]. Furthermore, while limb withdrawal reflexes are not yet fully modality-specific at term age, an increased specificity to noxious stimuli is already observed between preterm and term neonates [64]. These reflexes can be quantified via surface electromyography (EMG) recordings of the biceps femoris (a knee flexor muscle) [65]. Their duration is longer in premature than term neonates, and longer in neonates than adults in response to a heel lance [64].

From the spinal cord, the information on noxious stimuli can be transmitted to higher centres. At the brainstem level, facial expressions to noxious stimuli are processed [65]. A differential expression of facial responses to noxious or innocuous stimuli is observed from approximately 33 weeks GA [66], and grimacing appears with shorter latency with increasing postmenstrual age (PMA) [67]. The ascending somatosensory pathways convey information on peripheral stimulation to the thalamus before the third trimester [58]. The subplate, a transient brain structure, plays an important role in the development of brain connectivity [68], including thalamocortical connections [58]. It is formed approximately at 15 to 17 weeks GA [59], and regresses in the first few postnatal months [58, 69].

Thalamocortical connections first synapse in the subplate, and later directly in the prospective somatosensory cortex in the cortical plate, earlier than in the occipital and frontal areas [69, 70]. Thalamocortical connections are functional at term age and continue to be refined beyond term age [59, 70]. This is reflected in changes in EEG patterns [71–73]. Noxious input can induce cortical activity from roughly 25–27 weeks GA [69, 74, 75], in parallel or shortly after the development of the anatomical substrate required for processing of sensory input [69, 70]. Somatosensory-evoked potentials (SEP) are used clinically to assess the processing of sensory stimuli; they can be recorded with EEG already below 30 weeks GA and provide useful information on the maturation of the nervous system [58, 76]. Brain activity recorded with EEG begins to discriminate between non-noxious touch and noxious stimulation from roughly 35–37 weeks GA [66, 77]. Patterns of brain activity are less modality-selective in premature neonates, as delta brushes (a slow delta wave with superimposed fast frequencies [78]) can be seen in response to heel lances and innocuous stimuli [77]. The ability of the brain to encode stimulus intensity was observed in functional magnetic resonance imaging (fMRI) studies of term neonates [79]. Goksan and colleagues reported a similar pattern of noxious-evoked brain activity in term-born neonates in the first week of life compared to adults, suggesting that thalamocortical connections are likely functional at birth; yet, some of the regions which are involved in emotional processing of noxious stimuli in adults were not yet activated in neonates [58, 80]. Furthermore, it was reported that the signal was more bilateral in term-born neonates compared to adults [80].

Development of pathways for processing of emotional quality of pain appear to develop slightly after pathways for sensory quality [81]. The balance of descending modulation via the rostroventral medulla, which is one of the key structures involved in pain modulation and located in the brainstem, shifts from facilitating spinal excitability to a primarily inhibitory function and continues to refine itself until adult age is reached [81]. In rat pups the rostroventral medulla initially promotes signal transmission, and from the fourth rodent postnatal week until adulthood it starts to perform both signal inhibition (to higher intensity stimuli) and facilitation (to

lower intensity stimuli)[82]. The presence of a descending pain modulatory system was reported in an fMRI study alongside the observation that higher connectivity strength within the descending pain modulatory system at rest inversely correlates with noxious-evoked brain responses [83]. Brain resting state networks reported in adults can be observed, albeit with some differences, in premature neonates [84], and resting-state activity could predict noxious-evoked fMRI activity in healthy neonates [85]. Despite some similarities in noxious-evoked brain activity and resting networks between adults and neonates, the neonatal nervous system is in a period of rapid development and not yet mature, and it is vulnerable to the detrimental short-term effects, as well as long-term effects, that can be induced by exposure to clinically-required painful procedures.

2.4.2 Adverse effects of pain in early life

Noxious stimuli during the neonatal period can have detrimental effects in the short and long-term period. Noxious stimuli can induce acute cardiorespiratory changes such as relevant increases in heart rate and decreased oxygen saturation levels and hormonal responses, depending on the intensity and duration of the stimuli as well as concomitant neonatal clinical factors; some of the adverse effects can partly be reduced with the administration of analgesics [17].

Exposure to noxious stimuli in a period of rapid development such as early life can alter the development of nociceptive pathways, inducing long-term effects [81, 86–88]. Neonates born very premature are particularly vulnerable to the negative consequences of pain exposure in this critical period of maturation of the nervous system [70, 89]. Associations between premature birth and changes in brain structure and function and neuroendocrine responses have been drawn in the premature population [89]. Studying the effect of pain in early life on long-term outcomes is complicated by confounding factors. For example, neonates with the highest pain exposure also tend to be the babies who are born youngest or have more severe co-morbidities [90]. Disentangling the effect of pain from that of other factors such as co-morbidities and drugs received during the NICU stay is

challenging; to this end, some studies assessed the association between number of skin-breaking procedures with neurodevelopmental outcomes while also adjusting for clinical factors [89]. Furthermore, animal models can provide more confidence in the observations made in the human neonatal population by controlling for some of the confounding factors [91].

Structural and functional changes in the brain have been identified in association with higher exposure to procedural pain and surgery during the neonatal period [89]. In neonates born very premature (below 32 weeks GA), the number of skin-breaking procedures remained significantly associated with lower body weight and head circumference at 32 weeks postconceptional age, even after accounting for illness severity and other relevant clinical factors [92]. A higher exposure to painful procedures, counted again as the number of skin-breaking events, was associated with reduced maturation of white matter and subcortical grey matter, after correction for clinical confounding factors such as illness severity [93]. An association between a higher burden of skin-breaking procedures and structural brain development was observed as late as 7 years following birth below 32 weeks GA: after adjusting for relevant clinical factors (gestational age, illness severity, infection, mechanical ventilation surgeries and morphine exposure), a heavier burden of neonatal painful procedures was associated with a reduction in cortical thickness in various regions, suggesting a persistent effect of pain-exposure [94]. Furthermore, higher exposure to painful procedures was linked to altered development of the corticospinal tract, assessed with magnetic resonance imaging (MRI) at term equivalent age [95]. An increased number of painful procedures was also found to correlate with reduced total brain [96], basal ganglia, [96, 97], and thalamic volumes [96–98] as well as with slower thalamic growth [99], and reduced subregional volumes in the cerebellum [100].

Neonatal injury can also have effects on behavioural and cognitive outcomes. In a further study from the longitudinal Canadian cohort using magnetoencephalography (MEG), a higher exposure to skin-breaking procedures in neonates born up to 28 weeks GA was linked to altered spontaneous cortical oscillatory activity, which was itself associated with reduced visual-perceptual abilities at seven years from birth;

these changes were seen only in the cohort of children born at 24–28 weeks and not in the cohort of children born at 29–32 weeks GA [101]. A heavier burden of painful procedures was linked directly to lower neurodevelopmental scores at 8 [102] and 18 months corrected age [96, 102], or indirectly via reduced thalamic growth at three years corrected age [99], and is associated to lower IQ at school age [103]; all these studies controlled for relevant clinical factors, including morphine exposure and GA. The number of skin-breaking procedures undergone by premature neonates was found to be associated with internalising behaviours at 18 months corrected age [104, 105], three [105], 4.5 [105] and eight years [105, 106]. Furthermore, following correction for multiple comparisons, the number of skin-breaking procedures in a premature cohort remained significantly associated with hyperconnectivity at term age within the cerebellum, as well as interregional connectivity (cerebellum to association, paralimbic, and limbic, as well as subcortical-limbic). In the same cohort, a higher number of skin breaks positively correlated with risk of autism at 18 months [107]. Accordingly, in rodent models of neonatal injury, worse performance was reported for spatial learning, recognition memory, cognitive flexibility, as well as diminished social interactions and exploratory behaviour [108, 109].

Alterations in sensory paths have been studied assessing sensory thresholds following neonatal injury in humans and various models of injury in rodents. While not all rodent neonatal injury models lead to the same long-term consequences, the consequences of neonatal injury across various rodent models have been summarised as a generalised increase in baseline sensory thresholds (hypoalgesia), and a localised decrease in sensory thresholds (hyperalgesia) in response to noxious stimuli of higher intensity or longer duration [88, 108, 110]. However, the intricate interplay of numerous factors can affect the development of nociceptive pathways towards heightened or decreased sensitivity [91]. Observations made in studies investigating the impact of early pain in human neonates revealed similar patterns to those observed in rodents of generalised hypoalgesia and local hyperalgesia [111], such as decreased thermal sensitivity to mild stimuli but increased reports of pain to more intense thermal stimuli in children who had been admitted to the NICU and

were born preterm [90]. Neonatal exposure to tissue injury can shift the balance of nociceptive signalling at the level of the primary afferents, as well as at the spinal cord level, towards signal facilitation, contributing to enhanced pain sensitivity [88, 90, 110]. Furthermore, peripheral sensitisation and hyperinnervation of the skin have been suggested as mechanisms underlying hyperalgesia [110]. In rodent models, full-thickness skin wounds can lead to increased innervation of the injured skin area, as well as increase in the receptive field size of dorsal horn neurons in the spinal cord [108]. The increased local sensitivity develops first, followed by the generalised hyposensitivity [110], which corresponds to the later maturation of descending modulation [110]. Increased responses to subsequent painful procedures have been reported in the neonatal period [112, 113] up until young adulthood: in the proximity of neonatal surgical scars, 18 to 20-year-old adults born extremely preterm reported a mixture of increased and decreased sensitivity, with some participants declining brush stimulation due to fear of pain [114]. Furthermore, increased analgesia was required for surgery in infants who had undergone major surgery in the same dermatome within the first three months of life [115].

The timing of injury has been shown to have an important impact on the development of long-term effects of early pain in most rodent models of neonatal injury [108, 110]: while the critical windows vary depending on the injury model, they tend to occur in the first three postnatal weeks and in particular in the first postnatal week in the rat [110], which is roughly comparable to the preterm period in human in terms of CNS development [111]. In humans, factors at the time of the neonatal injury, including timing of pain exposure [99, 101], sex, gestational age and pain-relieving interventions, and later in life (such as illness or anxiety) have been reported to modulate the development of long-term effects following pain exposure in early life [90, 111].

The effect of analgesia in preventing the long-term effects of pain exposure is being investigated. Numerous studies in rodents model of pain reported a dampening of enhanced responses to subsequent noxious stimuli when morphine or a local anaesthetic were administered at the time of the initial noxious stimulus [88, 116].

Nevertheless, reduction of long-term effects via analgesia is not consistent across all rodent pain models [108], and data from clinical studies does not yet provide a clear picture. Irrespective of the ongoing research related to the dampening of long-term effects of neonatal pain via analgesic provision, there is an ethical duty to reduce harm incurred during clinically-required procedures in neonates via appropriate analgesia. In order to provide appropriate analgesia, the mechanisms via which noxious stimuli affect the developing pain system require further elucidation [90, 108] in order to develop the best strategies to reduce the burden of early life pain.

Painful procedures not only affect neonates, but also their families. It still remains to be clarified how to best manage neonatal pain and support families during their baby's painful procedures. Yet, in order to understand whether an intervention is successful in relieving neonatal pain, there is a need for establishing pain assessment tools which, ideally, are reliable, valid, responsive and interpretable [117].

2.5 Assessing pain in a rapidly developing body

Challenges related to accurate assessment of pain in a rapidly developing body, presented in the previous sections, have hindered the establishment of a 'gold standard' for neonatal pain assessment. While verbal reports are considered gold standard and recommended primary end points in adults [118], measuring pain in preverbal infants and neonates inherently has to rely on proxy measures. Via neural activation in the spinal cord and brainstem, noxious stimuli can elicit fast protective responses such as reflexes, as well as changes in facial expressions [81]. Additionally, changes in cardiorespiratory patterns, hormonal levels and cortical activity can occur in response to noxious stimuli. Of the range of responses elicited by noxious stimuli, numerous are used as proxy indicators to facilitate assessment of acute pain for research and clinical purposes. Over forty neonatal pain assessment tools have been developed [119, 120], which are based on the neonate's responses to noxious stimuli; yet measurement properties such as validity of pain assessment tools display large variations even in the context of randomised trials [117, 119]. In the past two decades, brain-derived measure of pain-related activity have been

developed, offering a window into the processing of noxious stimuli at the cortical level. Given that pain is an experience occurring in the brain [53], brain-derived outcome measures can provide valuable insights into neonatal pain. This section will provide a brief overview of neonatal pain assessment methods, with a particular focus on those based on brain activity.

Establishing valid, reliable, responsive and interpretable outcome measures — as per COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) definitions [117] — to assess neonatal pain is paramount to be able to assess the analgesic efficacy of interventions. Considering that pain responses can be influenced by numerous factors, e.g. previous pain exposure, GA, postnatal age (PNA), sex, and wake-sleep state, pain assessment tools need to carefully define which population and situation they have been developed for [121–125]. Regarding the impact of contextual factors, some of the neonatal pain assessment methods take these into account [121]. In addition to neonatal factors, where pain is assessed by another person, personal beliefs and attitudes of the observer can influence pain assessment [121]. Objective outcome measures could prevent bias caused by subjective assessment, and are currently being researched [121]. These include, among others, hormones such as cortisol [121], heart rate indices [126], skin conductance [127], automated facial assessment [128], indices of parasympathetic activity such as the Newborn Infant Parasympathetic Evaluation (NIPE) index [129] and brain responses [130]. Methods which can be calculated in an objective manner such as brain-derived methods hold great promise in improving how pain is assessed and managed in neonates [121]. Their scope is currently still primarily within the research remit [119]; validating objective outcome measures for pain assessment in neonates is a crucial step to be able to provide neonates with appropriate analgesia and protect their neurodevelopmental trajectories from the detrimental effects of pain exposure in early life [30, 131].

2.5.1 Pain scales

Numerous pain assessment tools have been developed, which are primarily based on the neonate's behavioural and physiological responses to noxious stimuli and some contextual factors [132]. The number of neonatal pain scores plateaued around 2014 [120, 133], and numerous tools were validated before a consensus was established on how to validate outcome measures [119]. The work of the COSMIN initiative is generating agreement on how to assess measurement instruments [117, 134], which is helping establish the measurement properties of the instruments used to assess neonatal pain. This is a much needed area of research, considering that a recent review of the risk of bias according to COSMIN identified a moderate to high risk in a large proportion of neonatal pain assessment tools [135], and a review of methodological studies on pain assessment tools' responsiveness, a measurement property, revealed a generally low study quality [120]. The pain scale used most often in randomised neonatal trials is the Premature Infant Pain Profile-Revised (PIPP-R), which is composed of contextual factors, as well as behavioural and physiological indicators [119, 136].

2.5.2 Behavioural indicators

Facial expression, crying, body movements, and sleep-wake state are used as behavioural indicators of pain [125]. Limb withdrawal reflexes in response to noxious stimuli can be observed in both premature and term neonates. Behavioural responses to noxious stimuli were described extensively already in the 1980s and 1990s [137]. Tools have been developed to measure behavioural pain responses such as, for example, cry duration and pitch [14, 138], facial expression [139] and body movements [137]. Behavioural measures can be assessed at the cot-site but may be subject to observer bias if not estimated objectively, e.g. via automated methods. Furthermore, as it is generally the case with neonatal responses to noxious stimuli, they can be affected by various clinical and contextual factors, such as neurodevelopmental maturation [64, 67, 124]. Therefore, while behavioural indicators can inform pain assessment and are often included in pain scales [135],

relying exclusively on behavioural measures may not be sufficient to accurately depict whether a neonate is in pain or whether an intervention aimed at pain relief is effective.

2.5.3 Cardiorespiratory parameters and skin conductance

Acute noxious stimuli can alter physiological parameters such as heart rate and heart rate variability [14, 126], blood and intracranial pressure, breathing patterns, oxygen saturation and skin conductance [125, 140]. A systematic review of cardiovascular indices in response to acute noxious stimuli in the first year of life identified an increase in heart rate in most infants, though differences were noted depending on GA and PNA [126]. Skin conductance is increased by noxious stimuli such as clinically-required heel lances [141]. As for behavioural tools, changes in autonomic system parameters alone constitute one facet of the complex response to noxious stimuli.

2.5.4 Brain activity

Measures of noxious-evoked brain activity can also inform the assessment of neonatal pain [142]. Cortical activity in response to clinically-required noxious stimuli has been detected already at 25–27 weeks GA using near-infrared spectroscopy (NIRS) [74, 75]; these were also the first studies reporting cortical activity in response to noxious stimuli in neonates [130]. Considering that pain is 'an unpleasant sensory and emotional experience' which emerges in the brain [27, 53], brain-derived measures can provide valuable insights into nociceptive processing [130]. As of October 2015, a systematic review identified nineteen studies implementing neurophysiological outcomes of acute pain in term or preterm infants within the first year of life: nine thereof used NIRS, eight EEG and two fMRI [143]. Techniques which can detect haemodynamic signals such as NIRS and fMRI, as well as techniques which can directly detect electrical neuronal activity such as EEG, are shedding light on how noxious stimuli are processed in the brain. Furthermore, MRI is providing valuable information on the anatomy of the pain system, and how brain structure and responses to noxious stimuli relate [85].

2.5.4.1 NIRS

NIRS can measure changes in the concentration of oxygenated and deoxygenated haemoglobin, as well as global cerebral blood flow and volume via sensors, called optodes placed on the scalp [144]. NIRS is an indirect measure of neuronal activity, and relies on neurovascular coupling to provide information on neuronal activity. This is a process where increased neuronal activity is linked with haemodynamic changes such as increased blood flow and changes in oxygenation [144, 145].

Upon clinically-required heel lances and venepunctures, an increase in oxygenated haemoglobin or total haemoglobin concentrations has been observed over the contralateral somatosensory cortex in preterm and term neonates [74, 75, 146, 147]. The response is greater following clinically required heel lances than following control or tactile stimulation [146]. Although the cortical response generally correlates well with the facial expression component of the PIPP-R score, it was noted that some neonates do not respond to the noxious stimulus with a change in facial expression, despite the presence of a cortical response [74].

2.5.4.2 EEG

While NIRS detects haemodynamic changes related to neural activity, EEG can directly detect the activation of large populations of neurons [148]. In comparison to NIRS and fMRI, EEG has a higher temporal resolution of one millisecond or even less [148]. However, spatial resolution is low, so it is difficult to localise the source of the signal [148]. Neonatal responses to noxious stimuli based on EEG data have been investigated using event-related potential (ERP) (for example, [77, 149, 150]), and frontal asymmetry [151, 152] [143]. Sensory evoked potentials to auditory and visual stimuli are used clinically in paediatrics to determine the maturation and integrity of the nervous system; visual, somatosensory and auditory evoked potentials can already be detected in premature and term neonates [153, 154].

EEG data is used to identify ERPs, which can be assessed based on extracted characteristics such as amplitude or latency, and via time-frequency analyses [148]. Among neurophysiological assessment methods of neonatal pain, a scoping review

published in 2017 identified ERP-based methods as the most promising ones [143]. Noxious-evoked potentials in response to clinically-required heel lances have been reported in neonates in 2010 by Slater and colleagues [149], and have since been reported in over fifteen studies [155]. These noxious-evoked potentials were observed in a group of neonates with PMA ranging between 35 and 39 weeks [149]. In this seminal study, principal component analysis (PCA) was used to identify components which differed in response to a heel lance compared to a sham heel lance and a background period. The sham stimulus mimics a clinically-required heel lance but omits the noxious component by rotating the lancet by 90°, so that it fires into the air [149]. With PCA the dataset is simplified by extracting uncorrelated components that together can best account for data variance, i.e. the EEG data is decomposed into variables which still retain a large proportion of the original information [156]. The process of selection of principal components is hierarchical, whereby the first component accounts for the largest data variance, and the following components can best account for residual variance [157]. The central Cz and CPz electrode sites emerged as locations where the principal component weights differed significantly between stimulation type [149]. Principal components at these sites were investigated and led to the isolation of a component that best discriminated between heel lance and sham heel lance or background. This was referred to as N420-P560, where N refers to negative voltage and P to positive voltage, while 420 and 560 indicate the post-stimulus latency in ms. An earlier component which was not specific to the noxious clinically-required heel lance (N150-P260-N430) was also identified in the same study [149]. The earlier potential is sometimes referred to as N2P2, while the later noxious-evoked potential is occasionally referred to as N3P3 [146, 155].

A similar response was observed in a subsequent study comparing the magnitude of the noxious-evoked principal component at term age between a group of neonates born prematurely and having spent at least 40 days in intensive or special care and a group of healthy neonates born at term [158]. The authors observed that the magnitude of the later potential was larger in the group of neonates who had been born prematurely [158]. The noxious-evoked response is seen in neonates already at

32 weeks GA [77, 159]; it was noted that this corresponds to the period when direct connections are established between thalamus and cortex while the subplate is disappearing [159]. The occurrence of noxious-evoked brain activity increases with GA: while preterm neonates more often respond to noxious stimuli with non-specific neuronal burst activity (delta brushes), from 35–36 weeks GA a specific pattern of noxious-evoked response is more likely to occur than a non-specific pattern of neuronal activity [77]. With an evolving morphology and larger amplitude, noxious-evoked negative-positive peaks have been observed at Cz in infants up to one year old in response to routine immunisation [160], but not in adults [161].

The relation between noxious-evoked brain activity and other pain responses, as well as with some clinical factors has been reported in numerous studies. The noxious-evoked potential was found to correlate well with the intensity of an experimental mildly noxious stimulus, as well as with withdrawal reflexes quantified with electromyography in a group of term infants in the first ten postnatal days [162]. In contrast, the clinical pain score did not significantly correlate with the intensity of the stimulus [162]. This was interpreted as possibly being due to the relatively low intensity of the stimulus, which lead to low pain scores even in response to the highest stimulus intensity applied, and to a significantly smaller amplitude of the noxious-evoked brain response in comparison to a heel lance [162]. In a later study by Hartley and colleagues, an inverse pattern of increasing magnitude of noxious-evoked brain activity and decreasing reflex magnitude, latency and duration was observed with increasing GA [159]. It was suggested by the authors that this might reflect a maturation of the descending modulatory system inhibiting the limb withdrawal reflexes [159]. In addition to gestational and postmenstrual age [66, 77, 158, 159], sex [163], mode of delivery [164] and inflammation[165], can also influence the magnitude or distribution of the noxious-evoked brain response. Additionally, stress has been linked to higher magnitudes of noxious-evoked brain activity and a dissociation between behavioural and brain responses [166]. Noxious-evoked brain activity has been implemented as primary outcome measure in a randomised controlled trial (RCT) investigating the analgesic efficacy of sucrose for clinically-required heel

lances [167]. After the development of a standardised template for quantification of noxious-evoked response [168], as detailed in the following paragraphs, it served as co-primary outcome measure together with a clinical pain score in an RCT assessing morphine as pain-relieving intervention for acute procedural pain [169].

Properties of n-NRF outcome measure

The noxious-evoked response described above has been observed across multiple studies. In terms of quantifying the response, in the initial studies this was generally done by performing PCA in each study dataset, therefore creating a study-specific principal component for each study. Hartley and colleagues advanced this approach by characterising the noxious-evoked response: using PCA, they isolated a noxious neurodynamic response function (n-NRF) in a population of term neonates in channel Cz-Fz [168]. A neurodynamic response function is generally a fixed shape waveform that can be fit to an evoked signal. The n-NRF is a fixed shape waveform that can be fit to the signal evoked by a noxious stimulus. The n-NRF (previously termed 'template') resembles the later component of noxious-evoked brain activity identified in earlier studies [77, 149, 158]. This standardisation of the component assessed in response to the noxious stimulus brings the large advantage over study-specific components that it allows for this outcome to be validated across studies. In the same study, Hartley and colleagues assessed some properties of the n-NRF, showing that (i) the n-NRF magnitude evoked by noxious stimuli was significantly larger than that evoked by visual, auditory and tactile stimuli [168]. This observation was maintained after limiting the dataset to responses with highest heart rate changes, providing some evidence that this observation was not due to different stimulus saliency [168]. Furthermore, (ii) the n-NRF magnitude was larger in response to noxious compared to non-noxious stimuli also in premature neonates born at 34—36 weeks GA, (iii) and it was significantly associated with facial expressions (Pearson correlation coefficient 0.39, p=0.038, n=28). Importantly, in line with other studies, the authors highlighted that the presence of cortical activity was not always reflected in behavioural responses, and vice versa. Finally, the n-NRF

amplitude was significantly reduced upon application of local anaesthetics [168]. A comparable nociceptive-specific principal component was derived in a separate dataset following the same process [170].

The n-NRF derived by Hartley and colleagues was implemented in the Procedural Pain in Premature Infants (Poppi) trial, an RCT assessing the analgesic efficacy and safety of morphine for acute procedural pain published in *The Lancet* [169]. In addition, it was implemented as outcome measure in various observational trials [66, 164, 165, 171–174]. Following a similar process to Hartley and colleagues [168], a transitory principal component related to noxious stimulation was identified in younger premature neonates (30–33 PMA), which may be further investigated as an outcome measure for this younger population [174].

2.5.4.3 MRI

MRI is a non-invasive technique which relies primarily on the magnetic properties of hydrogen nuclei forming water molecules [175]. While it has worse temporal resolution and it is more costly than EEG and NIRS, it offers higher spatial resolution, providing an excellent opportunity to investigate the spatial distribution of brain activity in response to noxious stimuli. Furthermore, MRI provides whole brain coverage, which is a large advantage over EEG and NIRS. MRI can provide functional information in response to tasks or stimuli and about spontaneous brain activity during rest [175, 176]. Functional MRI often relies on the blood oxygenation level dependent (BOLD) effect, which links neural activity to the haemodynamic changes detected with MRI [175, 177]. Additionally, MRI can inform about brain structure and anatomical connectivity via structural and diffusion imaging [175]. MRI scans in neonates are challenging, due to, among other factors, large changes in the magnetic properties of the brain in the first postnatal months, smaller brain size compared to adults, as well as the logistics of scanning a newborn baby [178]. Nevertheless, they have been providing valuable insights into pain and somatosensory processing in neonates. It was observed that mild noxious stimuli activate in neonates most of the brain regions which are activated in adults [80].

Distinct patterns of brain activity were observed in response to different types of stimuli including noxious stimuli [79], and the magnitude of the response to mildly noxious stimuli was inversely related to the connectivity strength within the descending pain modulatory system [83]. Patterns of brain activity which in adults relate to pain intensity (which can be assessed using fMRI models like the neurologic pain signature (NPS)) were observed in term neonates [179]. Furthermore, resting state activity and structural characteristics could predict the magnitude of the noxious-evoked response, unveiling associations which could explain part of the variability in neonatal noxious-evoked brain activity [85].

Considering the different temporal and spatial resolutions, EEG, NIRS, and MRI provide useful complementary information. MRI is shedding light on the encoding of pain and individual variability in humans and non-human animals [56, 57, 180]. Currently, the use of MRI to assess pain or nociception remains primarily limited to a research context due to, among other reasons, higher costs and more complex logistics [118]. Currently, most MRI scanners are not available at the cot-side. Although portable devices are being developed, these have lower field strengths and currently yield much more limited information, primarily on large intracranial abnormalities visible on structural scans [181].

2.5.4.4 Brain-derived outcome measures as clinical trial endpoints

The establishment of validated pain outcome measures for neonatal RCTs is crucial to the advancement of neonatal pain assessment and management [30, 131]. Brain-derived outcome measures may offer objective endpoints for such trials. Despite the general reliance on verbal reports as gold standard for pain in adults, indicators based on brain activity alongside others have been suggested as a complementary endpoint in adults [176, 182], and could be particularly useful in situations where verbal reports are not available such as in unconscious patients or infants [176, 183].

No pain biomarker has yet been approved by the FDA [184, 185]. The establishment of pain biomarkers – where a biomarker is ‘a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes,

or biological responses to an exposure or intervention, including therapeutic intervention' according to the Food and Drug Administration (FDA) [186] – complying with the high validation standards of FDA and COSMIN will be central to advancing the field [118, 182]. Biomarkers are further categorised by the FDA depending on their purpose: for example, as a safety, diagnostic or response biomarker to assess an individual's response to an intervention [186]. In line with this categorisation of biomarkers, Mouraux and Iannetti underscore the importance of thoroughly reflecting on the intended use of a biomarker and the construct it represents prior to claiming that a biomarker is a general representation of 'pain' [183]. Furthermore, they highlight the importance of using appropriate control stimuli with comparable saliency to differentiate between biomarkers related to pain *vs* saliency [183]. Depending on the intended use, different biomarkers would prove most beneficial. For instance, neonates could profit from objective response biomarkers to evaluate the effect of interventions [182].

Potential MRI and EEG biomarkers are being investigated in the adult and paediatric populations [118, 176, 182]. Large collaborations have been established both for adult and paediatric populations aiming at identifying the best candidates for pain biomarkers [118, 131, 187]. While neuroimaging biomarkers including the n-NRF approach cannot be equated to a representation of the whole pain experience, they can shed light onto the way the brain processes noxious stimuli and complement verbal reports where available [143, 168, 176]. The n-NRF detected in EEG studies in neonates described above (2.5.4.2) has the advantage that, being a clearly defined waveform, it standardises the quantification of noxious-evoked brain activity across trials and can be validated [168]. Previous studies highlighted that noxious-evoked brain activity quantified via n-NRF (i) is responsive to analgesic interventions such as the administration of paracetamol or local anaesthetic [168, 173], (ii) it shows a borderline weak-moderate strength of association with facial expressions in response to a clinically-required heel lance ($r=0.39$, $p=0.038$, $n=28$)[168, 188] (iii) and it is reproducible across datasets collected by different research teams (manuscript under review - see the pre-registered study's manuscript with in principle acceptance from

Cortex [189]). Additionally, the way the raw EEG data is processed and analysed to calculate the magnitude of the n-NRF is being standardised in the form of an EEG analysis pipeline which is going to be publicly available as a plug-in for EEGLAB (manuscript under review). Furthermore, the reliability, validity and interpretability of the n-NRF is being assessed within the framework of a systematic review [190]. Alongside outcome measures assessing other dimensions of the pain response, the n-NRF approach can provide an indication of the noxious stimulus processing at the cortical level, where pain perception emerges [53, 191].

2.5.4.5 Multimodal pain assessment

Considering that pain is 'an unpleasant sensory and emotional experience' [27], a single biomarker cannot capture the whole pain experience [118, 182]. Pain-related outcome measures capture parts of the activation of the pain system, and do not always correlate with each other [130, 162, 167, 168]. Relying exclusively on behavioural and physiological indicators may lead to an underestimation of pain, as brain noxious-related pain activity can be observed in the absence of behavioural or physiological changes [192]. For example, while the magnitude of n-NRF tends to correlate with the facial expressions components of the Premature Infant Pain Profile (PIPP) score in preterm and term neonates (34–42 weeks PMA)[168], Hartley and colleagues observed that approx. 40% of neonates (11/28) did not display a facial expression in response to a clinically required heel lance; yet, 45% of the neonates lacking a facial response had a significant noxious-evoked brain activity [168]. Conversely, among the neonates who did display a change in facial expression following the heel lance, 24% did not have significant noxious-evoked cortical response [168]. In this study, significant noxious-evoked brain activity was defined by the authors as a template magnitude — the weight of the principal component in the 400–700 ms window following the heel lance — above 80% of the distribution of weights in the background epochs [168].

The observation of discrepancy across neonatal pain-related outcome measures has been reported in numerous studies, highlighting the importance of a multimodal

pain assessment [193, 194] which is not limited to behavioural observation and physiological indicators but also includes brain-derived indicators [172, 191, 195, 196]. Further research is required to determine the optimal measure or combination of measures of acute and chronic pain and stress in infancy [132, 194].

2.6 Current pain management strategies

Despite the lack of a generally agreed instrument to measure pain, efforts have been made to identify effective pain-relieving interventions. As of 2023, the authors of a Cochrane review on a subset of pain management interventions highlighted that 'it is clearly evident that infant pain is still under-managed and unmanaged' [197]. This situation was also previously described in a policy statement by the American Academy of Pediatrics published in 2016 [198].

The first principle of pain management is to prevent pain wherever possible by restricting the number of painful procedures to a minimum [198, 199]. It has been suggested that recent epidemiological studies are providing some preliminary evidence of a decrease in the number of painful procedures that neonates undergo [43], although this is based on data stemming from different countries and settings with limited comparability. Where a painful procedure is clinically required, pain management should be offered for minor as well as more invasive procedures [198]. In contrast, in a recent retrospective chart review in Canadian NICUs, pain relief was reported for 58.5% of painful procedures [200], and in a large prospective epidemiological study in France, some form of analgesia was provided for 75% of heel sticks [35].

Pain management interventions are often categorised as pharmacologic, such as sucrose or opioids, and non-pharmacologic, such as swaddling, non-nutritive sucking, or skin-to-skin contact [198, 201]. Furthermore, environmental measures such as reduction of lights and noise have been suggested in neonatal clinical guidelines on the management of neonatal acute procedural pain [201]. Although recommendations vary from guideline to guideline, breastfeeding, skin-to-skin care (also referred to as

kangaroo care) and sucrose are often recommended for mild procedural pain, while other non-pharmacological interventions may be recommended as adjuncts [199].

2.6.1 Pharmacological interventions

The WHO three-step analgesic ladder is sometimes used in children even though it was developed for adults, but concerns related to the safety of some pharmacologic agents further reduce its applicability in the neonatal population [202]. Off-label analgesics are often given to infants [30], and off-label prescriptions are common in the NICU [131, 203–205], and more generally across paediatrics [206]. The translation of efficacy and safety from adults or older children is complicated by developmental differences, e.g. large changes in pharmacokinetics and pharmacodynamics [195], and only few RCTs on analgesic interventions have been performed in children [30].

Among the most used pharmacological pain-relieving interventions are opioids, sucrose and paracetamol [202]. Sucrose is often given to neonates to prevent mild to moderate pain [198], and is recommended for minor painful procedures and in combination with another intervention for more painful procedures [207]. According to a recent Cochrane review, sucrose reduces some pain scores compared to a control group with moderate-certainty evidence, but the evidence is very uncertain when comparing sucrose to other interventions such as non-nutritive sucking, breastfeeding or facilitated tucking [208]. Although it has been reported that sucrose may decrease pain scores such as the PIPP-R, it has been underscored that other pain-related outcomes may not be beneficially affected by sucrose [208, 209]. In a sample of 59 neonates enrolled in an RCT comparing sucrose against a control group, sucrose did not significantly reduce limb withdrawal reflexes or noxious-evoked brain activity with a trend towards higher magnitude of noxious-evoked brain activity and reflexes in the intervention group despite lower PIPP scores [167]. Many questions remain in relation to the administration of sucrose in the neonatal population; for example, the recent Cochrane review underscored the need for studies to assess long-term neurodevelopmental outcomes upon repeated sucrose administration [208], as concerns of possible brain alterations were linked to sucrose

administration in mice [210], and a protecting effect of sucrose on neurodevelopment could not be detected in neonates [96].

While 55 RCTs were included in the recent Cochrane review on sucrose, only two studies were eligible for a recent review on nonsteroidal anti-inflammatory drugs or NMDA receptor antagonists for procedural pain in neonates, thus no conclusions could be drawn [211]. Paracetamol may reduce opioid use following major surgery and be effective for minor to moderate pain, yet it is unclear whether it is effective in dampening acute procedural pain such as that evoked by heel lances [212]. A small observational study investigated the effect of paracetamol on noxious-evoked brain response following immunisations; the authors compared a cohort of neonates who did not receive paracetamol to a later cohort of neonates enrolled after the local implementation of paracetamol administration into clinical routine, and reported a significant reduction of noxious-evoked brain activity, suggesting that paracetamol may have an effect on cortical noxious-evoked activity [173]. Information on long-term effects of paracetamol is currently limited [212].

Opioids are often used as first-line for moderately to severely painful procedures [207, 209]. A recent Cochrane review on the use of opioids for procedural pain in neonates suggested that they might reduce pain scores and underscored that the evidence grade of the studies was low to moderate [213]. Furthermore, the authors highlighted that episodes of apnoea might increase due to opioid administration, prompting caution in their prescription [169, 213]. Concerns related to adverse events [214] turn opioid administration into a difficult balancing act between providing adequate analgesia and minimising adverse events [215]. An improved understanding of which neonates might profit from morphine administration, for example based on genotypes, could be beneficial [30, 202, 215]. Concerns related to drug use are not limited to sucrose or opioids, but other medicinal agents too, such as the sedative midazolam [216]. Worry about adverse effects of drugs such as analgesics and sedatives is one of the reasons that hinder their use in NICUs [202].

2.6.2 Non-pharmacological interventions

Non-pharmacological interventions for neonatal pain management encompass a wide range of options [217] including skin-to-skin contact (kangaroo care) [218], massage [197], breastfeeding [219], and using a pacifier [197]. The use of non-pharmacological interventions is strongly encouraged for mild and moderately painful procedures based on observed reduced pain scores [198]. Breastfeeding, sweet tasting solutions (e.g. sucrose) and kangaroo care have been recommended as primary interventions during heel lances, venepunctures or injections in a comprehensive narrative review of non-pharmacological interventions [217]. In this review, sucrose was listed among the non-pharmacological interventions, in contrast to its grouping under pharmacological interventions in the policy statement published by the American Academy of Paediatrics in 2016 [198]. Although numerous studies have investigated non-pharmacological interventions for minor acute procedural pain, it was noted that the heterogeneity of the intervention protocols limits their implementation into clinical practice [209]. The ideal combination of non-pharmacological interventions is yet to be established [132], and various combinations of non-pharmacological interventions are recommended in clinical guidelines on the management of acute procedural pain in neonates [201].

Kangaroo care consists of holding a baby with a diaper and cap on the caregiver's chest. It was implemented in Colombia to replace incubators, and was associated with improved neonatal outcomes [217]. A recent Cochrane review on its effect for acute procedural pain relief in neonates listed its beneficial effects on behavioural and physiological outcome measures [218]. The effect of kangaroo care as an analgesic intervention has also been assessed with brain-derived outcome measures. Jones and colleagues performed a prospective cross-sectional study, comparing maternal skin-to-skin contact (n=9), being held by the mother with clothing (n=9), and individualised care in the cot or incubator (n=9) during a clinically-required heel lance. Noxious-evoked brain activity (global field power of the P3 potential measured with EEG) was significantly smaller in the kangaroo mother care group compared to being held with clothing [220]. There was no significant difference

between the kangaroo mother care group and individualised care group, which was interpreted as success of individualised care [220]. Other outcome measures (behavioural score, heart rate) did not significantly differ between groups [220]. Olsson and colleagues assessed maternal skin-to-skin care against being in the crib or incubator during a venepuncture in a prospective cross-over study enrolling ten preterm neonates [221]. They observed significantly smaller increases in cerebral oxyhaemoglobin in the intervention group, which was interpreted as a reduction of cortical pain-related responses. Non-significant results were reported for PIPP-R scores, oxygen saturation and heart rate increase in the 30 s following the procedure; the authors noted that the concomitant use of glucose may have dampened the behavioural responses [221]. Wang and colleagues enrolled 73 preterm neonates in an RCT assessing the analgesic efficacy on repeated heel lances of kangaroo mother care compared to a control group [222]. Kangaroo care was deemed superior to a nesting position in the absence of mothers in dampening neonatal pain responses based on PIPP score, regional cerebral tissue oxygenation saturation measured with NIRS and physiological indicators. A further study assessing the analgesic efficacy of kangaroo care upon repeated procedural pain reported a sustained beneficial effect of kangaroo care equivalent to that of sucrose and of a combination of sucrose and kangaroo care, based on PIPP and neurobehavioural assessment [223]. Kangaroo care is still underused and its beneficial effects go beyond neonatal procedural pain management, as it has further important benefits for neonates such as reduced mortality and breastfeeding establishment, and also reduces depression risk in mothers and fathers and facilitates bonding [224]. Almost all studies have focussed on the mother as the provider of skin-to-skin care [218], and the recent WHO guideline focusses on 'kangaroo mother care' and recommends that mothers perform kangaroo care in the first line [225].

Massage is another intervention which has been investigated for neonatal procedural pain management. Massage studies tend to focus on premature rather than term neonates, and reduced stress hormones, decreased pain scores and improved weight gain are among the beneficial effects of massage summarised in reviews

[207, 226, 227]. A recent Cochrane review on non-pharmacological interventions for neonatal acute procedural pain concluded that massage interventions may be effective in reducing pain-related responses, but the evidence certainty is very low to low [197]. Different massage protocols are reported in the literature, using different techniques, locations and durations [227]. Among frequently used techniques are 'Field massage', which involves 15 minutes of moderate pressure massage [226, 227], 'gentle human touch', which involves placing one hand around the neonate's head and the other hand on the neonate's bottom or abdomen [197], or Yakson, a Korean massage technique composed of 5 minutes of steady touch, 5 minutes of caressing and 5 minutes of steady touch again [197]. Sepsis [228–230] or even suspected infection [228] are sometimes listed as exclusion criteria in massage studies, thus caution should be warranted when generalising safety and effectiveness results to babies with suspected or confirmed infection. Suggested mechanism through which massage might reduce pain-related responses are the gate control mechanism and descending inhibition [197, 227].

Beneficial effects have also been reported for other interventions including touch components, such as non-nutritive sucking, facilitated tucking and swaddling. However, the evidence certainty was very low for these three interventions [197]. Mechanisms underlying non-pharmacological interventions are generally unclear [197]. Multiple potential mechanisms have been proposed for interventions such as breastfeeding and kangaroo care, including warmth, the caregiver's heartbeat and scent, the sweet taste of breast milk, and tactile stimulation [199]. Non-pharmacological interventions such as kangaroo care might activate the endogenous opioid system, or act via gate control mechanism [231]. Massage and kangaroo care studies in neonates tend not to aim at unveiling the mechanisms by which the beneficial effects are generated [230, 232]. It has been suggested that massage as a form of touch with higher pressure may be primarily conveyed via Aβ fibres, while light touch such as a caress can activate C-tactile (CT) fibres [233]. A further potential mechanism behind the analgesic effects reported for touch-based non-pharmacological interventions in the neonatal population could be the activation

of CT fibres, a population of nerve afferents which is preferentially activated by a stroking motion such as that of a caress and which has been linked to reduced verbal pain reports in adults and beneficial effects in neonates [51, 234, 235]. An improved understanding of the mechanisms underlying the beneficial effects reported for non-pharmacological interventions could improve defining the population and situations where the interventions would be most valuable.

2.6.3 Potential role of C-tactile fibres in mediating pain-relieving effects of touch interventions

CT fibres are a type of C-fibre [236] nerve afferent. C-fibres have unmyelinated, thin-diameter axons, and can transmit information about noxious stimuli, itch, temperature and gentle touch [51, 236]. The latter population is referred to as CT fibres in humans, or C-low threshold mechanoreceptive afferents in non-human animals [237]. It is found primarily in human hairy skin [238], and more sparsely in glabrous skin [239]. CT-fibres have been studied with microneurography in adults, a technique where an electrode tip of approx. 5 µm size is placed into a peripheral nerve via percutaneous insertion, allowing recording of bursting patterns of single CT fibres [236, 240, 241]. In contrast to myelinated afferents, which show a linear correlation between brushing velocity and firing, CT fibres display an inverted U-shape with preferential activation in the range of 1–10 cm/s (peaking at approximately 3 cm/s) [242] compared to slower or higher velocities, matching the range of speeds deemed more pleasant [235, 243]. A further observed characteristic of CT fibres is that they respond preferentially to gentle touch at skin temperature (32 °C) compared to colder or warmer temperatures, matching the verbal reports of pleasantness [244]. Stroking of a partner's skin and maternal or paternal stroking of their babies is reported to naturally occur at CT-optimal speeds, and slower than stroking inanimate objects [245–248]. A recent study assessed the hand movement in 3D and suggested that previously reported velocities might have been somewhat underestimated, as previous measurements have generally occurred only in one dimension, even though the hand does not move exactly along a line [249].

The preferential activation of CT fibres during gentle touch at speeds akin those of interpersonal stroking led to the 'affective touch hypothesis' and 'social touch hypothesis'. Both hypotheses attribute a role to CT fibres in the encoding of positive affective input and social touch, respectively [51, 240, 242]. Forms of touch such as a gentle caress by a parent or partner are conveyed via myelinated Aβ, as well as CT fibres [51]. In a recent review by Schirmer, Croy and Ackerley on CT fibres, where they reviewed the literature on CT fibres since their discovery, they investigated the semantic association between the terms 'CT fibres' and 'affective touch', observing a strong co-occurrence of the terms [237]. The authors also warned about a logical fallacy in the literature: the observations that CT fibres firing is linked to the perceived pleasantness of touch should not be equated to CT fibres being the only afferents conveying information about touch pleasantness [237]. The authors reiterated that Aβ fibres, which are generally seen as conveying discrimination touch information, may be involved in the transmission of affective touch information [237].

Based on the characteristics of CT fibres and their neuroanatomy, it was suggested that they might play a role not only in conveying information about gentle touch, but also in pain modulation [242]. Studies in adults have shown pain-modulating properties of CT fibres, as long as the fibres are intact [238]. Based on similar brain activation patterns on fMRI scans between the conditions of brushing at CT-optimal speed and experimental heat pain compared to heat pain only, the authors suggested that pain modulation via CT fibres might occur at the spinal cord level [238]. A recent study in rats indicated a role of the endogenous opioid system at the spinal cord level in the modulation of nociceptive transmission [250]. An analgesic effect of touch at CT-optimal speed was also reported when touch was provided in the form of stroking by a romantic partner in a different area (forearm) compared to the experimental noxious stimulus (dorsum of the hand) [251]. The mechanism through which the activation of CT fibres can provide analgesic efficacy is not yet clear. In the study involving 32 couples and assessing noxious-evoked brain activity, partners brushed their partner's skin at CT-optimal (3 cm/s) and higher speed (18 cm/s). CT-optimal touch by the romantic partner reduced verbal

pain reports and evoked potentials in response to laser noxious stimuli compared to stroking at higher speed [251]. Further studies assessed the effect of experimental brushing at CT-optimal speed on brain-derived outcome measures. In a study in fifty young women, Krahé and colleagues observed reduced EEG peaks related to nociceptive processing when women's arms had been stroked at CT optimal compared to sub-optimal speed in response to laser stimulation [232]. Liljencranz and colleagues confirmed that brushing at CT-optimal speed could reduce pain reports in response to laser stimulation, whereas faster brushing or vibration could not [252]. Furthermore, in the same study the authors observed that CT-optimal brushing was effective in significantly reducing verbal pain reports not only when applied simultaneously to the noxious stimuli, but also when applied beforehand. However, the longer the delay between brushing and noxious stimulus, the smaller the effect of the intervention. Finally, brushing for a longer duration (20 s) was more effective than a shorter period (8 s) [252].

Studies aiming at activating CT fibres have been performed in the paediatric population as well, yet without the use of microneurography due to ethical reasons. The CT fibre population is thought to be mature in neonates [230, 253]. Similar brain activation patterns were reported in two-month-old infants compared to adults in response to slow stroking, which was interpreted by the authors as an indication of the infant's ability to encode social touch in a similar way to adults [254]. Children aged 5–12 years reported the same preference as adults for stroking at CT-optimal speed of 3 cm/s compared to 0.3 or 30 cm/s using visual pleasantness ratings[255]. Brushing at CT-optimal speed was found to decrease the heart rate of nine-month-old infants more than brushing at sub-optimal speeds [256, 257], as well as the heart rate of infants in the first few postnatal months [248]. Manzotti and colleagues recruited a cohort of premature neonates within a week from birth and investigated the effect of dynamic touch in the form of stroking compared to static touch on the baby's back. Both forms of touch were provided by researchers over a period of five minutes. Based on the beneficial effect of CT-optimal stroking in reducing premature neonates' heart rate, the authors concluded that more research into frequency and

duration, as well as long-term effects of CT touch intervention was needed [230]. Püschel and colleagues also reported a mean reduction in heart rate in preterm neonates stroked by their mothers, who naturally stroked at CT-optimal speed [246].

Based on the analgesic effect reported for touch-based interventions such as kangaroo care and massage in neonates and infants, as well as for interventions aimed at activating CT fibres in adults and infants, two pilot studies assessing the analgesic efficacy of experimental brushing at CT-optimal speed for acute procedural pain in neonates were conducted at the University of Oxford [171, 173]. In the study by Gursul and colleagues, brushing at CT-optimal speed (n=16) led to a significant reduction of approximately 40% in noxious-evoked brain activity assessed with EEG in response to a clinically-required heel lance compared to a control group (n=16) [171]. Noxious-evoked brain activity was quantified with the n-NRF approach described in section 2.5.4.2. In contrast, reflex withdrawals were not significantly altered by brushing at CT-optimal speed [171]. Although the proportion of neonates who displayed facial grimacing was similar in the two groups, their duration was longer in the control group [171]. Following this study, a similar reduction in the n-NRF magnitude in response to a clinically-required heel lance was reported for brushing at CT-optimal speed (n=16) compared to a control group (n=15) in an independent dataset of premature and term-born neonates [173]. In both studies, brushing using an experimental brush stimulus was performed by a researcher on the leg ipsilateral to the heel lance [171, 173].

2.6.4 Parental involvement in pain management

While research on CT fibres has mainly involved experimental stimuli, it would be valuable to expand research on interventions aiming at activating CT-fibres to a more naturalistic setting, e.g. between parents and infants [234]. Numerous non-pharmacological interventions offer an opportunity to involve parents in their baby's care, which is encouraged by the American Academy of Pediatrics and included in numerous neonatal pain management guidelines [198, 199, 201]. Investigating stroking of the baby's skin as a pain-relieving intervention offers an opportunity

to involve parents. The importance of parental involvement is underscored in a variety of approaches to improve neurodevelopmental outcomes and parental experiences. For example, parental involvement forms one of the core measures of the Neonatal Integrative Developmental Care Model (NIDCAP) [258], and family-centred approaches where the parents are primary caregivers in the NICU are effective in improving important neonatal outcomes, such as breastfeeding rates and weight gain, as well as in reducing parental anxiety and stress [259]. The neonatal UK charity Bliss has developed a Bliss Baby Charter to support neonatal units in the process of implementing family-centred care [260].

However, parent-led interventions are under-used [200]. Although they can be time-consuming in every setting, non-pharmacological pain management interventions offer the advantage to be available in low-middle income settings, where their use may be more feasible than the administration of drugs such as sucrose [261, 262]. There is potential to increase parental involvement in their baby's care via inter-professional collaboration and offering more training to parents [263–265]. Furthermore, parents could be involved more in the development of pain management guidelines, considering that a recent systematic review of clinical guidelines to manage acute procedural pain in neonates reported that only three out of seventeen eligible guidelines involved parents as stakeholders during either development or revision stage [201]. While the importance of partnering with families is being increasingly underscored, progress can be made on how best to support families, in particular with conflicting feelings such as generally wanting to be involved in their baby's care, but also feeling distressed by looking at their baby undergoing a clinically-required painful procedures [263, 266–271]. A better understanding of how to enhance and support parental involvement in their baby's pain management is needed [217].

2.7 Challenges related to obtaining evidence on neonatal pain-relieving interventions

Limited inter-professional collaboration and parental involvement alongside methodological issues when developing neonatal pain management guidelines can hinder the implementation of pain-relieving interventions [201, 261]. Large variations in pain management practices are observed internationally [46], and even within the same country [35, 36]. Furthermore, Cochrane reviews on interventions for acute procedural pain in neonates have often reported low-moderate certainty [208, 213, 218, 219, 272] or even a very low evidence grade [197, 211]. Sweet solutions such as sucrose and glucose have been extensively studied providing moderate-certainty evidence that it can reduce neonatal pain scores following a heel lance [199, 208]; yet, despite over two-hundred trials important questions remain unanswered, such as recommended dosages depending on the procedure and neonatal characteristics, long-term effects of repeated administration, or mechanism underlying diverging effects of sucrose on different modalities of pain responses [208, 209]. Improving the methodology of neonatal pain trials would help answering some of the open questions related to neonatal pain management [261].

Generating high quality evidence for pain management interventions in the neonatal population remains particularly challenging. At the design stage, choosing appropriate outcome measures is complicated by the fact that the majority of instruments used to assess pain and sedation in pre-verbal children have not been assessed for central instrument qualities such as validity or reliability [117, 135]. Furthermore, minimally clinically significant differences for calculation of sample sizes are yet to be established [195]. Given that analgesic interventions can differentially affect different modalities of pain responses, multimodal outcome measures can provide a more comprehensive picture than relying on unimodal outcome measures [172, 191]. While in adults interventions can be compared to a placebo arm, this would generally not be ethically acceptable in the neonatal population [195, 273]. The choice of a comparator arm requires careful planning to

ensure equipoise [195]. Enrolment occurs by proxy in a very special and stressful moment in parents' lives, and thus particular ethical and legal issues are intrinsically linked to enrolling neonates [205, 274]. It was noted by Baer and colleagues that the reason why most clinical trials fail is the inability to enrol the planned sample size [205]. Another consideration that complicates neonatal trials compared to adults is the reliance on a single noxious event, such as a clinically-required heel lance, whereas adults can provide consent to repeat noxious stimuli beyond what is clinically necessary and thus improve signal-to-noise ratio [65]. Furthermore, the choice of eligibility criteria should take into consideration substantial developmental differences even within the neonatal period [187, 273]; thus the recruitment of more homogeneous samples has been recommended [187, 273]. The study eligibility criteria should be carefully chosen, as increasing homogeneity among participants decreases generalisability of the observations to the population. The need for more emphasis on study designs is one of the points listed in the European Medicines Agency (EMA) document highlighting the necessity of a revision of the 'Guideline on the investigation of medicinal products in the term and preterm neonate' [275]. Not many RCTs on paediatric pain management have been performed [30], despite their higher value in determining the efficacy and safety of interventions compared to observational studies.

Pharmaceutical companies struggle with performing trials on paediatric pain management not only in the neonatal period but generally in the paediatric population [276], leading to only few analgesics approved in the first few years of life [273]. The general paucity of paediatric trials has prompted regulatory agencies such as FDA and EMA to introduce regulations to increase the number of paediatric trials [273]. Furthermore, they introduced guidelines and regulations to increase the number of pharmaceutical agents tested and approved for neonates. For example, since 2012 paediatric trials in the US should include neonates unless they obtain an exemption from the FDA [187]. To improve the quality of evidence available within paediatrics, including on paediatric and neonatal pain management, large international collaborations were established in recent years [131].

These include the International Neonatal Consortium [277], the Newborn Drug Development Initiative [195], or the Lancet Child & Adolescent Health Commission on 'Delivering transformative action in paediatric pain' [30]. The importance of increased collaboration and data sharing among different stakeholders has been highlighted, and might encompass parents, pharmacologists, trialists, clinicians, teams developing biomarkers and policy makers [30, 131, 201].

The RCT presented in this thesis involves parents and assesses a non-pharmacological intervention, namely stroking at the optimal speed to activate CT fibres. Outcome measures were multimodal: the primary outcome measure was brain activity measured with the n-NRF approach; secondary outcome measures were a neonatal pain score, neonatal development of tachycardia, and parental anxiety. The stroking was performed by parents, who also shared their feelings and views on participation in their baby's pain management via an anonymous survey. The activation of CT fibres may be one of the mechanisms underlying the beneficial effects of touch-based pain-relieving interventions. This trial tested whether parental stroking at CT-optimal speed could dampen neonatal pain responses using a multicentre RCT design.

Chapter 3

Developing clinical trial methodology to assess analgesic efficacy with a brain-derived outcome measure

This chapter is primarily based on two manuscripts which I co-authored. The protocol of the trial was published in July 2022 in *BMJ Open* under a CC BY 4.0 license with copyright retained by the authors [1]. It follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement [278].

- Cobo, M. M., Moultrie, F., Hauck, A. G. V., Crankshaw, D., Monk, V., Hartley, C., Evans Fry, R., Robinson, S., van der Vaart, M., Baxter, L., Adams, E., Poorun, R., Bhatt, A., & Slater, R. (2022). Multicentre, randomised controlled trial to investigate the effects of parental touch on relieving acute procedural pain in neonates (Petal). BMJ Open, 12(7), e061841-e061841. doi:10.1136/bmjopen-2022-061841

The SAP was published online on ISRCTN on 31 March 2023, prior to unblinding the data on 4 April 2023. It was later submitted to Wellcome Open Research as an article of which I am co-first author [2, 279]. The article is currently undergoing peer review and was developed according to the relevant International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines and the guideline on SAP for clinical trials published by Gamble

and colleagues in *JAMA* in 2017 [280]. It was submitted under a CC BY 4.0 license with copyright retained by the authors [2].

- Baxter, L.*, Hauck, A. G. V.*, Bhatt, A., Cobo, M. M., Hartley, C., Marchant, S., Poorun, R., van der Vaart, M.‡ & Slater, R.‡ (2023) Statistical Analysis Plan for the Petal Trial: The Effects of Parental Touch on Relieving Acute Procedural Pain in Neonates [version 1; peer review: 2 approved with reservations] Wellcome Open Res, 8:402. doi:10.12688/ wellcomeopenres.19819.1

*equal contributions ‡ equal contributions

The content of the manuscripts mentioned above was restructured and expanded to align with the structure of the thesis. The initial idea of testing an intervention that activates CT fibres for neonatal pain relief stems from research conducted prior to the time I joined the research team. While the backbone of the trial had been defined when I joined this project, numerous processes and details which were core to conducting the trial and analysing the dataset had not yet been fully established. I created with Dr Maria Cobo the guidance sheets that describe trial procedures and were used to train research teams at both trial sites. Furthermore, I performed multiple site visits and a large proportion of data collection, and incrementally assumed responsibility for trial management up to a leading role. I performed numerous data quality checks and contributed to identifying improvement areas and addressing these throughout the study. Building on the initial statistical analysis plan included in the ethics application, in collaboration with colleagues (in particular Dr Luke Baxter and Dr Marianne van der Vaart) I defined the outcome measures and their assessment in much more detail, and amended and expanded the plan for comparative analyses between trial arms. This work forms the core of the SAP manuscript [2]. We thank the OxUSC (Oxford University Statistical Consulting) group for reviewing our statistical analysis plan, helping to ensure an appropriate analysis plan was in place prior to data unblinding.

3.1 Background and rationale

Based on observations that brushing or stroking at CT-optimal speed can reduce verbal pain reports and noxious-evoked brain activity in adults [232, 251, 252], and pilot studies showing a reduction of noxious-evoked brain activity in neonates [171, 173], an RCT investigating the effect of parental stroking at the optimal speed to activate CT fibres was designed: the Parental touch trial (Petal).

In addition to the evaluation of parental touch as a potential analgesic intervention for acute procedural pain (clinically-required heel lance) in neonates, this trial is part of an effort to establish the objective, brain-derived n-NRF approach (detailed in section 2.5.4.2) as an outcome measure for analgesic trials in the neonatal population. The regulatory agencies FDA and EMA are calling for an increase in paediatric pain trials [273], and developed specific guidelines for any trial performed in the neonatal population due to complexities linked to this period of life [275, 281]. Indeed, designing and conducting RCTs in the neonatal or more generally paediatric population is challenging but also fundamental to improve clinical practice [205, 282]. Brain-derived outcome measures for analgesic trials in neonates are gathering increasing attention in the scientific community and from regulatory agencies [195], and approaches including the n-NRF have been discussed in a recent FDA workshop [283]. This trial was developed and described according to the relevant ICH guidelines: *ICH E3 Structure and Content of Clinical Study Reports* [284], *ICH E6 Guideline for Good Clinical Practice* [285], and *ICH guideline E8 (R1) on general considerations for clinical studies* [286].

To minimise the risk of bias in this trial, and facilitate future risk of bias assessment by authors of systematic reviews and meta-analyses, the statistical analysis plan aims to provide information on all domains of the Cochrane risk-of-bias tool for randomized trials (RoB) 2, which is a tool that authors of Cochrane reviews are encouraged to use when assessing studies [287]. Where applicable to the Petal trial, elements from the Consolidated Standards of Reporting Trials (CONSORT) extension for non-pharmacological interventions were detailed in

the SAP and alongside the trial results [288]; these included who provided the intervention, accurate details on blinding, and whether the intervention planned and performed matched. While the Petal trial protocol already contained a preliminary plan for the statistical analysis of the dataset, this plan was refined where needed and described in much more detail in a separate document. This SAP document was developed to reflect the ICH E9 guidance document on *Statistical Principles for Clinical Trials* [289, 290] and the guideline on SAPs for clinical trials published by Gamble and colleagues in *JAMA* [280]. The SAP was published online prior to unblinding the data [2, 279].

The low certainty grade of evidence on pain-relieving intervention has been a recurring issue in neonatal pain management, and it is indeed particularly challenging to conduct studies in this population. For example, the Cochrane 2023 review on non-pharmacological interventions for procedural pain management in infants and young children concluded that 'most analyses were based on very low- or low-certainty grades of evidence and none were based on high-certainty evidence. Therefore, the lack of confidence in the evidence would require further research before we could draw a definitive conclusion.' [197]. In the Petal trial, we aimed at implementing the available guidelines and minimising risk of bias while investigating a potentially pain-relieving intervention for the neonatal population. Furthermore, parents were at the centre of this trial as the person providing the intervention, and their feelings and motivations to take part in the trial were investigated in the attempt to identify how to best support parents in comforting their baby and what would potentially motivate them to participate in future studies.

3.2 Designing a clinical trial to assess analgesic efficacy in neonates

3.2.1 Trial design

Petal is a multicentre, randomised, two-arm, parallel, controlled, superiority trial. It tests the hypothesis that parental stroking at CT-optimal speed prior to a

clinically-required heel lance reduces noxious-evoked brain activity compared with a control arm. The clinical heel lance is one of the two acute pain models for clinical analgesic trials in the neonatal population listed in the Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks (ACTTION) recommendations [195], and one of the most common painful procedures undergone by neonates whilst hospitalised [41]. In a recent prospective longitudinal observational study conducted in four Canadian NICUs, heel lances constituted over half of the recorded painful procedures premature neonates underwent [43]. Heel lances are thought to activate Aδ and C-fibre nociceptive afferents based on studies in adults, who consider them painful [291]. Laudiano-Dray and colleagues pooled evidence from numerous studies to quantify the pain severity associated with common NICU procedures, and estimated the pain severity of a heel lance to fall into the moderate group [42]. The trial protocol described in the following sections includes the items listed in the SPIRIT 2013 statement, which is endorsed by the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) Network for transparent reporting of clinical trials' protocols [278, 292]. A SPIRIT checklist is appended to in the published study protocol [1]. The study received approval from the London-South East Research Ethics Committee of the National Research Ethics Service (reference 21/LO/0523) and conformed to the Declaration of Helsinki and Good Clinical Practice (GCP) standards. To standardise the trial across sites and personnel involved during the study, guidance sheets were developed. These covered recruitment, randomisation, performing the intervention, equipment set up and monitoring, test occasion overview, data analysis and entry, safety and incident reporting, withdrawal, training and delegation, documentation and data storage. The guidance sheets are included in Appendix F.

3.2.2 Eligibility criteria and recruitment of participants

Participants were recruited from two centres in the UK: the John Radcliffe Hospital (Oxford University Hospitals NHS Foundation Trust, Oxford) and the Royal Devon and Exeter Hospital (Royal Devon University Healthcare NHS Foundation Trust,

Exeter). Neonates were assessed for eligibility at the time of recruitment, and their eligibility was reassessed at the time of randomisation. Inclusion and exclusion criteria are listed below.

Inclusion criteria

1. Participants born at the John Radcliffe Hospital, Oxford or the Royal Devon and Exeter Hospital, Devon
2. Neonates born at or after 35+0 weeks gestation
3. Neonates with a postnatal age of $\leq$7 days
4. Neonates who require a heel lance as part of clinical care
5. Neonates for whom parents/guardians have given written informed consent for participation.

Exclusion criteria

1. Intraventricular haemorrhage (IVH) > grade II
2. Hypoxic Ischaemic Encephalopathy
3. Received any analgesics or sedatives in the last 24 hours
4. Congenital malformation or genetic condition known to affect neurological development
5. Born to mothers who have a history of substance abuse.

Parents were initially asked if they might be interested in being involved in research. If this was the case, they were verbally informed about the study and provided with written information via a participant information leaflet (Appendix B, C). After some time to consider study participation, parents were asked again if they wished to participate in the trial. Recruitment generally occurred on the day of the test occasion, because the timing of each study was centred around the clinically required heel lance, which was often scheduled by the clinical team on the day. The timing and location of the test occasions were jointly agreed upon by the research and clinical teams in order to optimise the integration of the test occasion with the rest of clinical care. Written informed parental consent was obtained for all neonates

(sample consent forms for each site are available in Appendix D, E). Parents were informed that they could withdraw their baby from the trial at any point without any obligation to provide a reason. In the event that parents decided to withdraw their baby after the study had begun, they were asked if data already collected may be retained and analysed in the trial. Parents were informed that a withdrawal had no impact on their baby's care. The attending healthcare professional could withdraw the neonate from the trial if this was in the best interest of the neonate's health and wellbeing. If exclusion criteria manifested after parents had provided informed consent, the neonate was withdrawn from the trial.

Regarding the two study sites chosen, the Oxford site (Oxford University Hospitals NHS Foundation Trust) is a larger hospital at level 3 care offering medical care to neonates needing the highest level of medical care, whereas the Exeter site (Royal Devon University Healthcare NHS Foundation Trust) is a level 2 care neonatal unit. The research team at the Oxford site was larger than the team at the Exeter site. At the Exeter site, rooms were generally a little larger than at the Oxford site. Multiple site visits occurred to optimise standardisation of trial procedures during the trial.

Impact of the COVID-19 pandemic on trial progress:

Recruitment for this trial and other similar research projects was largely impacted by the COVID-19 pandemic. Recruitment at the John Radcliffe Hospital, Oxford, UK for our research studies was halted for numerous months, properly resuming only towards the late spring of 2021, shortly before recruitment for Parental touch trial (Petal) began. While enrolling participants and conducting studies was allowed and supported when recruitment started in September 2021, concerns related to emerging virus variants and ever-changing governmental regulations likely negatively affected enrolment in this trial. For example, the Omicron variant was first detected in the UK at the end of November 2021 [293]. Concomitantly, we noticed a drop in the rate of parents agreeing to enrol their baby in Petal, which might have been linked to concerns related to increasing the number of contacts during the hospital stay.

3.2.3 Intervention and trial procedures

3.2.3.1 Intervention and control groups

Neonates were randomised in equal proportions to receive either parental touch just before the heel lance (intervention group) or parental touch after the heel lance (control group). Comfort care including swaddling and non-nutritive sucking, in line with local practice guidelines, was offered to all participants, irrespective of trial arm assignment, in addition to the pre- or post-procedural stroking. In both groups, parental touch was delivered in the form of stroking at the CT-optimal speed of approximately 3 cm/s for a duration of ten seconds. The duration of the stroking was chosen to match the pilot studies, where the intervention significantly reduced the magnitude of noxious-evoked brain activity [171, 173]. Parental stroking did not differ between trial arms with regard to the mode of delivery (parental hand), duration (10 s), approximate speed (3 cm/s), number of stroking motions (3 times), location (neonate's lower leg ipsilateral to the heel lance) and direction of stroking (along the limb). The trial arms differed in respect to the timing of the stroking: in the intervention group, this was just before the heel lance; in the control group, this was at least 30 s after the heel lance, at a time considered appropriate by the clinician performing the procedure to ensure that blood collection was not disrupted.

Parental stroking as well as standard of care were provided to neonates in both intervention and control groups. At the timepoint of measuring neonatal outcomes (primary: noxious-evoked brain activity, secondary: PIPP-R and tachycardia) only neonates in the intervention group had received parental touch in addition to standard of care; neonates in the control group had received standard of care only. The choice of these trial arms allowed assessment of neonatal outcomes between standard of care and parental touch intervention, and standard of care only. In the control group, parental touch was provided shortly after the measurement of the primary and secondary neonatal outcomes. This allowed parents to be physically involved in the care of their baby around the time of the procedure regardless of trial arm, which we therefore considered to be an ethically acceptable comparison arm. It was deemed potentially beneficial for both neonates and parents

to receive and provide touch, respectively. The State Trait Anxiety Inventory-State (STAI-S) (parental secondary outcome) was recorded after parental touch had been administered in both trial arms.

Active collection of the blood sample, which can involve applying gentle pressure to the baby's foot to collect an adequate quantity of blood, commenced at least 30 s after the heel lance. This ensured that the blood collection process did not impact the time window used to assess neonatal outcomes, which occurred within the 30-second period following the heel lance. During the intervention, parents were guided by a computer animation developed with PsychoPy software which was displayed on a screen [294]. The animation showed a 3 s countdown timer to identify the start of the stroking motion. This was followed by a progress bar that extended 3 times at a speed of 3 cm/s over a 10 cm distance for a duration of 10 s. Parents were trained verbally by a researcher on how to perform the stroking intervention, and the same animation was shown during training and during the intervention. Parents demonstrated to the researchers that they knew how to follow the animation and stroke their baby according to the trial protocol before commencing the stroking intervention.

3.2.3.2 Heel lance and sham heel lance procedures

All heel lances performed as part of this trial were clinically required. No additional heel lance was performed for the purpose of the study. Where neonates required multiple heel lances, data from the first heel lance was included in the analysis, conditional on data quality. Before the heel lance, we performed a non-noxious sham heel lance. During the sham heel lance the lancet was placed against the baby's foot but rotated at 90° so that the blade fired away from the baby and was released into the air. The sham heel lance mirrors a heel lance with the exception of the noxious skin-breaking component. The heel lances used were those in use by the clinical team at the time of the trial. All neonatal outcomes were recorded in response to both sham heel lance and heel lance. The sham heel lance is an

important validity check to demonstrate that the outcome measures are assessing the noxious component of the procedure.

3.2.4 Outcomes and recordings

An overview of the trial objectives and outcome measures is available in Tables 3.1 and 3.2. The primary outcome measure was the magnitude of noxious-evoked brain activity in response to the heel lance. Two of the three secondary outcomes were neonatal: a pain score, the PIPP-R, and the development of tachycardia [136]. A third secondary outcome measure assessed parental anxiety with the STAI-S score [295].

Objectives	**Outcome measures**
Primary objective	**Primary outcome measure**
To test whether parental touch prior to the clinically required heel lance reduces noxious-evoked brain activity assessed using EEG following a heel lance.	Magnitude of noxious-evoked brain activity following a heel lance (EEG data recorded in the 1000 ms period following each heel lance).
Secondary objectives	**Secondary outcome measures**
1. To test whether parental touch prior to the heel lance reduces clinical pain scores (PIPP-R) following a heel lance.	1. PIPP-R score during the 30 s period after the heel lance.
2. To test whether parental touch prior to the heel lance reduces incidence of tachycardia activity following a heel lance.	2. Tachycardia in the 30 s period post-heel lance.
3. To test whether parental touch prior to the heel lance reduces parental anxiety, compared with post-procedural touch.	3. STAI-S scores post procedure.

Table 3.1: Primary and secondary objectives and outcome measures. EEG: electroencephalography, PIPP-R: Premature Infant Pain Profile-Revised, STAI-S: State Trait Anxiety Inventory-State, STAI-T: State Trait Anxiety Inventory-Trait.

An overview of the study timeline and recordings is depicted in Figure 3.1. Each neonate was studied in a single test occasion during approximately one hour, with no follow-up. Baseline demographics and characteristics were recorded based on information reported in the medical records. Ethnicity was recorded based

Objectives	**Outcome measures**
Exploratory objectives	**Exploratory outcome measures**
1. To explore how parental touch impacts background brain activity.	1. Changes in brain activity during the touch intervention.
2. To explore whether parental touch prior to the clinical procedure reduces the duration of time for heart rate to return to baseline after a heel lance.	2. Time taken for heart rate to return to baseline post heel lance.
3. To explore how parental touch prior to the clinical procedure affects respiratory stability.	3. Post-procedural respiratory dynamics and incidence of apnoea.
4. To explore parental anxiety and distress, and their experience of the trial and infant research.	4. Scores for individual parameters from the STAI-T and STAI-S; four- point distress questionnaire score; responses to survey about participation in Petal and infant research.

Table 3.2: Exploratory objectives and outcome measures. STAI-S: State Trait Anxiety Inventory-State, STAI-T: State Trait Anxiety Inventory-Trait.

on information in the medical notes and, where unclear, on parental report. It was grouped based on the categories used in the census of England and Wales [296]. The time of the clinically-required heel lance was coordinated with the clinical team, and the test occasion was timed around this procedure. Data recorded includes neonatal EEG, neonatal physiological monitoring (electrocardiogram (ECG) and pulse oxymetry), video recording of the neonate, and parental questionnaires. Relevant demographic information from the medical notes was stored in the research electronic database by qualified members of the research team.

At the beginning of the test occasion, the parent who signed the consent form completed the first group of anxiety questionnaires (STAI-T and STAI-S). These responses were collected prior to the heel lance and stroking intervention, and served as measures of the anxiety levels at baseline. Physiological monitoring lasted from approximately 30 minutes before the heel lance until approximately 30 minutes after. The vital signs were used to calculate the occurrence of tachycardia (a secondary neonatal outcome) in response to the heel lance and to calculate the heart rate and oxygen saturation components of the PIPP-R score. EEG data was recorded for at

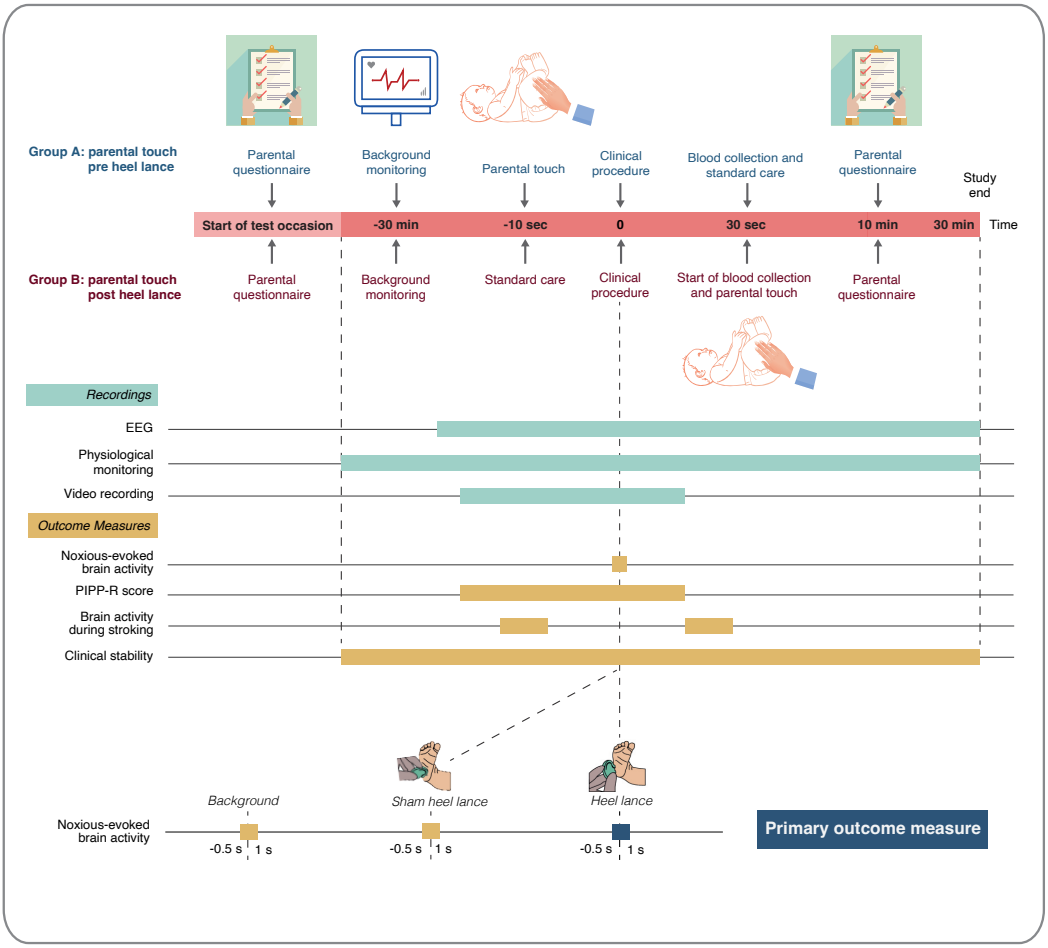


Figure 3.1: Overview of participant timeline. In the upper part of the figure, the two trial arms are depicted: the sequence of events in the intervention group is indicated in blue, while the sequence of events in the control group is indicated in red. The timings of recordings and outcome measures are shown. EEG: electroencephalography, PIPP-R: Premature Infant Pain Profile-Revised. This figure is reproduced with minor modifications from the published trial protocol [1] under a Creative Commons Attribution 4.0 (CC BY 4.0) license.

least 10 minutes before and after the heel lance. The EEG recordings were used to assess the magnitude of noxious-evoked brain activity, which was the primary outcome measure. Video recording of the baby's facial expressions for PIPP-R scoring lasted from about 30 s before to 30 s after the sham heel lance and heel lance procedures. The baby's foot was held by a clinical member of the research team in the same way independently of trial arm and during both sham and clinical heel lance procedures. The videos were used to calculate the secondary neonatal outcome PIPP-R score. Furthermore, they were used to report the behavioural

state at baseline; we used the four behavioural states of the PIPP-R score, and grouped them as either awake or asleep. After the procedure, parents completed a second set of questionnaires, including the same STAI-S questionnaire they had answered at baseline. Parental questionnaires were used to calculate the STAI-S anxiety scores before and after the heel lance.

3.2.4.1 Primary outcome

3.2.4.1.1 Magnitude of noxious-evoked brain activity

The magnitude of noxious-evoked brain activity in response to the heel lance was chosen as the primary outcome measure, as it can be assessed objectively. The magnitude is derived from EEG data recorded 400–700 ms after the heel lance at the central Cz electrode, and is quantified by the n-NRF. The n-NRF, named 'template' in previous work by our team, is a fixed-shape waveform which is fitted to each neonate's EEG data via linear regression. The n-NRF magnitude is the regression coefficient. The n-NRF is conceptually similar to a haemodynamic response function (HRF) in fMRI data [177, 297]. The n-NRF has been developed to quantify the characteristic noxious-evoked waveform reported in late preterm and term neonates [168]. While the primary outcome measure does not encompass the whole pain experience, it is related to the noxious input [168]. The magnitude of noxious-evoked brain activity correlates with the intensity of the nociceptive input [162]. Furthermore, it is scaled so that the average response in a group of control term neonates has a value of approximately one [168]. It is reduced with interventions such as the application of a local anaesthetic, paracetamol, and experimental brushing [168, 171, 173].

Electroencephalographical (EEG) recording

EEG data was recorded with the SynAmps RT 64-channel headbox and amplifiers (Compumedics Neuroscan) at the Oxford site, and with the Compumedics Grael V2 EEG system (Compumedics Neuroscan) at the Exeter site. The sampling rate was 2000 Hz at the Oxford site and 2048 Hz at the Exeter site, with an amplifier bandwidth of DC (direct coupled) to 400 Hz. CURRY 7 and CURRY 8

Neuroimaging Suite (Compumedics Neuroscan) were used to record the activity at the Oxford and Exeter sites, respectively. Eight surface EEG recording electrodes were positioned on the scalp at Cz, CPz, C3, C4, FCz, T3, T4 and Oz according to the modified international 10–20 System (Figure 3.2). Reference and ground electrodes were placed at Fz and Fpz, respectively. The recording electrode used to derive the primary outcome measure is located centrally at the vertex, at position Cz. The surface electrodes (Ambu Neuroline cup, Ambu®) consist of cups made of silver/silver chloride with no needles. All impedances were reduced to approximately 5 kΩ by rubbing the skin with EEG preparation gel (Nuprep Skin Prep Gel, Weaver and Company) prior to electrode placement. EEG conductive paste (Elefix V, Nihon Kohden) was used to optimise contact with the scalp. An ECG electrode was placed on the left clavicle to record heart rate.

During the sham heel lance and heel lance, the release of the blade triggers an accelerometer connected to an automatic detection interface which time-locks these procedures to the EEG recordings [196]. Start and end times of parental stroking were manually annotated to the EEG recordings by a researcher pressing a button. Background periods were manually annotated in the recordings to later be able to compare the magnitude of noxious-evoked brain activity in response to the heel lance to values obtained during background periods, when no procedure occurred and neonates were generally resting. Background EEG epochs were extracted from the period before the sham heel lance.

Quantification of noxious-evoked brain activity

EEG data were analysed with EEGLAB [298] as illustrated in Figure 3.3. Data rejection because of insufficient data quality, such as data corrupted by artefacts, was performed by two investigators blinded to trial arm, with discrepancies resolved by discussion. The magnitude of the noxious-evoked brain activity was calculated in response to heel lance as the primary outcome measure, and in response to the sham heel lance and during a background period as part of data quality checks. The magnitude in response to the noxious stimulus (heel lance) was expected to be larger than the magnitude in response to the sham stimulus.

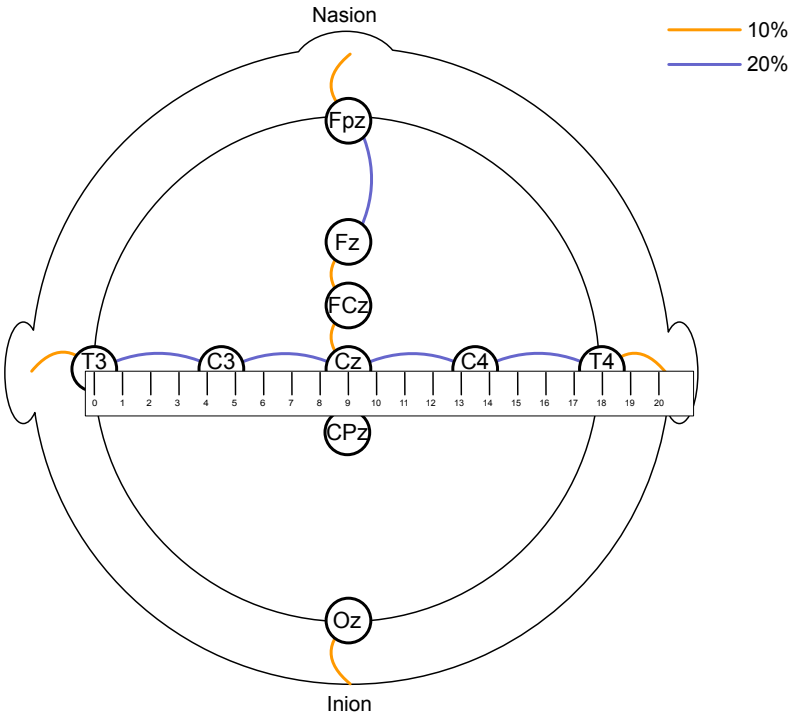


Figure 3.2: Overview of EEG electrode placement according to the modified international 10–20 System. The electrodes are placed over the frontal (F), central (C), parietal (P), occipital (O), and temporal (T) regions, with odd numbers indicating the left side and even numbers indicating the right side of the head. The 'z' indicates the midline. Therefore, Cz is placed centrally and at the midline, i.e. at the vertex. The distance between electrode T3 and the left pre-auricular point corresponds to 10% of the distance between the two pre-auricular points. The distance between Oz and the inion (the occipital protuberance of the skull) represents 10% of the distance between inion and nasion (the bridge of the nose). EEG: electroencephalography.

3.2.4.2 Secondary outcomes

Secondary outcomes were (i) PIPP-R scores in the 30 s following the heel lance; (ii) the development of neonatal tachycardia in the 30 s following the heel lance; and (iii) parental anxiety scores after the heel lance measured with the STAI-S questionnaire. Physiological recordings and videos were used to assess the secondary neonatal outcomes PIPP-R and tachycardia. Parental questionnaires were used to assess the secondary parental anxiety outcome.

3.2.4.2.1 Premature Infant Pain Profile-Revised (PIPP-R)

The PIPP-R is the most commonly used pain scale in randomised and quasi-randomised neonatal pain trials [119]. It is composed of contextual (PMA and

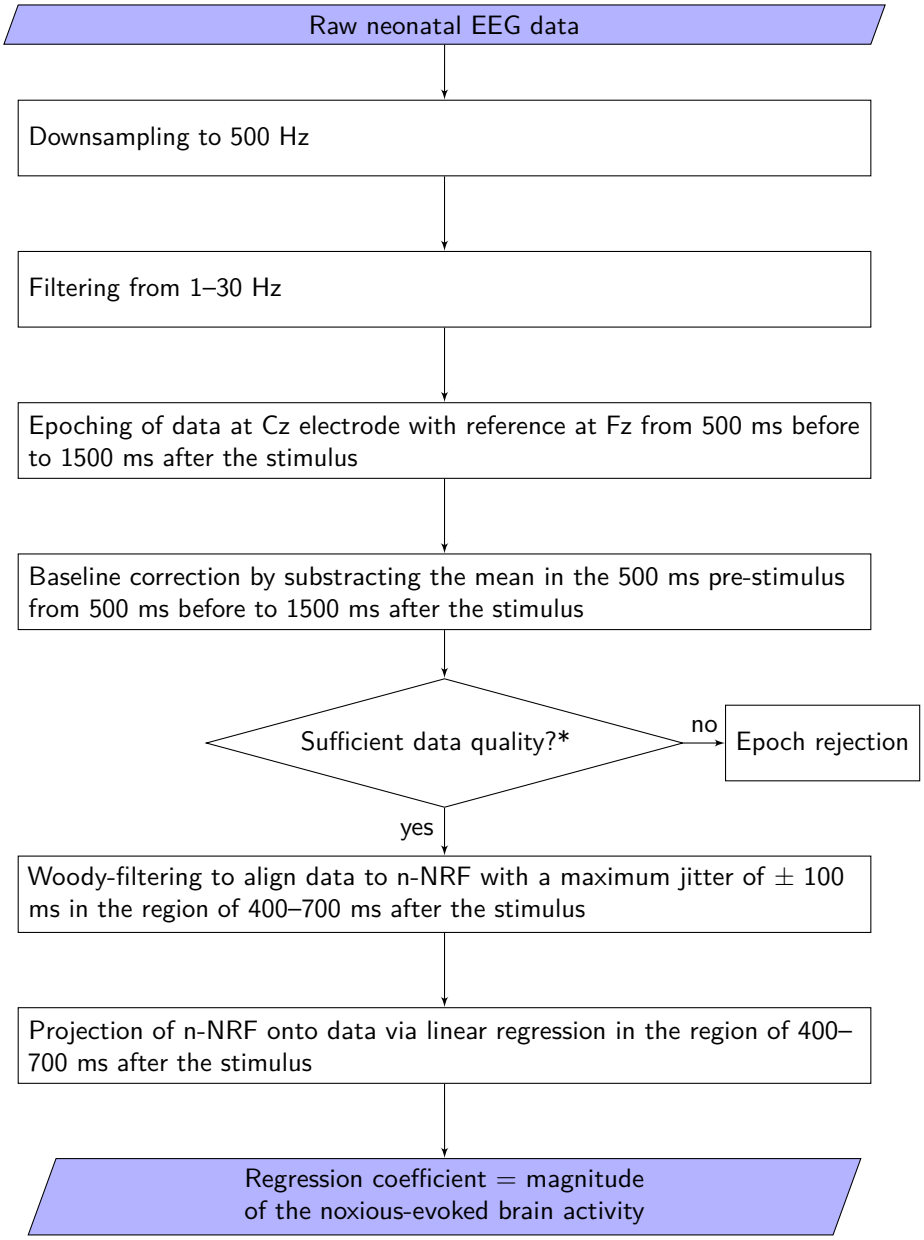


Figure 3.3: Pre-processing and analysis of EEG data. Data was filtered using Hamming-windowed sinc FIR filters (high-pass -6 dB cut-off = 0.5 Hz, transition band width = 1 Hz, low-pass -6 dB cut-off = 33.75 Hz, transition band width = 7.5 Hz). *Data quality assessment: individual EEG traces were rejected if the point of stimulation was not marked on the EEG recording due to technical failure or if there was artefact (for example, gross movement artefact, electrical interference) during the immediate baseline or post-stimulus period. Woody-filtering involves finding the maximum cross-correlation of the n-NRF with the data [168]. EEG: electroencephalography, FIR: finite impulse response, n-NRF: noxious neurodynamic response function.

behavioural state), physiological (heart rate change and oxygen saturation reduction) and behavioural indicators (duration of brow bulge, eye squeeze and nasolabial

furrow) [136]. Each of these items can be assigned a score from 0 to 3, with a total score ranging from 0 to 21 (Table 3.3) [136]. Contextual parameters are included in the total score only when the sub-total on any of the other indicators (physiological or behavioural) is other than 0 [136]. Total PIPP-R scores can indicate no pain (0), or mild (1–6), moderate (7–12) and severe (>12) acute biobehavioural responses [299].

Recordings to assess PIPP-R and tachycardia outcomes

Physiological Monitoring (ECG and Pulse Oximetry)

Heart rate, respiratory rate and oxygen saturation were recorded continuously throughout the study period of approximately one hour using ECG and pulse oximetry. The heart rate was used to calculate a physiological component of the PIPP-R and the tachycardia outcome; the oxygen saturation values were used to calculate another physiological component of the PIPP-R. Three ECG electrodes were placed on the neonate's chest (Ambu WhiteSensor 40554, Ambu®), while the probe for oxygen saturation monitoring was placed on the foot. Physiological data was recorded with Philips IntelliVue monitors (MX750, MX800) and ixTrend software (ixTrend Express version 2, ixexcellence GmbH). The events were time-locked to the vital signs recordings either through an automated detection interface (Oxford site) [300], or manually by a researcher pressing a button (Exeter site).

Video Recording

Video recordings were used to assess items of the PIPP-R score: the behavioural responses, i.e. duration of facial expressions during the sham stimulus and clinically-required heel lance, and the infant baseline behavioural state. Video recordings were performed by research personnel specified on the delegation log with a digital video camera (Sony RX100). The video footage was transferred to the secure trial electronic database. If parents provided optional additional consent for videos and images recorded during the study to be used for publications and presentations, stills from the video footage may be taken at a later date as required. In the videos, the timings of the sham heel lance and heel lance were marked by the activation of a bespoke LED light. The light was activated by the clinical researcher at the time of the procedures by pressing a pedal.

PIPP-R score calculation

Table 3.3 shows how the PIPP-R score is calculated. The PIPP-R assesses the behavioural and physiological responses occurring in the 30 seconds following the stimulus. The physiological data recorded from the neonates' vital signs monitors was downloaded and stored in our research database. Physiological traces were assessed for quality by a researcher not involved in the specific test occasion and blinded to arm allocation. The physiological components of the PIPP-R score were calculated via a bespoke MATLAB script run on the data and the values were stored on our electronic research database prior to unblinding. The videos recorded during the study were used to assess the duration of the facial expressions and the neonate's behavioural state. The score related to the PMA item was calculated automatically on the electronical research database. The total PIPP-R score for each neonate was generated automatically on the database for both sham heel lance and heel lance stimuli. Twenty percent of total PIPP-R scores in response to the heel lance were rescored to assess intra- and inter-rater reliability with intraclass correlation coefficient (ICC). Inter-rater ICC was 0.98 (95% CI 0.95 to 0.99), intra-rater ICC was 0.99 (95% CI 0.98 to 0.99).

3.2.4.2.2 Tachycardia

The cut-off for the tachycardia outcome was defined based on the Advanced Paediatric Life Support guidelines, and reflects values above the 90^{th} centile [301, 302] in the first week of life. This outcome was defined as a binary outcome to reflect the clinical definition of tachycardia.

Assessment of tachycardia outcome

This outcome was assessed using the ECG recordings described in '3.2.4.2.1 Recordings to assess PIPP-R and tachycardia outcomes'. For each neonate, the tachycardia outcome was dichotomous, i.e. 'yes/no'. It was defined as a development of tachycardia (heart rate increase over 160 beats per minute (bpm)) in the 30 s following the heel lance if the mean heart rate in the baseline period was less than

	Indicator Score			
	0	1	2	3
Change in Heart Rate (bpm)	0–4	5–14	15–24	>24
Decrease in Oxygen Saturation (%)	0–2	3–5	6–8	>8 or increase in oxygen supply
Brow Bulge (s)	None (<3)	Minimal (3–10)	Moderate (11–20)	Maximal (>20)
Eye Squeeze (s)	None (<3)	Minimal (3–10)	Moderate (11–20)	Maximal (>20)
Nasolabial Furrow (s)	None (<3)	Minimal (3–10)	Moderate (11–20)	Maximal (>20)
PMA at Study (weeks + days)*	≥36+0	32+0–35+6	28+0–31+6	<28+0
Baseline Behavioural State*	Active and Awake	Quiet and Awake	Active and Asleep	Quiet and Asleep

Table 3.3: Premature Infant Pain Profile-Revised (PIPP-R) scoring system. The physiological indicator 'Change in heart rate' records the largest change, which can be either an increase or decrease, between the maximal heart rate (HR) value during a 15-s baseline period and the minimal or maximal HR value occurring in the 30 s following the stimulus. The physiological indicator 'Decrease in oxygen saturation' assesses the percental decrease from the lowest oxygen saturation (SpO2) value in the 15-s baseline period to the lowest value in the 30 s following the stimulus. The duration of the facial expressions (brow bulge, eye squeeze and nasolabial furrow) is calculated in the 30 s following the stimulus. *The contextual indicators (PMA at study and baseline behavioural state) are scored only if any of the other indicators is scored other than 0. bpm: beats per minute, PMA: postmenstrual age. This table is adapted from [136].

160 bpm. The period used as baseline was 15 seconds long and before the start of the stroking in the intervention group, and a sham marker before the heel lance in the control group. Each neonate's outcome was calculated via a bespoke MATLAB script run on the data downloaded from the neonate's vital signs monitor and recorded in the trial database prior to unblinding the data.

3.2.4.2.3 State-Trait Anxiety Inventory

The State Trait Anxiety Inventory (STAI) is composed of two scales: the STAI-T, which measures general propensity towards anxiety, and the STAI-S, which measures the anxiety level at a specific time point. The secondary outcome of parental anxiety was assessed with the State component of the STAI, but the STAI-T at baseline was also recorded. In each scale, twenty items are rated on a four-point Likert scale, with a total score ranging from 20 to 80 for each sub-scale. Mean STAI-S scores of approximately 35 are described in working adults [295], whereas scores of approximately 50 are reported by parents of neonates admitted to neonatal intensive care units in the UK and US [267]. State items assess feelings in a precise moment, and are ranked from 'not at all' through 'somewhat', 'moderately so', to 'very much so'. Trait items are related to general frequency of certain feelings, and items are ranked from 'almost never', through 'sometimes', 'often', to 'almost always' [295]. Higher scores indicate higher anxiety. The STAI was initially published in 1970 as STAI-X, and its revised version STAI-Y was published in 1983. The latter was used in the Petal trial. Strengths of the STAI include being widely used, fairly brief—lasting approximately ten minutes—and easy to administer [303]. The COSMIN panel defined validity as 'the degree to which an health-related patient-reported outcome instrument measures the construct(s) it purports to measure', and reliability as 'the extent to which scores for patients who have not changed are the same for repeated measurement under several conditions' [117]. The validity and reliability of the STAI have been assessed, and higher reliability and validity were reported in comparison to other similar instruments [304]. Regarding responsiveness of the instrument, which is 'the ability of an health-related patient-reported outcome instrument to detect change over time in the construct to be measured' according to COSMIN panel, the STAI-S scores generally respond to threat, while the STAI-T tend to be more stable, as it can be expected considering that they assess general propensity towards anxiety rather than anxiety at a specific time point [117, 304]. The STAI is copyrighted and available from the publisher, Mind Garden Inc. (URL: https://www.mindgarden.com).

Recording of parental questionnaires

The parent administering the stroking intervention was asked to complete questionnaires assessing anxiety and distress at the specific time points during the study (Table 3.4). Parents completed the Trait and State components of the STAI prior to the stroking and heel lance [295]. After the heel lance, parents completed the STAI-S for a second time. Furthermore, parents completed a questionnaire investigating four emotional constructs at the time of the heel lance. They rated how much they perceived each of these feelings ('worried', 'upset', 'anxious', 'sad') from 'not at all' (0) to 'extremely' (10). Responses to the questionnaires were stored in an electronic case report form. At the end of the test occasion, parents completed a web-based anonymous survey investigating their motivations for participating in the trial, as well as their feelings and experience related to trial involvement. Survey responses included information on the trial arm and site of the respondent, in addition to their responses.

Questionnaire section	**Topic**	**Timing**	**Questionnaire administrator**
20-point STAI-T	Trait anxiety	Start of test occasion	Administered verbally by researcher
20-point STAI-S	State anxiety before and after the heel lance	Start of test occasion, as well as after the procedure and intervention	Administered verbally by researcher
Four-point distress questionnaire	Emotional constructs at the time of the heel lance	After the procedure and intervention	Administered verbally by researcher
Anonymous survey	Motivations to participate and trial participation experience	End of test occasion	Completed by parent

Table 3.4: Parental questionnaires.

Calculation of STAI-S scores

The STAI-S scores after the heel lance were compared between trial arms. The STAI-T and STAI-S questionnaires completed at the start of the test occasions, as well as the STAI-S completed after the heel lance, were recorded into an electronic database at the time of test occasion, and the full scores computed according to the STAI manual [295]. The manual indicates that in the case of responses missing to up to three items per participants, the participant's total score can be derived based on the answered items by inflating the score to account for the proportion of missing items. For example, if only 19 out of 20 items were answered, the total sub-score based on 19 items would be multiplied by $\frac{20}{19}$ [295].

3.2.5 Assignment of interventions and blinding

3.2.5.1 Allocation

Neonates were randomised to receive parental touch either before (intervention group) or after (control group) the clinically-required heel lance. The allocation ratio was 1:1, and a web-based minimisation algorithm including a random element and provided by Sealed Envelope Ltd (London, UK) was implemented to balance five demographic characteristics at baseline [305]. The five minimisation criteria were (i) GA at birth, (ii) PNA at study, (iii) sex (based on information recorded in the medical notes), (iv) site, and (v) primary reason for blood test. GA was categorised as 35+0 to 36+6/37+0 to 42+0/42+1 or later; PNA at study was grouped as 0–2/3–4/5–7 postnatal days, sex was grouped as female/male/indeterminate, site as Exeter/Oxford, and primary indication for blood sampling as newborn screening/jaundice/glucose monitoring/suspected sepsis/other. Randomisation was managed centrally at the Oxford site, and the research team had no insight into the allocation of the next participant. Sex was determined based on the information provided in the medical notes. When there is a relatively high number of randomisation criteria in relation to the number of participants, a covariate adaptive randomisation method such as minimisation can help achieve balance between groups [205, 289].

3.2.5.2 Blinding

Assessment of all primary and secondary outcomes occurred prior to unblinding the data for statistical analysis, and followed the detailed descriptions included in the statistical analysis plan [2]. Researchers who collected the data could not be blinded to trial arm allocation. However, the primary outcome measure was a pharmacodynamic biomarker, so recording the outcome was not influenced by participant or researcher judgement. Automated bespoke MATLAB scripts were used to quantify the magnitude of the n-NRF, the physiological components of the PIPP-R score, the development of tachycardia, and the STAI-S score. The rejection of EEG or physiological data due to artefacts involved subjective assessment and was thus performed by two blinded researchers, with any discrepancies resolved by discussion. The scoring of the facial expression components of the PIPP-R was performed by investigators who had not been involved in the specific test occasion. The EEG recordings contained markers for stroking before and after the heel lance irrespective of trial arm to further ensure blinding. For participants in the intervention group, the EEG recordings included real stroking markers before the heel lance, as well as sham stroking markers after the heel lance. For participants in the control group, the sham stroking markers were placed before the heel lance and the real stroking markers after the heel lance. The timing of fake and real stroking markers in relation to the heel lance was similar, ensuring that blinded investigators could not distinguish group allocation based on the annotations in the EEG data.

The videos presented to the scorer of the PIPP-R facial expression components had been previously clipped to include a 15 s baseline, the moment of the stimulus, and the 30 s following the stimulus. The 15 s baseline was taken before the start of stroking in the intervention group, and before the heel lance in the control group. A brief gap of up to a few seconds between baseline and stimulus was introduced in all videos, so that all videos would briefly skip forward just before the stimulus, independently of trial arm and stimulus type. Videos were scored without sound and were assigned random numbers for scoring, so that the participant study identifiers were not known to the scorers. Researchers scoring the videos were blind to trial

arm allocation for that test occasion and to the stimulus type, i.e. sham heel lance or heel lance. All outcome measures were stored in our research database prior to locking the dataset and unblinding the data.

Parents were unaware of trial arm allocation at the time of answering the first set of questionnaires (STAI-T and STAI-S at baseline). Since parents performed the stroking, they could not remain blinded to trial arm allocation for the whole duration of the study. They were informed of trial arm allocation after completion of the first set of questionnaires so that the questionnaires could assess how the timing of stroking influenced parental anxiety.

3.2.6 Statistical analysis

The content of the statistical analysis plan reflects guidelines and recommendations written by the ICH and other entities, as well as the CONSORT 2010 statement [306]. The statistical analysis plan is based on ICH E9 and its addendum [289, 290], ICH E3 [284], and ICH E6 [285]. It includes the items listed in *Guidelines for the Content of Statistical Analysis Plans in Clinical Trials* published in JAMA in 2017 by Gamble and colleagues [280]. The National Institute for Health and Care Research (NIHR) lists three documents (ICH E9, the CONSORT statement and the guideline by Gamble and colleagues) in their clinical trials toolkit, and encourages their use as reference documents when writing SAPs [307].

The SAP (v 1.0, dated 30/03/2023) was uploaded to the ISRCTN registry on 31 March 2023. On 4 April 2023 the database was hard locked, and data were unblinded later on the same day.

3.2.6.1 Sample size

A 40% reduction in the intervention group was considered realistic based on the two pilot studies, where the magnitude of noxious-evoked brain activity in response to a clinical heel lance or mild noxious stimulus was significantly reduced by 39–40% following experimenter-led brushing of the baby's skin at CT-optimal speed [171, 173]. Furthermore, it was considered clinically significant based on similar reductions

of noxious-evoked brain activity in adults corresponding to significantly reduced pain scores [251, 308]. The required sample size was estimated with G*Power by entering a mean (SD) magnitude of noxious-evoked brain activity of 1.07 (0.66) in the control group, and 0.642 (0.66) in the intervention group, which corresponds to a Cohen's D effect size of 0.648 [309]. The power was set to 90%, the two-sided significance level to 5%, and the allocation ratio to 1:1. The resulting sample size was 102 neonates, which was inflated by approximately 10% to account for potential data loss, leading to an estimated final sample size of 112 neonates.

3.2.6.2 Timing of final analysis and outcome assessment

The statistical analysis occurred only after completing data collection from the final participant, online publication of the SAP on ISRCTN, hard data locking and data unblinding [279]. The timings of outcome assessment varied depending on the outcome, and are presented in Fig 3.1. No interim analysis was performed.

3.2.6.3 Statistical principles

3.2.6.3.1 Confidence intervals and P values

The statistical significance level was set at 5% for the primary outcome. A total alpha of 5% is shared among the three secondary outcomes, with correction for multiple comparisons via Holm method [310].

For the three linear regression analyses, we present the mean, SD, and 95% confidence interval (CI)s for the effect size of interest, i.e. the difference in mean magnitudes of noxious-evoked brain activity, PIPP-R scores, and STAI-S scores, respectively, between groups. For the binary tachycardia outcome, we present the odds ratio with a 95% CI. For completeness, the risk ratio (RR) is also reported.

3.2.6.3.2 Analysis populations

Neonates in both groups received standard care and parental stroking: while the neonates in the intervention group were stroked before the heel lance, neonates in the control group were stroked after the heel lance. The primary outcome and

the two secondary neonatal outcomes (PIPP-R and tachycardia) were measured within 30 seconds from the time of the heel lance. Therefore, when these outcomes are assessed, only the babies in the intervention group had been stroked by their parents. The analysis quantified the per protocol effect in the full analysis set by extracting the effect of the intervention among compliers. When referring to the 'full analysis set', this reflects the definition included in the *ICH E9 Statistical Principles for Clinical Trials* [289], and comprises all randomised participants with an available outcome measure [311].

A CONSORT flow diagram detailing the number of approached parents, recruited participants, and analysed outcome measures is included in the trial results [306]. Baseline characteristics of neonates enrolled in the trial are presented by randomisation arm in the results.

3.2.6.4 Analysis methods

3.2.6.4.1 Estimating the effect of the intervention

We assessed the effect of adhering to the stroking intervention, i.e. the per protocol effect, as specified in the SAP. The per protocol effect can be estimated in different ways. One approach, named naïve per protocol analysis, is to simply exclude non-compliers from the analysis. Naïve per protocol analyses can lead to biased estimates because they ignore the fact that prognostic factors may have influenced compliance, and are thus generally discouraged [287]. Non-compliance can be appropriately addressed when estimating the complier average causal effect (CACE). In a CACE analysis, the effect of the intervention in compliers is estimated through appropriate use of instrumental variables in the full analysis set [312].

The aim of a CACE analysis is to compare the outcome of the compliers in the intervention group with the outcome of participants in the control group who would have taken the intervention, had they been randomised to the intervention group. This type of analysis is based on a causal modelling framework that specifies four groups of potential behaviour of participants with regard to treatment compliance: 'always-takers', 'never-takers', 'compliers' and 'defiers' [313]. 'Always takers' will

always implement the intervention, irrespective of their trial arm assignment. In the context of Petal, this would be participants that would perform pre-procedural stroking no matter their trial arm assignment. In contrast, 'never takers' will refuse to implement the intervention irrespective of their trial arm assignment. This would be participants that would never perform pre-procedural stroking, irrespective of their trial arm assignment. The 'compliers' are participants who will adhere to the assigned group. Therefore, these are participants that would perform pre-procedural stroking if assigned to the intervention group, and post-procedural stroking if assigned to the control group. Lastly, the 'defiers' will always do the opposite of their assigned intervention, namely perform pre-procedural stroking if assigned to the control group, and perform post-procedural stroking if assigned to the intervention group.

Given that the four groups are defined based on participant's theoretical behaviour in the case that they are assigned to the intervention as well as in the case that they are assigned to the control group, but in reality they are only assigned to either group in a parallel trial design, it is not possible to classify a participant into these four theoretical groups merely based on observed data. Therefore, assumptions are made that allow to specify which of these groups a participant belongs to [312]. Based on the assumptions, it is possible to estimate the outcome among the control group participants who would have complied to the intervention had they been assigned to the intervention arm, and compare it to the outcome among compliers in the intervention group [312].

In summary, the CACE isolates the effect of the intervention in the group of 'compliers', and yields a causal estimate of the effect of the intervention among 'compliers' [312]. In contrast, intention-to-treat (ITT) assesses the effect of being assigned to a treatment arm [287]. Where perfect compliance is achieved, the CACE and ITT effect are identical [312]. Where some non-compliance occurs, the ITT effect can underestimate CACE, as some participants assigned to the intervention arm will not have received the intervention and vice versa, those assigned to not perform the intervention may still perform it. In an additional

analysis performed for the purpose of completeness, the ITT effect was estimated in the full analysis set. The per protocol effect (e.g. estimated via CACE) is useful to estimate the effect an intervention has under idealised conditions (i.e. efficacy) allowing insight into whether the intervention itself, if properly applied and fully adhered to, actually works; the intention-to-treat effect is useful to estimate the effect an intervention has under real world conditions (i.e. effectiveness) which combines information on efficacy with factors affecting compliance (e.g. intervention tolerance, side effects, realistic regimens).

3.2.6.4.2 Statistical models

CACE was calculated via the two-stage least squares (2SLS) approach, which I will describe in brief. The group allocation variable was the instrumental variable in all cases, and both regression stages involved multiple regression. In the first stage, the treatment received was regressed on the treatment assigned and five covariates, namely the baseline characteristics used as minimisation criteria for randomisation (GA at birth, PNA at the time of study, site, sex, and primary reason for blood test). This yields a predicted treatment received for each participant. In the second stage, the outcome is regressed on the predicted treatment received as well as baseline characteristics. Multiple linear regression analysis was used to assess the effect of parental stroking on the primary outcome measure and on the secondary outcome measures PIPP-R score and STAI-S score. The effect of the intervention on the development of tachycardia, which was a binary secondary outcome, was assessed using a logistic regression. In all regression models, the group allocation variable was adjusted for the five minimisation variables. In the linear regression model for the STAI-S score the group allocation variable was additionally adjusted for the STAI-S score collected at baseline, i.e. before the heel lance.

3.2.6.4.3 Inference methods

Relevant assumptions for the regression models were assessed. For all three multiple linear regression models, we assessed homoscedasticity and normality

of residuals. The normality of residuals was assessed by plotting a histogram of residuals, by creating a normal probability plot of the raw residuals and by performing a Lilliefors test. Homoscedasticity is the homogeneity of variance of the residuals [314]. The variance of the residuals should not differ depending on the fitted value. This was tested by plotting the residuals against the fitted values and observing if a pattern emerged. Heteroscedasticity, the non-constant variance of the residuals, can be identified when the residuals plot shows a funnel shape (e.g. larger residuals associated with larger fitted values) [314]. This funnel shape was tested for by assessing the Spearman correlation between the spread (i.e. SD) of the residuals and the fitted values. For large deviations from normality, we pre-specified that we would consider deriving non-parametric p-values via permutation testing. For large deviations from homoscedasticity, we stated that we would consider robust standard error linear regression.

In addition to normality of residuals and homoscedasticity, further assumptions for the three multiple linear regression models assessing the primary outcome measure and the secondary outcomes PIPP-R and STAI-S were linearity, independence of residuals and exogeneity of variables. Linearity describes a linear relationship between the independent variables and the dependent variable. Independence of residuals occurs when the residual terms are independent of each other. The assumption of exogeneity of variables is met when the independent variables are not dependent on the dependent variable. For these analyses, we did not expect issues with linearity, independence of residuals, or exogeneity of variables based on the design, models, and variables of interest. The underlying assumptions for the logistic regression model for the binary tachycardia outcome were linearity between log odds ratio (OR) and explanatory variables, independence of residuals, exogeneity of variables and the absence of perfect separation. Perfect separation occurs when some covariates can perfectly separate the outcome groups [315]. We did not expect issues with linearity between log OR and explanatory variables, independence of residuals, or exogeneity of variables given our design, model, and

variables of interest. The absence of perfect separation was assessed by visually inspecting plots depicting the dependent variable and the independent variables.

We tested the linearity assumption for the linear models with multivariable fractional polynomialss (FPs) using the *mfp* package in R [316, 317]. This algorithm identifies the most appropriate functional form for continuous predictors in a linear model. We fitted linear regressions for the magnitude of heel lance-evoked brain activity, PIPP-R, and STAI-S outcomes and a logistic regression for the tachycardia outcome. GA, PNA, sex, site and the primary reason for blood test were included in all models as covariates. In the STAI-S model, we also included baseline STAI-S as a covariate. In brief, for each continuous predictor in turn (GA, PNA and baseline STAI-S), the mfp algorithm estimates the most appropriate functional form in an iterative way, which includes testing the FP against a straight line. The p-value for this comparison is based on the difference in deviances between the FP models and the linear model.

3.2.6.4.4 Exploratory intention-to-treat analysis

This exploratory analysis assessed the ITT effect, i.e. the effect of being assigned to the intervention. The ITT analysis was performed on the full analysis set. The ITT analysis assessed the effect of the group assigned at randomisation, regardless of any subsequent non-compliance, on the outcome measures. In contrast, the main CACE analysis assessed the effect of adherence to the intervention (per protocol effect). The primary outcome (magnitude of the n-NRF) and the secondary outcomes PIPP-R and STAI-S were compared between the intervention and control groups using multiple linear regression analysis. The development of tachycardia (binary secondary outcome) was compared between the groups using a logistic regression. To estimate the ITT effect, the regression models were the same as described above for the second stage regression, but using the treatment assigned at baseline as the predictor, rather than the predicted treatment received derived from the first stage regression. In the regression models, the variable of group allocation at randomisation was adjusted for the five minimisation variables (gestational age,

postnatal age, site, sex and primary reason for blood test). In the analysis of the STAI-S outcome, the group allocation variable was additionally adjusted for the STAI-S at baseline (i.e. before the heel lance).

3.2.6.4.5 Risk ratio for the secondary tachycardia outcome

While the effect of the intervention on the development of tachycardia (binary secondary outcome) was reported as OR in the main analysis, the development of tachycardia was additionally compared between the groups using a robust Poisson regression to allow reporting of risk ratio outcomes [318]. This was implemented in R using the sandwich package [319].

3.2.6.5 Missing data

In the SAP published online prior to unblinding the data, we pre-specified that missing data might occur for various reasons, such as equipment failure, artefacts in the neurophysiological or physiological recordings, or changes in the neonate's clinical care plan, such as instances when the heel lance might not be needed any more after the participant has been enrolled. Missing data can occur in different patterns and via three different mechanisms [320]. Data may be missing completely at random (MCAR) where the probability of an observation being missing does not systematically depend on observed or unobserved values [320, 321]. This mechanism is generally unlikely to occur [321]. Another mechanism is missing at random (MAR), where the probability of an observation being missing depends on the observed data only [322]. Therefore, the observed values can be used to predict the missing values [320, 321]. The third mechanism is missing not at random (MNAR), where the distribution of the missing variables depends on unobserved variables, making a prediction of what the missing values would be rather challenging [320, 321]. In brief, both MAR and MNAR indicate data missing in a systematic pattern; in the case of MAR the observed data can be used to explain data loss, while in the case of MNAR data loss cannot be explained with variables recorded during the study. Our

main analysis used the full analysis set and assumes MCAR. Sensitivity analyses can be used to test the sensitivity of our main result to this MCAR assumption [322].

We planned to assess the impact of missingness with a description of the reasons for missing outcomes, where available. Furthermore, we intended to assess the extent of missingness presented by trial arm, as well as by covariates with a potential influence on the missing variable or on the pattern of missingness. Moreover, sensitivity analyses were suggested for the primary outcome only. Sensitivity analyses can assess whether the conclusions of the primary analysis would change when using different assumptions [323], i.e. they can assess whether the conclusions are robust to a different set of assumption [290, 324]. Where the sensitivity analyses yield similar results to the primary analysis, the conclusions based on the primary analyses are lent more credibility [323]. The assumptions made in the sensitivity analyses should be different than those made in the primary analysis [325]. Two sensitivity analyses were proposed for the primary outcome: multiple imputation (which assumes MAR) to assess the main assumption of MCAR, and a pattern mixture model to test the MAR assumption used for multiple imputation [2]. Through multiple imputation, the missing data are replaced using an appropriate stochastic model, yielding a complete set [322, 326]. This process is repeated and the parameter estimates combined to generate a point estimate and standard error based on the uncertainty of the imputation process [322, 326]. In pattern mixture models, parameters can vary in line with the missingness patterns, and the final estimate is derived from combining the estimates by weighting the proportion of individuals in each pattern [327].

3.2.6.6 Software

All statistical analyses were performed in R (version 4.2.2 or newer) or MATLAB (Mathworks, version 9.14, R2023a or newer). Permutation testing to derive non-parametric p-values was performed using Permutation Analysis of Linear Models (PALM) [328]. The MATLAB code for testing the assumptions pre-specified in the SAP, and to generate the results of the group comparisons for primary and secondary

outcomes is publicly available (https://zenodo.org/record/8429864). Linearity assumption testing was performed upon request of the statistical peer-reviewer, using multiple FPs (function *mfp*) in R. The plots testing the normality and homoscedasticity assumptions of the statistical models were generated in MATLAB (using a bespoke script, as well as function *plotResiduals*). When comparing the outcome measures in response to the clinically required heel lance to the sham stimulus, figures were generated in R using the *dabestr* package, which also generates non-parametric CIs via bootstrapping [329, 330].

3.2.7 Administrative information, data management, data monitoring, and patient and public involvement

An overview of the trial administrative information is available in Table 3.5.

Short title	Parental touch trial (Petal)
Long title	A randomised controlled trial to investigate the effects of parental touch on relieving acute procedural pain in neonates
IRAS	Reference 291213
Protocol version and date	v5.0 26-01-2023
Ethics	London-South East Research Ethics Committee of the National Research Ethics Service 21/LO/0523
ISRCTN	14135962
ClinicalTrials.gov identifier	NCT04901611
Funding	This work is supported by a Wellcome Senior Fellowship awarded to Professor Rebeccah Slater (grant number 207457/Z/17/Z) and by Bliss (a UK charity) via a research grant. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Table 3.5: Parental touch trial administrative information. IRAS: Integrated Research Application System, ISRCTN: International Standard Randomised Controlled Trial Number

3.2.7.1 Data management and monitoring

Detailed information on data management is available in the full trial protocol. The Project Management Group (PMG) met regularly over the course of the trial to monitor data quality and adherence to the trial procedures. Blinded

investigators performed periodic data quality assessments. Internal guidance sheets for standardisation of trial procedures across sites and research teams were created at the start of the trial to maximise the standardisation of procedures, as recommended in ICH E9 [289], and training and problem-solving sessions were regularly organised by the PMG.

3.2.7.1.1 Adherence and protocol deviations

Researchers qualitatively assessed adherence to the intervention by observing parents stroking their baby. In preparation to delivering the intervention, parents were trained by researchers on how to stroke at approximately 3 cm/s by following an animation displaying a bar filling at 3 cm/s three times in a row, for a total of ten seconds. Parents delivered the intervention to their own arm and received feedback by the researchers. During the intervention, the same animation was shown: the animation included a three seconds countdown prior to the start, and also displayed the start and end times. The same animation and training were implemented at both sites. Deviations from the assigned intervention are reported alongside the results. If a participant was withdrawn from the study, the reason for withdrawal is reported. Furthermore, we report the timing of withdrawal in relation to communicating to parents their child's allocation arm and in relation to the trial's timeline, wherever possible.

The delay between the stroking intervention and the painful procedure can impact the pain-relieving effect of the intervention. In a study in adults, Liljencrantz and colleagues showed that a delay of as little as 5 or 10 seconds reduces the pain-relieving effect of stroking compared to 1 s based on verbal reports [252]. Data quality assessments by blinded investigators prior to unblinding the data suggested that delays between the stroking and the heel lance might be longer than a few seconds. During those quality assessments, it was not possible for blinded investigators to distinguish between real and sham markers indicating the times of stroking on the EEG and physiological recordings. Blinded investigators suggested including a 45 s threshold between the start of the intervention and the

heel lance as a cut-off for non-compliance (non-adherence). This cut-off applies to the neonatal outcomes only, as there is a clear rationale as to why we would expect a reduced effect of the pain relieving intervention on the neonatal outcomes with a longer delay. In contrast, there is no clear evidence suggesting the direction of a potential effect of a delay on the parental anxiety levels. Therefore, the 45 s cut-off does not apply to the analysis of the parental STAI-S outcome. This non-adherence definition for the analysis of the neonatal outcomes was included in the SAP published online prior to unblinding the data.

3.2.7.1.2 Harms

Serious adverse events occurring during the study were recorded and assessed by a senior clinician. The senior clinician classified the severity of the event and assessed whether this was causally linked to trial participation. Serious adverse events were defined in the full trial protocol as an untoward occurrence that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, consists of a congenital abnormality or birth defect, or is otherwise considered medically significant by the investigator. Besides serious adverse events, no pre-specified adverse events were provided in the trial protocol.

As described in the safety monitoring section of the study protocol (Ethics Ref: 21/LO/0523), there were no expected or foreseeable SAEs in this population or with the intervention chosen. In the trial protocol, possible risks to the baby related to the application of the EEG electrodes were listed as skin irritation or reaction to the conductive paste used to apply the electrodes to the skin. The heel lance was performed exclusively by experienced members of the research team who regularly perform heel lances as part of their clinical duties, and only when a blood test was required for clinical purposes. Regarding interim analyses, according to the ICH E9 guideline on Statistical Principles for Clinical Trials [289] 'most clinical trials intended to support the efficacy and safety of an investigational product proceed to full completion of planned sample size accrual; trials should be stopped early only

for ethical reasons or if the power is no longer acceptable'. Given the nature of the intervention (10 s of parental touch) and to control Type I error, no interim analysis was planned. Similarly, due to the nature of the intervention and the extremely low risk of safety concerns, the project management group did not consider a data monitoring committee (DMC) necessary. However, oversight was provided through regular project management group meetings which included safety updates and thorough review of the conduct and quality of the trial.

3.2.7.2 Patient and public involvement (PPI)

Parent-facing materials were reviewed by the charity Supporting sick newborn and their parents (SSNAP). The neonatal UK charity Bliss partly funded the trial and was regularly updated on the progress of the trial, as well as involved in the dissemination of trial outputs.

3.3 Discussion

Few paediatric trials investigating analgesic interventions are ongoing, and these trials are complex to perform, in particular in the neonatal population [30, 331]. FDA and EMA have implemented legislation to boost the number of paediatric trials and have dedicated particular attention to the neonatal population with bespoke guidelines [275].

The Parental touch trial follows established guidelines and consensus statements, such as recommendations published in 2018 following a consensus meeting on pain assessment and trial methodology for analgesic trials from birth to adolescence held by representatives from academia, industry and governmental agencies [195]. This trial implements an objective, brain-derived outcome measure recently discussed in an FDA workshop as primary outcome. Parallel efforts to establish this outcome measure are ongoing, such as assessing its reliability, validity and interpretability, its reproducibility, and standardising the way it is generated. Further neonatal outcome measures from other domains (clinical pain score, development of tachycardia) were chosen to provide a wider picture of the effect of the intervention on pain-related

outcomes. In addition, a secondary outcome assessed parental anxiety. Parents were involved in the trial as the providers of the touch intervention, and their feelings and motivations to take part in the trial were investigated to better understand how being involved in their child's care affected them and inform future trials aiming to involve parents. Importantly, fathers could also provide the intervention, in contrast to a high number of trials which focus exclusively on mothers.

The trial was registered on ISRCTN and ClinicalTrials.gov and its protocol published and peer-reviewed [1]. Guidance sheets were developed and implemented to optimise standardisation of procedures across sites and team members (Appendix F). The statistical analysis plan was developed with the support of statisticians and published online prior to unblinding the data [2, 279]. There are multiple known issues with not pre-specifying and sharing SAPs prior to unblinding, which we aimed at preventing [332, 333].

The published documents (protocol, SAP), analysis scripts and sharing of dataset upon request can inform the development of future trials implementing brain-derived methods such as the n-NRF and investigating parental-led interventions.

Chapter 4

Effect of gentle touch on neonatal outcomes and parental anxiety

This chapter is primarily based on the manuscript reporting the trial results published in *The Lancet Child & Adolescent Health* in 2024, of which I was co-first author. The article is published under a CC BY 4.0 license with copyright retained by the authors [3]. The methods are described in detail in the previous chapter. The results reflect those published with some minor additions, while the introduction and discussion of this chapter have undergone more conspicuous adaptation.

- Hauck, A. G. V.*, van der Vaart, M.*, Adams, E., Baxter, L., Bhatt, A., Crankshaw, D., Dhami, A., Evans Fry, R., Freire, M. B. O., Hartley, C., Mansfield, R. C., Marchant, S., Monk, V., Moultrie, F., Peck, M., Robinson, S., Yong, J., Poorun, R., Cobo, M. M.‡, & Slater, R.‡ (2024). Effect of Parental Touch on Relieving Acute Procedural Pain in Neonates and Parental Anxiety (Petal): A Multicentre, Randomised Controlled Trial in the UK. The Lancet Child & Adolescent Health. doi.org/ 10.1016/S2352- 4642(23)00340-1.

*equal contributions ‡ equal contributions

The content of the manuscript mentioned above was restructured and expanded to align with the structure of the thesis. In addition to my role in the management of the trial and leading data collection underpinning this project, I performed extensive data quality checks with a colleague prior to hard locking the database.

I had a core role in the small data analysis team, as well as in the creation of figures. I have written most of the first manuscript draft, integrated edits throughout, and coordinated responding to reviewer feedback while responding to a large proportion of the feedback.

4.1 Introduction

Forms of parental touch such as skin-to-skin care [218] and massage [197, 227] have been reported to reduce pain-related responses to a painful procedure. Nevertheless, the certainty level of the evidence could be improved: while the evidence quality for skin-to-skin care for procedural pain is graded as low to moderate by the authors of a Cochrane review on the topic [218], the evidence grade for touch-related interventions (excluding skin-to-skin care) is described as very low to low by the authors of a recent Cochrane review on non-pharmacological interventions for procedural pain [197].

Studies that assessed the effect of parent-led touch as a potential pain-relieving intervention with brain-derived outcomes focussed on maternal skin-to-skin care for pain relief during clinically-required venepunctures and heel lances [220–222]. Wang and colleagues conducted an RCT in a group of 73 neonates born at 31 to 33 weeks GA, who were randomised to either kangaroo mother care or being nested while prone. They reported beneficial effects of kangaroo mother care across all outcomes (cerebral tissue oxygenation saturation measured with NIRS, PIPP-R score, HR and oxygen saturation levels), which was maintained over multiple heel lances [222]. Olsson and colleagues performed a cross-over study in a group of ten infants born at 26 to 35 weeks GA undergoing venepuncture, and assessed kangaroo mother care against laying in the incubator or crib. Glucose was provided to all neonates irrespective of trial arm. The authors concluded that kangaroo mother care was pain-relieving based on its beneficial effect on the NIRS outcome (smaller increase in oxygenated haemoglobin) [221]. No significant differences were reported for the PIPP-R score and physiological outcomes. Jones and colleagues enrolled neonates in a cross-sectional study investigating kangaroo mother care (n=9), being held with clothing (n=9), and individualised care in the cot (n=9) during heel sticks. One of

the outcomes was a noxious-evoked potential which, as also stated by the authors, had a latency and morphology compatible with the N3P3 potential described earlier (section 2.5.4.2). The authors described a significantly reduced noxious evoked potential in the kangaroo mother care group compared to the held with clothing group, whereas the reduction between kangaroo mother care and individualised care was not significant, which was interpreted as a reflection of the beneficial effects of individualised care [220]. Overall, the authors of these three studies assessing the impact of parent-led touch (maternal skin-to-skin care) reported a beneficial effect of the intervention on brain-based pain-related responses.

In adult studies, a specific population of neurons (CT afferents) has been linked to affective touch and has been investigated for its potential pain-relieving effects [237]. Studies in adults showed that touch at 1–10 cm/s—the speed that optimally activates CT fibres—is associated with a reduction in verbal pain reports and noxious-evoked brain activity [232, 251, 252]. The link between stroking at 1–10 cm/s and activation of CT fibres is established in adults with microneurography, which is not performed in neonates due to ethical reasons. Therefore, the potential activation of CT fibres in studies in neonates is postulated based on the fact that the same intervention has been shown to activate CT fibres in adults. In two studies in nine-month-old infants, brushing of the skin at CT-optimal speed was associated with a HR reduction, whereas lower and higher velocities did not lead to a significant HR decrease [256, 257]. Studies in neonates showed that mothers tend to naturally stroke at CT-optimal speed, and that maternal stroking of their baby's skin is associated with a HR reduction [246].

A potential pain-relieving effect of stroking at CT-optimal speed on a brain-derived outcome measure has been assessed in neonates. Two studies in independent datasets assessed the effect of brushing of the baby's leg for ten seconds just before a clinically-required heel lance [171, 173]. The stroking intervention was delivered by a trained researcher with a calibrated brush. These studies implemented noxious-evoked brain activity (n-NRF magnitude) as an outcome measure. Compared to

the control groups, experimental brushing led to a significant reduction in noxious-evoked brain activity (quantified as n-NRF magnitude) of 39–40% [171, 173].

Given the importance of involving parents in their baby's care [334], the Petal trial aimed at assessing the effect of parent-led touch in the form of stroking of their baby's skin at approximately 3 cm/s for ten seconds. The speed and duration of the intervention were chosen to match the two pilot studies [171, 173].

4.2 Methods

Methods are described in detail in chapter 3.2. In summary, Petal is a multicentre, randomised, two-arm, parallel, controlled, superiority trial. The trial was conducted at two sites in the UK: the John Radcliffe Hospital in Oxford and the Royal Devon and Exeter Hospital in Exeter. Eligible neonates were born at least 35 weeks GA at either site, and required a heel lance for clinical reasons in the first week of life. Exclusion criteria were hypoxic ischaemic encephalopathy, intraventricular haemorrhage above grade II, receipt of analgesic or sedatives in the 24 h before the study, born with a congenital malformation or genetic condition known to affect neurological development, or born to a mother with a history of substance abuse. Neonates were randomised to receive parental touch either before or after a clinically-required heel lance with an allocation ratio of 1:1. In addition, comfort measures were offered to all neonates irrespective of trial arm in line with local practice guidelines, and included swaddling and non-nutritive sucking. Randomisation occurred via minimisation algorithm with five minimisation criteria: sex, GA at birth, PNA at study, site, and primary reason for blood test. The intervention was parental stroking for ten seconds at approximately 3 cm/s, a speed that is considered to activate CT fibres. Beside the timing of parental touch, which occurred before the heel lance in the intervention group and after the heel lance in the control group, the methodology of parental stroking was the same. The primary outcome was the magnitude of noxious-evoked brain activity quantified with a previously defined n-NRF [168] in the first second following the heel lance. Secondary outcomes were the neonatal PIPP-R score and the development of tachycardia, which were

both assessed in the 30 s following the heel lance. A further secondary outcome was parental anxiety measured with the STAI-S after the heel lance. For the three continuous outcome measures, the effect of the intervention was assessed via multiple linear regression. For the binary tachycardia outcome, the effect of the intervention was assessed via logistic regression. In all regression models, the effect of the intervention was adjusted for the five minimisation criteria. In the STAI-S multiple linear regression, the effect of the intervention was additionally adjusted for the STAI-S score at baseline, which was collected prior to the communication to the parent of their baby's allocation arm.

4.3 Results

4.3.1 Enrolment of trial participants

Between the 1st of September 2021 and the 7th of February 2023, 159 parents were approached about trial participation. Upon parental consent, 112 neonates were enrolled and randomised: 56 neonates were allocated to the intervention arm (pre-procedural stroking), whereas 56 were allocated to the control arm (post-procedural stroking). The CONSORT flow diagram (Figure 4.1) illustrates participant numbers at enrolment, allocation and analysis stages. In each group, two neonates were withdrawn prior to both heel lance and stroking due to unsettled behaviours or the clinical team cancelling the scheduled heel lance. Therefore, 54 neonates in each group received the allocated intervention. In the analysis stage, some outcomes were excluded due to technical faults, artefacts, or missing outcomes. The numbers of the full analysis sets are listed at the bottom of Figure 4.1. Full analysis sets, as per ICH E9 description, include all randomised participants with available outcomes irrespective of non-adherence [289].

4.3.2 Baseline characteristics of trial participants

Baseline characteristics and demographics of the neonates randomised to either trial arm were similar (Table 4.1). Baseline characteristics of the full analysis set used for the analysis of the primary outcome, as well as for each of the secondary outcomes,

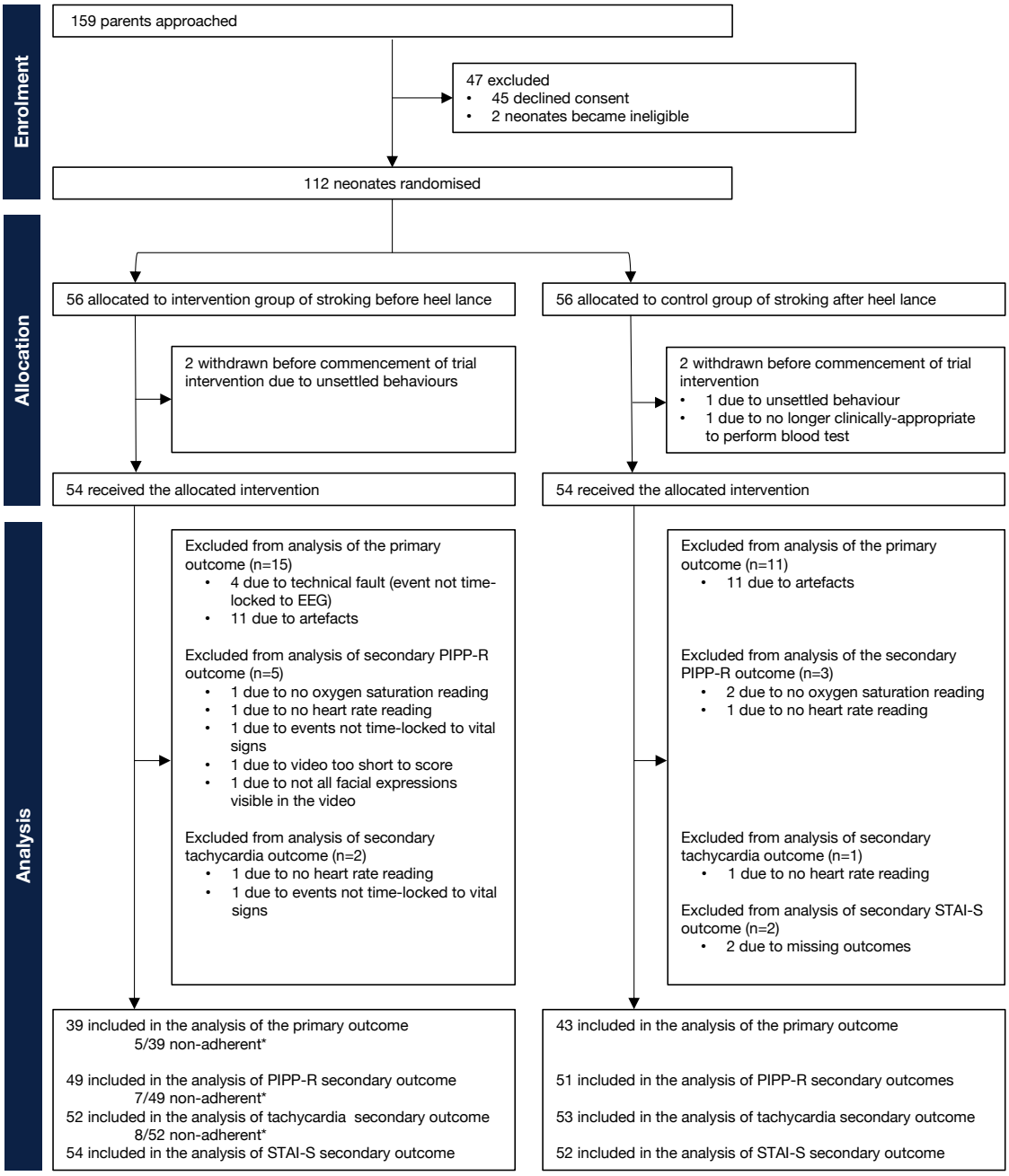


Figure 4.1: CONSORT flow diagram. Trial profile reflecting the CONSORT 2010 statement [306]. Artefacts that led to exclusion of primary outcomes were either movement or electrical artefacts. *non-adherence: the stroking start was longer than 45 s before the heel lance (affects only neonatal outcomes, only the intervention group). Four neonates clinically required a second heel lance: for one participant, no outcomes were recorded due to technical failure of time-locking the heel lance event; for all other three neonates, the outcomes from the first heel lance were used. EEG: electroencephalography, PIPP-R: Premature Infant Pain Profile-Revised, STAI-S: State-Trait Anxiety Inventory.

are available in Appendix A.1 and A.2, respectively. Among all randomised neonates, the parent who planned to perform the stroking intervention was the biological mother in 65% (73/112) of cases, and the biological father in the remaining 35% (39/112) of cases. Across groups, the median neonatal PMA at study was 38.6 weeks (interquartile range (IQR) 37.2 to 40.3), and median weight at birth was 3299 g (IQR 2765 to 3767). The majority (68/112, 61%) of randomised neonates were male, whereas the other participants were female (44/112, 39%). Prior to the test occasion, the estimated median cumulative number of skin-breaking blood tests, oral and endotracheal suctions were 4 (IQR 2 to 6). The reason for blood test was recorded for all randomised participants. The majority of neonates (54/112, 48%) required a heel lance to measure serum bilirubin as part of a jaundice assessment. The second most common primary reason for blood test was measuring infection markers to assess suspected sepsis in 33 (29%) neonates. Further primary reasons for a heel lance were newborn screening (8/112, 7%), glucose monitoring (6/112, 5%) and reasons classified as 'other' (11/112, 10%). The latter category included urea and haemoglobin measurements. The majority of neonates were White (91/112, 81%), and most randomised neonates were in postnatal wards (86 out of 112, 77%). *

4.3.3 Example of data recorded in an infant assigned to the intervention group

An example of the data used to calculate the neonatal primary and secondary outcomes is illustrated in Figure 4.2.

*Following variables were included in the minimisation algorithm: (i) GA at birth, (ii) PNA at study, (iii) sex (based on information recorded in the medical notes), (iv) site, and (v) primary reason for blood test. For each variable, neonates were split into categories. GA was categorised as 35+0 to 36+6 (n=16 in intervention group, n=16 in control group), 37+0 to 42+0 (n=38 in intervention group, n=39 in control group, or 42+1 or later (n=2 in intervention group, n=1 in control group). PNA at study was grouped as 0–2 (n=28 in intervention group, n=26 in control group), 3–4 (n=13 in intervention group, n=14 in control group), or 5–7 postnatal days (n=15 neonates in intervention group, n=16 in control group). Sex was grouped as female, male or indeterminate (see Table 4.1), no neonates in latter category) site as Exeter or Oxford (see Table 4.1), and primary indication for blood sampling as newborn screening/jaundice/glucose monitoring/suspected sepsis/other (see Table 4.1). GA: gestational age, PNA: postnatal age.

	Intervention group (stroking pre-procedure; n=56)	**Control group (stroking post-procedure; n=56)**
Parent stroking*		
Biological father	20 (36%)	19 (34%)
Biological mother	36 (64%)	37 (66%)
Gestational age at birth (weeks)**	38.8 (36.9–40.1)	38.0 (36.7–39.4)
Postmenstrual age at time of study (weeks)	38.9 (37.2–40.5)	38.4 (37.2–40.1)
Postnatal age at time of study (days)**	3 (1–5)	3 (1–5)
Birthweight (g)	3423 (2765–3817)	3230 (2770–3722)
Sex**		
Female	22 (39%)	22 (39%)
Male	34 (61%)	34 (61%)
Mode of delivery		
Normal vaginal	23 (41%)	20 (36%)
Breech vaginal	1 (2%)	0
Elective C-section	14 (25%)	9 (16%)
Emergency C-section	12 (21%)	15 (27%)
Ventouse or forceps	6 (11%)	12 (21%)
Apgar score at 1 min	9 (7–10)	9 (8–10)
Apgar score at 5 min	10 (9–10)	10 (9–10)
Primary reason for blood test**		
Glucose monitoring	3 (5%)	3 (5%)
Jaundice	26 (46%)	28 (50%)
Newborn screening	4 (7%)	4 (7%)
Suspected sepsis	17 (30%)	16 (29%)
Other	6 (11%)	5 (9%)
Behavioural state at baseline before the heel lance		
Awake	15 (27%)	15 (27%)
Asleep	38 (68%)	39 (70%)
NA	3 (5%)	2 (4%)
Site**		
Exeter	15 (27%)	14 (25%)
Oxford	41 (73%)	42 (75%)
Estimated cumulative prior pain exposure	4 (2–6)	4 (2–6)

Table 4.1: Baseline characteristics of all randomised participants. Data are median IQR or count (%). NA: not available. * For the four neonates withdrawn before commencement of the trial intervention, the parent who had planned to perform the stroking is indicated. **Minimisation criteria: a summary of these variables in categorical form is provided in the footnote in the previous page. Sex was determined based on information provided in the medical notes. The estimated cumulative prior pain exposure refers to skin-breaking blood tests, as well as oral and endotracheal suctions.

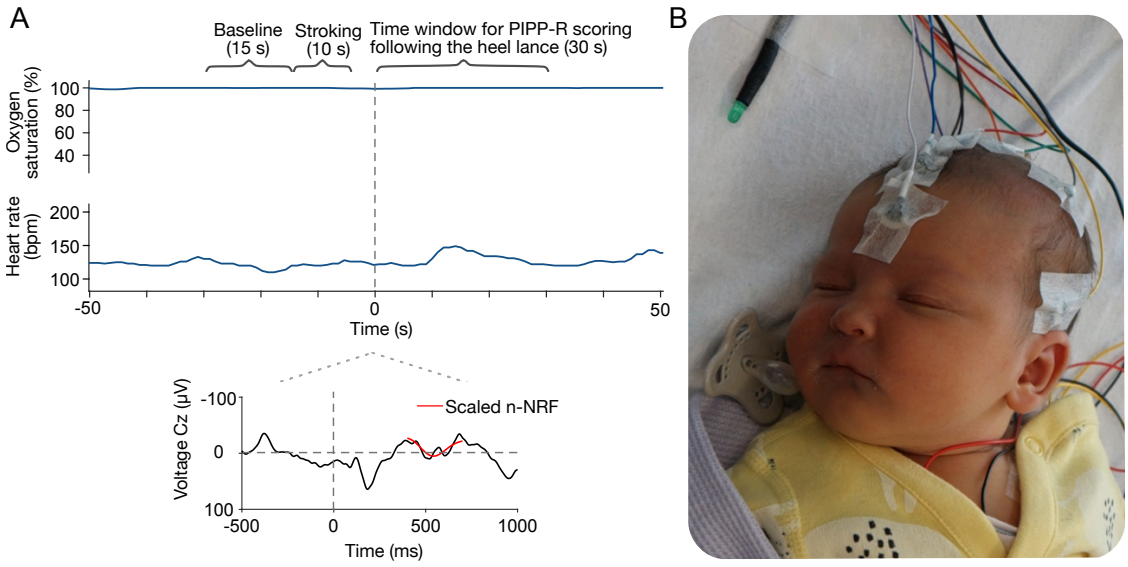


Figure 4.2: Example of data recorded in a trial participant. A. Data recorded in a participant assigned to the intervention group showing physiological data used for the calculation of the PIPP-R score, as well as the scaled n-NRF projected onto the participant's EEG data recorded at the central electrode Cz with reference at Fz. The plots are centred around the time of the clinically-required heel lance, more precisely when the movement of the spring in the lancet triggered the accelerometer attached to the lancet **B.** A frame of a participant's video used to assess facial expressions for calculation of the PIPP-R score. On the top left, the light to mark the timing of the heel lance is visible. This image is reproduced with parental permission. The layout of the figure is modified from the article by Hartley and colleagues [169], which was published under a CC BY 4.0 license. bpm: beats per minute, n-NRF: noxious neurodynamic response function, PIPP-R: Premature Infant Pain Profile-Revised.

4.3.4 The responses to the heel lance are significantly greater than to the sham stimulus

The same neonatal outcomes used as primary and secondary outcomes were calculated in response to a sham stimulus as well. This sham stimulus—a sham heel lance—resembles a heel lance except for its noxious component, as the lancet is rotated by 90° so that it fires into the air. The responses to the sham stimulus were used in a quality assessment to verify that the trial outcomes are related to the nociceptive input. The expectation was thus that the responses to the heel lance would be significantly greater than to the sham heel lance. This was confirmed for all neonatal outcomes (magnitude of noxious-evoked brain activity, PIPP-R, and development of tachycardia), as illustrated in Figure 4.3 and Figure 4.4.

For the primary outcome measure of noxious-evoked brain activity, values obtained during a background period when no procedure occurred were also included in the data quality assessment. The mean (SD) magnitude of the n-NRF was 0.45 arbitrary unit (a.u.) (0.34, n=105) during background recordings, 0.51 a.u. (0.46, n=94) following the sham heel lance, and 0.88 a.u. (0.73, n=82) following the clinically-required heel lance (Figure 4.3B).

The mean n-NRF during the background period and in response to the sham heel lance did not significantly differ (paired t-test, mean difference 0.06 a.u., non-parametric p=0.31, 95% bootstrap CI -0.05 to 0.18, n=93) (Figure 4.3B). Magnitudes of the n-NRF differed significantly between sham heel lance and heel lance responses at Bonferroni corrected alpha level of 0.05/2=0.025 (paired t-test, mean difference 0.37, non-parametric p<0.001, bootstrap 95% CI 0.19 to 0.59, n=78) (Figure 4.3B).

PIPP-R scores in response to the sham heel lance and heel lance, paired by participant, are illustrated in Figure 4.4A. The mean (SD) PIPP-R score following the sham heel lance was 4.81 (2.53, n=98). The mean (SD) PIPP-R score following the heel lance was 7.63 (3.39, n=100). The PIPP-R scores following the sham heel lance and heel lance differed significantly (paired t-test, mean difference 2.77, non-parametric p<0.001, bootstrap 95% CI 2.03 to 3.51, n=96).

The development of tachycardia following the sham heel lance and heel lance, paired by participant, is shown in Figure 4.4B. Following the sham heel lance, 4 out of 105 participants with an available outcome (4%) developed tachycardia, whereas following the heel lance 19 out of 105 participants (18%) developed tachycardia. The occurrence of tachycardia was significantly greater following the heel lance than following the sham stimulus (difference in proportions 0.14, non-parametric p<0.001, bootstrap 95% CI 0.06 to 0.22, McNemar's chi-squared 11.53 with one degree of freedom, n=104).

The average EEG traces in the full analysis sets are shown during background periods, in response to the sham stimulus and to the heel lance in Figure 4.5. They are presented by trial arm assigned and trial site for visual quality assessment. In contrast to Figure 4.3A, Woody-filter was not applied on EEG data presented in

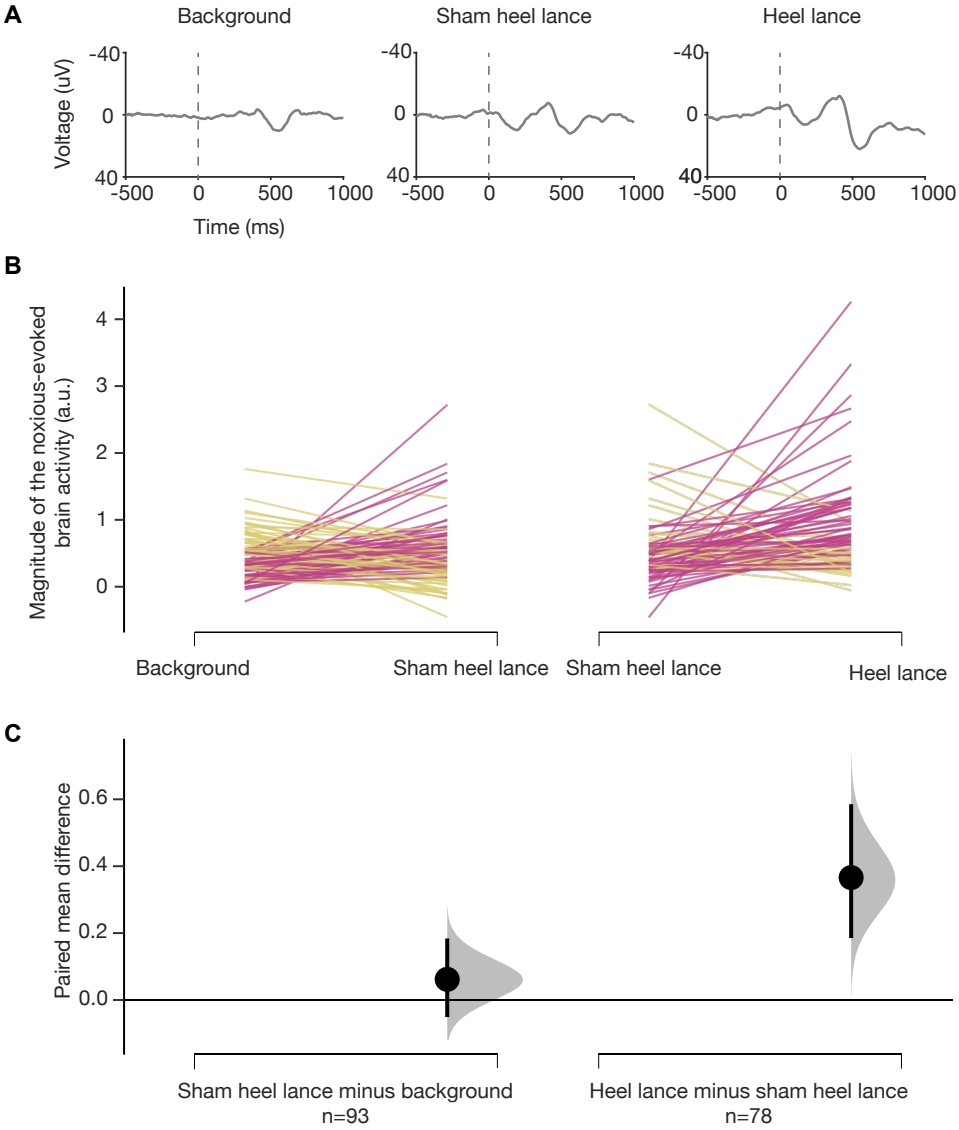


Figure 4.3: Magnitude of n-NRF during background period, in response to sham heel lance and heel lance. Data from both trial arms is included. **A.** Average EEG traces during background periods (left, n=105), in response to the sham stimulus (middle, n=94) and in response to the heel lance (right, n=82). EEG traces are shown for epochs starting 500 prior to the annotated event and ending 1000 ms thereafter. EEG data is processed as described in the Methods (section 3.2.4.1) including Woody-filtering to the n-NRF used in this study. **B.** Magnitudes of noxious-evoked brain activity as quantified by the n-NRF, paired by participant. The pink connecting lines indicate a stable or an increase in n-NRF magnitude value, whereas yellow lines indicates a decrease. **C.** Paired mean difference of the n-NRF magnitude during background period and in response to sham heel lance and heel lance. EEG: electroencephalography, n-NRF: noxious neurodynamic response function.

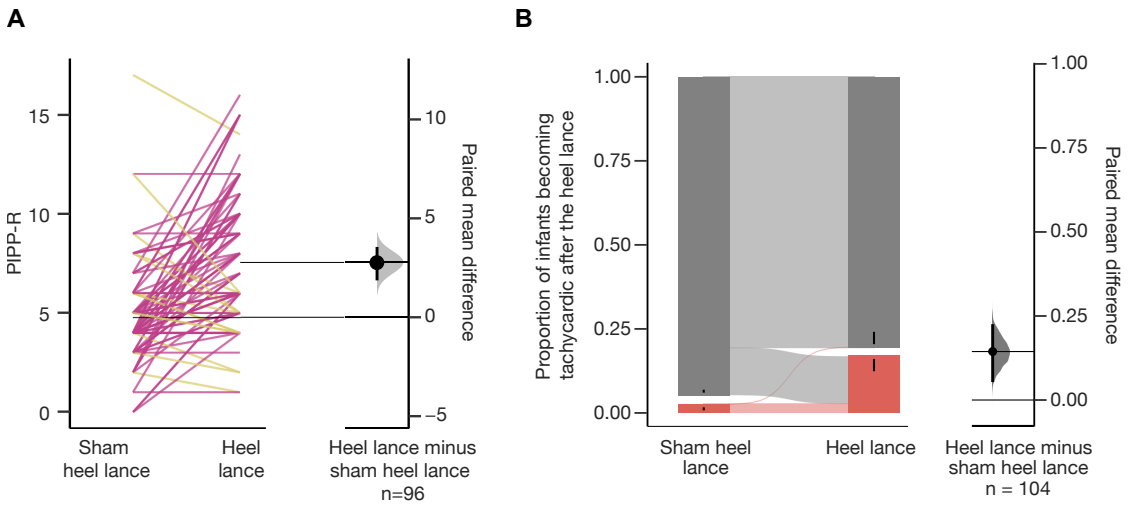


Figure 4.4: PIPP-R scores and tachycardia in response to sham heel lance and heel lance. A. PIPP-R scores in response to the sham heel lance and heel lance, paired by participant. The lines connecting the PIPP-R score in response to the sham heel lance and heel lance indicate whether the PIPP-R score of the neonate remained the same or increased (pink lines), or decreased (yellow lines) between sham heel lance and heel lance. **B.** Development of tachycardia following the sham heel lance and heel lance, paired by participant. The proportion of infants developing tachycardia is indicated by the red bar.

Figure 4.5. Therefore, the background traces are relatively flat, as expected. The other plots in Figure 4.5 generally align with expectations in terms of morphology and latency. Both the sham heel lance and the heel lance evoked an early positive deflection, as previously described [149]. The characteristic noxious-evoked positive deflection occurring later between 400 and 700 ms is visible in response to the heel lance, but not to the sham heel lance. Waveform morphology was consistent across the two research sites (Figure 4.5B).

4.3.5 Testing of assumptions underlying statistical models

The assumptions underlying the statistical models were tested as pre-specified in the SAP and described in the Methods chapter 3.2.6.4.

First, the normality assumption was tested for the three linear regression models. Residuals of the three linear regression models (magnitude of heel-lance evoked brain activity, PIPP-R, and STAI-S outcomes) were not normally distributed (Figure 4.6), so non-parametric p-values derived using permutation testing are reported.

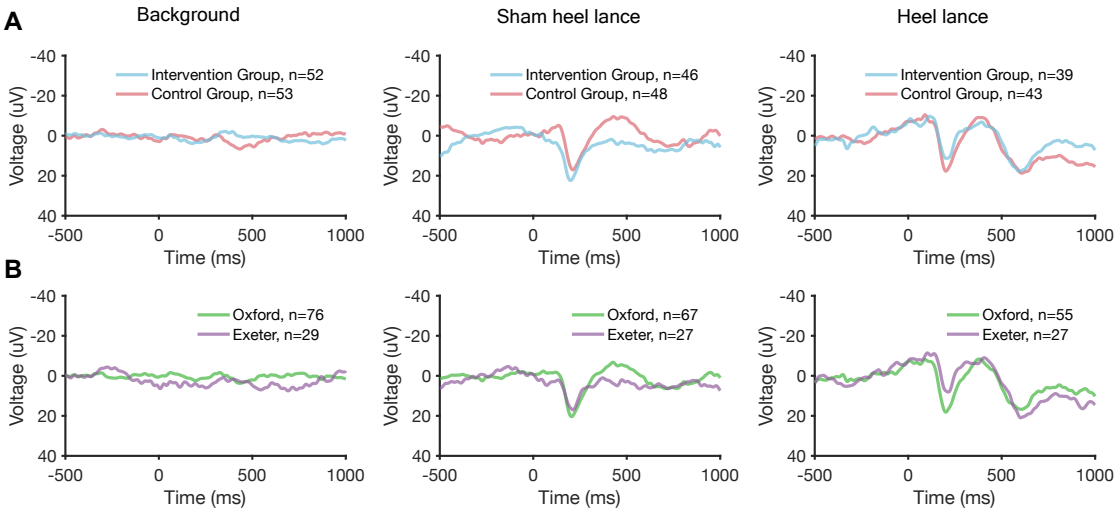


Figure 4.5: Average EEG traces by intervention arm and site in the full analysis set. Average EEG traces during background periods (left), in response to the sham stimulus (centre) and in response to the heel lance (right). Raw EEG traces were processed as outlined in section 3.2.4.1.1 except for Woody-filtering. **A.** Average traces are presented by the intervention arm assigned to the participant. **B.** Average traces are presented by site. EEG: electroencephalography

Second, the homoscedasticity assumption for the three linear regression models was tested. Of the three linear regression analyses, residuals were heteroscedastic for both magnitude of heel-lance evoked brain activity and STAI-S outcomes (Figure 4.6A,C), so robust linear regression was used. While the PIPP-R outcome had homoscedastic residuals (Figure 4.6B), for consistency robust linear regression was used for the PIPP-R analysis. However, using ordinary least squares (OLS) linear regression for PIPP-R data produces results very closely matched the robust analysis, with slightly larger effect sizes identified using robust regression. Lastly, the linearity assumption was tested for the linear models. This test was non-significant for all the regression models and all continuous variables (Table 4.2), thus confirming the linearity assumption.

4.3.6 Effect of parental touch on primary and secondary outcomes

The number of participants with an available outcome is listed in the bottom rectangles in Figure 4.1. The numbers listed there refer to the full analysis sets, i.e. the sets with all randomised participants with an available outcome. The main

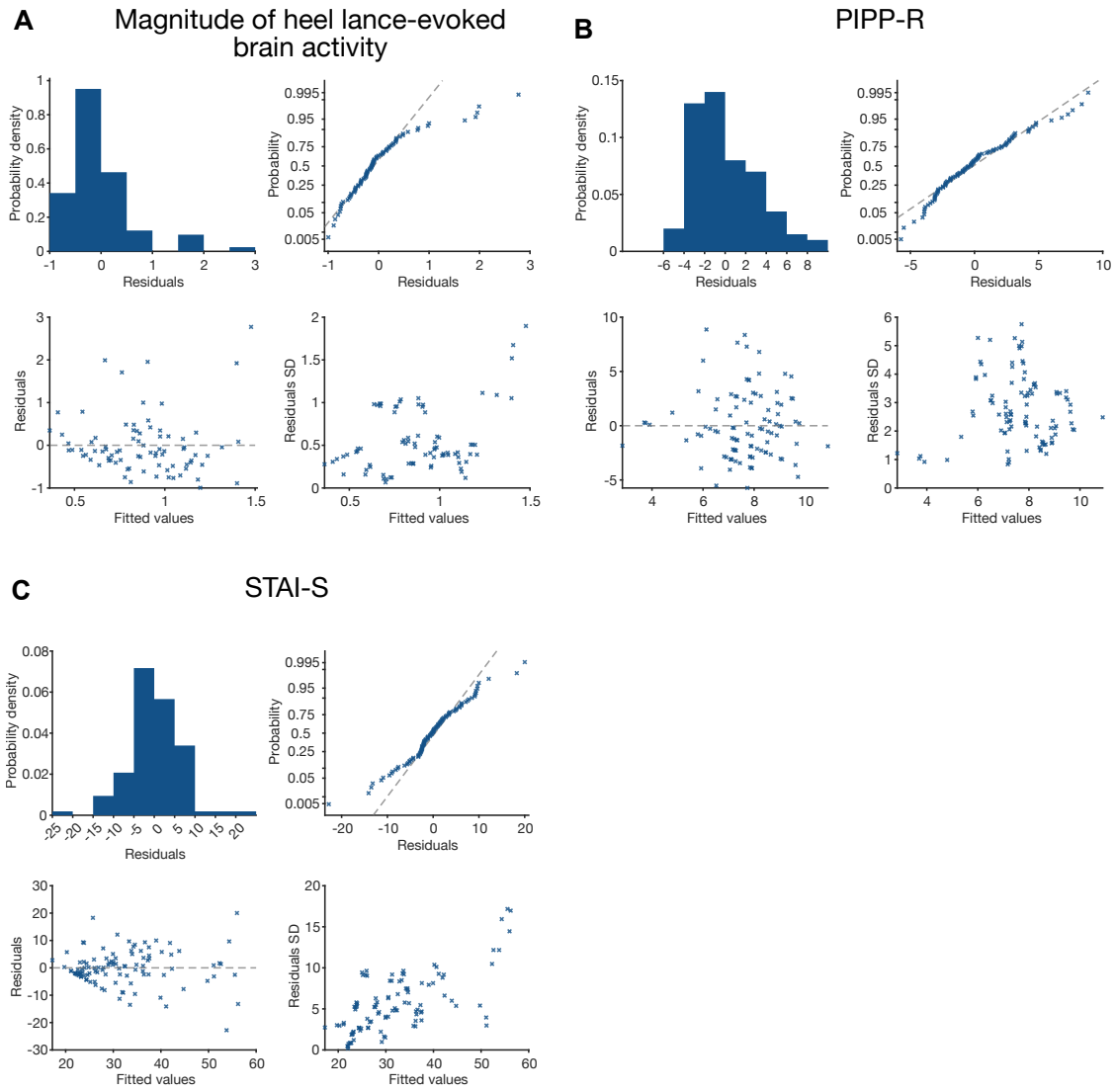


Figure 4.6: Testing of assumptions underlying statistical models. For each outcome, the two top plots were used to visually assess the normality of residuals, while the two lower plots were used to visually assess the homoscedasticity of residuals of the fitted regression model. The top left plot shows a histogram of the residuals of the fitted linear regression model using a probability density function. The top right plot is a normal probability plot of residuals. The bottom left plot depicts residuals against fitted values. The bottom right plot shows standard deviations (calculated over a moving window of five subsequent ordered values of residuals) against the fitted values. **A.** Normality of residuals (Lilliefors test p=0.001, non-normal) and homoscedasticity assumption testing (Spearman's ρ =0.24, p=0.033, heteroscedastic) for the primary outcome regression model. **B.** Normality of residuals (Lilliefors test p=0.005, non-normal) and homoscedasticity assumption testing (Spearman's ρ =-0.08, p=0.44, homoscedastic) for the secondary outcome PIPP-R regression model. **C.** Normality of residuals (Lilliefors test p=0.001, non-normal) and homoscedasticity assumption testing (Spearman's ρ =0.62, p<0.001, heteroscedastic) for the secondary outcome STAI-S regression model. PIPP-R: Premature Infant Pain Profile-Revised, STAI-S: State Trait Anxiety Inventory-State.

Predictor outcomes	GA	PNA	Baseline STAI-S
Magnitude of heel lance-evoked brain activity	0.87	0.29	NA
PIPP-R	0.79	0.97	NA
Tachycardia	0.99	1.00	NA
STAI-S	0.62	0.95	0.87

Table 4.2: Linearity assumption testing. P-values for the test of linearity for the continuous predictors in each of the four regression models, as obtained by the multivariate fractional polynomials algorithm. GA: gestational age at birth, NA: not available, PNA: postnatal days at study, STAI-S: State Trait Anxiety Inventory-State.

analysis assessed the CACE, the effect of the intervention isolated among compliers in the full analysis sets. The 45 s threshold between the start of stroking and the time of the heel lance was selected to define non-adherence with regard to neonatal outcomes as described in the Methods 3.2.6.4. After accounting for other reasons for unavailable outcomes such as missing data, non-adherence affected five participants with regard to the analysis of the primary outcome, seven participants with regard to the analysis of the secondary PIPP-R outcome, and eight participants with regard to the analysis of the secondary tachycardia outcome. The CACE analysis was performed in the full analysis sets, which includes all available outcomes irrespective of compliance (adherence).

4.3.6.1 Effect on the primary outcome of noxious-evoked brain activity

The primary outcome of the trial, the magnitude of heel lance-evoked brain activity quantified via n-NRF, did not significantly differ between trial arms (Figure 4.7). In the intervention group, the mean n-NRF magnitude in response to the heel lance was 0.85 a.u. (SD 0.70, n=39). In the control group, the mean was 0.91 a.u. (SD 0.76, n=43). Via CACE analysis, we identified a difference in means between groups of -0.11 a.u. (lower in intervention group; SD 0.77, 95% CI -0.42 to 0.20, p=0.38, n=82). In the intervention group, the median time between the start of stroking and the heel lance was 16.9 s (IQR 11.6–33.0, n=39). This delay

was calculated across compliers and non-compliers in the full analysis set for the analysis of the primary outcome. Given that the intervention lasted approximately ten seconds, this corresponds to roughly a median of seven seconds between the end of the pre-procedural stroking and the time of heel lance.

4.3.6.2 Effect on the secondary outcomes PIPP-R, tachycardia and parental anxiety

None of the secondary outcomes differed significantly between the intervention and control groups (Figure 4.8). There were no differences between the PIPP-R values in each group following the heel lance. The mean PIPP-R score in the intervention group was 8.0 (SD 3.17, n=49) and in the control group was 7.20 (SD 3.56, n=51). The difference in means between the two groups was 1.10 (higher in the intervention group, SD 3.26, 95% CI -0.42 to 2.61, p=0.15, n=100, Figure 4.8A). As mentioned in the assumption testing section (4.3.5), robust multiple linear regression was performed for the PIPP-R outcome for consistency with the other regression models, even though the residuals of the PIPP-R model were homoscedastic. The regression performed with OLS for the PIPP-R outcome led to a similar result compared to the robust regression: the difference in means was 1.04 (higher in intervention group, SD 3.25, 95% CI -0.48 to 2.55, p=0.15, n=100). For reference, PIPP-R scores can indicate no pain (0), or mild (1–6), moderate (7–12) and severe (>12) acute biobehavioural responses [299], and heel lances were estimated to be of moderate pain severity in the study by Laudiano-Dray and colleagues [42].

The number of neonates who became tachycardic did not differ significantly between groups (11 [21%] of 52 *vs* eight [15%] of 53). The OR was 2.08 (95% CI 0.46 to 9.46, p=0.34, n=105, Figure 4.8B) for the intervention group in reference to the control group. For completeness, the risk ratio (RR) for the secondary tachycardia outcome is also presented. The RR was 1.47 (greater in the intervention group, 95% CI 0.62 to 3.48, p=0.38, n=105).

For parental anxiety, the mean STAI-S score was 33.81 (SD 12.21, n=54) in the intervention group and 30.06 (SD 9.87, n=52) in the control group. The mean

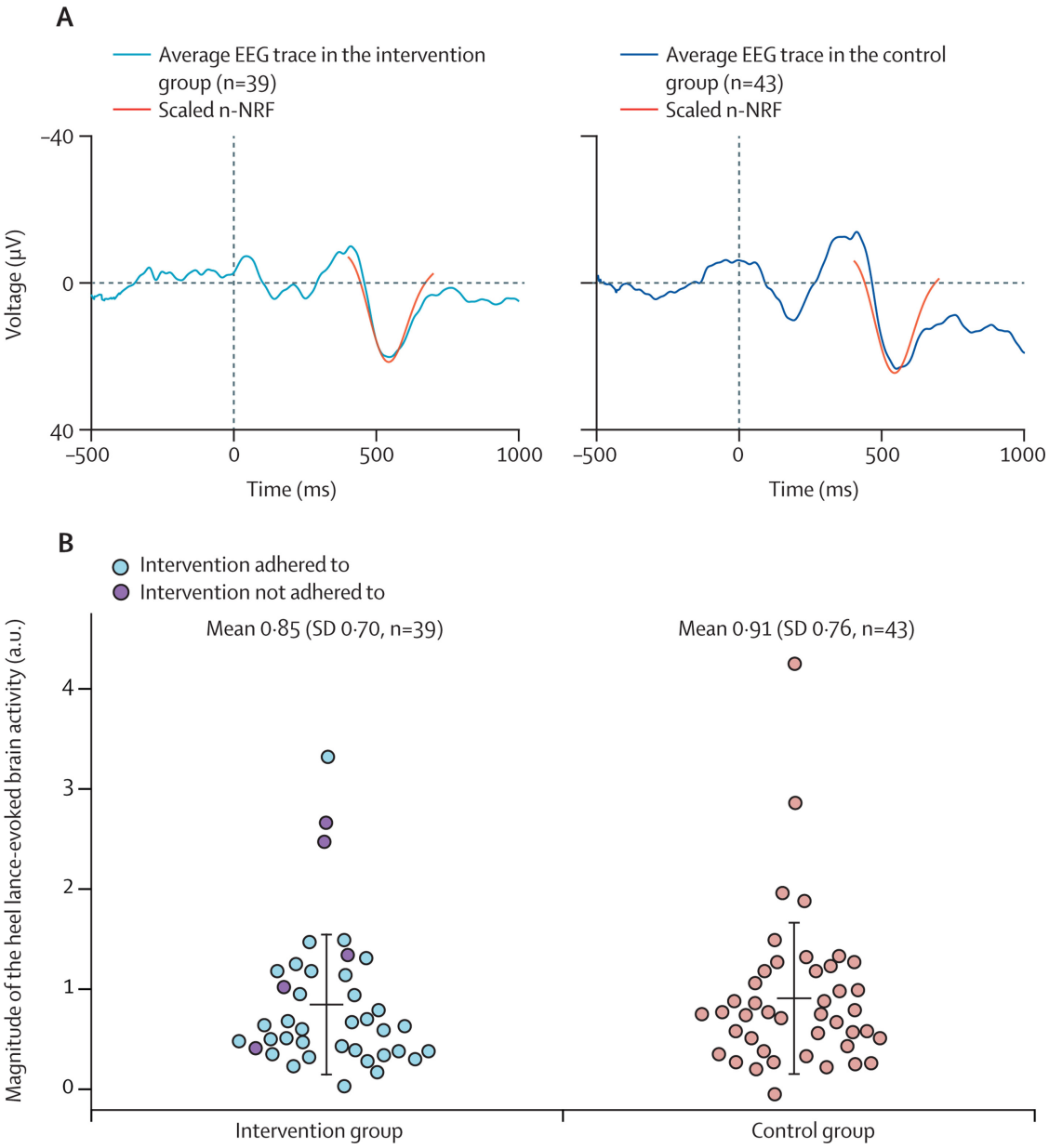


Figure 4.7: Primary outcome of heel lance-evoked brain activity (n-NRF magnitude) in intervention group and control group. A. Average EEG traces recorded at electrode Cz between 500 ms before and 1000 ms after the heel lance in intervention and control groups (full analysis set, groupings as randomised, n=82). The scaled n-NRF is shown overlaid in red. **B.** Magnitudes of the n-NRF. Each point in the two scatter plots represents the primary outcome measure for a single neonate. The error bars illustrate mean and SD of the n-NRF magnitudes. EEG raw data was processed as described in section 3.2.4.1.1. This figure is reproduced from Hauck and colleagues [3], under a CC BY 4.0 license with copyright maintained by the authors. a.u.: arbitrary units, EEG: electroencephalography, n-NRF: noxious neurodynamic response function, SD: standard deviation.

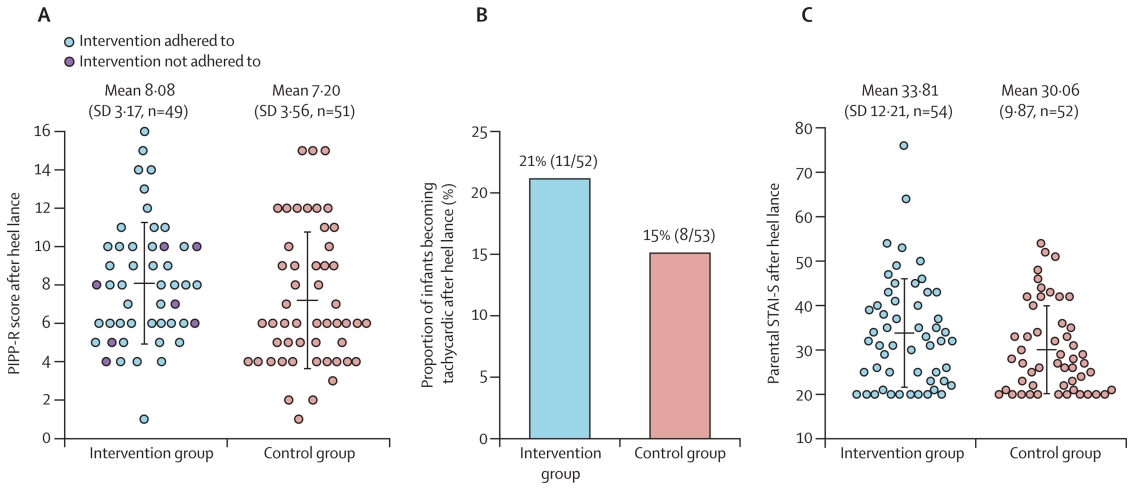


Figure 4.8: Secondary outcomes in the intervention group and control group. **A.** Total PIPP-R score for each neonate. **B.** Proportion of neonates who developed tachycardia after the heel lance. **C.** Total STAI-S score after the heel lance for each parent who stroked their baby. The mean difference between groups adjusted for baseline STAI-S and minimisation criteria was -0.44 (higher in control group, SD 6.85, n=106). In the scatter plots in (A) and (C), each dot represents the outcome measure for a single baby or their parent, respectively. The error bars in (A) and (C) illustrate mean and SD. This figure shows the full analysis set grouped as randomised. This figure is reproduced from Hauck and colleagues [3], under a CC BY 4.0 license with copyright maintained by the authors. PIPP-R: Premature Infant Pain Profile-Revised, STAI-S: State Trait Anxiety Inventory-State.

difference between groups was -0.44 (higher in the control group, SD 6.85, 95% CI -2.91 to 2.02, p=0.72, n=106; Figure 4.8C).

4.3.6.3 Exploring the intention-to-treat (ITT) effect

The ITT analysis was carried out to assess the effect of assignment to the intervention and used the full analysis set. As is expected, the effect of non-compliance resulted in a smaller ITT effect size compared to the per protocol effect size, and was similarly non-significant. Averages and SD of the outcomes are reported below for groupings as randomised. There was no significant difference between groups in the primary outcome measure: the difference in means was -0.10 a.u. (smaller in intervention group; SD 0.77, 95% CI -0.37 to 0.18, p-value 0.37, n=82). None of the secondary outcomes differed significantly between intervention and control group. For the PIPP-R outcome, the difference in means between the two groups was 0.94 (greater in intervention group; SD 3.26, 95% CI -0.35 to 2.23, p-value 0.15,

n=100). For the tachycardia outcome, the OR was 1.86 (greater in the intervention group; 95% CI 0.52 to 6.64, p-value 0.34, n=105). For the parental anxiety STAI-S outcome, the mean difference between groups was -0.44 (greater in control group; SD 6.85, 95% CI -2.91 to 2.02, p-value 0.72, n=106).

4.3.7 Exploring the neonatal heart rate during stroking

Having observed a non-significant trend in the PIPP-R and tachycardia outcome measure of more tachycardia and higher PIPP-R in the intervention group, it was hypothesised that this might be due to increased arousal of the neonate following parental touch. To explore whether stroking might have aroused neonates, the heart rate during the 10 s of parental stroking was explored (see Figure 4.9). The average heart rate trace during the pre-procedural stroking in the intervention group is plotted in Figure 4.9A. Given that the post-procedural stroking in the control group could occur in parallel to blood collection, which can affect the neonatal heart rate, the average heart during the stroking was plotted for the intervention group only. As a further data quality assessment, we expected that average heart rate during a control period without any intervention to remain roughly unchanged. The control period chosen was the ten seconds following a sham stroking marker added to the recordings before the heel lance in the control group. In the control group, sham stroking markers were placed before the heel lance to maintain blinding of the investigators assessing EEG. The sham and real stroking markers were exactly the same, and could only be distinguished upon unblinding based on the participant's study identifier. The times between the markers indicating the actual start of the stroking intervention and the heel lance in the intervention group, and the times between the sham stroking start and heel lance in the control group were similar, as expected (Figure 4.9B). The average heart rate change from the start to the end of pre-procedural stroking was an increase of 2.1 bpm (SD 9.1, n=49, Figure 4.9A).

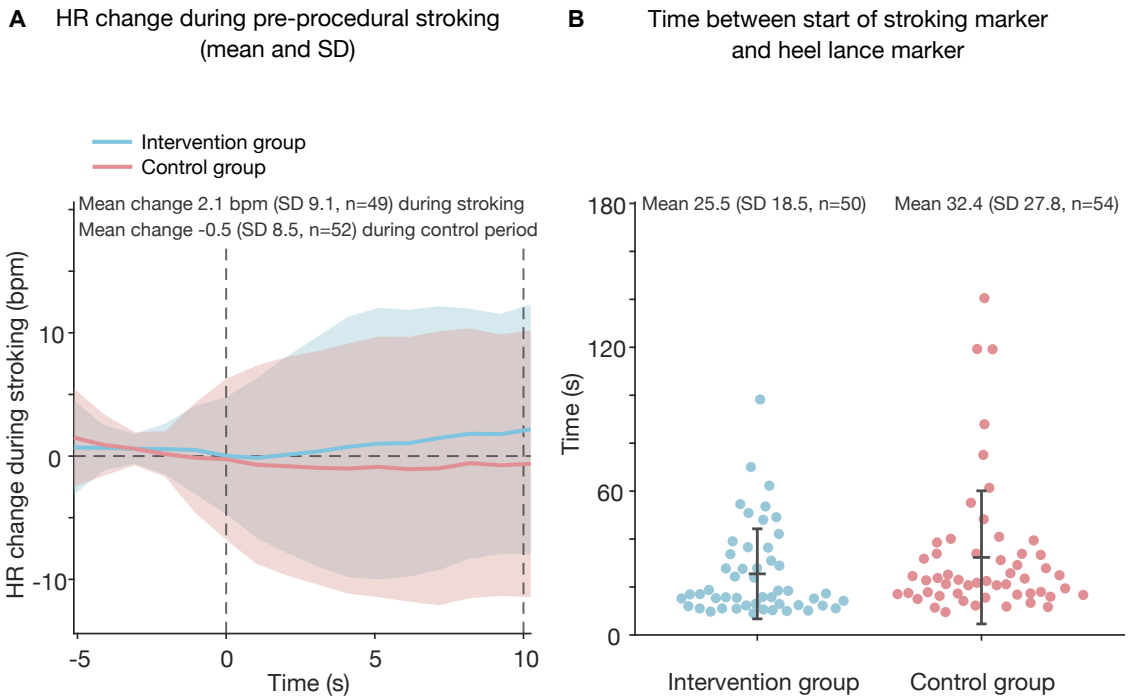


Figure 4.9: Neonatal heart rate (HR) during parental stroking. A. Neonatal heart rate during stroking in the intervention group, compared to neonatal heart rate during a comparable period where no intervention occurred in the control group. In the intervention group, the HR data depicts the actual stroking that occurred before the heel lance. In the control group, a sham stroking marker was used to plot the neonatal HR data. The line depicts the group average, while the shaded area is enclosed within the standard deviation of the group's HR. Each participant's HR data is demeaned based on the HR values in the 5 s preceding the marker indicating the start of stroking. The horizontal dashed line indicates the average HR value at the start of the stroking in the intervention group. The vertical dashed line indicates the start and end of the stroking, at t=0 and t=10 s. **B.** Each dot represents the time between the marker indicating the start of the stroking and the marker indicating the heel lance for a single baby. For neonates in the intervention group, these times reflect the real times between the start of parental stroking and the heel lance. For neonates in the control group, no stroking occurred before the heel lance. Thus, this time reflects the time between a sham marker 'stroking start' and the actual heel lance. The sham markers before the heel lance ensured that the trial arm could not be recognised based on the timing of the 'stroking start' markers compared to the heel lance in the EEG recordings. EEG: electroencephalography, HR: heart rate. The grey bars overlying the scatter plots show mean and standard deviation of the data.

4.3.8 Missing data patterns

We have investigated the missing data patterns by assessing the standardised mean difference [335] between the group of subjects with available outcome data and the subjects with missing outcome data. This was performed in R with the package

	Primary outcome available	**Primary outcome missing**	**p**	**SMD**
n	82	30		
GA (mean (SD))	38.38 (2.08)	38.45 (1.86)	0.867	0.037
PNA (mean (SD))	0.40 (0.26)	0.50 (0.26)	0.088	0.367
Primary indication for blood sampling (%)			0.586	0.346
Glucose monitoring	5 (6.1)	1 (3.3)		
Jaundice	39 (47.6)	15 (50.0)		
Newborn screening	6 (7.3)	2 (6.7)		
Suspected sepsis	26 (31.7)	7 (23.3)		
Other	6 (7.3)	5 (16.7)		
Site = Oxford (%)	55 (67.1)	28 (93.3)	0.010	0.698
Sex = Male (%)	50 (61.0)	18 (60.0)	1.000	0.020
Parent stroking = Biological mother (%)	56 (68.3)	17 (56.7)	0.358	0.242
Ward (%)			0.574	0.357
HDU	11 (13.4)	5 (16.7)		
ITU	4 (4.9)	0 (0.0)		
LDU	5 (6.1)	1 (3.3)		
Postnatal ward	62 (75.6)	24 (80.0)		
Behavioural score (mean (SD))	2.15 (1.20)	1.60 (1.29)	0.053	0.439
STAI-T (mean (SD))	34.26 (8.69)	33.86 (8.49)	0.832	0.047

Table 4.3: Pattern of missing primary outcome data. T-tests and chi-squared tests were performed for continuous and categorical variables, respectively. P-values were not corrected for multiple comparisons. Continuous variables: GA, PNA, behavioural score and STAI-T. Categorical variables: primary indication for blood sampling, site, sex, parent stroking, ward. The behavioural score refers to the behavioural score at baseline of the PIPP-R, with more active neonates having a lower score: active and awake (score 0), quiet and awake (score 1), active and asleep (score 2), quiet and asleep (score 3). GA: gestational, HDU: high dependency unit, ITU: intensive therapy unit, LDU: low dependency unit, PNA: postnatal age, SD: standard deviation, SMD: standardised mean difference, age, STAI-T: State Trait Anxiety Inventory-Trait.

tableone, t-tests and chi-squared tests were used for continuous and categorical variables, respectively, and the results are presented below in Tables 4.3, 4.4, 4.5 and 4.6 [336]. Across outcomes, a potential imbalance between the group of participants with missing and those with available outcomes seems to be related to site differences, as well as to neonates being more awake (which leads to more likely movement artefacts across recordings, affecting in particular the primary EEG outcome).

	Secondary PIPP-R outcome available	**Secondary PIPP-R outcome missing**	**p**	**SMD**
n	100	12		
GA (mean (SD))	38.45 (1.97)	37.96 (2.39)	0.431	0.222
PNA (mean (SD))	0.42 (0.27)	0.49 (0.24)	0.383	0.279
Primary indication for blood sampling (%)			0.514	0.644
Glucose monitoring	5 (5.0)	1 (8.3)		
Jaundice	46 (46.0)	8 (66.7)		
Newborn screening	7 (7.0)	1 (8.3)		
Suspected sepsis	32 (32.0)	1 (8.3)		
Other	10 (10.0)	1 (8.3)		
Site = Oxford (%)	72 (72.0)	11 (91.7)	0.262	0.528
Sex = Male (%)	63 (63.0)	5 (41.7)	0.264	0.437
Parent stroking = Biological mother (%)	68 (68.0)	5 (41.7)	0.137	0.549
Ward (%)			0.775	0.383
HDU	15 (15.0)	1 (8.3)		
ITU	4 (4.0)	0 (0.0)		
LDU	5 (5.0)	1 (8.3)		
Postnatal ward	76 (76.0)	10 (83.3)		
Behavioural score (mean (SD))	2.08 (1.20)	1.14 (1.46)	0.052	0.699
STAI-T (mean (SD))	34.67 (8.64)	29.10 (6.69)	0.051	0.721

Table 4.4: Pattern of missing secondary PIPP-R outcome data. T-tests and chi-squared tests were performed for continuous and categorical variables, respectively. P-values were not corrected for multiple comparisons. Continuous variables: GA, PNA, behavioural score and STAI-T. Categorical variables: primary indication for blood sampling, site, sex, parent stroking, ward. The behavioural score refers to the behavioural score at baseline of the PIPP-R, with more active neonates having a lower score: active and awake (score 0), quiet and awake (score 1), active and asleep (score 2), quiet and asleep (score 3). GA: gestational, HDU: high dependency unit, ITU: intensive therapy unit, LDU: low dependency unit, PIPP-R: Premature Infant Pain Profile-Revised, PNA: postnatal age, SD: standard deviation, SMD: standardised mean difference, age, STAI-T: State Trait Anxiety Inventory-Trait.

	Secondary tachycardia outcome available	**Secondary tachycardia outcome missing**	**p**	**SMD**
n	105	7		
GA (mean (SD))	38.41 (1.98)	38.18 (2.63)	0.772	0.099
PNA (mean (SD))	0.42 (0.26)	0.55 (0.29)	0.194	0.487
Primary indication for blood sampling (%)		0.390	1.012	
Glucose monitoring	5 (4.8)	1 (14.3)		
Jaundice	50 (47.6)	4 (57.1)		
Newborn screening	7 (6.7)	1 (14.3)		
Suspected sepsis	33 (31.4)	0 (0.0)		
Other	10 (9.5)	1 (14.3)		
Site = Oxford (%)	76 (72.4)	7 (100.0)	0.242	0.874
Sex = Male (%)	64 (61.0)	4 (57.1)	1.000	0.078
Parent stroking = Biological mother (%)	70 (66.7)	3 (42.9)	0.384	0.493
Ward (%)			0.863	0.461
HDU	15 (14.3)	1 (14.3)		
ITU	4 (3.8) 0 (0.0)			
LDU	6 (5.7)	0 (0.0)		
Postnatal ward	80 (76.2)	6 (85.7)		
Behavioural score (mean (SD))	2.08 (1.20)	0.00 (0.00)	0.004	2.439
STAI-T (mean (SD))	34.37 (8.61)	30.50 (8.29)	0.286	0.458

Table 4.5: Pattern of missing secondary tachycardia outcome data. T-tests and chi-squared tests were performed for continuous and categorical variables, respectively. P-values were not corrected for multiple comparisons. Continuous variables: GA, PNA, behavioural score and STAI-T. Categorical variables: primary indication for blood sampling, site, sex, parent stroking, ward. The behavioural score refers to the behavioural score at baseline of the PIPP-R, with more active neonates having a lower score: active and awake (score 0), quiet and awake (score 1), active and asleep (score 2), quiet and asleep (score 3). GA: gestational, HDU: high dependency unit, ITU: intensive therapy unit, LDU: low dependency unit, PNA: postnatal age, SD: standard deviation, SMD: standardised mean difference, age, STAI-T: State Trait Anxiety Inventory-Trait.

	Secondary STAI-S outcome available	Secondary STAI-S outcome missing	p	SMD
n	106	6		
GA (mean (SD))	38.43 (2.03)	37.83 (1.82)	0.482	0.310
PNA (mean (SD))	0.42 (0.27)	0.43 (0.20)	0.971	0.017
Primary indication for blood sampling (%)			0.509	0.762
Glucose monitoring	5 (4.7)	1 (16.7)		
Jaundice	51 (48.1)	3 (50.0)		
Newborn screening	7 (6.6)	1 (16.7)		
Suspected sepsis	32 (30.2)	1 (16.7)		
Other	11 (10.4)	0 (0.0)		
Site = Oxford (%)	77 (72.6)	6 (100.0)	0.313	0.868
Sex = Male (%)	65 (61.3)	3 (50.0)	0.902	0.229
Parent stroking = Biological mother (%)	70 (66.0)	3 (50.0)	0.718	0.329
Ward (%)			0.520	0.647
HDU	14 (13.2)	2 (33.3)		
ITU	4 (3.8)	0 (0.0)		
LDU	6 (5.7)	0 (0.0)		
Postnatal ward	82 (77.4)	4 (66.7)		
Behavioural score (mean (SD))	2.04 (1.23)	1.00 (1.41)	0.241	0.783
STAI-T (mean (SD))	34.38 (8.68)	29.40 (5.41)	0.207	0.689

Table 4.6: Pattern of missing secondary STAI-S outcome data. T-tests and chi-squared tests were performed for continuous and categorical variables, respectively. P-values were not corrected for multiple comparisons. Continuous variables: GA, PNA, behavioural score and STAI-T. Categorical variables: primary indication for blood sampling, site, sex, parent stroking, ward. The behavioural score refers to the behavioural score at baseline of the PIPP-R, with more active neonates having a lower score: active and awake (score 0), quiet and awake (score 1), active and asleep (score 2), quiet and asleep (score 3). GA: gestational, HDU: high dependency unit, ITU: intensive therapy unit, LDU: low dependency unit, PNA: postnatal age, SD: standard deviation, SMD: standardised mean difference, age, STAI-S: State Trait Anxiety Inventory-State, STAI-T: State Trait Anxiety Inventory-Trait.

4.3.9 Protocol deviation monitoring

Beside an instance in which the parent switched from the correct (ipsilateral leg) to the contralateral leg between the first and the second stroking movement, no protocol deviations occurred. As this baby was in the control group, this deviation does not affect the study.

4.3.10 Serious adverse event monitoring

One serious adverse event occurred in a neonate randomised to the post-procedural stroking group. The event (desaturation) occurred prior to the stroking and was deemed of mild severity (i.e. of mild intensity) as well as unrelated to study participation by the medically-qualified safety delegate.

4.4 Discussion

The pain-relieving effects of touch-based interventions such as skin-to-skin care and massage have been reported in numerous studies. However, according to recent Cochrane reviews, there is low to moderate evidence quality for skin-to-skin care [218] and very low to low certainty of evidence for massage [197] for dampening neonatal responses to procedural pain. It was noted that massage protocols vary widely from study to study [227], and that mechanisms underlying skin-to-skin care and massage interventions are not fully understood [218, 227]. Clarifying the underlying mechanisms could inform the interpretation of the differences observed among studies, and support the refinement of interventions towards their tailoring to the situations and populations where they can be most beneficial. Studies in adults have reported that the activation of CT afferents via experimental brushing of the skin is associated with reductions in verbal pain reports and noxious-evoked brain activity [232, 251, 252]. Similarly, in two pilot studies in independent groups of neonates, experimenter-led brushing resulted in a reduction of heel lance-evoked brain activity [171, 173]. The Petal trial built on this evidence and assessed the effect of parental stroking at an optimal speed to activate CT fibres on neonatal pain

responses. Neonatal outcomes were the magnitude of noxious-evoked brain activity (primary), as well as PIPP-R score (secondary) and the development of tachycardia (secondary) in response to the heel lance. Furthermore, parental anxiety levels were assessed as an additional secondary outcome. The primary and secondary outcomes did not significantly differ between intervention and control groups. However, the CIs of the primary outcome measure crossed the threshold of 40% reduction used as clinical significance threshold in the calculation of the sample size, as well as the threshold that would need to be excluded to reject the null hypothesis. Hence, a pain-relieving effect cannot be excluded based on the results.

Primary and secondary outcomes (as presented in Table 3.1) have been fully assessed and presented in this thesis. Regarding the exploratory objectives presented in Table 3.2, these have been partly investigated, focussing on the aspects deemed of most relevant to families, clinicians and researchers, in order to produce these results within the timeline of this thesis. We deemed the investigation of exploratory objective 4, 'To explore parental anxiety and distress, and their experience of the trial and infant research' as the most relevant to accompany the main study results. These results formed a manuscript published in *PAIN* [4], of which I am first co-author and are presented in Chapter 5. My contributions to the paper are highlighted at the beginning of Chapter 5. Regarding exploratory objectives 2–3, which focus on cardiorespiratory responses to the heel lance, the objectives listed are currently being investigated by the research team. Given that both these outcomes were measured past the initial 30 s used to record the PIPP-R, therefore in a period where multiple concomitant events occurred, these outcomes can provide valuable information to develop future hypotheses, but it will be harder to disentangle the effect of parental touch from that of the concomitant events. However, I did explore the heart rate in terms of its trend during the 10 seconds of parental stroking (section 4.3.7 Exploring the neonatal heart rate during stroking). The reason why I decided to explore this is that given the results of the primary and secondary outcomes, where a slight, non-significant increase in PIPP-R scores and tachycardia rates was observed in the pre-procedural parental stroking group, it was suspected that some

of the results might be driven by a slight arousal during the stroking prior to the lance. As a general trend, the heart rate of neonates increased on average by 2.1 bpm during parental stroking. Another outstanding exploratory objective is to investigate changes in EEG during parental stroking, which is currently being investigated by the research team. Given the short amount of time (10 s) during which the stroking occurred and potential confounders as well as electrical and movement artefacts, this exploratory objective might be harder to interpret. In summary, in this thesis all primary and secondary objectives are included as well as the exploratory objective which was deemed most relevant and informative alongside the primary and secondary results, while the other exploratory objectives are currently being investigated by the research team. Regarding multiple imputation analysis, this sensitivity analysis was not performed due to limitations in study resources.

Despite the inconclusive results, the trial has numerous strengths. It implemented outcomes across multiple modalities, and used an objective brain-derived primary outcome measure. The trial was successfully completed at two sites in the UK, with 112 neonates randomised out of 159 parents approached (70%). For comparison, the consent rate of an RCT investigating kangaroo care and sucrose for management of acute procedural pain throughout hospitalisation in a slightly younger group (born below 37 weeks GA), was 54% [223]. The consent rate of Petal suggests acceptability of assessing pain using multimodal outcomes and noxious-evoked brain activity as primary outcome in a multicentre RCT context. Regarding the two study sites chosen, they differed in the level of care (level 3 neonatal unit in Oxford vs level 2 neonatal unit in Exeter) as well as in the larger research team involved at the Oxford compared to the Exeter site. The study procedures were similar at the two sites, with small differences due to partly different recording equipment (as listed in 3.2.4 Outcomes and recordings) included automatic time-locking of the vital signs events in Oxford whereas events had to be manually marked in the vital signs in Exeter, a slightly different sampling rate of the EEG recording in Exeter and Oxford (2048 *vs* 2000 Hz, respectively, downsampled to 500 Hz during pre-processing), and a slightly more compact recording system in Exeter compared to Oxford (one computer

trolley *vs* two trolleys). Regarding the study participants, another research team member assessed whether the index of multiple deprivation based on the postcodes of the study participants reflected the index of multiple deprivation of the Exeter and Oxford areas. The socioeconomic status of the single participants was not collected, nevertheless the postcode was collected within the remit of this research trial and could be linked to the publicly available index of multiple deprivation [337]. The index of multiple deprivation is a measure which comprises seven domains of deprivation (income, employment, health deprivation and disability, education and skills training, crime, barriers to housing and services, and living environment). The distribution of the index of multiple deprivation of the study participants at the Oxford and Exeter sites reflected the distribution of the index of multiple deprivation in the Oxford and Exeter areas, respectively, suggesting that the participants were roughly representative of the local areas with regard to socioeconomic status. While the trial protocol was the same at both sites, differences between study sites such as those related to the local population as well as study personnel involved increase the external validity of the trial results. Site differences in relation to missing data are explored in chapter 4 (section 4.3.8 Missing data patterns), whereas site differences in terms of primary outcome are being investigated as part of ongoing research on reproducibility of the primary outcome measure [189].

The full statistical analysis plan was developed and published online prior to unblinding the data. It follows the relevant ICH and additional guidelines, and was developed to minimise the risks of bias and enable reproducibility of the trial's methodology. The focus of the trial was not only on neonates, but also on parents and their feelings while being involved in their baby's care, which will be described in the following chapter. Although most of the literature assessing parent-led interventions for neonatal pain management focusses on mothers only, in the Petal trial fathers could also provide the gentle touch intervention to their babies.

In the effort to accurately reproduce the pilot studies and assess stroking at the optimal speed to activate CT fibres lies also a limitation of the trial. To maintain a stroking speed of approximately 3 cm/s, which is within the 1–10 cm/s

range to which CT fibres preferentially respond, an animation with a bar filling at 3 cm/s was shown to parents. Although a similar visual metronome approach has been reported, trained researchers rather than parents followed the visual animation [230, 338]. In a study where mothers performed a touch intervention guided by a metronome, the metronome implemented provided acoustic cues. In this study, mothers were guided by a metronome to massage their babies at three different velocities within the range to which CT fibres preferentially respond CT. However, massage lasted approximately 15 minutes, which likely allowed mothers to become more used to the acoustic metronome and rendered their movement more natural over time compared to the shorter intervention used in Petal [339]. In the Petal trial, parents were trained with a visual metronome animation at the start of the study, which also guided them during the intervention. This might have affected an otherwise naturally occurring interaction between parents and their babies. Given that social touch tends to have a more variable movement pattern compared to stroking of inanimate object [249], using the computer animation might have made the movement more mechanical. This limitation could be overcome in future studies, as it was recently shown that mothers tend to naturally stroke their babies at CT-optimal speeds. In future studies, the approach used by Püschel and colleagues could be considered, where researchers retrospectively assessed the speed of parental stroking: the authors of the study placed a ruler next to the cot and recorded a video of the parental movement, which they used to calculate the speed of the stroking movement [246]. Other approaches recently reported are to reconstruct the stroking velocity after the study by attaching a sensor to the hand [249]. However, it could be argued that a sensor on the hand could again interfere with the natural parent-baby interaction, and that it would be more suitable for a more experimental setting, whereas recording a video for retrospective assessment of stroking velocities might be less invasive and allow for a natural interaction to unfold. Due to the short duration of the intervention, parents and neonates did not have much time to become accustomed to the touch, potentially leading to a slight arousal of the babies (Figure 4.9). Speed and duration of the intervention

were chosen to match the pilot studies, where brushing of the baby's skin led to a significant reduction of noxious-evoked brain activity [171, 173]. However, in the pilot studies the intervention was delivered by a trained researcher with a calibrated brush, while in the Petal trial each parent provided the intervention only once, beside the initial training session. Parental stroking of their baby's skin for a longer period would have potentially reproduced some of the beneficial results reported in the literature. The study by Püschel and colleagues mentioned above, which was published while the Petal trial was ongoing, showed that the group's mean HR decreases from about 1–2 minutes into the stroking movement in premature neonates [246], reflecting similar findings by Manzotti and colleagues [230]. This is considerably later when compared to reports of HR reductions in nine-month-old infants after experimenter-led brushing of their skin for periods of ten seconds [257]. A longer intervention would potential have been helpful also in minimising the delay between the end of the stroking movement and the heel lance. In the pilot studies, experimenter-led brushing occurred just before the heel lance [171, 173]. In the Petal trial, there was a median delay of 16.9 s (IQR 11.6 to 33.0, n=39) between the start of the pre-procedural stroking and the heel lance, which is roughly equivalent to a median delay of 7 s between the end of the stroking and the heel lance. In a study conduced in adults investigating experimental brushing, a delay as little as 5 or 10 s between the end of brushing and the noxious stimulus led to a significantly less effective pain reduction compared to 1 s [252]. It is conceivable that a longer intervention would have allowed parents to become more acquainted with the stroking, and could thus have facilitated a shorter delay between parental touch and the heel lance procedure. With regard to the location of parental stroking, recent preliminary evidence suggests that CT fibres might be more scarce in the leg compared to the forearm [340], where studies involving experimental brushing typically deliver the intervention [234]. The location of the stroking—the lower leg ipsilateral to the heel lance—was chosen to match the pilot studies. Of the three studies reporting a reduction of noxious-evoked brain activity in adults upon experimental brushing of the skin, in one brushing and noxious stimulation occurred

in the same dermatome [252], while in the other two they occurred in different dermatomes [232, 251]. Whether pain relief is more effective when CT fibres are activated in proximity of the noxious stimulus site compared to elsewhere is yet to be established [233]. Meijer and colleagues recently investigated the effect of experimental brushing at CT-optimal and non-optimal speed performed on the ipsilateral or contralateral dorsal forearm on verbal pain reports. The noxious stimulus was repeated heat pulses on the ventral side of the participant's wrist. In line with previous studies, they reported reduced pain scores with brushing at CT-optimal compared to non-optimal speed. With regard to the application site of the brushing, ipsilateral and contralateral site stimulations led to similar pain reductions [341]. Although these findings have yet to be replicated and might not apply to the neonatal population, they suggest that the site of gentle touch at CT-optimal speed may not play a major role in pain modulation in adults. A key limitation that needs to be overcome is the high degree of data loss due to data quality affected by noise. In the missing data pattern analysis, there is a recurrent imbalance with more data missing in the Oxford site compared to the Exeter site, and in neonates who were more awake at baseline. While the latter could be linked to a higher degree of movement artefacts affecting the data recording, the site difference might be due to differences in personnel, as well as in the room set up, with electrical interference being more likely in rooms with more electrical devices. Refining the methodology to reduce data loss is a key research goal, as this is a known challenge, particularly in pain studies that rely on single-event recordings. Refined methodology, using an inverse-variance weighted least squares approach, should reduce data loss to less than 5%, which is typically considered acceptable in RCTs [287].

Concomitant non-pharmacological interventions were permitted during the trial in line with local practice guidelines. This included breastfeeding, kangaroo care and non-nutritional sucking. Sucrose was not administered, as this is not a standard strategy implemented at the trial sites nor recommended within local guidelines. Feeds took place as and when deemed appropriate by parents. The timing of the test occasion was planned with parents and clinicians to minimise disruption to clinical

practice and parental cares. Concomitant non-pharmacological interventions may have impacted the outcome measures recorded; disentangling their effect from that of the intervention is challenging. Use of these interventions was context-specific to each participant as appropriate according to the clinical team and parents. Each baby's reaction to concomitant interventions is unknown, as is how to assess the impact of the timing of the concomitant intervention in relation to the timing of the measured outcome. Concomitant interventions were however annotated in the documentation of the test occasion, and investigating their impact could be explored in future projects.

Future studies may aim to standardise procedures further e.g. using an explanatory trial framework, where variance is minimised to maximise likelihood of observing an intervention effect [342]. One approach could be implementing a protocol for additional pain-relieving interventions e.g. first line non-nutritive sucking, then breastfeeding, etc. This would reduce variance and therefore required sample size. However, it would also reduce generalisability of the results and may therefore not be the preferred approach. Conversely, future studies may implement a more pragmatic design with small changes to usual care in order to increase generalisability, e.g., standard of care plus an intervention compared to standard of care only [342]. This may be preferable to regulatory agencies by assessing effectiveness in conditions more alike to a real-life scenario. However, this less standardised approach may dilute intervention effects, reducing power, thus requiring larger sample sizes. A pragmatic design, such as an 'add-on' design, where standard of care plus an intervention is compared to standard of care only, is recommended by the FDA for neonatal pain trials [276, 343]. The use of further interventions ('rescue measures') can be considered within the study design as a main outcome for infant pain studies [343]. Ultimately, the positioning of a trial in the continuum between explanatory and pragmatic trials should be based upon the intended purpose [342].

Ethics of use or non-use of concomitant interventions for pain is central in the development of a trial aimed at assessing a pain-relieving intervention in a vulnerable population such as neonates, in particular considering that neonates

cannot provide informed consent and this only occurs via proxy (parent). One of the principles of research is to 'do no harm' and, equipoise between trial arms has to be warranted [195]. There is evidence that other non-pharmacological measures (such as breastfeeding and non-nutritive sucking) can reduce pain-related outcomes (see chapter 2 Background), and these interventions are included in local practice guidelines. While local practice guidelines vary between regions, they must comply with ethical standards. Concomitant measures considered within standard of care were provided to the neonates enrolled in the trial according to local guidelines. Adding further concomitant interventions beyond standard of care would deviate from real-life conditions and reduce generalisability of the results. We deemed the use of standard of care according to local practice guidelines and the implementation of an 'add-on' intervention (parental touch) in the intervention group to be ethical and reflect the suggestions of the FDA with regards to clinical trial designs [343]. While providing concomitant measures (according to local practice guidelines) may have reduced the ability to detect an effect of parental stroking, it more closely resembled standard of care in a non-trial setting, adding to the external validity and generalisability of results.

In summary, The Parental touch trial investigated the potential pain-relieving effect of parental stroking at the optimal speed to activate CT fibres in neonates in a hospital setting. Although we did not observe a significant effect of the intervention, the trial contributed to the field of neonatal pain management in numerous ways. It was noted in a recent review that a large proportion of studies aiming at activating CT fibres are performed in experimental settings, whereas less is known about more natural interactions such as those between parents and their children [234]. The Parental touch trial investigated the potential pain-relieving effect of parental stroking at the optimal speed to activate CT fibres in neonates who clinically required a heel lance. Although the use of a metronome likely reduced part of the instinctive component of parental touch, the trial design was performed in a hospital setting with inpatient newborn babies who clinically required a heel lance and their parents, rather than in an experimental room with calibrated brushes and experimental

noxious stimuli. Recording multimodal outcome measures during the parent-baby interaction was feasible and the consent rate suggested parental acceptability of the trial design. While future studies may focus on longer interventions and more natural types of interactions, such as skin-to-skin care or stroking for a longer period of time and in a more natural fashion, the trial methodology developed for Petal could be used as a blueprint. Furthermore, the statistical analysis plan outlines how outcomes were measured and compared between groups, and may provide a useful resource for future trials. Finally, involvement of both mothers and fathers was central in this trial and aligns with concepts such as family-centred care, where parental involvement and pain management are among the main pillars to support babies and their families [271].

Chapter 5

Parental experience of neonatal pain research while participating in the Parental touch trial (Petal)

This chapter is primarily based on the following manuscript published in *PAIN* in 2024 [4], of which I am co-first author. The article is published under a CC BY 4.0 license with copyright retained by the authors [4]. The methods and results section reflect those published with some minor additions, while the introduction and discussion of this chapter have undergone more conspicuous adaptation.

- van der Vaart, M.*, Hauck, A. G. V.*, Mansfield, R., Adams, E., Bhatt, A., Cobo, M. M., Crankshaw, D., Dhami, A., Hartley, C., Monk, V., Evans Fry, R., Moultrie, F., Robinson, S., Yong, J., Poorun, R., Baxter, L.‡, & Slater, R.‡ (2024). Parental experience of neonatal pain research while participating in the Parental touch trial (Petal). Pain. doi:10.1097/j.pain.0000000000003177

*equal contributions ‡ equal contributions

The content of the manuscript mentioned above was restructured and expanded to align with the structure of the thesis. In addition to my role in the management of the trial and leading data collection underpinning this project, I have performed a large proportion of the analysis and generated three of the main figures reported in the publication, whereas the correlation matrix with dendrogram was generated

by colleagues. I contributed to writing part of the first manuscript draft and to the reviewing and editing stage.

5.1 Introduction

Neonatal pain can affect not only neonates, but also their families, as seeing one's own baby undergoing painful procedures has been reported as a source of significant parental stress and feelings of helplessness [266–270, 344]. When parents are asked about their feelings while being involved in their baby's care during a clinically required painful procedure, some report negative feelings, such as feeling uncomfortable [270, 345, 346]. However, parents may prefer to be involved in their baby's care despite feeling uncomfortable, and generally studies have underscored parental desire to be involved in their baby's comfort care [264, 270, 271, 345, 346].

Nevertheless, the amount of parental involvement can largely differ depending on the context, and parents are not always given the opportunity or information they desire to be involved in their baby's care during a painful procedures [344, 347]. A strong partnership between health care professionals and parents is one of the core elements of current programmes to optimise neonatal and parental wellbeing and increase parental involvement, such as *Family Integrated Care* [348] or the *Bliss Baby Charter* [260]. Parental involvement in neonatal pain relief can help parents to establish their caregiver role [263, 349]. Parents can use a wide-range of comforting techniques to support their babies during painful procedures, such as breastfeeding [219], kangaroo care [218] and further non-pharmacological interventions [197]. However, as concluded by Ullsten and colleagues in their recent review on parent-delivered pain interventions for neonates, little is yet known about parental experiences while delivering pain-relieving interventions [271]. Furthermore, the review's authors highlighted that, while there is sufficient evidence suggesting that parents generally want to be involved in comforting their babies, the motivational factors underlying parents' decision to be actively involved in their baby's care require further elucidation [271]. A caveat of the current literature on parental involvement in their baby's care is that the focus is often placed on mothers

[350], despite the fact that fathers have also expressed feelings of helplessness and a wish to be more involved in their baby's care [351, 352].

Parental involvement was central in the Petal trial, which investigated parental touch as a potential pain-relieving intervention. Fathers and mothers provided the touch intervention. The effect of the intervention was not only assessed on neonatal outcomes (magnitude of noxious-evoked brain activity, PIPP-R and tachycardia), but also on parental anxiety. Parental anxiety was measured with the STAI questionnaire, and the state component (STAI-S score) after their baby's clinically required heel lance was one of the secondary outcomes. Furthermore, the trial was designed to explore parental feelings. After trial participation, parents were asked to complete an anonymous survey. Based on the survey, the following was explored: (i) the parent-reported motivations in deciding to enrol in the trial, (ii) parent-reported feelings while they delivered the gentle touch intervention, (iii) the parent-reported experience of participating in the trial, and (iv) parental willingness to participate in future studies.

5.2 Methods

This chapter focusses on one of the exploratory objectives of the Petal trial specified in the preregistered protocol: the exploration of parental feelings and experience while participating in the trial. The multicentre RCT Petal investigated whether parental touch before a clinically required heel lance could dampen neonatal pain responses to the procedure and reduce parental anxiety. The trial enrolled at two sites in the UK: the John Radcliffe Hospital in Oxford, and the Royal Devon and Exeter Hospital in Exeter. A detailed description of the trial protocol and SAP is provided in Chapter 3. The primary objective of the Petal trial was to investigate whether parental touch—in the form of stroking delivered at a speed which optimally activates CT fibres–could reduce the magnitude of heel lance-evoked brain activity in neonates. Secondary objectives were whether the intervention could reduce the neonatal PIPP-R score and the development of tachycardia. In addition, a secondary outcome measure assessed parental anxiety with STAI-S

scores, which capture anxiety levels at a specific time point. In the intervention group parental touch was provided before the heel lance, whereas in the control group it was provided after the heel lance. Further comfort care (e.g. swaddling and non-nutritive sucking) was offered to all babies irrespective of trial arm. Parents were trained on how to stroke their baby's skin at CT-optimal speed following an animation on a computer screen. Further sensory stimulation such as talking to their baby could occur if parents wished to do so, but it was not consistently documented whether further sensory stimulation was provided besides parental touch. Fathers and mothers could provide the intervention and the parent delivering the intervention was asked to complete an anonymous survey towards the end of the test occasion. The survey was approved as one of the components of the Petal trial by the ethics committee (section 3.2.7), and parents did not receive financial remuneration for participating in the study.

5.2.1 Anonymous survey

Parents were presented with the online survey on an electronic device by a member of the research team. Responses were collected on Jisc Survey (Jisc, Bristol, United Kingdom, https://www.onlinesurveys.ac.uk). Survey questions were developed and piloted in an independent ongoing study (Investigating Pain in the Developing Brain; ethics ref: 12/ SC/0447) to assess appropriateness and then tailored to this study. The survey was conducted in English. The first part was completed by the researcher to document the research site (Exeter, Oxford) and trial arm (parental touch before the heel lance or parental touch after the heel lance). The second part was completed by the parent and included four compulsory questions listed in Box 5.1.

The survey's first question explored motivations for taking part in the trial and presented five possible options, which reflect reasons provided by parents who provided consent for taking part in neonatal trials. The second question focussed on how parents felt while being actively involved in their baby's care at the time of the heel lance. The descriptors chosen included both positive and negative feelings. They were presented in the same order to all participants, and all adjectives were

presented at the same time. No limit was imposed on the maximal number of answers. The bespoke group of adjectives was selected by the research team when setting up the study. The third question focussed on whether parents were pleased in having taken part in the trial, and the final question asked whether they would take part in future studies.

Box 5.1: Parental Touch Trial: Parental Survey.

1. How important were the following factors in helping you decide to take part in the Petal trial?
   (5 options, which could be graded as very important/important/neutral/not very important/not considered)
   (a) Contributing to science
   (b) The potential benefit to your baby
   (c) The potential benefit to future babies
   (d) Knowing that your baby would be monitored closely by the trial team during the blood test
   (e) Concern that your baby might be in pain from the blood test
2. Being actively involved in my child's care at the time of the blood test made me feel...
   (a list of descriptors where multiple options could be selected out of a list of ten adjectives)
   - anxious
   - stressed
   - relaxed
   - empowered
   - helpless
   - calm
   - frustrated
   - useful
   - upset
   - reassured
3. Are you pleased that your baby took part in the Petal trial?

(a) Yes
(b) No

4. Would you consider taking part in future research studies?

(a) Yes
(b) Maybe
(c) No

Descriptive statistics (percentage of respondents for each multiple-choice answer) are reported for each survey question and generated in MATLAB (version 2022b or newer). For survey question number two, correlations between parent-reported feelings were explored using Pearson correlation coefficients and hierarchical clustering using Ward method with Euclidian distances, implemented in MATLAB (Mathworks, version 2022b). The network plot was produced in R (Figure 5.5) by adapting publicly available code originally created for text analysis [353]. Furthermore, the plot showing how often each combination of feelings was chosen (Figure 5.6) was generated using package UpSetR in R (R Core Team, version 4.3.0) [354]. Differences in the frequency of each parent-reported feeling between trial arms were explored using Fisher exact test of contingency at an alpha level of 0.05 implemented in MATLAB (Mathworks, version 2022b or newer). As the analysis was exploratory, no correction for multiple comparisons was made. The code used in the exploratory analyses to generate the published figures is available through https://github.com/mvandervaart/petal_survey_code.

5.2.2 STAI and four-point distress questionnaire

In addition to the anonymous survey described above, parental anxiety and distress scores were collected. Parental anxiety was assessed with the STAI, which is composed of a trait (STAI-T) and state (STAI-S) scale. The STAI-S recorded after the intervention and procedure was one of the secondary outcomes. The same questionnaire was completed also at baseline, and the baseline STAI-S was used to adjust the effect of the intervention on the post-procedural, secondary

outcome STAI-S. Additionally, the trait score STAI-T was collected at baseline. Parental questionnaires implemented in Petal are described in more detail in section 3.2.4.2.3. Briefly, the STAI-S and STAI-T scales each consist of twenty items to be rated on a 4-point Likert scale. The total score of each scale can range from 20 to 80, with higher scores indicating higher anxiety levels at either a specific time or more generally, respectively. Additionally, parents were asked to complete a four-point distress questionnaire. Parents rated how intense they perceived four feelings (worried/upset/anxious/sad) on a scale of 0–10. The total four-point distress score could thus range between 0 and 40.

To assess the change in parental anxiety after the clinical procedure (regardless of trial arm), STAI-S change scores were computed for each parent. Two general linear models (GLMs) adjusted for STAI-S scores at baseline were used: one to calculate the mean change in STAI-S scores, and another one to explore differences in anxiety change scores between mothers and fathers. As the residuals of the models were not normally distributed, p values for both GLMs were derived non-parametrically via permutation testing using the tool PALM with 10,000 permutations [328]. The difference in parental anxiety between trial arms is reported in Chapter 4 (section 4.3.6.2), and did not significantly differ between trial arms. The mean difference between groups was -0.44 (higher in the control group, SD 6.85, 95% CI -2.91 to 2.02, p=0.72, n=106; Figure 4.8C).

5.3 Results

Between September 2021 and February 2023, 159 parents were approached about participating in the Petal trial. The majority (114, 72%) provided written informed consent to participate. As two neonates became ineligible before randomisation, a total of 112 neonates were randomised with a 1:1 ratio to either pre-procedural or post-procedural parental touch. Out of 112 randomised parent-baby dyads, 106 parents completed the anonymous survey at the end of the test occasion. Two parents did not complete the survey, and four neonates were withdrawn. Of the parents who

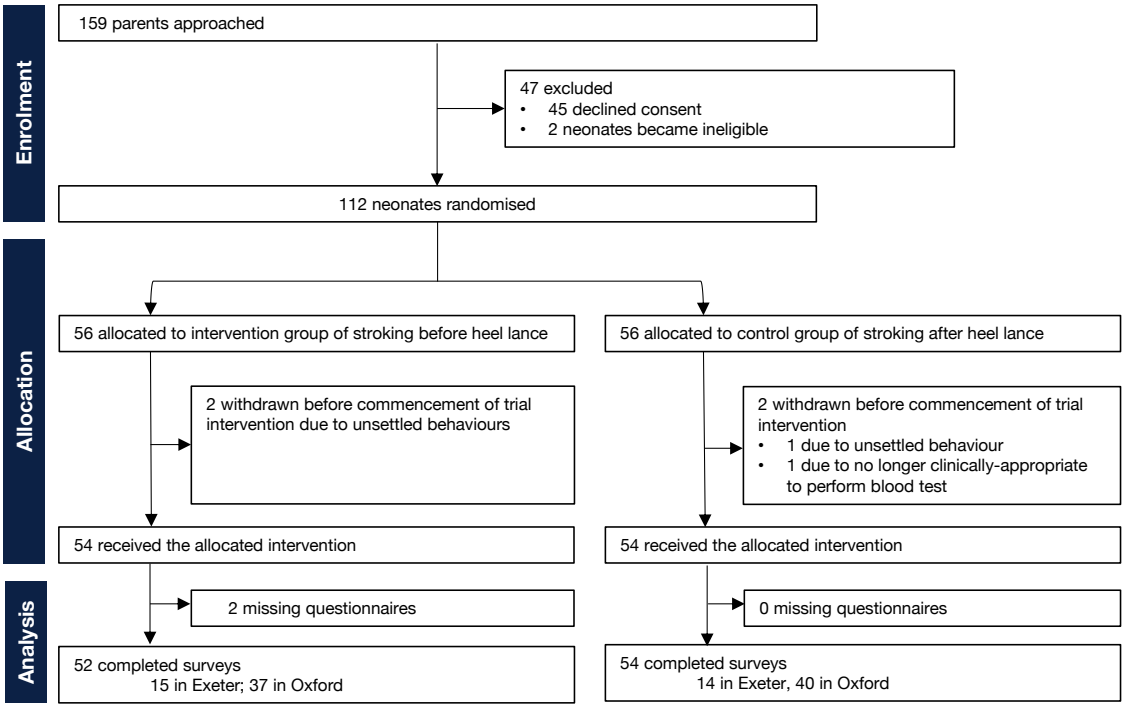


Figure 5.1: Flow diagram of parental survey respondents.

completed the survey, 29 were in Exeter and 77 in Oxford. Regarding the assigned trial arm, 52 had been assigned to pre-procedural and 54 to post-procedural stroking.

Regarding the STAI-S scores, 106 parents had complete pairs of scores available (one score at baseline before the intervention and clinically-required heel lance, and one score after the intervention and procedure). For the four withdrawals after randomisation, in three cases only the STAI-S after intervention and procedure was missing, while in another case both STAI-S values (at baseline and after the intervention and procedure) were missing. All parents of withdrawn babies had given consent to use the data collected up to the point of withdrawal. Baseline STAI-S of one further participant, and a different participant's score after the intervention and procedure were missing. Baseline characteristics of all randomised participants are included in 4.1. The withdrawals occurred before the intervention and procedure (Figure 4.1). Excluding withdrawals, there was a total of n=108 parent-baby dyads. Fathers delivered the stroking in n=36 (33%) cases, while mothers delivered the stroking in n=72 (67%) cases. A flowchart of parents included in the anonymous survey is illustrated in Figure 5.1.

5.3.1 Parental motivations to participate in the Petal trial

Parents were asked to rate the importance of five factors when considering their decision to allow their baby to take part in Petal. The survey identified that the two primary motivators were (i) the potential benefit to babies in the future and (ii) contributing to science, which were both considered 'very important' or 'important' by 99% of parents. Figure 5.2 summarises the relative importance of each of the five factors related to the parents' decision to take part in the study. Factors directly relating to care needs for their baby also influenced parents' decision to take part, albeit to a lesser extent. Overall, 73% of parents considered the potential benefit to their baby to be 'very important' or 'important', and 77% of parents considered the additional clinical observations that were provided as part of the trial (which included continuously monitoring the infants' heart rate, breathing rate, and oxygen saturation during the heel lance procedure) as 'very important' or 'important'. Concerns that their baby might be in pain was a 'very important' or 'important' motivator for just more than half of parents (55%). Overall, 98% of parents were pleased that they took part in the Petal trial, and 98% responded that that they would consider (75%) or maybe consider (24%) taking part in future research trials.

5.3.2 Parental feelings when being involved in their baby's care during the blood test

As part of the Petal trial, parents were asked to gently stroke their baby's leg either before or after a clinically required heel lance. Parents were given the opportunity to select up to ten words which best described their experience in being involved in their baby's care at the time of the blood test out of a list of ten descriptors. Parents commonly chose positive descriptors, and most commonly reported feeling (i) 'useful' (64%), (ii) 'reassured (53%), and (iii) 'calm' (44%). Negative descriptors such as feeling (i) 'frustrated' (4%), (ii) 'helpless' (11%), and (iii) 'stressed' (12%) were chosen much less often (Figure 5.3).

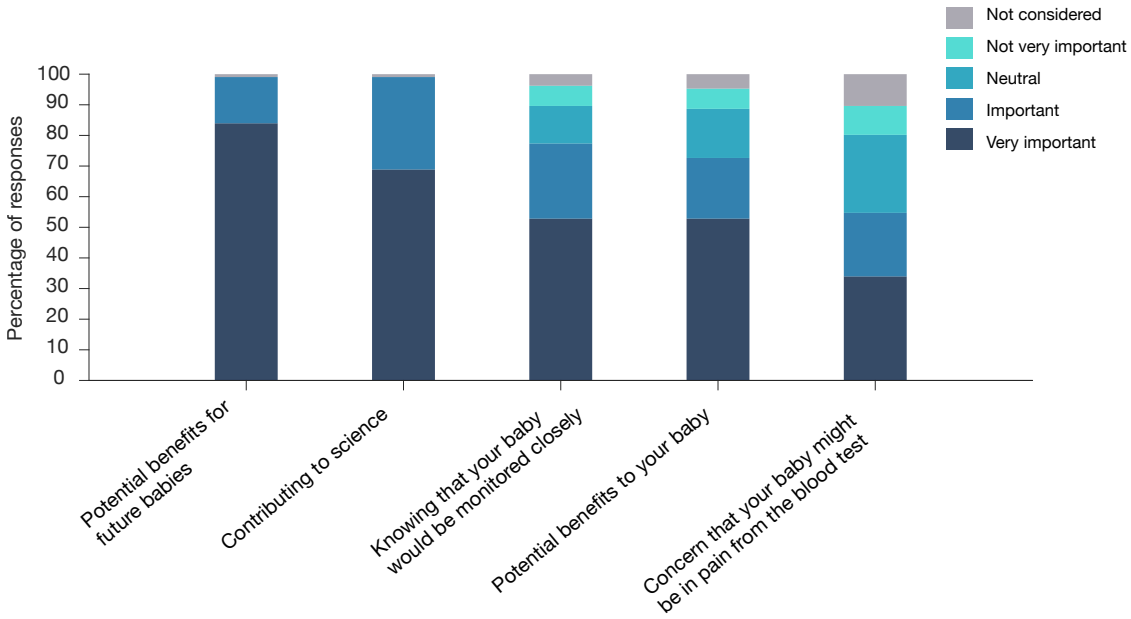


Figure 5.2: The importance of five factors that influenced parents' decisions to participate in the trial. Parents rated each of the five reasons as 'very important', 'important', 'neutral', 'not very important', or 'not considered'. The plots shows the reasons graded from those more often deemed 'very important' to those less often considered so. Potential benefits for future babies and contributing to science were rated as most important in the decision to participate in the trial, but individual factors (additional monitoring, potential benefits, and concerns about pain from the clinically-required heel prick) were also considered 'important' or 'very important' by most parents.

The correlation matrix and dendrogram show that, in general, the positive and negative descriptors formed separate data clusters (Figure 5.4). Nevertheless, it is visible that the two clusters are not completely separated.

The network graph (Figure 5.5) illustrates how often adjective pairs were chosen together by a parent. The size of the circles (nodes) illustrates how many of the 106 survey respondents stated that they felt that particular feeling—matching the data presented in Figure 5.3. The weights (thickness) of the connecting lines (edges) shows how many parents chose a specific combination of two adjectives. The lines connecting negative and positive feelings show that positive and negative feelings were not mutually exclusive. For example, parents who may have felt stressed or anxious can also report feeling useful or reassured at the same time.

The network plot depicts how many times each combination of two adjectives was chosen, but does not take into account the number of adjectives chosen by

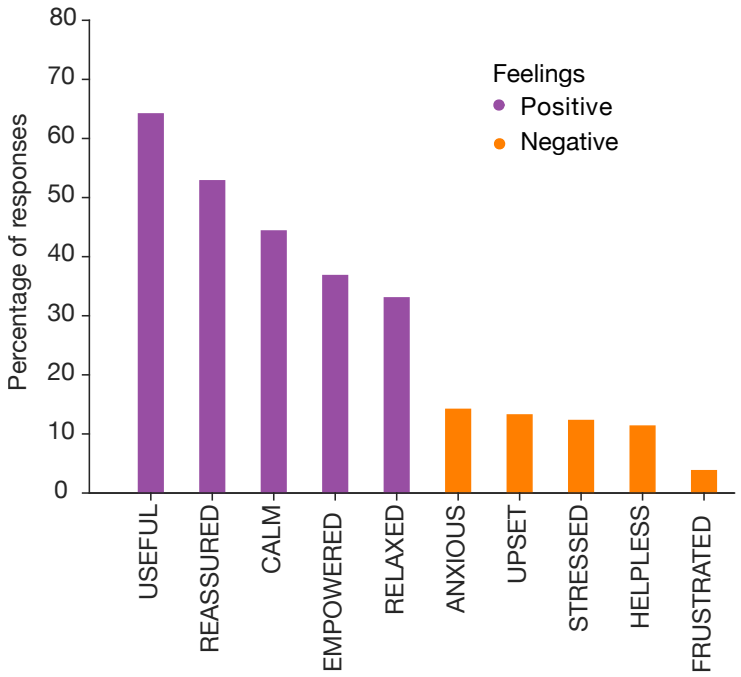


Figure 5.3: Parent-reported feelings about being actively involved in their child's care at the time of the blood test. Parents were asked to select up to ten descriptive words to describe their experience. This plot shows the percentage of parents who reported each feeling, ranked from the most commonly selected feeling to the least commonly selected feeling. Positive feelings (purple) were selected more often than negative feelings (orange).

each parent. For example, if a parent selected only two adjectives, this would be illustrated in one connecting line, whereas if a parent selected three adjectives, their choice would be reflected in three connecting lines (adjective 1 to adjective 2, adjective 2 to adjective 3, and adjective 1 to adjective 3). Therefore, the balance of the graph is shifted towards representing parents who reported a higher number of concomitant feelings. For completeness, the frequencies of all chosen combinations are illustrated in the histogram at the top of Figure 5.6. This plot mirrors the network plot (Figure 5.5) in that it shows connections between positive and negative clusters, while also providing more granular information on each participant's set of feelings. In total, 77 parents reported positive feelings only (purple, 73%), eight parents selected negative feelings only (orange, 8%), and 21 parents reported a mixture of feelings from both positive and negative groups (grey, 20%)(Figure 5.6) about being actively involved in their baby's care at the time of the blood test. Where a mixture of feelings from both positive and negative groups were

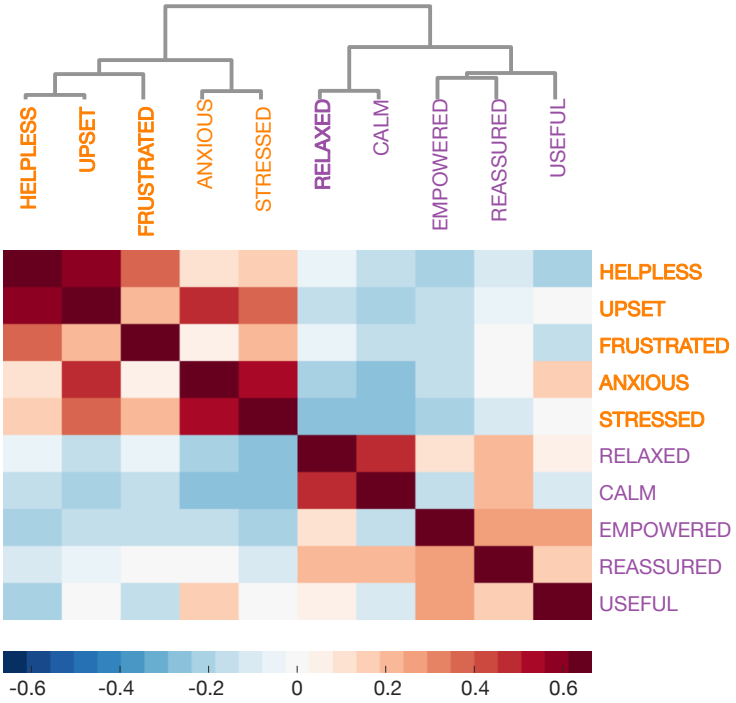


Figure 5.4: Dendrogram and correlation matrix of parental feelings about being involved in their baby's care during the clinically-required blood test. Dendrogram and correlation matrix showing the correlations between parent-reported feelings during the clinical procedure. Negative feelings (orange, left) and positive feelings (purple, right) are clustered. Colour bar indicates Pearson correlation coefficient. Clustering was performed using Ward method based on Euclidean distances.

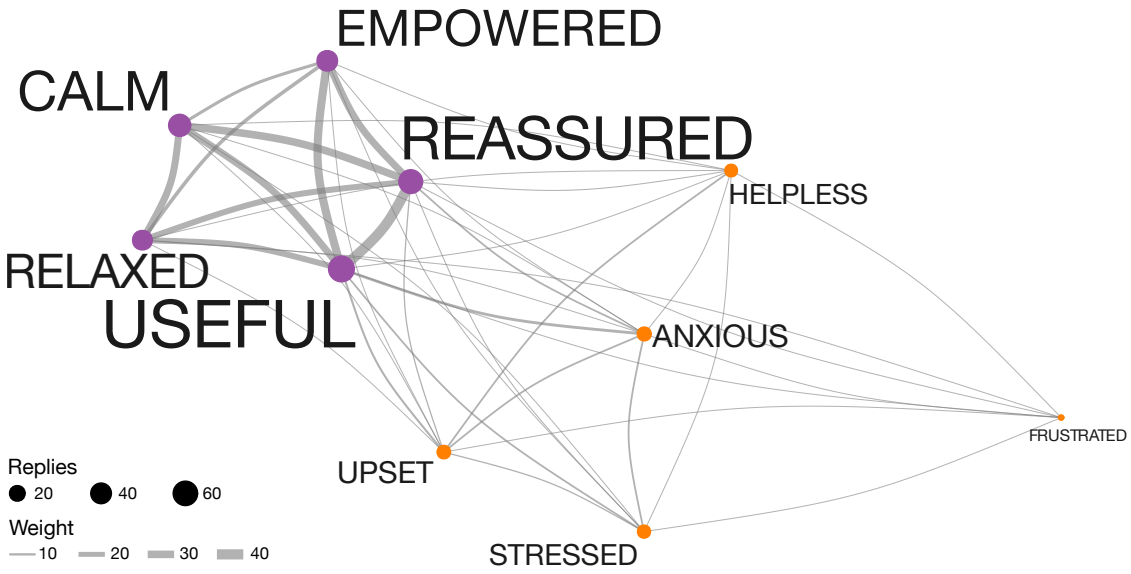


Figure 5.5: Network graph depicting parent-reported feelings. The size of the nodes (circles) represents how many times parents chose each adjective as an answer. The weights of the lines reflect how often each pair of adjectives was chosen in combination.

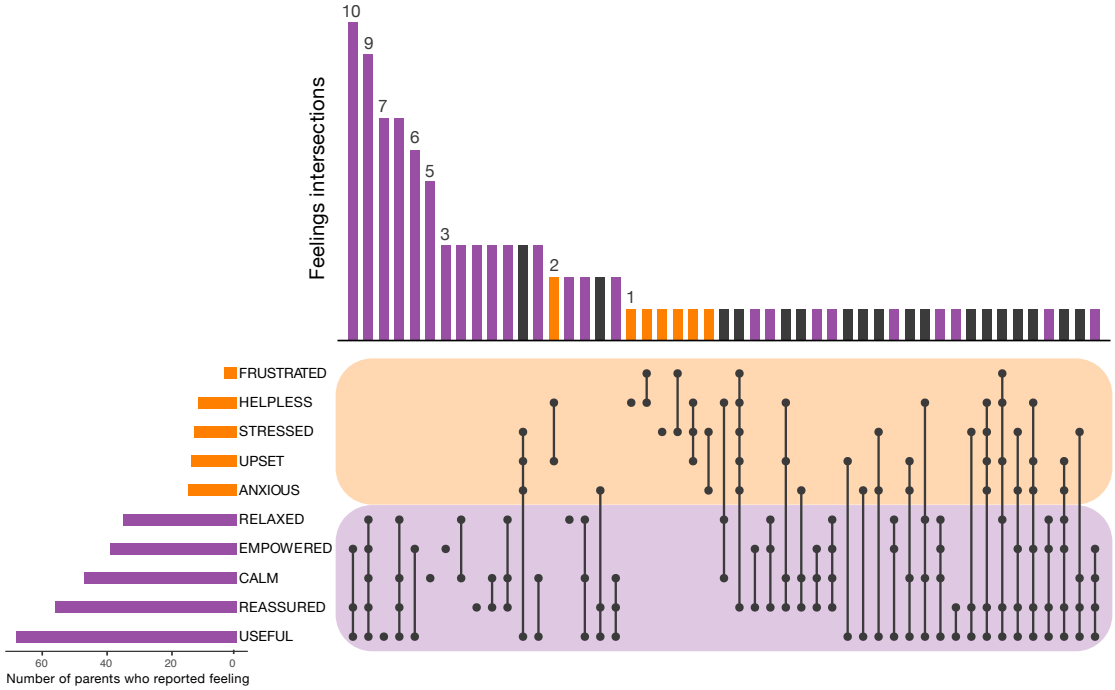


Figure 5.6: Parental feelings about being involved in their baby's care during the clinically-required blood test. The histogram on the bottom left matches the histogram of Figure 5.3, except for the fact that here the number of parents reporting a certain feeling is provided as absolute number rather than percentage. In total, 106 parents completed the anonymous survey. At the bottom, all chosen combinations are illustrated. Positive descriptors are indicated in purple, while negative descriptors are indicated in orange. In the histogram at the top, where the combinations of chosen descriptors was composed of exclusively positive feelings, the bar is filled in purple (n=77), whereas it is filled in orange where all adjectives came from the group of negative descriptors (n=8). Grey bars indicate a mixture of feelings from both positive and negative groups (n=21).

reported, the most commonly used descriptors from the positive group were feeling useful (17/21, 81%) and reassured (12/21, 57%).

Overall, parents reported feelings similarly often regardless of whether they were asked to stroke their baby before or after the heel lance (Figure 5.7). The descriptor 'helpless' was more frequently selected by parents in the control group who provided reassuring tactile stimulation after the heel lance (10/54, 19%) compared to parents in the intervention group who provided pre-emptive gentle touch before the clinical procedure (2/52, 4%, Fisher exact test, p=0.03, not corrected for multiple comparisons).

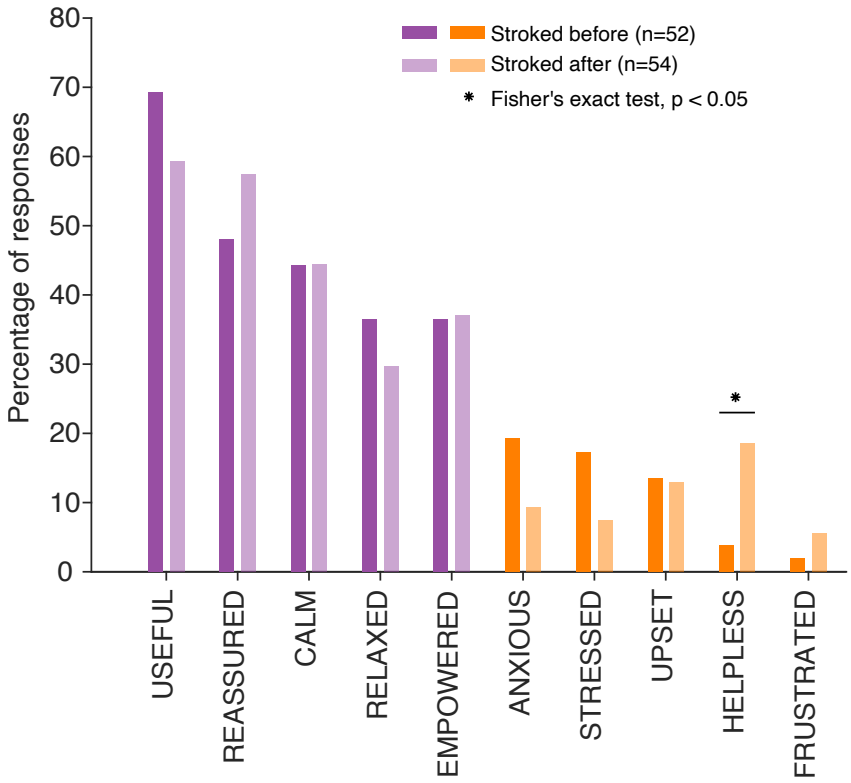


Figure 5.7: Percentage of parents who reported each feelings, shown by trial arm. There were no significant differences between the intervention and the control groups, except for the feeling of helplessness, which was more prevalent in the post-procedural stroking control group. As this was an exploratory analysis, no correction for multiple comparisons was applied.

5.3.3 Parental anxiety and distress during their baby's painful procedure

General anxiety levels, assessed with the STAI-T, were recorded at the start of the test occasion. The median STAI-T was 32.5 (IQR 28 to 40, n=106). Parental anxiety at specific time points, as assessed by STAI-S score, significantly decreased following the clinical procedure during which parents were actively involved. The median STAI-S was 32 (IQR 24 to 42, n=107) before the heel lance, and 30 (IQR 22 to 40, n=107) after the heel lance. The mean STAI-S change score was -3.07, t-statistic -4.77, p=0.0001, n=106).

The change in anxiety levels did not significantly differ between mothers and fathers. The median STAI-S score change was -1.5 (indicating a decrease from before to after the heel lance, IQR -4.5 to 0, n=36) in fathers, and -2 (IQR -7 to 1,

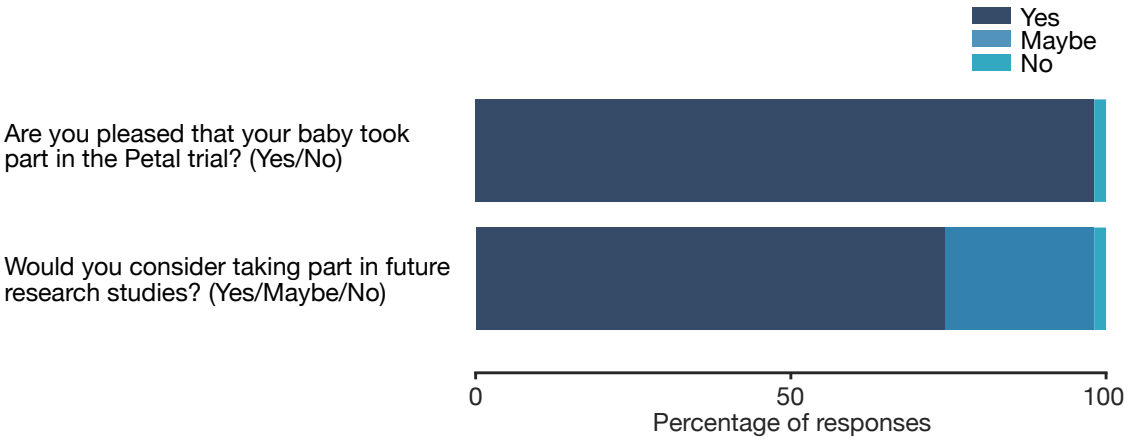


Figure 5.8: Experience of trial participation and willingness to participate in future studies. Nearly all parents (98%) answered affirmatively to the question as to whether they were pleased to have participated to the Petal trial. Similarly, 98% stated that they would consider (75%) or maybe consider (24%) taking part in future research trials.

n=70) in mothers. The adjusted mean difference in STAI-S change score was 0.97 (mothers - fathers, t-statistic 0.70, p=0.44, mothers n=70, fathers n=36).

Parents were asked how anxious, upset, sad and worried they felt at the time of the heel prick by rating each of these four adjectives on a 0–10 scale. The total score could thus range between 0 and 40. The median total score in the intervention group was 6 (IQR 0 to 18, n=54) and 8 (IQR 0.5 to 18, n=52) in the control group. The total score did not significantly differ between intervention and treatment groups (two-sample t-test, t-statistics -0.0027 [lower in the control group], 95% CI -4.2 to 4.2, p=0.9978, df=104).

5.3.4 Experience of having taken part and willingness to participate in future research studies

The final two questions of the survey asked if parents were pleased to have taken part in the Petal trial, and whether they would participate in future studies. The responses were overwhelmingly positive, with over 98% of parents stating that they were pleased to have taken part, and 98% stating that they would consider, or maybe consider, taking part in future studies (Figure 5.8).

5.4 Discussion

In the Petal trial, parents delivered a gentle touch intervention to their baby at the time of a clinically-required blood test. As part of the trial, parental feelings were explored. Parents were asked to fill out an anonymous survey about their motivations for and experience while participating in the trial, as well as their feelings while being involved in their baby's care at the time of the heel lance. Furthermore, they were asked whether they were pleased to have taken part in the trial, and whether they would consider participating in future research studies. The response rate was nearly 100% (106 out of 108 parents who completed trial procedures completed the survey). Of the 159 parents approached about participation in the Petal trial, 114 (72%) agreed to take part, which is consistent with other studies in the neonatal population [355].

Parents rated how important they considered five reasons when deciding to participate in the trial. The reasons considered 'very important' or 'important' by 99% of parents (105/106) were altruistic: the potential benefit for future babies and contributing to science. The high importance attributed to altruistic reasons is supported by previous observations in neonatal studies [356–358] and paediatric research [359]. Most parents were also motivated because their baby would be closely monitored and, possibly linked to the increased monitoring, because of a perceived potential benefit to their babies. These factors have been previously reported in the literature, and the importance attributed to them by parents in the Petal trial aligns with observations by other researchers [355, 357, 358]. The potential concern that their baby might be in pain from the blood test was deemed less important than the other factors mentioned, with approximately half of the surveyed parents rating concerns about pain from the heel lance as important or very important.

Non-pharmacological interventions such as skin-to-skin care or facilitated tucking can be delivered by parents and the current evidence suggests that they are likely effective in relieving pain [197, 218]. Parents can feel stressed when seeing their baby in pain [269] and may feel uncomfortable when delivering comfort care during

a painful procedure [270, 271, 345, 346]. However, parents have often expressed a desire to be more actively involved in their baby's care, as well as to receive more information on how to comfort their baby [360]. Reviews have highlighted the importance of partnering with parents in neonatal pain management [201, 334], and the need to better understand parental feelings while involved in their baby's care [271]. This knowledge gap was also underscored in the Cochrane review on opioids for procedural pain in neonates published in 2023, where none of the included RCT assessed parental satisfaction [213]. As part of Petal anonymous survey, parents were asked to choose from a list of descriptors what they felt while being actively involved in their baby's care—by providing the gentle touch intervention—at the time of their baby's blood test. The majority of parents reported positive feelings, such as feeling useful (64%) and reassured (53%). However, a smaller number of parents reported negative feelings, such as feelings stressed (12%), or helpless (11%). The clusters of parents who reported positive and negative feelings were not completely separated, as 20% parents (21 out of 106 survey respondents) reported feelings from both positive and negative groups. In the group of parents who reported feelings from both groups, 17 out of 21 felt 'useful', and 12 out of 21 felt 'reassured', despite the concomitant negative feelings, while being actively involved in their baby's care. This is consistent with previous studies where parents reported feelings uncomfortable but also wanting to be actively involved in their baby's care [345].

The timing of parental involvement may also influence negative perceptions. In our exploratory analysis, we observed that feelings of 'helplessness' were less prevalent in the group of parents who performed the parental stroking intervention before the clinical procedure, as compared with the parents who performed the parental stroking afterwards. Although this observation is preliminary, it might be an indication that pre-emptive touch makes parents feel more useful compared to post-procedural touch. For future research, it would be valuable to further explore the roots of these feelings, for example via structured interviews and by collecting more data about the parents which could aid the interpretation of the results, e.g. previous experiences in the NICU and number of previous children. Irrespective of trial arm,

there was a reassuring overall decrease in parental anxiety following the blood test. This suggests that despite observing the painful clinical procedure, parents were not more anxious following their involvement in their baby's comfort care than before. Because the trial did not include an arm where parents were not present for the clinical procedure, this decrease in anxiety cannot be directly attributed to the study intervention. However, this observation indicates that it is important not to assume that presence during clinical procedures will heighten parental anxiety.

It should be noted that the observations reported in this exploratory analysis are not necessarily generalisable to trials investigating pharmaceutical drugs, or to families of babies who require longer hospitalisation or were born more prematurely than the late preterm and term babies enrolled in Petal. The median STAI-S of Petal participants' parents was 30 (IQR 22 to 40, n=107, after the heel lance) which is consistent with STAI-S scores reported in a large cohort of mothers during their postpartum neonatal stay (mean 31.0, SD 8.6, n=1123 [361]) and lower compared to STAI-S reported in parents of babies hospitalised in the NICU (mean 48.4 (SD 14.9 in control group, n=85 and mean 42.7 SD 16.5, n=84 in intervention group) [349]). Furthermore, while our consent rate was similar to other studies in the neonatal population, future studies may consider implementing in the study protocol the permission to ask parents about the reason they decide not to participate in the trial. Examples exist of studies where this information has been collected [355, 357], which could inform the design of future trials and be used to tailor interventions for parents whose perspectives might not be as widely represented in the literature as those of parents who participated in research trials. Regarding the psychometric properties of the questions in the anonymous survey, the survey was not tested according to the thorough standards of the COSMIN guidelines but developed in a bespoke fashion for the Petal trial by the research team [117]. The motivations proposed for taking part in the trial reflected those previously reported in the literature and included both factors related to one's own baby and altruistic factors. Regarding the adjectives listed, the selection of proposed adjectives was bespoke and made by the research team when setting up the study offering a balanced mix

of negative and positive adjectives (five and five, respectively). For future trials implementing similar surveys, content validity defined as 'the degree to which the content of a measurement instrument is an adequate reflection of the construct to be measured' could be improved by performing a pilot study allowing parents to describe their feelings in their own words, and creating a list of answers based on those answers [117]. In a future version, it might be valuable to present a consistent list of antonyms, as some adjectives presented were antonyms and others were not. For a future study integrating a survey component, an assessment of the psychometric properties beforehand would be valuable.

One of the strengths of the Petal trial is the involvement of both fathers and mothers. In our study, approximately 35% of babies were stroked by their fathers, while the remaining 65% were stroked by their mothers. The mean decrease of anxiety score of fathers and mothers was similar, suggesting that the experience was comparable for both. In a recent systematic review of father-led interventions to their premature babies in the NICU, only few studies focussed on fathers [350], potentially reflecting a perspective of mothers being the primary caregiver [362]. This is in contrast to models where healthcare professionals partner with all parents [259]. When asked, fathers have reported a wish to be provided with more opportunities to be involved in their baby's care [352]. One of the main non-pharmacological interventions, skin-to-skin care, has been the focus of a recent position paper and implementation strategy written by the WHO. Although the beneficial effects of skin-to-skin care provided by another parent than the birthing mother are underscored in these documents, the intervention is often primarily referred to as 'kangaroo mother care', including in the title of these documents [224]. This also reflects the literature on skin-to-skin care included in the Cochrane review for neonatal procedural pain management: of 25 included studies, only two compared mothers to another caregiver (father, another female) [218]. The importance of engaging fathers in neonatal care has been underscored [351], as well as the need to further explore parental feelings while being involved in their baby's pain management [271].

Future work might further extend the exploration of reasons taken into account when deciding about trial participation to parents who declined participation. Furthermore, factors that contribute to parental anxiety warrant further investigation, given the wide range of anxiety and distress scores reported by parents. Despite the fact that most parents felt exclusively positive feelings while being involved in their baby's care, a minority of parents reported negative feelings. Further research could aid in identifying potential drivers and find the best approach to support and partner with parents in neonatal care. Within the rich Petal dataset, associations between neonatal medical history, e.g. the reason for the blood test and parental anxiety and distress could be further explored. Further exploring potential roots of parental positive and negative feelings perceived by parents while being actively involved in their baby's care could facilitate tailoring the support provided by healthcare professional to parents. Although the anonymous survey data does not include information on which parent provided the gentle touch intervention, an overwhelmingly high percentage (98%) of parents were pleased to have participated in the trial, and the same proportion of parents would consider taking part in future research studies. This is highly encouraging, and underscores parental acceptability of recording multimodal outcome measures including a brain-derived primary outcome measure, which is a strength of the trial. Furthermore, these parental responses highlight the desire to be involved in their baby's pain management and to contribute to this important area of research.

Chapter 6

General discussion and conclusion

6.1 Thesis summary

Most neonates require painful clinical procedures, such as routine blood tests or vaccinations. Hospitalised neonates can undergo numerous painful procedures, with some neonates facing over 300 painful procedures in the first two weeks since NICU admission [34, 41]. Although pain in the neonatal period can have negative short and long-term consequences on neonates and their families [90, 109, 267], analgesia is provided inconsistently [41]. The current repertoire of pain management interventions encompasses pharmacological interventions, such as sucrose and opioids, and non-pharmacological interventions, such as skin-to-skin care and swaddling. However, given the subjective nature of the pain experience, assessing the analgesic efficacy of interventions is particularly challenging in the pre-verbal neonatal population. In the absence of a gold standard for neonatal pain assessment, a large number of different pain assessment tools are being use in clinical studies, which is partly hindering pooling into meta-analyses to generate recommendations with high confidence [208, 218, 219, 272]. Numerous pain scales exist, often based on behavioural responses (e.g. facial expressions) and vital signs changes. Given that pain perception emerges in the brain [53, 58, 81] brain-based methods to assess analgesic efficacy in neonates can provide further valuable information compared to relying solely on behavioural responses and vital signs changes. Neonatal pain-related outcome measures do not always correlate [130, 162, 168], and studying their divergence can shed light on how different interventions

and stages of maturation of the nervous system differentially affect pain responses [91, 143]. For example, significant noxious-evoked brain activity is not always accompanied by facial expressions [168], underscoring the importance of collecting a spectrum of relevant outcome measures when assessing the analgesic efficacy of an intervention in neonates. Another reason to record multimodal outcome measures is that they can be differentially modulated by interventions. For example, current evidence suggests that sucrose can dampen pain-related behavioural responses, whereas a beneficial effect on further outcome measures including brain-based outcome measures has not consistently been reported [132, 208]. A combination of neonatal pain responses across multiple modalities can provide a more informed picture of the analgesic efficacy of interventions [132, 143, 172]. Brain-based outcome measures are gathering increasing attention in the neonatal pain management research field [125, 143], and were recently discussed in an FDA workshop on how to improve analgesic clinical trials in children below the age of two [283]. However, only a minority of the interventions has been assessed on brain-based outcomes.

The Petal trial is a multicentre randomised controlled trial investigating the analgesic efficacy of a non-pharmacological intervention—gentle parental touch—prior to a clinically required heel lance. Parental touch was provided in the form of stroking at the optimal speed (3 cm/s) to activate CT fibres. In adults, it was observed that (i) CT fibres are preferentially activated by brushing of the skin at 1–10 cm/s, the speed range of soft brush stroking that is also perceived as most pleasant [235]. Furthermore, it was shown that (ii) brushing of the skin at CT-optimal speed prior to an experimental noxious stimulus can reduce verbal pain reports (iii) and noxious-evoked brain activity [232, 251, 252]. In the intervention group of the Petal RCT (n=56), parents stroked their baby before the clinically required heel lance, whereas in the control group they did so after the heel lance (n=56). Comfort care in line with local practice guidelines was offered to all neonates irrespective of trial arm. The neonatal outcome measures were recorded at the time of the heel lance. The primary outcome measure was the magnitude of noxious-evoked brain activity, whereas secondary neonatal outcome measures were the PIPP-R pain

score, and the development of tachycardia. A further secondary outcome focussed on parents by assessing their anxiety levels with the STAI-S score. The magnitude of noxious-evoked brain activity was chosen as a primary outcome measure as it is a pharmacodynamic biomarker which can be objectively measured, and because it was responsive to the touch intervention in the pilot studies [171, 173]. The two pilot studies were performed in independent groups of neonates where a reduction of approximately 40% in the magnitude of noxious-evoked brain activity was observed following brushing of the skin at CT-optimal speed [171, 173]. In the Petal trial, none of the outcome measures significantly differed between intervention and control group. This is in contrast to results shown for other touch-based interventions such as skin-to-skin care which have been linked to decreases in neonatal pain-related responses [218]. However, skin-to-skin care is a multisensory intervention, which comprises auditory and olfactory components, as well as static touch and dynamic touch, which may activate CT fibres. Reasons as to why a decrease of neonatal pain-related responses was not shown are discussed in Chapter 4. In short, the study design was chosen to match studies in adults and the pilot studies where a brush intervention was applied for ten seconds prior to the noxious input, leading to a decrease in verbal pain reports in adults, and a decrease in noxious-evoked brain activity in adults and neonates. Given the value of involving parents in their baby's pain management [271, 334], and the fact that CT fibres respond optimally to touch at skin temperature [244], in the trial parents provided the touch intervention in contrast to the pilot studies where trained researchers use a calibrated brush [171, 173]. Parents were guided to stroke their baby at the right speed by an animation displayed during the touch intervention. A study published during the trial showed that mothers naturally stroke their babies at CT-optimal speed, and that the HR decrease during stroking of their baby occurs later in neonates compared to older infants where the effect is already observed after ten seconds of stroking, suggesting that an important next step is to implement a longer self-led parental touch intervention [246, 257].

Based on a systematic literature search [3], the Petal trial is, to my knowledge, one of the first very few studies investigating a parent-led intervention using a primary brain-derived outcome measure, and the first in this group with a multicentre RCT design. Furthermore, it is the first trial among those assessing parent-led interventions on brain-derived outcomes that also assesses parental feelings while being involved in their baby's care. The trial was designed to involve parents, and included questionnaires and an anonymous survey to explore their motivations to participate and feelings while being involved in their baby's care at the time of the blood test. A recent review on parental involvement in neonatal pain management concluded that, while there is sufficient evidence that parents generally want to be informed and involved in their baby's comfort care, little is yet known about their motivations to engage in their baby's pain management and their feelings while doing so [271]. In the anonymous Petal survey reported in *PAIN*, 99% of parents (105 out of 106 survey respondents) rated altruistic reasons such as contributing to science and potential benefits for future babies as very important or important [4] factors when deciding to participate in the trial. Overall, further reasons directly related to care needs of their baby were rated as important by parents, albeit to a lesser extent. These included the additional physiological monitoring implemented in the study and potential benefits to their baby, which were considered important by most parents, which is consistent with previous studies [355, 357, 358]. Most parents reported positive feelings such as feeling useful and reassured while being involved in their baby's care at the time of the blood test, with fewer parents reporting negative feelings (e.g. stressed or anxious). The majority of parents who reported some negative feelings also concomitantly reported positive feelings, such as being reassured and feeling useful. These observations underscore the importance of further investigating reasons behind negative parental feelings reported by some parents, in order to tailor and optimise the support to each parent. In an exploratory analysis, the feeling of helplessness was less prevalent in the intervention group providing pre-emptive touch compared to the control group, potentially suggesting that parents may feel more useful when being involved before painful procedures.

The vast majority of parents (98%) were pleased to have taken part in the trial, and the same proportion would consider participating in future trials.

The Petal trial was designed following the relevant FDA and EMA guidelines and the recommendations made by the ACTTION consortium [195]. The SAP was developed to minimise the risks of bias and provide clarity on how the outcome measures were calculated. It follows the relevant guidelines by regulatory agencies and the guideline for SAPs published in *JAMA* in 2017 [280]. The trial provides a framework for conducting RCTs that can objectively assess the analgesic effect of parent-led strategies to reduce neonatal pain. The FDA has underscored the importance of conducting more paediatric analgesic trials, as many analgesics remain understudied in this population [276]. Furthermore, it was highlighted that measuring analgesic efficacy is particularly challenging in the youngest children [276], and the FDA organised a workshop on analgesic clinical trials in 2021, where brain-derived approach to assess neonatal pain were also discussed [283]. The Petal trial is part of a larger effort to establish the objective, brain-derived, primary outcome measure as an outcome measure of analgesic efficacy in neonates, to improve the methodology of neonatal analgesic trials, and to foster the partnership between researchers, clinical teams and families.

6.2 Current limitations and future avenues

A limitation of the trial was the short duration of the intervention, which was chosen to match the pilot studies and was discussed in detail in Chapter 4. Field reviewed the literature on touch aiming at activating CT fibres, and noted that most studies are performed in experimental settings, where the participants' skin is brushed by a trained researcher using a calibrated brush. She underscored the need to evaluate interventions aiming at activating CT fibres in a more naturalistic setting. In the Petal trial, parents provided the touch intervention in a hospital setting when their baby's clinically required heel lance was scheduled, moving closer to a naturalistic setting. However, the use of a visual metronome guiding parents in delivering the stroking at CT-optimal speed may have prevented the

beneficial effects of the intervention from unfolding by leading to a more mechanical movement than what would have naturally occurred. The rationale behind using a visual guide was to reproduce the same speed as in the pilot studies and adult literature, albeit via parental hand rather than an experimental soft brush. A potential consideration for future studies would be to include a rating by parents of the ease of providing the intervention. While most parents reported positive feelings only while being involved in their baby's care at the time of the blood test, in future trials it would be informative to know whether parents think an intervention is easy to implement, and whether feelings such as 'usefulness' correlate with how easy an intervention is perceived to be or how confident parents felt. Although information on the ease of delivering the intervention was not collected in the Petal trial, a training session occurred in the first part of each test occasion, where parents became acquainted with the animation.

Another factor to consider when planning future trials is how to account for comfort measures provided to neonates in addition to the studied intervention. In the trial, additional comfort measures were not recorded systematically. However, documenting accompanying comfort measures so that their impact can be sensibly quantified and taken into account when estimating the effect of the intervention remains challenging. It remains to be established how to best collect information on comfort measures, given that the type of comfort measure, the timing in relation to the heel lance, the duration, and its effect on the baby could potentially all have an impact on the pain-related response outcome. Uncertainty about the effect of comfort measures could mean that recording additional variables would not necessarily lead to a significant improvement in estimating the effect of an intervention despite the increased complexity of recording additional variables during the study. An alternative approach could be to establish in advance a hierarchy of interventions to standardise the care received by babies enrolled in the trial. The hierarchy of interventions would be based on local practice guidelines and could, for example, consider additional sucrose or non-nutritive sucking. As planned in an ongoing RCT [363], the use of 'rescue sucrose'—or of another rescue

intervention—could be used as an outcome measure [187, 282]. While the use of comfort measures may be standardised in a trial, provided that it follows local practice guidelines and is aligned with parental preference, other factors are difficult to standardise across participants. For example, each baby might be on a different feeding routine, and to minimise disruption of the baby's care the outcome measures will likely be measured at different times in relation to the last feed, which can also affect the pain-related response. However, the time in relation to the last feed is a variable that might be worth recording and may aid the interpretation of the results.

The focus is increasingly shifting towards integrating parents in the clinical care team and involving parents in their baby's pain management [258, 259, 347]. For example, the protocol of the SWEpap trial (parents as pain management in Swedish neonatal care) describes a multicentre RCT investigating a multisensory combination of interventions against standard care [364]. The intervention includes skin-to-skin care, breastfeeding and live parental lullaby singing [364]. The SWEpap trial uses the PIPP-R as primary outcome measure and includes multiple parent-reported outcomes; the results have not yet been published. Similar studies investigating the synergy of intervention bundles—where a validated brain-derived outcome measure could also be implemented—could advance the understanding of the analgesic efficacy of such interventions. To my knowledge, only few trials have investigated the effect of skin-to-skin care provided by other caregivers than the mother [218, 365], no study has yet assessed the effect of kangaroo care provided by other parents than the mothers on brain-based pain-related outcomes, and no study has yet assessed a dose response curve of kangaroo care on this outcome. Expanding kangaroo care research to other providers than the mother and establishing dose response curves were listed as future areas of research in the 2017 Cochrane review on skin-to-skin care for the management of neonatal procedural pain [218]. Combinations of interventions should maximise the baby's comfort and minimise the safety risk profile. For example, caution is warranted when using solely sucrose for pain relief with high frequency, given that high exposure to oral sugar solutions has been linked with poorer neurodevelopmental outcomes in premature neonates and brain

alterations in neonates and mouse pups [96, 210, 366], which led the American Academy of Pediatrics to recommend documenting all sucrose administrations [132, 198]. Interventions which can prevent high sucrose administration might therefore be beneficial.

In order to be able to weigh analgesic efficacy against the safety profile of an intervention, robust evidence on both should be available. However, neonatal analgesic trials are intrinsically challenging given the vulnerable population involved and the complexities related to assessing analgesic efficacy without being able to rely on verbal reports [205, 273, 276]. In the absence of verbal expression, pain management relies on surrogate measures [198]. One of the challenges in assessing analgesic efficacy in neonates is not the number of pain assessment tools—considering than more than 40 pain measurement instruments exist—but rather the limited number of studies assessing their measurement properties [120]. For example, a recent systematic review assessing the responsiveness of neonatal pain measurement instruments reported that this property had been investigated in only a small proportion of the instruments [120]. In a systematic review of the neonatal pain scales used in RCTs, Olsson and colleagues reiterated that there is likely no need to develop further scales, but rather for more studies reporting the properties of such measurement instruments, such as validity and responsiveness, given the relative lack of studies on measurement properties [117, 119]. In line with the conclusions of these reviews, investigations of the measurement properties of the n-NRF are ongoing, and some of the limitations previously mentioned with regard to the n-NRF approach have been overcome in the Petal trial [143]. For example, further evidence was provided to the reproducibility of the outcome measure by running a multicentre trial, and the smaller sample size of previous studies was largely increased in Petal. Work is ongoing on multiple fronts to assess and improve the measurement instrument properties of this outcome measures, such as its reliability, validity and interpretability. Future work will be needed to refine the minimal clinically important differences of neonatal pain assessment tools including the n-NRF magnitude [195]. The pain experience emerges in the

brain, thus assessing multimodal outcome measures including a brain-based outcome measure can provide a better understanding of the analgesic efficacy of interventions to improve neonatal pain management.

Beside the refinement of n-NRF magnitude outcome measure, the evidence on neonatal pain management could be improved by consistently defining the outcome measures used in trials and by specifying a set of outcome measures and their intended use [187]. One of the issues encountered by the authors of various Cochrane reviews was the large number of heterogenous outcomes measured, which can hinder the pooling of data into reliable meta-analyses [208, 218, 219, 272]. Similarly, an issue encountered in Cochrane reviews on neonatal pain management is the inconsistent reporting of adverse events [218]. The International Neonatal Consortium (INC) has highlighted the importance of standardising the assessment and reporting of harms in neonatal trials as part of an effort to improve the quality of neonatal clinical trials [367]. Inconsistent sets of outcomes can be found across neonatal research, not just in the field of pain research [368], and efforts are ongoing to establish relevant neonatal outcomes across neonatology [369]. With regard to analgesic trials, Pediatric Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (PedIMMPACT), defined outcomes for paediatric pain trials in 2008 by generating consensus among key stakeholders (researchers, government funding and regulatory agencies, and representative of the pharmaceutical industry), albeit excluding the neonatal population due considerable developmental differences [370]. Although the authors of the PedIMMPACT outcomes reported in 2021 that their uptake was insufficient, such initiatives can pave the way to pooling more evidence into larger meta-analyses. The ACTTION recommendations published in 2018 were generated by a joint effort from academics, governmental agencies and industry representatives and led to the definition of appropriate pain models for various paediatric groups, including neonates [195]. However, to my knowledge, the definition of a set of neonatal outcome measures for analgesic trials is yet to be established. The COSMIN initiative has extensively defined measurement instrument properties, and the COSMIN definitions can prove particularly useful

when assessing neonatal outcome measures [117]. The ongoing Cochrane review by Bruschettini and colleagues assesses the measurement properties of clinical pain rating scales according to COSMIN, and holds the potential to provide crucial information to inform the definition of a core outcome set of neonatal outcome measures for analgesic trials [371]. Clearer evidence on therapeutic effect and risk profile of an intervention based on reliable meta-analyses would be beneficial and inform clinical decisions which are based on careful weighing of beneficial effects and potential risks.

The FDA has underscored the need to increase the number of paediatric analgesic trials [282], and a workshop has recently been held by the FDA to discuss how to improve the outcome measures and more generally the methodology of neonatal analgesic trials [133, 283]. Neonatal clinical trials are challenging yet essential to improve clinical practice [205]. Neonatal analgesic trials should not only aim to implement valid, reliable, responsive and interpretable neonatal outcome measures, but also assess parental views and feelings. Non-pharmacological interventions can often be provided by parents, and understanding their feelings and perspectives on being involved in their baby's care can help improve not only their baby's but also parental wellbeing. In the Petal trial, neonatal pain-related and parental anxiety outcome measures were assessed, and parental feelings and motivations to participate in the trial were explored. The trial protocol and SAP were developed to minimise the risks of bias and are publicly available [1, 2]. The multicentre RCT Petal was described as 'an example of excellence in research' by Professor Ruth Guinsburg in her commentary published in *The Lancet Child & Adolescent Health* alongside the main trial results [372], and may serve as a framework for future RCTs assessing analgesic efficacy in neonates.

Appendices

Appendix A

Supplementary tables to chapter 4

A.1 Baseline characteristics of neonates included in the analysis of the primary outcome (full analysis set)

	Primary outcome	
	Intervention group (n=39)	Control group (n=43)
Parent stroking*		
Biological father	12 (31%)	14 (33%)
Biological mother	27 (69%)	29 (67%)
Gestational age at birth (weeks)	38.1 (36.9–40.0)	38.1 (36.7–39.4)
Postmenstrual age at time of study (weeks)	38.3 (37.1–40.4)	38.3 (37.2–39.9)
Postnatal age at time of study (days)	2 (1–5)	3 (1–5)
Birthweight (g)	3385 (2696–3757)	3225 (2690–3762)
Sex		
Female	14 (36%)	18 (42%)
Male	25 (64%)	25 (58%)
Mode of delivery		
Normal vaginal	16 (41%)	16 (37%)
Breech vaginal	1 (3%)	0
Elective C-section	10 (26%)	5 (12%)
Emergency C-section	8 (20%)	13 (30%)
Ventouse or forceps	4 (10%)	9 (21%)
Apgar score at 1 min	9 (7–10)	9 (8–10)
Apgar score at 5 min	10 (9–10)	10 (9–10)
Primary reason for blood test		
Glucose monitoring	2 (5%)	3 (7%)
Jaundice	17 (44%)	22 (51%)
Newborn screening	3 (8%)	3 (7%)
Suspected sepsis	13 (33%)	13 (30%)
Other	4 (10%)	2 (5%)
Site		
Exeter	13 (33%)	14 (33%)
Oxford	26 (67%)	29 (67%)
Estimated cumulative prior pain exposure	4 (2–6)	4 (2–6)
Time (s) between stroking start (pre-heel lance) and heel lance	1.9 (11.6–33.0)	NA
Time (s) between heel lance and stroking start (post-heel lance)	NA	46.5 (42.1–106.3)

Table A.1: Baseline characteristics of neonates included in the analysis of the primary outcome (full analysis set). Median (interquartile range (IQR)) or count (%) are reported. NA: not available, PIPP-R: Premature Infant Pain Profile-Revised, STAI-S: State-Trait Anxiety Inventory-State.

A.2 Baseline characteristics of neonates included in the analysis of the secondary outcomes (full analysis sets)

	PIPP-R		Tachycardia		STAI-S	
	Intervention group (n=49)	Control group (n=51)	Intervention group (n=52)	Control group (n=53)	Intervention group (n=54)	Control group (n=52)
Parent stroking						
Biological father	17 (35%)	15 (29%)	18 (35%)	17 (32%)	19 (35%)	17 (33%)
Biological mother	32 (65%)	36 (71%)	34 (65%)	36 (68%)	35 (65%)	35 (67%)
Gestational age at birth (weeks)	38.7 (36.9–40.0)	38.1 (36.7–39.4)	38.8 (36.9–40.0)	38.1 (36.7–39.4)	38.8 (36.9–40.0)	38.1 (36.7–39.6)
Postmenstrual age at time of study (weeks)	38.7 (37.3–40.3)	38.6 (37.3–40.2)	38.9 (37.3–40.4)	38.6 (37.3–40.1)	38.9 (37.3–40.4)	38.6 (37.2–40.3)
Postnatal age at time of study (days)	2 (1–5)	3 (1–5)	3 (1–5)	3 (1–5)	3 (1–5)	3 (1–5)
Birthweight (g)	3390 (2740–3765)	3240 (2745–3762)	3387 (2740–3755)	3235 (2710–3737)	3392 (2740–3760)	3237 (2700–3750)
Sex						
Female	18 (37%)	19 (37%)	20 (38%)	21 (40%)	21 (39%)	20 (38%)
Male	31 (63%)	32 (63%)	32 (62%)	32 (60%)	33 (61%)	32 (62%)
Mode of delivery						
Normal vaginal	21 (43%)	19 (37%)	22 (42%)	20 (38%)	22 (41%)	19 (37%)
Breech vaginal	1 (2%)	0	1(2%)	0	1(2%)	0
Elective C-section	13 (27%)	8 (16%)	14 (27%)	8 (15%)	14 (26%)	8 (15%)
Emergency C-section	8 (16%)	13 (25%)	9 (17%)	14 (26%)	11 (20%)	15 (29%)
Ventouse or forceps	6 (12%)	11 (22%)	6 (12%)	11 (21%)	6 (11%)	10 (19%)
Apgar score at 1 min	9 (8–10)	9 (8–10)	9 (7–10)	9 (8–10)	9 (8–10)	9 (8–10)
Apgar score at 5 min	10 (10–10)	10 (9–10)	10 (9–10)	10 (9–10)	10(10–10)	10 (9–10)
Primary reason for blood test						
Glucose monitoring	2 (4%)	3 (6%)	2 (4%)	3 (6%)	2 (4%)	3 (6%)
Jaundice	21 (43%)	25 (49%)	24 (46%)	26 (49%)	25 (46%)	26 (50%)
Newborn screening	4 (8%)	3 (6%)	4 (8%)	3 (6%)	4 (7%)	3 (6%)
Suspected sepsis	17 (35%)	15 (29%)	17 (33%)	16 (30%)	17 (32%)	15 (29%)
Other	5 (10%)	5 (10%)	5 (10%)	5 (9%)	6 (11%)	5 (10%)
Site						
Exeter	14 (29%)	14 (28%)	15 (29%)	14 (26%)	15 (28%)	14 (27%)
Oxford	35 (71%)	37 (73%)	37 (71%)	39 (74%)	39 (72%)	38 (73%)
Estimated cumulative prior pain exposure	4 (2–6)	4 (2–7)	4 (2–6)	4 (2–6)	4 (2–6)	4 (2–6)
Time (s) between stroking start and subsequent heel lance	17.3 (12.8–31.7)	NA	17.2 (12.6–32.3)	NA	17.0 (12.3–31.7)	NA
Time (s) between heel lance and subsequent stroking start	NA	50.8 (42.2–125.2)	NA	50.8 (42.2–122.7)	NA	49.4 (42.1–117.6)

Table A.2: Baseline characteristics of neonates included in the analysis of the secondary outcomes (full analysis sets). Median (interquartile range (IQR)) or count (%) are reported. NA: not available, PIPP-R: Premature Infant Pain Profile-Revised, STAI-S: State-Trait Anxiety Inventory-State.

Appendix B

Parent Information Leaflet Exeter

Parental touch trial

Parent Information Leaflet

Royal Devon and Exeter Hospital

DEPARTMENT OF PAEDIATRICS

UNIVERSITY OF OXFORD

NHS
Royal Devon University Healthcare
NHS Foundation Trust

You and your child are eligible to take part in a research study. Before you decide, it is important that you understand why the research is being done and what it involves. Please read the following information and ask us if anything is unclear or if you would like more information.

Study title: **P**ar**ental** touch trial (*Petal*)
A randomised controlled trial to investigate the effects of parental touch on relieving acute procedural pain in neonates

1. What is the purpose of the study?
Babies in hospital often require clinical procedures as part of their routine medical treatment. As babies cannot tell us how much these procedures hurt, it is difficult to make sure that they are receiving the right pain-relief treatments. We know babies can experience discomfort and pain, and we have developed a method to measure changes in brain activity that occur when a baby undergoes a clinical procedure. We also know that babies display specific facial expressions when they are in pain and that their heart rate and breathing rate can increase.

Touch is important for parent and child bonding, and research in adults has shown that stroking the skin at the right speed can reduce pain experienced during some procedures. Stroking and parental touch activates special fibres in the skin that we think can make procedures feel less painful. Some studies have shown that close skin-to-skin contact between babies and their parents can reduce pain during procedures (such as blood tests).

The aim of this research is to understand if parental touch can reduce how much pain their babies experience during a blood test. We also want to know how parents feel when they stroke their baby's leg during a blood test. We would like to see if there are any differences in how a baby responds if a parent strokes their child's leg before or after a blood test.

2. Why have I been invited?
You and your child have been invited to take part in this study because your child requires a blood test for clinical purposes. We are recruiting parents and their children who were born at least 35 weeks gestation, who need to have a clinical blood test. We hope to recruit in total 112 babies.

3. Do we have to take part?
No, it is your decision whether or not you and your child take part. If you decide to take part, you will be asked to sign a consent form. If you decide you do not want to take part, this will not affect your child's care.

If you decide you would like to take part, you can change your mind at any time and withdraw you and your child from the study by telling the research team. You do not have to give a reason. You will be asked if we can use the data/images that have already been collected for analysis and if we can publish the anonymised results.

4. What is involved in the study?
We would like to understand how parental touch (in the form of stroking) may affect how babies respond to a clinically-required blood test. **No blood tests will be carried out solely for research purposes.** We will only study your child during a blood test that is needed for clinical purposes. Blood tests will be scheduled according to clinical need and thus you may have less than 24 hours to consider your participation in this trial. Blood tests will be completed in the routine way. On some occasions more than one heel lance is required to collect a sufficient blood sample. If this is the case, the research monitoring equipment will not be removed from your baby between heel lances in order to ensure that we do not interfere with the clinical procedures. As part of the study, we will also administer a 'sham' heel lance: this is not a real blood test and will not pierce your baby's skin

or cause any pain. This is a control stimulus to simulate the blood test without the 'painful' part. The heel lance is placed against your baby's foot but angled away so that the sharp fires into the air rather than the foot. The study will not interfere with your child's clinical care, nor will there be any delay if an emergency procedure is required.

As part of the study, we will ask you to stroke your child's leg for 10 seconds before, or 10 seconds after, their blood test. Half of the babies in the study will be stroked by a parent before the blood test, and half will be stroked afterwards. Before the study, your baby's details will be entered into a computer programme that will randomly select whether you should stroke your baby before or after the blood test. Where required we will demonstrate on a doll the speed, location and duration of the stroking. Before we start the study we will make sure you know how to complete the stroking in the correct way. As part of the study, we will also ask you to complete a short questionnaire; the first part will be before the study, the second part at the end. The questionnaire overall should take approximately 15 minutes.

We will assess your child's responses to the blood test by measuring their brain activity. We will also video your child's face, and measure other responses such heart rate, breathing rate and oxygen saturations. We will monitor your child before, during and after the blood test for approximately one hour.

We will use the following recording measures for your child:

Measuring brain activity

Electroencephalography (EEG)**:** EEG is a portable imaging system to measure brain activity. It involves gently placing electrodes (small discs) on the head using a paste that can be washed off with soap and water. EEG is routinely used on the neonatal unit, children's wards and clinics.

Measuring other responses

Vital sign monitoring: Small adhesive discs will be placed on your child's chest to measure changes in breathing rate and heart rate (this is called an ECG).

Videoing your child: We will also video your child during the study. This is so that we can assess changes in facial expression and body movements, and record the exact timing of the blood test.

We may ask if you are happy for us to use these images for teaching, publicity and/or in scientific journals. If you agree, we will take separate consent for this as your child's face would be present in the video footage. This is an optional part of the study and is not essential. If we do not use the images, this will not affect your child's care or stop your child participating in this research.

5. Are there any additional risks or benefits for my child?

Recording a video of your child is non-invasive and does not present any risk. EEG and ECG have been used clinically for over 20 years without any adverse effects. All studies have a dedicated team of healthcare professionals and researchers that will ensure the safety of your child at all times. We are not aware of any risks for your child taking part in this study.

The research data collected will not be routinely reviewed by a doctor. If any clinically significant findings are identified at the time of the study then the research team will report these to the clinical care team for further review.

There are no direct benefits of taking part in the study. This study is designed to gather information, to help improve the care we provide for babies in the future. If your child becomes distressed, the research study will be paused or stopped. Any clinically required procedures will still go ahead if the clinician looking after your child feels that this is appropriate.

6. What information will be collected about me and my child?
We will collect relevant medical (e.g. age), environmental (e.g. medical ward where study was conducted), demographic (e.g. post code) and social (e.g. ethnicity) information about your child from their medical notes. This information helps us to determine which factors may influence the way a baby copes with pain. We will also collect vital sign data (such as heart rate and breathing rate), recordings of their facial expressions and body movements and changes in brain activity caused by the blood test. We will ask you to complete a questionnaire.

All information and videos that are collected during this research study will be stored confidentially. Each baby will be allocated a study number which will be used to label the data. This study forms part of an educational programme.

7. What will happen to the results?
Results will be analysed and published in a journal. All publications will be made available on our website **https://neuroimaging.paediatrics.ox.ac.uk**. The findings may also be used for teaching or academic research presentations. No identifying information will be presented about you or your child, unless you have provided specific consent for us to use videos or images of your child in this way.

8. What will happen to my data and my child's data?
We will use the information about you and your child in order to conduct this study. Research is a task that we perform in the public interest. The University of Oxford, based in the United Kingdom as Sponsor, is the data controller. This means that we, as University of Oxford researchers and collaborators, are responsible for looking after the information collected and using it properly. We will use the minimum personally-identifiable information possible. We will keep identifiable information about you and your child for up to 12 months after the study has finished. This excludes any research documents with personal information, such as consent forms and facial expression recordings, which will be held securely at the University of Oxford for 21 years after the end of the study.

UK data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Further information about your rights with respect to your personal data is available at https://compliance.web.ox.ac.uk/individual-rights. You can find out more about how we use your information from the contacts in section 13.

Research data may be shared with other researchers, both here and abroad. Responsible members of the University of Oxford or the Royal Devon University Healthcare NHS Foundation Trust may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations.

9. Who is organising and funding this research?
This study is sponsored by the University of Oxford and has been funded by the Wellcome Trust and the charity BLISS. Your doctor will not be paid for including you in this study.

10. Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' interests. This study has been reviewed and given favourable opinion by the London - South East Research Ethics Committee.

11. Comments or concerns during the study

The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which your child is provided. If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Dr Ravi Poorun (details below) or the University of Oxford Clinical Trials and Research Governance, Ethics & Assurance (RGEA) office (tel: 01865 616480, email: rgea.complaints@admin.ox.ac.uk).

The Patient Advisory Liaison Service (PALS) is a confidential NHS service that can provide you with support for any complaints or queries you may have regarding the care you receive as an NHS patient. PALS is unable to provide information about this research study. The Royal Devon and Exeter Hospital PALS team can be contacted on: Tel: 01392 402093, Email: rde-tr.PALS@nhs.net, https://www.rdehospital.nhs.uk/patients-visitors/patient-advice-liaison-service-pals/#

12. Participation in future research

As we are interested in how your child's response to pain changes as they grow, we may ask if we can contact you in the future, to ask if you would be happy for your child to take part in other similar research studies run by our research team. If you agree that we can contact you about other research studies we will ask you to complete an optional additional consent item on the Consent Form used when you agree for your child to participate in the study. We will record your contact details, and these will be kept in a separate electronic database from the rest of the research data. This database is password-protected and can only be accessed by members of the research team.

Your contact details will not be passed onto anyone outside of the research team. All contact will come from the research team in the first instance. You can opt-out of this at any point by contacting Prof Rebeccah Slater (details below). Your agreement for us to contact you does not form any obligation to participate in future research.

What will happen to my data?

If you have provided optional additional consent to be contacted about future studies, we will store your contact indefinitely unless you choose to opt-out at any point.

13. Contact for further information

Chief Investigator: Dr Eleri Adams eleri.adams@ouh.nhs.uk 01865 221356
Principal Investigator: Dr Ravi Poorun r.poorun@exeter.ac.uk 01392 406980

You can also access further information about research or parent support from the following groups:

- BLISS: UK based charity https://www.bliss.org.uk/
- SSNAP (Support for the Sick Newborn And their Parents): Oxford based charity https://www.ssnap.org.uk



Picture shows example of an EEG study.

Thank you for reading this leaflet.

Appendix C

Parent Information Leaflet Oxford

Parental touch trial

Parent Information Leaflet

John Radcliffe Hospital

DEPARTMENT OF PAEDIATRICS

UNIVERSITY OF OXFORD

Oxford University Hospitals NHS
NHS Foundation Trust

You and your child are eligible to take part in a research study. Before you decide, it is important that you understand why the research is being done and what it involves. Please read the following information and ask us if anything is unclear or if you would like more information.

Study title: **P**ar**ental** touch trial (*Petal*)
A randomised controlled trial to investigate the effects of parental touch on relieving acute procedural pain in neonates

1. What is the purpose of the study?
Babies in hospital often require clinical procedures as part of their routine medical treatment. As babies cannot tell us how much these procedures hurt, it is difficult to make sure that they are receiving the right pain-relief treatments. We know babies can experience discomfort and pain, and we have developed a method to measure changes in brain activity that occur when a baby undergoes a clinical procedure. We also know that babies display specific facial expressions when they are in pain and that their heart rate and breathing rate can increase.

Touch is important for parent and child bonding, and research in adults has shown that stroking the skin at the right speed can reduce pain experienced during some procedures. Stroking and parental touch activates special fibres in the skin that we think can make procedures feel less painful. Some studies have shown that close skin-to-skin contact between babies and their parents can reduce pain during procedures (such as blood tests).

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2. Why have I been invited?
You and your child have been invited to take part in this study because your child requires a blood test for clinical purposes. We are recruiting parents and their children who were born at least 35 weeks gestation, who need to have a clinical blood test. We hope to recruit in total 112 babies.

3. Do we have to take part?
No, it is your decision whether or not you and your child take part. If you decide to take part, you will be asked to sign a consent form. If you decide you do not want to take part, this will not affect your child's care.

If you decide you would like to take part, you can change your mind at any time and withdraw you and your child from the study by telling the research team. You do not have to give a reason. You will be asked if we can use the data/images that have already been collected for analysis and if we can publish the anonymised results.

4. What is involved in the study?
We would like to understand how parental touch (in the form of stroking) may affect how babies respond to a clinically-required blood test. **No blood tests will be carried out solely for research purposes.** We will only study your child during a blood test that is needed for clinical purposes. Blood tests will be scheduled according to clinical need and thus you may have less than 24 hours to consider your participation in this trial. Blood tests will be completed in the routine way. On some occasions more than one heel lance is required to collect a sufficient blood sample. If this is the case, the research monitoring equipment will not be removed from your baby between heel lances in order to ensure that we do not interfere with the clinical procedures. As part of the study, we will also administer a 'sham' heel lance: this is not a real blood test and will not pierce your baby's skin or cause any pain.

This is a control stimulus to simulate the blood test without the 'painful' part. The heel lance is placed against your baby's foot but angled away so that the sharp fires into the air rather than the foot. The study will not interfere with your child's clinical care, nor will there be any delay if an emergency procedure is required.

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We will assess your child's responses to the blood test by measuring their brain activity. We will also video your child's face, and measure other responses such heart rate, breathing rate and oxygen saturations. We will monitor your child before, during and after the blood test for approximately one hour.

We will use the following recording measures for your child:

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5. Are there any additional risks or benefits for my child?

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All information and videos that are collected during this research study will be stored confidentially. Each baby will be allocated a study number which will be used to label the data. This study forms part of an educational programme.

7. What will happen to the results?
Results will be analysed and published in a journal. All publications will be made available on our website **https://neuroimaging.paediatrics.ox.ac.uk**. The findings may also be used for teaching or academic research presentations. No identifying information will be presented about you or your child, unless you have provided specific consent for us to use videos or images of your child in this way.

8. What will happen to my data and my child's data?
We will use the information about you and your child in order to conduct this study. Research is a task that we perform in the public interest. The University of Oxford, based in the United Kingdom as Sponsor, is the data controller. This means that we, as University of Oxford researchers and collaborators, are responsible for looking after the information collected and using it properly. We will use the minimum personally-identifiable information possible. We will keep identifiable information about you and your child for up to 12 months after the study has finished. This excludes any research documents with personal information, such as consent forms and facial expression recordings, which will be held securely at the University of Oxford for 21 years after the end of the study.

UK data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Further information about your rights with respect to your personal data is available at https://compliance.web.ox.ac.uk/individual-rights. You can find out more about how we use your information from the contacts in section 13.

Research data may be shared with other researchers, both here and abroad. Responsible members of the University of Oxford and Oxford University Hospitals NHS Trust may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations.

9. Who is organising and funding this research?
This study is sponsored by the University of Oxford and has been funded by the Wellcome Trust and the charity BLISS. Your doctor will not be paid for including you in this study.

10. Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' interests. This study has been reviewed and given favourable opinion by the London - South East Research Ethics Committee.

11. Comments or concerns during the study
The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which your child is provided. If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Prof

Rebeccah Slater (details below) or the University of Oxford Research Governance, Ethics & Assurance (RGEA) office (tel: 01865 616480, email: rgea.complaints@admin.ox.ac.uk).

The Patient Advisory Liaison Service (PALS) is a confidential NHS service that can provide you with support for any complaints or queries you may have regarding the care you receive as an NHS patient. PALS is unable to provide information about this research study. The John Radcliffe Hospital PALS team can be contacted on: Tel: 01865 221473, Email: PALS@ouh.nhs.uk, http://www.ouh.nhs.uk/patient-guide/pals.aspx.

12. Participation in future research

As we are interested in how your child's response to pain changes as they grow, we may ask if we can contact you in the future, to ask if you would be happy for your child to take part in other similar research studies run by our research team. If you agree that we can contact you about other research studies we will ask you to complete an optional additional consent item on the Consent Form used when you agree for your child to participate in the study. We will record your contact details, and these will be kept in a separate electronic database from the rest of the research data. This database is password-protected and can only be accessed by members of the research team.

Your contact details will not be passed onto anyone outside of the research team. All contact will come from the research team in the first instance. You can opt-out of this at any point by contacting Prof Rebeccah Slater (details below). Your agreement for us to contact you does not form any obligation to participate in future research.

What will happen to my data?

If you have provided optional additional consent to be contacted about future studies, we will store your contact details indefinitely unless you choose to opt-out at any point.

13. Contact for further information

Chief Investigator: Dr Eleri Adams eleri.adams@ouh.nhs.uk 01865 221356
Principal Investigator: Prof Rebeccah Slater rebeccah.slater@paediatrics.ox.ac.uk 01865 234229

You can also access further information about research or parent support from the following groups:

- BLISS: UK based charity https://www.bliss.org.uk/
- SSNAP (Support for the Sick Newborn And their Parents): Oxford based charity https://www.ssnap.org.uk



Picture shows example of an EEG study.

Thank you for reading this leaflet.

Appendix D

Consent Form Exeter

Consent Form (Exeter)

Parental touch trial

Study ID: | | | | | | | | | |

Infant's name:

Study Title: Parental touch trial (Petal)

Chief Investigator: Dr Eleri Adams **Principal Investigator:** Dr Ravi Poorun

Please initial each box

Please complete in black ballpoint pen.

1	I confirm that I have read and understood the information sheet (**v. , dated / /**), for the above study. I have had the opportunity to ask questions and have had these answered satisfactorily.	☐
2	I understand that my participation and my child's participation is voluntary and that me and my child are free to withdraw at any time, without giving any reason, without our medical care or legal rights being affected.	☐
3	I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the University of Oxford, Oxford University Hospitals NHS Trust or Royal Devon University Healthcare NHS Foundation Trust, where it is relevant to my child's taking part in this research. I give permission for these individuals to access to my child's records.	☐
4	I agree to my child being videoed during the study. I understand that recorded images will not be used for public use, only analysis. No identifiable information, including video recordings or imaging, will be used in any publications/presentations. Only anonymised data will be published or presented at meetings.	☐
5	I agree for the collected data to be used for teaching or academic research presentations.	☐
6	I agree to me and my child taking part in the above study.	☐
7	I agree to complete a parental questionnaire related to my child's study.	☐
	OPTIONAL	
8	I consent to being approached in the future about other similar research studies, by the research team, that my child may be eligible for. I understand that agreeing to be contacted does not oblige me or my child to participate in any further studies.	☐
9	I agree to the images/videos of my child recorded during this study being used for publications and presentations.	☐

Name of parent:

Relationship to baby:

Signature:

Date:

Name of investigator taking consent:

Signature:

Date:

1 to be kept as part of the study documentation (original)
1 copy for parent
1 copy with hospital notes
1 copy with PI

Appendix E

Consent Form Oxford

Consent Form (Oxford)

Parental touch trial

Study ID: | | | | | | | | | |

Infant's name:

Study Title: Parental touch trial (Petal)

Chief Investigator: Dr Eleri Adams **Principal Investigator:** Prof Rebeccah Slater

Please initial each box

Please complete in black ballpoint pen.

1	I confirm that I have read and understood the information sheet (**v. , dated / /**), for the above study. I have had the opportunity to ask questions and have had these answered satisfactorily.	☐
2	I understand that my participation and my child's participation is voluntary and that me and my child are free to withdraw at any time, without giving any reason, without our medical care or legal rights being affected.	☐
3	I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the University of Oxford or Oxford University Hospitals NHS Trust, where it is relevant to my child's taking part in this research. I give permission for these individuals to access to my child's records.	☐
4	I agree to my child being videoed during the study. I understand that recorded images will not be used for public use, only analysis. No identifiable information, including video recordings or imaging, will be used in any publications/presentations. Only anonymised data will be published or presented at meetings.	☐
5	I agree for the collected data to be used for teaching or academic research presentations.	☐
6	I agree to me and my child taking part in the above study.	☐
7	I agree to complete a parental questionnaire related to my child's study.	☐
	OPTIONAL	
8	I consent to being approached in the future about other similar research studies, by the research team, that my child may be eligible for. I understand that agreeing to be contacted does not oblige me or my child to participate in any further studies.	☐
9	I agree to the images/videos of my child recorded during this study being used for publications and presentations.	☐

Name of parent:

Relationship to baby:

Signature:

Date:

Name of investigator taking consent:

Signature:

Date:

1 to be kept as part of the study documentation (original)
1 copy for parent
1 copy with hospital notes

Appendix F

Internal Guidance Sheets

The Petal guidance sheets are presented below with permission from Prof Rebeccah Slater. Where relevant, with permission from Prof Rebeccah Slater, these have also been adapted to remove any sensitive or confidential information.

PETAL

GUIDANCE SHEET 01: RECRUITMENT

SCOPE

This Guidance Sheet describes the processes involved in recruiting a participant to the Petal trial.

PROCEDURE

Eligibility

- Babies should be considered eligible if they meet the inclusion criteria:
    a. Neonates born at the John Radcliffe Hospital, Oxford or the Royal Devon and Exeter Hospital, Devon
    b. Neonates born at or after 35+0 weeks gestation
    c. Neonates with a postnatal age of ≤ 7 days
    d. Neonates who require a heel lance as part of routine clinical care
    e. Neonates for whom parents/guardians have given written informed consent for inclusion in the trial.
- Babies are ineligible if the meet any of the exclusion criteria:
    a. Intraventricular haemorrhage (IVH) > grade I
    b. Received any analgesics or sedatives in the last 24 hours
    c. Congenital malformation or genetic condition known to affect neurological development
    d. Born to mothers who have a history of substance abuse.

Screening

- Babies that are screened should be entered onto a Screening Log (initials only, no full names) only if the parents have been approached. The live Screening Log should be kept in the Site File (Exeter)/TMF (Oxford), blank refills can be added from the Document Box.
- Make sure you liaise with the clinical team to ensure that the baby will definitely require a blood test, and when it is likely to be needed.

Parental approach

- Once a baby is identified as potentially eligible (and this has been confirmed by a clinical member of the team), the researcher should approach the parents to explain the study.
- The baby's initials should be entered onto the Screening Log.
- Provide the parents a PIL and ensure that all aspects of the study have been described.
- Make sure they understand:
    - The intervention and their involvement
    - The timing around a blood test for clinical reasons
    - All the recording measures we will use, in particular the facial video. Ensure it is clear what procedures would be conducted as part of the study that are additional to standard care.
    - The questionnaire they will be asked to complete – whichever parent signs the consent form should be the one to administer the intervention and complete the questionnaire.
- Give them the opportunity to ask questions, and then allow them time to read the PIL and consider if they are interested.
- Record in the medical notes that the baby was considered eligible, and that parents have been approached about the Petal trial.
    - Exeter: upload an electronic copy of the PIL to the medical notes
    - Oxford: place a paper copy of the PIL into the medical notes.

- Parents who do not speak English should only be approached if an unrelated adult interpreter is available.

Consent

- After a suitable amount of time, return to the parents to answer any further questions they may have and discuss the study in more detail.
- Make sure that parents understand they can withdraw their consent at any time, and that they do not have to give a reason why.
- If they are happy to enrol their child, allocate the baby a Study ID
    - [****** confidential information redacted **********]

- The Study ID should be entered onto the Consent Form, which should then be completed by a parent using a black ballpoint pen. Blank Consent Forms are stored in the Document Box.
- Only the mother or father, or person designated formally by legal process, may sign the Consent Form. In law, unmarried fathers do not automatically have parental responsibility for their child, unless they are named on the birth certificate, or have been granted parental responsibility through a court order or parental responsibility agreement. Please check this before allowing a father to provide informed consent for the child.
- In the case of twins or triplets, **each baby must** have a **separate signed consent form**.
- Please ensure the following:
    - Full names of both parent and Health Professional are clearly recorded
    - Dates are fully completed for both parent and Health Professional (these must be the same)
    - Parent name and date is not pre-populated by Health Professional
    - Parents have initialled each box (**not ticked**).
- The Health Professional taking informed consent must be **delegated this duty** by the PI on the Delegation Log and have **valid GCP training** (the Delegation Log and copies of valid GCP training certificates should be kept in the Site File).
- Exeter:
    - The Consent Form should then be photocopied, with a copy given to the parents for their records and a copy placed in the Site File
    - A scan of the completed Consent Form should be uploaded to the medical notes
    - The PI should arrange to transfer the original signed Consent Form to the Oxford research team.
- Oxford:
    - The top copy of the Consent Form (the original signed) should be placed in the TMF
    - One of the remaining NCR parts should be placed in the medical notes, and one given to the parents.
- The Screening and Enrolment Log should be updated to confirm that the baby was enrolled onto the study.
- Recruitment should be noted on the local system for monthly accrual data and uploaded to Siteline/CPMS.

Optional consent items

On the Consent Form there are 4 optional consent items. Explain to the parents that these are not mandatory, and if not agreed to will not affect their child's care or ability to take part in the study.

1. *I consent to being approached in the future about other research studies that my child may be eligible for. I understand that agreeing to be contacted does not oblige me or my child to participate in any further studies.*
   When this is completed, parents should be asked to also complete a Contact Details Form (blank copies available in the Document Box). The completed form should be kept in the Site File with the copy of the Consent Form (Exeter)/in the TMF with the original Consent Form (Oxford). The details should be entered onto the personal details database.

2. *I agree to the images/videos of my child recorded during this study being used for publications and presentations.*
   When this is completed, the facial video recordings used for PIPP-R scoring may be used for publications and presentations (i.e. not just the PIPP-R scores as part of the research data). Please ensure that parents understand that their child's face will be visible in these recordings.

Once the baby has been consented, they should be entered onto the randomisation system and allocated to an arm (see Guidance Sheet 02).

Training and Delegation

Informed consent can only be taken by individuals who have completed their GCP training/refresher within the last 3 years and who have been delegated this duty on the Delegation Log by the PI. Exeter: please email scanned copies of the GCP certificates and completed Delegation Log to petal@paediatrics.ox.ac.uk and store the originals in the ISF).

Individuals should only be delegated this duty after they have received appropriate training from a medically qualified individual who has been delegated this training duty on the Delegation Log (or the PI). Training should initially involve watching the PI/appropriate individual providing training complete the parental approach and take informed consent. The PI/trainer should also ensure that the trainee is clear what information needs to be recorded in the medical notes at which time points. The individual being trained should then complete the parental approach and take informed consent whilst being observed by the trainer/PI (the trainer/PI will need to countersign the Consent Form). When the trainer/PI is content that the trainee can independently take informed consent, they should complete an Informed Consent Training Certificate. This should be stored in the ISF and a scan emailed to the Oxford team at petal@paediatrics.ox.ac.uk (Exeter)/stored in the TMF (Oxford). The trainee can then be delegated the duty of taking informed consent on the Delegation Log.

PETAL

GUIDANCE SHEET 02: RANDOMISATION

SCOPE

This Guidance Sheet describes the processes for randomising a participant to the Petal trial.

PROCEDURE

Eligibility

- Babies should be considered eligible if they meet the inclusion criteria:
    a. Neonates born at the John Radcliffe Hospital, Oxford or the Royal Devon and Exeter Hospital, Devon
    b. Neonates born at or after 35+0 weeks gestation
    c. Neonates with a postnatal age of ≤ 7 days
    d. Neonates who require a heel lance as part of routine clinical care
    e. Neonates for whom parents/guardians have given written informed consent for inclusion in the trial
- Babies are ineligible if the meet any of the exclusion criteria:
    a. Intraventricular haemorrhage (IVH) > grade II
    b. Received any analgesics or sedatives in the last 24 hours
    c. Congenital malformation or genetic condition known to affect neurological development
    d. Born to mothers who have a history of substance abuse.

Screening

- Babies that are screened should be entered onto a Screening Log (initials only, no full names). The live Screening Log should be kept in the Site File (Exeter)/TMF (Oxford), blank refills can be added from the Document Box.
- Make sure you liaise with the clinical team to ensure that the baby will definitely require a blood test, and when it is likely to be needed.

Parental approach

- Once a baby is identified as potentially eligible (and this has been confirmed by a clinical member of the team), the researcher should approach the parents to explain the study
- Provide the parents a PIL and ensure that all aspects of the study have been described.
- Make sure they understand:
    - The intervention and their involvement
    - The timing around a blood test for clinical reasons
    - All the recording measures we will use, in particular the facial video
    - The questionnaire they will be asked to complete – whichever parent signs the consent form should be the one to administer the intervention and complete the questionnaire
- Give them the opportunity to ask questions, and then allow them time to read the PIL and consider if they are interested
- Record in the medical notes that the baby was considered eligible, and that parents have been approached about the Petal trial.
    - Exeter: upload an electronic copy of the PIL to the medical notes
    - Oxford: place a paper copy of the PIL into the medical notes.
- Parents who do not speak English should only be approached if an unrelated adult interpreter is available.

Consent

- After a suitable amount of time, return to the parents to answer any further questions they may have and discuss the study in more detail
- Make sure that parents understand they can withdraw their consent at any time, and that they do not have to give a reason why
- If they are happy to enrol their child, allocate the baby a Study ID (this should be the next consecutive ID for the relevant research project)
    - [****** confidential information redacted ******]
    - To ensure that the same study ID is not accidentally allocated to two different babies at each site, the person randomising will communicate with the rest of the Petal team at both sites as soon as a study ID has been assigned.
- The Study ID should be entered onto the Consent Form, which should then be completed by a parent using a black ballpoint pen. Blank Consent Forms are stored in the Document Box
- Only the mother or father, or person designated formally by legal process, may sign the Consent Form. In law, unmarried fathers do not automatically have parental responsibility for their child, unless they are named on the birth certificate, or have been granted parental responsibility through a court order or parental responsibility agreement. Please check this before allowing a father to provide informed consent for the child.
- In the case of twins or triplets, **each baby must** have a **separate signed consent form**.
- Please ensure the following:
    - Full names of both parent and Health Professional are clearly recorded
    - Dates are fully completed for both parent and Health Professional (these must be the same)
    - Parent name and date is not pre-populated by Health Professional
    - Parents have initialled each box (**not ticked**)
- The Health Professional taking informed consent must be **delegated this duty** by the PI on the Delegation Log and have **valid GCP training** (the Delegation Log and copies of valid GCP training certificates should be kept in the Site File)
- Exeter:
    - The Consent Form should then be photocopied, with a copy given to the parents for their records and a copy placed in the Site File
    - A scan of the completed Consent Form should be uploaded to the medical notes
    - The PI should arrange to transfer the original signed Consent Form to the Oxford research team
- Oxford:
    - The top copy of the Consent Form (the original signed) should be placed in the TMF
    - One of the remaining NCR parts should be placed in the medical notes, and one given to the parents
- The Screening and Enrolment Log should be updated to confirm that the baby was enrolled onto the study
- Recruitment should be noted on the local system for monthly accrual data and uploaded to Siteline/CPMS

Optional consent items

On the Consent Form there are 4 optional consent items. Explain to the parents that these are not mandatory, and if not agreed to will not affect their child's care or ability to take part in the study

1. *I consent to being approached in the future about other research studies that my child may be eligible for. I understand that agreeing to be contacted does not oblige me or my child to participate in any further studies.*
   When this is completed, parents should be asked to also complete a Contact Details Form (blank copies available in the Document Box). The completed form should be kept in the Site File with the

copy of the Consent Form (Exeter)/in the TMF with the original Consent Form (Oxford). The details should be entered onto the personal details database.

2. *I agree to the images/videos of my child recorded during this study being used for publications and presentations.*
   When this is completed, the facial video recordings used for PIPP-R scoring may be used for publications and presentations (i.e. not just the PIPP-R scores as part of the research data). Please ensure that parents understand that their child's face will be visible in these recordings.

Once the baby has been consented, they should be entered onto the randomisation system and allocated to an arm (see Guidance Sheet 5).

Training and Delegation

Informed consent can only be taken by individuals who have completed their GCP training/refresher within the last 3 years and who have been delegated this duty on the Delegation Log by the PI. Exeter: please email scanned copies of the GCP certificates and completed Delegation Log to petal@paediatrics.ox.ac.uk and store the originals in the ISF).

Individuals should only be delegated this duty after they have received appropriate training from a medically qualified individual who has been delegated this training duty on the Delegation Log (or the PI). Training should initially involve watching the PI/appropriate individual providing training complete the parental approach and take informed consent. The PI/trainer should also ensure that the trainee is clear what information needs to be recorded in the medical notes at which time points. The individual being trained should then complete the parental approach and take informed consent whilst being observed by the trainer/PI (the trainer/PI will need to countersign the Consent Form). When the trainer/PI is content that the trainee can independently take informed consent, they should complete an Informed Consent Training Certificate. This should be stored in the ISF and a scan emailed to the Oxford team (Exeter)/stored in the TMF (Oxford). The trainee can then be delegated the duty of taking informed consent on the Delegation Log.

PETAL

GUIDANCE SHEET 03: INTERVENTION

SCOPE

This Guidance Sheet describes the processes for training the parents to administer the intervention, and how to ensure consistency when the intervention is administered during the test occasion.

PROCEDURE

The touch intervention should be administered by the parent who has signed the consent form. The intervention involves stroking the lower limb of the leg where the heel lance will be administered at 3cm/s for 10s.

Training parents

Approximately 30 minutes prior to the clinical procedure, the research team should explain to the parents how to administer the intervention correctly. Parents should be told which group their baby is in and therefore when the touch intervention should be administered.

The research team should demonstrate the speed, location, and duration of the intervention.

Explain that they should stroke the leg, and that strokes should be in one direction down the limb towards the foot. The research team will also make sure the to show the parents the PsychoPy animation.
Explain that PsychoPy is a software that provides visual cues on a computer screen to guide a consistent speed of stroking at 3cm/s for a 10 second duration. Where appropriate, place on a flat surface like the position the baby is likely to be in during the test occasion and explain the intervention by showing how to stroke on a doll or arm. The research team will also explain what guidance the parent will receive during the test occasion too.

After the demonstration, ask the parent wishing to administer the intervention to demonstrate this on their own arm using the PsychoPy software for cues and the guidance that the research team will be giving during the test occasion. The research team should observe and ensure that this is done correctly before proceeding to the test occasion.

Intervention administration during the test occasion

During the test occasion the parents will be asked to start and stop stroking at the defined points. If parents require additional guidance during the stroking intervention, the guidance given should be documented in the Study comments section of the database.

PETAL

GUIDANCE SHEET 04: EQUIPMENT SET UP AND RECORDING

SCOPE

This Guidance Sheet describes the processes for the equipment set up and recordings that should be taken during the Petal test occasions.

PROCEDURE

EEG

- Refer to NIPI Guidance Sheets 06, 07 & 08 (Exeter)/Paediatric Neuroimaging SOPs 01 & 04 (Oxford) for equipment set up and data transfer instructions
- Eight EEG recording electrodes should be positioned on the scalp at Cz, CPz, C3, C4, FCz, T3, T4 and Oz according to the modified international 10-20 System. Reference and ground electrodes should be placed at Fz and the forehead respectively.
- An ECG electrode will be placed on the left clavicle to record heart rate and respiration.
- EEG should be recorded for approximately 10 min before, and 30 min after, the control and clinical heel lance.

Physiological monitoring

- Refer to NIPI Guidance Sheets 04, 05 & 08 (Exeter)/Paediatric Neuroimaging SOPs 07, 08 & 20 (Oxford) for equipment set up and data transfer instructions
- Heart rate, respiratory rate and oxygen saturation will be measured using ECG and pulse oximetry
- Physiological monitoring should be recorded for approximately half an hour before, and half an hour after, the control and clinical heel lance.

Facial video recordings

- The facial video camera should either be set up using a clamp to record the baby's facial expressions, or by a member of the research team
- Facial video recordings will be used to assess change in facial expression, for the PIPP-R scores.
- Facial video recording should be continuous from approximately 30s before the control heel lance to 30s after the clinical heel lance.
- Refer to NIPI Guidance Sheet 10 (Exeter)/Paediatric Neuroimaging SOP 14 (Oxford) for information about video epoching and uploading.

PETAL

GUIDANCE SHEET 05: TEST OCCASION OVERVIEW

SCOPE

This Guidance Sheet provides an overview of the Petal test occasion.

PROCEDURE

Prior to the test occasion

- Take consent for participant
- Add participant to the database (this must be done in advance so that the parent questionnaire responses can be added directly to the database)
- Randomise participant

At the beginning of the test occasions

- Set up the physiological monitoring and EEG equipment
- Set up the facial video camera
- Provide the parent education for administering the intervention
- Set up PsychoPy ready for the test occasion

During the test occasion

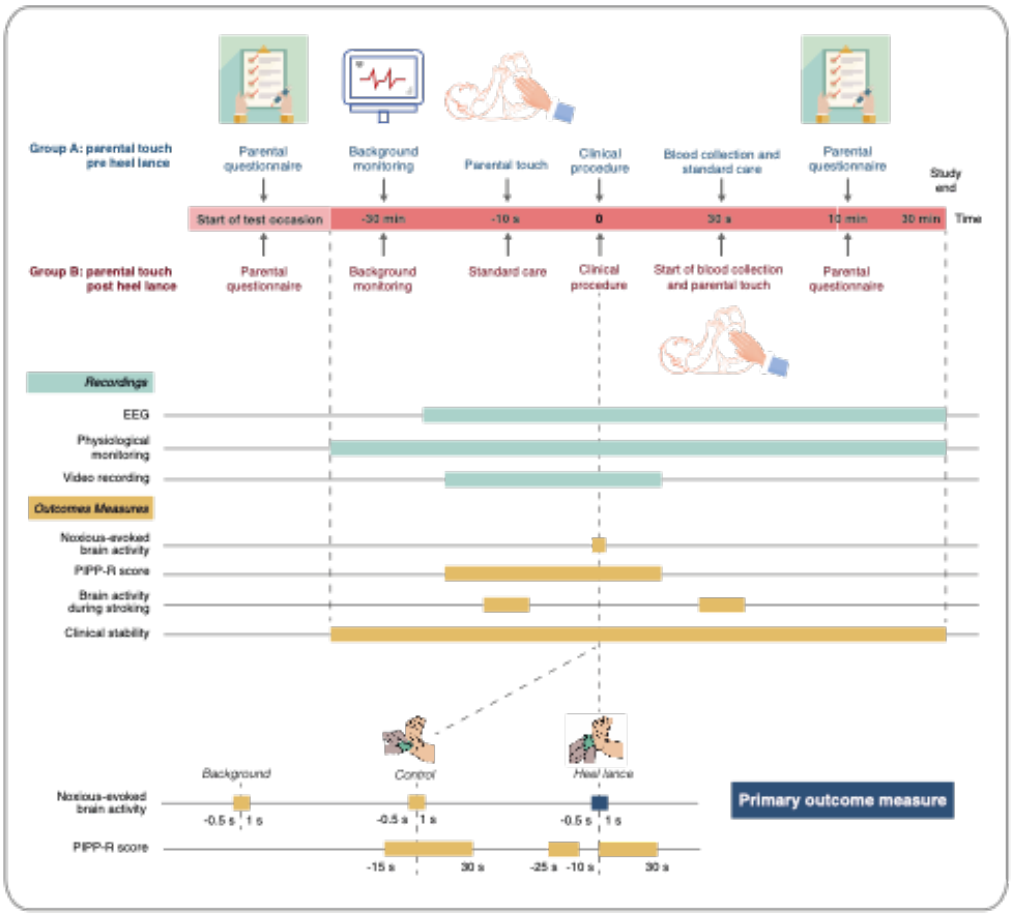
- Record physiological data for approximately half an hour before and after the control and clinical heel lance. At least 10 minutes of physiological data should be acquired before EEG setup.
- As soon as possible verbally ask parents the pre lance questionnaires.
- Record EEG for approximately 10 min before and at least 10 min after the control and clinical heel lance. You can continue recording EEG until 30 minutes since the heel lance, which is when the vital signs recording is stopped.
- Tell the parents which arm their baby is in**.**
- Record facial video for approximately 30 seconds before and **at least 30 seconds** after the control and clinical heel lance
    - The PIPP-R score baseline measurements should be taken for the time period of 30 to 15 seconds before the control and clinical heel lances – this will mean the 15 seconds immediately prior to each lance is clear for the stroking intervention is required.
- Parents should administer the intervention:
    - Pre-lance stroking: the parents should stroke their baby in the 10s immediately before the clinical heel lance
    - Post-lance stroking: the parents should stroke their baby at least 30s after the clinical heel lance (this will allow time for blood collection, and so that the post-lance stroking doesn't impact facial expression change during the time that PIPP-R scores are being calculated)
- Verbally ask the parents the post lance questionnaires.

At the end of the test occasions

- Log into the survey and ask the parent to complete the final section (remind them that this part is anonymous and only recorded by trial arm) link: [Link to password-protected anonymous survey]

Parent questionnaire

- The whole parent questionnaire should be completed directly onto an electronic device.
- **Important:** the researcher should ask all questions of STAI-T, STAI-S to the parent in a private room. The STAI-T and STAI-S should be completed as soon as possible after taking informed consent.
- One member of the research team should log into the research database on the electronic device and load up the relevant participant. Then verbally ask the STAI-S and STAI-T questions, entering the responses directly into the database.
- Following the stroking the parents should be asked by the research team how the heel lance (4-point-questionnaire), they were assigned to, made them feel.
- Towards the end of the test occasion, once the baby is calm and parents are settled, the researcher should again log into the database and verbally ask the STAI-S questions– directly entering the responses into the database.
- Once the questionnaires tabs on the electronic database are complete, log out of the database and load up the anonymous survey (this comprises section 4 of the parent questionnaire). Log in using your log in details and complete the first part for the researcher (site and randomisation arm). Then pass the electronic device to the parent and ask them to complete the part for the parent and then press submit.



PETAL

GUIDANCE SHEET 06: DATA ANALYSIS AND ENTRY

SCOPE

This Guidance Sheet describes the process for entering the data on the database and analysing the data.

PROCEDURE

Analysis

1. **EEG data**

EEG data should be analysed using ad hoc MATLAB scripts [********** location of internal scripts **********].

2. **Physiological data**

Physiological data should be analysed using ad hoc MATLAB scripts [****** location of internal scripts **********].

3. **PIPP-R scores**

The Oxford team will complete PIPP-R scoring for all the videos in the Petal trial. However, the site collecting the data is responsible for uploading the full and epoched videos to the database. Further information about epoching is in NIPI Guidance Sheet 10 (Exeter)/Paediatric Neuroimaging SOP 14 (Oxford). The videos recorded in Exeter should be uploaded to MedSciNet. The Exeter site should communicate to Oxford which videos need to be scored. A researcher at the Oxford site will download the videos from the database and save them on a password-protected hard drive in Oxford. The blinded person performing the PIPP-R scoring will receive the list of numbers of videos which need to be scored and will perform the PIPP-R scoring and enter the scores on MedSciNet under the corresponding test occasion and stimulus.

Only individuals who have completed PIPP-R training and have been delegated this duty can complete it during the trial. They must not be members of the data acquisition team, to remain blinded to arm allocation. Further information about PIPP-R training and scoring is available in Paediatric Neuroimaging SOP 15.

4. Questionnaire scoring

The questionnaires questions will be asked by the researcher to the parent and the answers directly entered onto MedSciNet by the researcher. A sum score will be automatically generated by MedSciNet.

Data entry

All background data, analysis outputs for EEG and physiological monitoring, PIPP-R scores and sections 1-3 of the parent questionnaire should be entered into the Paediatric Neuroimaging research database.

If you are already a user for the NIPI study, you can use the same login details. The database can be accessed at [link to the trial database].

[********** Setting up a new user for the trial database **********
**********]

All data entry should be completed ideally within 24 hours of the test occasion.

PETAL

GUIDANCE SHEET 07: SAFETY AND INCIDENT REPORTING

SCOPE

This Guidance Sheet describes the process for safety and incident reporting in the Petal trial.

PROCEDURE

Safety reporting

A **SERIOUS ADVERSE EVENT (SAE)** is any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Consists of a congenital anomaly or birth defect
- Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

SAEs occurring during the one-hour trial period should be recorded on a paper SAE Report Form as soon as the research team become aware of them. This includes SAEs which are unlikely to have any association with the research study. Any member of staff can report an SAE.

SAEs can be reported by anyone, however they must be assessed for causality by a medically qualified investigator named on the Delegation Log. The SAE CRF includes this assessment in the final section. The definitions for assessing causality are:

- **Unrelated** – where an event is not considered to be related to the research study
- **Possibly** – although a relationship to the research study cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible
- **Probably** – the temporal relationship and absence of a more likely explanation suggest the event could be related to the research study
- **Definitely** – the research study is the most likely cause.

No SAEs are expected in this study, therefore any occurring are considered 'unexpected'.

Exeter

SAEs will be reported to the Oxford team within **24 hours** of the research site becoming aware of the event. The individual reporting the SAE should telephone the Oxford team to inform them:

[******* Contact details **********]

All SAEs will also be assessed by the CI/Safety Delegate. This can be documented on a File Note (must be signed by the SI/Safety Delegate) and placed into the TMF.

Where an SAE is considered to be both related to the research activities and unexpected, the CI will report this to the REC and Sponsor within 15 calendar days of becoming aware of the event.

Follow-up Information

Any new, updated, or corrected information on previously reported SAEs must be reported on a separate SAE form when the information becomes available, with 'Follow-up information' selected instead of 'Initial report'. The outcome of events 'Resolving' or 'Not Resolved' must be followed up until 'Resolved'. All information on any SAE Report Form (i.e. both 'initial report' and 'follow-up information') should be entered into the corresponding participant's record in the database. This is important as cumulative numbers of related SAEs need to be included in the annual safety reports to the REC.

Incidents/serious breaches

Any deviations from the protocol, trial procedures, Good Clinical Practice (GCP), or regulatory requirements will need to be notified to Paediatric Neuroimaging team (**petal@paediatrics.ox.ac.uk**) as an **incident** using an Incident Report Form. These should be reported to as soon as practical. This should not contain any personal identifiable information. Any member of staff can report incidents. Exeter should send a scan of the Incident Report Form to the Oxford team by email, then place the original form in the ISF. Oxford should place all Incident Report Forms (from both sites) into the TMF.

Examples of possible incidents include:

- Problems with recording and exporting data
- Parent complaints

An incident can also be considered to be a **serious breach**. This is a breach in the protocol or Good Clinical Practice (GCP) that is likely to significantly affect either:

- The safety, physical or mental integrity of the subjects of the trial
- The scientific value of the trial

An example of a serious breach is an infant being recruited and studied without written informed consent from the parent. All incidents should be assessed by a member of the core team in Oxford using and Incident, Deviation and Serious Breach Assessment Form. This includes the above assessments. This form should be stored with the Incident Report Form in the TMF. Any corrective or preventative action to be taken centrally should be recorded on this form, along with any related email correspondence with site (e.g. corrective of preventative action the site should complete).

If the study team consider the report to encompass a serious breach, the CI or delegate must report this to the Sponsor within 1 working day and to the REC, HRA and R&D department within 7 calendar days.

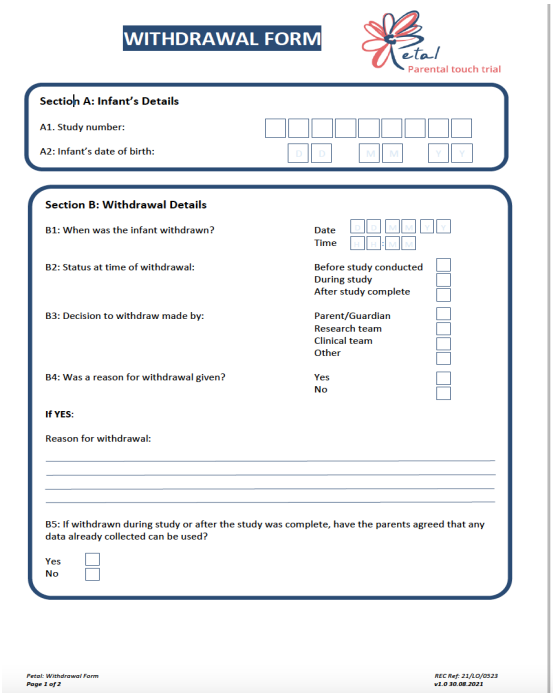
GUIDANCE SHEET 08: WITHDRAWAL

SCOPE

This Guidance Sheet describes how to withdraw a participant from the 'Investigating pain in the developing human brain' study. A participant can be withdrawn at any time (before, during or after data collection) by either a parent or a member of the research/clinical team. Parents do not need to give a reason for the withdrawal.

PROCEDURE

1. Fill in the paper Withdrawal Form (any staff member can use this form).
2. The form will ask if a reason for withdrawal has been provided, and if the parents have agreed that data already collected can still be used.
3. It is important to reiterate to parents that clinical care will not be affected by the decision to withdraw. All clinical care should continue as planned.
4. The completed paper form should be stored in the Site File with the participant's Consent Form etc.
5. **The time and date of the withdrawal should be documented in the medical notes, together with any other necessary information.**
6. The information from the Withdrawal Form should be entered onto the database.
7. Participants who have been withdrawn should still be entered onto CPMS/Siteline, as rate of withdrawal is an outcome of this study.



WITHDRAWAL FORM

Parental touch trial

Section A: Infant's Details

A1. Study number:

A2: Infant's date of birth: D D M M Y Y

Section B: Withdrawal Details

B1: When was the infant withdrawn? Date / Time

B2: Status at time of withdrawal: Before study conducted / During study / After study complete

B3: Decision to withdraw made by: Parent/Guardian / Research team / Clinical team / Other

B4: Was a reason for withdrawal given? Yes / No

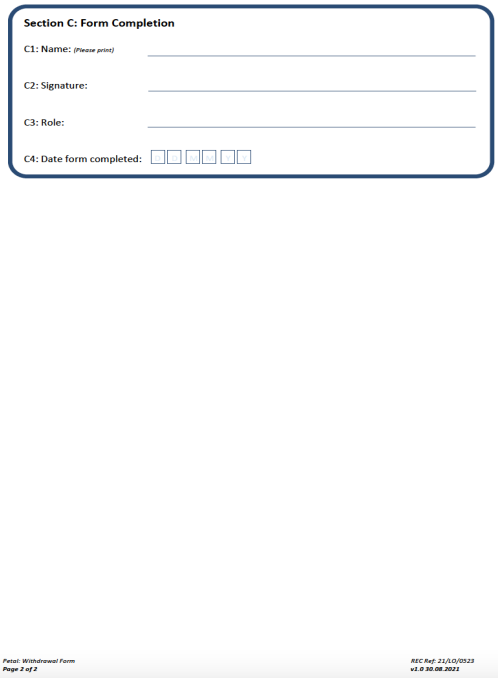
If YES:

Reason for withdrawal:

B5: If withdrawn during study or after the study was complete, have the parents agreed that any data already collected can be used?

Yes / No

Petal: Withdrawal Form Page 1 of 2 REC Ref: 21/LO/0523 v1.0 30.08.2021



Section C: Form Completion

C1: Name: *(Please print)*

C2: Signature:

C3: Role:

C4: Date form completed:

Petal: Withdrawal Form Page 2 of 2 REC Ref: 21/LO/0523 v1.0 30.08.2021

PETAL

GUIDANCE SHEET 09: TRAINING AND DELEGATION

SCOPE

This Guidance Sheet describes the processes involved in training and delegating the training of a research team member engaged in the Petal trial.

PROCEDURE

- Training should involve watching the trainer performing the task, then practising the task under supervision and finally being assessed by a qualified person while performing the task.
- The definition of 'qualified person' changes depending on what is appropriate for the task:
  - **Taking informed consent**: this has to be signed off by a medical investigator.
  - **EEG placement:** in Oxford, this should be signed off by [names of qualified personnel]
    - **Providing parent intervention education/PIPP-R scoring/Other (if non-clinical):** [names of qualified personnel ********]

- The Health Professional taking informed consent must be **delegated this duty** by the PI on the Delegation Log and have **valid GCP training** (the Delegation Log and copies of valid GCP training certificates should be kept in the Site File).
- When the trainer/PI is content that the trainee can independently perform the task, they should complete a Training Certificate clearly stating what the trainee is being signed off for.
- Training Certificates are available for:
  - Taking informed consent
  - EEG placement
  - Providing parent intervention training
  - PIPP-R
  - Other
  - Data capture
  - PIPP-R scoring
  - Randomisation
  - SAE review
  - The Training Certificates should be stored in the ISF and a scan emailed to the Oxford team at petal@paediatrics.ox.ac.uk (Exeter)/stored in the TMF (Oxford). The trainee can then be delegated the duty of taking informed consent on the Delegation Log.
  - *Exeter*
    - Please email scanned copies of the GCP certificates and completed Delegation Log to petal@paediatrics.ox.ac.uk and store the originals in the ISF). The Exeter site should use the same Training Certificates as Oxford, while they can create a site-specific training log if they wish.
- **Note:** Please be aware that NIPI certificates are not valid for the PETAL trial. PETAL specific Training Certificates need to be issued.

PETAL

GUIDANCE SHEET 10: DOCUMENTATION AND DATA STORAGE

SCOPE

This Guidance Sheet describes the processes involved in documenting and storing the data recorded for the Petal trial.

PROCEDURE

- The database used for the PETAL dataset is the general Paediatric Neuroimaging database developed and hosted by MedSciNet. [********** internal location of documentation ********** ********** **********

Identifiable information about the parent and child will be kept for up to 12 months after the study has finished. This excludes any research documents with personal information, such as consent forms and facial expression recordings, which will be held securely at the University of Oxford for 21 years after the end of the study.

Sharing

Research data may be shared with other researchers, both here and abroad. Responsible members of the University of Oxford and Oxford University Hospitals NHS Trust may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations.

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