Parental touch trial

Trial Protocol

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
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2 Synopsis

Long Title	A randomised controlled trial to investigate the effects of parental touch on relieving acute procedural pain in neonates	
Short Title	**P**ar**ental** touch trial (*Petal*)	
Trial Design	Randomised-controlled clinical trial	
Trial Participants	Neonates born at or after 35+0 weeks' gestation with a postnatal age of ≤7 days	
Planned Sample Size	112 neonates: 56 will be randomised to the pre-procedural touch intervention, 56 will be randomised to the post-procedural touch intervention	
Trial duration	Each neonate will be included in the trial for approximately one hour	
Planned Trial Period	29 months total	
Planned recruitment period	18 months 01/09/2021 – 01/03/2023	
	Objectives	**Outcome Measures**
Primary	To test whether parental touch of the limb prior to a clinically-required heel lance reduces noxious-evoked brain activity.	Magnitude of noxious-evoked brain activity following a heel lance.
Secondary	To test whether parental touch of the limb prior to a clinically-required heel lance reduces clinical pain scores (PIPP-R). To test whether parental touch of the limb prior to a clinically-required heel lance reduces post-procedural tachycardia. To test whether parental touch of the limb prior to a clinically-required heel lance reduces parental anxiety.	PIPP-R score (baseline assessed for 15s before the heel lance and rest of score assessed during the 30 seconds period following a heel lance). Percentage of neonates who develop post-procedural tachycardia (in the 30 seconds following a heel lance). Difference in State Trait Anxiety Inventory (STAI)-S scores pre- and post-procedure.
Exploratory	To explore how parental touch may impact background brain activity. To explore whether parental touch prior to a clinically-required heel lance reduces the duration of time for heart rate to return to baseline. To explore how parental touch prior to a clinically-required heel lance affects respiratory stability.	Changes in brain activity elicited during the touch intervention. Time taken for heart rate to return to baseline following a heel lance. Respiratory dynamics and incidence of apnoea following a heel lance.

	To explore parental anxiety and distress, and their experience of the trial and infant research.	Scores for individual parameters from the STAI-trait (T) and STAI-state (S); 4-point distress questionnaire score; responses to survey about participation in Petal and infant research.
Intervention	Parental touch in the form of a stroking intervention, at 3cm/s for 10 seconds down the lower limb receiving the heel lance.	

3 Abbreviations

CI	Chief Investigator
CRF	Case Report Form
CT	C-Tactile
EEG	Electroencephalography
GA	Gestational Age
GCP	Good Clinical Practice
HIE	Hypoxic Ischaemic Encephalopathy
IRAS	Integrated Research Application System
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
IVH	Intraventricular Haemorrhage
KC	Kangaroo Care
NEC	Necrotising Enterocolitis
NHS	National Health Service
PC	Principal Component
PI	Principal Investigator
PIL	Parent Information Leaflet
PIPP-R	Premature Infant Pain Profile – Revised
PMG	Project Management Group
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RMS	Root Mean Squared
SAE	Serious Adverse Event
SD	Standard Deviation
SSC	Skin-to-Skin Contact
SSNAP	Supporting the Sick Newborn And their Parents
STAI	State Trait Anxiety Inventory

4 Background and Rationale

4.1 Background

Newborn infants undergo painful procedures as part of routine postnatal care. Sick or premature infants experience an average of 10 painful procedures per day as part of life-sustaining treatment (Carbajal et al., 2008). Measuring pain in non-verbal infants is challenging, and few safe and effective analgesics have been tested and approved for use in infants. Non-pharmacological pain-relieving strategies have been introduced over the last few decades including sweet-taste solutions, breastfeeding and swaddling. Sweet-taste solutions such as sucrose are effective in relieving behavioural responses following minor painful procedures (Stevens et al., 2013). However, this commonly promoted pain-relieving strategy does not reduce noxious input to the brain (Slater et al., 2010a), causing concern that it may not mitigate long-term consequences of early life pain, and it may have long-term neurodevelopmental effects with repeated use (Ranger et al., 2019; Schneider et al., 2018; Tremblay et al., 2017). Breastfeeding also reduces behavioural and physiological responses to pain in full-term infants undergoing heel lancing, intramuscular injection, and venepuncture (Benoit et al., 2017). However, this strategy can be challenging for new mothers and is not always practical to implement, for example in premature and critically ill infants, in mothers with transmissible infections, and due to maternal concerns regarding potential negative impact on breastfeeding when it is coupled to a painful procedure (Tansky and Lindberg, 2010). Other comfort measures include swaddling and facilitated tucking of infants, which, although useful, are less effective in reducing pain (Meek and Huertas, 2012). Despite guidelines recommending the use of nonpharmacological interventions for pain relief, uptake of these practices is poor and inconsistent (Courtois et al., 2016; Losacco et al., 2011).

Untreated repetitive pain in neonates can cause short-term physiological instability as well as long-term neurodevelopmental consequences such as reduced growth, altered structural and functional brain development, and reduced school-age academic performance (Walker, 2019). Poor management of neonatal pain also has a negative impact on parents. Mothers of hospitalised infants report feeling emotionally and psychologically traumatised due to having to allow their infants to undergo clinically-necessary painful procedures, and due to feelings of helplessness from being unable to protect or comfort their child (Franck et al., 2005; Gale et al., 2004; Kyololo et al., 2019). Actively involving parents in care relieves parental distress (Franck et al., 2012) and increases the likelihood that infants receive treatment for pain (Carbajal et al., 2008; Johnston et al., 2011; Kyololo et al., 2019). Furthermore, there is evidence to suggest that parents of healthy infants are increasingly refusing clinically-recommended interventions such as vitamin-K intramuscular injections at birth (Nicholls and Southern, 2013) and immunisations (Harrington et al., 2000; Mills et al., 2005; Tickner et al., 2010) due to anxiety about pain. Avoidance of key interventions in early life could have drastic consequences for child health and this issue must be addressed. Indeed, parental anxiety and attitudes during painful procedures can impact neonatal distress and subsequent pain experience during clinical procedures in later life (Racine et al., 2016). Parental anxiety regarding pain could be alleviated by empowering parents to provide safe and effective pain relief for their child. Parental touch through stroking is an instinctive parental behaviour which may provide a simple parent-led intervention to alleviate procedural pain in infants by activating their innate C-tactile (CT) fibre system to naturally reduce noxious input reaching the brain.

4.2 Existing Evidence

4.2.1 Pain-relieving tactile interventions

Skin-to-skin contact (SSC) or kangaroo care (KC) is a comfort measure consisting of ventral skin contact of the newborn with the caregiver's chest. SSC has been shown to reduce behavioural and physiological indicators of infant pain to acute noxious procedures including heel lancing (de Sousa Freire et al., 2008; Gray et al., 2000; Johnston et al., 2003), venepuncture (Akcan et al., 2009), and

intramuscular injection (Chermont et al., 2009), and has been recommended for pain relief during blood sampling (Howard et al., 2008). A Cochrane review of 25 studies concluded that SSC is safe and effective in reducing physiological (heart rate) and behavioural (crying time) indicators of pain following clinically-required painful procedures (Johnston et al., 2017). However, the quality of evidence for an effect on acute pain response was low. Infant massage is another tactile comfort measure in which stroking and passive movements are performed at intervals (Field et al., 1986). Across a range of painful procedures including heel lancing, venepuncture and tape removal, infant massage has been shown to decrease facial expressions and heart rate responses in both term and premature infants (Abdallah et al., 2013; Bellieni et al., 2007; Chik et al., 2017; Diego et al., 2009; Hathaway et al., 2015; Jain et al., 2006; Zargham-Boroujeni et al., 2017). While many studies report the potential pain-relieving effects of tactile interventions in the context of minor painful procedures, these simple non-pharmacological interventions are scarcely used in maternity and neonatal units (Field et al., 2006) and the mechanisms underpinning the effectiveness of these interventions are still being established. Barriers to use likely include environmental issues, negative staff attitudes, lack of help from staff and limited awareness of the potential benefits (Seidman et al., 2015).

4.2.2 Stroking and C-tactile (CT)-fibres

Maternal touch behaviours are instinctive, evolutionarily conserved amongst mammals (Hertenstein et al., 2006) and enhance infant growth (Field et al., 2011) and development (Vickers et al., 2004). Stroking, by repeatedly applying gentle pressure to the skin, can activate CT fibres, a subclass of slow-conducting unmyelinated sensory neurons, mostly found on hairy skin (Liu et al., 2007; Vallbo et al., 1993, 1995). These fibres project to brain regions associated with affective processing such as the insular cortex, prefrontal cortex, superior temporal sulcus, and cingulate cortex (Bennett et al., 2014; Gordon et al., 2013; McGlone et al., 2012; Morrison et al., 2011a; Olausson et al., 2002) and are thought to have evolved to promote affiliative behaviours and social touch (McGlone et al., 2014; Morrison et al., 2011b; Olausson et al., 2010; von Mohr et al., 2017). CT fibres are optimally activated by stroking at a velocity of 3cm/s (optimal range 1-10cm/s) (Essick et al., 2010; Löken et al., 2009; Triscoli et al., 2014). In adults, activation of CT-fibres using gentle brushing or stroking paradigms has been shown to reduce pain. Stroking the skin at a CT-optimal rate prior to an experimental thermal pain stimulus reduces pain ratings compared to CT non-optimal touch (velocity 30cm/s) (Liljencrantz et al., 2017). Using EEG, a study has also demonstrated that CT optimal touch reduces noxious-evoked brain activity arising from laser stimulation, in addition to reducing pain ratings (von Mohr et al., 2018). CT-optimal stimulation therefore could provide a natural and safe pain-relieving intervention.

4.3 Clinical Measures of Pain in Infants

Pain assessment in infants primarily relies on measuring changes in infant behaviour in response to noxious events. In premature infants the most common validated clinical pain tool is the Premature Infant Pain Profile (PIPP) (Stevens et al., 1996, 2014). The PIPP primarily relies on the quantification of behavioural and physiological responses that are evoked by a painful procedure. As PIPP is a composite multimodal measure, incorporating measures of heart rate, oxygen saturation and facial expression change, it allows for different aspects of the infant pain experience to be captured. It is well-validated for pain assessment in premature infants and has been widely used as the primary outcome measure for infant pain in many clinical trials (Campbell-Yeo et al., 2012; Simonse et al., 2012; Taddio et al., 2008). If parental touch prior to a heel lance significantly reduces subsequent pain scores, there will be a strong rationale for its use in clinical practice.

4.4 Measures of Noxious-evoked Brain Activity

While behavioural measures are presently the gold standard for the assessment of neonatal pain, new electrophysiological techniques have more recently been developed to identify patterns of noxious-evoked brain activity (Slater et al., 2010c; Worley et al., 2012). This objective and quantifiable

neurophysiological measure has been used as the primary outcome measure in a clinical trial published in *The Lancet,* providing evidence to suggest that sucrose may not provide analgesia during clinical heel lancing (Slater et al., 2010a). It would be highly advantageous to use these electrophysiological measures of nociceptive brain and spinal cord activity in clinical trials investigating the efficacy of parental touch. Noxious-evoked patterns of brain activity have been extremely well-characterised following clinical heel lancing (Hartley et al., 2017; Slater et al., 2010c, 2010b; Worley et al., 2012). Heel lancing is a clinical procedure frequently performed in neonates to provide blood samples for the monitoring of jaundice, blood sugar, electrolytes and other haematological parameters.

4.5 Benefits and Risks

The benefit of this trial is that we hope to determine whether pre-emptive parental touch prior to a heel lance (through stroking) provides effective pain relief for acute procedural pain in neonates. Not only would effective pain relief make infants more comfortable during these procedures, but the provision of effective parental-led pain relief may prevent the long-term structural white matter damage and deficits in cognitive ability that have been directly linked to the number of painful experiences that hospitalised infants receive during their neonatal care (Vinall and Grunau, 2014).

Parental participation in this trial may also have a positive impact on parental wellbeing. The simple tactile intervention we propose could improve parental wellbeing by empowering them to take an active role in providing comfort and pain relief, reducing their anxiety and stress, increasing confidence (Skene et al., 2012) and addressing commonly experienced feelings of helplessness and lack of involvement (Kyololo et al., 2019). Unlike other non-pharmacological interventions, this strategy could be implemented regardless of feeding status of the infant or availability of a resource like sucrose.

Infant massage, a tactile comfort measure which involves patterns of stroking, has been shown to improve mother-infant bonding and improve postnatal depression (Onozawa et al., 2001), a condition affecting at least one in ten UK mothers in the first year post-delivery (Petersen et al., 2018). Furthermore, maternal stroking of infants has been shown to moderate the behavioural and physiological effects of maternal depression on infants (Sharp et al., 2012). Promoting parental stroking and exploiting this natural tactile behaviour in order to provide evidence-based pain-relief would be beneficial to both mothers and infants.

Greater parental involvement in comfort care can improve a family's experience of healthcare (Cooper et al., 2007) and strengthen the parent-physician relationship (Skene et al., 2012). Reducing parental anxiety regarding procedural pain could reduce parental refusal of clinically-necessary minor painful procedures such as blood tests and vaccinations, providing a considerable public health benefit.

4.6 Trial Justification

In the UK, more than 100,000 babies receive neonatal intensive care every year as a result of prematurity or illness (NICE, 2019). All newborns are exposed to clinically-necessary painful procedures. Even healthy neonates on postnatal wards can require repeated painful procedures beyond routine Newborn Screening, such as blood tests for glucose monitoring or jaundice, which can be distressing for both neonates and parents. As such, improving the management of pain is recognised as a top neonatal UK research priority (Duley et al., 2014) and a major concern amongst parents and neonatal nurses (Wielenga et al., 2015). This trial investigates a simple, free, low-risk, non-pharmacological pain-relieving intervention, which could be delivered by parents in partnership with physicians. Following evidence of clinical benefit, this intervention could be rolled out almost immediately through standard NHS clinical guideline adoption processes.

We previously conducted the only study of pre-procedural stroking for pain relief in neonates (Gursul et al., 2018), in which we demonstrated that CT-optimal stroking (at 3cm/s) prior to an experimental noxious stimulus or clinical heel lance significantly reduced noxious-evoked brain activity in term neonates compared to non- CT-optimal stroking. We replicated this study in an independent sample of term neonates and showed consistent results and a similar effect size in the group receiving the stroking intervention (Cobo et al., 2021). However, in this study stroking was delivered by the researcher using a soft experimental brush with a known force. Although the study did not identify a significant effect of the intervention on a behavioural pain score, it was notably not powered to investigate this. Considering CT-optimal stroking is a natural maternal behaviour and there is evidence to suggest CT fibres respond optimally to touch at human skin temperature (Ackerley et al., 2014), hands-on parental stroking has the potential to provide even greater benefit than CT-optimal brushstrokes. It is hoped that this clinical trial would validate the results of this small mechanistic study and crucially translate the experimental paradigm into a simple and effective hands-on parent-led intervention. Our pilot work demonstrates that stroking a neonate has similar efficacy to researcher-led experimental brushing.

4.7 Trial Description

This is a multicentre randomised-controlled interventional trial, with two research sites (John Radcliffe Hospital, Oxford, and Royal Devon and Exeter Hospital, Devon). In this study we aim to determine whether parental touch prior to a painful clinical procedure provides effective analgesia.

The primary objective is to determine whether parental touch of the limb prior to a clinically-required heel lance reduces noxious-evoked brain activity. In addition, our secondary and exploratory objectives include investigating the effect of touch on behavioural and physiological measures evoked by a heel lance. Neonates will be randomised to receive parental touch either prior to or post a clinically required heel lance. We will also investigate how parental touch impacts background brain activity and post-procedural clinical stability. We will lastly explore whether this intervention affects parental anxiety and distress.

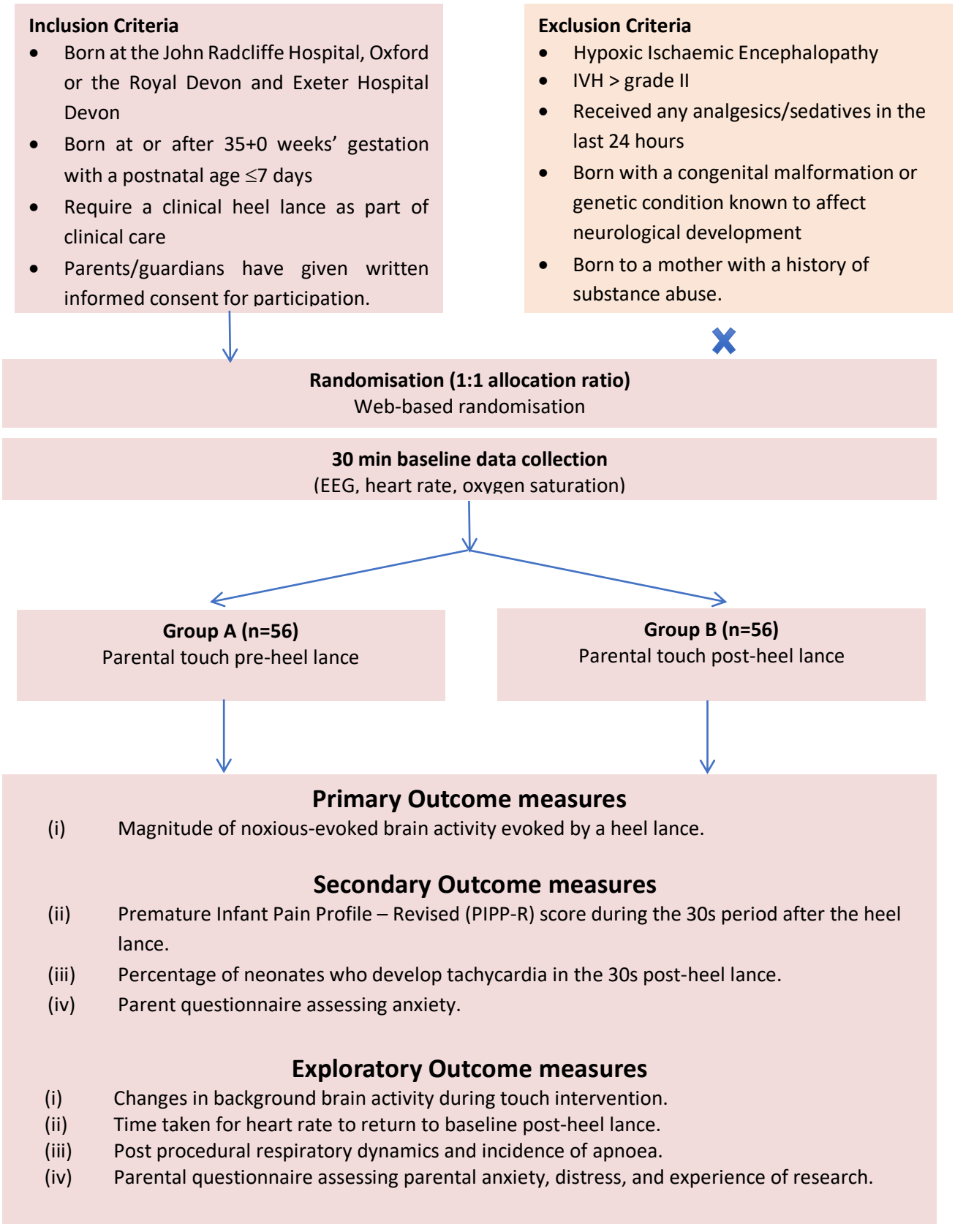
Participants will be recruited during an 18-month period. Participants will be studied on a single test occasion while they are in hospital, when they require a heel lance as part of routine clinical care. **No extra blood tests or noxious procedures will be performed for the purpose of the study.** A control heel lance (non-noxious sham procedure, described in more detail in section 7.6) will be administered to participants, and recording measures used for outcome measures include an EEG, pulse oximetry and facial video recording (described in more detail in section 7.9). Participants will be included in the study for approximately an hour period. This will be approximately 30 min before and after the time when the heel lance is performed. Each participant will only be included in the trial once. If a participant requires additional heel lances as part of routine clinical care, to acquire sufficient blood for clinical assessment at the time of the test occasion, we will also record the responses to this. The research monitoring equipment will not be removed from the participant between heel lances (if more than one heel lance is required for clinical purposes) in order to ensure we do not interfere with the clinical procedures.

5 Objectives and Outcome Measures

Objectives	Outcome Measures
Primary Objective	**Primary Outcome Measure**
(i) To test whether parental touch prior to the clinical procedure reduces noxious-evoked brain activity following a heel lance.	(i) Magnitude of noxious-evoked brain activity following a heel lance (EEG data recorded in the 1000ms period following each heel lance).
Secondary Objectives	**Secondary Outcome Measure**
(i) To test whether parental touch prior to the clinical procedure reduces clinical pain scores (PIPP-R) during the 30 second period after the heel lance.	(i) PIPP-R score during the 30s period after the heel lance.
(ii) To test whether parental touch prior to the clinical procedure reduces incidence of post-procedural tachycardia activity following a heel lance.	(ii) Percentage of neonates who develop tachycardia in the 30s post-heel lance.
(iii) To test whether parental touch prior to the clinical procedure reduces parental anxiety, compared with post-procedural touch.	(iii) Difference in STAI-S scores pre- and post-procedure.
Exploratory Objectives	**Exploratory Outcome Measures**
(i) To explore how parental touch impacts background brain activity.	(i) Changes in brain activity during touch intervention.
(ii) 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.	(ii) Time taken for heart rate to return to baseline post-heel lance.
(iii) To explore how parental touch prior to the clinical procedure affects respiratory stability.	(iii) Post-procedural respiratory dynamics and incidence of apnoea.
(iv) To explore parental anxiety and distress, and their experience of the trial and infant research.	(iv) Scores for individual parameters from the STAI-T and STAI-S; 4-point distress questionnaire score; responses to survey

	about participation in Petal and infant research.

5.1 Trial Flowchart



6 Participants

6.1 Eligibility Criteria

Inclusion criteria:

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

Exclusion criteria:

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

7 Trial Procedures

7.1 Recruitment

The research team will identify neonates who are eligible for inclusion in the study shortly after birth, against the inclusion and exclusion criteria. They will then inform the neonate's parents about the study.

7.2 Informed consent

Written and verbal versions of the Parent Information leaflet and Informed Consent form will be presented to the parent(s) detailing no less than: the exact nature of the study; what it will involve for the parent and participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the parent/participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The parent(s) will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, the clinical team or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of parent-dated signature and dated signature of the person who presented and obtained the Informed Consent. Either parent (with legal parental responsibility) may provide written informed consent. The parent providing informed consent will be the parent administering the intervention; this will be specified on the Consent Form. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator. In Oxford, a copy of the signed Informed Consent will be given to the parent(s), a copy retained at the study site and the original signed form will be placed in the participants' medical notes. In Exeter, since the patient's notes are electronic, a copy of the signed Informed Consent will be given to the parent(s), the original will be retained at the study site and an electronic copy will be placed in the participants' medical notes.

Parents who do not speak English will only be approached if an adult interpreter is available. Relatives will not interpret.

Parents will also be asked to consent to being contacted about participation in additional studies in the future; if consent is given parental contact details will be collected for this purpose. Parents will also be asked to consent to images/video footage collected during the study being used for publications and presentations. These aspects of the Consent Form will be optional and will not affect the parents' rights for their child to participate in the original study. Declining these optional additional consent items will not affect the care of the neonate.

7.3 Enrolment

Each approach will be documented onto an Enrolment Log, where it will state whether parents provided informed consent. After informed consent has been obtained, the participant will be allocated a study number. The participant will then be randomised (see section 7.4), at which point they will be considered included in the sample size.

7.4 Randomisation

Randomisation of participants to receive parental touch either before or after a clinically-required heel lance will be managed via a secure web-based randomisation facility provided by Sealed Envelope. Participants will have a roughly equal chance of being allocated to either arm. The randomisation program will use a minimisation algorithm to ensure approximate balance between the groups with respect to post-menstrual age at birth, post-natal age at time of randomisation, sex, the indication for blood sampling, and centre. The users of the system will have no insight into the next allocation.

Participants will be randomised as soon as parental consent has been provided.

7.5 Blinding

This will be a single-blinded study. As parents are key to implementing the intervention, they cannot be blinded. The researchers conducting the test occasion similarly cannot be blinded as they will be present and observe the timing of the intervention. However, the research team members responsible for outcome assessment will be blinded to arm allocation.

When the study has officially ended and data analysis is complete, the randomisation code will be released to the primary researchers so that the treatment groups can be correctly identified.

7.6 Trial Design Summary

Each participant will participate in the trial for approximately one hour and will not require further follow-up. The research team will liaise with the clinical team to identify when the clinically-required heel lance is needed, and will time the test occasion around this event. Participants will be randomised to receive parental touch either before, or after, the clinically-required heel lance.

At the time of the heel lance, a control heel lance and clinical heel lance will be performed. The control heel lance is a sham procedure. It is not a blood test and not noxious. The lancet is placed against the baby's foot but angled away – upon release, the sharp is fired into the air rather than into the foot. This is to simulate the experience of having a blood test without the noxious input – i.e. the baby's foot being held by the clinical researcher, the feel of the lancet placed against the foot, and the click sound when the button is pressed and sharp released. Brain activity is recorded in response to the control heel lance in addition to the clinical heel lance, to ensure that we are assessing responses to the noxious aspect of having a blood test. This is an important validity check for the integrity of our recording measures.

All outcome measures will be recorded for both the clinical heel lance and control heel lance stimulus.

The parental touch intervention will involve one parent stroking the lower limb of their baby for 10 seconds before, or after, the clinically-required heel lance. When the intervention is conducted before the heel lance, it will be commenced 10s prior to the heel lance. When implemented after the heel lance it will commence after the start of blood collection (minimum of 30s post heel lance), when deemed appropriate by the clinician performing the heel lance, to ensure that blood collection is not disrupted.

At least 30 minutes before the heel lance and control stimulus (and after randomisation), the research team will set up the electroencephalography (EEG), physiological monitoring (electrocardiogram (ECG) – for heart rate and respiratory rate; pulse oximeter – for oxygen saturations), and video monitoring (for facial expression change). Physiological monitoring will continue from approximately 30 minutes prior to the heel lance and control stimulus until approximately 30 minutes afterwards. Noxious-evoked brain activity (measured using EEG) will be recorded for a minimum of 10 minutes prior and 10 minutes after the heel lance and control stimulus. Video monitoring will continue from approximately 30 seconds before to 30 seconds after the heel lance and control stimulus. The neonate's foot will be held by the clinical researcher during the video recording for PIPP-R scoring, the control heel lance stimulus and the heel lance. The foot will be held in the same manner in both trial arms.

Clinical stability of the neonates will be assessed throughout the hour trial period using the physiological recordings. These measures will be calculated from ECG recordings to monitor heart rate and respiratory rate. These data will be monitored and recorded on our data logging equipment for approximately 30 minutes before and 30 minutes after the heel lance and control stimulus.

7.7 Intervention

Approximately 30 minutes prior to the clinical procedure, the research team will explain to the parents how to administer the intervention correctly. Parents will be told which group their baby is in and therefore when the touch intervention should be administered. Where appropriate, the research team will demonstrate on a doll the speed (3cm/s), location (lower limb of the leg where the heel lance will be administered), and duration (10 seconds) of the touch intervention; they will explain that this should involve the whole hand and that strokes should be in one direction down the limb towards the foot. During the intervention demonstration and the test occasion, PsychoPy software (Peirce et al., 2019) will be used: this software provides visual cues on a computer screen to guide a consistent speed of stroking at 3cm/s for a 10 second duration.

After the demonstration from the research team, the parent wishing to administer the intervention will be asked to demonstrate this on their own arm. The research team will observe and ensure that this is done correctly before proceeding. During the test occasion the research team will also continue to guide the parent as necessary. If parents require additional guidance during the stroking intervention, the guidance given will be documented.

7.8 Heel Lance Procedure

All heel lances performed will be clinically-necessary and requested for blood collection purposes by the clinical team responsible for the neonate's care. In the event of a neonate requiring multiple heel lances, data will only be included from the first heel lance (conditional on data quality). The heel lance and control stimulus will be linked electronically to the recording equipment, using methods that have been used in previous studies (Slater et al., 2010c, 2010a; Worley et al., 2012). This methodology provides an opportunity to record the precise timing of when the heel lance occurs without interfering with clinical practice.

7.9 Recording Techniques

We will use a range of techniques to characterise the responses to noxious stimulation, and to collect data on parental experience:

Recording Methods	Measurement	Relevant Outcome Measure	Outcome type
EEG	Brain activity evoked by heel lance	Magnitude of noxious-evoked brain activity	Primary
	Brain activity evoked by non-noxious heel lance control	Magnitude of brain activity evoked by heel lance control	Quality Check
	Brain activity during stroking intervention	Changes in background brain activity associated with parental touch	Exploratory
Physiological monitoring (ECG and Pulse Oximetry)	Heart rate	PIPP-R score Clinical stability	Secondary Exploratory
	Respiratory rate	Clinical stability	Exploratory
	Oxygen saturations	PIPP-R score	Secondary
Video recording	Facial expression change	PIPP-R score	Secondary
Parent questionnaire	Parental anxiety	Difference in STAI-S scores pre- and post- procedure STAI-T, STAI-S scores	Secondary Exploratory
	Parental distress	4-point questionnaire score	Exploratory
	Parental experience	Parental experience of trial and research	Exploratory

7.9.1 Electroencephalography (EEG)

Electrophysiological activity will be acquired with the SynAmps RT 64-channel headbox and amplifiers (Compumedics Neuroscan) or with the Compumedics Grael V2 EEG system, with a bandwidth from DC - 400 Hz and a sampling rate of 2000 or 2048 Hz. CURRYscan7 or CURRYscan8 neuroimaging suite (Compumedics Neuroscan) will be used to record the activity. All equipment will conform to the electrical safety standard for medical devices, IEC 60601-1. Eight EEG recording electrodes will 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 will be placed at Fz and the forehead respectively. EEG conductive paste will be used to optimise contact with the scalp. All impedances will be reduced to approximately 5kΩ by rubbing the skin with EEG preparation gel prior to electrode placement. An ECG electrode will be placed on the left clavicle to record heart rate and respiration.

7.9.2 Physiological Monitoring (ECG and Pulse Oximetry)

Heart rate, respiratory rate and oxygen saturation will be measured throughout the one-hour study period using ECG and pulse oximetry and downloaded to a computer to assess clinical stability. Heart rate and oxygen saturation data will be used to calculate the clinical pain scores following the heel lance and control stimulus and to assess clinical stability across the hour test occasion. Data will be

analysed offline, and episodes of artefact (i.e. neonate handling or essential clinical procedures) will be documented throughout the recordings.

7.9.3 Video Recording

Video recording will be used to measure behavioural responses i.e. changes in facial expression during the control stimulus and clinically-required heel lance. Video recordings will be performed with a handheld digital video camera (Sony RX100). Video recordings will be performed by research personnel who are specified on the delegation log. The video footage will be transferred to the secure trial electronic database as specified in Section 10.2. A synchronised LED flash will be activated by the researcher simultaneously with each stimulation as a marker for the time of stimulation.

If parents have provided optional additional consent for videos/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. Still images will not be taken during the test occasion for any outcome measures.

7.9.4 Parental Questionnaire

The parent administering the stroking intervention will be asked to complete a short series of validated electronic questionnaires assessing anxiety and distress at the specific time points in the test occasion (see Table 4).

Questionnaire section	Topic	Timing of administration	Questionnaire administrator
20-point State-Trait Anxiety Inventory (STAI)-T	Trait anxiety	Start of test occasion	Administered verbally by researcher
20-point State-Trait Anxiety Inventory (STAI)-S	State anxiety pre-heel lance State anxiety post-heel lance	Start of test occasion After the procedure and intervention are completed	Administered verbally by researcher Administered verbally by researcher
4-point distress questionnaire	Emotional constructs experienced at time of the clinical heel lance	After the procedure and intervention are completed	Administered verbally by researcher
Anonymous survey	Views on the trial and infant research	End of test occasion	Completed by parent

The researcher will directly record the responses to the STAI-T, STAI-S and distress questionnaire into the relevant electronic Case Report Form on the research database. The electronic device will then be presented to the parent for a short survey about participating in this study and general infant research, which the parent will be invited to complete and submit their answers before returning the electronic device to the researcher. The survey will be completed anonymously, responses will be stored by trial arm with no link to study IDs.

The completion of parental questionnaires should take approximately 15 minutes in total.

7.9.4.1 STAI

The STAI is the gold standard assessment for state anxiety (Spiegelberger et al., 1983) and it is well validated. The STAI has a trait (STAI-T) and a state version (STAI-S). The STAI-T scale consists of 20 statements exploring general feelings of anxiety. The STAI-S scale also consists of 20 statements and

requires subjects to rate their anxiety at a particular moment in time. The STAI-S scale can be used to determine the actual levels of anxiety induced by stressful procedures. Each question is rated on a 4-point scale (not at all, somewhat, moderately so, very much so). The scores range from a minimum of 20 to a maximum of 80 on both the STAI-T and STAI-S subscales.

7.9.4.2 4-point distress questionnaire

Parents will be asked four questions related to the emotions they felt during the clinical heel lance procedure (Caes et al., 2011; Vervoort et al., 2019). Each of the four emotional constructs (worried, upset, anxious and sad) will be rated on an 11-point scale ranging from "not at all" (0) to "extremely" (10). A total score between 0 and 40 will be calculated, where higher scores are indicative of higher levels of parental distress.

This score has been selected because it is frequently used by researchers who are interested in parent/child interactions during painful procedures.

7.10 Outcome Measures

7.10.1 Noxious-evoked Brain Activity

Noxious-evoked brain activity following a clinical heel lance has been well characterised in previous studies (Hartley et al., 2017; Slater et al., 2010a, 2010c; Worley et al., 2012). In this study we will investigate whether parental touch administered prior to a clinically-required heel lance reduces the noxious-evoked brain activity compared to no intervention prior to the lancing (and parental touch afterwards). An EEG template based on the Principal Component (PC) that reflects the noxious-evoked brain activity in neonates has been defined and validated in independent data sets (Hartley et al., 2017). This template will be projected onto the EEG data recorded in the 1000ms period following each heel lance and heel lance control stimulus and the relative weight of the component calculated for each neonate. A greater weight indicates a stronger noxious-evoked response. While the brain activity characterised here is directly related to noxious input, it does not reflect all noxious-evoked activity that takes place across the brain or all aspects of the pain experience. The response to the heel lance control stimulus is being recorded so that we can confirm that it differs significantly from the brain activity evoked by the heel lance. This forms an important data quality control check (Slater et al., 2010c).

7.10.2 PIPP-R Score

Clinical pain scores will be calculated using the validated Premature Infant Pain Profile - Revised (PIPP-R) (Stevens et al., 2014). PIPP-R is a composite measure encompassing behavioural, physiological and contextual indicators involved in the pain response. These include gestational age, behavioural state, heart rate, oxygen saturation, and duration of brow bulge, eye squeeze, and nasolabial furrow. Each indicator in the PIPP-R is rated on a 4-point scale (0, 1, 2, and 3); these are summed together to produce a maximum possible score of 21. In the revised PIPP-R, the scores for the contextual indicators (gestational age and behavioural state) are only included if a non-zero score is recorded for either the physiological or behavioural variables (Stevens et al., 2014).

PIPP-R score will be calculated on two occasions: (i) during the heel lance control procedure; and (ii) during the heel lance procedure. Videos of facial expression will be recorded throughout the heel lance and control stimulus and scored offline from single frames using the PIPP-R facial coding system. Changes in heart rate and oxygen saturation will be downloaded from the pulse oximeter and used to calculate the PIPP-R score. Heart rate, oxygen saturation and facial expression will be recorded in the 15-second period before and 30-second period after the heel lance and heel lance control (Stevens et al., 1996, 2014). In neonates randomised to pre-procedural stroking, the 15-second baseline period of

assessment for the PIPP-R score will be recorded prior to the stroking intervention, which will be immediately followed by the heel lance.

20% of PIPP-R scores will be re-assessed by two blinded investigators to estimate the inter-rater and intra-rater reliability.

7.10.3 Clinical Stability

Clinical stability will be assessed in the 30 min period before and after the heel lance. Measures will include:

Secondary outcome measure

- Percentage of neonates who develop post-procedural tachycardia (in the 30 seconds post-heel lance)

Exploratory outcome measures

- Time taken for heart rate to return to baseline values after the heel lance
- Respiratory dynamics and incidence of apnoea in the 30 min pre- and post- heel lance

Tachycardia will be defined as a heart rate >160 beats per minute as per Advanced Paediatric Life Support guidelines, reflecting heart rate values >90th centile for newborns in the first week of life (Fleming et al., 2011; Schwartz et al., 2002). An episode of apnoea will be defined as the cessation of breathing on impedance pneumography for at least 20s (Adjei et al., 2021).

7.10.4 Parental Experiences

Secondary outcome measure

- Difference in Anxiety scores from 20-point STAI-S pre- and post-procedure

Exploratory outcome measures

- Parental experience of their child's heel lance, general state, and views on infant research, using:
    - Individual parameters from the STAI-T and STAI-S
    - Scores for emotions experienced during the heel lance from the 4-point distress score
    - Responses to survey about participation in trial and infant research

7.11 Unblinding

An unblinding procedure is not required as this is a single blind study where the research team administering the study and the parent applying the intervention will be aware of arm allocation.

7.12 Withdrawal

Parent(s) may withdraw their neonate from the trial at any time and they are not obliged to give a reason. If parents choose to withdraw their child after the study has begun, they will be asked whether data already collected may be retained and used for the purposes of the trial. Parents will be made aware that this decision has no impact on any aspects of their infant's continuing care.

The attending clinician may withdraw the neonate from treatment if they consider this to be in the best interest of the neonate's health and well-being. If any of the exclusion criteria manifest after consent but prior to data collection, the participant will be withdrawn.

7.13 Definition of the End of the Trial

The end of the trial will be defined as the date six months after the recruitment of the last participant. The trial database will be locked when recruitment is completed. An end of trial declaration will be made to the approving REC and HRA.

8 Safety Reporting

8.1 Definitions

SAEs are defined 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
- is otherwise considered medically significant by the investigator.

8.2 Recording SAEs

SAEs occurring during the one-hour trial period will be recorded using a trial SAE form, including the following information: description, time and date of onset, severity, assessment of relatedness to trial procedures (medical investigator only), other suspect drugs or devices and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe. SAEs considered related to the research activities as judged by a medically qualified investigator or the Sponsor will be followed up either until resolution, or the event is considered stable.

8.3 Causality

The relationship of each SAE to the research activities must be determined by a medically qualified individual according to the following definitions:

Unrelated – where an event is not considered to be related to the research activities.
Possibly – although a relationship to the research activities 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 activities.
Definitely – the research activities are the most likely cause.

All SAEs labelled possibly, probably or definitely will be considered as related to the research activities. There are no expected or foreseeable SAEs in this population or with this intervention.

8.4 Reporting Procedures for SAEs

SAEs can be reported by anyone, however they must be assessed for causality by a medically qualified investigator. There are no expected SAEs in this trial, so all reported SAEs will be considered 'unexpected'. The SAE CRF includes these assessments. SAEs will be reported to the CI within 24 hours of the research site becoming aware of the event. The individual reporting the SAE should telephone the Oxford team to report the SAE, and then send a scan of the SAE form to the Oxford team by secure email. The information should also be entered onto the electronic CRF on the research database.

All SAEs will also be assessed by the CI/Safety Delegate. 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. This will be reported according to current REC/HRA guidance (at present this is using an SAE form for non-CTIMPs, available on the HRA website).

8.5 Urgent Safety Measures

An urgent safety measure is a procedure which is not defined by the protocol that can be put in place with immediate effect without needing to gain prior authorisation by the REC, in order to protect clinical trial participants from any immediate hazard to their health and safety. In the event of an urgent safety measure the CI will inform the REC immediately by telephone, and in writing within three calendar days (setting out the reasons for the urgent safety measures and the plan for further action). The CI will also inform the Sponsor, host organisation and PIs.

9 Statistics and Analysis

9.1 Sample Size Determination

Approximately 6500 infants are born at the John Radcliffe Hospital each year and approximately 4000 per year at the Royal Devon University Healthcare NHS Foundation Trust. These infants frequently require heel lances within the first seven days of life for indications including Newborn Screening, jaundice, suspected sepsis, and blood sugar monitoring. For recruitment to be completed in 9 months, approximately 15 babies need to be recruited to the trial per month (allowing for loss to follow-up etc.). This would be achievable if 3-4 neonates were recruited per week from a single centre, or 2 neonates were recruited per week from each of the two centres. There will therefore be a sufficient number of eligible infants and the recruitment plan is highly feasible.

9.2 Power Calculation

The assumptions for these calculations are based on data from mechanistic studies investigating the effect of (experimenter-led) soft brushing of the skin at CT-optimal rate on the response to noxious stimulus or clinical heel lance in term neonates (Cobo et al., 2021; Gursul et al., 2018).

The mean (SD) brain activity evoked by heel lancing in the control group is estimated to be 1.07 (0.66). A 40% reduction in the intervention group is considered to be clinically significant and realistic from other studies, and using other interventions (Gursul et al., 2018; Hartley et al., 2018). With 90% power and a two-sided 5% significance level, to observe a 40% reduction in brain activity, a sample size of 102 would be required. Allowing for 10% loss due to technical difficulties or clinical issues, this increases to 112.

9.3 Significance Levels

For the analysis of the primary outcome measure, a P-value of 0.05 (two-sided 5% significance level) will be used to indicate statistical significance. Comparisons of all other outcomes will be reported in full for completeness and transparency. Significance levels for secondary outcomes (excluding the sensitivity analysis) will be corrected for multiple comparisons and the method will be specified in the statistical analysis plan. Two-sided statistical tests and corresponding P-values will be presented throughout; however, for the purposes of interpretation of results, confidence intervals will dominate, rather than P-values.

9.4 Missing Data

Missing data may occur in our trial. For example, this could be due to equipment failure, artefacts within the EEG recording, or clinical issues if for example the heel lance is deemed clinically

inappropriate after a participant has been randomised. To account for missing data we have inflated our sample size by 10%. The analysis will be conducted using the available data.

9.5 Analysis of outcome measures

The analysis and presentation of results will follow the most up-to-date recommendations of the CONSORT group. The primary results will be presented unadjusted. To perform sensitivity analysis, the minimisation variables will be used to make statistical adjustments to the primary analysis and the sensitivity analysis results will be presented as secondary results. A statistical analysis plan will be finalised before any comparative analyses are performed. Where the trial protocol and the statistical analysis plan differ, the analysis will follow the statistical analysis plan.

MatlabR2020a or an updated version will be used for statistical analysis.

9.5.1 Primary

9.5.1.1 Noxious-evoked brain activity

The magnitude of noxious-evoked brain activity will be compared between the two groups using a parametric two-sample t-test if the residuals are normally distributed. If the residuals are non-normally distributed, a Wilcoxon rank-sum test will be used. If appropriate, and depending on the distribution of residuals and the test used, we will present the mean and standard deviation or the median and IQR (or entire range, whichever is appropriate) for each group. We will estimate the unadjusted mean or median difference between groups with a corresponding 95% confidence interval. P-values will also be reported as described in section 9.3.

9.5.2 Secondary

9.5.2.1 PIPP-R score during the 30 second period after heel lance

PIPP-R scores (during the 30 second period after a heel lance) in the two groups will be compared using a two-sample t-test if the residuals are normally distributed. If the residuals are skewed, a Wilcoxon rank-sum test will be used. If appropriate, and depending on the distribution of residuals and the test used, the mean and standard deviation or the median and interquartile range (or entire range, whichever is appropriate) will be presented for each group, and the unadjusted mean or median difference between groups with a corresponding 95% confidence interval will be reported. P-values will also be reported as described in section 9.3.

9.5.2.2 Clinical Stability Analysis (Tachycardia)

The tachycardia outcome per infant will be dichotomous (i.e. 'yes/no' per infant). The percentage of neonates experiencing tachycardia will be compared between the parental pre-procedural stroking group and post-procedural stroking group using a logistic regression. If appropriate, adjustment for the 30 min baseline period will also be made. We will report the proportion of tachycardia for each group, as well as the difference in proportions between the groups.

9.5.2.3 Parental anxiety

The difference between pre-procedural and post-procedural STAI-S scores will be compared between the two arms using a two-sample t-test if the residuals are normally distributed. If the residuals are skewed, a Wilcoxon rank-sum test will be used. If appropriate, and depending on the distribution of residuals and the test used, we will present the mean and standard deviation or the median and interquartile range (or entire range, whichever is appropriate) for each group, and the unadjusted mean or median difference between groups with a 95% confidence interval. P-values will also be reported as described in section 9.3.

9.5.3 Exploratory

Exploratory analyses will be conducted to investigate:

- How parental touch impacts brain activity
- If parental touch prior to the clinical procedure reduces the duration of time for heart rate to return to baseline after a clinically-essential heel lance, compared with post-procedural touch
- How parental touch prior to the clinical procedure affects variability in respiratory rate, incidence of apnoea, and change in respiratory stability within the duration of the study
- The parental experience of their child's heel lance, general state, and feelings on infant research.

10 Data Management

10.1 Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, video footage acquired for the purpose of the trial, physiological data (e.g. EEG recordings and other physiological data) and correspondence. Baseline medical (e.g. age), environmental (e.g. medical ward where study was conducted), demographic (e.g. post code) and social (e.g. ethnicity) information will be recorded from the medical notes.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions.

10.2 Data Handling and Record Keeping

The CI will take overall responsibility for ensuring that each participant's information remains confidential. All records will be stored securely and kept in strict confidence in compliance with data protection regulation. All data handling will be conducted in accordance with the University of Oxford, the John Radcliffe Hospital and the Royal Devon and Exeter Hospital data protection requirements. Paper CRFs, Consent Forms and Parent Contact Details forms will be stored on university property in locked filing cabinets (Oxford), and Trust property in the locked research office (Exeter). At the Oxford site, paper records are stored in a secure area of the John Radcliffe Hospital with swipe card access, and individual rooms are kept locked when unoccupied. At the Exeter site, offices where paper records are stored are in a secure area with a locked door, swipe card access and behind a security desk. Original Consent Forms will be transferred in person from the Exeter PI to the Oxford PI. Raw data which is not entered onto the database (e.g. EEG traces) will be transferred from the Exeter team to the Oxford team by a secure shared drive with restricted access. The parental responses to questions about infant research will be completed anonymously using Jisc (password-protected, only members of the research team will have access); results will be saved by trial arm on the secure shared drive with restricted access.

Data from paper CRFs, and direct parental responses from the 20-point STAI and 4-point distress questionnaire, will be entered onto an electronic database on a restricted area of a file server. An Internet-accessed, secure, central database is supplied by MedSciNet to perform the study. The database was designed using Microsoft.NET Framework and Microsoft SQL Server technologies to provide data storage. The database has high security systems to meet the regulations, including password protection, tracking of all personnel who access the database, and a full audit trail of all data changes. The levels of access to this database are restricted according to user identity and role within the study. The database also has built in checks to reduce errors. The database is approved and

validated according to regulations in the EU and USA. The IT-solution created complies with GCP and predicates rule requirements, laws, as well as regulations for clinical trial conduct and Food and Drug Administration FDA CFR 21 part 11 for electronic record and signature use. The IT-solution will also be compatible with HIPPA, NIH and HL7 as well as the European (CENTC-251) recommendations and requirements. Anonymised video files are stored on a password-protected hard drive and linked through the database; these will be identified using only the participant's study number.

A separate electronic database is used for the participant's name and contact details of the parents. This is an internet-accessed database also supplied by MedSciNet. This database has separate password and login details from that which is used for the research data. Only members of the research team have access to this database.

Data will be processed on a workstation by authorised staff. All desktop and laptop computers are password protected. Backing up of data will take place at least monthly.

The University of Oxford, as per their requirements for paediatric studies, will keep essential documents for the time period of 3 years after the youngest subject reaches 18 years old or 5 years after the last participant has completed the study, whichever is longer. Research data will be stored for a period of no less than 21 years in order to follow up health related issues that may become relevant in the future. After the trial has been completed and the reports published, the data will be archived in a secure physical or electronic location with controlled access.

11 Quality Assurance Procedures

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. The CI and PIs, with the support of the Project Management Group (PMG) will be responsible for the day-to-day smooth running of the trial at the site. They will encourage recruitment, provide staff education and training, and monitor data completeness and quality.

11.1 Monitoring

The PMG will meet regularly (approximately monthly) to discuss the progress of the trial. The core PMG will consist of the following: CI, PIs and Trial Manager. Where necessary, the extended PMG will meet and will include the following in addition to the core PMG: PPI representative, member(s) from the recruitment and data acquisition team, member(s) from the data management team, statistician, and any other invited clinical or research individual.

11.2 Patient and Public Involvement (PPI)

The research team will work closely with the on-site local Oxford charity Supporting the Sick Newborn And their Parents (SSNAP) during the design, conduct and dissemination of the trial. The charity's remit is to provide family support for parents with infants on the newborn care unit and to support neonatal medical research. SSNAP have reviewed all parent-facing materials, will also review manuscripts for reporting results, and will be involved in disseminating results to the public once the trial is complete.

Bliss: for babies born premature or sick, a national UK charity promoting better care and improved quality of life for babies who are born premature or sick, are partially funding this trial. They will also be involved in promoting the trial across their various channels, as well as dissemination of results. The PMG will regularly report back to Bliss with trial updates.

A PPI member will also be included in the extended PMG group and will be invited to join specific PMG meetings to discuss trial progress and developments.

12 Serious Breaches

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree:
(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial".

The research team will notify the REC, Sponsor and host organisation of any serious breaches within seven calendar days of becoming aware of the event.

13 Ethical Considerations

13.1 Safety

EEG is a safe tool used routinely in clinical practice to measure brain activity. The electrodes are temporarily placed on the baby's head for the duration of the clinically required procedure. The electrodes are non-invasive and are not fixed in place with glue. Possible risks to the baby are skin irritation or reaction to the conductive paste used to apply the electrodes to the skin.

13.2 Clinical Care

The heel lance observed in this study will be clinically required and will be performed as a part of clinical care; this will not be performed solely for the purpose of the study. The control stimulus does not break or damage the skin. There will be no changes made to the routine clinical care of the baby. Every effort will be made to minimise inconvenience or intrusion. There are no additional risks to the neonates participating in this study. No additional drugs (other than those clinically required) will be given to the participants of the study.

13.3 Participant Confidentiality

The research team will ensure that the participants' confidentiality is maintained. The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be anonymised as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique nine-character study number only on all study documents and any electronic database(s). Identification may be possible from hospital numbers, but only through using secured password-protected hospital computers. All information, videos and results will be kept strictly confidential. Patient identifiable data will not be published, other than videos/images where explicit additional written consent has been provided from the parent (as described in section 7.2).

13.4 Expenses

Neither trial participants nor their parents will be given financial or material incentive or compensation to take part in this trial.

14 Regulatory

14.1 Declaration of Helsinki

The CI will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

14.2 Guidelines for Good Clinical Practice

The CI will ensure that this study is conducted in full conformity with relevant regulations and with GCP guidelines.

14.3 Approvals and Reporting

Following Sponsor approval the protocol, informed consent form and participant information sheet will be submitted to an appropriate Research Ethics Committee (REC), and HRA (where required) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

A summary of the final report (as required by the REC) will be submitted within one year of the conclusion of the research. The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

14.4 Transparency in Research

Prior to the recruitment of the first participant, the trial will be registered on a publicly accessible database. Trial information will be kept up to date on the multiple public platforms on which it has been registered, and the CI or their delegate will upload results to public registries within 12 months of the end of the trial declaration.

15 Finance and Insurance

15.1 Funding

The Wellcome Trust and the charity Bliss: for babies born premature or sick are funding the trial.

15.2 Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment which is provided.

15.2.1 Contractual Arrangements

Appropriate contractual arrangements will be put in place with all third parties.

16 Publication Policy

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the Wellcome Trust funded the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

The success of the trial depends on a large number of neonatal nurses, neonatologists, and parents. Credit for the study findings will be given to all who have collaborated and participated in the study.

At the time of recruitment, parents will be directed to the Paediatric Neuroimaging website for trial updates and results once available; this will include a reference to the full publication. A copy of the journal article will be available on request from the PI.

17 Development of a new product/process or the generation of intellectual property

Not applicable.

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19 Appendix A: Amendment History

Amendment No.	Protocol Version	Date Issued	Author(s) of Change	Details of Changes
1	2.0	03-08-2021	Rebeccah Slater	Amendments following ethics committee issue of a favourable opinion with additional conditions
2	3.0	11-11-2021	Fiona Moultrie, Maria Cobo, Annalisa Hauck	Minor amendments for 1) addition of ISRCTN Number 2) clarity, 3) consistency across the protocol, 4) correction of typographical errors 5) Exeter site contact number updated
3	4.0	15-03-2022	Maria Cobo	Minor amendments for 1) extension of planned recruitment period (primary completion date: the date on which the last participant in the trial will be studied) until 1 March 2023, 2) correction of the end of the trial definition (section 7.13). The end of the trial date will be 6 months after the completion of the recruitment.

4	5.0	26-01-2023	Annalisa Hauck Maria Cobo Vaneesha Monk	Clarity and further detail about Consent Form storage, enrolment status on the trial, and technical information for EEG recording. Correction to state hypoxic ischaemic encephalopathy as an exclusion criterion. Further detail of planned statistical analyses and confirmation that a separate statistical analysis plan document will be used. Updated name of Sponsor office and email. Updated Trust name for the Exeter site. Correction of typographical errors. Addition of references.