**1. Summary of methods**

**Experimental design**

Brain activity in response to 3 stimuli (noxious heel lance, sham control, and auditory control) was monitored using electroencephalography (EEG). All stimuli were performed using a lancet and time-locked to the ongoing EEG recording (Worley et al., 2012). Behavioural and physiological measures were also collected as part of the Premature Infant Pain Profile (PIPP). A more detailed description of these procedures has been published elsewhere (Jones et al., 2017; Slater et al., 2009, 2010). Infant pain history and relevant infant and maternal medical information was then collected by a research nurse.

**Stimulation**

The noxious stimulus was a heel lance that was clinically required to collect a blood sample. The heel was cleaned with sterile water using sterile gauze and the site allowed to air dry. The lancet was placed against the heel for at least 30s prior to the release of the blade. This was to obtain a baseline period free from other stimulation in order to identify evoked changes using EEG and the PIPP. The heel was then gently and intermittently compressed 30s after the release of the blade, again to ensure a post-stimulus period free from other stimuli. Babies were soothed as and when required. The control stimulations (sham and auditory) followed the same procedure as the heel lance, however the blade was facing away from the heel upon its release (sham), or the lancet was not touching the foot at any point (auditory – due to the audible click that is produced following the release of the blade).

**Electroencephalography**

EEG was recorded from up to 20 electrodes (disposable Ag/AgCl cup electrodes) in addition to the ground and reference electrodes. Recording electrodes were positioned according to the modified international 10/10 electrode placement system, with high density central-parietal and posterior temporal coverage, overlying primary visual (O1, O2,), primary auditory (T7, T8), association (F7, F3, F4, FCz, F8, P7, P8, TP9, TP10, POz), and somatosensory cortices (C3, Cz, C4, CP3, CPz, CP4). Reference and ground electrodes were respectively placed at Fz and FC1/2. ECG was recorded from both shoulders and respiratory movement was monitored with an abdominal transducer. Electrode/skin contact impedances were kept to a minimum by gently rubbing the skin with a prepping gel and then applying the electrodes with a conductive paste. A soft bonnet was then secured over the electrodes. EEG activity, from DC to ≥500 Hz, was recorded using the Neuroscan SynAmps2 EEG/EP recording system. Signals were digitised with a sampling rate of 2 kHz and a resolution of 24 bit.

The EEG recordings were converted into EEGLAB data structure and segmented into 4 second epochs (2 seconds pre- and post-stimulus) using MATLAB (MathWorks) and EEGLAB (Swartz Center for Computational Neuroscience). No pre-processing was performed except for one file (253501L01) which was downsampled from 5 kHz to 2 kHz using EEGLAB. Each file contains the data of the EEG electrodes, ECG, and respiration, with a trigger (denoted with “4” or “8”) indicating the onset of the stimulus at 0s. These can be found in the parent folder “EEG” in the related Figshare item.

**Premature Infant Pain Profile (PIPP)**

A PIPP score was calculated for each of the stimulations by combining behavioural (3 facial expressions) and physiological measures (heart rate and blood oxygen saturation) (Stevens et al., 1996). Infant facial behaviour was recorded on video with an LED light placed within the frame. When the blade from the lancet was released, the light flashed allowing for accurate timing of stimulus onset. Beat-by-beat blood oxygenation and heart rate were monitored with a pulse oximeter (Nellcor Oximax). Scoring details are provided below.

**Patient notes**

Detailed notes were retrieved from numerous sources (1) Nursing charts that logged blood gas results, serum bilirubin levels (SBR), haematology results, microbiology results, observations chart (e.g. vital signs and urine output), feeding times and volume, and medications chart. (2) Cranial ultrasound scan results and any other imaging results (MRI). (3) Echocardiography results. (4) Specialist reports in case of referrals, from within or outside the department. (5) Contemporaneous notes (logging of each event as and when it occurs). All sources were used to record each painful procedure up to the day of the study. As we were testing the responses to a heel lance, additional information regarding previous heel lances was also obtained. No infants in this sample had undergone any surgery prior to the day of the study.

Following additional consent from parents we recorded the mother’s medications and conditions during pregnancy. This information was collected using the electronic drug charts, and maternal patient notes if available on the ward. If the notes were not available the midwife was able to provide the information. Maternal information was only collected if the baby was <3 days old or breast feeding and therefore likely to be influenced by maternal factors.

**Missing data**

There is some missing data within the database. Due to technical faults or the baby moving and covering their face some PIPP scores are not complete. There are fewer control and auditory stimulation entries and corresponding EEG files, as the original pilot study involved the noxious heel lance only. Information regarding the relevant infant and maternal medical histories could not always be obtained from the sources listed above.

**2. Database guide**

Below is a guide to the contents of the excel sheets within the parent folder “Database”. The name of the excel file is in bold capitals (**FILENAME**), the name of the sheets within that file are in bold (**Sheet**), and the name of each variable within those sheets are in italics (*Variable*). The first column in each sheet contains the “Record identifier”. There is one identifier per infant studied so this links the information across all the separate sheets and excel files.

**INFANT DEMOGRAPHICS**

**Demographics (N=112)**

* *Gestational age at birth* (number of weeks from the first day of the mothers last menstrual cycle to the birth)
* *Sex*
* *Birth weight*
* *Head circumference* - as measured at birth
* *Heel to crown* - length as measured at birth
* *Weight centile* - based on the UK-WHO paediatric growth charts for boys and girls

**Delivery details**

* *Time of birth*
* *Delivery method*
* *Multiple births* – if infant was part of a single or multiple birth
* *Resuscitation methods* - at time of birth (if needed)
* *Apgar score at 1 min* – clinical condition post birth (out of 10) - <http://www.datadictionary.nhs.uk/data_dictionary/nhs_business_definitions/a/apgar_score_de.asp?shownav=1>
* *Apgar score at 5 min* – clinical condition post birth (out of 10)

**SNAP scores (N=112)**

Assessment of illness severity, often used in neonatal intensive care units to establish appropriate treatment plans (Richardson et al., 2001).

Number of completed SNAP II and SNAPPE II scores are 28 and 27, respectively. Some overall scores are available but the details of the individual components of the score are not. This was subject to the information provided within the patient notes.

* *Blood pressure*: Mean blood pressure and score according to SNAP II/SNAPPE II guidelines (see link above)
* *Serum pH*: Lowest pH within the first 12 hours since birth and score
* *Temperature*: Lowest temperature and score
* *Seizures*: Is there evidence of multiple seizures and score
* *PO2/FIO2 ratio*: PO2,FIO2,PO2/FIO2 ratio, and score
* *Urine*: Urine output and score
* *Apgar*: Apgar score at 5 min post birth (out of 10) and score
* *Birth weight*: weight as measured at birth and score
* *Gestational age*: Is baby small (weight) for their gestational age and score – for definition of small for gestational age see Richardson et al., 2001
* *Total scores*: SNAP II score and SNAPPE II score

**STUDY DETAILS**

**Study context (N=112)**

* *Gestational age* at study (gestational age at birth + number of days since birth)
* *Postnatal age* (number of days since birth)
* *Time study commenced*
* *Time study finished*
* *Duration of study* (including electrode placement)
* *Ward location* – the ward on which the infant was at the time of the study
* *Weight* – at time of study
* *Time of last feed* – the time of the infant’s last feed may be during the study
* *Oral feeding* – this refers to breast or bottle feeding (infant may have both oral and naso gastric tube (NGT feeds) – refer to **INFANT PATIENT NOTES:** **Painful procedures** to see presence of NGT during study)
* *Type of oral feeding* (breast milk or formula)

**EEG details (N=112)**

Based on the EEG recorded throughout the duration of the study and not just at the time of stimulation. This was performed by an experienced clinical physiologist. All infants tested had an EEG within normal limits for their corrected age based on synchronicity and symmetry of EEG activity between hemispheres, absence of electrographic seizures, and age-appropriate features (André et al., 2010; Tsuchida et al., 2013).

* *Recorded electrodes for EEG* – list of electrodes recorded
* *ECG reference* – some studies used a bipolar ECG (right shoulder referred to left shoulder) before the reference electrode Fz was so that each ECG channel used thesame reference as the EEG
* *Delta brushes* – presence in the EEG
* *Temporal sharpened theta* – presence in the EEG
* *Bilateral synchronicity* – presence in the EEG
* *Symmetry of amplitudes* – presence in the EEG

**STIMULATION INFORMATION**

**Heel lance (N=112)**

* *EEG file name* – this is the name of the corresponding EEG file (.mat) located in the parent folder “EEG” within an infant sub-folder (folders name corresponds to record identifier)
* *Stimuli order* – the order in which the 3 stimulation types were conducted
* *Time of stimulation*
* *Stimulation site* – which heel was lanced
* *Infant position* (e.g. prone vs supine)
* *Infant location* (e.g. cot vs held)
* *Pre-stimulus vigilance state* *(EEG)* – vigilance state determined by electrographic and respiratory criteria, presence or absence of rapid eye movements (appreciable on the F8/F7 electrode recordings), and behavioural observation by the clinical scientist and research nurse (Grigg-Damberger, 2016)
* *Post-stimulus vigilance state (EEG)*
* *Blood taken for* – specific blood test/s being conducted; Blood gas (estimation of acid-base balance and adequacy of ventilation), Urea & Electrolytes (kidney function test), FBC (full blood count, i.e. number of white and red blood cells), Reticulocyte count (how fast red blood cells are being made in order to judge need for blood transfusion), SBR (serum-bilirubin – jaundice level), Guthrie (Newborn Blood Spot Screening (NBSS)), Drug assay, CRP (C-reactive protein - marker for inflammation), TFT (Thyroid function test), DAT (Direct anti-globulin test – blood group compatibility with mother)
* *Blood quantity* – approximate amount of blood taken
* *Distraction used* – A list of any techniques employed during the test to soothe the infant; Swaddling (wrapped in a blanket), Skin to skin (bare parent’s chest against bare baby’s chest), Kangaroo care (chest to chest but baby or parent is dressed), Containment (cocooning using boundaries e.g. fabric or hands), Non-nutritive sucking (e.g. pacifier), Active distraction (e.g. patting, rocking)

Premature Infant Pain Profile (PIPP)

For the description of how the PIPP is scored see Stevens et al., 1996

* *PIPP score – facial expression*: Brow bulge (number of seconds out of 30), Brow score (percentage of time exhibited during the 30s post-stimulus), Eye squeeze, Eye score, Nasolabial, Nasolabial score, Expression change during baseline (any of the 3 expressions observed during the 15s pre-stimulus period), Latency of the 1st expression (how long before one of the expressions was observed), Total face expression score (Brow score + Eye score + Nasolabial score)
* *PIPP score – heart rate and blood O2 saturation*: Baseline heart rate (average heart rate during the 15s pre-stimulus period), Max heart rate (maximum heart rate during the 30s post-stimulus period), Heart rate score (based on the increase in beats from the average baseline heart rate to the maximum heart rate post-stimulus), Baseline O2 sats (average blood oxygen saturation during the 15s pre-stimulus period), Min O2 sats (minimum blood oxygen saturation during the 30s post-stimulus period), O2 sat score (based on the decrease in O2 from the average baseline O2 to the maximum O2 post-stimulus)
* *PIPP score*: Vigilance state score (based on which vigilance state the infant is in during the 15s pre-stimulus period, according to behaviour), Gestational age score, Total PIPP score (Total face expression score + Heart rate score + O2 sat score + Vigilance state score + Gestational age score)

**Sham control (N=99)**

* *EEG file name*
* *Time of stimulation*
* *Stimulation site* – the location of the lancet (with the blade facing away from the skin)
* *Infant position*
* *Infant location*
* *Pre-stimulus* vigilance *state (visual)*
* *Pre-stimulus* vigilance *state (EEG)*
* *Post-stimulus* vigilance *state (EEG)*
* *PIPP score – facial expression*: Brow, Brow score, Eye squeeze, Eye score, Nasolabial, Nasolabial score, Expression change during baseline, Latency of the 1st expression, Total face expression score
* *PIPP score – heart rate and blood O2 saturation*: Baseline heart rate, Max heart rate, Heart rate score, Baseline O2 sats, Min O2 sats, O2 sat score
* *PIPP score*: Vigilance state score, Gestational age score, Total PIPP score

**Auditory control (N=99)**

As there is an audible click when the blade is released from the heel lance, the lance is held near the foot but not touching the infant.

* *EEG file name*
* *Time of stimulation*
* *Stimulation site* – the location of the lancet (with lance not touching the skin at any point)
* *Infant position*
* *Infant location*
* *Pre-stimulus* vigilance *state (visual)*
* *Pre-stimulus* vigilance *state (EEG)*
* *Post-stimulus* vigilance *state (EEG)*
* *PIPP score* *– facial expression*: Brow, Brow score, Eye squeeze, Eye score, Nasolabial, Nasolabial score, Expression change during baseline, Latency of the 1st expression, Total face expression score
* *PIPP score – heart rate and blood O2 saturation*: Baseline heart rate, Max heart rate, Heart rate score, Baseline O2 sats, Min O2 sats, O2 sat score
* *PIPP score*: Vigilance state score, Gestational age score, Total PIPP score

**INFANT PATIENT NOTES**

**Ventilation (N=112)**

* *Ventilation* – any form of respiratory support on the day of study; self-ventilating in air (no ventilation), low flow oxygen, high flow oxygen, continuous positive airway pressure, Biphasic positive airway pressure, and mechanical ventilation
* *#Days of mechanical* – number of days on mechanical ventilation from birth until day of study (not necessarily consecutive)
* *#Days of BiPAP/CPAP/HF* – number of days on Biphasic positive airway pressure, continuous positive airway pressure, or high flow oxygen from birth until day of study (not necessarily consecutive)
* *#Days of LF* – number of days of low flow oxygen from birth until day of study (not necessarily consecutive)

**Diagnosis (N=112)**

If an infant had any of the listed diagnoses they are subsequently defined as either: resolved (had diagnosis at some point between birth and up to 3 days prior to the day of study), current within last 3 days (had the diagnosis within the 3 days prior to the study and/or on the day of study), yes – unknown (has had the diagnosis but notes were unclear as to when and if resolved or not).

* *Any diagnosis?* - yes/no
* *Small for gestational age* – based on weight centile at birth <https://www.rcog.org.uk/globalassets/documents/guidelines/gtg_31.PDF>
* *Placental insufficiency*
* *Clinically wasted*
* *Surfactant deficiency* – babies who developmentally are deficient or babies who temporarily had a deficiency at birth
* *Lung disease*
* *Pleural effusion*
* *Pneumonia*
* *Hypertension*
* *Hypotension*
* *Cardiac abnormality*
* *Adrenal insufficiency*
* *Hypothyroidism*
* *Jaundice*
* *Hypoglycaemia*
* *Hyperglycaemia*
* *Infection*
* *Temperature instability*
* *Neonatal abstinence*
* *Seizures*
* *Patent ductus arteriosis*
* *Pneumothorax*
* *Necrotising enterocolitis*
* *Nec entero\_severity* – the severity of the necrotising enterocolitis based on the Modified Bell’s Staging (Gregory et al., 2011) (Stage I & IIa: mild, Stage IIb: moderate, Stage III: severe)
* *Respiratory distress*
* *Gastro oesophageal reflux*
* *Gastro reflux\_severity* - the severity of the Gastro oesophageal reflux (mild: occasional reflux corrected with adequate positioning, moderate: frequent reflux and showing signs of physiological instability, severe: profound physiological instability and requiring pharmacological treatment)

**Cranial scans (N=112)**

Presence of hypoxic ischemic encephalopathy (HIE) was also recorded. None of the infants in this study had evidence of HIE so the variables were removed from this database. Details regarding multiple scans were only completed for those with any previous or current abnormal results. The reported results regarding multiple scans are those that were deemed the most significant or concerning.

* *No scans* – if this is “yes” the baby did not have any scans in the patient notes to report
* *Cranial ultrasound scan (latest)*: Summary (normal or abnormal), Intraventricular hemorrhage (IVH), Periventricular leukomalacia (PVL), Days old at the time of the scan
* *Multiple cranial ultrasound scans (significant):* Multiple scans? (yes or no), IVH, PVL, Days old at scan
* *MRI scan (latest):* MRI result (normal, abnormal, or uncertain), Days old at the time of the scan
* *Multiple MRI scans (significant):* Multiple MRI (yes or no), MRI result (normal, abnormal, or uncertain), Days old at scan

**Medication (N=112)**

* *Any medication* (yes or no)
* *Renal impairment* (yes or no)
* *Analgesics/Anaesthetics:* Details (specific medication type), Method (Bolus or via a naso-gastric tube), Time since last dose
* *Broncho/Stimulants:* Details, Time since last dose
* *Anticonvulsants/Sedatives:* Details, Time since last dose
* *Muscle relaxants:* Details, Time since last dose
* *Blood pressure medication*: Details, Time since last dose
* *Muscarinic receptor blocker*: Details, Time since last dose
* *Other medication*: Details, Time since last dose
* *Medication comments*: more information on other medications not listed or if 2 medications within one of the above categories are being administered, details on time since last doses are provided, details of long term morphine
  + Abbreviations: OD (once daily), IV (Intravenous), NGT (naso-gastric tube), ETT (endotracheal tube), TDS (three times a day), BD (twice a day), po (per oral medication), QDS (four times a day), d (day)

**Heel lances (N=112)**

Both heels were inspected at the time of the study for bruises, swelling, and previous lance marks. In some instances the number of lance marks on the heel is missing. This was due to a visual restriction such as a cannula or dressing over the heel.

* *Total lances* – based on the patient notes (since birth and up to the time of the study)
* *Visible lances* (right heel) – number of marks from previous lances determined by the research nurse
* *Visible lances* (left heel)
* *Time since last lance (days)* – based on patient notes
* *Time since last lance (hours)*
* *Heel inflamed* – (yes or no)
* *Crying on handling* – (yes or no) did the infant cry when the heels were touched
* *Bruising* – bruising of either heel (yes or no)

**Painful procedures (N=112)**

This refers to procedures that are invasive and potentially painful other than a lance. No infants in this sample had undergone any surgery prior to the day of the study so these variables were removed from the database.

* *Any other procedures* – refers to any other painful and invasive procedure (other than the heel lance) experienced by the infant from birth up until the time of the study.
* *Venesections*: Number (total since birth until the time of the study), Days old (at the time of the most recent procedure)
* *Vitamin K injection* (intramuscular injection): Number, Days old (most recent)
* *Immunisation* (intramuscular injection): Number, Days old (most recent)
* *Intravenous line*: Number (insertions), Days old (most recent)
* *Long line*: Number (insertions), Days old (most recent)
* *Arterial line*: Number (insertions), Days old (most recent)
* *Umbilical line*: Number (insertions), Days old (most recent)
* *Intubation*: Number (insertion), Days old (most recent)
* *Naso-gastric tube*: Number (insertions), Days old (most recent), present during study (if not present then can assume each count refers to an insertion and removal, if present the number of removals will be Number-1)
* *ROP* (retinopathy of prematurity) screen: Number, Days old (most recent)
* *Lumbar puncture*: Number, Days old (most recent)
* *Cranial tap*: Number, Days old (most recent)
* *Pleural tap*: Number, Days old (most recent)
* *Chest drain*: Number (insertions), Days old (most recent), Duration – how many days was the drain in place
* *Peritoneal drain*: Number (insertions), Days old (most recent), Duration
* *Therapeutic cooling*
* *Extravasation*: Number, Days old (most recent)

**Injuries (N=112)**

* *Ischaemic necrotic digits*: Number, Days old (when first occurred)
* *Fractures*: Number, Days old
* *Nasal injury from CPAP* (Continuous positive airway pressure ventilation):Number, Days old
* *Total number of injuries*
* *Detailed notes* – notes on all references to any injury within the patient notes, or any injuries observed by the research nurse at the time of the study
  + Abbreviations: IV (intravenous line), PNA (postnatal age – days old), TC (transcutaneous gas monitoring), ECG (electrocardiogram)

**MATERNAL PATIENT NOTES**

**Maternal (N=112)**

In some instances there is no data due to lack of consent from mother or unable to retrieve maternal patient notes.

* *< 3 days old or breast feeding* – If the infant younger than 3 days or breast feeding, maternal medications may have an effect on the infant
* *Medications* – list of any medication that the mother is taking
* *Conditions* – list of any maternal diagnoses/conditions

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