NeuroImage 52 (2010) 583–589

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Premature infants display increased noxious-evoked neuronal activity in the brain compared to healthy age-matched term-born infants

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ARTICLE INFO

Article history:
Received 2 November 2009
Revised 20 April 2010
Accepted 26 April 2010
Available online 8 May 2010

Keywords:
Neonate
Pain
EEG/ERPs
Cortex
Development

ABSTRACT

This study demonstrates that infants who are born prematurely and who have experienced at least 40 days of intensive or special care have increased brain neuronal responses to noxious stimuli compared to healthy newborns at the same postmenstrual age. We have measured evoked potentials generated by noxious clinically-essential heel lances in infants born at term (8 infants; born 37–40 weeks) and in infants born prematurely (7 infants; born 24–32 weeks) who had reached the same postmenstrual age (mean age at time of heel lance 39.2 ± 1.2 weeks). These noxious-evoked potentials are clearly distinguishable from shorter latency potentials evoked by non-noxious tactile sensory stimulation. While the shorter latency touch potentials are not dependent on the age of the infant at birth, the noxious-evoked potentials are significantly larger in prematurely-born infants. This enhancement is not associated with specific brain lesions but reflects a functional change in pain processing in the brain that is likely to underlie previously reported changes in pain sensitivity in older ex-preterm children. Our ability to quantify and measure experience-dependent changes in infant cortical pain processing will allow us to develop a more rational approach to pain management in neonatal intensive care.

Introduction

Throughout the developed world it has become routine to resuscitate and offer neonatal intensive care to infants as young as 24 postmenstrual weeks. Increasing survival rates provide strong justification for this approach (Dani et al., 2009) but there are concerns that children born extremely prematurely have an increased risk of cognitive, behavioural, emotional and learning deficits (Bhutta et al., 2002; Marlow et al., 2005). Furthermore, while technical advances in neonatal intensive care may improve cognitive and motor outcomes, there is increasing evidence that the adverse sensory environment of intensive care, for which the preterm baby is poorly prepared, leads to other more subtle disabilities (Johnson et al., 2009). One frequent adverse sensory experience in the neonatal intensive care unit (NICU) is the painful, tissue-damaging stimulation caused by repeated but essential clinical procedures that, for some babies, can last for months. The increasing evidence that exposure to pain in early infancy leads to long-term changes in pain sensitivity in later life (Fitzgerald and Walker, 2009) led us to hypothesise that the neonatal intensive care experience alters the development of central pain pathways and changes the neuronal responses to noxious stimulation in the infant brain. It is plausible that by the time a premature infant reaches term-age their early life experience may alter the central nervous system processing of noxious events.

A number of techniques can be used to assess infant responses to noxious stimulation. Traditional pain measurements in preterm and newborn infants have relied on the characterisation of behavioural and autonomic responses to noxious stimulation (Stevens and Franck, 2001). Cortical haemodynamic activity has also been recorded in infants in response to noxious events, including heel lance, venipuncture, and endotracheal tube suctioning and repositioning (Bartocci et al., 2006; Limperopoulos et al., 2008; Slater et al., 2006; Slater et al., 2008). More recently, we have developed an electroencephalic (EEG) technique to measure evoked neuronal activity following noxious stimulation (Slater et al., 2010), which has provided electrophysiological evidence that the immature infant brain can discriminate between noxious and non-noxious stimulation from 35 postmenstrual weeks. The aim of this study was to assess whether noxious and non-noxious stimulation is processed differently in premature infants who have reached their due date compared to healthy newborn infants.

Design

The blood sampling procedure used to extract blood from hospitalised infants offers a unique opportunity to study pain in infancy. Infants routinely have their heels lanced with a spring-loaded blade so that blood samples can be extracted for clinical analysis. In this study, time-locked EEG was recorded during noxious heel lance and during non-

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doi:10.1016/j.neuroimage.2010.04.253

noxious (touch) stimulation of the heel and compared between two groups of infants. The first group were healthy newborn infants who were born and studied at term ('term-term'). The second group were premature infants who had reached a postmenstrual age equivalent to term at the time of the study ('prem-term').

Method

Subjects

Two groups of infants, recruited from the special care baby unit at the Elizabeth Garrett Anderson and Obstetric Hospital, participated in the study. The first group (no. of infants = 8; age range: 37–40 weeks PMA at birth) were normal term infants who were less than 7 postnatal days ('term-term'). The second group (no. of infants = 7; age range: 24–32 weeks PMA at birth) had been born prematurely and were studied when they reached a PMA equivalent to term ('prem-term') (Table 1). All infants in the 'prem-term' group were older than 40 postnatal days and these infants had a range of conditions associated with their prematurity. Table 2 shows the previous surgery, EEG, head ultrasound and brain MRI results for the 'prem-term' infants. All the 'term-term' infants had not had previous surgery, had a normal EEG and had not been diagnosed with any brain abnormalities. Further clinical characteristics for each infant are given in Supplementary Table 1. Two additional infants who fulfilled the eligibility criteria were not included in this final sample of infants because movement artefact was identified in the EEG recordings.

PMA was determined from antenatal ultrasound scans taken at 19–20 weeks gestation or from the maternal report of the last menstrual period. No infants had congenital abnormalities or were receiving sedatives, analgesics or other psychotropic agents. Ethical approval was obtained from the UCLH ethics committee and informed written parental consent was obtained prior to each study. The study conformed to the standards set by the Declaration of Helsinki.

Non-noxious stimulation

Innocuous tactile stimulation was applied by lightly tapping a rubber bung (surface area = 177 mm^2) against the heel of the infants. The rubber bung was attached to an impedance head (Bruell Kjaer, Type 8001, Denmark), which in turn was attached to the arm of a tendon hammer so that a controlled and measurable force could be applied. Touch stimuli were presented to each infant with an inter-stimulus interval of 14.7 ± 6.5 s and a force 25.5 ± 7.5 N (no. stimuli/infant = 11.1 ± 3.7). An average waveform for each series of tactile stimulation was produced after traces were aligned to correct for latency jitter (Bromm and Scharein, 1982; Woody, 1967). Alignment was obtained by maximizing the correlation between traces in the time interval between 100 and 400 ms poststimulation (allowed jitter: −50 to +50 ms). The non-noxious tactile stimulation was always performed prior to the heel lance.

Table 1
Demographic characterisation of the sample.

	Group 1 'prem-term' (n = 7)	Group 2 'term-term' (n = 8)
Mean PMA at birth (weeks)	26.9 (3.4); range 24.0–32.6	38.6 (1.2); range 37.0–40.6
Mean PMA at time of study (weeks)	39.3 (1.2); range 37.6–41.4	39.1 (1.2); range 37.7–41.0
Mean postnatal age at time of study (days)	87 (28); range 40–116	4 (2); range 0–6
Mean birth weight (g)	933 (403); range 540–1692	3215 (407); range 2700–4125
Mean weight at time of study (g)	2258 (355); range 1696–2744	3099 (484); range 2481–4125
No. multiple gestation infants	3	0
No. of males	3	6
No. of days ventilated	22 (22.1); range 0–69	0(0); range 0

Noxious stimulation

The noxious stimulus was a clinically-essential heel lance. No heel lances were performed solely for the purpose of the study. Following the heel lance, the foot was not squeezed for a period of at least 30 s to ensure that the recorded responses were solely due to the lance. All studies were conducted during normal working hours.

EEG recording

17 recording electrodes (Ambu Neuroline disposable Ag/AgCl cup electrodes) were positioned according to the modified international 10/20 electrode placement system positioned at Fz, Fp1, Fp2, F7, F8, Cz, CPz, C3, C4, CP3, CP4, T3, T4, T5, T6, O1 and O2. In one case a reduced number of electrodes were used due to parental request. Reference and ground electrodes were placed at FCz and the chest respectively. Electrode/skin impedance was kept to a minimum by rubbing the skin with an EEG prepping gel (NuPrep gel D.O.Weaver and Co). Conductive EEG paste (Ten20 D.O.Weaver and Co) was used to optimise contact with the electrodes. Electrodes were held in place by a Surgifix elastic bandage (Fagron, Italy) and leads were tied together to minimise electrical interference. Electrodes were disposed of after each study. EEG activity, from DC (or 0.05 Hz in 3 cases) to 70 Hz, was recorded using the Neuroscan (Scan 4.3) SynAmps2 EEG/EP recording system (Compumedics US Co.). A 50 Hz notch filter was used and signals were digitised with a sampling rate of 2 kHz and a resolution of 24 bit.

Assessment of the state of the infants at the time of study

Medical charts were reviewed and infants were assessed as clinically stable. The EEG record was assessed offline for normality considering factors such as symmetry, synchronicity, epileptiform activity and normal background rhythms appropriate for age. Sleep states were characterised using EEG criteria and behavioural data from the video recording. Regularity of respiration was measured using a movement transducer (Unimed) placed on the abdomen and heart rate was measured using lead 1 ECG. To check that the evoked activity was not generated by stimulus-triggered electro-oculographic activity the video footage and EEG recordings (activity at electrode site Fp1 and Fp2) were assessed to ensure that concurrent eye movement did not occur following the stimulation.

Evoked potential analysis

EEG epochs corresponding to a noxious heel lance, innocuous touch of the heel and no stimulation (background control EEG) were considered for analysis. The heel lance was time-locked to the EEG recording using an accelerometer (K-shear accel., Kistler Instruments Ltd.) attached to the superior surface of the lancet (Worley et al., 2006). The innocuous touch was time-locked using the signal generated by the impedance head. The segment of background EEG activity was manually event-marked prior to all stimulation during the EEG recording.

The EEG epochs were 1500 ms segments which included activity recorded 500 ms prior to and 1000 ms post each event re-referenced to a common average. The segments were baseline corrected and high pass filtered above 0.5 Hz. Data files were exported to MATLAB (Version 7.2 Mathworks, Inc.) for further analysis.