Brief Communications

Cortical Pain Responses in Human Infants

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Despite the recent increase in our understanding of the development of pain processing, it is still not known whether premature infants are capable of processing pain at a cortical level. In this study, changes in cerebral oxygenation over the somatosensory cortex were measured in response to noxious stimulation using real-time near-infrared spectroscopy in 18 infants aged between 25 and 45 weeks postmenstrual age. The noxious stimuli were heel lances performed for routine blood sampling; no blood tests were performed solely for the purpose of the study. Noxious stimulation produced a clear cortical response, measured as an increase in total hemoglobin concentration [HbT] in the contralateral somatosensory cortex, from 25 weeks (mean Δ[HbT] = 7.74 μmol/L; SE, 1.10). Cortical responses were significantly greater in awake compared with sleeping infants, with a mean difference of 6.63 μmol/L [95% confidence interval (CI) limits: 2.35, 10.91 μmol/L; mean age, 35.2 weeks]. In awake infants, the response in the contralateral somatosensory cortex increased with age (regression coefficient, 0.698 μmol/L/week; 95% CI limits: 0.132, 1.265 μmol/L/week) and the latency decreased with age (regression coefficient, −0.9861 μmol/L/week; 95% CI limits: −1.5361, −0.4361 μmol/L/week; age range, 25–38 weeks). The response was modality specific because no response was detected after non-noxious stimulation of the heel, even when accompanied by reflex withdrawal of the foot. We conclude that noxious information is transmitted to the preterm infant cortex from 25 weeks, highlighting the potential for both higher-level pain processing and pain-induced plasticity in the human brain from a very early age.

Key words: **nociception; analgesia; neonatal; pediatric; spectroscopy; cerebral oxygenation**

Introduction

The postnatal development of pain processing in both man and rodents has been the subject of considerable research in the last few years (Fitzgerald, 2005). Robust nociception has been demonstrated in the youngest infants using a wide range of physiological, biochemical, and behavioral measures (Franck et al., 2000; McNair et al., 2004) and both "wind up" to repeated noxious stimulation and hypersensitivity after superficial and deep tissue injury have been demonstrated using spinal withdrawal reflexes (Andrews and Fitzgerald, 1999; Andrews et al., 2002) and grimacing (Taddio et al., 2002).

However, very little is known about the development of higher pain processing in man. The strong responses to noxious stimulation observed in preterm infants could be entirely mediated at spinal or brainstem level, with little or no cortical involvement. Indeed, <32 weeks postmenstrual age, key behavioral and autonomic pain responses are comparable in normal infants and infants with brain injury associated with white matter damage (Oberlander et al., 2002). It is possible, therefore, that despite some anatomical evidence of thalamocortical projections in the human brain from 24 weeks (Lee et al., 2005), functional nociceptive connections with cortical cells and circuits are not formed until much later. Because the true experience of pain includes emotional and affective components which require higher level cortical processing, knowledge of the maturation of infant cortical responses to noxious stimulation would be a major step toward understanding the infant pain experience.

A number of studies have been undertaken on somatosensory evoked potentials in infants from 27 weeks (Klimach and Cooke, 1988; Pihko et al., 2004) but they have focused on investigations of the integrity of somatosensory pathways and prognostic indicators of neurological impairment. Brain-imaging methods that have proved so useful in the analysis of cortical pain processing in adults (Brooks and Tracey, 2005) are impractical for neonates in intensive care units. We have therefore used near-infrared spectroscopy to directly measure the cortical hemodynamic response and have shown, for the first time, that preterm infants display cortical responses to noxious stimulation.

Materials and Methods

Participating infants. Studies were undertaken on inpatients in the Neonatal Intensive Care Unit and the Special Care Baby Unit at University College London Hospital (UCLH), London, United Kingdom. Ethical approval was obtained from the UCLH ethics committee and informed written parental consent was obtained for each infant. Eighteen infants aged between 25 and 45 weeks postmenstrual age (PMA) were tested on 31 occasions during clinically required routine heel lancing. Table 1 de-

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Table 1. Demographic characterization of the neonatal population

Mean gestational age at birth (weeks), $n = 18$	28.2 (4.3); range, 24.0–37.3
Mean PMA at time of study (weeks), $n = 31$	35.0 (5.2); range, 25.7–45.6
Mean postnatal age at time of study (days), $n = 31$	7.8 (38.5); range, 5–134
Mean birth weight (g), $n = 18$	1212.0 (574.3); range, 547–2350
Mean weight at time of study (g), $n = 31$	1914.9 (952.9); range, 520–4184
No. of multiple gestation infants, $n = 18$	7
No. of infants with chronic lung disease, $n = 31$	7
Mean no. of days on mechanical ventilator, $n = 18$	9.4 (16.7); range, 0–54
Mean no. of test occasions, $n = 18$	1.7 (1.1); range, 1–5
Mean no. of days between consecutive studies, $n = 13$	27.3 (20.7); range, 1–69

SDs are in parentheses. No., Number.

tails the demographic characteristics of the infants. In a separate study, the cortical response to nonpainful mechanical stimulation of the foot using von Frey hairs was studied in 11 infants, aged 26–35 weeks PMA. PMA was determined from antenatal ultrasound scans or from the maternal report of the last menstrual period. Infants included in the study were tested on one or more occasions. All infants had normal appearances on cranial ultrasound scans. At the time of the study, all infants were assessed as clinically stable. Infant handling was kept to a minimum; hence, infants were studied in a variety of positions and sleep states. Two infants were receiving morphine at the time of heel lance.

Sleep state was assessed according to the Premature Infant Pain Profile using the behavioral-state indicator, which categorizes an infant into one of four states: active/awake, quiet/awake, active/sleep, or quiet/sleep (Stevens et al., 1996). Infants in the first two categories were classified as awake and those in the second two categories were classified as asleep.

Near-infrared spectroscopy. Cortical activity is associated with increased localized cerebral blood flow. In this study, a double-channel near-infrared spectrophotometer (NIRO-200; Hamamatsu Photonics, Hamamatsu City, Japan) was used to measure regional changes in oxygenated and deoxygenated hemoglobin concentration. This is a noninvasive technique that has been widely used in neonatal research to measure functional activation of the cortex (Sakatani et al., 1999; Kusaka et al., 2004). The technique depends on the transparency of biological tissue to near-infrared light and uses the fact that the absorption of near-infrared light by oxygenated and deoxygenated hemoglobin depends on the oxygenation state (Owen-Reece et al., 1999). The light emitters and detectors (optodes) were positioned symmetrically on either side of the head over the somatosensory cortex, using the international 10–20 EEG placement system to identify key landmarks. Figure 1*A* shows a photograph of an infant with the optodes placed over the somatosensory cortex. Optodes were placed on the head at least 15 min before recording and real-time changes in cerebral hemodynamic activity were monitored. Changes in oxyhemoglobin [HbO_2] and deoxyhemoglobin [HHb] were measured. Total hemoglobin [HbT] concentration change was calculated [HbT] = [HbO_2] + [HHb].

Heel-lance protocol. The heel lance was performed when heart rate, oxygen saturation, and hemodynamic cerebral activity were stable. After heel lance the foot was not squeezed for a period of 30 s to ensure that the evoked response occurred only as a result of the initial stimulus. The baseline was determined 20 s prestimulus and the cortical response to the stimulus was measured. The maximum change in [HbT] was defined as the maximum change from the mean prestimulus recording in the 20 s period post heel lance. In five studies, only unilateral data were obtained because of loss of contact of an optode on one side while the other optode remained in good contact. Data analysis was undertaken by a blinded observer using a prewritten Matlab script (Matlab version 7.0).

Tactile stimulation. In a second series of measurements, the selectivity of the cortical response was investigated using non-noxious stimulation. Von Frey hairs were used to apply a known calibrated force to the plantar surface of the foot as described previously (Andrews and Fitzgerald, 1994). The force applied to the foot was increased using graded von Frey hairs. Each von Frey hair was applied to the plantar surface of the foot five times in each infant at intervals >60 s. The reflex withdrawal threshold was defined as the stimulus at which a distinct movement of the foot away from the same von Frey hair occurred in at least three of five occasions. The maximum force applied was one von Frey hair above reflex withdrawal threshold. The range of force applied by the von Frey hairs (0.096–25.81 g) was similar to that previously reported (Andrews and Fitzgerald, 1999). For each hair, a hemodynamic response was defined as >10% of the poststimulus response lying two SDs outside the prestimulus baseline in three of five responses.

Statistical analyses. The maximum change in [HbT], defined as the maximum change from the mean prestimulus recording in the 20 s period post stimulus, is referred to as the stimulus response and is used in all the statistical analyses. To assess whether the stimulus response was significantly different from the prestimulus baseline within an individual subject, the maximum poststimulus value was compared with the prestimulus sample mean (two-sample *t* test). To establish whether there was a significant difference between the cortical response in the awake and asleep infants, restricted maximum-likelihood mixed-model regression was used (Hand and Crowder, 1996). The regression analysis provides an estimate of the mean stimulus response in the awake and asleep states as a function of age; it takes into consideration the nonindependence among observations made in infants where more than one measurement was acquired. The analysis was performed using SAS PROC MIXED (SAS version 9.1; SAS Institute, Cary, NC).

A linear dependence on age was adopted, and an (age/state) interaction term allowed for a potential difference in age dependence between the awake and asleep states. The likelihood ratio test was used to test whether the response to heel lance was different in the ipsilateral and contralateral cortices. A number of calculations, including sensitivity analysis and the usual regression diagnostics were performed to ensure that the regression results were robust. The results were satisfactory. The inclusion of infants receiving morphine did not substantially change the statistical assessment; however, these infants were excluded from the statistical analysis used to calculate regression coefficients.

Results

Noxious stimulation produces a cortical response in preterm infants from 25 weeks

Figure 1*B* shows a typical somatosensory hemodynamic response to noxious stimulation of the heel during routine blood sampling in a preterm infant. An increase in the total hemoglobin concentration over the contralateral somatosensory cortex after heel lance was observed in all but one infant. The mean increase in [HbT] in the contralateral somatosensory cortex, calculated from the whole sample of infants, was 7.74 μmol/L (SE, 1.10; $n = 30$), whereas a mean decrease in [HbT] of -0.10 μmol/L (SE, 1.10; $n = 26$) was recorded on the ipsilateral side. Although the magnitude of the ipsilateral response was variable and sometimes positive, it was always smaller than the contralateral response (Fig. 2*A*). The likelihood ratio test, used to compare the responses in the ipsilateral and contralateral cortices, showed a highly significant difference between the two sides (the likelihood ratio: 34.6 with 4 degrees of freedom, where 0.05 level χ^2 critical value = 9.49). This confirms that the cortical response to heel lance was a localized effect that did not occur as a result of a global hemodynamic change.

The magnitude of cortical responses to noxious stimulation increases with postmenstrual age

Figure 2*B* illustrates that the cortical response to noxious heel lance increases with PMA. Mixed-model regression was used to estimate the mean response in the waking and sleeping states, as a function of postmenstrual age, allowing for the nonindependence among repeated observations made in the infants where more than one measurement was acquired. Analysis shows that this age dependence in the magnitude of the stimulus response occurs only in awake infants [PMA regression coefficient, 0.698 μmol/L/week; 95% confidence interval (CI) limits: 0.132, 1.265 μmol/L/week]. Age dependence was not observed in the sleeping state (PMA regression coefficient in the asleep state, -0.291 μmol/L/week; 95% CI limits: -1.004, 0.423

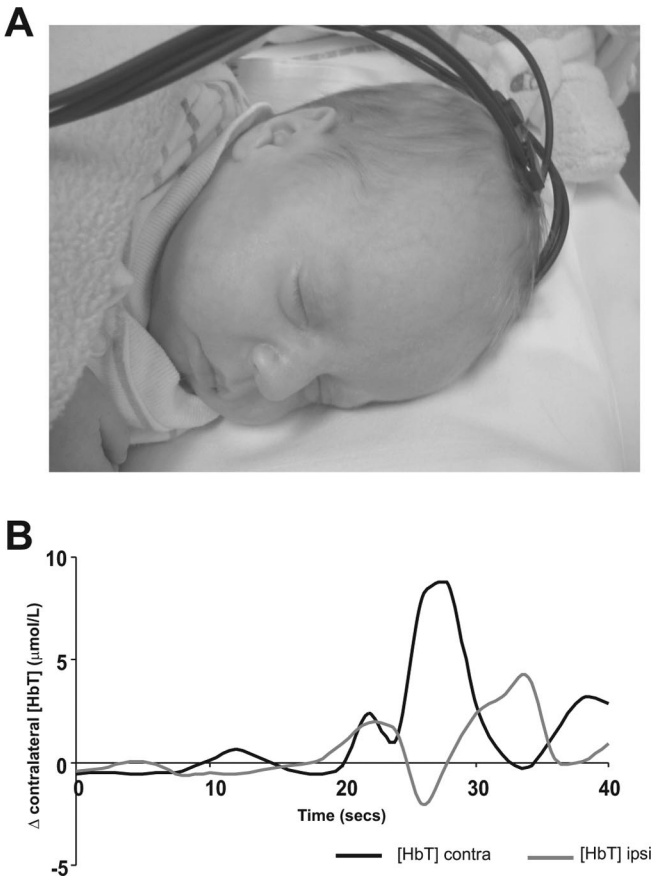

Figure 1. ***A***, Optode placement. A photograph of a premature infant (33 + 4 weeks PMA) with optodes positioned over the somatosensory cortex is shown. ***B***, Hemodynamic response. A sample trace in a single infant (29 + 5 weeks PMA) is shown, demonstrating the evoked change in [HbT] in the contralateral and ipsilateral somatosensory cortex after a painful stimuli given at $t = 20$ s. Sampling frequency, 2 Hz.

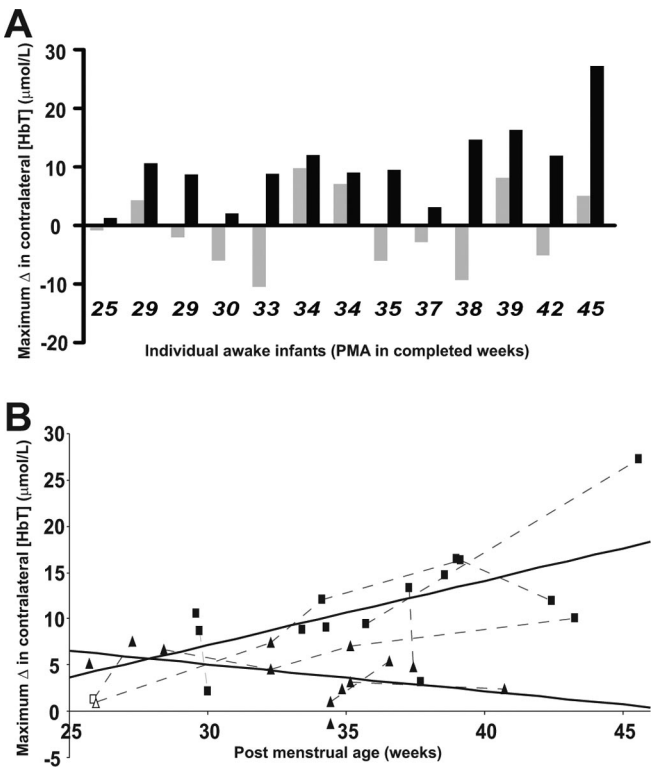

Figure 2. ***A***, Group results for awake infants. A bar chart shows individual ipsilateral and contralateral maximum Δ[HbT] after heel lance in each awake infant. The age of each infant (completed postmenstrual weeks) is shown. ***B***, Effect of age on hemodynamic response. A developmental profile of the maximum Δ[HbT] in the contralateral somatosensory cortex after heel lance in infants at different PMAs is shown [regression coefficient for awake infants (squares): 0.698 μmol/L/week, 95% CI limits: 0.132, 1.265 μmol/L/week; asleep infants (triangles): −0.291 μmol/L/week, 95% CI limits: −1.004, 0.423 μmol/L/week]. Connected points represent repeated measures in individual infants. Unfilled points represent infants receiving morphine; these infants are excluded from the regression analysis.

μmol/L/week). Furthermore, as illustrated in Figure 2*B*, there was no age dependence in the ipsilateral responses. It should be noted that although the youngest infants displayed the smallest responses, even the very youngest infant (25 + 5 weeks PMA) demonstrated a clear and significant response above the prestimulus baseline ($p < 0.005$, two-sample t test). Figure 3 shows the cortical response to heel lance in the youngest infant in our sample.

Awake infants have larger cortical responses to noxious stimulation than sleeping infants

The magnitude of the response was dependent on sleep state. At the mean PMA (35.2 weeks) the least-squares mean stimulus-response estimates in the waking and sleeping states are 10.20 μmol/L and 3.57 μmol/L (95% CI limits: 7.31, 13.09, and 0.31, 6.83 μmol/L, respectively). The mean difference between these two estimates is 6.63 μmol/L, which achieves statistical significance (95% CI limits: 2.35, 10.91 μmol/L).

Latency between noxious stimulation and peak response is greater in younger infants

The latency of the maximum response in the contralateral somatosensory cortex from the time of the heel lance stimulus is dependent on age but the relationship is nonlinear over the whole age range (25–45 weeks PMA) with a greater dependence in the younger subjects. Individual latencies range from 2–19 s [mean 8.02 s (SD, 4.74)] with the longest latencies recorded in the

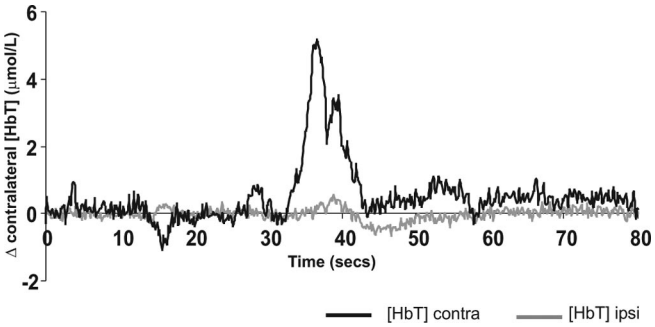

Figure 3. Hemodynamic response in the youngest infant. A sample trace in the youngest infant in our sample (25 + 5 weeks PMA) is shown, demonstrating the evoked change in [HbT] in the contralateral and ipsilateral somatosensory cortex after a painful stimuli given at $t = 20$ s. Sampling frequency, 6 Hz.

youngest infants. In the age range from 25–38 weeks PMA, regression analysis, used to estimate the time to peak response as a function of PMA, demonstrates a clear increase in latency with age (regression coefficient, −0.9861 μmol/L/week; 95% CI limits: −1.5361, −0.4361 μmol/L/week).

Non-noxious tactile stimulation does not evoke infant cortical responses

Non-noxious mechanical stimulation of the plantar surface of the foot did not elicit a measurable change in the hemodynamic

activity in either the contralateral or ipsilateral somatosensory cortex ($n = 11$, 26–35 weeks PMA). In each case a stimulus intensity was used that produced a visible foot withdrawal, demonstrating that the sensory input was sufficient to produce a reflex motor response. Despite this, no measurable cortical hemodynamic response was recorded.

Discussion

Interpreting cortical hemodynamic pain responses

Our results show that noxious stimulation can evoke specific hemodynamic changes in the cortex of the youngest infants recorded at 25 weeks PMA. These changes were measured using near-infrared spectroscopy, which allowed us to perform continuous and noninvasive monitoring of blood oxygenation of the brain at the bedside after a clinically required heel lance. Many studies have confirmed the cerebral origin of such optical hemodynamic recordings from the surface of the head and the feasibility of its use to detect neural activity (Gratton et al., 2005). It has been used previously in infants, from neonates to 12-month-olds, to measure the hemodynamic correlates of neural activity during visual, memory, and language tasks (Baird et al., 2002; Pena et al., 2003; Taga et al., 2003).

It is reasonable to suppose that increasing hemodynamic responses correlate with increasing cortical activity. The magnitudes of the evoked responses are similar to those obtained in infants using visual and olfactory stimuli (Meek et al., 1998; Bartocci et al., 2000). The smaller cortical responses to noxious stimulation in younger infants are likely to reflect lower energy requirements because of less neuronal activity (Kostovic and Judas, 2002). In adults, there is a linear relationship between subjective pain intensity and regional cerebral blood flow in the contralateral somatosensory cortex (Bornhovd et al., 2002). Our results therefore imply that infants undergoing intensive care process painful experiences at the cortical level but that the activity associated with this process increases with postmenstrual age. This is a specific effect, restricted to the contralateral cortex and cannot be ascribed to age-related changes in global cerebral blood flow. However, a true confirmation of cortical pain processing in preterm infants would require electrophysiological measures such as somatosensory evoked potentials.

The development of spinal and supraspinal pain processing in human infants

In contrast to the cortical responses reported here, spinally mediated reflex limb withdrawal from a noxious stimulus is greater in both magnitude and duration in the youngest infants and decreases with age (Fitzgerald et al., 1988; Andrews and Fitzgerald, 1994, 1999). Our data therefore highlights the difference between spinal and cortical nociceptive processing in human infants and shows that the strong reflex responses in preterm neonates do not necessarily mean more perceived pain. Reflexes may have a protective function for an organism that is less able, through cortical immaturity, to perceive and organize a more directed response. Reflex thresholds are also lower threshold in infants than in adults and can be evoked by von Frey hair mechanical stimulation, as observed here. The postnatal development of cutaneous flexion reflexes in rat pups and preterm human infants shows remarkable parallels (Fitzgerald et al., 1988; Fitzgerald and Jennings, 1999) and it is likely that this reflex excitability is attributable to an altered balance of local and descending excitatory and inhibitory processing in the developing dorsal horn (Fitzgerald, 2005). Here, we show that von Frey hair mechanical stimulation of the foot, sufficient to evoke a flexion reflex, did not elicit a measurable hemodynamic response in the somatosensory cortex, again highlighting the differences between spinal and cortical pain pathways at this stage and suggesting that early cortical pain processing may be more specific than spinal processing.

The onset and maturation of cortical pain processing

Much has been written about when exactly a human preterm infant or fetus may begin to process pain (Lee et al., 2005). It would in principle, with more data, be possible to establish whether there was a well defined age of onset in the cortical pain response. However, as the age of onset may precede the limits of viability (23–24 weeks PMA) we cannot assume that cortical responses seen in neonates are directly translatable to the fetus *in utero* (Mellor et al., 2005).

The long latencies of the cortical responses in the youngest infants are likely attributable to the low conduction velocities and slow synaptic responses in the nociceptive circuitry, which is consistent with the long latency reflex responses observed at the same age (Andrews and Fitzgerald, 1999). Despite the long latencies, their cortical responses were well defined with a clear onset. The responses were even evident in the two extremely premature infants that were receiving morphine at the time of the study. This is interesting in light of recent reports that show that morphine has no effect on behavioral and physiological pain scores after heel lance (Carbajal et al., 2005) and requires further investigation.

Higher pain processing does not only take place in the somatosensory cortex. Functional imaging studies in adults have provided a picture of a "pain matrix" in the brain, subdivided into a medial and a lateral system, based on the projection sites of the lateral and medial thalamic structures to the cortex. The somatosensory cortices, SI and SII, in the lateral system are thought to subserve a discriminatory role in detecting pain localization and intensity, whereas the medial system involving the anterior cingulate cortex and the insula are thought to mediate the cognitive-evaluative component of pain (Apkarian et al., 2005; Brooks and Tracey, 2005). The results show that the cortical response is significantly attenuated during sleep, which in adults has been interpreted as evidence of cognitive processing of pain (Wang et al., 2004) but could be because of a general reduced firing ability of cortical neurones during sleep and a widespread impairment of sensory processing (Rosanova and Timofeev, 2005). Behavioral states are more undifferentiated in preterm infants and a large proportion of their sleep is classified as indeterminate, that is neither active nor quiet sleep (Lehtonen and Martin, 2004), which complicates interpretation of these data on the basis of cognitive processing.

The significance of cortical pain processing in human infants

Preterm infants are exposed to painful procedures as part of their essential medical care. The adverse effects are both immediate and potentially long-term, affecting future sensation and behavior (Anand, 2000; Grunau et al., 2001; Fitzgerald and Walker, 2003). However, because infants are unable to report pain directly, indirect physiological and behavioral methods are required to assess its existence and severity (Stevens and Franck, 2001). These methods have demonstrated the ability of the youngest infants to mount a strong and organized response to noxious stimulation, however, it is not clear at what level of the CNS these responses are produced. Many complex physiological and behavioral responses can be mediated through spinal cord and brainstem reflex pathways, whereas true perception of pain requires cortical processing of noxious stimulation (Treede et al.,

1999). The advantage of investigating the hemodynamic activity in the infant brain is that it provides, for the first time, a direct measure of cortical activation in response to noxious stimulation in the preterm infant. It has highlighted the dissociation between spinal reflex responses and cortical activity and therefore may be a more sensitive way to assess the effectiveness of analgesic strategies.

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