Pain 122 (2006) 109–117

PAIN

www.elsevier.com/locate/pain

Pain activates cortical areas in the preterm newborn brain

Marco Bartocci [a,b,*], Lena L. Bergqvist [a,c], Hugo Lagercrantz [a], K.J.S. Anand [d]

[a] *Neonatal Research Unit, Astrid Lindgren's Children's Hospital, Karolinska University Hospital, Karolinska Institute, SE-17176 Stockholm, Sweden*
[b] *Department of Pediatrics, Neonatal Intensive Care, University of Genoa, Gaslini Institute, I-16147 Genoa, Italy*
[c] *Research and Development Unit, Department of Internal Medicine, Ostersund Hospital, Jämtland County Council, SE 831 25 Östersund, Sweden*
[d] *Departments of Pediatrics, Anesthesiology, Pharmacology, Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, and Pain Neurobiology Laboratory, Arkansas Children's Hospital Research Institute, Little Rock, AR 72202-3591, USA*

Received 13 June 2005; received in revised form 30 October 2005; accepted 17 January 2006

Abstract

To study the patterns of supraspinal pain processing in neonates, we hypothesized that acute pain causes haemodynamic changes associated with activation of the primary somatosensory cortex. Forty preterm neonates at 28–36 weeks of gestation (mean = 32.0) and at 25–42 h (mean = 30.7) of age were studied following standardized tactile (skin disinfection) and painful (venipuncture) stimuli. Changes in regional cerebral haemodynamics were monitored by near infrared spectroscopy (NIRS) over both somatosensory cortices in 29 newborns, and over the contralateral somatosensory and occipital areas in 11 newborns. Heart rate (HR) and peripheral oxygen saturation (SaO_2) were recorded simultaneously with NIRS parameters: oxygenated [HbO_2], deoxygenated, and total hemoglobin. Tactile stimulation produced no changes in HR or SaO_2. HR increased in the first 20 s ($p < 0.001$), while SaO_2 decreased during the 40 s after venipuncture ($p < 0.0001$). Following tactile or painful stimulation, [HbO_2] increased bilaterally regardless of which hand was stimulated ($p < 0.0001$). Pain-induced [HbO_2] increases in the contralateral somatosensory cortex ($p < 0.05$) were not mirrored in the occipital cortex ($p > 0.1$). Pain-related [HbO_2] increases were more pronounced in male neonates ($p < 0.05$ on left, $p < 0.001$ on right), inversely correlated with gestational age ($r = -0.53$ on left, $p < 0.01$; $r = -0.42$ on right, $p < 0.05$) and directly correlated with postnatal age ($r = 0.75$ on left, $p < 0.0001$; $r = 0.67$ on right, $p < 0.0001$). Painful and tactile stimuli elicit specific haemodynamic responses in the somatosensory cortex, implying conscious sensory perception in preterm neonates. Somatosensory cortical activation occurs bilaterally following unilateral stimulation and these changes are more pronounced in male neonates or preterm neonates at lower gestational ages.

Keywords: Pain; Tactile; Infant–newborn; Near infrared spectroscopy; Cerebral cortex; Consciousness; Sensory perception

1. Introduction

Recurrent neonatal pain and stress occur routinely during neonatal intensive care, particularly among the extremely low birth weight preterm neonates (Johnston et al., 1997; Simons et al., 2003; Stevens et al., 2003). Analgesic or anesthetic drugs, whose long-term effects are not yet fully understood (Anand and Soriano, 2004), may attenuate prolonged pain but may not be

Abbreviations: bpm, beats per minute; BW, birth weight; fMRI, functional magnetic resonance imaging; GA, gestational age; HbH, deoxygenated hemoglobin; HbO_2, oxygenated hemoglobin; Hbtot, total hemoglobin (=HbO_2 + HbH); HR, heart rate; NICU, Neonatal Intensive Care Unit; NIRS, near infrared spectroscopy; PNA, postnatal age; r-CBV, regional cerebral blood volume; SaO_2, peripheral arterial oxygen saturation.

* Corresponding author. Tel.: +46 8 51777 354; fax: +46 8 51777 353.
E-mail address: marco.bartocci@kbh.ki.se (M. Bartocci).

0304-3959/$32.00
doi:10.1016/j.pain.2006.01.015

effective for the acute pain caused by invasive procedures (Liu et al., 2004; Carbajal et al., 2005). Moreover, a painful procedure like venipuncture for blood sampling is not routinely preceded by any analgesic measures (Simons et al., 2003) and its repercussions on the development of the cerebral cortex or sub-cortical structures are not yet understood.

Recent studies on adult subjects report that painful stimuli are associated with circulatory and metabolic changes in specific cortical and subcortical areas (Ringler et al., 2003; Ohara et al., 2004). Objective evidence for the supraspinal processing of pain in human neonates, particularly those born preterm, is currently lacking. Further, investigation of the mechanisms regulating cerebral haemodynamics in the cortical areas processing pain stimuli might be of great help in understanding the influence of pain on neonatal brain plasticity and their cognitive or behavioral outcomes (Winberg, 1998; Anand et al., 1999b; Anand, 2000; Anand and Scalzo, 2000).

A non-invasive technique, near infrared spectroscopy (NIRS), can detect subtle changes in the concentration of natural chromophores such as oxygenated [HbO_2] and de-oxygenated hemoglobin [HbH]. NIRS was used simultaneously with functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) scans, which demonstrated the reliability of the NIRS technique in monitoring cortical activation in functional studies (Kleinschmidt et al., 1996; Hock et al., 1997; Punwani et al., 1997). NIRS was used both in adults (Meek et al., 1995) and newborn infants (Sakatani et al., 1999; Bartocci et al., 2000; Meek, 2000) to monitor haemodynamic and oxygenation adjustments related to the cerebral cortical processing of specific stimuli. Franceschini et al. (2003) showed that NIRS can detect cortical haemodynamic changes related to tactile stimulation. Functional MRI and EEG studies provide consistent evidence that painful and tactile stimuli are associated with distinct patterns of response within the human cerebral cortex (Chen et al., 2002).

We hypothesized that acute pain activates the somatosensory cortex in preterm neonates, associated with significant metabolic changes in the cortical areas associated with pain processing, which can be measured by the NIRS technique. To compare the cortical processing of tactile and painful stimulation, we recorded the responses during gentle cleaning of the skin (tactile stimulation) and during venipuncture (acute pain). The NIRS device used in this study recorded from two channels, placed bilaterally over both somatosensory areas. To test for the specificity of these responses, we compared the somatosensory cortex with the occipital cortex, which is not primarily involved in sensory processing for painful or tactile stimuli.

2. Methods

2.1. Study group

This prospective study included 40 vaginally delivered newborn infants, who fulfilled the inclusion criteria and required blood sampling in the NICUs at Karolinska Hospital (Stockholm, Sweden) or Gaslini Hospital (Genoa, Italy). Cranial and cardiac ultrasonography were performed on all subjects. The inclusion criteria selected neonates born after 26 weeks of gestation, postnatal age (PNA) more than 24 hours (h), absence of congenital malformations affecting the brain circulation or the cardiovascular system, ongoing intubation, and mechanical ventilation, who had not received analgesic, anesthetic, or sedative drugs over the preceding 24 h (e.g., morphine, fentanyl, phenobarbital, and midazolam). The Local Ethics Committee approved the research protocol. Informed consent was obtained from the parents of all enrolled subjects and around 90% of the parents approached for this study gave their consent.

2.2. NIRS settings

A double-channel near infrared spectroscopy device (NIRO 300, Hamamatsu Photonics, Hamamatsu, Japan) was used to monitor the changes in oxy-hemoglobin [HbO_2], deoxy-hemoglobin [HbH], and total hemoglobin [Hbtot] concentrations. The basic principles of this technique (Jöbsis, 1977), its use in functional studies (Villringer et al., 1997; Benaron et al., 2000), and its reliability in studying cerebral cortical responses in newborn infants (Sakatani et al., 1999; Bartocci et al., 2000; Meek, 2000) have been described in detail elsewhere.

Briefly, the NIRO 300 device produces light at four different wavelengths (775, 810, 850, and 910 nm) by means of four pulsed laser diodes. Each diode has a pulse frequency of approximately 2 kHz, each pulse lasts about 100 ns, and average output power is about 1 mW. Through an optical fiber cable, the emitter probe provides near infrared light, which is detected by the receiver probe. The emitter and receiver probes constitute one pair of optodes, located in a black semi-rigid rubber holder, with an inter-optode distance of 4 cm and a differential path-length factor (DPF) of 4.2 (Wyatt et al., 1990).

2.3. Study procedures

The study interval was divided into 3 main periods, period 0 (P_0 = baseline), period 1 (P_1 = tactile stimulus) for cleaning, and period 2 (P_2 = painful stimulus) for the venipuncture. Neonates were gently handled in their cot, the NIRS probes were placed on the head, and the venipuncture site was identified visually. When the infant was in a quiet, awake, and stable condition (behavioral state 3 according to Prechtl) (Prechtl, 1974) the various haemodynamic signals including NIRS were recorded for a baseline period of at least 60 s (P_0). During period 1, the dorsum of the right or left hand was stimulated for 30 s by disinfecting the skin with an alcohol-soaked, cotton pad at room temperature. The onset of tactile stimulation was marked on the NIRS recording and the following 60 s were stored (P_1). During this period, the neonate was quiet and not moving. Venipuncture was performed by an expert

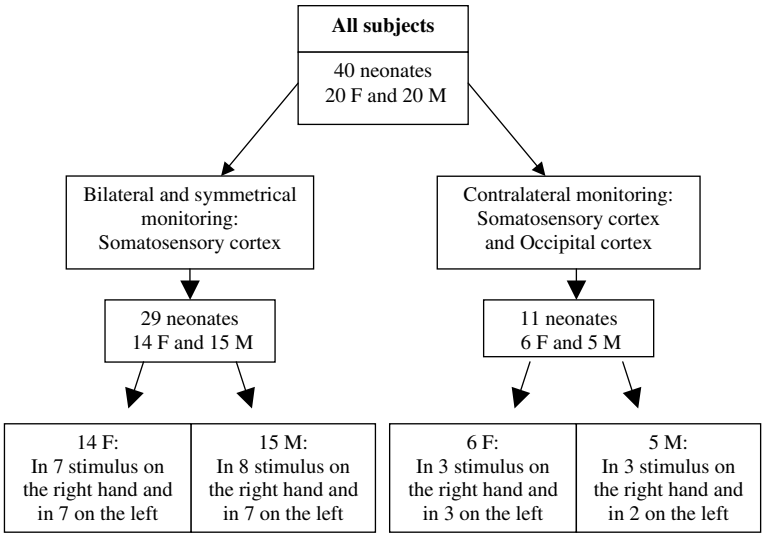

Fig. 1. Flow diagram describing the study population (F, females; M, males).

neonatal nurse using a standard needle size (24 gauges, 0.75 in.) for all newborns and a 60 s period following the needle insertion was recorded for analysis (P_2). The environment around the infant during the experiment was kept as quiet as possible, avoiding any kind of visual, auditory, and olfactory external stimuli. Those newborns that showed prolonged episodes of crying and movements affecting the NIRS signal were not considered for further analysis, thus excluding 6 of the 46 subjects monitored.

We analyzed the recordings from 40 subjects (20 females and 20 males), as described in Table S1 and Fig. 1. For 29 neonates (15 females), the 2 pairs of optodes were positioned over the somatosensory cortex symmetrically on each side of the head. The emitter optode was placed about 2 cm below and slightly posterior to the C3/C4 position [according to the international EEG 10–20 system (Mehagnoul-Schipper et al., 2002; Schroeter et al., 2002; Kuboyama et al., 2005; Suzuki et al., 2005)]. The receiver optode was consequently positioned 4 cm above the emitter optode in order to illuminate the primary somatosensory cortex and underlying structures (Fig. 2 and Figure S1 in supplementary material on-line). Eleven newborns were monitored with the 2 pairs of optodes placed on the same side of the head: one pair overlying the somatosensory cortex (as described above) and the other pair overlying the occipital cortex. In these 11 newborns, the tactile and painful stimuli were applied contralaterally to the side where the optodes were placed.

2.4. Recording and data analyses

2.4.1. Heart rate and arterial oxygen saturation

Heart rate (HR) and arterial oxygen saturation (SaO_2) data were recorded by a HP monitoring system (Hewlett–Packard, Boeblingen, Germany) simultaneously with the NIRS data. Respiration was observed during the procedure. HR and SaO_2 average values were averaged at baseline and at 10, 20, 30, 40, 50, and 60 s after the tactile and painful stimuli, respectively. Repeated-measures ANOVA and Newman–Keuls post

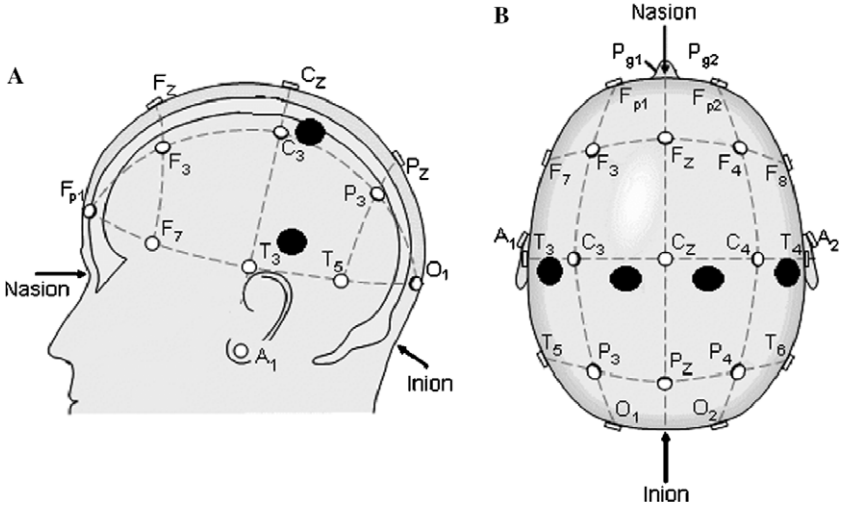

Fig. 2. Position of NIRS optodes (dark circles) placed in reference to the international EEG system (open circles). (A) Viewed from the left side. (B) Viewed from the top.

hoc tests were used to compare these values throughout the experiment. Differences between males and females were analyzed by Student's *t*-test for independent samples.

2.5. Near infrared spectroscopy

NIRS data were sampled every second during the baseline (P_0), tactile (P_1), and pain stimulation (P_2) periods and exported to a computer via RS232 digital output. As the sampling occurred every second, each of these periods contained 60 numerical values corresponding to the [HbO_2] and [HbH] concentrations. [Hbtot] was calculated off-line by adding the [HbO_2] and [HbH] values (Wyatt et al., 1990). Values during the period P_0 were subtracted from the P_1 and P_2 values for each neonate, to calculate the $[HbO_2]_{diff}$, $[HbH]_{diff}$, and $[Hbtot]_{diff}$ values for tactile vs. baseline and painful vs. baseline periods, respectively. An average for each measurement (from the 60 measured values per period) was calculated for each neonate, to describe the haemodynamic changes in the left and right hemispheres during each study period (P_0, P_1, and P_2).

Using repeated-measures ANOVA and Newman–Keuls post hoc comparisons, the P_0 curves for each side were compared to the P_1 and P_2 curves of the same side, for [HbO_2], [HbH], and [Hbtot]. Student's *t*-tests for independent variables were used to compare $[HbO_2]_{diff}$ values between the parietal and occipital cortex (in neonates where both sets of optodes were positioned contralateral to the stimuli), to compare $[HbO_2]_{diff}$ values between the ipsilateral and contralateral side, to evaluate inter-hemispheric differences depending on whether the left or the right hand was stimulated, and to compare the differences between male and female infants. Correlations between GA, BW, PNA, and duration of the venipuncture, and magnitude of the NIRS response were performed by linear regression analyses. Data were analyzed by the program Statistica® (6.0, StatSoft 2001, Inc., Tulsa, USA), and *p*-values of less than 0.05 were considered significant.

3. Results

3.1. Study population

The neonates were born at 28–36 weeks of gestation (mean 32.0) and were studied at 25–42 h (mean 30.7) after birth (Table 1 in supplementary material on-line). The painful stimulation lasted for 35–60 s. No differences occurred between male and female infants in the GA (males 31.4 vs. females 32.7 weeks), PNA (males 32.0 vs. females 29.5 h), BW (males 1841 vs. females 1958 g), or duration of venipuncture (males 46.7 vs. females 49.8 s). The GA, PNA, BW, and duration of the venipuncture were also similar among neonates receiving left- or right-hand stimulation.

3.2. Heart rate and arterial oxygen saturation

During tactile stimulation, no significant changes occurred in the HR ($p > 0.05$) and SaO_2 ($p > 0.05$) values. The HR increased significantly with acute pain (repeated-measures ANOVA, $p < 0.001$) with differences from baseline level noted at 10 and 20 s following venipuncture, but not at 30 s (baseline 138.5 bpm (±11.5); after venipuncture, at P_{10}: 171.8 bpm (±11.2) and P_{20}: 174.9 (±15.5); post hoc Newman–Keuls tests, $p < 0.001$) (Figure S2 in supplementary material on-line). No differences occurred in the HR responses between female and male infants or between neonates receiving painful stimulation on the right vs. the left hand ($p > 0.05$). The SaO_2 values decreased significantly (repeated-measures ANOVA, $p < 0.0001$), with differences noted from 10 to 40 s following venipuncture (baseline 96.1% (±2.3); after venipuncture, at P_{10} 92.3% (±1.9), at P_{20} 89.2% (±1.0), at P_{30} 91.6% (±2.3), and at P_{40} 91.4 (±1.9); post hoc Newman–Keuls tests, $p < 0.001$) (Figure S2 in supplementary material on-line). No differences occurred in SaO_2 values between male and female neonates or between the two sides of the venipuncture ($p > 0.05$). None of the subjects showed apnoeic episodes during the experiment. No correlation between the magnitude of HR increase and GA or PNA was found.

3.3. NIRS data

Neuronal activation within cortical areas is coupled with regional changes in cerebral blood volume (r-CBV), thus reflected in the [HbO_2] changes measured by NIRS. We found increases in the [HbO_2] concentrations in both hemispheres after tactile stimulation, with further increases following painful stimulation (Fig. 3 and Figure S2 in supplementary material on-line). The response to pain was greater in the male neonates than in female preterm neonates ($p < 0.05$ for the left hemisphere and $p < 0.001$ for the right hemisphere; Fig. 4). Following tactile stimulation, no differences were recorded between the [HbO_2] changes on the right and left sides ($p > 0.5$ after stimulation of the right hand; $p > 0.5$ after stimulation of the left hand). In contrast, venipuncture on the right hand stimulated greater [HbO_2] increases in the left hemisphere as compared to right hemisphere ($p < 0.01$), whereas venipuncture in the left hand showed no differences between the right and left hemispheres ($p > 0.1$; Fig. 5). The latency between the skin puncture and a significant increase in [HbO_2] compared to the baseline was 2 s both in females and in males (repeated-measures ANOVA, $p < 0.0001$ and post hoc Newman–Keuls test, $p < 0.01$).

The [HbO_2] increases after pain were directly correlated with postnatal age ($r = 0.75$ for the left hemisphere, $p < 0.0001$; $r = 0.67$ for the right hemisphere, $p < 0.0001$). The magnitude of pain-induced [HbO_2] increases was negatively correlated with the GA ($r = -0.53$ for the left hemisphere, $p < 0.01$; $r = -0.42$ for the right hemisphere, $p < 0.05$) (Figure S4 in supplementary material on-line). No correlation occurred

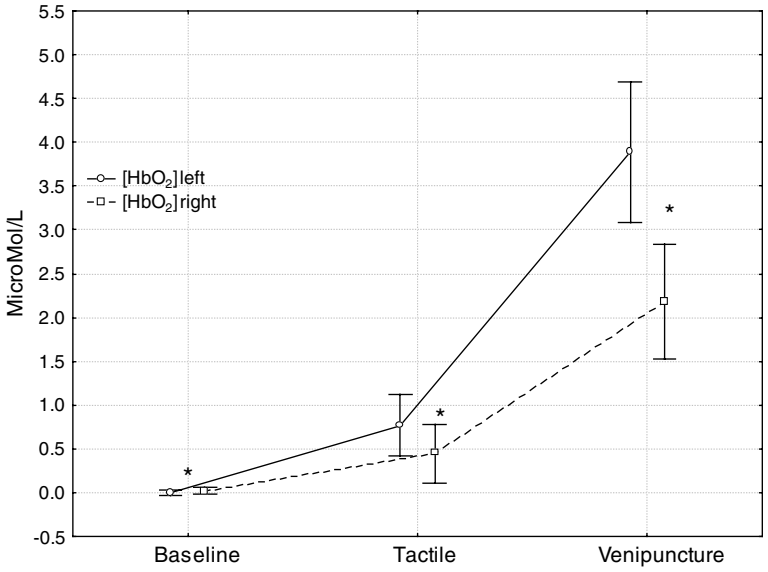

Fig. 3. Changes in [HbO_2] values occurring on the left side (circles and continuous line) and on the right side (squares and dotted line) in all subjects, regardless of which hand was being stimulated. The figure shows significant [HbO_2] increases from baseline in both hemispheres following tactile and painful stimulation (does not denote differences between the two hemispheres) (Wilk's lambda = 0.1945, $F_{(4,36)} = 37.28$, $p < 0.0001$; Newman–Keuls post hoc tests: baseline vs. tactile $*p < 0.001$; baseline vs. venipuncture $*p < 0.001$; tactile vs. venipuncture $*p < 0.001$). Vertical bars denote the 95% confidence intervals and the middle points denote the mean values.

between the [HbO_2] increases and the duration of venipuncture ($r = -0.17$ for the left hemisphere, $p > 0.1$; $r = -0.12$ for the right hemisphere, $p > 0.5$), or between [HbO_2] increases and BW ($r = -0.34$ for the left hemisphere, $p > 0.05$; $r = -0.18$ for the right hemisphere, $p > 0.1$).

Neonates monitored with NIRS on the contralateral side ($n = 11$) from venipuncture showed significant [HbO_2] increases over the somatosensory cortex (ANOVA, $p < 0.01$, baseline vs. tactile $p < 0.05$; baseline vs. pain $p < 0.01$; tactile vs. pain $p < 0.01$, Newman–Keuls post hoc tests), but no significant changes occurred over the occipital cortex (ANOVA, $p > 0.05$; baseline vs. tactile $p > 0.1$; baseline vs. pain $p > 0.1$; tactile vs. pain $p > 0.5$; Newman–Keuls post hoc tests) (Fig. 6).

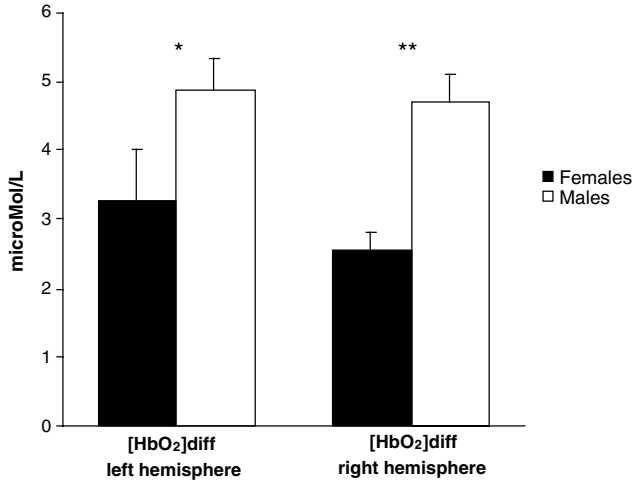

Fig. 4. Comparison of the cortical [HbO_2] increases between the female (black columns) and male (white columns) neonates following venipuncture. Males showed a more pronounced increase in [HbO_2] compared to females ($*p < 0.05$ on the left and $**p < 0.001$ on the right; Student's t-tests). Columns denote mean values and bars standard deviations.

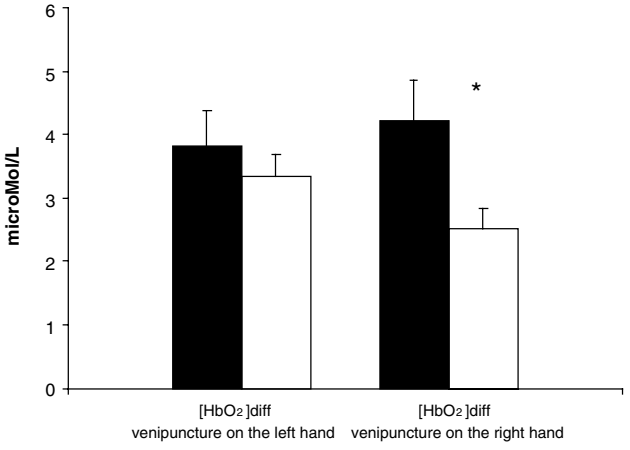

Fig. 5. Differences in cortical [HbO_2] changes following venipuncture on the left or the right hand. Black bars denote [HbO_2] increases on the left hemisphere and white bars on the right hemisphere. When the venipuncture occurred on the right hand, there was a more pronounced increase in [HbO_2] over the left hemisphere ($*p < 0.01$, Student's t-test), but no significant differences occurred following venipuncture on the left hand ($p > 0.05$). Columns denote mean values and bars standard deviations.

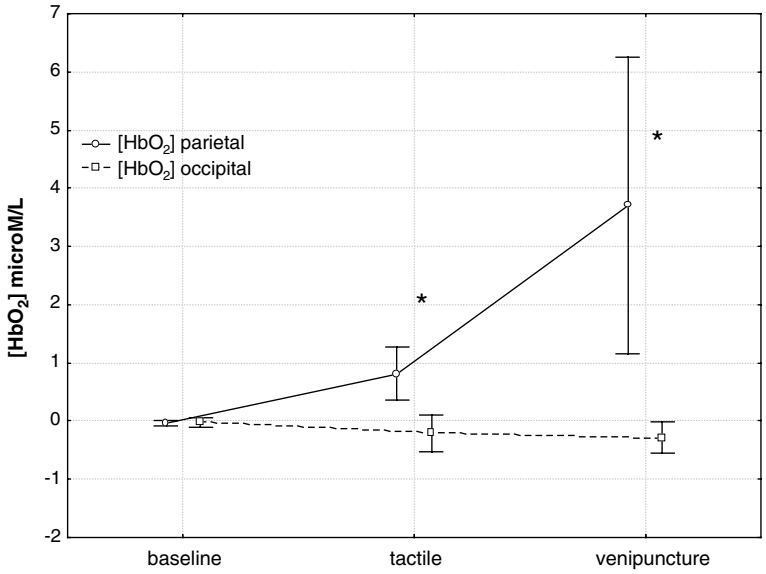

Fig. 6. Differences between cortical [HbO_2] values during simultaneous recordings from pairs of optodes located over the somatosensory cortex (continuous line) and the occipital cortex (dotted line). Significant changes occurred over the parietal area (repeated-measures ANOVA, *$p < 0.01$; baseline vs. tactile $p < 0.05$; baseline vs. pain $p < 0.01$; tactile vs. pain $p = 0.01$, Newman–Keuls post hoc tests), but not over the occipital area (repeated-measures ANOVA, $p > 0.05$; $p > 0.1$ baseline vs. tactile; $p > 0.1$ baseline vs. pain; $p > 0.5$ tactile vs. pain Newman–Keuls post hoc tests). Data from 11 neonates presented as mean values and 95% confidence intervals (vertical bars).

4. Discussion

In preterm newborn infants, unilateral tactile or painful stimuli elicited bilateral increases in cortical [HbO_2] concentrations, implying increases in regional cerebral blood flow that resulted from changes in metabolic activity within the somatosensory cortical areas. Responses to pain were greater in preterm boys than in girls and were greater over the left hemisphere when venipuncture occurred on the right side. The latency between venipuncture and cortical activity was comparable to that of adults (Spiegel et al., 1996) and was similar in male and female neonates. A negative correlation occurred between pain-induced cortical activity and gestational age, consistent with the ontogeny of pain thresholds in preterm neonates (Fitzgerald et al., 1988), but a positive correlation occurred with the postnatal age, consistent with a postnatal decay of fetal inhibition (Greenough et al., 1990).

Bucher et al. (1995) reported that r-CBV, as reflected by [Hbtot] variations, may increase, decrease or remain unchanged after heel puncture in preterm babies, with wide differences among subjects. In contrast, Brazy (1988) showed that [Hbtot] and [HbH] increased during crying in newborn infants. In abstract form, Meek (2000) reported unilateral somatosensory activation by NIRS in full-term infants, whereas Slater et al. (2004) recently presented functional cortical activity following heelsticks in seven neonates at 29–42 weeks GA. In contrast, our studies included a larger sample size and data were obtained only from preterm neonates, with tightly controlled gestational and postnatal ages.

The NIRS methods can detect blood flow fluctuations in the cerebral micro-vasculature, that are coupled with cortical neuronal activation in newborn and adult subjects (Kleinschmidt et al., 1996; Obrig and Villringer, 1997; Meek et al., 1998; Sakatani et al., 1999; Bartocci et al., 2000; Benaron et al., 2000). Although multi-channel NIRS prototypes have been introduced with encouraging results (Boas et al., 2001), most of the commercially available devices only allow the use of two pairs of optodes. This may represent a limitation when studying the responses to painful stimuli, which simultaneously involve multiple areas of the brain (Narsinghani and Anand, 2000; Hofbauer et al., 2001).

In our studies, NIRS optodes were placed over the post-central gyrus (Fig. 2 and Figure S1 in supplementary material on-line) bilaterally to illuminate the somatosensory cortex and contiguous cortical areas, that are postulated to be involved in pain processing from adult studies (Narsinghani and Anand, 2000; Price, 2000). We also studied 11 newborns by placing pairs of optodes over the contralateral parietal and occipital cortex, to exclude the possibility that haemodynamic changes in the somatosensory cortex may reflect global increases in cerebral blood flow, resulting from an increased cardiac output following pain (Morison et al., 2001). Auditory (Sakatani et al., 1999), visual (Meek et al., 1995, 1998), and olfactory (Bartocci et al., 2000) stimuli, which can confound the pain-induced NIRS responses, were reduced as much as possible during these studies and recordings with movement artifact were discarded.

The changes in HR and SaO_2 following pain are consistent with previous studies (Van Cleve et al., 1995;

Morison et al., 2001), but do not explain the [HbO_2] changes noted over the somatosensory cortex. The [HbO_2] changes persisted over the entire 60-s period which was analyzed, whereas changes in HR and SaO_2 were transient, lasting 20–30 s only. Moreover, following venipuncture, SaO_2 were decreased, whereas cortical [HbO_2] values increased significantly. In many neonates, though not reported in these results, the [Hbtot] values remained significantly above the baseline for 2–3 min, well after the changes in HR and SaO_2 had subsided.

Gender differences in pain-related cortical activation also occur in adult subjects. Noxious thermal stimulation, as recorded by PET in the contralateral prefrontal cortex, insula, and thalamus, was perceived with greater intensity and produced greater cortical activity in adult women than in men (Paulson et al., 1998), whereas visceral pain caused greater activation of supraspinal areas in men than in women (Berman et al., 2000). Gender-based differences in pain behavior occur during the neonatal period (Guinsburg et al., 2000), but do not persist in pre-pubertal children (Hogeweg et al., 1996). Female preterm neonates showed more robust facial expressions of pain than males following acute pain, but no differences occurred with multidimensional assessments of pain in the same study (Guinsburg et al., 2000). The greater cortical activation observed in males, both contralateral and ipsilateral to the venipuncture, implies increased neuronal activation in the underlying brain regions. This activation can either lead to surround-activation or surround-inhibition of adjacent neurons (Derdikman et al., 2003), or may be coupled to other neuronal circuits involved in pain modulation or pain affect (Hofbauer et al., 2001; Coghill et al., 2003; Singer et al., 2004). Another factor that has to be kept in mind as possible explanation of gender-related neurovascular difference is linked to hormonal differences. Reproductive hormones may potentially affect pain perception, including estrogen, oxytocin, or other hormones. In adult subjects, estrogens play a role in migraine headaches by affecting dural vasculature directly and perhaps indirectly by altering the levels of ionized magnesium (Lichten et al., 1996). Multiple studies show that estrogens can have either excitatory or inhibitory effects within the CNS, mediated via different estrogen receptors activating different types of neurons; for example, with inhibition of EAA-induced activity in the solitary tract nuclei by 17-β-estradiol (Woolley, 1999a,b; Kelly and Levin, 2001; Xue and Hay, 2003). Oxytocin and hypocretin also may modulate sensory input, particularly in regions of the brain and spinal cord related to pain perception and autonomic tone (Bodnar et al., 1984; van den Pol, 1999). Further investigations using fMRI or other imaging techniques are required to identify specific structures involved in the gender-based differences of supraspinal pain processing in preterm neonates.

Crying begins to influence cerebral haemodynamics after a time span of about 5 min, perhaps related to changes in venous return or intrathoracic pressure, rather than to a functional response. Our observations show a functional activation of the somatosensory cortex during the initial, "silent" phase, between the pain stimulus and a behavioral response. The latency and duration of these responses are also consistent with the parallel–serial model of affective pain processing (Price, 2000) and with recent mapping of cortical activity following acute pain in adult subjects (Ibinson et al., 2004; Ohara et al., 2004).

The number of painful procedures in the NICU may determine the patterns of subsequent pain processing in preterm neonates (Johnston and Stevens, 1996). Therefore, we studied all neonates between 25 and 42 h after birth, so that they were not exposed to widely disparate numbers of painful stimuli. A gradual loss of fetal inhibition or an increased excitability resulting from early pain exposure may explain why the postnatally older newborns responded with more pronounced increases in [HbO_2] (Taddio et al., 2002; Liu et al., 2004). Multiple lines of clinical evidence, anatomical and neurophysiological data indicate lower pain thresholds in early development (Anand, 1998; Fitzgerald et al., 1988), consistent with greater cortical [HbO_2] responses in the more immature preterm neonates following venipuncture.

Bilateral activation of cortical areas occurred in all infants, regardless of the side of venipuncture. We postulate that the NIRS beam was not exclusively confined to the S1 area, but also sampled other regions such as S2 cortex/anterior insula, the ventral premotor area, and the anterior cingulate cortex, areas that are implicated in bilateral pain processing (Coghill et al., 1999; Ibinson et al., 2004; Ohara et al., 2004). Changes in the NIRS signal are coupled to the neuronal firing activity in synaptic terminals, regardless of whether excitatory or inhibitory neurotransmitters are released, therefore any correlation with pain intensity or other perceptual qualities of acute pain is speculative and cannot be based on these data.

There is, however, widespread uncertainty about the cognitive ability or sensory awareness of newborn infants (Zelazo, 2004). Clinicians or scientists may characterize premature infants essentially as unconscious automata, capable of reflexive responses only (Anand et al., 1999a; Zelazo, 2004). The lateralization of pain processing, the latency and duration of these responses, their gradations across gestational age and postnatal age, and the neuroanatomical location of these responses (parietal vs. occipital) suggest that preterm infants may be consciously processing the acute pain from venipuncture. These data suggest a reconsideration of the prevalent notions regarding pain and consciousness in early human life (Anand and Maze, 2001).

In conclusion, using a non-invasive technique to monitor preterm neonates, we found that tactile and painful stimuli specifically activate somatosensory cortical areas. Male neonates show greater responses to pain than female neonates, whereas all preterm newborns at lower gestations and older postnatal ages showed progressively increasing cortical responses to venipuncture. We propose that collateral pain circuits are activated simultaneously in the preterm neonate and that future correlations with behavioral responses and also with other methods of studying functional cortical activity will elucidate the clinical importance of these findings.

Acknowledgments

The study was supported by The Wallenberg Foundation, Sällskapet Barnvård Foundation, Samariten Foundation, Freemasons and the Swedish Science Foundation grant nr 5234, all in Stockholm, First of May Flower Foundation, Gothenburg, and The Research and Development Unit, Jämtland County Council, Östersund. We are most thankful to the nurse staff of both NICUs at Karolinska Hospital in Stockholm and Gaslini Hospital in Genoa, whose generous and skillful help made this work possible. We also thank our colleagues BSN Elinor Ihre, Dr. Tina Berglin, Dr. Josef Milerad, and Prof. Giovanni Serra for significant contributions and guidance in the performance of these studies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pain.2006.01.015.

References

Anand KJS. Clinical importance of pain and stress in preterm neonates. Biol Neonate 1998;73(1):1–9.

Anand KJS. Pain, plasticity, and premature birth: a prescription for permanent suffering. Nat Med 2000;6(9):971–3.

Anand KJ, Maze M. Fetuses, fentanyl, and the stress response: signals from the beginnings of pain. Anesthesiology 2001;95(4):823–5.

Anand KJS, Scalzo FM. Can adverse neonatal experiences alter brain development and subsequent behavior. Biol Neonate 2000;77(2):69–82.

Anand KJS, Soriano SG. Anesthetic agents and the immature brain: are these toxic or therapeutic. Anesthesiology 2004;101(2):527–30.

Anand KJS, Rovnaghi C, Walden M, Churchill J. Consciousness, behavior, and clinical impact of the definition of pain. Pain Forum 1999a;8:64–73.

Anand KJS, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term effects of repetitive pain in neonatal rat pups. Physiol Behav 1999b;64(4):627–37.

Bartocci M, Winberg J, Ruggiero C, Bergqvist LL, Serra G, Lagercrantz H. Activation of olfactory cortex in newborn infants after odor stimulation: a functional near-infrared spectroscopy study. Pediatr Res 2000;48(1):18–23.

Benaron DA, Hintz SR, Villringer A, Boas D, Kleinschmidt A, Frahm J, et al. Noninvasive functional imaging of human brain using light. J Cereb Blood Flow Metab 2000;20(3):469–77.

Berman S, Munakata J, Naliboff BD, Chang L, Mandelkern M, Silverman D, et al. Gender differences in regional brain response to visceral pressure in IBS patients. Eur J Pain 2000;4(2):157–72.

Boas DA, Gaudette T, Strangman G, Cheng X, Marota JJ, Mandeville JB. The accuracy of near infrared spectroscopy and imaging during focal changes in cerebral hemodynamics. Neuroimage 2001;13(1):76–90.

Bodnar RJ, Nilaver G, Wallace MM, Badillo-Martinez D, Zimmerman EA. Pain threshold changes in rats following central injection of beta-endorphin, met-enkephalin, vasopressin or oxytocin antisera. Int J Neurosci 1984;24(2):149–60.

Brazy JE. Effects of crying on cerebral blood volume and cytochrome aa3. J Pediatr 1988;112(3):457–61.

Bucher HU, Moser T, von Siebenthal K, Keel M, Wolf M, Duc G. Sucrose reduces pain reaction to heel lancing in preterm infants: a placebo-controlled, randomized and masked study. Pediatr Res 1995;38(3):332–5.

Carbajal R, Lenclen R, Jugie M, Paupe A, Barton BA, Anand KJS. Morphine does not provide adequate analgesia for acute procedural pain among preterm neonates. Pediatrics 2005;115(6):1494–500.

Chen JI, Ha B, Bushnell MC, Pike B, Duncan GH. Differentiating noxious- and innocuous-related activation of human somatosensory cortices using temporal analysis of fMRI. J Neurophysiol 2002;88(1):464–74.

Coghill RC, McHaffie JG, Yen YF. Neural correlates of interindividual differences in the subjective experience of pain. Proc Natl Acad Sci USA 2003;100(14):8538–42.

Coghill RC, Sang CN, Maisog JM, Iadarola MJ. Pain intensity processing within the human brain: a bilateral, distributed mechanism. J Neurophysiol 1999;82(4):1934–43.

Derdikman D, Hildesheim R, Ahissar E, Arieli A, Grinvald A. Imaging spatiotemporal dynamics of surround inhibition in the barrels somatosensory cortex. J Neurosci 2003;23(8):3100–5.

Fitzgerald M, Shaw A, MacIntosh N. Postnatal development of the cutaneous flexor reflex: comparative study of preterm infants and newborn rat pups. Dev Med Child Neurol 1988;30(4):520–6.

Franceschini MA, Fantini S, Thompson JH, Culver JP, Boas DA. Hemodynamic evoked response of the sensorimotor cortex measured noninvasively with near-infrared optical imaging. Psychophysiology 2003;40(4):548–60.

Greenough A, Nicolaides KH, Lagercrantz H. Human fetal sympathoadrenal responsiveness. Early Hum Dev 1990;23(1):9–13.

Guinsburg R, de Araujo Peres C, Branco de Almeida MF, de Cassia Xavier Balda R, Cassia Berenguel R, Tonelotto J, et al. Differences in pain expression between male and female newborn infants. Pain 2000;85(1–2):127–33.

Hock C, Villringer K, Müller-Spahn F, Wenzel R, Heekeren H, Schuh-Hofer S, et al. Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS)–correlation with simultaneous rCBF-PET measurements. Brain Res 1997;755(2):293–303.

Hofbauer RK, Rainville P, Duncan GH, Bushnell MC. Cortical representation of the sensory dimension of pain. J Neurophysiol 2001;86(1):402–11.

Hogeweg JA, Kuis W, Oostendorp RA, Helders PJ. The influence of site of stimulation, age, and gender on pain threshold in healthy children. Phys Ther 1996;76(12):1331–9.

Ibinson JW, Small RH, Algaze A, Roberts CJ, Clark DL, Schmalbrock P. Functional magnetic resonance imaging studies of pain: an investigation of signal decay during and across sessions. Anesthesiology 2004;101(4):960–9.

Jöbsis FF. Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. Science 1977;198(4323):1264–7.

Johnston CC, Stevens BJ. Experience in a neonatal intensive care unit affects pain response. Pediatrics 1996;98(5):925–30.

Johnston CC, Collinge JM, Henderson SJ, Anand KJS. A cross-sectional survey of pain and pharmacological analgesia in Canadian neonatal intensive care units. Clin J Pain 1997;13(4):308–12.

Kelly MJ, Levin ER. Rapid actions of plasma membrane estrogen receptors. Trends Endocrinol Metab 2001;12(4):152–6.

Kleinschmidt A, Obrig H, Requardt M, Merboldt KD, Dirnagl U, Villringer A, et al. Simultaneous recording of cerebral blood oxygenation changes during human brain activation by magnetic resonance imaging and near-infrared spectroscopy. J Cereb Blood Flow Metab 1996;16(5):817–26.

Kuboyama N, Nabetani T, Shibuya K, Machida K, Ogaki T. Relationship between cerebral activity and movement frequency of maximal finger tapping. J Physiol Anthropol Appl Human Sci 2005;24(3):201–8.

Lichten EM, Lichten JB, Whitty A, Pieper D. The confirmation of a biochemical marker for women's hormonal migraine: the depo-estradiol challenge test. Headache 1996;36(6):367–71.

Liu JG, Rovnaghi CR, Garg S, Anand KJS. Opioid receptor desensitization contributes to thermal hyperalgesia in infant rats. Eur J Pharmacol 2004;491(2–3):127–36.

Meek J, Cerebral haemodynamic changes during pain measured by near infrared spectroscopy in newborn infants. In: Proceedings of the 5th International Symposium on Pediatric Pain. London June 18–22 2000.

Meek JH, Elwell CE, Khan MJ, Romaya J, Wyatt JS, Delpy DT, et al. Regional changes in cerebral haemodynamics as a result of a visual stimulus measured by near infrared spectroscopy. Proc R Soc Lond B Biol Sci 1995;261(1362):351–6.

Meek JH, Firbank M, Elwell CE, Atkinson J, Braddick O, Wyatt JS. Regional hemodynamic responses to visual stimulation in awake infants. Pediatr Res 1998;43(6):840–3.

Mehagnoul-Schipper DJ, van der Kallen BF, Colier WN, van der Sluijs MC, van Erning LJ, Thijssen HO, et al. Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. Hum Brain Mapp 2002;16(1):14–23.

Morison SJ, Grunau RE, Oberlander TF, Whitfield MF. Relations between behavioral and cardiac autonomic reactivity to acute pain in preterm neonates. Clin J Pain 2001;17(4):350–8.

Narsinghani U, Anand KJS. Developmental neurobiology of pain in neonatal rats. Lab Anim (NY) 2000;29(9):27–39.

Obrig H, Villringer A. Near-infrared spectroscopy in functional activation studies. Can NIRS demonstrate cortical activation? Adv Exp Med Biol 1997;413:113–27.

Ohara S, Crone NE, Weiss N, Vogel H, Treede RD, Lenz FA. Attention to pain is processed at multiple cortical sites in man. Exp Brain Res 2004;156(4):513–7.

Paulson PE, Minoshima S, Morrow TJ, Casey KL. Gender differences in pain perception and patterns of cerebral activation during noxious heat stimulation in humans. Pain 1998;76: 223–9.

Prechtl HF. The behavioural states of the newborn infant (a review). Brain Res 1974;76(2):185–212.

Price DD. Psychological and neural mechanisms of the affective dimension of pain. Science 2000;288(June 9):1769–72.

Punwani S, Cooper CE, Clemence M, Penrice J, Amess P, Thornton J, et al. Correlation between absolute deoxyhaemoglobin [dHb] measured by near infrared spectroscopy (NIRS) and absolute R2′ as determined by magnetic resonance imaging (MRI). Adv Exp Med Biol 1997;413:129–37.

Ringler R, Greiner M, Kohlloeffel L, Handwerker HO, Forster C. BOLD effects in different areas of the cerebral cortex during painful mechanical stimulation. Pain 2003;105(3):445–53.

Sakatani K, Chen S, Lichty W, Zuo H, Wang YP. Cerebral blood oxygenation changes induced by auditory stimulation in newborn infants measured by near infrared spectroscopy. Early Hum Dev 1999;55(3):229–36.

Schroeter ML, Zysset S, Kupka T, Kruggel F, Yves von Cramon D. Near-infrared spectroscopy can detect brain activity during a color-word matching Stroop task in an event-related design. Hum Brain Mapp 2002;17(1):61–71.

Simons SH, van Dijk M, Anand KS, Roofthooft D, van Lingen RA, Tibboel D. Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. Arch Pediatr Adolesc Med 2003;157(11):1058–64.

Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, Frith CD. Empathy for pain involves the affective but not sensory components of pain. Science 2004;303(5661):1157–62.

Slater R, Gallella S, Boyd S, Meek J, Fitzgerald M. Noxious stimulation causes functional activation of the somatosensory cortex in newborn infants. Summer meeting of the Neonatal Society, St John's College, Cambridge, UK, 2004. Early Hum Dev 2004;80:171–2.

Spiegel J, Hansen C, Treede RD. Laser-evoked potentials after painful hand and foot stimulation in humans: evidence for generation of the middle-latency component in the secondary somatosensory cortex. Neurosci Lett 1996;216(3):179–82.

Stevens B, McGrath P, Gibbins S, Beyene J, Breau L, Camfield C, et al. Procedural pain in newborns at risk for neurologic impairment. Pain 2003;105(1–2):27–35.

Suzuki M, Gyoba J, Sakuta Y. Multichannel NIRS analysis of brain activity during semantic differential rating of drawing stimuli containing different affective polarities. Neurosci Lett 2005;375(1):53–8.

Taddio A, Shah V, Gilbert-MacLeod C, Katz J. Conditioning and hyperalgesia in newborns exposed to repeated heel lances. JAMA 2002;288(7):857–61.

Van Cleve L, Johnsson L, Andrews S, Hawkins S, Newbold J. Pain responses of hospitalized neonates to venipuncture. Neonatal Netw 1995;14(6):31–6.

van den Pol AN. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J Neurosci 1999;19(8):3171–82.

Villringer K, Minoshima S, Hock C, Obrig H, Ziegler S, Dirnagl U, et al. Assessment of local brain activation. A simultaneous PET and near-infrared spectroscopy study. Adv Exp Med Biol 1997;413:149–53.

Winberg J. Do neonatal pain and stress program the brain's response to future stimuli? [comment]. Acta Paediatr 1998;87(7):723–5.

Woolley CS. Effects of estrogen in the CNS. Curr Opin Neurobiol 1999a;9(3):349–54.

Woolley CS. Electrophysiological and cellular effects of estrogen on neuronal function. Crit Rev Neurobiol 1999b;13(1):1–20.

Wyatt JS, Cope M, Delpy DT, Richardson CE, Edwards AD, Wray S, et al. Quantitation of cerebral blood volume in human infants by near-infrared spectroscopy. J Appl Physiol 1990; 68(3):1086–91.

Xue B, Hay M. 17beta-estradiol inhibits excitatory amino acid-induced activity of neurons of the nucleus tractus solitarius. Brain Res 2003;976(1):41–52.

Zelazo PD. The development of conscious control in childhood. Trends Cogn Sci 2004;8(1):12–7.