

Basic principles of optical imaging and application to the study of infant development

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Abstract

New optical methods of studying the infant brain have been developed over the last 20 years. These techniques make use of a window of transparency of biological tissue to near infrared light. Using spectrophotometry new insights have been gained into mechanisms of brain damage in newborn infants undergoing intensive care, and non-invasive functional activation studies have been pioneered in awake infants. Recently topographic mapping of activated cortex has become possible, and preliminary tomographic images of regional oxygenation in infants have been constructed. This is a rapidly changing field which offers exciting possibilities for studying both normal and abnormal child development.

Introduction

Optical imaging is a rapidly developing field which has provided enormous insights into the mechanisms of damage in the neonatal brain, as well as into the functional development of both the normal and abnormal brain. It is a technique that can be used in extremely fragile infants, because it is portable and non-invasive. It does not employ ionizing radiation, and is therefore repeatable. Its use rarely presents ethical dilemmas, and sedation of the subjects is not generally necessary.

Optical imaging of the human brain is possible because there is a window of transparency of biological tissue to light in the near infrared part of the spectrum. Light in the wavelength range 650–1000 nm is able to penetrate haemoglobin, so that absorption spectroscopy can be used to measure haemoglobin concentration in the brain, and deduce cerebral blood volume. In addition, oxy-haemoglobin (HbO₂) and deoxyhaemoglobin (Hb) have different absorption characteristics (see Figure 1), so that changes in blood oxygenation can be measured.

Description of methods

Absorption spectrophotometry

The technique was first applied to measurements in human tissue by Jobsis (1977), and has been widely used to study cerebral haemodynamics particularly in preterm infants (Wyatt, Cope, Delpy, Wray & Reynolds, 1986)

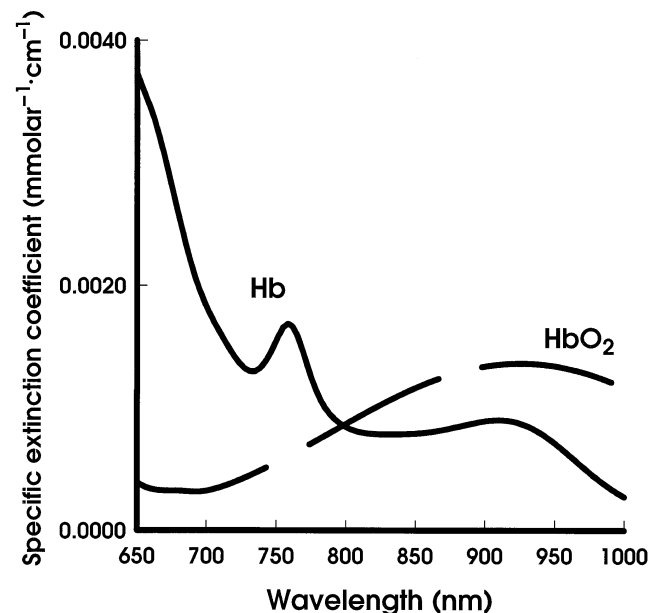


Figure 1 The absorption spectrum for oxygenated haemoglobin (HbO₂) and deoxyhaemoglobin (Hb) in the near infrared region, 650–1000 nm.

and children undergoing cardiac bypass (Fallon, Roberts, Kirkham, Edwards, Lloyd-Thomas & Elliott, 1994). These studies have all employed the simple, portable apparatus shown schematically in Figure 2. An emitting laser diode is coupled to the subject's head via a fibre optic cable. A sample of light emerging from the head is then collected

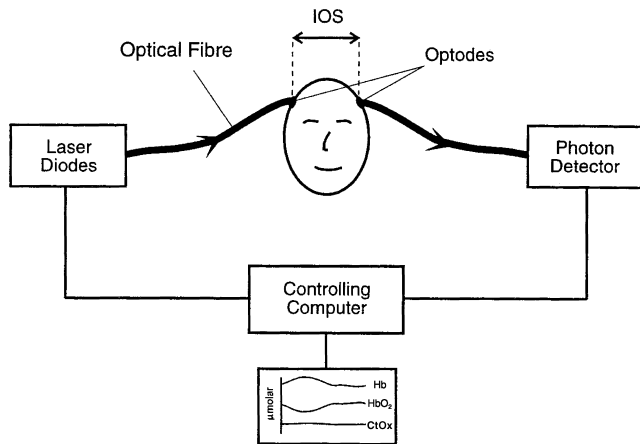


Figure 2 Schematic of the experimental set-up for NIRS measurements across the head. In practice the optodes are rarely positioned directly opposite each other.

by a detector, also coupled to a fibre optic bundle. Changes in optical density of the incident light are logged at a frequency of up to 50 Hz, and converted into changes in $[\text{HbO}_2]$ and $[\text{Hb}]$, using a modified Beer-Lambert law, which is described in the Appendix (Cope & Delpy, 1988). The consequence of applying spectroscopic methods to highly scattering, irregularly shaped matter, is that the absolute concentrations of $[\text{HbO}_2]$ and $[\text{Hb}]$ cannot be directly measured, and only their *changes* in concentration from an arbitrary baseline are derived. However, by applying physiological perturbations which change haemoglobin concentration or oxygenation, interesting information can be obtained. Global cerebral blood flow (CBF) can be measured by using a modified Fick Principle with either $[\text{HbO}_2]$ or indocyanine-green as an intravascular tracer (Edwards, Wyatt, Richardson, Delpy, Cope & Reynolds, 1988; Patel, Marks, Roberts, Azzopardi & Edwards, 1998). Global cerebral blood volume (CBV) and its change with carbon dioxide tension can be measured by applying small, gradual changes in inspired oxygen or carbon dioxide respectively (Elwell, 1995).

The technique is particularly useful in situations where physiological changes in $[\text{HbO}_2]$ and $[\text{Hb}]$ occur spontaneously. This forms the basis of its use in detecting regional evoked changes in haemoglobin concentration and oxygenation during cortical activation. Increases in local CBF are manifested as a rise in $[\text{HbO}_2]$ and a fall in $[\text{Hb}]$. Oxygen consumption during activation results in a decrease in $[\text{HbO}_2]$ and an increase in $[\text{Hb}]$. BOLD (blood-oxygenation-level-dependent) fMRI (Casey, Davidson & Rosen, this issue) makes use of the relative changes in $[\text{Hb}]$ and CBV, whereas optical methods have the advantage of measuring changes in both $[\text{HbO}_2]$ and $[\text{Hb}]$.

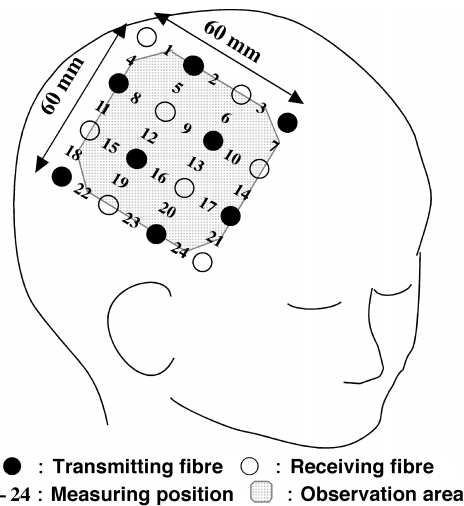


Figure 3 Probe position on the neonate's head, and measurement locations, over the sensorimotor cortex.

Optical topography

The logical extension of using more than one emitter detector pair (optodes) is to use an array of emitters and detectors (Chance, Luo, Nioka, Alsop & Detre, 1997; Hirth, Obrig, Villringer, Thiel, Bernarding, Muhlcnickel, Flor, Dirnagl & Villringer, 1996; van Houten, Benaron, Spilman & Stevenson, 1996). This arrangement permits topographical imaging. Topography is the simplest method of achieving an optical activation map, because it relies on absorption spectroscopy, and does not require sophisticated back-projection to derive an image. Topography yields improved optical information in two respects. First, a surface map of the activated area can be produced, giving spatial information about the development of function in both the normal and the damaged brain. Second, by isolating the region of maximum activation, more accurate quantitative measurements of evoked haemodynamic changes can be made than with a single set of optodes.

The most widely used mapping system is produced by Hitachi Ltd. (1-1-14, Uchi-kanda, Chiyoda-ku, Tokyo, Japan), and has been successfully used to study functional activation and epilepsy in adults (Watanabe, Yamashita, Maki, Ito & Koizumi, 1996). Figure 3 shows the size of an array relative to a neonate's head (Isobe, Kusaka, Nagano, Okubo, Yasuda, Kondo, Itoh & Onishi, 2001).

Optical tomography

Tomography is a method of acquiring 3-dimensional images by building up axial slices. This is achieved by shining radiation (X-rays, positrons or light) through tissue at several positions around the slice, and back-projecting

information about its absorption to form a picture. This is a complex mathematical task, which became possible in real time with the advent of fast computers in the 1970s. Tomography with near infrared light presents a difficult challenge because the light is so highly scattered in tissue (Hebden & Delpy, 1997). It is technically possible in the neonate because of the small size of the head and the thinness of the skull.

The ultimate goal of optical imaging is to provide spatially and temporally resolved information simultaneously about changes in $[\text{HbO}_2]$, $[\text{Hb}]$ and $[\text{total Hb}]$. Alternative techniques, such as BOLD MRI, provide information about $[\text{Hb}]$ only, which may be a result of several possible haemodynamic changes, such as a drop in saturation, an increase in oxygen consumption or a change in total blood volume.

The most basic reconstruction techniques still rely on absorption spectroscopy, encompassed within a more rigorous photon migration theory (Patterson, Chance & Wilson, 1989). Another exciting development is the use of time resolved detectors with an iterative reconstruction scheme to acquire images of both absorption and scattering properties of tissue (Hillman, Hebden, Schweiger, Dehghani, Schmidt, Delpy & Arridge, 2001). Light is scattered in the head by bone, and myelin, so that a combination of absorption and scattering images could yield information about haemodynamic changes related to structure and maturation.

Examples in developmental science

Mechanisms of brain damage in the preterm infant

Near infrared spectroscopy (NIRS) has been most successfully applied to the study of extremely premature infants. We have demonstrated that CBF is extremely low in preterm infants, and increases over the first 3 days of life as part of the normal adaptive response to extra-uterine life (Meek, Tyszcuk, Elwell & Wyatt, 1998). Low CBF during this period is associated with an increased risk of both intraventricular hemorrhage (Meek, Tyszcuk, Elwell & Wyatt, 1999) and periventricular leukomalacia, with subsequent neurodevelopmental impairment. Identifying avoidable causes of ischemia, such as the use of indomethacin (Edwards, Wyatt, Richardson, Potter, Cope, Delpy & Reynolds, 1990), and hypocarbia due to over-ventilation (Tyszcuk, Meek, Elwell & Wyatt, 1998) has improved brain centred care in the neonatal nursery. Conversely, demonstrating that CBF is independent of blood pressure in well preterm infants has prevented unnecessary treatment with potentially dangerous interventions (Tyszcuk *et al.*, 1998). NIRS

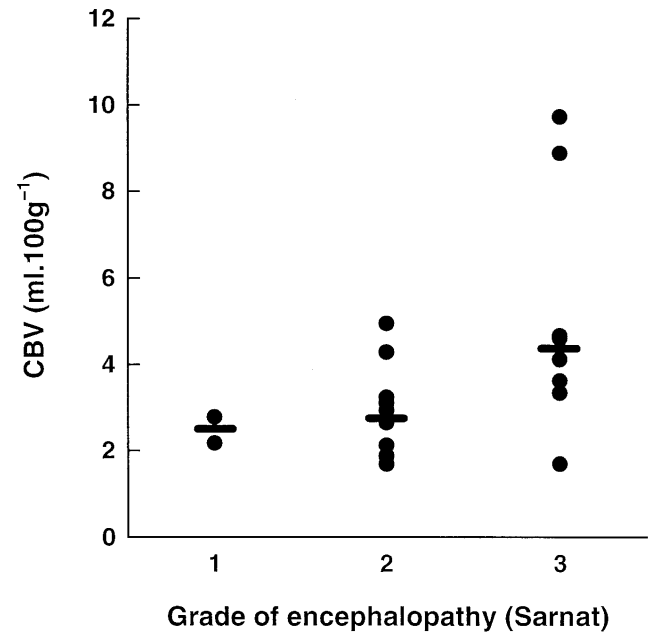


Figure 4 Cerebral blood volume on the first day of life related to severity of hypoxic-ischemic encephalopathy. The Sarnat scale measures an increasing severity of encephalopathy from 1 to 3.

has also been adapted to enable cotside measurement of cerebral oxygen saturation (Yoxall, Wiending, Dawani & Peart, 1995).

NIRS in the assessment of perinatal asphyxia

When the newborn brain is subjected to a hypoxic-ischemic insult, its circulation becomes vasodilated. This can be very clearly demonstrated using NIRS (Meek, Elwell, McCormick, Edwards, Townsend, Stewart & Wyatt, 1999), whereby measurements of cerebral blood volume are correlated with both the degree of encephalopathy (Figure 4) and the severity of neurodevelopmental impairment (Figure 5). These measurements are not sufficiently specific to be used clinically for prognostic information or for allocating cerebro-protective therapies.

Evoked haemodynamic responses

Once BOLD fMRI became widely used in the assessment of cerebral function, it became clear that NIRS was suitable for the same purpose. Activating a region of brain provides the ideal type and magnitude of changes in $[\text{HbO}_2]$ and $[\text{Hb}]$ for use with NIRS. Functional studies in the adult human brain were pioneered by several groups in the 1990s, mainly measuring responses to visual stimulation over the occipital region (Hoshi & Tamura,

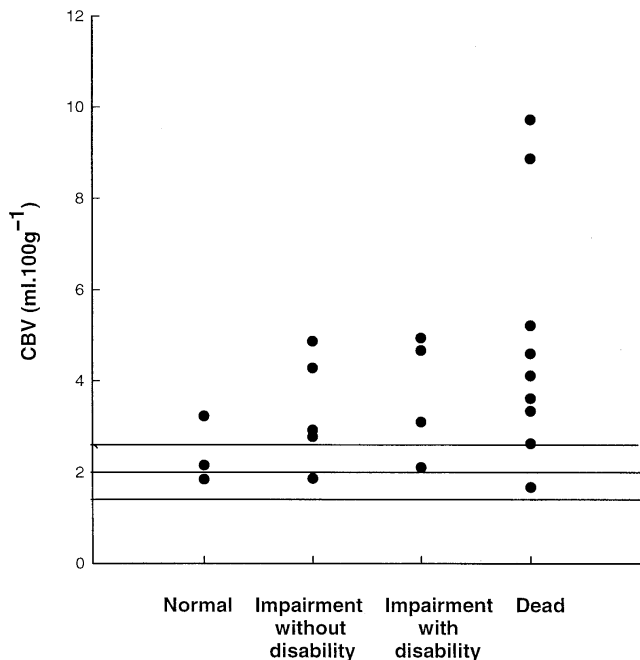


Figure 5 Cerebral blood volume on the first day of life related to neurodevelopmental outcome in asphyxiated neonates.

1993; Kato, Kamei, Takashima & Ozaki, 1993; Meek, Elwell, Khan, Romaya, Wyatt, Delpy & Zeki, 1995; Villringer, Planck, Stodick, Botzel, Schleinkofer & Dirnagl, 1993). These demonstrated localized increases in $[\text{HbO}_2]$ during stimulation with a corresponding decrease in $[\text{Hb}]$. This was consistent with the positive BOLD fMRI signal measured during activation. These findings were validated by simultaneous NIRS and fMRI studies (Kleinschmidt, Obrig, Requardt, Merboldt, Dirnagl, Villringer & Frahm, 1996). The overall change in total $[\text{Hb}]$ concentration, $[\text{total Hb}] = [\text{HbO}_2] + [\text{Hb}]$ was positive.

Visual activation in infants

Our studies in awake infants have demonstrated that the response of the immature brain is different (Meek, Firbank, Elwell, Atkinson, Braddick & Wyatt, 1998). Infants viewed a checkerboard with 5 Hz pattern reversal, alternating with a blank screen over 10-second cycles. Optodes were placed over the occipital region. In about half of the cases studied, the infants were sufficiently alert and still to enable successful data collection. Figure 6 shows the response of a 3-month-old infant, averaged over 9 cycles. The timecourse of the response, peaking between 6–8 seconds, is consistent with a haemodynamic change. The rise in $[\text{total Hb}]$ consists of increases in both $[\text{HbO}_2]$ and $[\text{Hb}]$, which is different from the decrease in $[\text{Hb}]$ seen in adults. This is consistent with the reversal

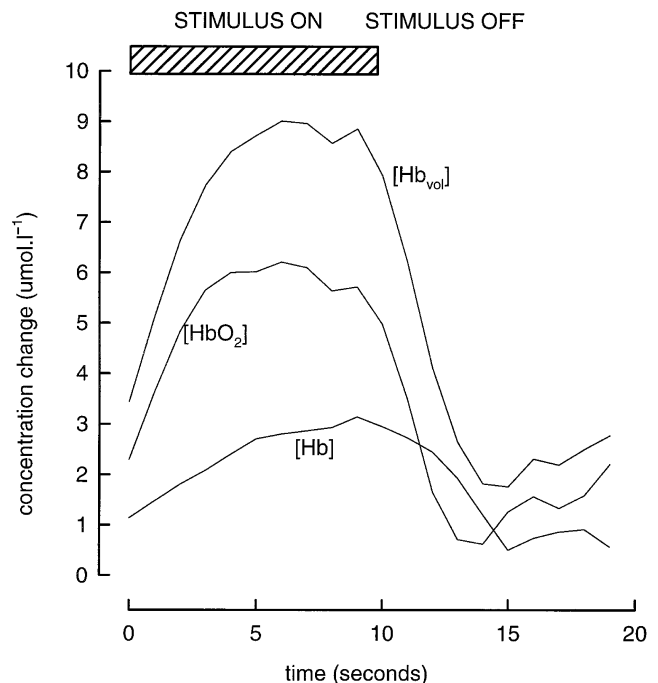


Figure 6 Changes of $[\text{HbO}_2]$, $[\text{Hb}]$ and $[\text{total Hb}]$ with visual stimulation.

of the BOLD fMRI signal seen in several studies (Born, Leth, Miranda, Rostrup, Stensgaard, Peiterson, Larsson & Lou, 1998; Martin, Joeri, Loenneker, Ekatodramis, Vitacco, Hennig & Marcar, 1999; Yamada, Sadato, Konishi, Timura, Tanaka, Yonekura & Ishii, 1997), which had previously been thought to be due to sedation (Joen, Huisman, Rumpel, Ekatodramis, Loenneker & Martin, 1996).

During cortical activation, increased regional cerebral blood flow (rCBF) causes an increase in $[\text{HbO}_2]$ and a decrease in $[\text{Hb}]$. However, increased oxygen consumption results in a decrease in $[\text{HbO}_2]$ and an increase in $[\text{Hb}]$. The relative proportions of these effects are different in the infant cerebral circulation to that of the adult. It appears that during activation in infants the rise in regional perfusion does not match the increase in oxygen consumption. This effect may be related to immaturity of vascular regulation, or to the greater metabolic demands of neurotransmission in unmyelinated white matter. There is BOLD fMRI evidence for this effect in children up to the age of 6 years (Martin *et al.*, 1999). Martin *et al.* and Born *et al.* (1998) also noted that the region of activation was more anterior and more lateral in the less mature group. Photon migration modelling estimates that the depth of penetration of the NIR light in the neonatal head is of the order of 2 cm (Okada & Delpy, 1996). It is not clear how a change in depth of the activated

area affects the relative proportions of perfusion and consumption in the interrogated field.

The increase in [Hb], or reversed BOLD signal, due to functional activation of the immature brain has been confirmed in most NIRS and BOLD fMRI studies, but there are several exceptions. Yamada *et al.* (1997) measured a BOLD signal increase in sedated infants undergoing photic stimulation, up to 8 weeks of age, and a reversed signal ([Hb] increase) thereafter. Isobe *et al.* (2001) have also measured a decrease in [Hb] in the motor cortex using NIRS due to contralateral knee movement. In both studies the infants were sedated.

Auditory activation in infants

This technique has also been successfully applied to studying systems which are relatively mature in the newborn human brain, by using auditory and olfactory stimuli. Sakatani, Chen, Lichty, Zuo and Wang (1999) studied 28 unsedated, newborn infants, and measured haemodynamic responses to music over both sides of the frontal cortex. Twenty-six infants responded with an increase in both [total Hb] and [HbO₂]. Seventeen infants also demonstrated an increase in [Hb] with stimulation. These results imply that the relative imbalance between regional perfusion and oxygen consumption with activation occurs in the frontal as well as the occipital cortex of the developing infant.

Zaramella, Freato, Amigoni, Salvadori, Marangoni, Supppei, Schiavo and Lino (2001) measured responses to a 2–4 Hz auditory sweep over the temporal area in pre-term and term neonates, and found that 69% of the infants studied had an increase in [total Hb] on stimulation, which the authors speculated may have been related to whether the infants were asleep or awake. No clear relation of response with age or with the evoked brainstem response was demonstrated.

Olfactory activation in infants

Bartocci, Winberg, Ruggiero, Bergqvist, Serra and Lagercrantz (2000) and Bartocci, Winberg, Papandieck, Mustica, Serra and Lagercrantz (2001) have demonstrated several interesting aspects of the response of the neonate to odours. Perception of smells is an essential feature of the adaptation of the newborn to extrauterine life. Exposure to vanilla and colostrum elicited an increase in [HbO₂] over the left orbito-frontal area in 23 term newborn infants (Figure 7). The magnitude of this response decreased over the first week of life for colostrum, but not for vanilla, possibly because the concentration or composition of colostrum changes during the first week of lactation. Unpleasant odours, such as

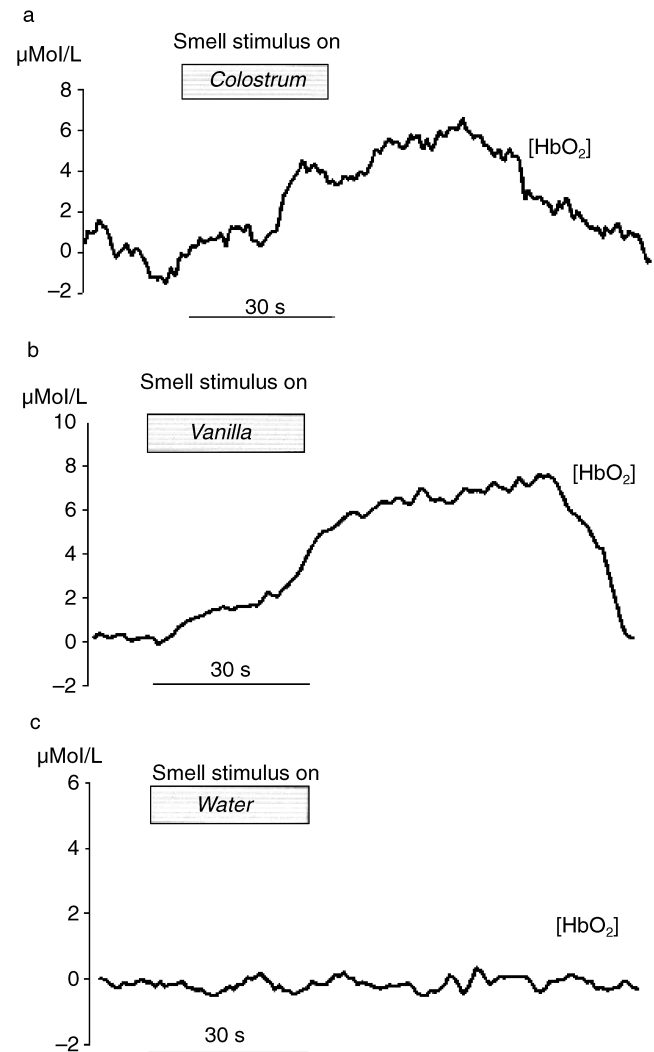


Figure 7 Changes in [HbO₂] during exposure to colostrum, vanilla and water in one baby 7 hours postpartum.

detergent and adhesive remover, elicited a decrease in [HbO₂] and [total Hb], which was greater over the right olfactory cortex than the left. The authors speculate that this may be due to cortical deactivation resulting from a repeated unpleasant stimulus, which adds to the body of evidence that adverse experiences during neonatal intensive care have long-lasting neurological effects.

Maturation of the haemodynamic response

We have used NIRS to study the response of awake infants to visual stimulation with a pattern reversal checkerboard, from 32 weeks gestation to 3 months of age. This is consistent with the well-established electrophysiological changes in latency and amplitude measured

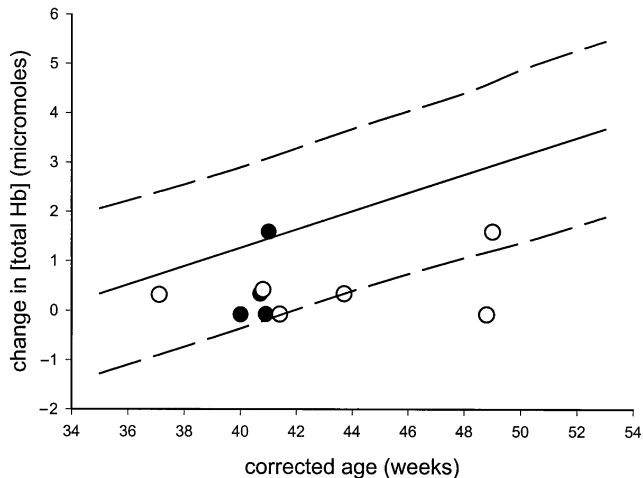


Figure 8 Response to visual stimulation of infants with abnormal neurological signs, compared with 95% confidence levels of regression line fitted to responses of normal infants. Closed circles represent infants with abnormalities on imaging, and open circles, those with normal imaging.

by visual evoked potentials (VEPs) measured in both preterm and term infants. It is interesting to compare this functional maturation in occipital haemodynamics with the increasing glucose metabolic rate in the primary visual cortex measured by positron emission tomography (Chugani & Phelps, 1986).

Before 36 weeks gestation there appears to be a maturational threshold for the onset of the response. This threshold may result from a decrease in visual acuity, whereby the checker size is too small for preterm infants to see (Harding, Grose, Wilton & Bissenden, 1989), or to the first appearance of cortical rather than subcortical processing of visual information, as rapid myelination in the optic radiation begins.

The relation of function to pathology

We have studied the visual response in infants with abnormalities on neurological examination in the neonatal period. They were studied when they had recovered sufficiently to view the checkerboard stimulus in an awake state (Meek, Noone, Elwell & Wyatt, 1999). Figure 8 shows the responses of these infants compared to the 95% confidence levels of the regression line fitted to the data for normal infants. Although it is clear that many of the impaired infants have decreased responses, the scatter of the normative data is so wide that a small response remains within the 'normal' range. This implies that using a single emitter and detector system does not provide quantitative results which can be used as a clinical tool.

Optical topography

Recently, exciting images of activation in the motor cortex of sedated newborn infants undergoing passive motor stimulation of the contralateral knee have been published (Figures 9A & B) (Isobe *et al.*, 2001). Dynamic topograms of $[HbO_2]$, $[Hb]$ and $[total\ Hb]$ are shown (Figures 9C & D). These graphs demonstrate a clear difference in the timecourse of the $[HbO_2]$ and $[Hb]$ responses. Both responses are slow, and the $[Hb]$ change is negative. The authors speculate that this may be related either to sedation, or to the greater metabolic activity in the motor cortex.

This technique has great potential for producing quantitative and clinically useful results, and has recently been used to measure regional CBF patterns in infants using indocyanine green as a tracer (Kusaka, Isobe, Nagano, Okubo, Yasuda, Kondo, Itoh & Onishi, 2001).

Optical tomography

Hintz, Benaron, Siegal, Stevenson and Boas (1999) have produced images in 8 neonates, of which 7 correlated very closely with ultrasound, computed tomography and magnetic resonance imaging. Benaron, Hintz, Villringer, Boas, Kleinschmidt, Frahm, Obrig, van Houten, Kermit, Cheong and Stevenson (2000) have used diffuse optical tomography (DOTS) to acquire real time images of activation in adults due to hand movement. Data acquisition was slow, restricting the technique to paralyzed or sedated infants, but this new technique offers exciting possibilities as faster algorithms are developed in the future. Boas, Gaudette, Strangman, Cheng, Marota and Mandeville (2001) have studied theoretical limitations to the ability of DOTS to localize focal changes in $[HbO_2]$ and $[Hb]$ and suggested methods for improving the standard NIRS analysis.

Discussion

Many of the limitations of the technique are inherent to the nature of the interaction of light with tissue. Because of the multiple scattering events each photon undergoes during its journey from the emitter to the detector, an estimate of the average differential pathlength is made. There is a 10–20% variation in this value for subjects at any given age (Duncan, Meek, Clemence, Elwell, Fallon, Tyszcuk, Cope & Delpy, 1995). The signal-to-noise ratio of the detector system limits the resolution of measurable concentration changes to the order of 1 micromol/litre.

The method is vulnerable to motion artifact, and secure fixation of the optodes on the infant or child's head

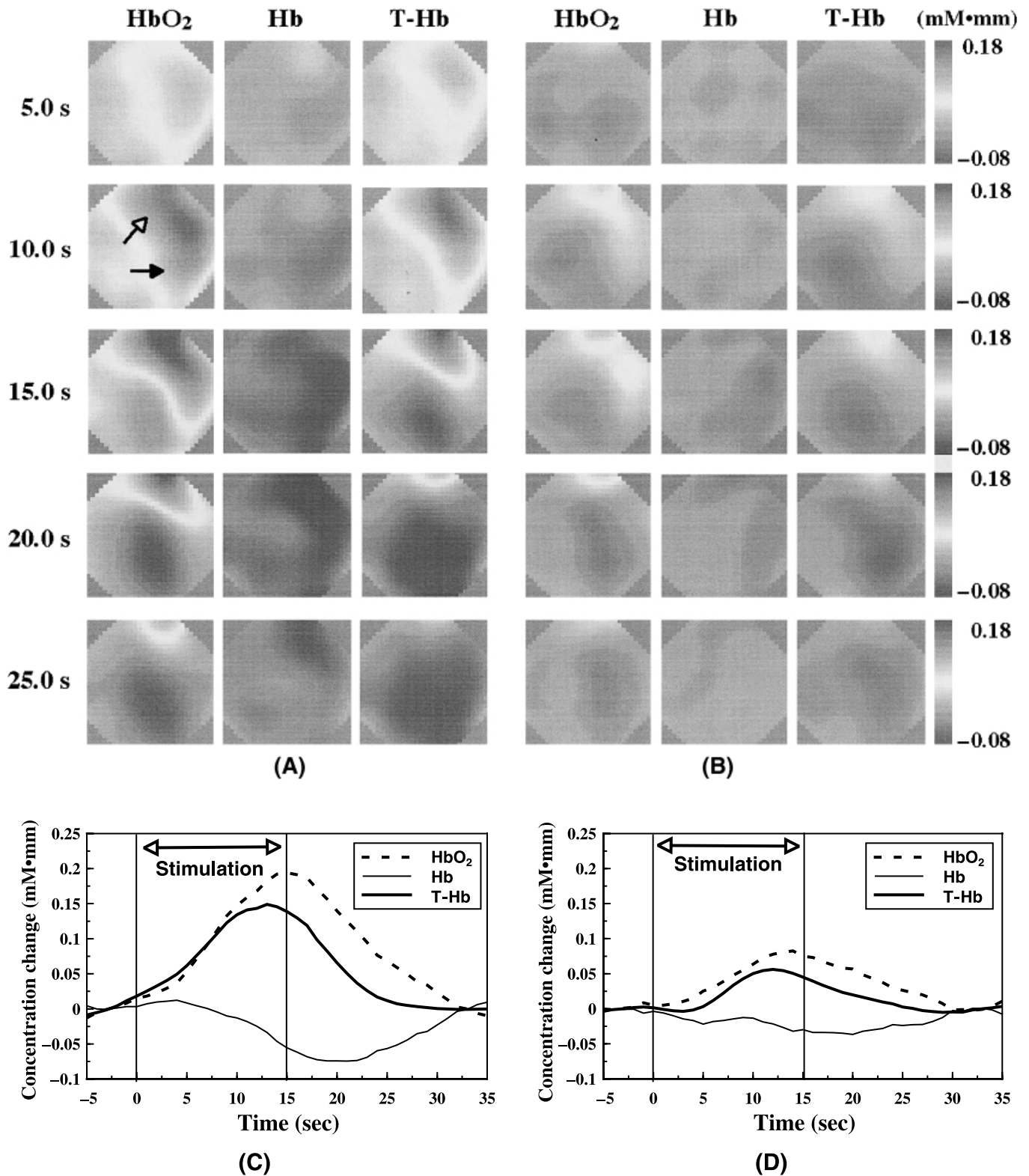


Figure 9 Dynamic topograms of [HbO₂], [Hb] and [total Hb]. (A) Topograms at 5 s intervals from 5 to 25 s after the start of contralateral knee movement. (B) Topograms at the same time after the start of ipsilateral knee movement. (C) & (D) timecourse of concentration changes in sensorimotor area, for contralateral and ipsilateral movement.

is difficult. Motion artifacts cause large spikes in the optical density measurement, which often do not return to the original baseline, which makes editing algorithms difficult to apply. Skill is needed to sustain infants in a state of comfortable, quiet alertness. Many studies are therefore performed on asleep, or even sedated infants. However, with patience and experience it is possible to study awake infants up to about 3 months of age.

Older infants and children are less cooperative. Hoshi, Chen, Liu and Tamura (2001) have designed a portable spectrophotometer for use in children, mounted in a backpack, which downloads signals by radio, to be analyzed in real time, remotely from the subject. Functional NIRS has been widely used in adults, and should not present difficulties in compliance with older children and adolescents. However, the signal is noisier, and a smaller proportion of the light traverses the cortex than in the infant (Okada & Delpy, 1996; Okada, Firbank, Schweiger, Arridge, Cope & Delpy, 1997).

Finally, the position of the optodes relative to the activated cortex has to be determined using bony landmarks. Simultaneous NIRS and BOLD fMRI studies have shown that a displacement of less than 1 cm can result in a substantial loss of NIRS signal (Kleinschmidt *et al.*, 1996). Functional NIRS studies in adults are enhanced by identifying the optode position using BOLD fMRI, but this is not possible in unsedated infants or young children.

Conclusion

The infant population is ideally suited to optical imaging because of the small size of their heads and the relatively low proportion of scattering material compared to adults. Optical techniques have provided insights into important aspects of brain development, both in normal infants and in high risk neonates undergoing intensive care. Future developments will include better filtering of movement artifacts and faster real time analysis. These techniques will then graduate from being used as research instruments, into becoming useful and non-invasive clinical tools.

Appendix

The modified Beer-Lambert law

The Beer-Lambert law is widely used in chemical spectroscopy, and defines the attenuation (A) of light in units of optical density. A is measured by comparing the incident light intensity (I_0) to the intensity of the emerging

transmitted light (I), and expressing this as the number of orders of magnitude that the light intensity has been reduced when traversing the medium.

$$A = \log [I_0/I]$$

If the light is absorbed by a compound whose concentration is c, and travels a distance d, then:

$$A = a \times c \times d$$

where a is the extinction coefficient of the compound, for light at that wavelength (see Figure 1). If a and d are known, the concentration (c) of the absorbing compound can be measured.

When spectrophotometry is applied to a highly scattering, irregularly shaped medium, such as the human head, this equation is modified as follows:

$$A = a \times c \times d \times B + G$$

where B is a differential pathlength factor which takes account of the mean extra distance photons travel due to scattering, and G is an additive term due to scattering losses. G is unknown, and depends on the geometry of the head, and on the composite scattering properties of the interrogated tissue. It is not, therefore, possible to deduce the concentrations of $[HbO_2]$ or $[Hb]$ directly from optical density measurements. However, because G remains constant for a given configuration of optodes on the head, it is possible to measure *changes* in chromophore concentration, by subtracting optical density measurements under two different sets of conditions. It is then possible to calculate absolute changes in $[HbO_2]$ and $[Hb]$. The sum of these changes, $\Delta[\text{total Hb}]$ is related to the change in cerebral blood volume.

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