Illuminating the developing brain: the past, present and future of functional near infrared spectroscopy

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Abstract

A decade has passed since near infrared spectroscopy (NIRS) was first applied to functional brain imaging in infants. As part of the team that published the first functional near infrared spectroscopy (fNIRS) infant study in 1998, we have continued to develop and refine both the technology and methods associated with these measurements. The increasing international interest that this technology is generating among neurodevelopmental researchers and the recent technical developments in biomedical optics have prompted us to compile this review of the challenges that have been overcome in this field, and the practicalities of performing fNIRS in infants. We highlight the increasingly diverse and ambitious studies that have been undertaken and review the technological and methodological advances that have been made in the study design, optical probe development, and interpretation and analyses of the haemodynamic response. A strong emphasis is placed on the potential of the technology and future prospects of fNIRS in the field of developmental neuroscience.

Keywords

Near infrared spectroscopy (NIRS), optical imaging, infant, developmental neuroscience, functional brain imaging

Contents

1 Introduction

A decade has passed since near infrared spectroscopy (NIRS) was first applied to functional brain imaging in infants. This review is offered in recognition of the work that has been achieved over the last ten years and the progress that has been made in developing this technique for the investigation of developmental cognitive neuroscience. Further refinement and application of NIRS over the next ten years will contribute significantly to the advancement of our understanding of the developing brain. We believe that functional NIRS provides an essential bridge between our current understanding of cortical activity in the developing brain and our knowledge of adult human brain function. Whilst there is now a multitude of behavioural research on infant development, the majority of which use looking time paradigms with preverbal infants, the number of developmental cognitive neuroscience studies still remain low. fNIRS will allow us to elucidate the connections between localized cortical activity and behavioural responses during early human development. Moreover, NIRS systems are inexpensive and portable, can accommodate a degree of movement from the infants enabling them to sit upright on a parent's lap, and essentially can measure spatially localized patterns of haemodynamic activity allowing comparisons with fMRI data of adult human brain function. fNIRS is ideally suited for infant research, as will be evidenced in the following review.

Neuronal activation originates in the neurons as electrical signals are passed between cells. During this activation the metabolic demand of neurons changes, provoking an increase in oxygen consumption, local cerebral blood flow (CBF) and oxygen delivery. A typical haemodynamic response (see Fig. 1) to cortical neuronal

activation in adults shows an increase in blood flow leading to an increase in oxy-haemoglobin (HbO_2) and a (relatively smaller) decrease in deoxy-haemoglobin (HHb) as it is displaced from the veins, leading to an increase in total-haemoglobin (HbT) (Villringer and Chance, 1997). Neuroimaging methods either detect the direct activation related to electrical activity of the brain (e.g. electroencephalography (EEG), magnetoelectroencephalography (MEG)) or the consequent haemodynamic response (e.g. positron emission tomography (PET), functional magnetic resonance imaging, (fMRI), functional near infrared spectroscopy (fNIRS)).

****INSERT FIGURE 1 ****

Many of these techniques, which are well established in adults, have limiting factors restricting or preventing their use in infants. PET requires the use of radioisotopes, whilst fMRI and MEG require the participant to remain very still, usually swaddled or restrained. There has been some infant research published using these techniques (i.e. Dehaene-Lambertz et al., 2002; Huotilainen et al., 2003; Imada et al., 2006; Tzourio-Mazoyer et al., 2002), however this work has generally been restricted to the study of sleeping, sedated or very young infants. For many years, the primary choice for functional imaging in awake infants has been EEG, a non-invasive technique with high temporal resolution but relatively poor spatial resolution. The advent of the new technology, fNIRS, was a welcome addition to a limited choice of neuroimaging methods suitable for use in infants.

The relative attributes of the functional neuroimaging techniques that have been and are currently used with infants are outlined in Fig. 2. EEG and fMRI bear

the closest relation to fNIRS, the former uses a similar experimental setting, whilst the latter measures the same haemodynamic response. The major advantage of fNIRS compared with EEG is that it is less susceptible to data corruption by movement artifacts and offers a more highly spatially resolved image of activation allowing the localization of brain responses to specific cortical regions. In addition, compared with fMRI, fNIRS has high temporal resolution, is silent allowing easy presentation of auditory stimuli, and can measure both oxy- and deoxy-haemoglobin chromophores (the molecules that are responsible for the colour of the blood due to their absorption of light at different wavelengths) providing a more complete measure of the haemodynamic response. Though fMRI and fNIRS measure the same haemodynamic response, generally fMRI techniques have an intrinsically limited acquisition rate usually at a minimum of one hertz (Huettel et al., 2003; but see Weishaupt et al., 2008), whereas fNIRS can acquire data rapidly, up to hundreds of hertz, thus providing a more complete temporal picture (Huppert et al, 2006). Naturally, as with any technique there are also limiting factors of NIRS; the temporal resolution is lower than EEG (the precision of which can reach up to a thousand hertz, Luck, 2005); the depth resolution is dependant on the age of the infant and the optical properties of the tissue (see Fukui et al., 2003); the technique offers lower spatial resolution compared with MRI; and there is no capacity for measuring brain structure for anatomical reference (see Minagawa-Kawai et al., 2008 for further discussion of these limitations). Despite these shortcomings a niche exists for fNIRS in developmental neuroimaging which should be explored by more researchers. Moreover, research is underway to provide optimized measurements of brain activation by combining the advantages of several neuroimaging methods for the study of infants (see section seven for a further discussion). This practice has

been successfully implemented in research with adult participants, e.g. combined fNIRS and EEG (Moosmann et al., 2003), fNIRS and MRI (Steinbrink et al., 2006) and fMRI and EEG (Dale and Halgren, 2001; Eichele et al., 2005). Aside from the advantages this presents for neurodevelopmental research, this multimodality approach has the potential to improve clinical neuromonitoring of preterm and term infants with acute brain injury (Toet and Lemmers, 2009).

****INSERT FIGURE 2 ****

The use of NIRS to study functional brain activation in infants is a rapidly increasing research area. Table 1a provides a summary of all of the infant fNIRS studies published over the last ten years and demonstrates a fivefold increase in the number of papers published per year since 1998. This summary table illustrates how the study design, hypotheses, and cortical areas of interest have evolved and diversified. Whereas in the early fNIRS studies the main aim was to detect the response to basic stimuli activating the primary cortical areas such as acoustic tone in the auditory cortex (i.e. Sakatani et al., 1999) or stroboscopic flashing light in the visual cortex (i.e. Hoshi et al., 2000; Zaramella et al., 2001), more recently researchers have focused on more complex stimuli activating multiple cortical regions (see Table 1a). Moreover, an increasing number of researchers have focused on the study of awake infants to address topics such as; object processing (Wilcox et al., 2005, 2008; Watanabe et al., 2008), social communication (Grossman et al., 2008; Minagawa-Kawai et al., 2009), biological motion processing (Lloyd-Fox et al., 2009), action observation (Shimada and Hiraki, 2006), and face processing (Blasi et al., 2007; Otsuka et al., 2007; Carlsson et al., 2008). In these studies,

fNIRS has been used to localize haemodynamic responses in specific regions of the cortex such as the superior temporal sulcus (eye gaze/biological motion processing), orbitofrontal cortex (maternal face/emotion recognition), sensorimotor areas (action observation), prefrontal cortex (object permanence), and occipitotemporal cortex (dynamic objects) (see Table 1a). This capacity for localizing the cortical region of activation, and the allowance for movement of the participant, are the defining attributes of the application of the fNIRS method for the study of the early developing brain.

The ambition and drive to use fNIRS for increasingly complex studies continuously fuels the development of technologies and methods specifically for infant research. Practical issues that have arisen include; 1) the development of the probe and head gear to reduce the effects of movement of the infant, particularly important when studying visual paradigms where the infant is awake; 2) the design of the study, considering the effects of boredom, anticipation and the synchronization of systemic/biorhythmic responses; 3) an understanding of the haemodynamic response in infants and how to interpret a significant result; 4) and coregistration between the haemodynamic response measured at the surface of the head and the underlying cortical anatomy (for a review of these issues see Aslin & Mehler, 2005; Meek, 2002). Recent work in various research labs has led to major progress in these areas. For example, the development of multiple source detector separation arrays to investigate depth discrimination of the haemodynamic response; an ever-increasing number of channels allowing for a wider coverage of the head; and advances in the design of the headgear providing improved quality of the optical signals. This review will consider these methodological and technological advances with particular focus on studies in awake infants.

Importantly we will highlight the advantages of using NIRS for the study of infant development and outline prospects for the future.

2 Near Infrared Spectroscopy: general principles and methods of measurement

The use of near infrared light to monitor intact organs began as a discovery at a dinner table with the passage of light being observed through a steak bone at a family supper (Jöbsis-vanderVliet, 1999). Biological tissue is relatively transparent to light in the near infrared part of the spectrum allowing several centimetres of tissue to be illuminated. This fortuitous "optical window" coincides with the favourable differential absorption spectra of oxy- and deoxy-haemoglobin, thus allowing near infrared absorption spectroscopy methods to provide a non invasive measure of tissue oxygenation and haemodynamics. From the outset of its practical application, NIRS found widespread use in monitoring the infant brain partly because of the convenient optical geometry of the infant head. Initially, infant work with NIRS took place within a clinical setting investigating cerebral oxygenation in preterm and term neonates. Studies focused particularly on infants at risk of brain injury and subsequent neurodevelopmental abnormalities (i.e. Bucher et al., 1993; Wyatt et al., 1986; see Nicklin et al., 2003 for review of early clinical work). Later, researchers realized the potential for NIRS as a monitor of functional brain activation during infancy.

With this optical technique, the light migrates from sources to detectors located on the head, by travelling through the skin, skull and underlying brain tissue (Elwell, 1995; Jöbsis, 1977). The attenuation (or loss) of this light (in the wavelength range 650-1000nm) will be due to both absorption and scattering effects

within these tissues. Further, blood oxyhaemoglobin (HbO_2) and deoxyhaemoglobin (HHb) chromophores have different absorption properties of near infrared light so that blood oxygenation can be measured. If scattering is assumed to be constant the measured changes in the attenuation of the near infrared light can therefore be used to calculate the changes in blood oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb) and total haemoglobin (HbT= HbO_2 + HHb) in the illuminated tissue. With knowledge of the optical pathlength in tissue (see Delpy and Cope, 1997; also see appendix 1 for a detailed description) the changes in HbO_2, HHb and HbT can be expressed in μmolar units. The changes in concentration of these chromophores can be used as surrogate markers of brain blood flow and hence provide a means of investigating brain function. Figure 1 shows the typical changes in HbO_2 and HHb during functional activation of the adult brain. Stimulus onset and neuronal activation induces an increase in the concentration of HbO_2, which is accompanied by a lesser decrease in HHb concentration. This activation induced vascular response is known as the haemodynamic response function (HRF). The shape of the signal may vary according to the evoking stimuli (i.e. differences in amplitude are observed between brief and prolonged stimulus presentation) as well as the underlying neural activity.

Previous work in adults suggests that the characteristics of the vascular response measured by NIRS are comparable to the BOLD (blood oxygen level dependence) response seen in fMRI. It is important to note that unlike fMRI BOLD, fNIRS provides a separate measure of quantified changes in both HbO_2 and HHb. Over the last decade, there has been some controversy over which chromophore change (HbO_2 or HHb) best correlates with the BOLD signal (for review see Obrig & Villringer, 2003; Ferrari, et al., 2004). Though the BOLD contrast reflects

stimulus-induced reductions in the concentration of deoxyhaemoglobin it is physiologically ambiguous due to the coupling of CBF, oxidative metabolism and cerebral blood volume (Logothetis, 2003). Simultaneous recordings of fNIRS and fMRI provide an insight into this controversy and have been given considerable attention over the last few years (Steinbrink et al., 2006; Ye et al., 2009). It has been shown that the HHb signal follows the BOLD signal more closely than does the HbO_2 signal (e.g. Huppert et al., 2006; Kleinschmidt et al., 1996; Toronov et al., 2001). For example, Huppert and colleagues showed correlation coefficients of r = 0.97 for HHb/BOLD compared with r = 0.71 for HbO_2/BOLD for measurements over the primary motor cortex during a motor task (Huppert et al., 2006). Conversely, Strangman and colleagues found high individual variation and after accounting for systemic errors in the signal found the highest correlation between the BOLD signal and HbO_2 rather than HHb (Strangman et al., 2002). They suggest this could reflect the higher signal to noise ratio for HbO_2 compared with HHb, rather than physiology related to BOLD signal interpretation. It is clear that in adults both HbO_2 and HHb changes are correlated with BOLD fMRI signal changes. However, while adult NIRS studies show HbO_2 and HHb responses related to BOLD fMRI signal changes, infant data reveals a less consistent pattern of activation (Baird et al., 2002; Hoshi et al., 2000; Meek et al., 1998; Sakatani et al., 1999; Wilcox et al., 2005; Zaramella et al., 2001) and simultaneous fNIRS/fMRI studies in infants would be a valuable addition to this field of research. These issues will be discussed further in sections six and seven.

2.1 Instrumentation

Several techniques have been developed to measure the haemodynamic response using NIRS: continuous wave, time resolved, spatially-resolved and frequency resolved spectroscopy (for review see Minagawa-Kawai et al., 2008; Wolf et al., 2007). The majority of fNIRS infant research has been undertaken using continuous wave (CW) systems (e.g.. Blasi et al., 2007; Hintz et al., 2001; Pena et al., 2003) as they provide the simplest approach (for a detailed report see Hebden, 2003). Beyond standard commercialized systems there is an opportunity to measure with multiple wavelengths to enhance spectral resolution not just of the haemodynamic chromophores but also of cytochrome oxidase, a chromophore which may provide a marker of cellular oxygen metabolism (Heerken et al., 1999). However, the CW-NIRS systems that have been used for functional activation studies in infants typically measure attenuation changes at only two wavelengths to provide changes in the concentration of the two chromophores HbO_2 and HHb (see Table 1b). The selection of these two wavelengths is an important issue and has been shown to affect the accuracy of the HbO_2 and HHb measurements. Several studies have suggested paired wavelengths of approximately 690nm and 830nm are optimal (Yamashita et al,. 2001, Strangman et al., 2003, Boas et al., 2004 and Sato et al., 2004). Uludag and colleagues (2004) determined that cross talk is reduced and separability is increased between the HbO_2 and HHb signals if one wavelength is below 720nm and the other is above 730nm. However it is important to note that these studies have used experimental data and theoretical models based on adult rather than infant head geometry. Further discussion of the influence of infant head geometry on the measured fNIRS signals is given in section three.

CW optical topography systems which use arrays of multiple sources and detectors (Fig. 1b and d) have been developed to provide two-dimensional maps of

the cortical haemodynamic response (and therefore brain activity) (Blasi et al., 2007 - Fig 1 c). Each detector records the amount of light coming from a subset of neighbouring sources, with each source-detector pair called a channel. To identify the source associated with a given detected signal either the sources are illuminated sequentially or are intensity modulated at unique frequencies. If using the latter method, the signals are then decoded in hardware using lock-in amplifiers or in software using a Fourier transform. In this way, measurements can be taken at a rate of several Hz, typically between 1 and 10 Hz, enabling the time course of the haemodynamic response to be accurately charted. As shown in Table 1b the majority of infant fNIRS studies have been performed using multi channel optical topography systems.

In addition to CW-NIRS systems, time-resolved (TR-NIRS) and frequency-resolved (FR-NIRS) spectrometers can be used to derive absolute concentrations of oxy and deoxy-haemoglobin., although these techniques are generally impractical for infant fNIRS studies. However these systems may be of use in determining the spatial and temporal variations in optical pathlength which could influence the measured haemodynamic response (Ijichi et al., 2005; Sakatani et al., 2006),

3 The development and use of fNIRS for infants

fNIRS studies with infants can lead to high drop out rates compared with adults. The proportion of infants who cannot be included in a NIRS study due to unsatisfactory data is approximately 40% (measure obtained from the published papers described in Table 1a). This can be attributed, in a large proportion, to the difficulty of designing an efficient and comfortable method by which to attach

multiple source and detector optodes to the infant head. Therefore, much time and many resources have been applied to their improvement to maximize the effectiveness of NIRS as a tool for infant research. The prerequisites for NIRS headgear for infant studies are that it must be comfortable, light-weight, easy to position in a very short space of time and must provide reliable continuous optical measurements from all channels. Unlike studies in adults, it is not possible to place the headgear on an infant, pause to assess the optical signals, adjust the position/fit of the headgear, and then begin the experiment. The headgear must fit securely on the head so that any movement of the infant does not alter the position of the source and detector fibres and lead to artifacts in the optical signal. The array of channels within the headgear must be contained within a semi-rigid structure allowing some flexibility to mold around the head while maintaining a fixed source-detector spacing across the array. The delicate optical fibres need to exit the head gear away from the infant's face and remain out of reach of their hands. (see Fig. 1). During the positioning of the headgear the infants must not be unduly disturbed (this could lead to them being unwilling to start the study) and during the recording the headgear must be sufficiently unobtrusive to ensure that it does not distract the infants from focusing on the task.

A promising area for future fNIRS infant research is in the investigation of differences between individuals, which for example could help identify markers of typical and atypical processing in infants at risk of developing disorders such as autism and thus contribute to diagnosis and intervention programs. Sample sizes are typically small in clinical population studies of this kind and so it is particularly important that headgear design is optimized to provide robust optical data and to minimize the proportion of excluded data.

The last decade has witnessed an increasingly sophisticated array of designs for NIRS probes and headgear for infant studies, with important developments from a number of research labs including those at Harvard (Franceschini et al., 2006; Siegel et al., 1999) and the University of London (Blasi et al., 2007; Lloyd-Fox et al., 2009). Developments have also been made in a number of commercial NIRS systems such as the ETG Optical Topography system (The Hitachi Medical Corporation, http://www.hitachimedical.com) and the Imagent (ISS Inc., http://www.iss.com).

An overview of these recent developments in relation to some of the factors that influence probe and headgear design is discussed below.

3.1 The number of channels, sources and detectors

The development of optical topography has enabled researchers to increase coverage over the cortex, allowing comparisons of activation within and between broad areas such as frontal and temporal or occipital cortices. Whereas at first, fNIRS studies used a restricted number of channels (the first five published studies used only 1-3 channels – see Table 1b), this number has steadily increased and been incorporated into the design of the probes. A major limitation of restricting measurements to just one or two channels is the level of uncertainty that the measurements are being made over the relevant cortical region. As the underlying brain anatomy is not imaged with NIRS it is essential to account for individual differences in brain structure by measuring the signal over a relatively wide area of the cortex. For example an array with one or two channels which produces unpredicted haemodynamic changes, may be attributable either to an unexpected functional response or misplacement of the optodes relative to the region of interest

(see section six for further discussion). It is therefore advisable to use optical topography with multiple channels wherever possible. Hitachi Medical Systems, has recently developed probes capable of providing between 48 and 128 measurement channels (ETG Optical Topography System, http://www.hitachimedical.com). A study by Watanabe and colleagues (2008) at the University of Tokyo used an 84-channel design to investigate the functional activation response across a wide area of brain regions to mobile objects and a black and white chequerboard in three month olds. The results revealed that whereas the occipital cortex responded to both types of stimuli, the occipitotemporal and prefrontal regions responded specifically to the mobile objects evidencing localized functional organization of the cortex at a young age.

The in-house UCL system is capable of measuring between 20 and 45 channels (Everdell et al., 2005). As well as enabling the comparison of activation across brain regions, a recent infant study from the research group at Birkbeck/UCL (Lloyd-Fox et al., 2009) highlighted the potential of fNIRS for the investigation of specific activation within a given brain region using multiple channels. An increase in HbO_2, was observed in response to dynamic social stimuli, and was localized to specific channels in a bilateral posterior area of the temporal cortex thought to be homologous to the superior temporal sulcus region.

The advantages of increasing the number of channels are clear. However this is confounded by the inevitable increase in weight and size of the headgear. This may explain the higher proportion of excluded optical data due to excessive movement artifacts in those fNIRS studies using a high number of channels. For example Watanabe and colleagues (2008) used up to 84 channels to measure multiple cortical regions but excluded data from almost 70% of the participants (see Table

1a). Similarly, Nakano and colleagues (2009) used a 48 channel system and excluded data from 56% of the participants. Given that the average proportion of excluded data for NIRS infant studies is around 40%, and can be as low as 12.5% (see Table 1a), these values are rather high. This is not to say that other studies where a lower number of channels were used have always reported a lower proportion of excluded data but rather that an increase in the number of channels is one factor that impacts the quality of the optical signal unless this is carefully considered within the headgear design.

3.2 The cortical area of interest

The cortical area of interest can affect the probe and headgear design in terms of the depth of the underlying cortex from the surface of the skin, the distribution and arrangement of the channels over the area of interest and the accommodation of the curvature of the skull.

Theoretically, if the source to detector distance is increased, the detected light is more likely to provide information about a haemodynamic response occurring in deeper tissue (Fukui, et al., 2003). However, this assumption is limited by the intensity of the source light and the diffuse nature of optical scattering in tissue. To reach the cortex the separation between source and detector should be, at minimum, double that of the distance between the skin surface and the surface of the cortex. To ensure that the measurement is taken from within the cortex, when studying adult brain responses, the channel separations are usually between 40 – 50mm (Okada et al., 1997). In infants, the skin, skull and CSF layer are all thinner and more translucent and so to reach the cortex the channel separations are usually less (Fukui et al., 2003), between 20 - 30mm (see Table 1b). The optimal separation

for infants is a matter of much debate amongst NIRS researchers as there are several factors affecting this choice. An increase in the separation between source and detector will also lead to a decrease in the number of channels over a given area and will in turn affect the design of the headgear. Taga and colleagues (2007) investigated the optimal channel separation (between 10 – 40mm) for recording the haemodynamic responses to auditory stimuli over the temporal lobes in three-month-old infants and concluded that 20mm provided the highest sensitivity to cortical activation. However it must be noted that the optimal separation may vary depending on the intensity of the source lights, the age of the infant and the area of the cortex under investigation. Firstly, the intensity of the light can effect measurements as the stronger the intensity, the more light will reach the detectors, and the better the signal to noise ratio will be in the optical signal. As the intensity of the sources can vary across NIRS systems, a separation that is ideal for one system may not be for another. Gervain and colleagues (2008) noted that an increase in the intensity of the light over two studies of auditory stimulation in infants had a causal relationship with the magnitude of the haemodynamic response. Secondly, over the first year of life the infant's brain and head rapidly increases in size causing the distances between anatomical layers to change over time, thus influencing the optimal channel separation. Thirdly, the anatomical structure of the head varies within and between individuals, for example in five month olds the cortex can be approximately 4mm from the skin surface in the temporal lobe but at a greater depth of 10mm in the frontal and occipital lobes (Salamon et al., 1990). Thus the optimal separation may vary across areas of the cortex and between individuals. In addition to using a single separation, one method to overcome the issue of choosing the ideal separation is to use a probe design that incorporates

several source-detector separation distances. This multi separation source-detector approach allows for several depths of the cortex to be measured simultaneously (see Blasi et al., 2007; Franceschini et al., 2007), leading to depth discrimination of cortical activation.

Finally, the curvature of the skull will have a dramatic effect on the design of the probe and headgear. As mentioned earlier, a major challenge is to ensure that the probe sits flat and securely against the head. Therefore, areas of the infant head that are more curved or have more individual variability, such as the forehead, are more difficult to accommodate. Despite these challenges, designs are now in use for occipital, frontal, temporal and parietal regional investigation.

3.3 Hair

Although the designs of the probes and headgear for fNIRS studies in infants have improved significantly there remains one issue that continues to frustrate researchers: the challenge of dealing with participants' hair. Firstly, hair reduces the grip of the headgear and probes on the head, lowering friction and increasing the effects of the movement of the infant. Secondly, the layering, differing orientation and colour of the hair lying between the probe and infant head will cause attenuation of the light and can lead to unreliable measurements. If the hair is particularly dark the optical data may simply be unusable. In adult studies, a great deal of time is spent moving hair away from the area directly under the optodes. This is largely impractical with infants as they will not tolerate much interference of the hair/headgear before becoming fussy. To combat the issue of hair in adults, designs such as the ETG system (Hitachi Medical Systems) spring-loaded optodes, capable of penetrating the hair layer, have been developed. However, the

spring-loaded optodes are usually too uncomfortable for use with unsedated infants. The effects of a moderate amount of hair can be overcome by ensuring the headgear is securely fitting, for example by using a chin strap and a silicon layer or similar gripping material on the probes to increase friction and prevent slippage of the headgear (the approach used in the most recent ETG system (Hitachi Medical) (Gervain et al., 2008) and the Birkbeck/UCL system (Lloyd-Fox et al., 2009)). In addition, by allowing a small space (a few mm) between the tip of the source/detector fibre and the scalp, the light will filter through the strands rather than being obstructed by a layer of flattened hair (as used in the Birkbeck/UCL system (Lloyd-Fox et al., 2009)).

3.4 The fNIRS headgear and probe development at Birkbeck/UCL

Our own work in this area has resulted from a multidisciplinary collaboration between the Centre for Brain and Cognitive Development (School of Psychology; Birkbeck, University of London) and the Department of Medical Physics and Bioengineering (University College London). As part of the team that published the first fNIRS infant study in 1998, we have steadily worked on developing and refining this technology. One of the priorities of the Birkbeck/UCL research group has been to develop probes and headgear to provide robust and reliable optical data over a high number of channels covering a relatively large area of the cortex. Whereas our first study using a high number of channels (30) (Blasi et al., 2007) resulted in the exclusion of 64% of the infant data, our most recent study (Lloyd-Fox et al., 2009) using 20 channels resulted in the exclusion of 20% of the data. The majority of the participants excluded in the most recent study were invalid because they did not attend to the stimulus for long enough and so we are confident

that this reduction in data exclusion reflects the improvements in our headgear, which have made it more robust to head movement. As the design of the headgear has been a serious methodological issue for fNIRS users studying infants, there follows a brief outline of the improvements and practical adjustments that have been made to the design of the infant headgear by the Birkbeck/UCL research group.

i) *The optode holder* was changed from an end-on approach to, initially a prism-ended optode, and then a right-angled optode. This enabled the fibres to sit flush against the head and prevented them from pulling on the probes and headgear (see Fig. 3a). The prism-ended and right-angled optodes are also used by other NIRS systems (Benaron et al., 2000; Franceschini et al., 2007; Hitachi, http://www.hitachimedical.com).

ii) A *cloth headband* was designed to hold the fixed arrays of channels in the desired location on the head, with adjustable straps for different head sizes. This was a significant improvement compared with the Coban™ bandage (3M™ Coban™ self-adherent wrap; http://solutions.3m.co.uk/) used in the 2007 study (Blasi et al., 2007) as it reduced the degree of movement of the headgear relative to the head. The most recent headgear design incorporates a *second layer of headband* composed of a purpose made stretchy silicon band which is fastened around the head providing even pressure over the base of the probes while the cloth layer provides pressure over the top of the optodes (see Fig. 3b).

iii) In the design currently being used at the Birkbeck/UCL research lab the original plastic *optic fibres* have been replaced with *glass optic fibres* to improve flexibility, reduce the weight of the fibres pulling on the headgear and provide improved optical measurements. The latter benefit is achieved because light is absorbed less in glass compared with plastic fibres, thus improving the

signal to noise ratio. This affect can be highly significant with at least one order of magnitude more light transmitted through glass fibres than plastic fibres (unpublished observations).

iv) In recent work (Grossman et al., 2008; Lloyd-Fox et al., 2009), the use of a *thin silicon layer* under the rigid array of optodes on the side in contact with the skin has increased the friction between the headgear and head and reduced slippage, which is particularly helpful when the infant has some hair. Silicon probes have also been used in the recent infant probes designed by Hitachi (i.e. Gervain et al., 2008).

v) The current design also offers *flexibility for channel arrangement and source-detector separation* as the glass fibres can clip in and out of the array to change the distribution of channels and the area of the cortex being investigated (see Fig. 3c). A similar technique is used by the team at Charité, University of Berlin (Boden, 2007).

At Birbeck/UCL the combination of these modifications has improved the quality of the optical signal and enabled the measurement of up to 45 channels covering the frontal and temporal regions of the brain. However it should be stressed that several of these modifications have also been devised concurrently at other research centres. These recent advances have enabled researchers to overcome some of the main challenges that fNIRS studies on the infant population have posed and have therefore further strengthened the role of NIRS as an important technology for studying infant cognitive development.

****INSERT FIGURE 3 ****

4 Study design: considerations and effects

First and foremost, the design of the study must take into consideration the temporal characteristics of the haemodynamic response (see Fig. 1a). With the exception of one study (Taga and Asagawa, 2007), the majority of infant NIRS work has been conducted using a block design for stimulus presentation. The common method is to present the experimental condition for a period of 3-30s (or longer with auditory stimuli during sleep) followed by a control condition typically of longer duration to allow the haemodynamic response initiated during the experimental condition to return to a baseline level. This control condition is usually either of minimal stimulation (e.g. silence during sleeping studies) or designed to cause stimulation to a lesser extent than the experimental condition (e.g. use a mechanical movement in contrast to a biological movement in a region of the brain related to social perception as in Lloyd-Fox et al., 2009). Consequently the haemodynamic response to the experimental condition is measured in relation to the control condition.

An important consideration when designing a fNIRS study is the effect of physiological oscillations in the optical signal (e.g. vasomotion see section five). Further, it has been noted that infants are able to anticipate the onset of a stimulus (Csibra et al., 2001), which could in turn influence the onset of the haemodynamic response. One approach to reduce such physiological and anticipatory effects in a block design is to jitter experimental stimulus onset by varying the duration of the control trials so that they do not follow a predictable pattern. Alternatively, an event related design can also overcome these effects. A recent study by Taga and Asagawa (2007) used short stimulus presentations of 3.2s for two types of

experimental stimuli; auditory and visual. These stimuli were asynchronously presented with differing control trial durations to maintain unpredictable intervals of presentation. In this way, they were able to assess the effects of both types of stimuli relative to control, and found that only the auditory stimuli caused haemodynamic activation in the temporal lobe.

Finally, there are two considerations to be taken into account that affect the required number of trials for a given experimental condition. Firstly, one must establish the number of trial repetitions required for a robust response. Under ideal experimental conditions a single trial would be sufficient to yield a significant response (for an adult study see Colier et al., 1999; a study with awake infants see Carlsson et al., 2007; or studies with asleep infants see Saito et al., 2007a; 2007b). However inadequate signal to noise and the presence of motion artifacts typically require the repetition of several trials. NIRS studies do not require as many repetitions as ERP studies where the proportion of excluded trials per experimental condition in infant studies can range from 30-50%. Secondly, the repetition of several trials can result in adaptation effects where neural responses to repeated stimuli can diminish over time (Krekelberg et al., 2006). In a recent infant fNIRS study by the Birkbeck/UCL research group of biological motion processing (Lloyd-Fox et al., 2009), the first five trials revealed a larger and more widespread pattern of activation than the analyses of all ten trials. If the signal to noise ratio is adequate fewer trials could yield more reliable data. It may therefore be possible to design paradigms where relatively few trials are used to test multiple experimental conditions within one study.

5 Methods: NIRS signal processing and analysis of the haemodynamic response

The signal processing steps required to extract useable haemodynamic changes from the optical data typically involve (i) conditioning or smoothing the data by means of low-pass filtering and correction of linear trends (very slow fluctuations that could be of physiological origin); (ii) conversion of attenuation into changes in haemoglobin oxygenation (Beer-Lambert law, Kocsis et al., 2006); (iii) detection and removal of movement artifacts in the signal; and (iv) incorporation of an assessment of infant compliance/attention to the stimulus (typically extracted from the video recording of the session). In general these steps can be applied in any order since they all involve linear transformations. The analysis sequence used for the data collected in the Birkbeck/UCL studies is illustrated in Fig. 4. Steps (iii) and (iv) allow the selection of only the valid trials for block averaging and statistical analysis of the data. They ensure that the conclusions extracted from the study will be based on the true responses of the infants to the task assigned and not on spurious random effects from movement or other stimuli activating the same cortical region. Therefore it is important to have in place a set of objective criteria for this stage of the analyses. This and other issues involving the stages of NIRS signal processing and the techniques that are used at different research labs are discussed in detail below.

****** INSERT FIGURE 4 ********

5.1 Processing the optical signal

Processing of the optical data usually starts with a channel by channel assessment of the quality of the data in order to immediately discard those channels affected by poor scalp coupling or instrumentation problems. All steps involved in the optical data processing summarised in Fig. 4, except the conversion from attenuation to haemoglobin oxygenation, are designed to eradicate the effects of various sources of noise in the data. There are three types of noise to consider when processing NIRS data: instrumentation, motion (from the infant), and physiological oscillations.

Signal noise originating from the instrumentation can be determined by performing NIRS studies on dynamic tissue mimicking phantoms (Koh et al., 2009) and can usually be removed using low pass filters.

Motion artifacts are usually characterized by abrupt changes in the signals occurring simultaneously in several channels - quite distinctive from the usual slow and smooth haemodynamic response. As previously discussed a secure design of the head gear can reduce the presence of such artifacts, although it is unlikely to completely solve the problem. One option to retrospectively deal with movement artifact is to use thresholding algortithms to remove sections of the data containing these abrupt changes (Lloyd-Fox et al., 2009; Pena et al., 2003). But thresholds must be defined carefully in order to preserve the changes that truly result from activation. There is no direct correlation between the type and strength of head movement and disruption of the optical data, therefore the use of a motion senor to detect portions of the data affected by movement is not very effective. However, it is possible to use a motion sensor to evaluate the performance of infant head gear designs in terms of their robustness to movement and also to define thresholds for the automatic detection of artifacts (Blasi et al., in press). Other filtering techniques

are available (see Matthews et al., 2008 for a review), for example the principal component analysis (PCA), as proposed by Wilcox and colleagues (2005), attempts to account for large movement artifacts across all measurement channels.

In contrast to movement artifacts, low frequency oscillations such as vasomotion (oscillations in vascular tone caused by local changes in smooth muscle constriction of the vascular wall unrelated to heart rate or respiration), can mimic in frequency and magnitude the haemodynamic response to stimulation (as demonstrated by Elwell et al., 1999; Katura et al., 2006; and Taga et al., 2000). This can be overcome by careful design of the paradigm and the rate of stimulus presentation to minimise the possibility of coupling between functional activation and spontaneous cerebrovascular oscillations. In addition, in adults there is evidence of stimulus related changes in systemic parameters (e.g. heart rate, blood pressure, skin blood flow) (Obrig et al., 2000; Tachtsidis et al., 2008). Options for decoupling these systemic vascular changes and the cerebral haemodynamic response include the acquisition of systemic data by means of an independent measurement system, and the sampling of multiple volumes with NIRS so that measurements at the surface layer (with small source-detector separations) can be subtracted from measurements at the cortex level (larger source-detector separations) (Saager and Berger, 2008). Changes in systemic variables can also be accounted for using principal components analysis (PCA), by characterizing the systemic physiology features during the pre-stimulus control period and then removing the systemic components in the experimental task period (Tachtsidis et al., 2008; Zhang et al., 2005).

Another important step in the data processing sequence is the removal of baseline trends across trials within the same session. Averaging the time courses of

trials with different baselines could diminish the mean haemodynamic response. Baseline correction can be done by means of high-pass filtering or by simply removing a fitted line either across the whole study or within each block (a block is typically defined as including the period from the last few seconds of the control trial immediately preceding the experimental trial, the experimental trial, and the control trial that follows).

5.2 Analyzing the haemodynamic response

Traditionally, and due to the limited number of channels available in the first fNIRS systems, analysis of NIRS data has been based on time series processing, with standard t-tests to evaluate the significance of stimulus-correlated changes in the signals, or repeated measures analysis of the variance (ANOVA) to compare the shape (in time) of the response to different stimuli and/or groups of participants. With the incorporation of higher density arrays of sources and detectors, corrections for spatial correlation between closely placed measurement points have also been incorporated (Singh and Dan, 2006). Moreover, simultaneous acquisition of data from these multiple channel arrays has enhanced the potential of NIRS by opening the field to data reconstruction of functional images (Gibson et al., 2005). Indeed, the similarities between fMRI and optical experimental designs are being exploited and analysis techniques usually applied to fMRI are being adapted to optical data such as statistical parametric mapping techniques based on the general linear model (GLM) (Koh et al., 2007; Ye et al., 2009).

Being a relatively novel technique, it has been common practice for different groups using NIRS to develop their own analysis procedures and software. As an

increasing number of research groups use optical topography for a wide range of functional studies that differ in their degree of complexity, age range, cortical region of interest, optode geometries and system configurations, it is paramount that we adopt a standard and robust analysis methodology (Schroeter et al., 2004). Several freeware packages are available for the analysis of optical signals each with the flexibility to accommodate different system configurations (HomER, Huppert et al., 2009; fOSA, Koh, et al., 2007; NIRS-SPM, Ye et al., 2008), however some unification and standardization of data analysis methodologies may be beneficial to this research community.

6 Interpretation of fNIRS data

fNIRS measures the quantitative regional haemodynamic change that results from a localized change in brain *activation* state relative to a *control* state. As described earlier, an increase in HbO_2 and a decrease in HHb concentration represent the standard adult haemodynamic change, however from the very first fNIRS infant study in 1998, it was proposed that there could be a different infant-specific pattern of haemodynamic response. Despite numerous publications since then, controversy still remains regarding the exact nature of this infant response. Table 1c summarizes the analysis methodologies used in the fNIRS infant publications and the resulting magnitude and directional changes seen in HbO_2 and HHb. HbO_2 and HbT changes related to cortical activation are highly consistent across studies and in general similar to that observed in adults. However, HHb results are far less consistent in infants, and consequently are often not reported in publications. In addition, individual infants and certain study paradigms have shown unpredicted decreases in HbO_2. The situation is further confused when authors do

not report results for both HbO_2 and HHb changes (43% of the published infant studies listed in Table 1c do not report both).

The majority of fNIRS infant studies (94% of the published infant studies listed in Table 1c) use HbO_2 as the preferred measure for cortical activation. This is probably because this chromophore typically has the highest signal to noise ratio (SNR). Additionally, the direction of the HbO_2 change is the most consistent in infants and similar to that of adults: out of the 94% that reported HbO_2 changes, an increase in HbO_2 was reported in 93% of the studies. Controversially, the remaining 7% of the studies describing HbO_2 change, reported a consistent decrease in HbO_2 in response to the experimental stimuli. In a study by Csibra and colleagues (2004), a strong decrease in HbO_2 was recorded from a single channel positioned over the occipital cortex. In this case a decrease in HbO_2 could be related to an unpredicted increase in activation in response to the animated cartoons (control condition) relative to the static face stimuli (experimental condition) so that the baseline correction causes the appearance of 'deactivation' during the experimental condition. Alternatively, the single recording channel may have not been positioned over the optimal cortical region for face processing, an issue which reinforces the importance of multiple channel arrays that can investigate wider cortical regions. In a study by Kusaka and colleagues (2000) a significant decrease in HbO_2 was reported in response to stroboscopic flashing lights projected over the eyelids of sleeping infants. Further, a newborn study with the same paradigm using three channels over the occipital cortex found that only one of these three channels showed a significant increase in HbO_2 whilst the other two showed significant decreases (Hoshi et al., 2000). Relatively little is known about cortical activation during sleep, the sleeping state could induce neural inhibition in the visual cortex. In

addition as these were infants of just 1-3 mths of age, the haemodynamic properties of cortical activation may change over the course of development – this is discussed in detail later in this section.

As an increase in HbO_2 with no significant HHb change, would not represent activation in a BOLD contrast study (Obrig & Villringer, 2003) it is interesting to note that only 59% of the published infant studies (cited in Table 1c) report significant changes in HHb in addition to changes in HbO_2. Moreover, the direction of the HHb haemodynamic change is not consistent across the studies. Only 25% of these studies reported a decrease in HHb, whereas 40% reported an HHb increase. In the remaining 35% the changes were not consistent enough to draw conclusions or did not reach statistical significance. It has been suggested that ambiguity in the measured HHb change in adult data could be due to the choice of wavelengths used as discussed in Section 2.1 (Boas et al., 2004), but clearly an explanation based on physiology should also be sought, especially since the balance of oxygen supply and demand may differ in infants compared with adults. The haemodynamic response measured by fNIRS depends upon the combined effects of activation related increases in oxygen consumption (producing an increase in HHb and decrease in HbO_2) and oxygen delivery (producing an increase in CBF characterized by a decrease in HHb and an increase in HbO_2). The balance of these mechanisms determines whether there will be an increase or a decrease in HHb/HbO_2 concentration. In adults, typically the increase in CBF exceeds the demand for oxygen (Obrig and Villringer, 2003; Raichle, 1998) leading to an fNIRS measured haemodynamic response as shown in Figure 1. However, in infants HHb/HbO_2 concentration changes related to neural activation do not follow a typical pattern as evidenced in Table 1c and may depend on age, the region studied,

and sleep and/or sedation state, among other factors (Martin et al., 1999, Marcar et al., 2004; 2006; Meek, 2002; Wilcox et al., 2008). fMRI studies of the infant brain have also found marked differences in the haemodynamic response to neuronal activation compared to that in the adult brain. The reason for this is not entirely clear although it has been hypothesised that an excess of superfluous synaptic connections in younger infants may play a role (Marcar et al., 2004).

In addition, brain maturation is not homogeneous throughout the brain, which may explain why some researchers have reported that different types of haemodynamic responses have been observed across different regions (Dehaene-Lambertz et al., 2006). In an object processing study by Wilcox and colleagues (2009), a differential change in HbO_2 concentration across regions, specific to a single experimental condition, was observed. Whereas the featural difference condition produced HbO_2 increases in both the temporal and occipital area, the spatiotemporal-discontinuity condition produced an increase in HbO_2 in the occipital area, and a decrease in HbO_2 in the temporal area. The authors suggested that this unpredicted decrease in HbO_2 could have occurred because the channel covered an inactive area and so the blood supply was redirected to neighbouring active areas of the cortex.

Further understanding of the infant haemodynamic response could come from infant physiological models of brain circulation and metabolism in accord with those proposed for adults (Banaji et al., 2008; Mintun et al., 2001). In addition, simultaneous data acquisition with NIRS and fMRI could provide structural localization of the origin of the signal changes observed in infants. For the present, it is paramount that researchers report both HbO_2 and HHb changes in infant studies

until the relative functional importance of the two chromophores and the inconsistent HHb changes observed in infants can be explored further.

7 Coregistration

One of the main difficulties of NIRS data interpretation is localizing the origin of the haemodynamic response. NIRS measurements are made from the surface of the scalp making it difficult to establish the exact spatial origin within the brain, of the haemodynamic response. In NIRS and EEG studies it is standard practise to use the 10/20 system (Jasper, 1958) to localize regions of interest using external landmarks, but this method does not inform the internal organization of the brain. Hence, NIRS can benefit greatly from the accurate structural cerebral information provided by brain imaging techniques such as MRI. Multimodal data acquisition could contribute crucial information about rapid brain development and changes in head shape and size in the early growth stages.

fMRI and fNIRS view the same physiological phenomenon (the haemodynamic response to neural activation) but measure it using different physical principles with different spatial, temporal and quantitative scales. This has been exploited to enhance the information provided by each technique. For example, the high temporal resolution in fNIRS has been used to explore the transients in the fMRI-BOLD, and the quantitative HbO_2 and HHb fNIRS signals have been used to investigate the physiological origin of the fMRI-BOLD signal in adults (Steinbrink et al., 2006). More recently, Huppert and colleagues (2008) have used multimodality fMRI-fNIRS fusion imaging not only to improve the accuracy of the spatial localization of the HbO_2 and HHb changes, but also to provide calibration

measurements of the BOLD signal (the percentage of BOLD signal change per micromolar change in deoxyhemoglobin - measured by a 3T MRI scanner). This offers the possibility of comparing quantitative changes across participants.

At present, fMRI-fNIRS studies in humans have only been performed in adults (for a review, see Steinbrink et al., 2006). Nevertheless, it would be of great interest to use this multimodal approach to more fully characterize the haemodynamic response in the developing brain. One of the primary objectives of such studies would be to investigate whether the conflicting adult and infant haemodynamic responses to similar stimuli are a result of the stimulation protocol, the measurement systems, or differences in neurovascular coupling related physiology. Moreover, it could be beneficial to combine Diffusion Tensor Imaging (DTI) (infant myelination has also been investigated using spin labeled perfusion MRI; Wang et al., 2008,and T1 and T2 measurements; Sano et al., 2007) with NIRS for the study of myelination in infants. As the infants are usually asleep when they have a myelination scan such as DTI, integrating fNIRS would be limited to the study of auditory or stroboscopic light stimuli. The rationale for using these techniques in parallel is the same as that for combining fMRI and DTI. Multimodality integration studies of brain connectivity can enhance our understanding of structure-function relationships (Rykhlevskaia et al., 2008). This knowledge can then be applied to clinical investigation of relationships between structural brain damage and functional connections and subsequent impairment (Seghier et al., 2004). Further, fMRI and fNIRS can assist DTI by focusing on the anatomical connections to a localized region of interest, substantially reducing data collection and computational resources, which are very high in fibre tracking procedures.

A final multimodality integration technique that will enhance our understanding of infant brain development is combined NIRS-EEG research. Though neither of these will inform the underlying structural anatomy they can provide complimentary functional information of the spatial and temporal localization of the signal. A study by Grossmann and colleagues (2008) used each technique to investigate social perception of eye gaze in two groups of infants. They found correlation between the spatial response measured by NIRS and by EEG and further, using the temporal information from the EEG recordings, they were able to deconvolve responses to different components of the social stimuli, which would not have been possible from NIRS measurements alone. Work is underway to provide a method of measuring NIRS and EEG simultaneously in infants to enhance this powerful method of functional investigation similar to that developed for combined NIRS-EEG research in adults (Cooper et al., 2009; Moosmann et al., 2003) .

In summary, coregistration could lead to better and more accurate interpretation of the information provided by brain imaging techniques. This could be used to define normal cognitive developmental milestones of the infant brain. Detection of abnormal developmental patterns could then lead to early diagnosis of behavioral and cognitive disorders with the option to inform, design and test intervention strategies.

7 Concluding remarks

As has clearly been shown in this review, developmental neuroscience researchers are now readily using fNIRS to measure haemodynamic responses to a

range of experimental stimuli. Each successive year the field widens to include research on object processing, social communication, action observation, face processing and biological motion processing. The capacity of fNIRS for localizing cortical regions of activation and for allowing movement of participants defines its status as one of the most promising neurodevelopment imaging techniques to appear over the last decade. Recent findings reflect this advantage, for example with cortical responses to biological motion processing vs mechanical motion processing localized in the posterior superior temporal region (Lloyd-Fox et al., 2009) and processing of a mother's face vs a strangers face localized in the right fronto-temporal region (Carlsson et al., 2008). A promising future for this technique includes the longitudinal investigation of spatially localized developmental changes in brain function. One such study used fNIRS to investigate the development of a language-specific phonemic contrast in infants from the age of three months to 28 months (Minagawa-Kawai et al., 2007), finding age-specific onset in varying regions of the cortex. This work will be further enhanced in the future by combined NIRS and MRI studies. Moreover, due to the ease with which this technique can be used with infants and young children (Wartenburger et al., 2007) and the capability of measurements within individuals, fNIRS is ideally suited for research with atypical populations.

As this field of research continues to rapidly expand, it is paramount that the interpretation of, and subsequent claims arising from, fNIRS data are informed by a clear understanding of the complexities of NIRS measurement systems. Further investigation of the characteristics of the infant haemodynamic response using experimental data, physiological models and multimodal studies is imperative, and relies upon multidisciplinary teams addressing relevant targeted research questions.

There have been remarkable accomplishments in infant fNIRS research over the last decade encompassing instrument design, probe development, protocol optimisation and data analysis and interpretation. These have set a firm foundation for the continued role of fNIRS as an essential tool for advancing developmental neuroscience research in the future.

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Appendix

The modified Beer Lambert law relates the change in light attenuation to the change in chromophore concentration, assuming homogeneous light absorption and constant optical scattering effects:

$$\Delta A = \alpha \cdot \Delta c \cdot L \cdot DPF$$

Where A is light attenuation, α is the specific absorption coefficient and c is the concentration of chromophore of interest. L is the source detector separation and DPF is the differential pathlength factor, which is dependent upon the tissue type and wavelength. DPF has been measured in the heads of infants of a range of ages (Duncan et al., 1995). If the tissue is illuminated with two wavelengths (λ_1 and λ_2), simultaneous equations can be constructed:

$$\Delta A^{\lambda_1} = L^{\lambda_1} \cdot DPF\,(\alpha^{\lambda_1}_{HbO_2} \Delta c_{HbO_2} + \alpha^{\lambda_1}_{HHb} \Delta c_{HHb})$$

$$\Delta A^{\lambda_2} = L^{\lambda_2} \cdot DPF\,(\alpha^{\lambda_2}_{HbO_2} \Delta c_{HbO_2} + \alpha^{\lambda_2}_{HHb} \Delta c_{HHb});$$

allowing for the changes in HbO_2 (Δc_{HbO2}) and HHb (Δc_{HHb}) concentration to be resolved:

$$\Delta c_{HbO_2} = \frac{\alpha^{\lambda_1}_{HHb} \frac{A^{\lambda_2}}{L^{\lambda_2}} - \alpha^{\lambda_2}_{HHb} \frac{A^{\lambda_1}}{L^{\lambda_1}}}{\alpha^{\lambda_1}_{HHb} \alpha^{\lambda_2}_{HbO_2} - \alpha^{\lambda_2}_{HHb} \alpha^{\lambda_1}_{HbO_2}} \qquad \Delta c_{HHb} = \frac{\alpha^{\lambda_1}_{HbO_2} \frac{A_{\lambda_2}}{L^{\lambda_2}} - \alpha^{\lambda_2}_{HbO_2} \frac{A_{\lambda_1}}{L^{\lambda_1}}}{\alpha^{\lambda_1}_{HbO_2} \alpha^{\lambda_2}_{HHb} - \alpha^{\lambda_2}_{HbO_2} \alpha^{\lambda_1}_{HHb}}$$

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Figure and Table Legends

Figure 1: An overview of the NIRS technique as applied to infants; a) A typical haemodynamic response showing an increase in HbO_2 (red) and a decrease in HHb (blue) resulting from cortical activation (Courtesy of L. Coutts and A. Wilkins); b) An infant wearing NIRS probes and headgear designed by the Birkbeck/UCL research team; c) A linear reconstruction of changes in HbO_2 showing the spatial localization of the haemodynamic response to an experimental stimulus; and d) the source-detector arrangement of the optical array used to collect the data shown in (c)

Figure 2: This figure shows the spatial and temporal resolution of NIRS compared with other infant functional neuroimaging methods. It also illustrates the relative degree of tolerance needed from the infant for each method, ranging from yellow (low) to red (high). EEG, electroencephalography; ERP, event-related potential; MEG, magnetoencephalography; NIRS, near infrared spectroscopy; fMRI, functional magnetic resonance imaging; DTI, diffusion tensor imaging; PET, positron emission tomography. (This figure was inspired by Walsh and Cowey, 2000).

Figure 3: A schematic of a) the right-angled optodes; a photo b) of an infant wearing the silicon and cloth layered headgear and a photo (c) of the glass optodes and fibres clipping into the probe. These were all designed at the Birkbeck/UCL research lab (figure (a) appears in Blasi et al, in press).

Figure 4: Flow chart of the data analysis process as it is performed at the Birkbeck/UCL research lab. Infant looking time (a) is coded from a video recording of the session. Data pre-processing (b) involves low-pass filtering, channel by channel data quality assessment, construction of the trial blocks, and baseline

correction within each trial block. The Beer-Lambert Law is used for the data conversion (c) from attenuation to haemoglobin oxygenation (HbO_2, HHb) changes. Post-processing (d) consists in the automatic removal of movement artifacts. Finally, block averaging (e) and statistical analysis are carried out only in those channels that have good quality data (see Section 5.1 for a definition of a block) and within these, using only the trial blocks not affected by artifacts.

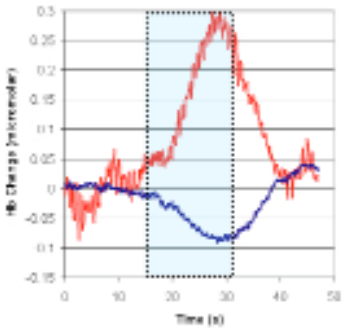
Table 1: An overview of all infant functional NIRS studies published since the first in 1998 (n/r indicates not reported). A further two studies have been published in Chinese and have not been included here; Hou et al, 2006a; Hou et al, 2006b. A table of (a) the experimental setup and cortical region of interest; b) the NIRS systems and specifications; and c) a summary of the analysis techniques (RM – repeated measure, FDR – false discovery rate, algorithm – automatic detection of artifacts, video – visual inspection of infant movement, visual – visual inspection of data)

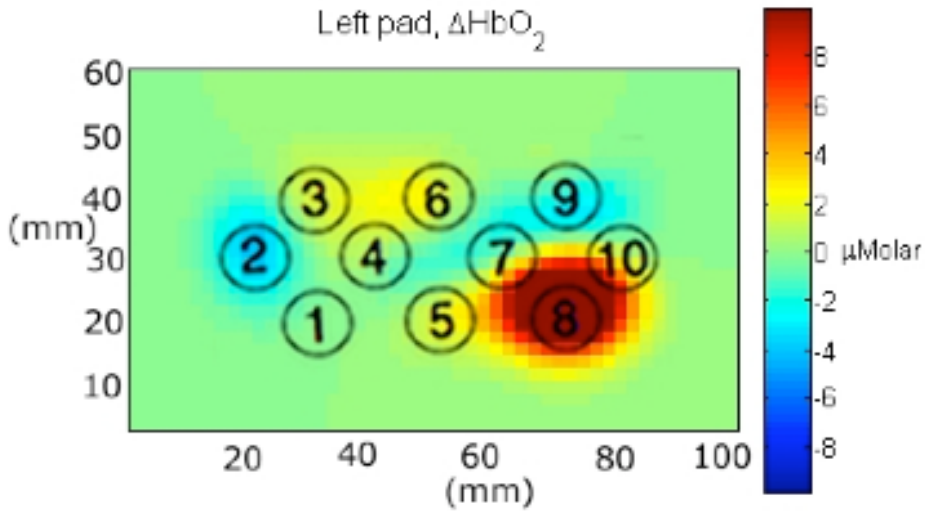
	First author	Year	Journal	Participants	Excluded infants	Cortical area Of Interest	Experimental Stimuli	Exp. Trial	Control Stimuli	Con. Trial	Significant Results		
											HbO2	HbT	HHb
1	Meek	1998	Ped Res	20 infants (2-3 mths)	38	Occipital and Parietofrontal	VISUAL- checkerboard reversal	10 s	blank dark screen	10 s	+	+	+
2	Sakatani	1999	Early Hum Dev	28 infants (neonates)	n/a	Frontal	AUDITORY- piano music	10 m	silence	10 mi	+	+	+(60%) - (32%)
3	Hoshi	2000	Ped Neurol	7 infants (neonates) asleep	n/a	Occipital	VISUAL - stroboscopic light flash	30 s	silence	1m 30s	↔	+	+
4	Bartocci	2000	Ped Res	23 infants (neonates)	7	Orbitofrontal and Olfactory	OLFACTORY own mother's colostrum, vanilla essence	30 s	distilled water	2 m	+		
5	Zaramella	2001	Ped Res	19 infants (1-49days) rest/sleep	n/a	Temporal	AUDITORY - tonal sweep of repeating sounds	2m 40 s	silence	4m	+	+	+ (62%)
6	Baird	2002	Neuroimage	12 infants (5-12mth)	n/a	Frontal	VISUAL object perm - toy hidden measure from point of occlusion	n/a	the previous visit when infant could not represent obj perm	n/a	+	+	↔
7	Pena	2003	PNAS	14 infants (neonates) asleep	2	Temporal	AUDITORY- motherese story, forward-FW or back-BW	15 s	silence (S)	25 – 35 s		+	
8	Taga	2003b	PNAS	7 infants (2-4mths)	13	Frontal and Occipital	VISUAL chequerboard (event relate)	3.2 s	red and black schematic face 7-15s. + beep sounds	7 - 15 s	+		- *
9	Taga	2003a	Early Hum Dev	25 infants (neonates) asleep	9	Frontal and Occipital	VISUAL stroboscopic light flash	3 s	silence	20 s	+		↔ *
10	Csibra	2004	J. of Ped Neurol	11 infants (4mths)	15	Frontal and Occipital	VISUAL face/scrambled face	8 s	cartoons (baseline calculated from 1s prestim)	10 s	-	↔	+
11	Kusaka	2004	Hum Bra Map	5 infants (1-3mths) asleep	n/a	Occipital	VISUAL stroboscopic light flash	15 s	silence	45 s	-	- (80%)	+
12	Kotilahti	2005	Neuroreport	20 infants (neonates) asleep	n/a	Temporal	AUDITORY 60 dB beeps sinussoidal	5 s	silence	25 s	+		
13	Wilcox	2005	J. of Bio Op	7 infants (6.5mths)	3	Temporal and Occipital	VISUAL live -ball moving behind a screen	30 s	no visual or auditory stimuli	10 s	+	+	- Occip + Temp
14	Shimada	2006	Neuroimage	13 infants (6-7mths)	5	Temporal	VISUAL live vs TV- exp. action with toy(AO) or invis action (OO).	20 s	pendullum.	5 s	+		
15	Homae	2007	Neurosci Res	21 infants (10mths) asleep	21	Temporal	AUDITORY flattened and normal speech	4.1 s	silence	12 - 15 s	+		
16	Saito	2007a	Arch Dis Child	20 infants (neonates) asleep	n/a	Frontal	AUDITORY speech, IDS or ADS (little red riding hood)	15 - 28s	computer generated white noise	1 m	+		
17	Blasi	2007	Phys Med Biol	12 infants (4 mths)	26	Occipital	VISUAL face v scrambled face	10 s	cartoons	10 s	+		
18	Bortfeld	2007	Neuroimage	35 infants (6-9mths)	5	Temporal and Occipital	VISUAL (V) ball & AUDIOVISUAL (AV) speech stimuli, 2 conditions	20 s	10s baseline of blank screen and silence	10 s	+		

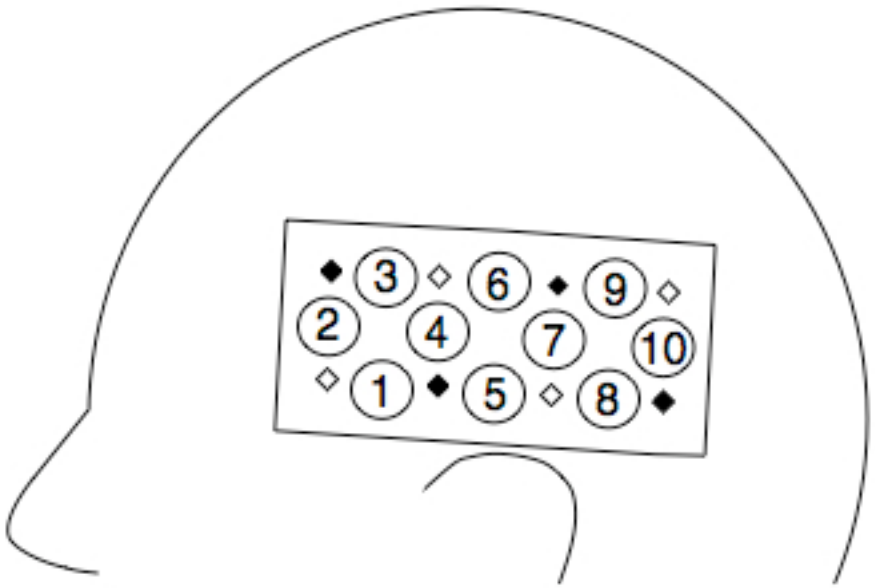
19	Minagawa -Kawai	2007	J of Neurosci	57 infants 4 age grps: 3-28mth	70	Temporal	AUDITORY pseudowords varied duration of vowel, 2 conditions.	20 s	stimA continuously repeated instead of alternate	20 s		+	
20	Otsuka	2007	Neuroimage	10 infants (5-8mths)	10	Temporal	VISUAL upright/inverted faces	5 s	vegetable photos	5 s	+	+	
21	Saito	2007b	Early Hum Dev	22 infants (neonates) asleep	n/a	Frontal	AUDITORY speech, emotive or monotone (little red riding hood)	30 s	computer generated white noise	1 m	+		
22	Taga	2007	Neuroimage	15 infants (2-4mths)	30	Temporal	VISUAL chequerboard or AUDITORY voice (event rel)	3 s (V) 3 s (A)	coloured shapes	12 s (V) 15 s (A)	+		-
23	Taga	2007	Neuroimage	9 infants (4-5mths) asleep	4	Temporal	AUDITORY speech sounds	3 s	silence	12 s	+		-
24	Carlsson	2008	Acta Paedia	19 infants (6-9mths)	8	Frontal and Occipital	VISUAL photos of mother and stranger face	15 - 20s	grey screen	15 s	+		↔
25	Karen	2008	Hum Bra Map	20 infants (neonates) asleep	n/a	Occipital	VISUAL flashing red lights	20 s	asleep - no sound or light	20 s	+(66%)		- (33%)
26	Wilcox	2008	Dev Sci	35 infants (6.5mths)	12	Temporal and Occipital	VISUAL live action, ball moving behind an occluder, changes	30 s	silence	10 s	+		
27	Grossman	2008	Pr Roy Soc B	12 infants (4mths)	10	Temporal and Frontal	VISUAL dynamic social faces with/without eye contact (CGI)	6 s	swirling cars (CGI)	12 s	+		↔
28	Gervain	2008	Pr Nat Aca Sci	44 infants (neonate) asleep/rest	n/a	Temporal and frontal	AUDITORY repetition sequences of syllables	18 s	auditory non repeat syllables and silence for rest period	35 s	+		↔
29	Watanabe	2008	Neuroimage	72 infants (3mths)	159	Frontal, Occipital And Temporal	VISUAL moving objects vs chequerboard stimuli (audio too)	5s	firework against black backdrop (audio as well)	10 s	+		
30	Nakato	2009	Hum Bra Map	20 infants (5 & 10mths)	15	Temporal and Occipital	VISUAL face direct/profile 5 types of photos	5 s	5 different vegetable images	5 s	+	+	+
31	Nakano	2009	Cerebral Cortex	80 infants (3-4mths) asleep	63	Temporal and Frontal	AUDITORY syllables test HABITUATION study	5 s	syllables but control no change in type ba	15 s	+		
32	Minagawa -Kawai	2009	Cerebral Cortex	18 infant-mother pair (9-13mths)	8	Frontal	VISUAL video smile infant or smile mother	20 s	video unfamiliar infant or mother	20 s	+		↔
33	Wilcox	2009	Neuropsychol	12 infants (6.5mths)	12	Temporal and Occipital	VISUAL live action, ball moving featural & spatiotemp change	30 s	silence	10 s	↔	↔	- (1 ch)
34	Lloyd-Fox	2009	Child Dev	36 infants (5mths)	9	Temporal	VISUAL dynamic social faces vs dynamic mechanical stimuli	16 s	Photos of transport Non-social stim	16 s	+		↔
35	Bortfeld	2009	Dev Neuropsych	21 infants (6-9mths)	7	Temporal	AUDIOVISUAL shapes + infant-dir speech, VISUAL dynamic shapes	20s	Black screen with silence	10s	+ AV - V		

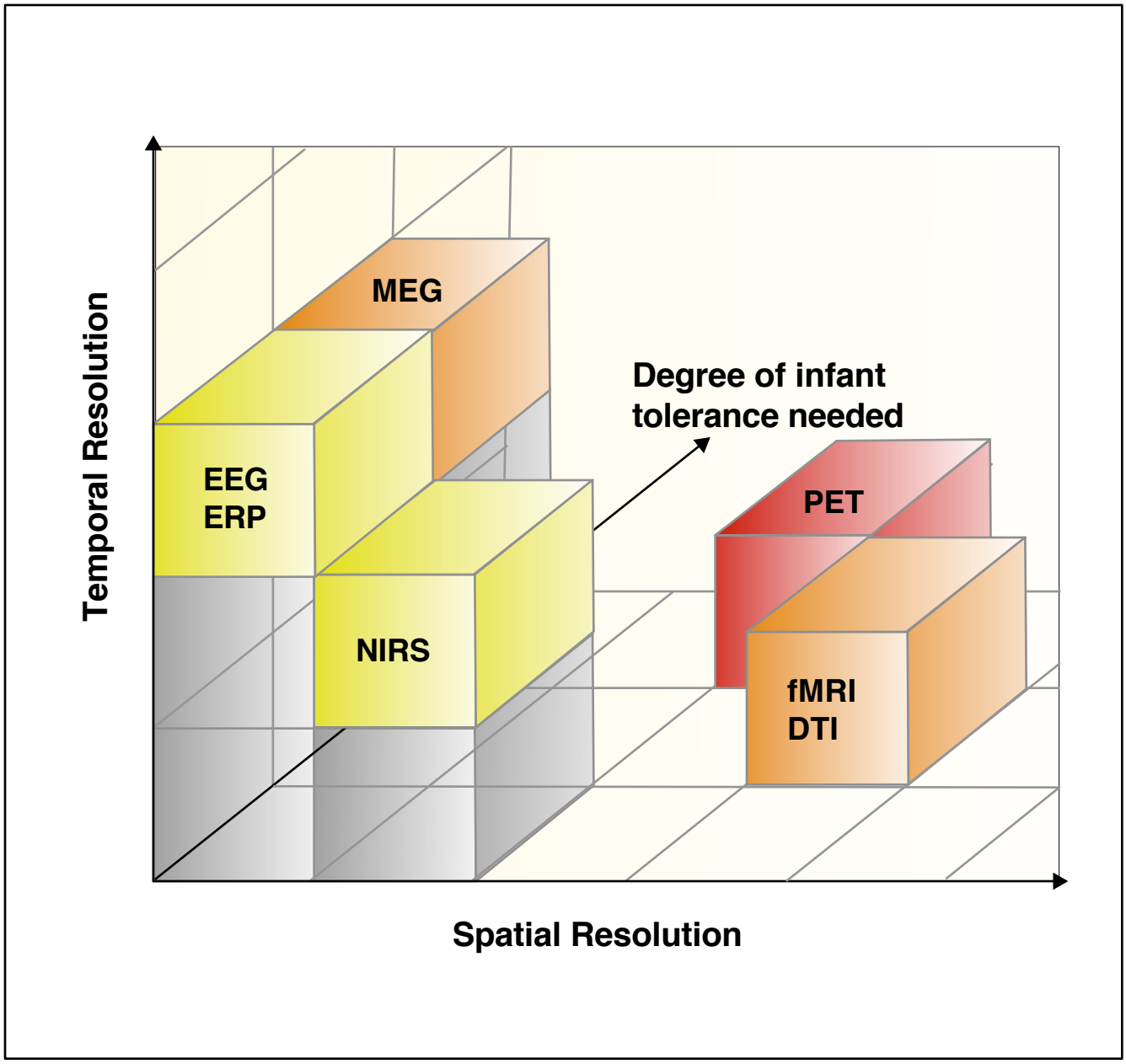
	First author	Year	NIRS System	Wavelengths (nm)	Sources	Detectors	Channels	Chan Sep (mm)
1	Meek	1998	Hamamatsu NIRO 500	775, 825, 850, 905	1	1	1	35
2	Sakatani	1999	Hammamatsu NIRO 500	775, 825, 850, 905	1	1	1	40
3	Hoshi	2000	Shimadzu OMM-100	780, 805, 830	3	3	3	25
4	Bartocci	2000	Hamamatsu NIRO 300	775, 810, 850, 910	1	1	1	40
5	Zaramella	2001	Hammamatsu NIRO 300	775, 810, 850, 910	1	1	1	35
6	Baird	2002	Hammamatsu C546001	785, 830	1	4	4	20
7	Pena	2003	Hitachi ETG-100	780, 830	10	8	20	30
8	Taga	2003b	Hitachi NIR OT	780, 830	10	8	12	20
9	Taga	2003a	Hitachi NIR OT	780, 830	10	8	12	20
10	Csibra	2004	Hammamatsu NIRO 300	775, 810, 850, 910	2	2	2	40
11	Kusaka	2004	Shimadzu OMM-1080S	776, 804, 828	8	8	24	20
12	Kotilahti	2005	Helsinki (in house system)	760, 830	?	?	18	25
13	Wilcox	2005	Harvard (in house system)	690, 830	2	4	4	20
14	Shimada	2006	Shimadzu OMM-1080S	780, 805, 830	3	3	7	20
15	Homae	2007	Hitachi ETG-100	780, 830	10	8	24	20
16	Saito	2007a	Hamamatsu NIRO -200	778, 812, 850	2	2	2	30
17	Blasi	2007	UCL NTS (in house system)	780, 830	8	8	30	14.3, 17.8, 22
18	Bortfeld	2007	Harvard (in house system)	680, 830	4	2	4	20
19	Minagawa-Kawai	2007	Hitachi ETG-100 & 7000	780, 830	2	2	4	30
20	Otsuka	2007	Hitachi ETG-100	780, 830	10	8	24	20
21	Saito	2007b	Hamamatsu NIRO -200	778, 812, 850	2	2	2	30
22	Taga	2007	Hitachi ETG-100	780, 830	10	8	24	20
23	Taga	2007	Hitachi ETG-100	780, 830	8	2	8	10, 20, 30, 40
24	Carlsson	2008	Hamamatsu NIRO - 300	775, 810, 850, 910	2	2	2	40
25	Karen	2008	MCP - II (in house system)	730, 830	4	4	10	25, 37.5
26	Wilcox	2008	Harvard (in house system)	690, 830	2	4	4	20
27	Grossman	2008	UCL NTS (in house system)	770, 850	11	12	27	20
28	Gervain	2008	Hitachi ETG-4000	695, 830	10	8	24	30
29	Watanabe	2008	Hitachi ETG-100 & 7000	780, 830 785, 830	20 21	16 21	48 84	20 20
30	Nakato	2009	Hitachi ETG-100	780, 830	10	8	24	20
31	Nakano	2009	Hitachi ETG-100 (x2)	780, 830	20	16	48	20
32	Minagawa-Kawai	2009	Hitachi ETG-7000	780, 830	2	2	7	30
33	Wilcox	2009	Harvard (in house system)	690, 830	2	4	4	20
34	Lloyd-Fox	2009	UCL NTS (in house system)	770, 850	8	8	20	20
35	Bortfeld	2009	Harvard (in house system)	680, 830	2	4	4	20

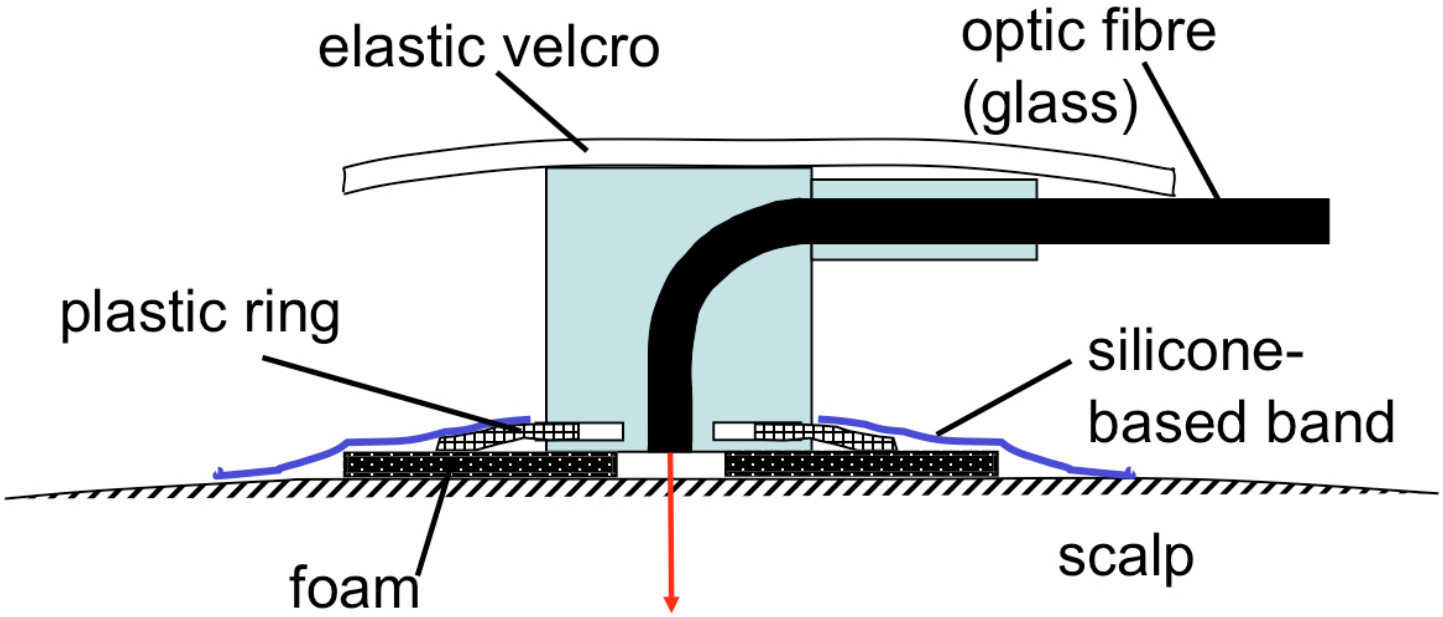
	First author	Year	Statistical Methods	Image reconstruction	Baseline correction	Treatment of artifacts
1	Meek	1998	Average change (t-test)	No	n/r	Algorithm
2	Sakatani	1999	Average change (t-test)	No	n/r	n/r
3	Hoshi	2000	Average change (t-test)	No	n/r	No
4	Bartocci	2000	Time course analysis (RM-ANOVA)	No	n/r	No
5	Zaramella	2001	Average change (t-test)	No	n/r	Algorithm
6	Baird	2002	Time course analysis (RM-ANOVA)	No	n/r	Algorithm
7	Pena	2003	Average change (RM-ANOVA + post-hoc comparisons)	Yes	Detrend - block	Algorithm
8	Taga	2003b	Time course analysis (RM-ANOVA)	Yes	High-pass-filter (0.002 Hz)	Algorithm
9	Taga	2003a	Time course analysis (RM-ANOVA)	No	Detrend - session	Algorithm
10	Csibra	2004	Average change (t-test)	No	Detrend - session	Video + Visual
11	Kusaka	2004	Average change (Wilcoxon's signed rank test, Mann-Whitney U test)	Yes	Detrend - block	n/r
12	Kotilahti	2005	Average around peak change (t-test)	No	n/r	Algorithm
13	Wilcox	2005	Average change	No	n/r	Algorithm
14	Shimada	2006	General Linear Model	Yes	n/r	Video
15	Homae	2007	Average change (t-test)	Yes	Detrend - block	Algorithm
16	Saito	2007a	Average change (t-test)	No	Detrend - block	Algorithm
17	Blasi	2007	Peak change (t-test)	Yes	Detrend - block	Algorithm
18	Bortfield	2007	Average change (ANOVA, t-test)	No	n/r	Algorithm
19	Minagawa-Kawai	2007	Peak change (t-test + Holm correction)	No	n/r	Visual
20	Otsuka	2007	Average change (t-test); Time course analysis (RM-ANOVA)	No	High-pass-filter (0.02 Hz)	Video + Algorithm
21	Saito	2007b	Average change (ANOVA)	Yes	Detrend - session	Algorithm
22	Taga	2007	Average change (t-test + FDR correction)	No	Detrend - block	Visual
23	Taga	2007	Time course analysis (F-test); Average change (t-test, RM-ANOVA)	No	Detrend - block	Video + Algorithm
24	Carlsson	2008	Average change (ANOVA)	No	Detrend - session	Video
25	Karen	2008	Average change (paired Wilcoxon sign rank test)	Yes	Detrend – block Low-pass-filter (0.25 Hz)	n/r
26	Wilcox	2008	Average change (t-test, ANOVA)	No	Detrend - block	Algorithm
27	Grossman	2008	Peak change (t-test + FDR correction)	No	Detrend - block	Algorithm
28	Gervain	2008	Average change (t-test, ANOVA)	No	n/r	n/r
29	Watanabe	2008	Average change (t-test + FDR correction)	No	Detrend - block	Video + Algorithm
30	Nakato	2009	Average change (Z-score, ANOVA)	No	High-pass-filter (0.02 Hz)	Video + Algorithm
31	Nakano	2009	Average change (t-test, ANOVA, + FDR corrrection)	No	Detrend - block	Video + Algorithm
32	Minagawa-Kawai	2009	Average change (t-test + Bonferroni correction)	No	n/r	Video + Visual
33	Wilcox	2009	Average change (t-test, ANOVA)	No	Detrend - block	Algorithm
34	Lloyd-Fox	2009	Peak change (t-test)	No	Detrend - block	Algorithm
35	Bortfeld	2009	Average change (ANOVA)	No	n/r	Video + Algorithm

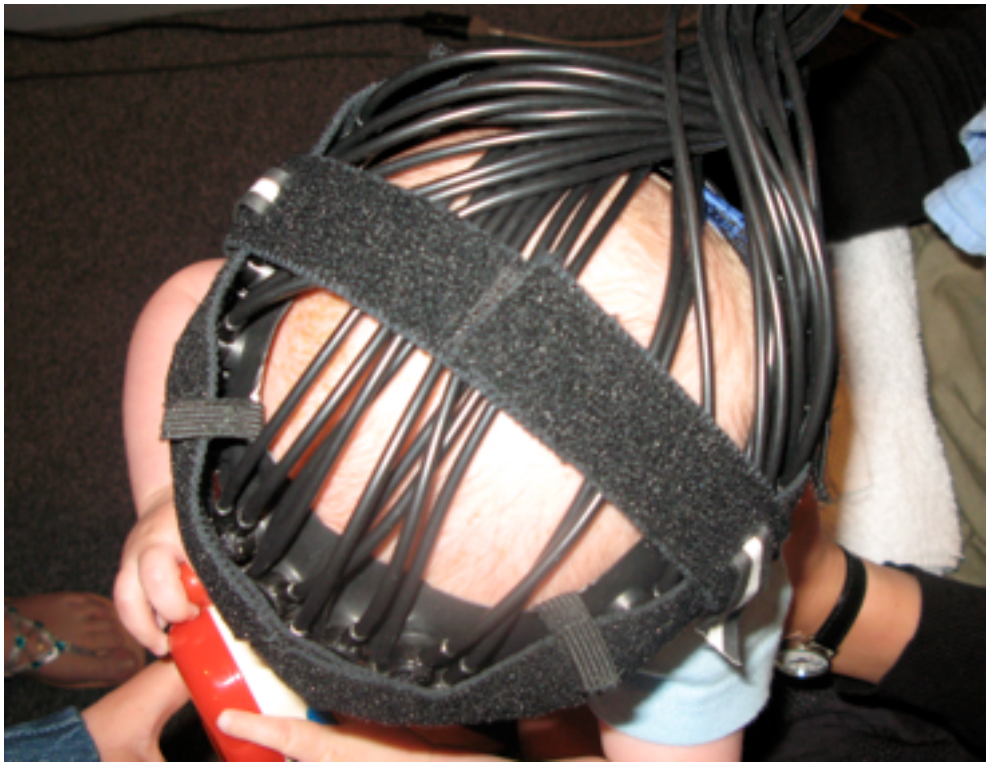

0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
0
10
20
30
40
50
Time (s)

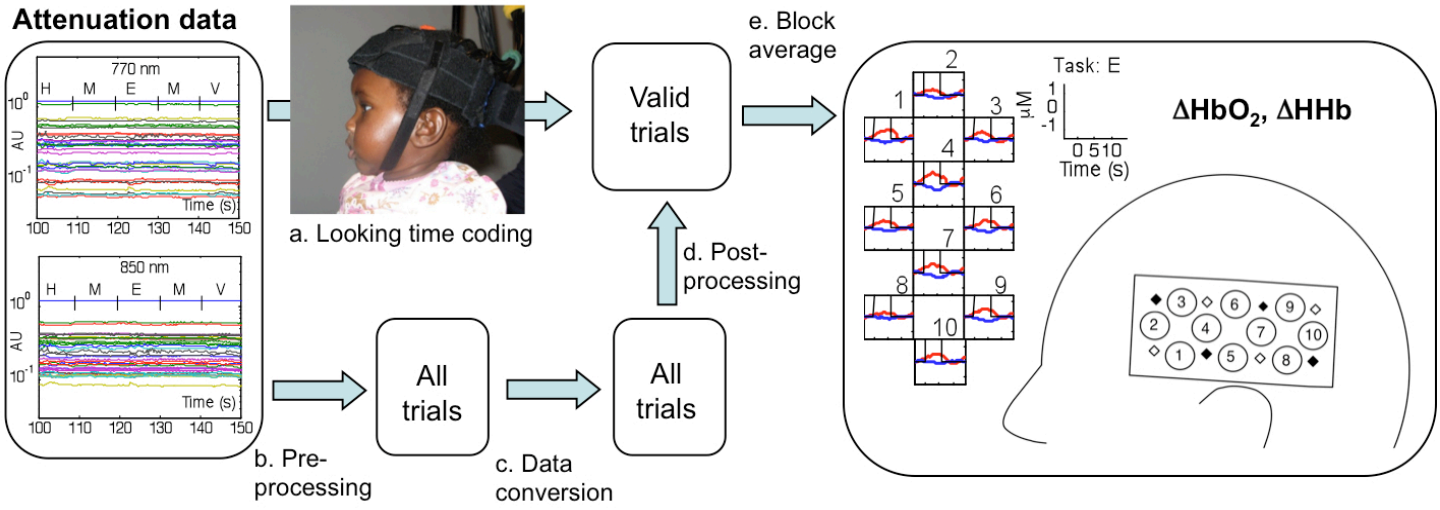

Left pad, ΔHbO2
60
50
40
30
20
10
(mm)
20
40
60
80
100
(mm)
1
2
3
4
5
6
7
8
9
10
6
4
2
0
-2
-4
-6
-8
µMolar


3
6
9
2
4
7
10
1
5
8


Temporal Resolution
MEG
EEG
ERP
NIRS
Degree of infant
tolerance needed
PET
fMRI
DTI
Spatial Resolution


elastic velcro
optic fibre
(glass)
plastic ring
silicone-
based band
foam
scalp




Attenuation data
770 nm
H M E M V
AU
Time (s)
100 110 120 130 140 150
850 nm
H M E M V
AU
Time (s)
100 110 120 130 140 150
a. Looking time coding
b. Pre-processing
All trials
c. Data conversion
All trials
d. Post-processing
Valid trials
e. Block average
Task: E
μM
Time (s)
ΔHbO2, ΔHHb