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**Are you talking to me? Neural activations in 6-month-old infants in response to being addressed during natural interactions**

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**Abstract**

Human interactions are guided by continuous communication among the parties involved, in which verbal communication plays a primary role. However, speech does not necessarily reveal to whom it is addressed, especially for young infants who are unable to decode its semantic content. To overcome such difficulty, adults often explicitly mark their communication as infant-directed. In the present study we investigated whether ostensive signals, which would disambiguate the infant as the addressee of a communicative act, would modulate the brain responses of 6-month-old infants to speech and gestures in an ecologically valid setting. In Experiment 1, we tested whether the gaze direction of the speaker modulates cortical responses to infant-directed speech. To provide a naturalistic environment, two infants and their parents participated at the same time. In Experiment 2, we tested whether a similar modulation of the cortical response would be obtained by varying the intonation (infant versus adult directed speech) of the speech during face-to-face communication, one on one. The results of both experiments indicated that only the combination of ostensive signals (infant directed speech and direct gaze) led to enhanced brain activation. This effect was indicated by responses localized in regions known to be involved in processing auditory and visual aspects of social communication. This study also demonstrated the potential of fNIRS as a tool for studying neural responses in naturalistic scenarios, and for simultaneous measurement of brain function in multiple participants.

**Keywords**

Social interactions, communication, ostensive signals, fNIRS, infant-directed speech

## 1. Introduction

Apart from rare exceptions, human interactions are guided by continuous communication among the parties involved. Human communication is ostensive: it advertizes itself as deliberate communication rather than just providing an information source for others (Sperber & Wilson, 1995). Beyond specifying that a certain act is meant to carry content for others, the ostensive nature of communication is also important in determining to whom the content is addressed. Indeed, ostensive signals serve both functions at the same time: they specify the addressee and mark the accompanying actions as communicative (Csibra, 2010). Such signals include eye contact, calling others by name, and adjusting one's actions temporally or spatially to the addressee, such as in turn-taking or blocking someone's path, etc. However, the inclusion of such signals is not compulsory because the addressee of a communicative act can also be inferred from the context or from the content communicated. For example, the dominant channel of human communication is vocal, and the linguistic content of speech can itself reveal whom it is meant to target.

This feat, however, is only available for addressees who can comprehend the verbal message embedded in the speech they hear. If they have to rely exclusively on speech content, some potential addressees, such as foreigners, non-human animals, and human infants would not be able to detect when someone is talking to them. For such addressees, sensitivity to ostensive signals that unambiguously define them as the addressee is the only way to notice communicative attempts directed at them. To overcome such difficulty, during periods of social interaction between adults and infants, adults often naturally adjust their communication to be explicitly infant-directed by including such ostensive signals. For example, they may communicate with the special intonation termed as infant-directed speech (IDS) — which Darwin (Darwin, 1877) referred to as “the sweet music of the species” — a tendency which seems to be independent of the culture, the language, or the experience of the parents (Albin & Echols, 1996; Fernald et al., 1989; Masataka, 2003; Panneton Cooper, Abraham, Berman, & Staska, 1997; Papousek, Papousek, & Symmes, 1991). Furthermore, adults often elicit eye contact, call an infant's name, or position themselves in an optimal

location for face-face communication, providing the infant with further ostensive signals prior to initiating periods of social interaction.

In turn, infants seem attuned to these signals from an early age. Newborns prefer to look at faces with direct gaze compared to averted gaze (Farroni, Csibra, Simion, & Johnson, 2002), and prefer to listen to infant-directed speech compared to adult-directed speech (Fernald, 1985; Panneton Cooper et al., 1997). By around five months of age infants can already extract infant-directed intonation patterns from background noise (Colombo, Frick, Ryther, Coldren, & Mitchell, 1995), and start to learn new ostensive signals, such as their name (Mandel, Jusczyk, & Pisoni, 1995). The behavioral responses with which infants respond to these signals are very similar: they smile, they pay enhanced attention to the source, and tend to follow its directional movement following the ostensive signals (Deligianni, Senju, Gergely, & Csibra, 2011; Senju & Csibra, 2008). Furthermore, these ostensive signals are known to activate certain regions of the frontal and temporal cortices (particularly in the right hemisphere) in infants (Grossmann, Parise, & Friederici, 2010; Grossmann et al., 2008; Grossmann, Johnson, Farroni, & Csibra, 2007; Imafuku et al., in press; Parise & Csibra, 2013; Parise, Friederici, & Striano, 2010; Saito et al., 2007; Zangl & Mills, 2007). Some of these regions match those that were identified in adults as responding to communicative signals (Kampe, Frith, & Frith, 2003). Recent electroencephalography (EEG) and functional near infrared spectroscopy studies (fNIRS) have highlighted cortical activation to various visual social cues during infancy. In particular, in the temporo-parietal region activation has been found to the perception of whole body movements (Hirai & Hiraki, 2005; Reid, Hoehl, & Striano, 2006), manual actions and gestures (Lloyd-Fox, Blasi, Everdell, Elwell, & Johnson, 2011; Lloyd-Fox, Wu, Richards, Elwell, & Johnson, 2013), eye contact and gaze direction (Grossmann, Parise, et al., 2010; Senju, Johnson, & Csibra, 2006). Furthermore, areas of the temporal lobes demonstrate stronger activation when infants listen to human-specific sounds, such as vocalizations (including speech, laughter, crying, coughing, etc.) compared with non-vocal environmental sounds (Grossmann, Oberecker, Koch, & Friederici, 2010; Lloyd-Fox, Blasi, Mercure, Elwell, & Johnson, 2012; Lloyd-Fox, Blasi, et al., 2013; Minagawa-Kawai et al., 2011) and forward versus backward speech (Pena et al., 2003).

In the present study we investigated whether various ostensive signals, which would disambiguate the infant as the addressee of a communicative act, would modulate the brain responses to speech and gestures in an ecologically valid setting. The existence of common indices of cortical activity from areas known to be sensitive to ostensive signals in adults, or in regions specialized to processing communicative acts (i.e., speech and/or gestures), would support the proposal that these stimuli are interpreted as ostensive signals and serve the function of orienting attention to potentially communicative acts. Natural infant-directed communication includes both visual ostensive signals, such as direct gaze, and auditory cues, such as infant-directed intonation. An earlier study compared brain responses of 5-month-old infants to various combinations of these signals and found that either of them separately, or both of them together, had the same effect (Parise & Csibra, 2013). Thus, the absence of eye contact (i.e., averted gaze) with infant-directed speech, or the absence of infant-directed intonation (i.e., adult-directed speech, ADS) in the presence of eye contact, did not prevent infants from interpreting the stimuli as addressed to them. However, this study employed short stimuli of less than a second in duration, and measured fast and phasic ERP responses as brain activation. It is possible that, just like adults when they overhear their name or experience fleeting eye contact that make them mistakenly think that they are being addressed, infants' first reactions to these ostensive signals also fail to take into account other cues that indicate otherwise. We thus created situations in which these signals were presented live and were available for a longer duration to allow infants to assess whether the speech and gestures were meant to target them. Live settings in neuroimaging studies are challenging but especially useful if the main question concerns responses during naturalistic social interactions rather than to disembodied stimuli. Because of the extended duration of these interactions, we chose fNIRS as the method for investigating brain responses to the combination of ostensive signals (Lloyd-Fox, Blasi, & Elwell, 2010).

In Experiment 1, we tested whether the gaze direction of the speaker modulates cortical responses to infant-direct speech. To provide a naturalistic “noisy” environment, two infants and their parents participated at the same time while fNIRS recordings were taken from each infant. In Experiment 2, we tested whether a similar modulation of the cortical response would be obtained by varying the intonation (IDS vs ADS) of the speech during face-to-face

communication, one on one. Because one kind of ostensive signal (IDS or infant-directed gaze, IDG) was present in either context, if 6-month-old infants only care about the presence of such a signal, but not about the contradicting nature of the other one, we should not find differential activation to the contrasted stimuli in either study. Alternatively, if infants take into account both sources of ostensive signals, we expect to find that the additional ostensive signal modulates the processing of speech and gestures, and does so similarly in both experiments.

## **2. Experiment 1**

Six-month-old infants watched a female experimenter communicating the same way but who looked towards either themselves or another baby. To provide a naturalistic environment, two infants and their parents participated at the same time. We measured their cortical responses using fNIRS to investigate the effect of ostensive signals (gaze and infant-directed speech) in the two modalities, also contrasted with a non-communicative baseline phase.

### **2.1 Methods**

#### **2.1.1 Participants**

Twenty-four full-term, healthy 6-month-old infants (7 female, age range = 164-199 days, mean age = 182.5 days) participated in the study. A further 28 infants participated, but were excluded because they failed to attend to the first four trials (n=18), were showing signs of distress themselves (heavily fussing or crying) or had a distressed infant next to them which distracted them from the study (n= 4), had signal quality problems because they pulled on the NIRS headgear (n=3), or experimental error (n=3). As the study was conducted with pairs of infants, we should note that valid data came from 16 infants in full pairs, and a further 8 infants whose partners' data were invalid. This attrition rate is at the high end of the standard range for infant fNIRS studies (Lloyd-Fox et al., 2010), because we applied strict inclusion criteria based on looking time and behavior (see below).

All parents gave written, informed consent prior to participation. The study design was approved by the United Ethical Review Committee for Research in Psychology (EPKEB), Budapest, Hungary and was conducted according to the Declaration of Helsinki.

### **2.1.2 Procedure**

Infants participated in the study in pairs. Each of them were seated on their parent's lap approximately 110 cm away from each other. A female experimenter sat in front of them midway between the two and at an equal distance (approximately 90 cm) from them (Figures 1 & 2). During the trials, the experimenter communicated towards one of the infants for approximately 15 seconds. She performed Hungarian nursery rhymes in infant-directed speech, accompanied by hand movements. For the infant who was addressed, the stimuli were considered to form a trial in the infant-directed gaze – infant-directed speech (IDG-IDS) condition, while for the other infant, who could observe and listen to the same communication while the experimenter was not looking at her, this trial belonged to the other-directed gaze – infant-directed speech (ODG-IDS) condition. During the baseline phase between trials (10 seconds), the experimenter looked down into a booklet on her lap as she was reading, with occasional body movements, such as moving her hair with her hand, turning the pages of the book, changing position in her chair (to approximately match the degree of movement with that during communication). Each trial started with the experimenter snapping her fingers to obtain the infants' attention. The trial length was timed by a second experimenter seated behind a curtain who tapped Experimenter 1 on the shoulder at the beginning/end of each trial and placed an event marker into the fNIRS recording at the same time. Experimental conditions alternated between the two infants in an ABAB format. The sequence of trials is shown in Figure 1. In the final dataset, 10 of the 24 infants saw the experimenter direct their speech and gestures to themselves on the first trial, while 14 of them started with observing the other infant being addressed. Parents were asked to refrain from interacting with their infant unless the infant sought it, and the experiment ended when infants became bored or fussy. Each session was video recorded for later off-line behavioral and looking time coding.

Behavioral coding assessed the duration of attending the experimenter in each trial. A trial was considered valid if the infant attended for at least 60% of its first 10 seconds as well as a minimum of 60% of the entire duration of the trial. Infants who made eye contact with their own parent at any point during the first four trials were excluded from the analysis. (In

contrast to in other studies with infants, in which parents are normally asked to close their eyes during stimulus presentation to avoid biasing their child's responses, we let them keep their eyes open but instructed them not to interfere. We acknowledge that the parents' postural responses to the stimuli might have affected the responses of the infants who were sitting on their lap. However, we chose to let the parents keep their eyes open because in our naturalistic procedure our participants would have noticed the closed eye of the parent of the other infant, which itself could have influenced their brain responses further, especially in the ODG-IDS condition.)

### ***2.1.3 Data recording and processing***

fNIRS measurements were recorded by the UCL-NIRS topography system (Everdell et al., 2005). This system uses continuous wavelength at 780 and 850 nm. Infants wore custom-built fNIRS headgear designed by the Centre for Brain and Cognitive Development (CBCD), Birkbeck University of London (Lloyd-Fox et al., 2010). Recordings were taken simultaneously from both infants with the source and detector fibres divided to generate two sets of fNIRS headgear. The headgear consisted of a fixed length headband with the two arrays (frontal and right lateral) attached within this headband in fixed positions (Figure 3). Because of the limited number of channels with this system, we focused our measures on the right hemisphere, given that previous evidence of activation to social cues often indicates greater involvement of the right compared with the left hemisphere. The midline of the frontal array was positioned over the glabella, covering the prefrontal region and consisted of two 2-cm and two 2.5-cm source-detector channels. The lateral array consisted of 13 2-cm source-detector channels. The midpoint of the lateral array was at a fixed distance, 11 cm from the midpoint of the prefrontal array, which on an average 6-month-old infant is centred above the right pre-auricular point (T4 according to the 10-20 system).

Based on an understanding of light transport, and given that the cortex is approximately 0.5 cm from the skin surface in this age group (measure taken from structural MRIs; Salamon, Raynaud, Regis, & Rumeau, 1990), the channel separation used in the current study was estimated to penetrate up to a depth of approximately 1 cm from the skin surface, potentially allowing measurement of both the gyri and parts of the sulci near the surface of the cortex.

Before the infants began the study, head measurements were taken to align the headgear with 10-20 coordinates. The head circumference, the lateral semi-circumference from ear to glabella to ear and the semi-circumference from ear to vertex to ear were measured.

Measurements from this group of infants showed that the average head circumference was 42.9 cm (s.d. = 1.0 cm). After the infant was fitted with the fNIRS headgear, pictures were taken from the front, and to the left and right. These pictures recorded positioning of the fNIRS arrays and headgear relative to the nasion, ears, and other fiducials.

As a single recording contained the datasets of two infants, the raw data was first separated into two data files for each infant and then converted into .nirs format for analysis through HOMER2 system (Huppert, Diamond, Franceschini, & Boas, 2009). The procedure of analysis followed a similar protocol to previous infant research (Wilcox, Bortfeld, Woods, Wruck, & Boas, 2005; Wilcox, Haslup, & Boas, 2010). First, we performed a principal component analysis (PCA) on the spatial covariance of the data. This method eliminates systemic physiological signals and motion artefacts common to all channels through filtering out the corresponding components (Zhang, Brooks, Franceschini, & Boas, 2005). Here, we removed the components that contributed 80% or more of the variance of the data. Following this first step, and given that infant data can often be contaminated by artefacts, we used a second form of artefact detection, wavelet analysis, to correct the data further (Cooper et al., 2012; Molavi & Dumont, 2012). After these corrections, the data were band-pass filtered (0.04–0.5 Hz, FIR digital filter) to attenuate slow drifts and high frequency noise. Then the filtered data for each of the two wavelengths were converted to relative concentrations of oxygenated (HbO<sub>2</sub>) and deoxygenated (HHb) haemoglobin using the modified Beer-Lambert law. Finally, relative changes in HbO<sub>2</sub> and HHb, were computed for 29-s-long epochs starting from 4 s before the onset of each trial. The mean concentration of the 4-s pre-experimental stimulus window was considered as baseline, and was subtracted from the signals of the whole epoch.

In preliminary analyses, we found that the differential response to the two conditions tended to diminish over repeated presentation of trials. We think that this effect was the result of the naturalistic situation, in which both infants were alternately addressed, generating the

impression that all communication targeted both of them after the first few trials. Therefore we decided to analyze only the first four trials, and only infants with valid data for all of the first four trials were included. First, we quantified the mean haemodynamic concentration changes during every 5-s sub-epoch following 5 s after the onset of the trial after averaging signals across trials for each channel and condition. We then compared these values to baseline (i.e., to zero), and selected channels and epochs with valid activation, i.e., where the HbO<sub>2</sub> concentration was significantly above or the HHb concentration was significantly below zero in either condition (Lloyd-Fox et al., 2010)<sup>1</sup>. Finally, paired t-tests were conducted on each of these pre-selected epochs to assess whether there were differences in the haemodynamic response between the two conditions (IDG-IDS vs. ODG-IDS).

## 2.2 Results

The initial analysis identified 9 epochs over 6 channels where there was a significant haemodynamic response during the trials compared to baseline (Table 1). Seven out of 9 of these epochs indicated activations to the IDG-IDS conditions, and we found only two epochs in which communication to the other infant (ODG-IDS condition) resulted in significant haemodynamic activations. When we compared the activations between conditions within these 9 epochs, we found a significantly greater increase of HbO<sub>2</sub> to the IDG-IDS condition relative to the ODG-IDS condition in channels 6 and 7 (Channel 6 – at 20-25 s window:  $t(23) = 2.23, p = .036$ ; Channel 7 at 10-15 s window:  $t(23) = 2.23, p = .036$ ). For the analysis of the HHb signal, there was also a significantly greater decrease to the IDG-IDS condition relative to the ODG-IDS condition in Channel 4 (at 10-15 s window:  $t(23) = 2.48, p = .021$ ), and a marginally significant decrease in Channel 8 (at 10-15 s window:  $t(23) = 1.99, p = .058$ ). These effects are depicted in Figure 4. No channels revealed a significantly greater activation to the ODG-IDS condition relative to the IDG-IDS condition. In additional analyses we found no effect of order of trials (IDG or ODG first) or interaction of this factor with condition.

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<sup>1</sup> In principle, the responses measured with the two chromophores should be coupled: whenever HbO<sub>2</sub> increases, HHb should decrease. However, empirically such clean responses are hardly found, and it is much easier to detect HbO<sub>2</sub> than HHb changes in fNIRS research (Lloyd-Fox et al., 2010). For transparency, here we report significant responses with both chromophores, but we treat HHb decrease without a corresponding significant HbO<sub>2</sub> increase cautiously.

While we found relatively late effects on some channels, this was partly due to the strict constraint we placed on statistical significance. For example, the HbO<sub>2</sub> increase on Channel 6 went significantly above baseline in the 20-25 s window, but it was close to that status in the previous two windows as well ( $p = .096$  and  $.064$ , respectively). Thus, the responses to the stimuli were developing gradually during the live interactions, but were reaching statistical significance earlier at certain sites (Channel 7) than at others (Channel 6). Note also that the two between-condition effects we found for HHb were accompanied by corresponding differences for HbO<sub>2</sub> (1.53  $\mu$ Mol and 1.73  $\mu$ Mol for Channels 4 and 8, respectively), but these differences were not significant ( $ps > .200$ ). We therefore remain cautious about the reliability of these results.

By using a standardized scalp surface map of fNIRS channel coordinates for this age range (Lloyd-Fox et al., 2014), the information from the head measurements and photos, and the known configuration of the CBCD-designed headgear we can approximate which cortical regions are underlying the channels that revealed significant responses. Channel 4 is positioned approximately over the inferior frontal gyrus, Channel 6 is on the border of the frontal and temporal cortices, and Channel 7 is positioned over the anterior superior temporal cortex. Channel 8, which showed marginally significant HHb responses, is positioned over the temporo-parietal region (which includes superior temporal to postcentral gyrus). Although earlier studies on infants' sensitivity to ostensive signals indicated the involvement of the orbito-frontal and pre-frontal region (Grossmann, Parise, et al., 2010; Grossmann et al., 2008, 2007), we could not confirm that result here.

Note also that our statistical approach expected activations (HbO<sub>2</sub> increase or HHb decrease) elicited by our experimental conditions. However, visual inspection of Figure 4 suggests that infants tended to produce the opposite pattern or responses (HbO<sub>2</sub> decrease and/or HHb increase) on some channels in the 5-10 s time window, and such a response was stronger in the ODG-IDS condition. Since we did not predict such 'deactivations,' we could perform only exploratory analyses on them. Indeed, within this time window, the concentration of the two chromophores deviated from baseline in the unexpected direction in many channels for

this condition, and in two of them (HbO<sub>2</sub> in Channel 5 over the inferior frontal cortex, and HbB in Channel 16 over the parietal cortex) the difference between conditions would have reached statistical significance. We cautiously interpret these unpredicted effects as potential deactivation responses to the termination of the baseline period, which also involved the observation of human behaviour, though without communication. Such deactivations might have been stronger when the attention of the experimenter turned to the other infant.

### 2.3 Discussion

Our statistical analyses suggested that the presence of two ostensive signals (IDG and IDS) elicited enhanced activation in inferior frontal and temporal regions relative to the presence of one ostensive signal (i.e., IDS). Furthermore, activation was not stronger in any of the measured regions when the experimenter directed their attention to the other infant rather than to the participant. Thus, direct gaze from the experimenter increased neural responses to the multimodal communicative actions (speech plus gestures). However, there are at least two different mechanisms that would explain such an effect.

First, it is possible that the speech and gesture stimuli elicited the same activation in the two conditions, and the difference we observed between conditions were due to the additional activation produced by the eye contact, which was only present in the IDG-IDS condition. Direct gaze has been reported to activate the posterior temporal cortex in young infants (Grossmann et al., 2008), and gaze direction has been shown to be processed by anterior temporal cortices in adults (Calder et al., 2002; Calder et al., 2007). Thus, additional activation due to direct gaze may account for increased responses in the IDG-IDS compared to the ODG-IDS condition. If this explanation holds, manipulating a different ostensive signal may result in a different activation pattern in the infant brain.

Alternatively, direct gaze could act to modulate the very response elicited by the experimenter's communicative actions. Observing intransitive manual gestures from a communicative agent activates both the posterior temporal (probably STS) and the inferior frontal (probably premotor) cortices (Lloyd-Fox, Wu, et al., 2013; Lloyd-Fox et al., 2009), and human vocalizations (including speech) are preferentially processed in various regions of

the anterior temporal lobe in infants (Grossmann, Oberecker, et al., 2010; Lloyd-Fox et al., 2012; Lloyd-Fox et al., 2014; Minagawa-Kawai et al., 2011). It is thus possible that the effect we found in this experiment was not directly due to the detection of direct gaze as an ostensive signal but to its impact on facilitating the processing of the accompanying communicative signals (speech and gestures). If this account is correct, we should find a similar pattern of activation when the presence of a different ostensive signal is manipulated while infants are exposed to speech and gesture stimuli.

### **3. Experiment 2**

To test whether different ostensive signals modulate the processing of communicative signals in the same way, we investigated whether the use of infant-directed (versus adult directed) speech and gestures would modulate brain activation the same way as direct gaze did in Experiment 1. In this study, an experimenter engaged in face-to-face communication with one infant and kept eye contact with her in both conditions.

#### **3.1 Methods**

##### **3.1.1 Participants**

Twenty-four full term, healthy 6-month-old infants (12 female, age range = 165-194 days, M age = 178.42 days) participated in the study. A further 27 infants participated, but were excluded because they failed to attend to the first four trials (n=7), were showing signs of distress (n= 5), their parent made eye contact, smiled and distracted them (n=2), signal quality problems: grabbing the headband or pushing against parent (n=4), low signal to noise ratio (n=4), or experimental error (n=5).

All parents gave written, informed consent prior to participation. The study design was approved by the United Ethical Review Committee for Research in Psychology (EPKEB), Budapest, Hungary and was conducted according to the Declaration of Helsinki.

##### **3.1.2 Procedure**

In Experiment 2, one infant participated per study. The infant sat on their parent's lap approximately 90 cm from a female experimenter. During the trials, the experimenter told the

infant Hungarian nursery rhymes (the same ones that had been used in Experiment 1) for approximately 15 seconds. During trials in the IDG-IDS condition, the experimenter acted the same way as in Experiment 1, using infant-directed intonation and gestures. During trials in the IDG-ADS condition, she gestured in an adult-directed way, without exaggerations, and used flat, adult-directed intonation. Conditions were alternated in an AABB format (Figure 1 & 2). Ten of the 24 infants were presented with IDG-IDS trials first, and 14 of them started with IDG-ADS trials. The baseline periods in between the trials were the same as in Experiment 1. A bell sound indicated the start and the end of each trial for the experimenter, and a second experimenter, who was hidden from view, placed event markers manually in the NIRS recording at the same time. Parents were asked to refrain from interacting with their infant unless the infant sought it. Each session was video recorded for later off-line behavioural coding and followed the same procedure as Experiment 1. Given the results of Experiment 1, we collected data for 4 trials only (2 trials per condition).

### ***3.1.3 Data recording and processing***

fNIRS measurements were recorded with the same UCL-NIRS topography system and headgear designed by CBCD. As only one infant took part per session in Experiment 2, we have more channels available for recording, and infants wore a custom-made headgear that covered the temporal areas above both hemispheres. The headgear consisted of a fixed length headband, with the three arrays (frontal, left lateral and right lateral) attached within this headband in fixed positions, as used in Experiment 1 (see Figure 3). Before the infants began the study, head measurements were taken to align the headgear with 10-20 coordinates. Measurements from this group of infants showed that the average head circumference was 43.0 cm (s.d = 1.1 cm).

Data processing and analyses of the fNIRS data followed the same procedure as in Experiment 1.

## **3.2 Results and Discussion**

In an initial analysis we assessed the differences in the haemodynamic response during the experimental conditions versus baseline (see Table 2). We found 18 epochs with significant

activations, half of them over the left and half over the right hemisphere. Adult-directed (IDG-ADS) communication resulted in reliable responses at 3 epochs only, all of them over the left hemisphere. Comparing these results to those of Experiment 1 (Table 1), it is clear that we replicated some of the previous results. We found significant activation to IDG-IDS communication in both experiments over channels 4, 6, 7 and 8 over the right hemisphere, and all of these activations emerged at least 10 s after the start of the trials.

To assess the responses to the infant-directed (IDG-IDS) condition relative to the adult-directed (IDG-ADS) condition, paired t-tests were conducted within the epochs identified in our initial analysis (see Figure 5 & 6). In the right hemisphere (which covered the same area as in Experiment 1), this analysis revealed significantly greater haemodynamic increases in HbO<sub>2</sub> to the IDG-IDS condition relative to the IDG-ADS condition in channels 4, 7 and 8 (Channel 4 – 20-25 s window:  $t(23) = 2.08, p = .049$ ; Channel 7 – 10-15 s window:  $t(23) = 2.40, p = .025$ ; 15-20 s window:  $t(23) = 2.95, p = .007$ ; and 20-25 s window:  $t(23) = 3.50, p = .002$ ; Channel 8 – 10-15 s window:  $t(23) = 2.54, p = .018$ ; 15-20 s window:  $t(23) = 2.29, p = .032$ ; and 20-25 s window:  $t(23) = 2.13, p = .044$ ). In the left hemisphere, this analysis revealed significantly greater haemodynamic increases in HbO<sub>2</sub> to the IDG-IDS condition relative to the IDG-ADS condition in channels 6 and 8 (Channel 6 – at 20-25 s window:  $t(23) = 2.69, p = .013$ ; Channel 8 – at 20-25 s window:  $t(23) = 2.25, p = .034$ ). For the analysis of the HHb signal, there was also a significantly greater haemodynamic decrease in HHb in the left hemisphere to the IDG-IDS condition relative to the IDG-ADS in channels 1 and 16 (Channel 1 – 20-25 s window:  $t(23) = 2.35, p = .028$ ; Channel 16 – 20-25 s window:  $t(23) = 2.60, p = .016$ ). No channels revealed a significantly greater response to the IDG-ADS condition relative to the IDG-IDS condition in either hemisphere.

The latency of the activation response appears to be earlier in Channels 7 and 8 on the right than on Channel 4 on the right and Channels 6 and 8 on the left. However, just like in Experiment 1, the activation started earlier in the left channels (Channel 6 – 10-15 s window:  $p = .112$ , 15-20 s window:  $p = 0.055$ ; Channel 8 – 10-15 s window:  $p = .061$ ), but failed to reach the required level of significance until the 20-25 s window. Nevertheless, the late activation of Channel 4 on the right may not be a reliable effect as this channel showed no

signs of earlier activation. In addition, the late relative HHb decrease over the left hemisphere (Channels 1 and 16) may not reflect real neural activation as they are not accompanied by corresponding HbO<sub>2</sub> differences — a pattern that is inconsistent with a haemodynamic response reflecting functional activation (Obrig & Villringer, 2003).

In accord with the findings from Experiment 1 — and referencing the standardized scalp surface map of fNIRS channel coordinates for this age range (Lloyd-Fox et al., in press) — the channels which revealed significant differences between conditions in the left and right hemisphere are positioned over the inferior frontal gyrus (channels 1 and 4), on the border of the temporal and precentral cortices (channel 6), over the anterior superior temporal cortex (channel 7), and the temporo-parietal region (channels 8 and 16).

Just like in Experiment 1, visual inspection of our results (Figures 5 & 6) indicated a potential unpredicted ‘deactivation’ response in the first 10 s after the start of the trials. Had this response been predicted, it would have been statistically significantly different from baseline in many channels and at two channels on the left (Channels 1 and 2, both over the lateral frontal area) the decrease of HHb concentration would have been stronger for trials with adult-directed than for trials with infant-directed speech. Since the baseline period in this study was the same as in Experiment 1, we offer the same speculation: this unpredicted effect might have been due to the termination of the quiet observation of the experimenter between experimental trials. However it is difficult to offer strong theoretical reasoning for these findings, as the locations of these significant effects do not form a consistent pattern across the two Experiments.

Our statistical analysis resulted in findings that are remarkably similar to those of Experiment 1. Infant-directed communication resulted in higher activation than adult-directed communication over the same channels (4, 7, 8) in the right hemisphere as the ones that were more active during observing the communicator with direct than averted gaze. Some corresponding activations were also observed in the left hemisphere, both over the anterior temporal cortex (channel 6) and over the temporo-parietal region (channels 8 and 16). There was also no effect of order of presentation of condition, the pattern of activation for those

infants presented with infant directed speech first did not differ from those presented with adult directed speech first.

#### **4. General Discussion**

In the current study we addressed the question of whether ostensive signals would enhance processing of speech and gesture stimuli in infants of six months of age in an ecologically valid setting. We approached this question by presenting infants with differing combinations of ostensive signals during live communicative interactions with an adult experimenter. In Experiment 1 infants were exposed to interactions, which employed infant directed speech and gestures, either directed towards themselves (making eye contact with the experimenter) or another infant (no eye contact). In Experiment 2 infants were exposed to interactions that involved direct eye contact from the experimenter, but employed either infant-directed or adult-directed speech and gestures. The results of both experiments indicated that the multimodal presentation of a combination of ostensive signals (infant-directed speech and infant-directed gaze) led to enhanced activation relative to the presentation of either ostensive signal alone. Furthermore, the responses in both Experiment 1 and 2 produced overlapping patterns of activation localized to a group of cortical areas — the inferior frontal, anterior temporal, and temporo-parietal regions — known to be involved in the processing of stimuli of communicative nature in infants (Grossmann, Oberecker, et al., 2010; Grossmann et al., 2008; Lloyd-Fox et al., 2011) and adults (Kampe et al., 2003; Lotze et al., 2006).

These findings allow us to draw several conclusions. Firstly, these responses cannot be attributed to unimodal stimulus-specific features, because a similar effect on cortical activation was seen across modalities (of visual and auditory ostensive signals) despite the physical parameters of the signals being more similar during presentation of their non-ostensive counterparts (i.e., visual change in gaze and auditory change in speech style). Also, the effects we identified corresponded only partly to the neural responses to ostensive signals reported earlier. For example, direct gaze (e.g., Grossman et al., 2007, 2008) and infant-directed speech (e.g., Saito et al., 2007) have been reported to activate orbito- or medial frontal areas in infants, but we failed to replicate these findings. Furthermore, in the rare cases where we found activations to single ostensive signals against baseline (Tables 1 & 2),

these activations did not overlap with the responses to the combined ostensive signals. This pattern of findings suggests that the localized cortical activations we found might not be related to the processing of the ostensive signal per se, but to the effect of the ostensive signals on enhancing speech and gesture processing. This interpretation is also supported by the fact that the sites of the activations we found correspond well to the brain regions that process vocal and gestural stimuli.

The second conclusion we can draw from our results is that they do not reflect a rigid obligatory response to the presence of ostensive signals, as the response was modulated by the presence of an additional ostensive signal. This suggests either that the presence of two ostensive signals had an additive effect on the response, or that the presence of conflicting signals (i.e., one signal indicating that the infant is being addressed, the other that she is not being addressed) had a reductive effect on the response.

It is difficult to elucidate which of these two hypotheses are more strongly supported by the current finding. The two experiments explored the effects of ostensive signals during highly ecologically valid situations of communicative interactions with infants. Experiment 1 represents a common situation for infants who have experienced scenarios where there is more than one infant present (i.e., at a nursery, family gatherings, child-friendly spaces such as a park). Experiment 2 represents a common situation in which an adult does not modulate her speech to provide an additional ostensive signal for infants during a face-to-face interaction (either because she is not used to talking to infants or because she may look at the infant while talking to someone else in an adult-directed manner). Therefore in ecologically valid scenarios infants may often hear speech or observe gestures that are not directed to them but nonetheless may be of interest according to the context in which it is portrayed. The presence of one ostensive signal could give an indication of communicative intent targeting the infant, and adding other ostensive signals could confirm this interpretation further, resulting in investing more effort into processing the accompanying speech and gestures. Thus, the two signals might have exerted additive effects on processing the multimodal stimuli.

However, some aspects of these results make such a conclusion unlikely. Especially, we rarely found activation in response to the partly ostensive stimuli (at least, this activation reached the level of statistical significance against baseline only at two epochs in Experiment 1 and only three epochs in Experiment 2), and when such activation was found, it occurred in channels that did not display further increasing activation in the presence of two ostensive signals (Table 1 & 2). This suggests that in the areas of the brain under investigation in the current study infants hardly processed the speech and gesture stimuli when the experimenter's gaze was not directed at them or when they were performed in an adult-directed manner. (It is possible that the presentation of contradictory communicative cues may have caused enhanced activation in brain areas in a different location to those interrogated by the fNIRS arrays used in the present study, and future work is needed to investigate this.) Furthermore, the inhibiting effect of the non-ostensive nature of potentially ostensive signals might have been partly due to the contrast with the fully ostensive interactions with which they alternated, though the fact that we did not find an effect of order of conditions on brain activations speaks against this explanation. Thus, we conclude that during extended naturalistic interactions 6-month-old infants can suppress the processing of communicative acts they see and hear, and do so even in the presence of an ostensive signal when some other cues indicates that the communicative acts may not be addressed to them.

A previous EEG/ERP study that investigated the effects of multimodal versus unimodal ostensive signals in five-month-olds did not find evidence of infants' ability to integrate ostensive signals (Parise & Csibra, 2013). Rather, they found that the presence of one ostensive signal elicited an equivalent response to multiple ostensive signals. However, the previous study differed from ours in several ways. Firstly, the stimuli were rapidly presented static images on a computer screen. Secondly, the dependent measures were fast, phasic responses to combinations of potentially ostensive signals (gaze direction upon opening the eyes, a single word in two different intonations). As a result of this design, the Parise and Csibra (2013) study probably identified initial responses to the ostensive signals rather than their effect on processing the whole interaction. The combined conclusion of the previous and the present study is therefore that the brain of young infants produces a quick obligatory response to the presence of any ostensive signal, but would invest enhanced processing of the

communicative acts of the source of these signals only if the nature of another potentially ostensive signal does not conflict with the interpretation that they are the ones who are being addressed by the communicator.

Since we found that combined ostensive signals facilitate the processing of the accompanying stimuli, one may raise the question of whether these signals, instead of being interpreted as communicative cues, simply enhance infants' attention. However, the very fact that the signals from the two sources interacted, rather than being additive in their effects, speaks against this explanation of the results. Interpreting a situation as ostensive should indicate to the infant the presence of information that is worthy of processing further (Csibra, 2010; Sperber & Wilson, 1995), and hence should enhance 'attention' to what is going on. However, if these signals had a direct (uninterpreted) effect on sensory processing, they would independently and additively generate 'attention' and produce corresponding cortical activation. This is not what we found. Thus, while we identified cortical activations that are not direct signatures of interpreting a situation as ostensive, but potential correlates of the products of such an interpretation, the pattern of results did demonstrate that infants took into account all available information to decide whether they were being addressed.

We wish to remain cautious about the precise underlying cortical mechanisms that produce the neural responses we reported here. Firstly, in contrast to adult research that suggests that activation in the pre-frontal cortex (PFC) is modulated by the degree of communicative intention towards the viewer (Enrici, Adenzato, Cappa, Bara, & Tettamanti, 2011; Walter et al., 2004; Enrici et al., 2011) — and previous work looking at ostensive signals in infants in gaze (Grossmann et al., 2008, 2007) and infant directed speech (Saito et al., 2007) — we did not find evidence of modulation of the PFC in response to these cues. However, the infant fNIRS studies (i) used arrays with a different layout to the current study and so may have covered different regions of the PFC, and (ii) used less naturalistic and more highly controlled disembodied stimuli. Therefore future work should investigate responses over a wider area of the prefrontal cortex to assess whether such naturalistic communicative interactions as those used in the current study, would also lead to the same differential activation in infants of this age.

## **5. Conclusions**

We used fNIRS to investigate infant's sensitivity to ostensive signals for speech and gesture processing during naturalistic communicative interactions. The results of both experiments indicated that only the multimodal presentation of combination of ostensive signals (infant-directed gaze and infant-directed speech) led to enhanced activation relative to baseline or to the presentation of either ostensive signal alone. This effect was indicated by responses localized in regions known to be involved in processing auditory and visual aspects of social communication. Thus, 6-month-old infants take into account all available information for figuring out whether they are being addressed by a communicative source, and invest more effort into the processing of vocal and gestural communicative acts when nothing contradicts this interpretation of the situation. In addition, this study demonstrated the potential of fNIRS as a tool for (i) studying infants in ecologically valid naturalistic scenarios, and (ii) the simultaneous measurement of brain function in multiple participants.

## Figure Legends

Figure 1: Experimental protocol: Experiment 1 (left panel) and Experiment 2 (right panel)

Figure 2: Infants' participating in the study and examples of gestures performed by the Experimenter for Experiment 1 (left panel) and Experiment 2 (right panel)

Figure 3: fNIRS channel layout for Experiment 1 (left panel) and 2 (right panel). The channels are highlighted in grey circles, and the 10-20 coordinates are superimposed on the diagram in red.

Figure 4: The results of Experiment 1. The centre panel shows the location of the fNIRS channels with significant increases in HbO<sub>2</sub> (red) and HHb (blue; white for marginally significant channel) for the IDG-IDS condition versus the ODG-IDS condition. The curves depict the time courses of the grand averaged haemodynamic responses in the same channels for each condition (greyed area indicates the interval where the difference in response was significant). Error bars indicate standard errors of averaged signals in corresponding 5-s epochs.

Figure 5: The results of Experiment 2 (left hemisphere): The centre panel shows the location of the fNIRS channels with significant increases in HbO<sub>2</sub> (red) and HHb (blue) for the IDG-IDS condition versus the IDG-ADS condition. The curves depict the time courses of the grand averaged haemodynamic responses in the same channels for each condition (greyed area indicates the interval where the difference in response was significant). Error bars indicate standard errors of averaged signals in corresponding 5-s epochs.

Figure 6: The results of Experiment 2 (right hemisphere): The top left panel shows the location of the fNIRS channels with significant increases in HbO<sub>2</sub> for the IDG-IDS condition versus the IDG-ADS condition. The curves depict the time courses of the grand averaged haemodynamic responses in the same channels for each condition (greyed area indicates

where the difference in response was significant). Error bars indicate standard errors of averaged signals in corresponding 5-s epochs.

**Table 1**

Significant activations from baseline in the IDG-IDS and ODG-IDS conditions in Experiment 1.

	Channel	Chromophore	Time Window (s)	<i>t</i> (23)	<i>p</i>
Right Hemisphere					
IDG-IDS > Baseline					
	4	HHb	10-15	2.25	.034
	4	HHb	15-20	2.18	.040
	4	HHb	20-25	2.60	.016
	6	HbO <sub>2</sub>	20-25	2.93	.008
	7	HbO <sub>2</sub>	10-15	3.64	.001
	7	HbO <sub>2</sub>	15-20	3.22	.004
	8	HHb	10-15	2.03	.058 <sub>a</sub>
ODG-IDS > Baseline					
	12	HbO <sub>2</sub>	15-20	2.49	.021
	17	HbO <sub>2</sub>	15-20	2.59	.017

<sub>a</sub> Note that this response is a trend to significance.

Table 2

Significant activations from baseline in the IDG-IDS and IDG-ADS conditions in Experiment 2.

	Channel	Chromophore	Time Window (s)	<i>t</i> (23)	<i>p</i>
Right Hemisphere					
IDG-IDS > Baseline					
	4	HbO <sub>2</sub>	20-25	2.59	0.016
	6	HbO <sub>2</sub>	20-25	2.65	0.015
	7	HbO <sub>2</sub>	10-15	3.72	0.001
	7	HbO <sub>2</sub>	15-20	3.94	<.001
	7	HbO <sub>2</sub>	20-25	4.13	<.001
	8	HbO <sub>2</sub>	10-15	2.17	0.040
	8	HbO <sub>2</sub>	15-20	2.16	0.042
	8	HbO <sub>2</sub>	20-25	3.10	0.005
	12	HbO <sub>2</sub>	20-25	2.33	0.029
Left Hemisphere					
IDG-IDS > Baseline					
	1	HHb	20-25	2.38	0.026
	6	HbO <sub>2</sub>	20-25	4.15	<.001
	8	HbO <sub>2</sub>	15-20	2.73	0.012
	8	HbO <sub>2</sub>	20-25	3.91	<.001
	8	HHb	20-25	3.69	0.001
	16	HHb	20-25	2.78	0.012
IDG-ADS > Baseline					
	7	HbO <sub>2</sub>	15-20	2.47	0.022
	12	HbO <sub>2</sub>	15-20	2.09	0.048
	12	HbO <sub>2</sub>	20-25	2.26	0.034

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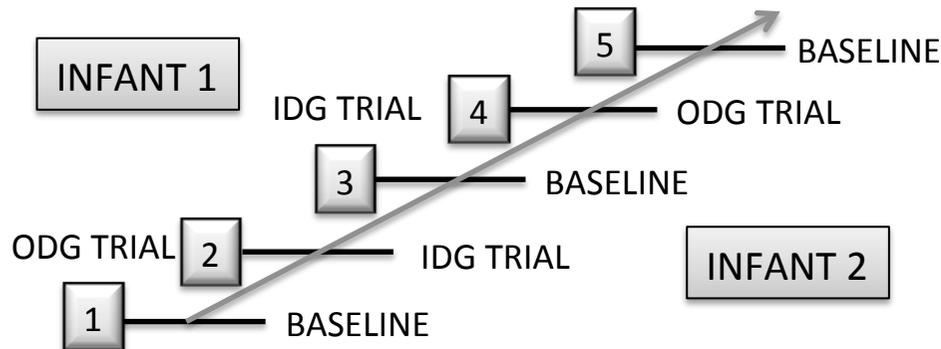
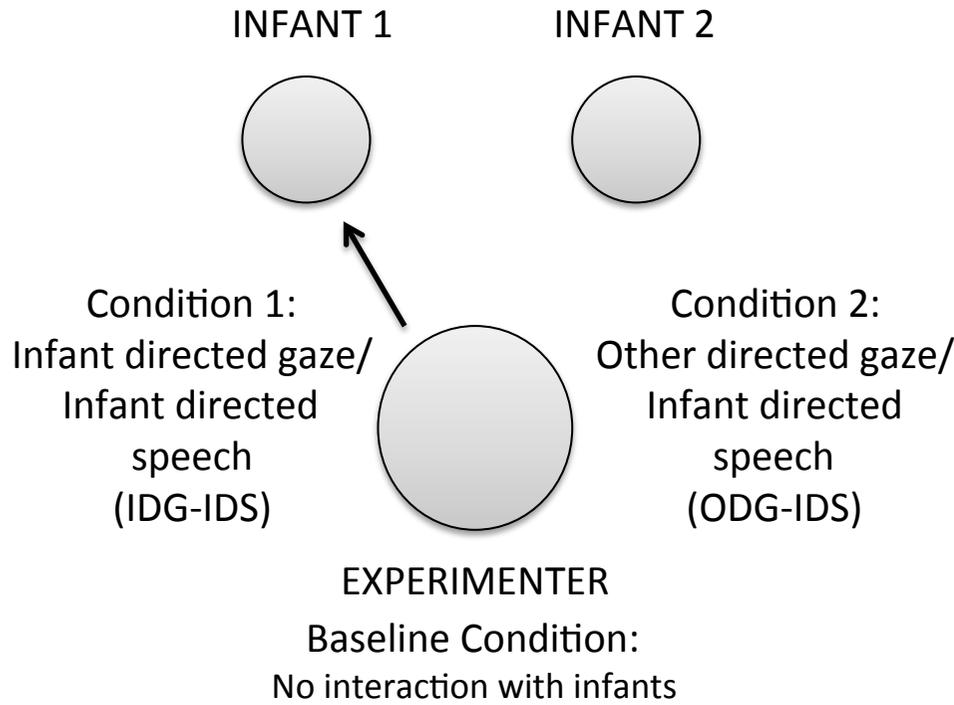
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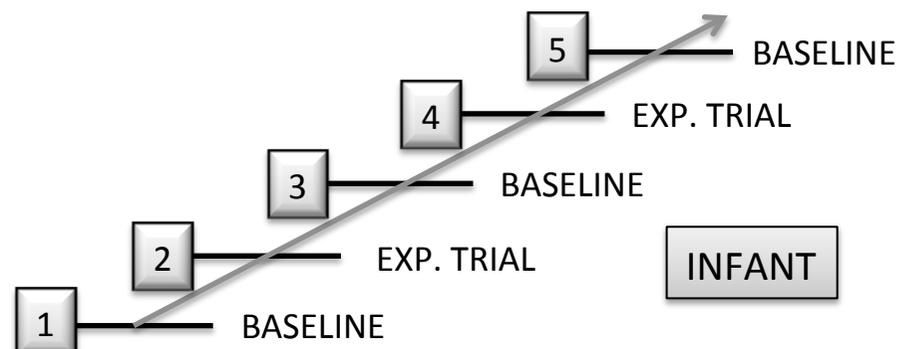
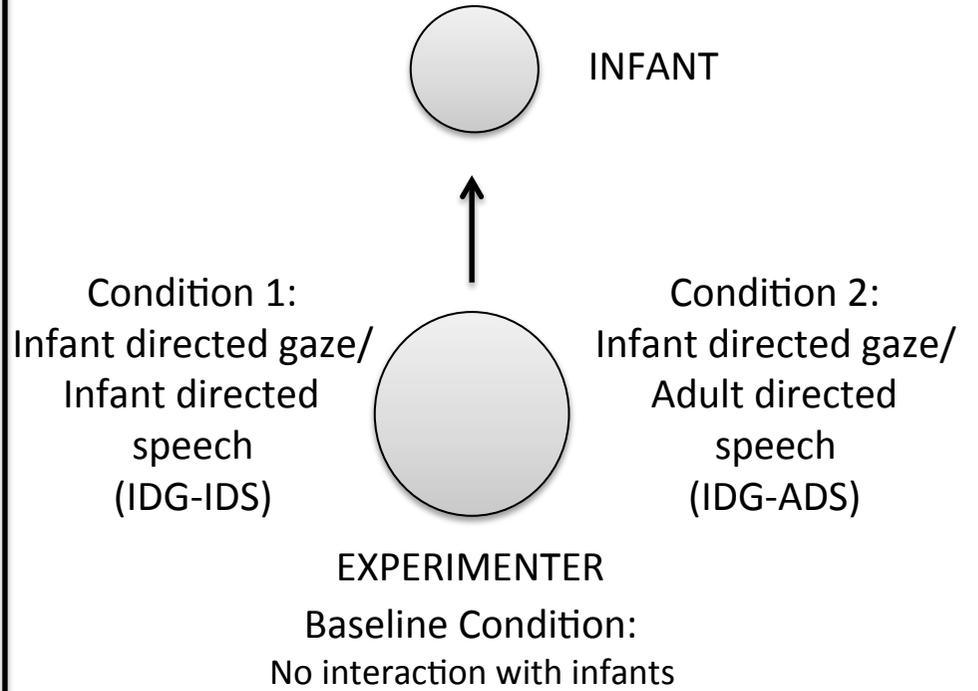
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### EXPERIMENT 1



### EXPERIMENT 2



EXP. TRIAL ORDER:

IDS, IDS, ADS, ADS or ADS, ADS, IDS, IDS

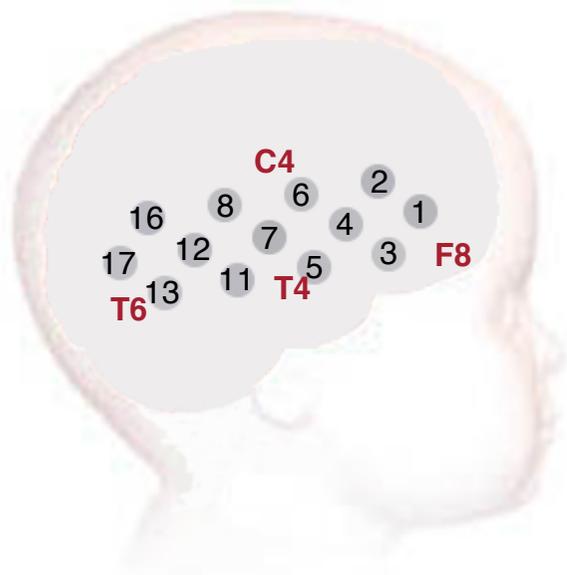
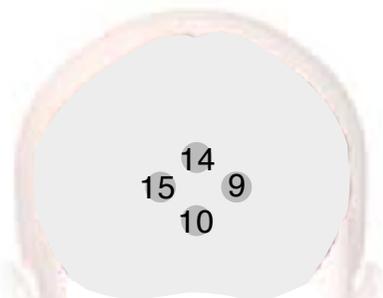
## EXPERIMENT 1



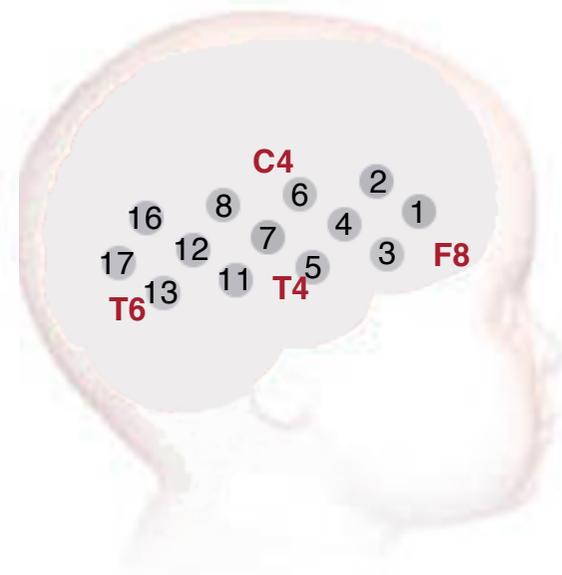
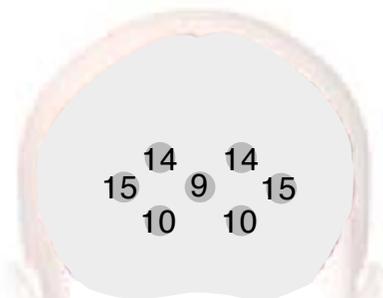
## EXPERIMENT 2



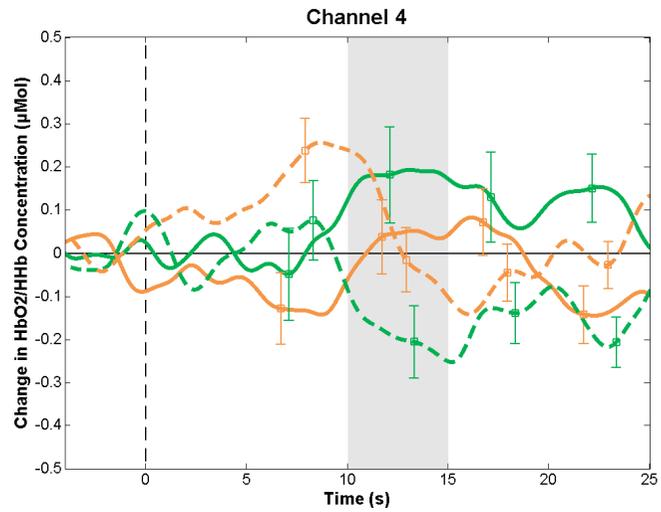
## Experiment 1



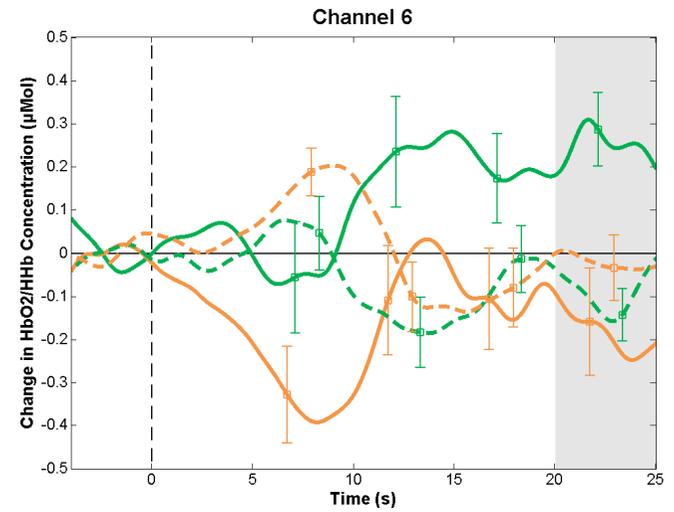
## Experiment 2



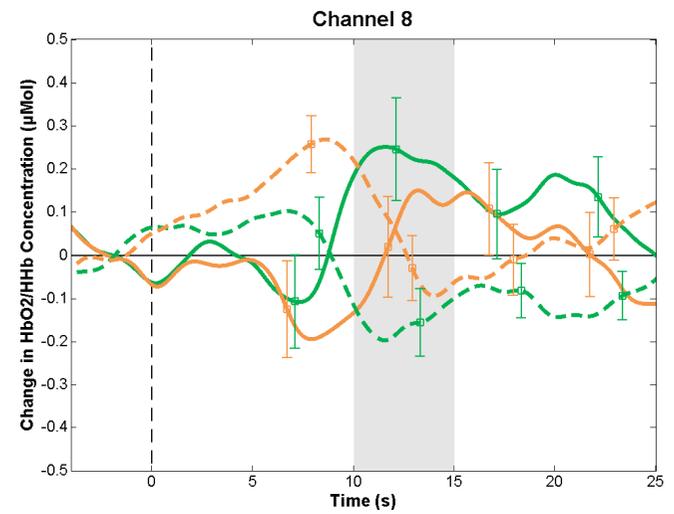
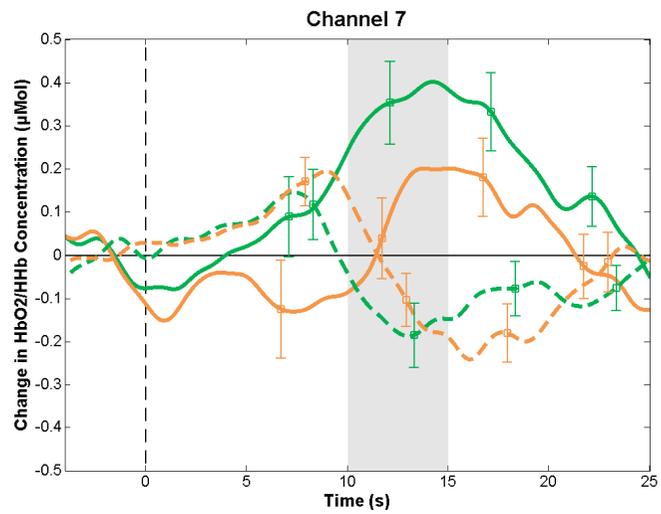
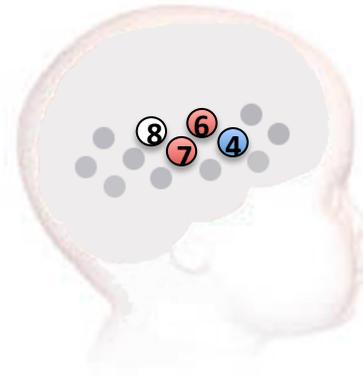
# Experiment 1: Right hemisphere



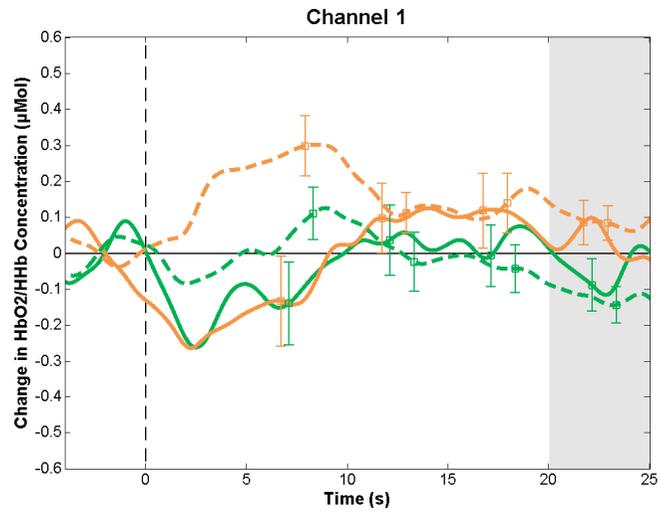
— IDG-IDS (HbO<sub>2</sub>)  
— ODG-IDS (HbO<sub>2</sub>)



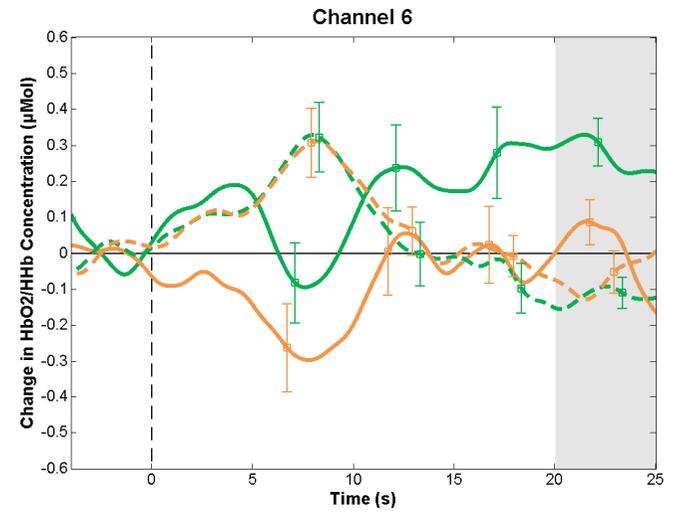
- - IDG-IDS (HHb)  
- - ODG-IDS (HHb)



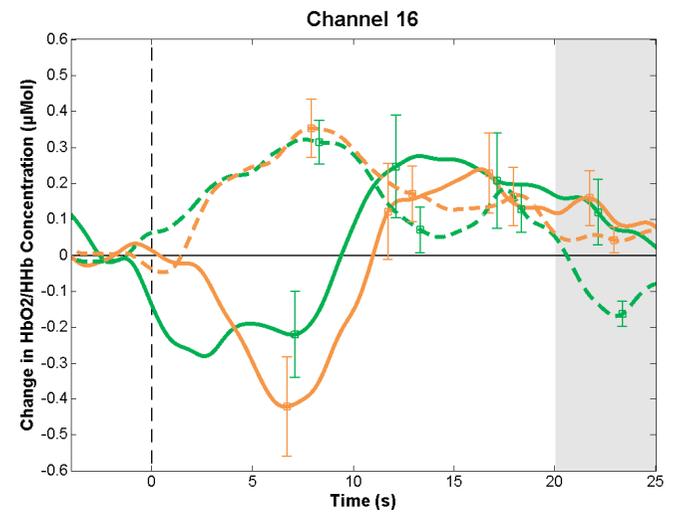
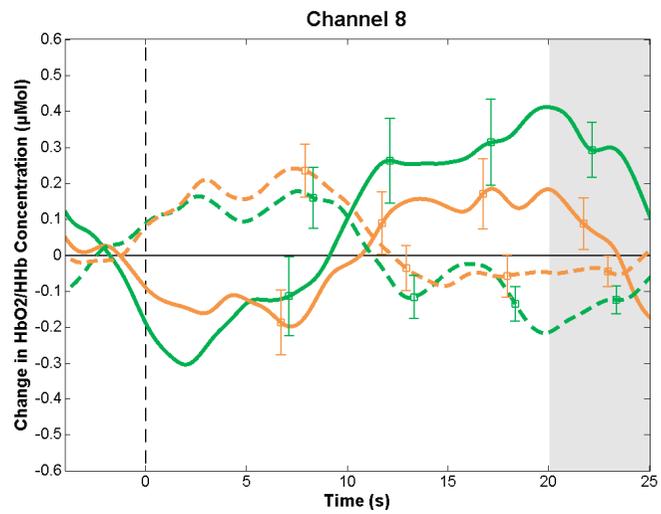
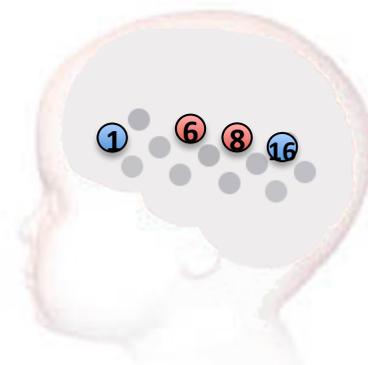
## Experiment 2: Left hemisphere



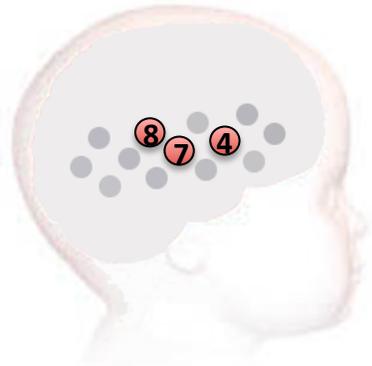
— IDG-IDS (HbO<sub>2</sub>)  
— IDG-ADS (HbO<sub>2</sub>)



— IDG-IDS (HHb)  
— IDG-ADS (HHb)



## Experiment 2: Right hemisphere



— IDG-IDS (HbO<sub>2</sub>)      - - - IDG-IDS (HHb)  
— IDG-ADS (HbO<sub>2</sub>)      - - - IDG-ADS (HHb)

