

## Assessing infants' cortical response to speech using near-infrared spectroscopy

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Sensitivity to spoken language is an integral part of infants' formative development, yet relatively little is known about the neural mechanisms that underlie the emerging ability to perceive and process speech. This is in large part because there are a limited number of non-invasive techniques available to measure brain functioning in human infants. Near-infrared spectroscopy (NIRS), an optical imaging technique that estimates changes in neuronal activity by measuring changes in total hemoglobin concentration and oxygenation, may be a viable procedure for assessing the relation between speech processing and brain function in human infants. While auditory processing data have been gathered from newborn and preterm infants using NIRS, such data have not been collected from older infants. Many behavioral measures used to establish linguistic sensitivity in this population are accompanied by visual stimuli; however, it is unclear how coupling of auditory and visual stimuli influences neural processing. Here we studied cortical activity in infants aged 6–9 months, as measured by NIRS, during exposure to linguistic stimuli paired with visual stimuli and compared this to the activity observed in the same regions during exposure to visual stimuli alone. Results dissociate infants' hemodynamic responses to multimodal and unimodal stimuli, demonstrating the utility of NIRS for studying perceptual development in infants. In particular, these findings support the use of NIRS to study the neurobiology of language development in older infants, a task that is difficult to accomplish without the use of attention-getting visual stimuli.

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Infants' ability to perceive speech begins in the womb and progresses dramatically during the first year of life (DeCasper and Fifer, 1980; Werker and Tees, 1984). Research indicates that, during the last trimester in-utero (Mehler et al., 1988) and in the first 12 months of life post-natally (Jusczyk, 1997), infants become aware of and adjust to regularities in their native language. By using various cues found in adult speech, infants gradually come to understand and use their native language. For example, between 6

and 9 months of age, infants become sensitive to patterns within their native language, such as prosodic cues (Cutler, 1990; Morgan, 1996), phonotactic cues (Hohne and Jusczyk, 1994; Jusczyk et al., 1994), and allophonic variation (Hohne and Jusczyk, 1994). Investigating and understanding the mechanisms of the trajectory of such advances are difficult tasks, and researchers have advanced the study of infant speech perception largely based on clever behavioral paradigms (e.g., Eimas et al., 1971; Trehub, 1973; Jusczyk et al., 1993; Saffran et al., 1996).

The behavioral methods used to understand language development across infants' first year are typically chosen based on the specific age group being studied and the particular question being asked. For example, with infants 4 months of age and younger, a useful method employs a pacifier to record changes in infant sucking patterns typically linked to discrimination of stimuli (Siqueland and DeLucia, 1969). Subsequent variations of this method focus on contingency sucking, where presentation of stimuli is dependent on the infant achieving a high, predetermined level of pacifier manipulation (Gottlieb and Krasnegor, 1985). Behavioral paradigms used with infants 4 months and older generally track head or eye movements and record duration of orientation time (Jusczyk, 1997). Like the pacifier method used for younger infants, such methods use a contingency dynamic where the infant receives additional stimuli in response to sustained looking time in a particular direction. Some paradigms use auditory stimuli alone, measuring duration of head-turns oriented towards the source of the stimuli (e.g., Kemler-Nelson et al., 1995), while others use both linguistic and visual stimuli, measuring duration of looking towards consistent and inconsistent pairings (e.g., Stager and Werker, 1997). Visual stimuli can also be used to maintain infants' attention, either between or during presentation of linguistic stimuli (e.g., Cooper et al., 1997). Results obtained from such behavioral methods are effective in that they yield consistent response patterns that researchers have used to suggest models of infant development, and results based on these methods have revealed remarkable cognitive advances within infants' first 12 months.

Recent investigations have increasingly used methods that assess neurological and hemodynamic parameters of infant

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perception and cognition (de Haan and Johnson, 2003). These methods show promise for providing insight on the neurobiological basis for infants' responses to stimuli. Direct measures of neural activity, such as event-related potentials, have provided information about both the timing and localization of neural responses to perceptual input. Hemodynamic indicators of neural activity offer exciting new and useful means for investigating infant development (e.g., Dehaene-Lambertz et al., 2002). While these neuroimaging techniques are allowing researchers to begin asking different questions about infants' development, they also have limitations. Methods such as positron emission topography (PET) and functional magnetic resonance imaging (fMRI) are generally considered impractical or unsafe for use with healthy infants (but see Dehaene-Lambertz et al., 2002). PET is more appropriate for clinical applications because it requires the injection of radioactive materials that are unsafe for repeated use. A technology for estimating hemodynamic changes that to some degree operates outside these limitations is near-infrared spectroscopy (NIRS). NIRS has several advantages over other hemodynamic-based techniques, in particular when investigations call for testing awake, behaving infants, as with language development. Although infants' movement must be somewhat reduced to control motion artifacts, it is a problem that is manageable with proper design and use of the equipment, as well as with good experimental design.

In NIRS, near-infrared light is projected through the scalp and skull into the brain, and the intensity of light diffusely reflected is recorded. The modulation of the recorded intensity by localized changes in the optical properties of the brain is used as an estimate of neural activation. The NIRS technique used in the present research utilizes changes in blood volume and hemoglobin oxygenation (i.e., hemodynamics) as an index of neural activation (see Meek, 2002; Grinvald et al., 1991; Strangman et al., 2002a; Villringer and Chance, 1997; Villringer and Dirnagl, 1995). This technique has been used with medically at-risk infants in the clinical setting (Chen et al., 2000; Hintz et al., 2001; Meek et al., 1998, 1999a, 1999b; Sakatani et al., 1999; Soul and du Plessis, 1999; Zaramella et al., 2001) and, more recently, its applicability in the experimental setting has been explored (Baird et al., 2002; Peña et al., 2003; Taga et al., 2003; Wilcox et al., 2005). The rationale for this approach rests on the concept that neural activation in response to a stimulus results in increased energy demands in the area activated. In response to the demand for energy, cerebral blood flow (CBF) increases to the activated brain areas, bringing both oxygen and glucose. Changes in blood flow lead to an increase in blood volume and can be assessed by measuring local concentrations of oxyhemoglobin (oxygenated blood) and deoxyhemoglobin (deoxygenated blood).

Typically, during cortical activation, local concentrations of oxyhemoglobin ( $\text{HbO}_2$ ) increase, whereas concentrations of deoxyhemoglobin (HbR) decrease (Bartocci et al., 2000; Hoshi and Tamura, 1993; Jaszewski et al., 2003; Obrig et al., 1996; Strangman et al., 2003). From the summated changes in HbR and  $\text{HbO}_2$ , total hemoglobin (HbT) can be computed and is found to increase following brain activation. To capitalize on these changes, the low tissue absorption of near-infrared light between approximately 650 and 950 nm is utilized. At these wavelengths, light is differentially absorbed by oxygenated and deoxygenated blood (Gratton et al., 2000; Villringer and Chance, 1997). Measuring the light intensity modulation during stimulus presentation and comparing it to the light intensity during a baseline event in which no stimulus is presented provides important information

about the hemodynamic response to brain activation (i.e., relative concentrations of  $\text{HbO}_2$  and HbR). Evidence that there is a relationship between hemodynamics and neural activity (Gratton et al., 2001) and that NIRS produces results consistent with other imaging techniques (fMRI and PET) used simultaneously (Kleinschmidt et al., 1996; Strangman et al., 2002b; Villringer et al., 1997) provides converging evidence that NIRS can provide a reliable estimate of brain activity. Occasionally, researchers find an increase, rather than a decrease, in HbR (Bartocci et al., 2001; Chen et al., 2002; Hoshi and Tamura, 1993; Jaszewski et al., 2003; Kato et al., 1993; Sakatani et al., 1999; Strangman et al., 2003) in targeted cortical areas. Although it is not entirely clear why an increase in HbR is sometimes observed, in infants, it is assumed to be a less mature hemodynamic response.

The application of methods for tracking hemodynamic correlates of brain activity, such as NIRS, is allowing further examination of the neural basis of perceptual and cognitive development. For example, recent experiments using NIRS (Peña et al., 2003) and fMRI (Dehaene-Lambertz et al., 2002) have indicated that the underlying neurological substrate necessary for language processing may already be present in very young infants. Results from both studies demonstrated significantly more activation in the left temporal cortex in response to forward speech, when compared to a corresponding location in the right hemisphere. Dehaene-Lambertz et al. (2002) tested 3-month-old infants using fMRI and observed increased activation in the left hemisphere relative to the right in response to forward playing speech relative to reversed speech. Similarly, Peña et al. (2003) tested neonates for hemispheric dominance using forward speech, reversed speech, and silence. Using a 24-channel NIRS imaging system, these researchers found that 2- to 5-day-old infants exhibited left hemisphere dominance for processing forward, infant-directed speech and not for reversed speech. These two studies highlight the left temporal region as a primary area of interest for understanding the neurobiology of language development. However, since the second half of the first year is the period of time during which infants begin to display a wealth of important perceptual abilities with regard to their native language, it is important to extend this research to older infants who are actively engaged with and attending to the experimental stimuli in question. While researchers appreciate that this subsequent stage of language development is important to document both behaviorally and neurophysiologically, research in the latter domain has been limited by the available technology. In light of its flexibility, NIRS provides an ideal mechanism by which to measure hemodynamic responses to linguistic stimuli in awake, behaving older infants.

The present study aimed to extend to an older age group the small body of findings that exist on the hemodynamic underpinnings of language processing in very young infants. Neural activation, as estimated based on relative changes in cerebral blood flow, was assessed in infants during exposure to speech coupled with visual stimuli (audiovisual condition) and compared to measurements taken during exposure to visual stimuli alone (visual only condition). Regions of interest were both the primary visual cortex and the left temporal cortex. We expected to observe a dissociation between cortical activity in the temporal region during the audiovisual condition from that observed in that region during the visual only condition. Conversely, we expected to observe comparable levels of neural activity in the primary visual cortex during exposure to both the audiovisual and visual only conditions.

## Methods

### Participants

Participants were 35 infants between the ages of 6 and 9 months, 20 male and 15 female ( $M$  age=7 months, 14 days, range=6 months, 3 days to 9 months, 0 days). Five additional infants were tested but eliminated from the sample because of large artifacts in the optical signal throughout the experimental run due to motion or hair obstruction ( $N=3$ ) and failure to obtain more than one useable block of trials ( $N=2$ ). Infants' names were obtained from birth announcements in the local newspaper and commercially produced lists. Parents were contacted by letter and follow-up phone calls. Infants and parents were offered a new toy as compensation for their participation.

Informed consent was obtained from the parents before testing began.

### Apparatus

During the experiment, each infant sat on a parent's or caretaker's lap in a testing booth. Infants were positioned facing a 53-cm flat panel computer monitor (Macintosh G4) 76 cm away ( $28.1^\circ$  visual angle at infants' viewing distance based on a 36 cm wide screen). The monitor was positioned on a shelf, immediately under which audio speakers and a low-light video camera were positioned, oriented towards the infants. The monitor was framed by a facade that functioned to conceal the rest of the equipment. The facade was made of three sections. The upper third was a dark black curtain that covered the wall from side to side and dropped

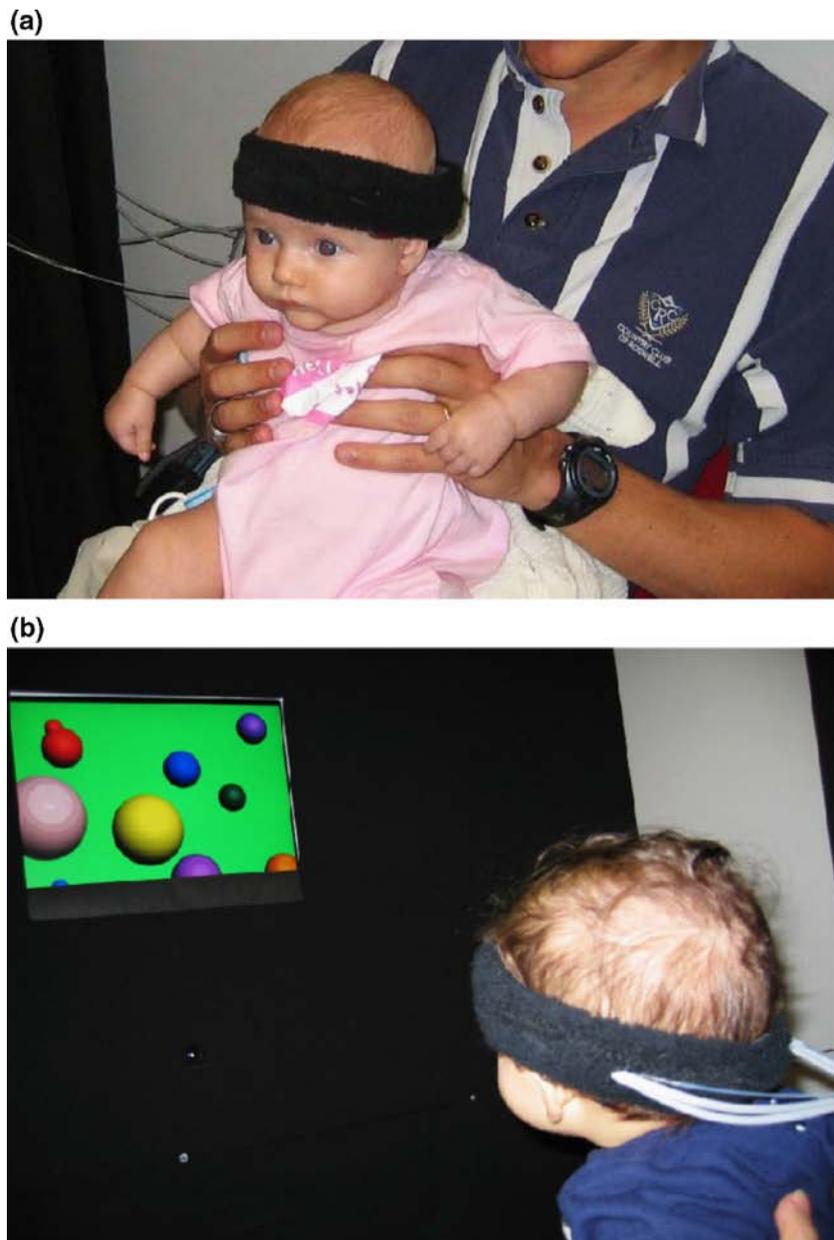


Fig. 1. Infant fitted with the NIRS probe (a); infant oriented towards visual stimuli in the testing booth (b).

down 84 cm from the ceiling. The middle section, measuring 152 cm (wall to wall horizontally)  $\times$  69 cm high, was constructed of plywood and covered with dark black cloth. The plywood had a rectangular hole cut out of its center that coincided with the size of the viewing surface of the computer monitor (48 cm diagonal). A dark curtain was hung from the bottom edge of this section to the floor. The testing area was separated by a curtain from the control area, where an experimenter operated the NIRS instrument out of infants' view. Fiber optic cables (15 m each) extended from the instrument to the testing booth and into a custom headband positioned on each infant's head during testing (Fig. 1). The cables were bundled into a single strand secured on the wall just over the parent's right shoulder.

The NIRS instrument consisted of three major components: (1) two fiber optic cables that delivered near-infrared light to the scalp of the infant (i.e., emitters); (2) four fiber optic cables that detected the diffusely reflected light at the scalp and transmitted it to the receiver (i.e., detectors); and (3) an electronic control box that served both as the source of the near-infrared light and the receiver of the refracted light. The signals received by the electronic control box were processed and relayed to a DELL Inspiron 7000™ laptop computer. A custom computer program recorded and analyzed the signal.

The imaging device used in this studies produced light at 680 and 830 nm wavelengths with two laser-emitting diodes (Boas et al., 2002, TechEn Inc.). Laser power emitted from the end of the fiber was 4 mW. Light was square wave modulated at audio frequencies of approximately 4 to 12 kHz. Each laser had a unique frequency so that synchronous detection could uniquely identify each laser source from the photodetector signal. Any ambient illumination that occurred during the experiment (e.g., from the visual stimuli) did not interfere with the laser signals because

environmental light sources modulate at a significantly different frequency. No detector saturation occurred during the experiment.

The light was delivered via fiber optic cables, each 1 mm in diameter and 15 m in length. These originated at the imaging device and terminated in the headband that was placed on the infant's head. The headband was made of elastic terry-cloth and was fitted with the two light-emitting and four light-detecting emitters and detectors. These were grouped into two emitter/detector sets, each containing two detectors placed at 2 cm distance on either side from the central emitter. One set delivered light to the temporal region at approximately position  $T_3$  according to the International 10–20 system, and the other set delivered light to the occipital region between positions  $O_1$  and  $O_2$  according to the International 10–20 system (see Fig. 2).

### Stimuli and design

The stimuli consisted of speech recordings and visual animations. Infants first saw a blank (dark) screen for 20 s before stimulus presentation began. Stimulus presentation proceeded as follows: 20 s of visual animation, 10 s of silence with a blank screen, 20 s of speech with visual animation, 10 s of silence with a blank screen. This sequence repeated five times over the course of the experiment. The initial visual trial and the baseline period preceding it served to familiarize the infant with the procedure and were not included in the analyses reported here. Likewise, the final audiovisual trial and baseline period were excluded. This design resulted in four 60-second blocks per infant, with each block consisting of an audiovisual trial (20 s), the subsequent baseline interval (10 s), a visual trial (20 s), and the subsequent baseline interval (10 s).

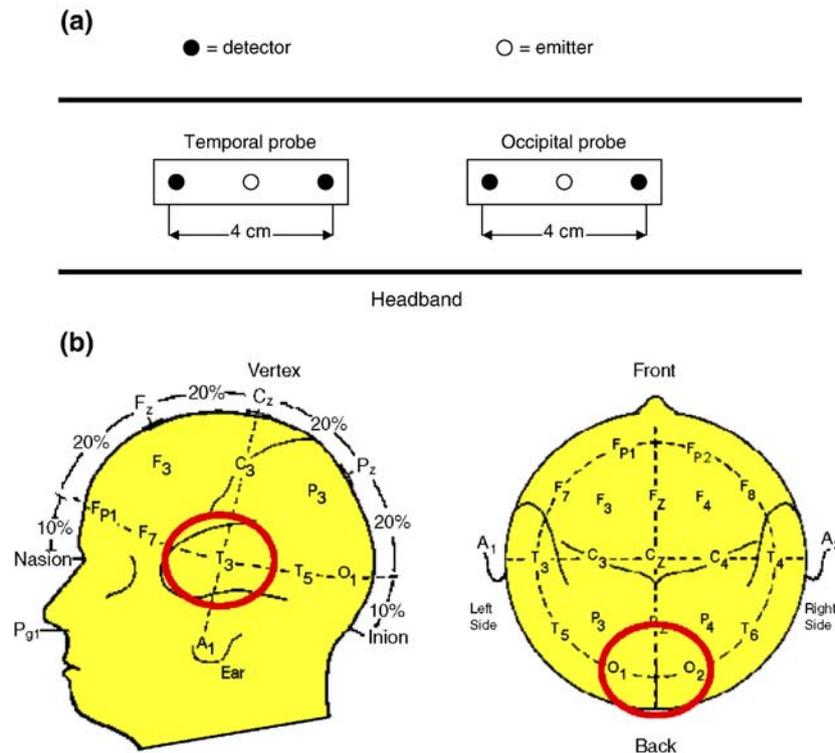


Fig. 2. Probe design for two regions of interest (a); localization of the probe over  $T_3$  (for left temporal measurements) and over  $O_1$  and  $O_2$  (for occipital measurements) was guided by International 10–20 EEG electrode position classification system (b).

The content of each speech segment was different, but all contained speech from the same speaker with the same general intonation variation and affective tone. The voice recordings were made by a female speaker relaying a children's story using highly animated, infant-directed speech. Recordings were made using a Sony digital camcorder and then converted to .wav sound files using Sound Forge 6.0™ audio editing software. The visual stimuli were distinct across trials, but each consisted of animations that were similar in color contrast and motion parameters. They consisted of simple 3-dimensional objects (e.g., spirals, circles, and rectangles) that rotated and moved slowly in front of a high-contrast colored background. The animations were produced using 3-D Studio Max™ computer graphics software. The auditory and animated digital files were combined using Adobe Premier 6.5™ video editing software, which produced .avi movie files that were then recorded onto a blank DVD. The recorded DVD was then played through the computer monitor and speakers. The hidden speakers were 82 cm from infants, facing them and producing audio stimuli at 75 dB when measured from the approximate location of the infant.

### Procedure

After the parent and infant were seated, a head circumference measurement was taken from the infant using a standard cloth tape measure. Parents were instructed to refrain from talking and interacting with their infants during the course of the experiment and to hold them up so that they were able to comfortably view the screen. Parents were also asked to guide infants' hands down and away from the headband if they began to reach up during the experiment. The experimenter then placed the NIRS headband on the infant. Following the 10–20 system, the occipital set was placed first on the nasion/inion center line of the scalp, about 1–2 cm above the inion, between O<sub>1</sub> and O<sub>2</sub> positions. The temporal set was then adjusted over the left temporal area, above and slightly anterior to the T<sub>3</sub> position. The experimenter moved to the control area. The room lights in both the experimental and control areas were turned off, leaving only a low intensity light and the computer monitor to sufficiently illuminate the experimental area, and light from the another computer monitor to light the control area. The emitter source lights of the imaging device were turned on, and optical recordings began. At this time, stimulus presentation began as well. Infants were video recorded for the duration of the experimental session for off-line coding of looking times.

## Results

### Looking time data

The length of time infants spent oriented towards the audiovisual and visual events was coded by two observers. Looking times were calculated for each 20-second trial, and a grand average was computed for visual and audiovisual conditions. Looking times during baseline periods were not calculated. The average looking time during the visual only condition was 17.8 s (SD=1.3). The average looking time during the audiovisual condition was 18.3 (SD=1.7). Interobserver agreement was measured for all infants and averaged 95% per test trial per infant. Because infants' failure to visually attend to the event could result in a decrease in hemodynamic response in the primary visual and/or temporal cortex, we rejected data blocks in which an infant (a)

cumulated less than 10 s total looking time per trial or (b) looked away from the display for more than 5 consecutive seconds. No infants who completed the experiment met these behavioral criteria, and therefore no trials were excluded for any of the infants based on looking times.

### Optical data

NIRS data collected from the two cortical regions of interest for each of the two stimulus conditions were analyzed the same way. The two detectors located over each region recorded the raw optical signals for subsequent digitization at 200 Hz for each of the four channels. The NIRS apparatus converted the signals to optical density units, which were digitally low-pass-filtered at 10.0 Hz for noise reduction, and decimated to 20 samples per second. The control computer then converted the data to relative concentrations of oxygenated (HbO<sub>2</sub>) and deoxygenated (HbR) hemoglobin using the modified Beer–Lambert law (Strangman et al., 2002a), which calculates the relationship between light absorbance and concentration of particles within a medium. HbO<sub>2</sub> and HbR were first plotted across the four 60-s time blocks composed of the following components: the entire 20 s audiovisual trial, the 10 s baseline period, the entire 20 s visual only trial, and the 10 s baseline period. Artifacts originating in infant physiology and movement were spatially filtered using a principal components analysis (PCA) of the signals across the four channels (Zhang et al., 2005; Wilcox et al., 2005). This approach is based on the assumption that systemic components of interference are spatially global and have higher energy than the signal changes evoked by the perceptual stimuli themselves (Zhang et al., 2005). Further filtering was conducted to eliminate “motion artifacts,” objectively defined as a signal change greater than 5% in a tenth of second. This second filtering step resulted in the removal of a total of 29 blocks (of 140 possible) for an average of 3.17 useable blocks per infant, with all infants providing at least two blocks. Optical signals were averaged across blocks for each infant and then grand averaged across infants. Concentration changes were then calculated to allow for statistical comparison of the average response to each stimulus condition (audiovisual and visual) in each cortical region (occipital and temporal).

### Hemodynamic response functions and analyses

Fig. 3 shows data collected from the left temporal and bilateral occipital regions of one infant across an entire experimental run. This figure illustrates optical density data across the experimental run and concentration conversions averaged across 60-s blocks within the run. On the left side of Fig. 3 are two horizontal illustrations showing the 4-block design flanked by the initial and final trials (not included in analyses) spanning 300 s (*x*-axis). Occipital and temporal regions each had one light emitter (emitting the two wavelengths of light) and two light detectors positioned over them, as indicated by the *y*-axis on the far left of the figures. The top left graph plots optical density changes in the infant's occipital region, while the bottom data plot optical density changes in the infant's temporal region. The red bars indicate each 20-s audiovisual trial, and the pink bars indicate each 20-s visual trial. No-stimulus baseline periods (10 s) follow each trial and are not marked.

The graphs on the right of Fig. 3 display the optical data once converted into relative concentrations of oxygenated and deoxygenated hemoglobin and averaged across each of the four blocks for

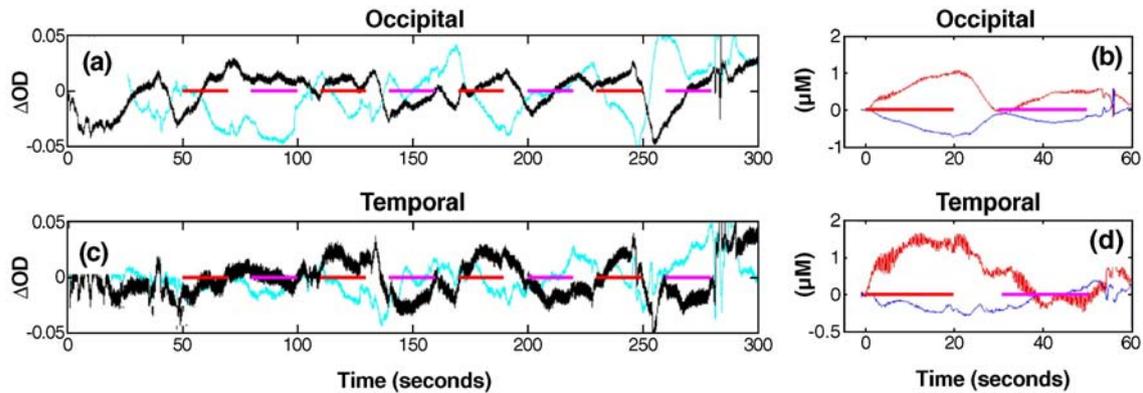


Fig. 3. An individual infant's data. The top left graph shows optical density changes from baseline in the occipital region over 300 s for 830 nm ( $\text{HbO}_2$ ) and 690 nm ( $\text{HbR}$ ). The bottom left graph shows optical density changes in the temporal region. Onset of each 20 s audiovisual trial is indicated by a red bar; onset of each 20 s visual trial is indicated by a pink bar. These alternate with 10 s no-stimulus baseline intervals (not marked). Graphs on the right reflect conversion of optical density to relative concentrations of oxygenated and deoxygenated hemoglobin, averaged across a maximum of four 60-s blocks (20 s audiovisual trial + 10 s baseline interval + 20 s visual trial + 10 s baseline interval). The top right graph shows the bilateral occipital response; the bottom right graph shows the left temporal response. The y-axes of both graphs indicate relative changes in concentration (micromolar) of  $\text{HbO}_2$  and  $\text{HbR}$ .

this infant. The y-axis of each graph on the right indicates relative concentration changes of  $\text{HbO}_2$  (in red, based on the 830 nm wavelength) and  $\text{HbR}$  (in blue, based on the 690 nm wavelength). The solid red bar indicates audiovisual trials, and the solid pink bar indicates visual trials. Both occipital and temporal regions show an increase in  $\text{HbO}_2$  during audiovisual trials, while only the occipital region shows an increase in  $\text{HbO}_2$  during the visual trials. Interestingly, the hemodynamic response in the temporal region during the audiovisual condition shows a delayed return to baseline, such that  $\text{HbO}_2$  remains elevated beyond the no-stimulus baseline, only recovering during the subsequent (visual only) condition. The basis for this late recovery will be addressed in the general discussion.

Since there was no indication that one channel was preferentially activated over another in either cortical region and because there remains some uncertainty about the relationship between the 10–20 coordinates and underlying brain coordinates, optical data were summed across the two channels per region. Fig. 4 illustrates the hemodynamic response function across the 60-s blocks for each region across infants. These functions are based on the average response across the two channels of data collected within each region for each infant. The overall hemodynamic response during the audiovisual segments (initial 20 s) is indicated by the solid red bar. This response was pronounced for both the occipital and the temporal regions, with a marked increase in  $\text{HbO}_2$ . The overall

hemodynamic response during the visual segments is indicated by the solid pink bar (onset at time 30 s), following the unmarked 10 s baseline. Notably, only the occipital region shows an increase in  $\text{HbO}_2$  during this condition, consistent with that seen in both regions during the audiovisual trials. The temporal region shows no such response during the visual only condition. It is worth noting that, as with the single infant data, while the average hemodynamic response in the occipital region completely returns to baseline prior to the onset of the subsequent (audiovisual) condition, the hemodynamic activity in the temporal region evoked during the audiovisual trials fails to completely recover during the baseline period and only does so during the subsequent visual trials. We will return to this issue in the general discussion.

Statistical analyses were performed on average changes in concentration of  $\text{HbO}_2$  within a cortical region, as this chromophore provided the most robust contrast-to-noise ratio across infants. Because changes in concentration began manifesting 2–3 s after stimulus onset and showed signs of abating near the stimulus offset, concentration change was calculated by measuring the average relative  $\text{HbO}_2$  concentration during the 10–20 second period for each stimulus trial and comparing it to the relative  $\text{HbO}_2$  concentration at time –2 to 0 s (baseline) prior to the trial. A 2 (cortical region)  $\times$  2 (stimulus condition) within-subjects ANOVA revealed significant changes in concentration of  $\text{HbO}_2$  by region,  $F(1,34)=6.49$ ,  $p<.05$ , and by stimulus condition,  $F(1,34)=6.74$ ,

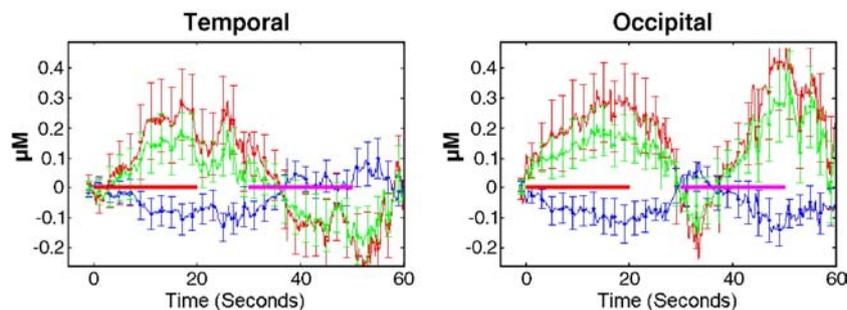


Fig. 4. Grand average hemodynamic ( $\text{HbO}_2$ ,  $\text{HbR}$ , and  $\text{HbT}$ ) response curves across 60-s blocks by cortical region. Audiovisual segments are indicated by the solid red bar (onset at time 0 s); visual segments are indicated by the solid pink bar (onset at time 30 s). Each trial was followed by a 10 s (no-stimulus) baseline interval. The y-axis indicates relative changes in concentration (micromolar) of the different chromophores.

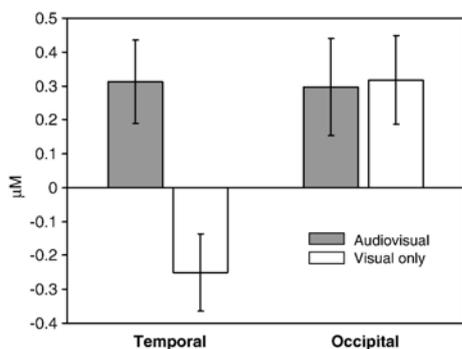


Fig. 5. Bar graph illustrates average change (and standard error) in HbO<sub>2</sub> concentration (micromolar) from time -2 to 0 s (baseline) to 10–20 s (second half of trial) for each stimulus condition and cortical region.

$p < .05$ . There was also a significant interaction between region of interest and stimulus condition,  $F(1,34)=27.71$ ,  $p < .01$ . Fig. 5 shows the average HbO<sub>2</sub> concentration change in the two regions (temporal and occipital cortex) given the two stimulus conditions (audiovisual and visual stimuli) relative to the no-stimulus baseline. Paired  $t$ -tests (two-tailed) were conducted to compare changes in HbO<sub>2</sub> concentration by region and by condition. A paired comparison revealed a significant difference in concentration change between the occipital and temporal regions during the visual only condition  $t(34)=4.49$ ,  $p < .001$ , as well as within the temporal region during the two different stimulus conditions  $t(34)=4.50$ ,  $p < .001$ . Together, these results demonstrate a dissociation of active regions in the infant brain given changes in processing modality.

## Discussion

Results from the present study support the feasibility of NIRS as a tool for tracking neural activity in awake, behaving infants. Results demonstrate robust and reliable patterns of activation in the left temporal and primary visual regions of the neocortex during exposure to sensory specific material. Significant increases in HbO<sub>2</sub> were observed in the left temporal region during exposure to continuous speech (e.g., in the audiovisual condition) relative to baseline periods (e.g., periods during which neither audio nor visual stimuli were presented), as well as relative to the hemodynamic changes observed during exposure to visual stimuli alone. The left temporal region is well established as a primary area for language processing in adults. The current investigation supports recent findings with very young infants (e.g., Dehaene-Lambertz et al., 2002; Peña et al., 2003) that exposure to linguistic stimuli results in increases in hemodynamic activity in the temporal region and that NIRS technology can be used to track such activity in this population. Our current findings extend the application of the technology to older infants. Furthermore, we have demonstrated a dissociation of sensory processing in two specific cortical regions, the temporal and occipital areas. By extending the application of NIRS to the processing of multiple modalities, we have demonstrated the utility of this technology for establishing the nature of the neurophysiological processes underlying a variety of perceptual abilities.

Data from the current study demonstrate local concentrations of oxyhemoglobin (HbO<sub>2</sub>) increasing in dedicated processing regions, a finding that is consistent with our previous work using this technology (e.g., Wilcox et al., 2005). This pattern of hemody-

amic activity is consistent with other research on the relationship between neural activity and cerebral hemodynamics (Bartocci et al., 2000; Hoshi and Tamura, 1993; Jaszewski et al., 2003; Obrig et al., 1996; Strangman et al., 2003). As was noted, however, researchers occasionally observe an increase, rather than a decrease, in HbR (e.g., Strangman et al., 2003). When seen in infants, such a result is generally attributed to their immature developmental status, and thus immature hemodynamic responses in particular cortical regions. Such a pattern emerges in the current data. Specifically, the hemodynamic response in infants' temporal region during exposure to audiovisual stimuli shows a late recovery. This manifests as HbO<sub>2</sub> returning to baseline only during the subsequent stimulus condition, while at the same time HbR appears to increase. The appropriate characterization of this pattern of responses remains unclear. The delayed return to baseline may reflect infants' reduced ability to switch focused attention from one modality to another, resulting in continued neural activity in the temporal cortex despite the cessation of the linguistic component of the stimuli. On the other hand, the apparent increase in HbR may be due to immature neurovascular coupling, whereby heightened demand for visual processing directs the supply of HbO<sub>2</sub> away from the temporal cortex and towards the visual cortex (the so-called plumbing problem). Such issues currently remain unresolved and highlight the importance of additional research to elucidate the nature of infant hemodynamics.

Another piece of information that emerges from the current findings is the importance of specific methodological manipulations to capture various stages of hemodynamic activity in the immature brain. Here we focused on two cortical regions, the left temporal and the primary visual areas. Incorporating two distinct stimulus conditions within a single experimental run allowed us to dissociate activity in these two cortical regions within the same group of infants. Given the problems inherent in making comparisons between groups of participants in this kind of research, this is an important methodological advancement. Furthermore, since visual stimuli are frequently used to maintain infants' attention in developmental research, understanding the interplay of different perceptual modalities, both attentionally and hemodynamically, is fundamental. While the current study is the first to dissociate hemodynamic activity in infants during exposure to both audiovisual and visual stimuli, future research will need to identify differences in patterns of activity given exposure to auditory stimuli with and without accompanying visual stimuli. Additional issues may also influence the hemodynamics of multimodal processing in infants, such as the perceptual coherence of the stimuli (e.g., Calvert, 2001).

The findings presented here raise important questions about the selection of cortical regions of interest in infants, given the specific perceptual questions being asked. In the current study, we aimed to establish the differential effects of visual stimuli with and without auditory stimuli on infant hemodynamics, and we were able to dissociate hemodynamic changes induced by exposure to linguistic stimuli from those induced by unrelated visual stimuli in the infant brain. This is an important first step in our examination of the neurobiology of perceptual development, in general, and language development, in particular. Further research with NIRS will be needed to examine other important issues, such as whether there is varying lateralization of auditory processing across the first year of life. Nonetheless, the current study demonstrates the wealth of information available through the application of this technology to the study of infant perception and cognition.

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