APPLYING NEAR-INFRARED SPECTROSCOPY (NIRS)

TO THE STUDY OF SPEECH PERCEPTION IN INFANTS

A Dissertation

by

ERIC MICHAEL WRUCK

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2005

Major Subject: Psychology

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Approved as to style and content by:

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ABSTRACT

Applying Near-Infrared Spectroscopy (NIRS)

to the Study of Speech Perception in Infants. (May 2005)

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Over recent decades, much has been learned about the perceptual capacity that enables infants to recognize and understand language. However, not until very recently have the neural mechanisms that are the substance of language learning been investigated. A recently developed optical imaging technique called near-infrared spectroscopy (NIRS) shows promise for being an acceptable alternative to invasive imaging techniques. NIRS measures correlates of neural activity by assessing hemoglobin concentration changes in the infant brain. The research presented here investigates neural activation in the left temporal and occipital cortex regions during exposure to speech and visual stimuli. As hypothesized, hemodynamic reaction was observed in both areas. Results indicate a significant activation in response to speech in the left temporal region, and an intriguing difference between uni- and bi-modally presented speech stimuli. These results have interesting implications for future multi-modal studies of infant speech perception.

TABLE OF CONTENTS

Page

LIST OF FIGURES

INTRODUCTION

Background

Behavioral methods for studying infant speech perception

Infants' ability to perceive speech begins in the womb, and progresses dramatically during the first year of life (DeCasper & Fifer, 1980; Werker & Tees, 1984). Research indicates that during the last trimester in-utero, and post-natally, in the first twelve months, infants become aware of and adjust to regularities in their native language (Jusczyk, 1997; Jusczyk, Cutler, & Redanz, 1993; and Saffran, Aslin, & Newport, 1996). Between 6 and 8 months of age, infants become sensitive to patterns within their native language, such as prosodic cues (patterns of intonation) (Cutler, 1990; Morgan, 1996), phonotactic cues (likelihood of phoneme pairing) (Jusczyk, Luce & Charles-Luce, 1994), and allophonic variation (phoneme pronunciation as a function of within-word location) (Hohne and Jusczyk, 1994). By using various cues found in adult speech, infants gradually come to understand and use their native language. Part of this difficult task is accomplished within the first six months as seen in infants' apparent recognition of familiar terms such as their own name, 'mommy' or 'cookie". Investigating and understanding the mechanisms of infant speech perception by researchers is also a difficult task. However, over recent decades, probing hypotheses and creative experimental designs have provided an accomplished view of how infants process speech.

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The present research aims to localize speech perception in infants by using a physiological method, and to examine changes in neurological activity as modulated by the different modalities of speech. The study of infant speech perception has been largely based on behavioral methods (Eimas et al., 1971; Trehub, 1973; Jusczyk, Cutler, & Redanz, 1993; Saffran, Aslin, & Newport, 1996; Bortfeld et al., 2004). With infants four months of age and younger, the most useful method employs a pacifier to record changes in infant sucking patterns that are typically linked to discrimination of visual or auditory stimuli (Siqueland & DeLucia ,1969). Subsequent variations of this method introduced contingency sucking, where presentation of stimuli is dependent on the infant achieving a high, predetermined level of pacifier manipulation. Paradigms used with infants four months and older generally track head or eye movements, and record duration of orientation time. Like the pacifier method used for younger infants, some methods used with older infants use a contingency dynamic where the infant receives additional stimuli in response to sustained looking time in a particular direction. Results obtained from such behavioral methods are effective in that they yield consistent response patterns that researchers use to suggest models of infant cognition. Occasionally however, the interpretation of results from these methods has been criticized for being ambiguous. For example, researchers are unable to know with certainty whether an infants looking time is based on a novelty or familiarity preference. Research by Hunter and Ames (1988) explored the underlying basis of infant preference and suggested that age of the infant and degree of difficulty in the experimental task guide the choice for novelty and familiarity. Beyond these broad parameters that frame the choice for novelty and

familiarity are hopes for a measure more capable of revealing specific underlying cognitive motivations.

Recent investigations of infant speech perception have increasingly used methods that assess neurological and hemodynamic parameters (Dehaene-Lambertz et al., 2002; Pena et al., 2003; Taga et al., 2003). These methods show promise for providing more precise determinations of stimulus responses. Research of this type employs a variety of neuro-imaging methods (EEG, fMRI, optical spectroscopy, PET) to achieve a more direct estimate of infant cognition than is possible with traditional behavioral methods. By accessing neuronal activity, or its hemodymanic correlates, researchers establish links between infant response and established functional areas of brain cortex. In so doing, correlates to existing behavioral data may be shown, thereby creating support between behavioral and neurological.methods.

Neurophysiological measures: Methods and limitations

While neuro-imaging methods offer exciting new and useful means for investigating infant cognition, they also have limitations. Neuro-imaging methods such as positron emission topography (PET) and functional magnetic resonance imaging (fMRI) scans may be impractical or unsafe for repeated use with infants. For example, fMRI requires infants to be motionless, and therefore are conducted when the infant is asleep or under sedation. PET is more appropriate for clinical applications because it requires the injection of radioactive materials that are unsafe for repeated use. Electroencephalography (EEG), and magnetoencephalography (MEG), are effective in identifying the temporal aspects of neural activity, but lack localization accuracy. A form

of neuro-imaging that to some degree operates outside these limitations is called near-infrared spectroscopy (NIRS). NIRS has advantages over other types of neuro-imaging techniques, such as better spatial localization than EEG or MEG, and does not use the radioactive tracers required by PET. In comparison to fMRI, NIRS offers slightly better temporal resolution, and is much less expensive. Additionally, the use of NIRS is particularly apt when the investigations call for awake, behaving, non-sedated infants. Though their movement must be partially constrained to reduce motion artifacts, it is a problem that is manageable with proper design and use of the equipment.

Emerging technology: Near-infrared spectroscopy (NIRS)

NIRS indirectly estimates neural activity by measuring changes in hemoglobin concentrations (oxygenated ([HbO]) and deoxygenated ([HbR])). Other hemodynamic parameters such as total hemoglobin concentration ([HbT]), cerebral blood volume (CBV), and regional cerebral blood flow (rCBF) are then calculated or deduced from [HbO] and [HbR] (Meek, 2002; Cope, et al., 1988). NIRS light sources enter the brain through the scalp, and then pass through various cranium tissues (skull, cerebral spinal fluid, vascular tissue, and cortex) in the brain. Measurements of changes in blood volume and concentrations of hemoglobin can allow researchers to identify regions of changing neural activity. By using NIRS technology, investigators can establish functional maps of neural activity in infants based on the hemodynamic changes observed during exposure to a variety of stimuli.

While NIRS is capable of indirectly estimating neural activity based on hemodynamic activity, it is also used by some researchers as a method of more directly

assessing neurological activity. The majority of investigations using NIRS examine hemodynamic response to stimulation. But, the true object of interest is neuronal activation. Thus, when researchers attempt to measure brain response to specific stimuli, NIRS is used to obtain these measurements indirectly by measuring hemoglobin levels, which are thought to be closely linked to neuronal activity. The link between neuronal activity and hemodynamics is framed by a 2-5 second latency of response. The latency of response between behavior and neuronal response is in the 10-100 milliseconds range. Thus, to get a more precise picture of the link between neuronal activity and behavior or cognition, researchers seek a method whose fundamental target of measure is the neuron. Specifically, such a method is available by using optical spectroscopy to at look at light absorption or scattering changes of neurons themselves, and not hemoglobin concentrations in the blood. Some researchers have called this unit of measure the fast-signal, or "event related optical signal" (EROS).

When a neuron fires, its membrane swells momentarily, causing a change in the membranes' light reflecting and scattering properties. Optical spectroscopy is able to measure light intensity and absorption changes in activated neurons, and thereby offer a closer link between behavior and neuronal activity than hemodynamics can. One major drawback of using spectroscopy to measure neuronal activity is that the signal is weak, and requires the inclusion of many hundreds of trials or samples to obtain a robust average. The signal to noise ratio is small compared to that obtained with hemodynamic measurements. Researchers who examine sc-called "fast-signals" with spectroscopy adjust their equipment and methodologies to obtain meaningful results. Nonetheless, a recent study by Franceschini and Boas (2004) found that, given stricter criteria for

assessing fast-signals, NIRS can be used effectively to measure neuron firing in response to motor tasks.

These researchers tested 10 adult subjects with an 8-emitter, 16-detector continuous-wave NIRS imager. Subjects were asked to finger-tap in a block design, with 20 seconds of stimulation and 20-second rest periods, for a total of 420 seconds. During each 20 second stimulation period, subjects tapped their finger continuously, thereby producing hundreds of stimuli events. The large number of stimulus events is important because the fast-signal is a weak one, and requires the averaging of many examples in order to produce an optically observable, statistically viable signal. Additionally, the fast-signal is much shorter (50-150 milliseconds) than typical hemodynamic signals (2000-5000 milliseconds). These considerations led the researchers to introduce more stringent signal detection significance criteria. With these criteria applied to the data analysis, the authors were able to detect significant neuronal response to tapping stimulation in 60% of the subjects. These results led them to report with confidence that NIRS can be used to directly detect neuronal activity. These authors point to other research (Gratton et al., 1997) that found good temporal agreement between fast-signal detection and a visual evoked potential (VEP) measurement. This result suggests that fast-signal detection is actually measuring neuronal activity, and not hemodynamic response.

Current research contrasts alternate NIRS methodologies, and illuminates promising features as well as some limitations of examining the “fast-signal”. Other spectrographic methods, such as PET and fMRI, are used for hemodynamic investigations, in which neuronal activity is deduced from hemodynamic information. In

such methods, the latency of the signal is measured in seconds, while latency of the signal in electrical methods such as EEG, MEG, and fast-signal NIRS are measured in milliseconds. The latency found by measuring brain activity with fast-signal spectroscopy is concordant with that found using EEG, thereby supporting the validity to fast-signal approach. However, these researchers point out that a major drawback of fast-signal spectroscopy is its low-amplitude signal. Such a weak signal is difficult to detect among peripheral physiologic and instrumentation noise. This results in a low signal to noise ratio (SNR) that requires manipulation to enhance. While the SNR limitation is challenging, these authors recommend expanding use of fast-signal analysis for brain activation studies. In particular, fast-signal NIRS can simultaneously measure hemodynamic and neuronal activity, which allows for cross-analysis of spatial information obtained from each line of inquiry. By directly examining neuronal activation, and the associated hemodynamic parameters, researchers gain a more revealing approach to understanding neurovascular coupling. However, collapsing data across neurological and hemodynamic domains is to be done cautiously, because many details of their inter-relatedness are not fully understood. These limitations aside, work examining the fast signal is considered promising because it gives localization results similar to those obtained with fMRI, and temporal results that coincide with those recorded by ERP (Gratton & Fabiani, 2001).

The hemodynamic and neurological investigative capabilities of optical spectroscopy in general, and NIRS specifically, allow for examination of neural correlates of behavioral activity through varied physiological approaches. As such, research done with such instruments establishes new parameters in the field of

neuroimaging. Ongoing discussions concerning NIRS and infant research continue to contribute to its promise as a preferable alternative to other less practical neuroimaging methods. Recently, a workshop was held that brought together specialists in a variety of NIRS related fields. Experts from optical physics, cognitive neuroscience, optical imaging, and developmental psychology discussed the state of NIRS and gave recommendations. Two attendees of that meeting wrote a summary article that is to be the forward of a special issue of Journal of Biomedical Optics. In that article (Aslin & Mehler, 2004), these researchers described NIRS as an exciting method for investigating infant cognition, but urged diligence in the development of safe and effective procedures. They point out that there is an acceptable intensity of laser power for use in human experimentation (.3-5 mW of near infrared light), deemed safe by the scientific community. However, it was revealed that to date, no studies have been done on tissue damage resulting from laser use in the infant brain.

Additional comments from the workshop contrast the use of NIRS with adults and infants. Factors such a hair density and quantity, skull thickness, and maturity of brain tissue, are significantly different in the two age groups. Therefore, when comparing data collected from the two populations, it is important to do so with caution. For example, infant frontal lobes are not fully myelinated until the later part of the first year, making NIRS data collected from that age group and brain location susceptible to understatement. Activation of infant frontal lobe neurons will be low due to immaturity of the vasculature and neuronal connectivity. Such results help to catalyze the evolution of new experimental methods and applications for NIRS. The infant neuroscience literature is increasingly composed of NIRS studies that demonstrate its viability as an

independent source of neuroimaging data. Currently, studies are showing its convergence with other neuroimaging methods.

NIRS: Areas of application

In a review of the current literature, it can be seen that NIRS has established itself as a valuable tool for measuring physiological changes and responses. NIRS has been used to investigate visual, auditory, olfactory, and cognitive areas of the brain. Such investigations have examined both adults (Villringer et al., 1993; Hoshi et al., 1994; Kato et al., 1993), and infants (Meek, et al., 1998; Wilcox et al., 2004; Chen et al., 2002; Zamarella et al., 2001; Sakatani et al., 1999, Taga et al., 2003; and Bartocci et al., 2000). In work with adults, Villringer et al. (1993) found significantly higher [HbO] and [HbT], and lower [HbR] in the left frontal cortex, than in the right, following performance of basic math operations. Hoshi et al. (1994) did a similar study with adults performing math operations, but found a decrease in [HbO]. Kato et al. (1993) performed one of first studies on adults using NIRS to examine hemodynamic response to visual stimuli. These researchers found responses that are typical of most NIRS studies (increased [HbO] and [HbT].

NIRS work with adults illustrates the relative ease with which data is collected from cooperative adult subjects. While much of the work done with NIRS has been done with adults, its application has increasingly been turned to the study of infant cognition. Many of the first studies using NIRS to investigate infant hemodynamics were conducted in clinical settings. Work conducted by Chen, et al. (2002) looked at infant response to auditory stimulation (piano music), and compared changes in CBO (cerebral blood

oxygenation) in normal and brain damaged awake newborns. Measurements of the frontal lobe CBO were made using a single laser emitting optode and a photon detector located 4 cm away. Recent studies suggest a more proximal placement of laser emitting optodes and photon detectors (less than 2cm apart) is helpful in improving the accuracy of readings. Placing the incident light source and the detector optode at greater distances increases the chance that the light will be affected by different tissue compositions (eg. bone, cell membranes, cerebral spinal fluid).

Results from the Chen, et al. (2002), study suggest different oxygenated and deoxygenated hemoglobin responses in normal and impaired infants. 19 of 20 normal infants showed increases in HbO (oxygenated hemoglobin) and HbT (total hemoglobin). In contrast, 14 of 22 impaired infants showed a decrease in HbO and HbT in response to the auditory stimuli. Additionally, among the impaired infants, severity of condition was negatively correlated with changes in HbT, such that increased severity was accompanied by larger decreases in HbT. The authors suggest that while the pattern of CBO in normal subjects was attributable to healthy neuronal responses to stimulation, the decrease in HbO and HbT in impaired infants was not known. They speculate that degenerated or incapacitated brain areas of impaired infants experienced decreases in overall blood influx due to surrounding areas, that were functioning and 'stealing' blood. These researchers conclude that NIRS is a valuable diagnostic tool in evaluating the development of normal and brain injured infants.

Once the safety and viability of NIRS was established through clinical studies, its application became widespread in academic environments. Currently, NIRS is being applied to infant studies in many leading universities. One of the first studies using NIRS

to study the visual response in very young infants was conducted by Meek et al. (1998). In her study, 20 non-sedated infants, aged 2 days to 14 weeks, were shown a checkerboard pattern for 10 seconds followed by a screen with the reverse pattern for 10 seconds. The incident light optode was placed on the occipital region, approximately 1 inch above the inion. These researchers found a significant increase in occipital cortical activity when compared to a control group. The control group had the incident light optodes placed on the right fronto-parietal region. [HbO], [HbR], and [HbT] increased in 9 out of 10 test subjects. In none of the control subjects was there a significant increase in any of these concentrations. Results from this study indicate a five-second latency period before peak hemodynamic response, and a five-second return-to-baseline latency period after offset of visual stimuli.

Further work using NIRS to detect hemodynamic changes in infants was performed by Zamarella, et al. (2001). These researchers used NIRS to measure hemodynamic changes in the right and left temporal region (T3 and T4 of the International 10-20 EEG system) in response to auditory stimuli (tonal sweep sounds of frequency 2-4 kHz) in sleeping and awake neonates. [HbR] (deoxygenated hemoglobin) and [HbO] (oxygenated hemoglobin) and cerebral blood volume (CBV) was measured at five-second intervals before, during and after the auditory presentation. Results indicate that during stimulation, 13 of 19 infants showed increased [HbT] (includes both oxygenated and deoxygenated hemoglobin) and increased CBV when compared to baseline. Of the 13 that showed increased [HbT], 8 showed an increase in [HbR]. The remaining six subjects showed decreased [HbO], [HbR], and [HbT] as compared to baseline. Additionally, of the 19 infants tested, 18 showed an increase in oxygenated

hemoglobin (HbO). These authors suggest that different patterns of cortical responses may be due to several factors. One may be the difficulty in locating the same functional areas on different subjects. Another reason for different response patterns may be varying rates of development between subjects. These authors suggest a way to increase confidence in NIRS results is to cross validate with other neuro-imaging methods such as PET or fMRI.

In a study of infants using NIRS to examine the developmental changes, Sakatani, et al. (1999), tested 28 neonates (mean age= 3 days) for frontal lobe activation following stimulation by music (popular piano music at 60 dB). Adults respond to this type of stimuli with increases in [HbO[and [HbT] accompanied by a *decrease* in [HbR]. These results are in contrast with infant studies that show an *increase* in [HbR] before approximately 7 weeks of age (Meek et al., 1998). In studying infants, these researchers found that 60% of the subjects showed increases in [HbR]. These authors suggest that maturational factors affecting oxygen consumption and delivery may be why results obtained here differ from those obtained in adult studies. The authors point out that in adults, oxygen consumption is replenished at an equal pace, but in infants, due to immature brain anatomy development, consumption outpaces replenishment.

Another group of researchers (Taga et al., 2003) tested 2-4 month old infants and focused on changes in blood oxygenation in the occipital cortex, after exposure to high contrast, visual stimuli. These researchers detected an increase in [HbO] in the occipital cortex area just above the inion in response to visual stimuli. According to Taga, differences in results between this study and others may be due to anatomical variation between their subjects, or differences attributable to using a multi-optode vs. a single

source opted light delivery system. These researchers conclude that NIRS is an effective system for inferring neural activation from hemodynamic measurements in infants. Additionally, they conclude that it is preferable to fMRI or PET scanning because of its safety features, relative low cost, portability, and non-invasiveness.

Other work with NIRS and infants was conducted by Wilcox et al. (2004). In this study, 6.5-month-old infant inferior temporal and primary visual cortex areas were investigated for neural activation in response to visual events. These infants were tested for their ability to differentiate, or individuate, objects based on featural information such as color, size and shape. Previous behavioral research on this topic suggests that there are two pathways in the brain that are involved in feature information processing. One such pathway is called the ventral route, and has been shown to support analysis of color, shape, and pattern information. The ventral route is an outer cortex structure that originates in the parvocellular layers of the laternal geniculate nucleus, and projects from the visual cortex to the inferior temporal cortex. In this experiment, the NIRS optodes were placed just above the inion of the occipital area (primary visual cortex), and on the T3 region (just above and behind the ear) of the infant scalp.

Typically, areas thought to be activated show an increase in [HbO] and a decrease in [HbR]. In response to the feature processing events in the Wilcox study, the occipital detectors showed the typical increases in [HbO] and [HbT], accompanied by a decrease in [HbR]. Conversely, activation in the inferior temporal region showed the typical increases in [HbO] and [HbT], but unexpectedly showed an increase in [HbR]. The authors suggest the unusual results may be due to the underdeveloped vasculature and blood delivery capacity of the infant brain. More specifically, as oxygen is consumed in

response to a stimulus, the deoxygenated hemoglobin is not removed as quickly as it is in an adult brain, thereby leaving its concentration elevated.

The collective view from researchers using NIRS is that this method is highly effective in measuring brain regions across adult and infant cortex. NIRS has been an important contributor to the neuroimaging literature, though it is only useful for cortical measurements. However, many functions of the brain are localized on the cortical surface and are therefore ideal for investigation by NIRS. Other physiological measures of neural activity such as EEG and fMRI offer methodological advantages, such as better temporal resolution and physical depth of analysis. These methods however also possess inherent disadvantages that NIRS overcomes. By offering a method that compliments, and is preferable to other neuro-imaging methods, NIRS applications become numerous. Imaging of all major brain areas with NIRS has produced results that in many cases coincide with other neuro-imaging methods. EEG, PET and fMRI scans reveal activation from auditory, visual, and cognitive stimuli that NIRS has replicated. As more regions of neural activity are investigated, the promise and potential of NIRS is realized.

One particular area of interest with respect to imaging infant cortical activity looks at language development in the first year. Infant speech processing has been localized mainly to the temporal regions of the left brain, with areas in the frontal lobes also showing activation in response to language stimuli. Typically, it is in the left temporal lobe, (anteriorly from Broca's area, posteriorly in Wernicke's area, dorsally from the superior temporal gyrus, and ventrally to the inferior temporal gyrus) that language response is identified. Behavioral and physiological methods testing infants

have revealed significant speech processing activation in all ages if infancy. It is this area that the present studies focus their investigation.

Neuroimaging data on infant speech perception

At birth, infants are capable of processing many aspects of human speech (Mehler et al., 1988). Throughout the first year, infants continue to develop the underlying neurological substrate necessary for language processing and learning. Research using neuroimaging methods has localized language processing in infants to the left temporal sulcus and gyrus regions (Binder et al., 2000). Recently, experiments by Pena et al., (2003) using optical topography, and Dehaene-Lambertz et al., (2002) using fMRI, found that very young infants show significantly more left temporal cortex activation in response to forward speech, when compared to a corresponding location in the right hemisphere. The Pena et al. (2003) study tested sleeping neonates with forward, reversed speech, and silence for hemispheric dominance. Using a 24-channel optical topography imager, these researchers found that 2-5 day old infants exhibit left hemisphere dominance for forward, infant directed speech. Localization of speech processing in the left hemisphere is also suggested by another brain imaging study. Dehaene-Lambertz et al., (2002) tested sleeping 3 month-old infants using fMRI and found increased activation in the left hemisphere when compared to the right side. These two studies point to the left temporal cortex region as a primary area of interest for language processing. However, in these studies, the infants were either very young, or were sleeping. Given the disposition of the infants tested, there is a chance to extend the literature in this area by testing older, awake and behaving infants. Infants four months

of age begin to move beyond discrimination of speech parts, to be able to recognize familiar words. This stage of language processing and cognitive development should be apparent in brain imaging studies of this age group. Therefore, the challenge of collecting hemodynamic data from older infants is a more methodological than developmental.

EXPERIMENT 1

Introduction

This experiment intended to investigate hemodynamic activation in response to speech stimuli in the temporal cortex. An emitter light was placed on the T3 area, with detectors aligned horizontally to the left and right of the emitter by 2 cm. An additional probe was placed on the occipital lobe as a control. There is no evidence that speech stimuli activate visual cortex in the occipital region. In addition to language investigation, this experiment aims to qualify NIRS as an effective tool for studying awake, behaving infants.

Method

Participants

Participants were 48 infants, aged 6 to 9-months, and were recruited by phone from birth records for use in the study described here. 30 infants were eliminated because of excessive motion artifacts, inadequate looking time, or fussiness. Of the 18 infants considered acceptable for the final analysis, 10 were male, and 8 were female (M age = 7 months, 17 days, range = 6 months, 7 days to 9 months, 14 days). Before the experiment began, a parent read and signed an informed consent page. Immediately following the experiment, parents were offered payment in the form of a new toy for their child.

If NIRS is to be used effectively with infants, there are equipment and procedural concerns that must be successfully addressed. In particular, the delivery of emitter light from the NIRS apparatus to the infant scalp is a task that requires special attention. NIRS transmits light through optic fibers that terminate in an elastic headband positioned

around the infants head. The tips of the optic fibers must rest cleanly on the infants scalp. Obstructions such as too much hair, or excessively dark hair, cause the union between optic fiber tip and scalp to be compromised, leading to blocks of both the light entering and leaving the infants scalp. Therefore, special care must be exercised when fitting the headband onto the infant to ensure proper contact. Having achieved an excellent surface contact with the skin, the optic fiber tips must then remain firmly located in one position. Any shifting from side to side, or twisting of the fiber tips, will cause disruptions in the delivery and recording of light signals. Solutions to these challenges include making sure the headband is sufficiently tight around the infants head, so that during periods of head movement, the fiber tips move as the head does. Additionally, an appropriate balance between tension and free play of the fiber lengths between the NIRS apparatus and the headband must be achieved to reduce swaying motion of the fibers.

Another method for reducing motion artifacts, and improving the quality of results obtained from NIRS, is in the development of engaging stimuli. Producing auditory and visual stimuli that cause the infant to maintain a forward gaze will help reduce head movement. Stimuli should be effective in holding infant attention during and between stimuli appearance. This improves the probability that data recorded by NIRS is due to stimulus response, and not peripheral distractions. And relatedly, special care should be taken to create a test area that is without ambient computer or technician noise. Having provided for these methodological and procedural contingencies, a high level of confidence can be assumed in using NIRS to collect infant speech perception data.

Apparatus

The stimulus delivery apparatus was located in the experimental area, which measured 5 ft. wide x 6 ft. deep x 7.5 ft. high, and was directly adjacent to the control panel area. In the experimental area, the infant sat in the parents lap, facing a computer monitor and hidden speakers. The experimental area was separated from the control panel area by a thick black curtain that hung from the ceiling to the floor. Both parent and infant faced the stimulus delivery apparatus (computer monitor and hidden speakers) throughout the experiment. The stimuli delivery apparatus was located on one wall, and was covered by a façade that functioned to conceal or make inconspicuous the stimulus delivery apparatus. The façade was made of three sections. The upper third was a dark black curtain that covered the wall from side to side, and dropped down 33 inches from its attachment at the ceiling. The middle section was constructed of plywood and covered with dark black cloth glued directly to its surface. This middle plywood section measured 5 ft. x 5 ft. (wall to wall horizontally) x 27 inches high. The plywood had a 13 inch x 9 inch rectangular hole cut out of its center that coincided with the size of the viewing surface of the computer monitor (Macintosh, G4 17 computer). Thus, visual items displayed on the computer screen could be viewed by the infant participants without distractions caused by the computers housing, control knobs or brand logos. From the bottom edge of the middle plywood section was attached a dark black curtain that hung 31 inches to the floor. Behind the lower black curtain were the speakers (standard computer speakers) that delivered the auditory stimuli. The speakers were measured to produce audio stimuli at 65 decibels at the approximate location of the infant's ears (32 inches from the speakers).

The Near-Infrared Spectroscope imaging device used in these studies produced light sources (emitters) of 680 and 830 nm wavelengths from two laser-emitting diodes (Boas et al. 2002). These emitter lights were delivered to the scalp of the infant subjects by fiber optic cables (1 mm in diameter) that originated in the Near-Infrared Spectroscope imaging device, and terminated in optodes that were sewn into a headband that was placed on the infants head. The headband was made of elastic terry-cloth and was fitted with two light-emitting and four light-detecting optodes (see Figure 1). The emitters and detectors were grouped into two optode sets (each set contained one emitter and two detectors). One set delivered light to the temporal region (approximately at position T3 according to the International 10-20 system), and the other set delivered light to the occipital region (approximately at position O2 according to the International 10-20 system). Detectors were located two centimeters to each side of the emitter. Following entry into the infants scalp and cortex area, the emitted light was reflected and then received by the detectors, and recorded by a custom computer program installed in the control computer (Dell Inspirion 7000 laptop). The light emitting and detecting optodes are illustrated below. The spectroscope and Dell laptop were located in the control area, just outside the experimental area. Fiber optic cables extended from the spectroscope to the experimental area, and onto the infant subjects head. The fiber optic cables came off the infant subjects' head to the rear, and were then bundled into a single strand that continued rearward, over the parents' right shoulder, along the rear wall, and back to the spectroscope.

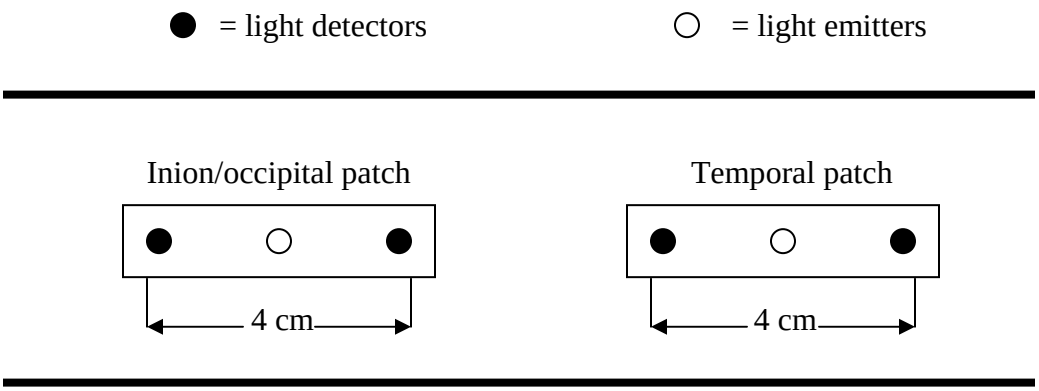

Figure 1. Detector and Emitter Placement in the Elastic Headband.

Stimuli

The stimulus were a combination of forward-speech auditory recordings and visual animations. The stimuli sequence proceeded as such: 10 seconds of silence with a black screen, 20 seconds of silence with visual animation, 10 seconds of silence with a black screen, and 20 seconds of speech with visual animation. This sequence repeated five times over the 5-minute length of the stimulus presentation. Speech segments were different, but were equivalent with one another in terms of intonation variation and phonetic content. The visual animations were all different, but were created to be generally similar in their color contrast and motion parameters. A central challenge in testing older awake infants is maintaining their attention during inter-stimulus intervals. First year infants find difficulty in attending to familiar stimuli for more than 30-40 seconds, or for experiments that last longer than 15-20 minutes. Creating engaging stimuli allows for reasonably uninterrupted data collection in first year infants.

The voice recordings were made by a female undergraduate, reading children's stories using highly intonated Infant Directed Speech (IDS). A complete text of the voice recordings can be found in Appendix A. The voice 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 animations were simple 3-dimensional objects (eg. spirals, circles, and rectangles) rotating and moving 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 for participants through the computer monitor and speakers as described above. The experiment area was illuminated by a low intensity light bulb located behind the curtained area of the stimuli projection wall to help maintain infant attention in the direction of the stimuli. Excessive light would illuminate the surrounding area and encourage infant attention to stray from the visual stimuli.

Procedure

After the parent and infant were seated, an infant-head circumference measurement was taken using a standard cloth tape measure. Next, the parent was instructed not to talk to the infant, to keep the infant seated upright, and to hold the infants' hands. The experimenter then placed the NIRS optode headband on the infant. Within the headband, were temporal and occipital optode sets. The occipital set was placed on the nasion/inion center line of the scalp, about 1-2 cm from the inion (between O1 and O2 positions of the International 10-20 EEG electrode position classification

system). The temporal set was placed on the T3 position of the 10-20 system (left temporal area). The occipital detectors were located 2 cm in either direction and on a horizontal plane from the occipital/inion emitter. The two temporal detectors were located 2 cm in either direction and on a horizontal plane from the temporal emitter.

Following successful placement of the NIRS headband onto the infants scalp, the experimenter moved to the control panel area and closed the dark curtains of the experimental area, thereby separating the two areas. The room lights in both the experimental and control areas were turned off, leaving only a low intensity light to sufficiently illuminate the experimental area, and light from the computer monitor to light to control area. Next, the emitter source lights of the Near-Infrared Spectroscope imaging device were turned on, and its timeline was set in motion by clicking on the Start button. At this time, the speech and visual stimuli DVD was started. Additionally, an audio/ video VHS recording of the participant was begun that taped the entire five-minute experiment. Throughout the experiment, the experimenter marked onset and offset times of the stimuli on the NIRS timeline. Following the five-minute duration of the experiment, the NIRS light emitters were turned off and the data saved. The room lights were turned on, the headband was removed from the infant, and the parent received compensation before leaving.

Data analysis

NIRS data was collected from the temporal and occipital regions. Data from each area was analyzed the same way. Two detectors in each brain region received the raw signals, and digitized them at 200 Hz for each of the four channels. The NIRS apparatus

then 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. Across the four channels, there were artifacts originating in the infant physiology, and also artifacts due to motion. These artifacts were reduced by performing a principle components analysis (PCA) of the signals across the four channels. PCA acts as a filter to remove the co-variance of the data. Three principle components were removed from the data, and it is believed that this procedure was successful at removing up to 85% of the co-variance. The NIRS computer then converted the data to relative concentrations of oxygenated (HbO_2) and deoxygenated (HbR) hemoglobin using the modified Beer-Lambert law (calculates the relationship between light absorbance and concentration of particles within a medium).

Results

Looking time data

Looking times were calculated for each 20-second trial, and a grand average was computed for visual and audiovisual conditions. Videotaped recordings of all subjects were viewed by an undergraduate infant lab student. Using a stop watch, the observer recorded the accumulated amount of time that each infant looked away from the stimuli during each 20 second trial. The visual only condition looking time average was M = 17.4 seconds. The audiovisual condition looking time average was M= 16.3. Trials were excluded if an infant looked away for more than 10 seconds during a 20 second trial.

NIRS imaging data

Data collected in the audiovisual and visual only conditions from the left temporal and bilateral occipital lobe are shown in Figures 2A-6. Trials were automatically eliminated based on the motion removal algorithm that detects excessive motion. Trials were also removed if an infant spent 10 or more seconds looking away from the visual stimuli. The two graphs above the midline of Figures 1 and 3 show the occipital response, while the two graphs below the midline show temporal activity. Occipital and temporal regions each had one light emitter and two light detectors, as indicated on the far right label of the y-axis. Detectors 1 and 2 received data from the temporal regions, while detectors 3 and 4 received light from the occipital area. The figure is representative of data analysis for each infant in the study, though specific results varied. On the left side of the figure are four horizontal illustrations showing the 5-block design that spanned 300 seconds (x-axis). The y-axis indicates relative concentration changes of HbO (830 nm wavelength) and HbR (690 nm wavelength). The thicker vertical lines throughout the graphs indicate onset of useable trials.

Audiovisual trials - Results from the audiovisual trials are presented for one individual (Figures 2A and 2B), and group data (Figure 3). Both occipital and temporal regions show an increase in [HbO] and a decrease in [HbR]. The changes in [HbO] and [HbR] in the occipital region are not significant, while the changes in the temporal region are ($p < .01$). Significance testing was performed between the baseline concentration measurement at -2 (two seconds prior to stimulus onset) to 0 (stimulus onset) seconds, and the 10-15 second interval following stimulus onset. Changes in concentrations begin 2-3 seconds after stimulus onset and begin to diminish close to the period of stimulus

offset at 20 seconds. As seen in the graphs of Figure 2a and 2B, the hemodynamic response lingers beyond stimulus offset. The resulting increase in oxygenated hemoglobin remains elevated well into the inter-stimulus interval, during which the infants' hemodynamics should be returning to baseline, and continues into the next stimulus period (that is, during the visual only stimulus beginning 10 seconds after offset of the audiovisual stimulus).

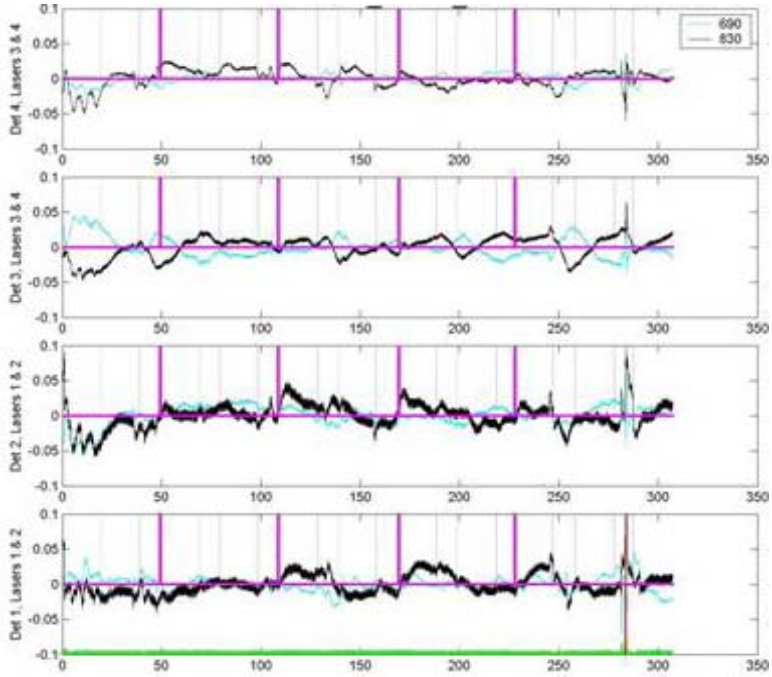

Figure 2A. Individual Results for Audiovisual Trials of Exp. 1. The top two graphs show [HbO/830 nm] and [HbR/690nm] occipital area changes from an arbitrary baseline (time -2 to 0 seconds) over 300 seconds. The bottom two graphs show temporal area changes.

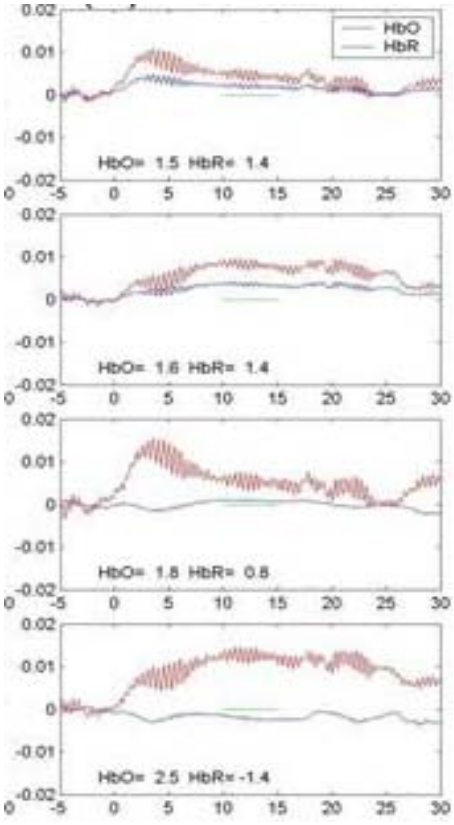

Figure 2B. Average of Individual Results from Figure 2A. Trials lasted 20 seconds, with 10 second inter-stimulus periods throughout the 300 second experiment. Inside the boxes are numerical values that indicate the change in concentration from time = 0 seconds to time 15-20 seconds. An average concentration was measured during the 15-20 second period and was compared for significance change from the level of concentration at time -2 to 0 seconds.

The grand average for changes in the audiovisual condition is illustrated in Figure 3. Overall, there is a significant increase in [HbO] in both occipital and temporal locations immediately following audiovisual stimulus onset. The larger subsequent increase in the temporal region compared to the occipital region supports earlier arguments that this region is relevant to auditory processing, even in very young infants,

and is particulary relevant to language processing. The slight decrease in temporal region [HbR] suggests that the area is participating in oxygen exchange associated with neural activity. It should be noted that since the visual stimuli appear in both audiovisual and visual only conditions, they should therefore elicit little to no change in the occipital region's Hb concentration. This is partially born out by these findings, as results indicate only slight and nonsignificant occipital area concentration changes.

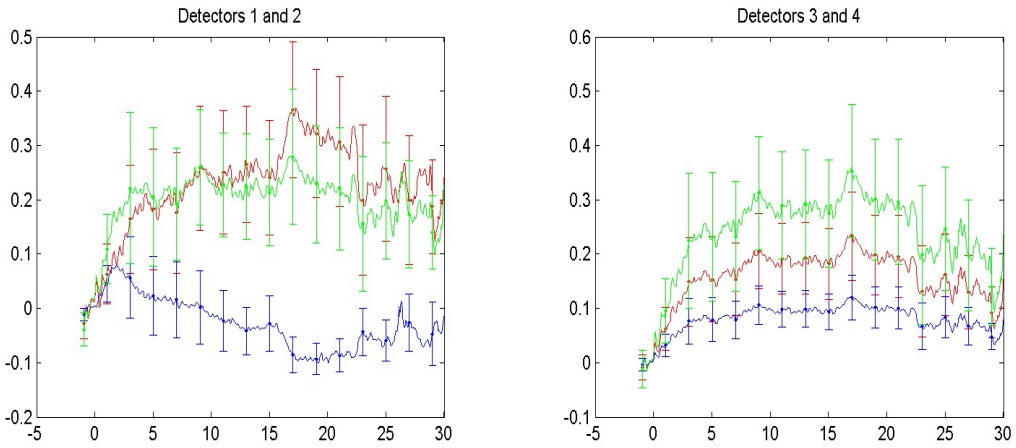

Figure 3. Grand Average for Audiovisual Trials in Exp. 1. Grand averages for temporal (left) and occipital [HbO] and [HbR] changes in the audiovisual condition.

Visual only trials.

Figures 4A and 4B show an individual subject's results. The changes in [HbO] and [HbR] from baseline in the occipital region are not significant in either of the occipital channels, however what change does manifest begins close after onset of the visual stimulus, and diminishes relatively quickly following stimulus offset. The temporal region results indicate a modest increase in [HbR] and a larger decrease in [HbO] in both channels. The direction of the change observed for both the occipital and

temporal regions is not in agreement with most neuroimaging studies, which show an increase in [HbO] and a decrease in [HbR] in response to stimuli. Possible causes for the atypical results are considered next.

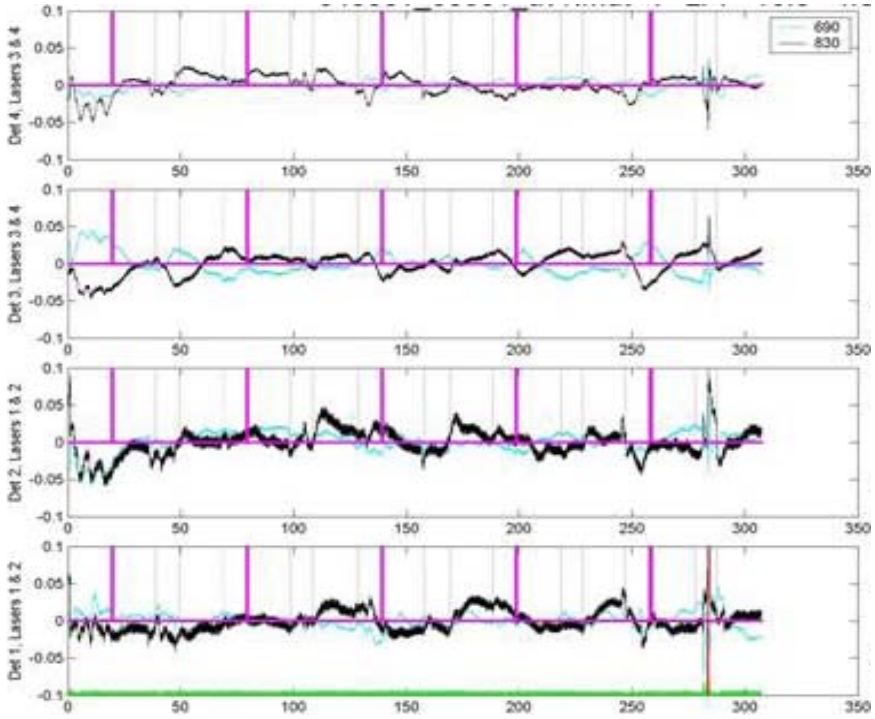

Figure 4A. Individual Results for Visual Only Trials in Exp. 1. Occipital response (upper two rows) shows little concentration change. Temporal response (lower two rows) shows uncharacteristic increase in [HbR], and a decrease in [HbO].

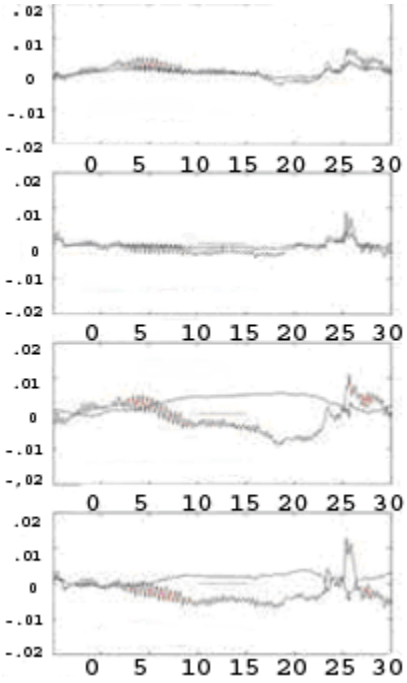

Figure 4B. Average of Individual Results from Figure 4A.

Grand average results (Figure 5) for the occipital and temporal regions show an uncharacteristic pattern for both [HbO] and [HbR]. The total concentration change ([HbO]} + [HbR]) in the left temporal region shows a large decrease. This is the result of adding a small increase in [HbR] and a large decrease in [HbO]. The occipital region grand average shows decreases in all species. The total hemoglobin concentration is found by summing [HbO] and [HbR]. Although the changes indicated in this grand average are substantial and indicate a real change across time in the hemodynamics of these regions, the relative position and direction of the changes are misleading and are the product of a methodological issue that will be addressed subsequently. Therefore, while results from the audiovisual condition of Experiment 1 are interpretable, the results from the visual-only condition must be treated with caution.

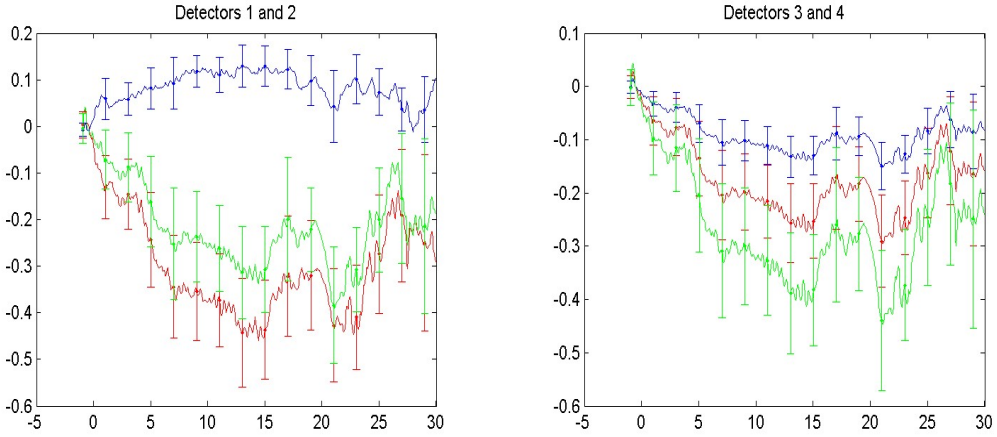

Figure 5. Grand Average for Visual Trials in Exp. 1. X-axis is time in seconds. Visual stimuli were presented from 0-20 seconds. Y-axis is relative changes in HbR (dark line), HbO (medium line), and HbT (light line) concentrations. Detectors 1 and 2 were placed on the temporal lobe (detector 1 = anterior, detector 2 = posterior). Detectors 3 and 4 were placed on the occipital lobe (detector 3 = subjects left side, detector 4 = subjects right side.

Discussion

Results from Experiment 1 demonstrate the feasibility of NIRS as an effective tool for inferring neural activation in the temporal and primary visual cortex regions following speech and visual stimulation. In the left temporal region, significant increases in [HbO] were recorded during exposure to sounds (e.g., in the audiovisual condition) relative to baseline periods (e.g., periods during which neither audio nor visual stimuli were presented). The left temporal region is an area well established as playing a role in language processing. The current investigation confirms that language exposure results

in heightened levels of hemodynamic activity in the left temporal region of awake infants and that NIRS technology can be used to track this activity.

A couple of methodological issues worth considering are 1) removal of variance (e.g., due to motion) from the raw signal, and 2) the setting of a unique and arbitrary baseline for each condition in a two condition design such as that used here. With regard to the first issue, comparing results obtained in each probe location highlights the heightened hemodynamic response in the left temporal lobe relative to the occipital lobe. The left temporal cortex has been identified as a fundamental language processing area across many studies and, on average, the results obtained here support results reported in the literature. However, this pattern of response was not universal across subjects. Some infants showed nonsignificant changes or no changes in [HbO] and [HbR]. Still other infants showed a decrease in [HbO] and an increase in [HbR]. The main variable affecting significance was variance removal due to principle component analysis. Variance due to infant pulse, breathing, and head motion could be removed to varying degrees. Caution had to be exercised in removing variance, because in the process, acceptable data may be lost. Removal of each additional principle components results in higher levels of variance removal. One principle component removes 40% of the variance, while two to three components removes up to 85%. Removing four principle components however excises a significant portion of the hemodynamic response. In this experiment, three principle components were removed. This PCA approach allows removal of noise associated with motion (thus eliminating variance), while retaining signals associated specifically with hemodynamic activity. Based on the data observed

here, this approach appears to sufficiently control for motion without contaminating measurement of the hemodynamic signal.

The second methodological issue, that of where to set what ultimately is an artificial baseline, motivates a reanalysis of the results observed for the visual stimulus condition in the current experiment. Grand average results for the occipital and temporal regions show an uncharacteristic pattern for both [HbO] and [HbR]. This unpredicted total concentration change ([HbO]} + [HbR]) in the left temporal region is actually an artifact introduced by adding a small increase in [HbR] and a large decrease in [HbO]. The pattern of hemodynamic response in the visual-only condition for both occipital and temporal regions was affected by this artifact. Figure 6 illustrates that the 10-second inter-stimulus interval between the visual-only and the audiovisual conditions was insufficient for hemodynamic changes in the infant population tested here to return to baseline. Therefore, the onset of the visual-only trial was affected (e.g., contaminated) by the lingering response to the audiovisual condition, particularly in the temporal lobe, but in the occipital lobe to some degree as well. As such, the hemodynamics upon which the baseline for the visual-only trials (e.g., -2 to 0 seconds prior to stimulus onset) were established was not, in fact, at baseline. The result of this inaccurate baseline cane be seen in both the individual and grand average figures, and varies according to the degree of [HbO] elevation at the time of visual-only trial onset. That is, at the point at which the visual-only trials begin, [HbO] is still elevated due to the activation induced by the audiovisual stimuli presented in the previous condition. It is probable that the visual-only trials did not elicit a significant hemodynamic change, given that visual stimuli are available in both conditions and the baseline period is relatively short. Therefore, for the

temporal region, the hemodynamic state has returned to baseline by the time the 10-second inter-stimulus period begins prior to the onset of the audiovisual trials. As such, measures taken during the audiovisual trials were not likewise affected by this artifact. In the audiovisual trials, the overall hemodynamic response was pronounced in the temporal region across subjects and, generally, typical of expected results. [HbO] increased and [HbR] decreased in response to audiovisual stimuli in temporal region, and the robust effect influenced hemodynamics in the occipital region as well, though not to the same degree. Whether this influence had to do with the audio stimuli alone or some interaction of the audio stimuli with the visual stimuli is unclear and is investigated further in the subsequent experiment.

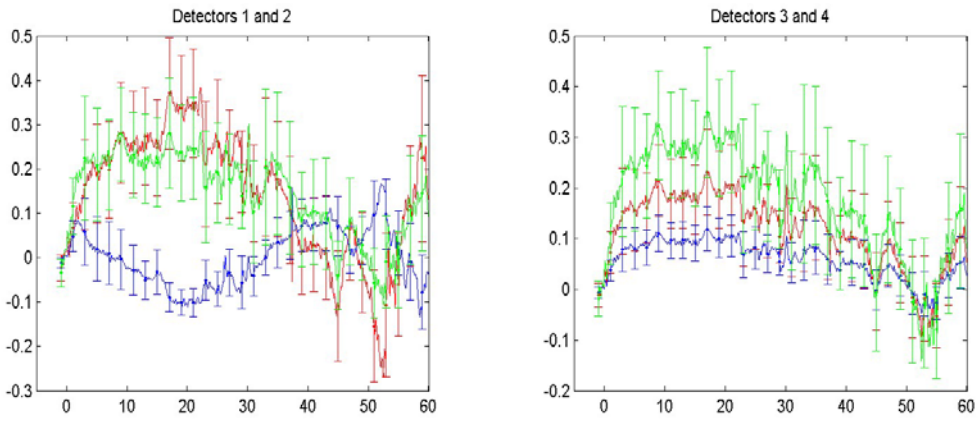

Figure 6. 60-second Analysis Showing Artifact in Exp. 1. Shows source of methodological artifact found in the visual only conditions. The hemodynamic response from the audiovisual trial (precedes visual only trial) continues at an elevated level, and does not return to baseline before the start of the visual only trials.

Overall, findings from Experiment 1 show an increase in [HbO] in the left temporal lobe of infants in response to speech stimuli relative to the response seen during

baseline (e.g., when no stimuli are presented). The level of this activation was significantly greater than during the visual-only conditions, though how to interpret this difference is unclear, given the methodological issues just discussed. However, it is possible that speech-induced hemodynamics may be influenced by factors other than simply the auditory component of speech.

EXPERIMENT 2

Introduction

To examine the auditory-visual interaction further, the character of the speech stimuli used in Experiment 2 was changed, such that more "meaningful" audiovisual speech stimuli were presented. There is some evidence that the coherence between audio and visual stimuli influences processing when such stimuli are presented jointly. The stimuli presented to infants in the audiovisual condition of Experiment 1 were unrelated (e.g., a woman's voice and geometric animations) and therefore somewhat incoherent. If the hemodynamic responses observed in temporal and occipital regions in the audiovisual condition of Experiment 1 are at all influenced by the multimodal nature of the stimuli, then presenting infants with coherent stimuli should have some effect (either qualitative or quantitative) on infants' hemodynamic response to such stimuli. Therefore, in Experiment 2, we presented the visual image of the woman's face while she produced the auditory narratives to create highly coherent audiovisual stimuli. We hypothesized that adding this form of visual stimulus would influence infants' hemodynamic responses at least as much as the audiovisual stimuli in Experiment 1 and perhaps more. This expectation is based on those characteristics of faces that interact with people's processing of spoken language. A face producing language influences the auditory signal in two ways: 1) it introduces synchronous, or coherent, visual and audio stimuli, 2) and it induces face-preferences/recognition effects that may catalyze additional neural and hemodynamic activity.

It is clear that our representation of speech is visual as well, due to lip and tongue movements and other facial gestures. Evidence for this comes from many sources, most

profoundly from the classic study by McGurk and MacDonald (1976) in which these researchers demonstrated that visual and acoustic elements of speech perception are linked at a very basic perceptual level. In this experiment, these researchers presented audio versions of 'ba' with visual versions of 'da', finding that subjects upon exposure to such ambiguous stimuli reported hearing 'ga'. In fact, adult (and grade school) participants were consistently incorrect in their identification of the sound actually produced. This effect, while still actively researcher and not completely understood, suggests that the two modalities (visual and auditory) are subject to one another's influence, and that speech is perceived multi-modally.

Research has demonstrated that speech is a complex stimulus in which information is represented in more than one modality. In work by Walton and Bower (1993), an attempt was made to investigate the link between face and voice perception in infants. These researchers tested 4- and 5-month-olds' visual preference for matched and mismatched voice and face pairings. The methodology used in this experiment was the high amplitude sucking procedure, allowing infants to indicate a preference for the matched and mismatched pairings through changes in sucking behavior. For example, by sucking on a pacifier at a particular rate or intensity, infants could control the presentation of combinations of face/voice pairings. The same combination would repeat if the infant kept sucking at a high rate, but would change to the next combination if there was a 1-second inter-suck interval. In this manner, the authors suggest, the infant can maintain, and thus indicate, preference for a particular combination. They can likewise stop sucking in order to change to the next face/voice pairing. Results from this study

indicate a significant preference for correctly matched voice and lip movement, thereby suggesting a coherence preference in infants.

These findings indicate that infants prefer a matched relative to an unmatched face-voice pairing, suggesting that synchrony between the modalities influences processing (and therefore preference). When the visual and the auditory portions of stimuli are not coherent, infant attention toward those stimuli decreases. Lewkowicz (1996) has specifically examined the timing of presentation of different modalities. Although his research did not focus on speech stimuli in particular, it is relevant to the current discussion in its general analysis of the temporal coincidence between different modalities. Lewkowicz (1996) compared infant sensitivity to synchronous and asynchronous events by varying the time lag between a visually-presented bouncing disk and an associated sound. Discrimination data indicated that infants have the ability to distinguish synchrony from asynchrony in multi-modal events. Lewkowicz described the amount of asynchrony between two stimuli that an infant will tolerate as an infant's "intersensory temporal synchrony window". Results indicate that cross-modal events occurring outside a 400 ms time lag are not perceived as unitary by 2- through 8-month-old infants. Lewkowicz (1996) suggests that awareness of temporal synchrony is necessary for the development of abilities to integrate other parameters of multi-modal events (e.g. duration, rate, and rhythm).

These results are particularly relevant when considering how infants process the speech signal. Work by Bahrick and Lickliter (2000), suggests that synchrony between separate modalities is a central factor in recruiting infant attention. These researchers suggest that multi-modal stimuli presented in synchrony provide redundant information

that guides perceptual learning. Their "intersensory redundancy hypothesis" states that 1) redundant information presented simultaneously by two or more senses causes those events to become foreground in perception, 2) those multi-modal events are processed and learned before other stimuli, and 3) perceptual processing of events that are not specified in more than one modality is constrained. These points suggest that perception of language stimuli presented naturally (e.g., emanating from a face) will be processed differently than speech presented uni-modally. In Experiment 1 of the present study, speech stimuli were presented to the infants in a non-ecologically valid manner (e.g., accompanied by animations that were not naturally linked to speech production). Therefore, probing the hemodynamic changes that occur when infants are exposed to synchronous, ecologically valid (e.g., emanating from a face) speech stimulus, may uncover a response that is distinct from that obtained with speech presented either uni-modally or with incoherent/asynchronous visual stimuli.

Previous neuro-imaging research with infants suggests that the hemodynamic response associated with both uni-modally and multi-modally presented speech stimuli stems from heightened neural activity located predominantly in the left temporal region. However, a difference in the hemodynamic response is predicted according to various researchers. For example, Calvert (2001) and Giard and Peronnet (1999) found a super-additive response to coherent, bimodal stimuli, where the quantity of response was larger than the sum of the uni-modal components. Conversely, Wasserhove et al. (2005) found an amplitude reduction in response to bi-modal stimuli. These findings suggest a need for additional investigations into the nature of the neural response to multi-modal events. In Experiment 2 of the current study, the hemodynamic response to coherent speech

stimuli is examined. By presenting speech stimuli in an ecologically valid, spatially and temporally coincident manner, a clearer picture of the underlying cognitive response may be obtained.

Method

Participants

Participants were 35 infants, aged 4 to 10-months, and were recruited by phone from birth records for use in the study described here. 24 infants were eliminated because of excessive motion artifacts, inadequate looking time, or fussiness. Of the 11 infants considered acceptable for the final analysis, 9 were male, and 2 were female (M age = 7 months, 13 days, range = 4 months, 12 days to 10 months, 4 days). Before the experiment began, a parent read and signed an informed consent page. Immediately following the experiment, parents were offered payment in the form of a new toy for their child.

Apparatus

The apparatus and experimental area used for Experiment 2 were exactly the same as used in Experiment 1 (see page 19).

Stimuli

The stimuli from Experiment 1 were altered by replacing the animated images in the audiovisual condition, with the actual face of the person speaking the speech stimuli. An audio-video recording of the face of a female research assistant was used for this

experiment. In Experiment 1, this portion of the stimuli was composed of a female voice recording played while animated computer graphics simulated objects in motion on the screen. In Experiment 2, the animated objects in motion were replaced with the actual face that produced the voice recordings. The same sequence of silence (10 seconds), visual-only (20 seconds), and audiovisual (20 seconds) that was used in Experiment 1, was used in Experiment 2.

Procedure

The procedure in Experiment 2 was exactly the same as in Experiment 1 (see page 22).

Data analysis

NIRS data was collected from the temporal and occipital regions. Data from each area was analyzed the same way. Two detectors in each brain region received the raw signals, and digitized them at 200 Hz for each of the four channels. The NIRS apparatus then 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. Across the four channels, there were artifacts originating in the infant physiology, and also artifacts due to motion. These artifacts were reduced by performing a principle components analysis (PCA) of the signals across the four channels. PCA acts as a filter to remove the co-variance of the data. 3 principle components were removed from the data, and it is believed that this procedure was successful at removing up to 85% of the co-variance. The NIRS computer then converted the data to relative concentrations of oxygenated

(HbO_2) and deoxygenated (HbR) hemoglobin using the modified Beer-Lambert law (calculates the relationship between light absorbance and concentration of particles within a medium).

Results

Looking time data

Looking times were calculated for each 20-second trial, and a grand average was computed for visual and audiovisual conditions. The visual only condition looking time average was16.5 seconds. The audiovisual condition looking time average was 17.3. Trials were excluded if an infant looked away for more than 10 seconds during a 20 second trial.

NIRS imaging data

Data collected in the visual only and audiovisual conditions from the left temporal and occipital lobe of a single infant is shown in Figures 7A-11. Excessive motion or insufficient infant looking time caused trials to be eliminated. The two graphics above the midline in Figures 7A and 9A show the occipital response, while the two graphics below the midline show temporal activity. Occipital and temporal regions each had one light emitter and two light detectors, as indicated on the far right label of the y-axis. Detectors 1 and 2 received data from the temporal regions, while detectors 3 and 4 received light from the occipital area. Figures 7A and 7B are representative of data analyses for each infant in the study, though specific results varied. The figure shows the 5-block design that spanned 300 seconds (x-axis). The y-axis indicates relative

concentration changes of HbO (830 nm wavelength) and HbR (690 nm wavelength). The thicker vertical lines throughout the graphs indicate onset of useable trials.

Audiovisual trials

In response to face-produced speech, the individual subject in Figure 7A shows a modest increase in occipital cortex activity, and a strong increase in the temporal region. For all channels, the increase peaks in the 15-20 second range. This individual shows a strong response, but does not sustain high concentrations is apparent in the grand averages shown in Figure 8.

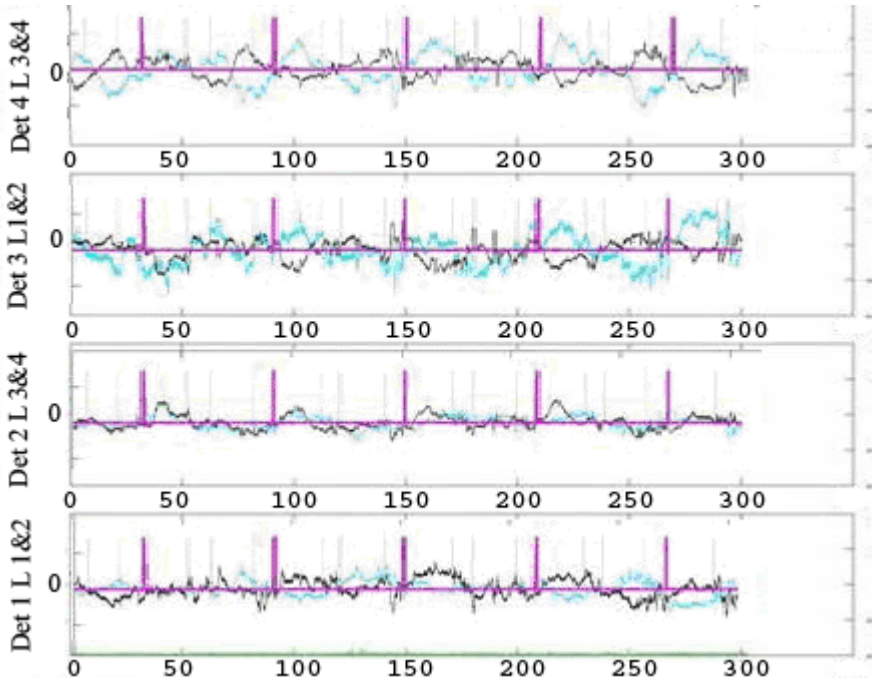

Figure 7A. Individual Results for Audiovisual Trials of Exp. 2. Individual subject data from the audiovisual (face) condition of Experiment 2. Note the consistent increase in [HbO] in the temporal detectors after each trial onset.

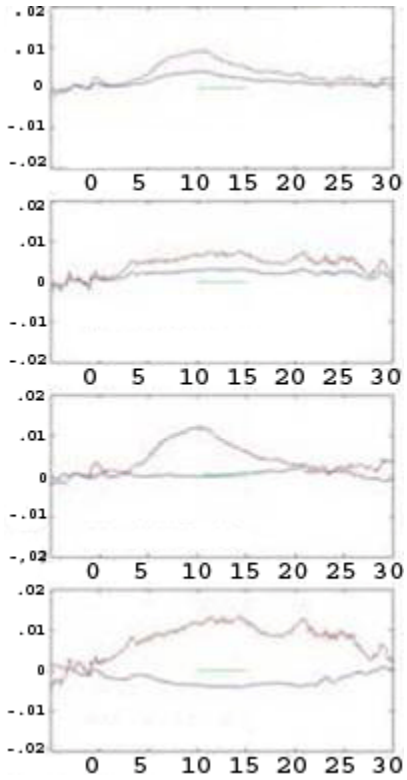

Figure 7B. Average of Individual Results from Figure 7A.

In the grand averages shown in Figure 8, after a period of low response, there is a strong increase in concentration beginning at approximately 10 seconds. In the temporal region, the change in hemodyamic concentrations relative to baseline is significant. The duration and sustained increase in temporal [HbO] following stimulus offset at 20 seconds suggests a unique response to facially produced speech stimuli. The occipital region shows an increasing concentration after stimulus offset, also suggesting a unique influence of coherence (e.g., of the sort that human faces producing speech have) on the hemodynamic response evoked.

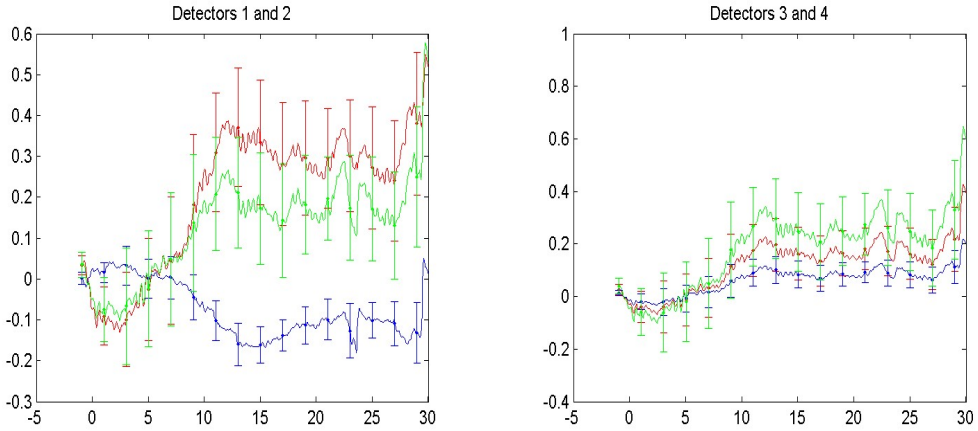

Figure 8. Grand Average for Audiovisual Only Trials in Exp. 2. Grand averages for temporal (left) and occipital [HbO] and [HbR] changes in the audiovisual condition.

Visual only trials.

Figures 9A and 9B show a non-significant change in [HbO] and [HbR] from baseline in the occipital region. The temporal data show a significant increase in [HbR] and a decrease in [HbO] that is not significant. These changes begin close after onset of stimulus, and appear to diminish quickly following stimulus offset. Results from the individual subject mirror those found in the grand average of all subjects in the visual-only trials (Figure 10). The results found for both the occipital and temporal regions are not in agreement with most neuroimaging studies, which show an increase in [HbO] and a decrease in [HbR] in response to stimuli. The cause for the atypical results is considered in the Discussion.

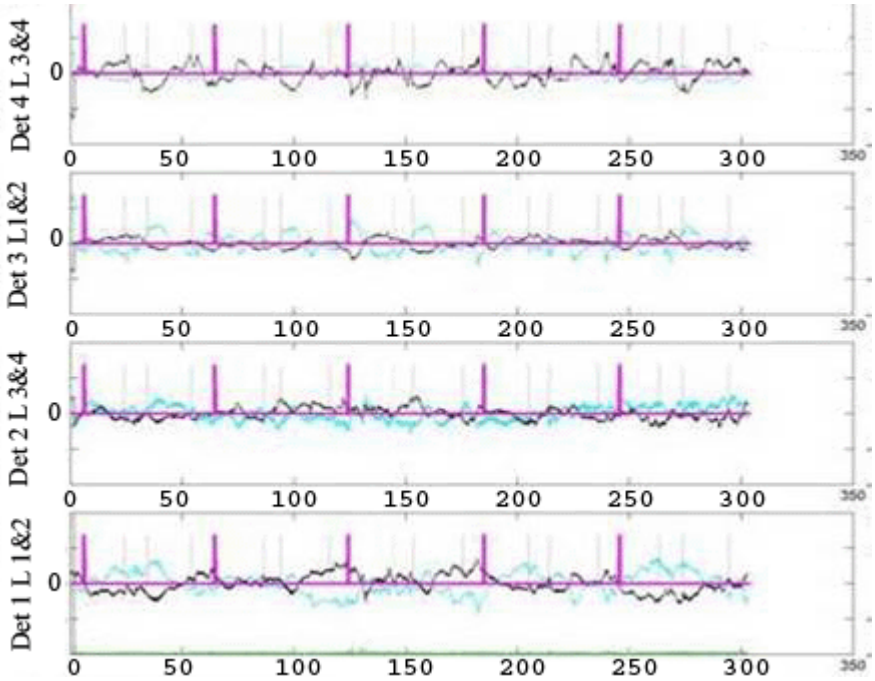

Figure 9A. Individual Results for Visual Only Trials in Exp. 2. Individual subject data for visual only condition of Experiment 2. The top two graphs show [HbO/830 nm] and [HbR/690nm] occipital area changes from an arbitrary baseline (time -2 to 0 seconds) over 300 seconds. The bottom two graphs show temporal area changes. Trials lasted 20 seconds, with 10-second inter-stimulus periods throughout the 300-second experiment.

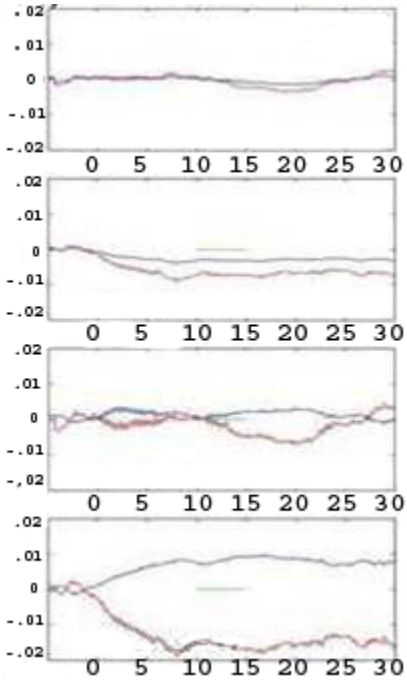

Figure 9B. Average of Individual Results from Figure The four graphs illustrate averages of the five corresponding trials from Figure 9A.

Grand averages for hemodynamic changes in the temporal and occipital regions across participants are shown in Figure 10. These results reflect the same methodological artifact discussed in Experiment 1. Reiterating, concentration changes due to stimuli are typically contrasted with a baseline measure taken at time –2 to 0 seconds from stimulus onset. However, in the visual only trials of both Experiment 1 and 2, the baseline measures are high due to an insufficient inter-stimulus interval between trials. Hemodynamic response remains elevated beyond the allotted 10-second silence period between trials. This errant baseline reading is then used in significance testing of post-stimulus activation averages. Because the baseline concentration is erroneously high, the post-stimulus concentration level is likewise erroneously low. This is seen in the depressed [HbO] of Figure 10. The converse is true for the [HbR]. It had levels that

were artificially low at baseline, and as they returned to the real baseline, the concentrations appear artificially high, as seen in the right graph of Figure 10. This issue will be dealt with further in the Discussion.

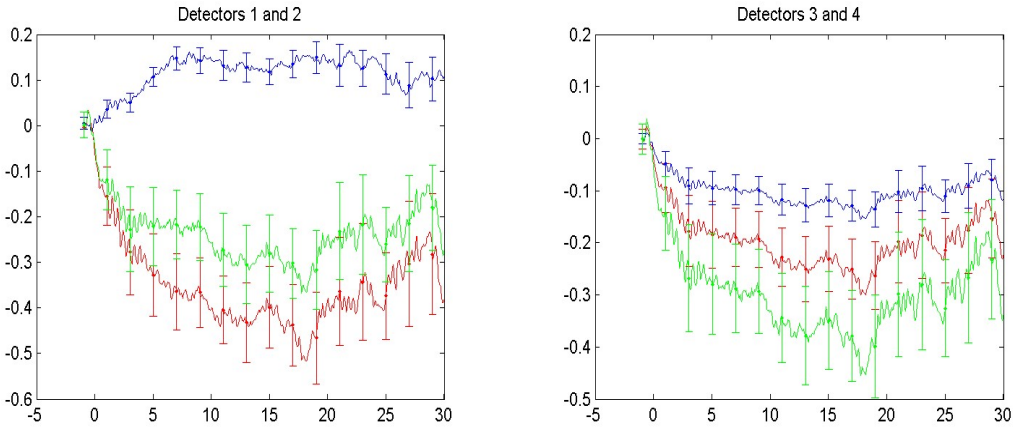

Figure 10. Grand Average for Visual Only Trials of Exp 2. Grand average of visual only condition of Experiment 2. X-axis is time in seconds. Visual stimuli were presented from 0-20 seconds. Y-axis is relative changes in HbO (dark line), HbR (medium line), and HbT (light line) concentrations. Detectors 1 and 2 were placed on the temporal lobe (detector 1 = anterior, detector 2 = posterior). Detectors 3 and 4 were placed on the occipital lobe (detector 3 = subjects left side, detector 4 = subjects right side.

Discussion

Recordings of hemodynamic response to the audiovisual (face) stimuli show patterns of response in the temporal region that are consistent with results from what other neuro-imaging studies have been done with infants in this age range. The grand average of hemodynamic change in the occipital and left temporal regions for audiovisual

trials show a signficant increase in [HbO] and decrease in [HbR] following stimuli presentation. These responses appear to be as substantial as those observed for the parallel condition in Experiment 1, though they are somewhat delayed relative to that set of findings. The source of this delay remains unclear though is something that merits further investigation. The stimuli used in the audiovisual trials (e.g., voice/face pairings) appear to elicit a strong neuronal response in the left temporal region, as seem in the large [HbO] increase. Language stimuli using human faces to convey the audio portion may aid in getting and maintaining infant attention during stimuli presentation. The influence that face/voice pairing has on occipital activation appears modest, as indicated by the slight increase in [HbO] as compared to the similar condition in Experiment 1 that had no face as part of the stimuli. The occipital areas increased concentration of [HbO] may be predicted from the exceptional interest infant's show toward human faces.

As in Experiment 1, the results of Experiment 2 were influenced by the lack of a sufficient baseline period. The time that was allotted between experimental trials was insufficient for infant hemodynamics to return to baseline. This manifested only in the visual-only condition, given the pronounced activation induced by the auditory stimuli in the audiovisual condition. Given that visual stimuli are presented in both conditions, we did not expect to see pronounced effects of the visual stimuli on the occipital region. The result was that all the immediately following trials (visual only) started with incorrectly high/low concentration levels (Figure 11). The concentration levels were still returning to baseline from the previous stimuli, and thereby gave incorrect concentration levels at the start of the next trial. These concentrations were then used in subsequent significance testing. Therefore, the data represented in Figures 4B and 9B is misleading. Ideally,

there should only be the influence of stimuli on the concentration levels. Instead, there is a physiological influence attempting to return the system to baseline, following stimulus offset.

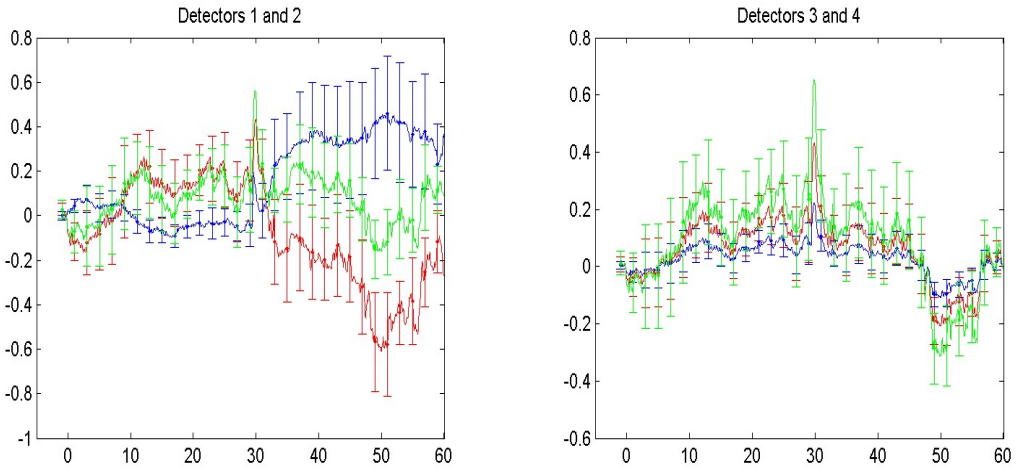

Figure 11. 60-second Analysis Showing Artifact in Exp. 2. Shows source of methodological artifact found in the visual only conditions. At the offset of the audiovisual trial at t = 20 seconds, the hemodynamic response continues through the inter-stimulus interval period, and into the next trial at t = 30 seconds (visual only). Nonetheless, the even more pronounced (though somewhat delayed) response in the temporal region in the audiovisual condition indicates at least some effect of perceptual coherence on neural processing, as indicated by the hemodynamic response.

CONCLUSIONS

The use of NIRS to study infant hemodynaimcis is a relatively new line of research. For this reason, these experiments attempted to replicate the results of two recent brain-imaging studies (Dehaene et al., 2002; Pena et al., 2003). Those studies used fMRI and optical spectroscopy to map language processing in infants. Here, NIRS was used to investigate the left temporal and occipital regions for language related hemodynamic activity. Evidence was found that supports the previous studies findings that the left temporal region is a language processing center. Additional intriguing evidence suggests that multi-modal speech stimuli provide a quantitatively unique hemodynamic response when compared to uni-modal speech. Through pilot studies and two experiments, NIRS proved itself to be an effective and independent source of neuro-imaging. The results obtained here demonstrate that NIRS is a powerful tool for investigating infant cerebral hemodynamics. To a degree, NIRS offers advantages where other neuro-imaging methods such as fMRI, PET, and EEG are lacking. fMRI and PET are best used in clinical settings where equipment risks are more acceptable. For example, PET scans require an injection of radioactive tracers before brain imaging can be done. This is not practical in a research setting where repeated trials are sometimes necessary. fMRI requires subjecting the infant to a magnetic field, and also requires that the infant be immobile. ERP provides excellent temporal resolution, but lacks adequate spatial definition. NIRS is a safe alternative imaging method that requires no radioactive materials or prohibitively expensive equipment. In addition, NIRS produces good spatial resolution (1-2 cm), affordability, and is safe for repeated use.

In the present study, NIRS was used to examine the left temporal and occipital regions of first year infants for hemodynamic response to “motherese” speech and visual stimuli. Compelling results were obtained that 1) support related work identifying the left temporal region as a language processing center by finding increased [HbO] in the speech conditions, and 2) suggest that multi-modal presentation of speech elicits a additional quality of response when compared to uni-modal presentation. In Experiment1, results from the left temporal region demonstrate a significant hemodynaimc response to audio recordings of speech, as compared to silence. Significant changes in [HbO] are seen shortly after stimulus onset, and decrease after stimulus offset. The grand average of temporal region activation in the audiovisual condition indicates a significant elevation in [HbO] and a modest decrease in [HbR]. This pattern of results is typical of results found in similar studies.

In addition to examining the left temporal region for activation, the occipital area was probed also. This area of observation functioned as a control for the speech measurements taken in the left temporal region, as well as a region of interest for hemodynamic measurement. Results from the audiovisual trials indicate a modest, but not significant increase in [HbO] and a slight increase in [HbR]. Typically accompanying an increase in [HbO] is a decrease in [HbR]. The results here showing a slight increase in [HbR] along with a relatively small increase in [HbO] suggest that source of activation was two-fold. First, the visual stimuli (computer animations) were presented during every trial. The results suggest that the visual stimuli appear to have been marginally, but continually engaging across the experiment, producing a slightly and consistently elevated concentration across the trials. It is possible that continual

exposure to the visual stimuli kept infant reaction at a near-constant level. This pattern can be seen in the grand average of these trials in Figure 3.

The visual only trials of Experiment 1 were influenced by a significant methodological artifact. Of the types of trials in the experiment (audiovisual and visual only), the audiovisual produced a larger and more sustained change in Hb concentrations. As such, there should have been a longer period between trials to allow the infants hemodynamics to return to baseline. In these experiments, 10 seconds was used as a between trial interval. However, hemodynamic response from the audiovisual trials continued beyond the 10-second inter-stimulus interval, and contaminated the visual trials. The result was that the baseline measure taken for the subsequent trials was artificially high, as it was still showing effects of the audiovisual trial. The solution is difficult in that a balance must be achieved in setting the duration of time between trials. Infant attention is a challenge to keep for extended periods. One way to maintain attention is to reduce the inter-stimulus interval. Reduce it too much, and the previous trials influence may bleed over into the next trial. The 60-second analyses of the audiovisual trials in Figure 6 and 11 clearly show a lingering, elevated Hb concentration, that continues beyond stimulus onset of the next trial.

Experiment 1 demonstrated the usefulness of NIRS as an effective and independent neuro-imaging method. Results shown here indicate that the hemodynamics of the left temporal region of first year infants is active in language processing. Related research suggests that language processing and representation formation occur in multiple modalities. In Experiment 1, the speech stimuli were presented uni-modally. In Experiment 2, the audiovisual stimulus was changed to include a face producing the

speech sounds. This inclusion added ecologic validity and temporal synchrony to the stimulus modalities. The presentation of modalities in synchrony has been shown to recruit and maintain infant attention more effectively than modalities out of synchrony. Thus, in this experiment, it was hypothesized that such stimuli would produce a response that was quantitatively different from the response obtained by uni-modal speech presentation.

The grand average of the audiovisual trials from Experiment 2 seen in Figure 8 demonstrates a response whose duration exceeds that found in the uni-modal presentation of Experiment 1. The concentration level appears to hold or rise in the face-included trials, while it is clearly decreasing in the animations trials of Experiment 1. This suggests the possibility of increased activation in the left temporal region due to synchronized modality presentation. An alternative explanation fro the increased activation is that infants are drawn to faces over computer graphics. Though the tendency was not quantified in these experiments, infants appeared to be more engaged with the face/voice, than the silent computer animations. Looking time data show no significant preference for trial type, though the average amount of time in Experiment 2, that infants looked at faces was slightly longer than the at the computer animations.

Though NIRS is a powerful imaging tool, it also has limitations, and methodological challenges. In part, the experiments performed here conducted to determine the useability of NIRS. There were significant obstacles to obtaining clean data. Foremost was the difficulty created by motion artifacts. Infant attention in the first year is a challenge to maintain. Pilot studies done before these experiments indicated that more engaging visual stimuli were needed to maintain infant attention. Coupled with a

10 second inter-stimulus interval that was silent and without visual, this caused the infants to become restless and fidgety. Such motion caused the NIRS probes to shift on the scalp, and to create artifact spikes in the concentration measurements. The headband into which the probes were sewn underwent a design revision during the pilot studies to reduce the artifacts from motion. The new design included small pads in the headband that helped grip the headband to the skin areas of the forehead. This design improved the data collection as it was more securely attached, and thus could tolerate slight head movements. Following these precautions, the data analysis included principle components analysis (PCA), which removed much of the co-variance due to motion. The PCA analysis was performed at a moderate level and as such removed what seemed to be the most appropriate amount of variance.

Another source of unpredictability comes from individual differences. The infants tested here ranged from 4-10 months. Significant development of neuronal, vascular, and cognitive brain features occur within this period. Though most of the infants studied were from the 6-9 month range, the outliers were tested and included as part of the useability investigations performed here. In addition to the age differences among the infants, anatomical differences also contributed to some uncertainty of probe placement. The left temporal optode was consistently placed on the T3 position of the International System. This is located directly above the ear. Though undoubtedly in the temporal region, it is unlikely that the underlying neuro and vascular anatomy would correspond between subjects. The occipital optode measurements seem more likely to be recording activity from the corresponding regions across subjects.

One possible next step in this line of research is to expand the modalities of speech and investigate the effect on infant attention. By varying temporal synchrony, speech and speaker variability, or motion, attempts could be made to identify brain regions localized for multi-sensory processing. Obtaining unique hemodynamic response patterns using NIRS and multi-modal stimuli may provide greater understanding of learning and memory as it relates to attention. It is also hoped that locating regions of multi-modal activation will add to an accumulating literature establishing NIRS as an effective neuro-imaging technology.

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APPENDIX

Hippo was very hot. He sat on the river bank and gazed at the little fishes swimming in the water. If only I could live in the water, he thought, how wonderful life would be. Then he jumped in and made a mighty splash.

One hot afternoon, the farmer and his animals were dozing in the barn. A warm breeze blew through the open doors. The only sound was the buzz-buzz of a lazy fly. Suddenly the buzzing stopped.

Robert was out riding his bike. He saw his friend Will by the old fence. Did you loose something?, he asked. I thought I saw a frog, said Will. I used to have a pet frog named Greenie. He'd wait for me by the pond near where I lived. He must miss me a lot.

Sometimes the chick and the other young penguins dig their beaks into the ice to help them walk up a slippery hill. They toboggan down fast on their fluffy bellies. The chick grows and grows. In a short while, he'll be a junior penguin.

While mama hangs the wash out and papa rakes the leaves, Oliver chases a big yellow leaf down the hill. He follows it under a twisty tree and all the way to the edge of the woods. From far away Oliver hears mama calling him. Oliver runs all the way home.

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