Cerebral Hemodynamic Response to Faces and Emotions in Infants at High Risk for Autism

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Sharon Elizabeth Fox

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ABSTRACT

The incidence of autism spectrum disorders (ASD) has risen alarmingly in the United States, and is now thought to affect approximately 1 in 110 live births. Early diagnosis and intervention is the only treatment proven effective in cases of autism, however the behavioral tests currently available cannot make this diagnosis until at least two years of age. A lack of normal attention to faces and abnormal face processing is a cognitive deficit common to nearly all individuals with autism spectrum disorder, and this deficit is likely present from a very early age. The primary goal of this dissertation is therefore to characterize the specific neural response of face processing in infants with near-infrared spectroscopy (NIRS), and to then apply these measures to the study of abnormal face processing in infants at high risk for autism.

In order to achieve these objectives, the work described herein aims to: 1) characterize the hemodynamic response to faces in normal infants at six months of age as measured by the Hitachi ETG-4000 functional Near-Infrared Spectroscopy (fNIRS) system; 2) Simultaneously measure orbitofrontal hemodynamic responses to social/emotional engagement and the response to faces in infants at high risk for autism as compared to low risk controls; and 3) Utilize a novel method of condition-related component selection and classification to identify waveforms associated with face and emotion processing in 6-7-month-old infants at high risk for ASD, and matched low-risk controls.

Our results indicate similarities of response waveforms, but differences in both the spatial distribution, magnitude, and timing of oxy-hemoglobin and deoxy-hemoglobin responses between groups. Our findings represent the first identification of neuroimaging markers of a functional endophenotype at six months of age that may be associated with high risk of ASD. These results support a model of altered frontal lobe structure through evidence of altered hemodynamic response and/or functional activity in the high risk infant group, and these changes may, in turn, contribute to the development of ASD in specific individuals.

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Chapter 1: Introduction to the Infant Sibling Project and Context of this Dissertation

1.1. The Infant Sibling Project

This dissertation describes a portion of a larger, collaborative study of risk markers for autism known as the Infant Sibling Project. In recent years, public concern has risen surrounding the apparent increase in the prevalence of autism spectrum disorder (ASD), with current estimates of a rate of one in 110 live births (Kim et al., 2011). Autism often goes unrecognized in children from disadvantaged backgrounds until they reach school age, though evidence has accumulated for the effectiveness of early intensive behavioral interventions for ASD (Dawson, 2008; Dawson et al., 2010). It is therefore increasingly necessary to develop methods for early diagnosis of autism – both for the purposes of studying those events correlating with the apparent onset of the disease, and for early intervention to improve cognitive outcomes. Zwaigenbaum et al. (2007) provide the rationale for employing prospective longitudinal studies of infants at high risk, usually defined on the basis of an older, diagnosed sibling. These infants, along with age-matched controls, are studied at a series of timepoints until 2-3 years of age, at which time approximately 20-30% meet criteria for an ASD (Ozonoff et al., 2011; Zwaigenbaum et al., 2005).

No studies to date have used neuroimaging methods to distinguish between high and low risk infants as young as 6 months of age, however studies of 4-6 month olds have found subtle differences in low-level visual processing (McCleery et al., 2007), as well as attentional or affective response to faces

(Cassel et al., 2007; Yirmiya et al., 2006). Both behavioral and imaging measures have shown promise as markers as early as 9-12 months of age (Bosl, 2011; McCleery, 2010). By 12 months, infants at risk show atypical patterns of object exploration, and can even show signs of autism as measured by standardized diagnostic behavioral tests (Ozonoff et al., 2008; 2011). These tests cannot be administered below one year of age, however, so our knowledge of the development of ASD during infancy must rely upon additional research tools.

It is important to note that differences between high risk infants and low risk controls may reflect familial traits, or endophenotypes (Szatmari et al., 2008), which do not necessarily predict the onset of the disease. The goal of the Infant Sibling Project is to identify a set of behavioral and brain measures that serve as a set of risk marker endophentypes during infancy. In addition, we hope that the profiles defined by aggregates of specific risk markers will differentiate between infants with and without clinical outcomes. Finally, the identification of endophentypes may inform personalized intervention programs aimed at halting disease development in infants at risk for ASD.

1.2. Overview of the Present Study

Over the past 15 years, mounting evidence has supported the human development of a face schema within the first few months of life (Carey & Diamond, 1994; de Haan and Nelson, 1997, 1999, & 2002; Mondloch, 2002). Face perception and discrimination is a key component of normal human cognitive development and may be significantly altered in cases of abnormal

development. It has been demonstrated, for example, that a lack of normal attention to faces, and abnormal face processing, are cognitive deficits common to nearly all individuals with autism spectrum disorder (Zwaigenbaum et al., 2007). Furthermore, it has been shown that deficits in face processing are present from an early age in autistic children and may be one of the first signs of ASD (Zwaigenbaum et al., 2007).

As part of the Infant Sibling Project, the primary goals of this dissertation are therefore to characterize and quantify the specific neural response of face processing in infants with near-infrared spectroscopy (NIRS), and to then apply these measures to the study of abnormal face processing in infants at high risk for autism spectrum disorders. The experiments described herein will characterize the infant response to faces using functional near-infrared spectroscopy, and then apply these findings to the study of face and emotion processing in the Infant Sibling Project. In addition, we will use novel methods of analysis to identify temporal waveforms associated with neural processing in each group, as well as the connectivity of these waveforms across brain regions. Our goal is to identify endophenotypes present in the high risk group at 6-7 months of age, which may later be combined with other markers of risk in the Infant Sibling Project as a whole.

Chapter 2: Current Neurodevelopmental Studies of Autism and the Role of Face Perception

2.1. Defining Autism Spectrum Disorders

The term "autism" was used in 1943 by Leo Kanner, a child psychiatrist, to define a disorder in which patients had difficulty with social interactions, difficulty processing and adapting to changes, good memory, developmental speech delay, sensitivity to sounds and other environmental stimulants, digestive issues, and good intellectual potential (Kanner, 1943). Over the subsequent decades, it was recognized that many children with autism were misdiagnosed on one extreme as "socially awkward," or on another, as mentally retarded. Further characterization of autistic traits led to the standardization of phenotypes that we now label as Autism Spectrum Disorders (ASD). In recent years, there has been an apparent increase in the prevalence of autism spectrum disorder, with current estimates of a rate of one in 110 live births (Kim et al., 2011). The diagnosis of autism is typically based on DSM-IV criteria, and standardized tools such as the Autism Diagnosis Observation Schedule (ADOS, Lord et al., 2000), and Autism Diagnostic Interview-Revised (ADI-R, Lord et al., 1994) are considered gold standards for research purposes. These tests are not standardized below the age of two years, however, which is currently the earliest age at which a definitive diagnosis of ASD can be made.

2.2. Clues to Autism Spectrum Disorders: Molecular to Anatomic

2.2.1. Genetics of Autism

The heritability of autism has led many investigators to explore the genetics of this disorder (Abrahams & Geschwind, 2010; Geschwind, 2011; Pinto et al., 2010). From these studies, it is clear that the genetic and molecular interactions involved in autism are complex, and it is unclear whether autism spectrum disorder (ASD) can be explained by single gene mutations or by multiple genetic or epigenetic interactions (Autism Genome Project Consortium et al., 2007; Cooper et al., 2011; Geshwind, 2011; Piggot et al., 2009; Weiss et al., 2009). Early twin studies revealed the heritability of autism to be more than 90%, and when only one identical twin is autistic, the other often has learning or social disabilities (Geschwind, 2011a). For adult siblings, the risk of having one or more features of ASD are estimated to be as high as 20-30% (Ozonoff et al., 2011), much higher than the risk in the general population (Kim et al., 2011; Zwaigenbaum et al., 2007). The distribution of autistic characteristics along a spectrum suggests the presence of "risk" alleles in the genome, which may interact with each other, or the environment, to cause a resulting phenotype. In addition, the effectiveness of early intervention for many individuals diagnosed with ASD suggests that the timing of these interactions throughout development may play a key role in the severity of the disease. Over thirty genes related to neural development have been implicated in the pathogenesis of ASD, and include SHANK3, PTEN, MET, neuroligins, and CNTNAP2 (Abu-Elneel et al., 2008; Autism Genome Project Consortium et al., 2007; Geshwind, 2009; Herbert,

2011; Mukamel et al., 2011; Pinto et al., 2010; Sanders et al., 2011; Scott-Van Zeeland et al., 2010; Smith et al., 2011; Voineagu et al., 2011;for recent review, see Geschwind, 2011a). Recent work has also implicated rare copy number variations (CNVs) on multiple chromosomes in the disease process of individuals with autism (Glessner et al., 2009; Pinto et al., 2010; Sanders et al., 2011; Smith et al., 2011). One such CNV, associated with the ubiquitin protein-ligase, has recently been used to create a mouse model with behavioral characteristics that mimic human ASD (Smith et al., 2011).

2.2.2 Histologic Findings

The development of high-resolution neuroimaging methods, as well as the post-mortem examination of autistic brains, has revealed a mixed array of abnormal histologic findings in individuals with ASD. Neuropathology findings have pointed to neuroinflammatory changes, as well as morphological changes in both neurons and glia during the development of autism (Pardo & Eberhart, 2007; Pickett & London, 2005). It has been suggested that astrocytes and microglia, which play important roles in neuroinflammatory and immune responses, may be overactivated and contribute to autistic neuropathology (Pardo, Vargas, & Zimmerman, 2005; Pardo & Eberhart, 2007; Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005). Yet another study found that cortical minicolulmns in Layer III of prefrontal, temporal, and cingulate cortex were more numerous in individuals with autism than controls, and contained smaller neuronal cell bodies (Casanova et al., 2006; Casanova, 2006). As Layer

III contains many commissural and connecting fibers it was suggested that this represents the proliferation of short-range fibers, perhaps with a reduction of long-range connections (Casanova, 2007). Additional studies have noted a decrease in the number of Purkinje cells within the cerebellum (Bailey et al., 1998), as well as more densely packed neurons in the frontal lobes (Bauman & Kemper, 2003; Courchesne et al., 2011).

2.2.3. Gross Anatomic Findings

Imaging methods have also proven useful in the study of brain structure of individuals with ASD throughout development. Autistic children between two and four years of age were found to have an increase in total brain volume, as well as a specific enlargement of dorsolateral and medial frontal cortex as compared to controls (Brambilla et al., 2003; Carper, Moses, Tigue, & Courchesne, 2002; Hardan et al., 2006; Hardan, Muddasani, Vemulapalli, Keshavan, & Minshew, 2006; Schumann et al., 2010). Greater head circumference was confirmed using direct measures in a large, prospective sample of high risk infants (Zwaigenbaum & Stone, 2008), suggesting that this atypical head growth may be a marker for ASD during infancy. This difference does not appear in adolescents (Courchesne et al., 2001), or in fetal ultrasound records (Hobbs et al., 2007), suggesting that important markers of the disease process may be present only at specific developmental timepoints. Volume increases in autistic adolescents have been found, however, in the right fusiform, right temporo-occipital, and various frontal

regions (Hardan et al., 2006; Haznedar et al., 2006; Hollander et al., 2005; Langen, Durston, Staal, Palmen, & van Engeland, 2007; Waiter et al., 2004). Other studies have suggested an increased basal ganglia volume, and reduced corpus callosum and medial temporal lobe volumes (Eigsti & Shapiro, 2003; Palmen & van Engeland, 2004). A rightward hemispheric asymmetry bias has also been implicated both structurally and functionally in autism, with higher order association and language areas most frequently described (Herbert et al., 2002; Herbert et al., 2004; Herbert et al., 2005). Such volumetric measurements can be difficult to interpret, however, as both the age at imaging, and the method of calculating regional volume, differs widely across studies.

2.2.4. Structural Connectivity and Synaptic Abnormalities

It is clear that autism is a structurally complex disease, and as a developmental disorder, it may involve multiple regions of the brain in a dynamic process of pathological change. The changes that have been observed may thus be due to a reorganization of brain networks (Muller et al., 2011), such that a focus on localized features may be insufficient to reflect the entire process of the disease. Mechanistically, it has been proposed that early overgrowth through aberrant neuronal migration and synaptogenesis, followed by errors in synaptic potentiation or neuronal apoptosis, may result in localized overconnectivity, whereas long-range connections, such as those integrating the frontal lobe with other structures in the brain, are underdeveloped (Courchesne et al., 2011;

Hendry et al., 2006; C. Schmitz & Rezaie, 2008). While both white and gray matter abnormalities have been found in autism (for reviews see Amaral, Schumann, & Nordahl, 2008; Brambilla et al., 2003; Eigsti & Shapiro, 2003), the heterogeneity of structural findings likely reflects differences in the timing of neuronal development, or the formation of specific synaptic connections and compensatory networks. As ASD is a phenotypically heterogenous disorder, a broad range of anatomical abnormalities is not inconsistent with a neuroanatomic correlate of disease.

2.2.4. Correlations between Structure and Function in Autism

While anatomical studies provide the physical clues to diseases along the autistic spectrum, these findings require correlation with cognitive profiles in order to develop an understanding of the etiology of ASD. Behavioral and functional imaging measures have therefore proven necessary in both the characterization and study of autism as a developmental disease. For example, Bigler et al. (2007) found a positive correlation between superior temporal gyrus volume and behaviorally assessed receptive language in controls, but not in ASD. Frontal regions have often been studied with morphometry, and performance on a reaction time task was positively correlated with frontal lobe volume and gray matter thickness in autistic individuals (N. Schmitz, Daly, & Murphy, 2007). Combined behavioral and structural measures have also been used to explore social cognitive function, with amygdala volume correlating with

social function in individuals with autism (Dziobek, Bahnemann, Convit, & Heekeren, 2010), and a reduction in frontal mirror neuron networks corresponding to greater severity of disease (Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2006).

Given the specific constellation of cognitive features that describe autism, as well as the wide distribution of affected brain regions, it has been hypothesized that autism is caused by an inability to integrate perceptual features into a whole (Frith & Happe, 1994). This is a theory known as "weak central coherence," and according to this top-down cognitive model, individuals with ASD will perform at a normal, or perhaps above normal, level on tasks involving localized perceptual processes, but poorly on tasks requiring perceptual integration. More recently, it has been suggested that deficits in integration may be an outcome of increased local processing (Happe & Frith, 2006). Possible anatomical explanations for this theory include deficient magnocellular pathways (Milne et al., 2002), right hemisphere impairments (Herbert et al., 2005; Isler, Martien, Grieve, Stark, & Herbert, 2010; McCleery, Akshoomoff, Dobkins, & Carver, 2009), dorsal visual pathway deficits (Pellicano, Gibson, Maybery, Durkin, & Badcock, 2005), or increasingly supported models of altered connectivity (Courchesne, Redcay, Morgan, & Kennedy, 2005; Hendry et al., 2006; Isler et al., 2010; Just, Cherkassky, Keller, & Minshew, 2004; Koshino et al., 2005; Muller et al., 2011; Scott-Van Zeeland et al., 2010). Brock et al. (2002) have proposed that reduced connectivity would result in deficits in synchronization between areas necessary for extracting coherence from

information. Similarly, Happe and Frith (2006) have suggested that "failure of early neural pruning" could cause a reduction in tuning by higher cognitive functions. In support of this theory, examination of long-range connections, such as the white matter tracts of the corpus callosum, has revealed decreased volume (Barnea-Goraly, Lotspeich, & Reiss, 2010; Casanova, 2007). At the same time, both examination of white matter tracts (Herbert et al., 2005), and cortical thickness (Hardan, Muddasani et al., 2006), have suggested a relative overgrowth of cortico-cortical short tracts as compared to long tract fibers. Likewise, diffusion tensor imaging studies of children with autism have indicated a disruption of white matter tracts in the anterior cingulate, corpus callosum, and prefrontal regions (Alexander et al., 2007; Barnea-Goraly et al., 2010; Lange et al., 2010). Further studies of functional connectivity are required, however, to determine whether these differences in white matter tracts are a cause or effect of the development of ASD.

2.3. Infant Face Perception

2.3.1. Face Perception as a Developmental Process

One of the principle characteristics of ASD is difficulty with social interactions, which is manifested by such behaviors as reduced eye contact, decreased attention to faces, and problems with responses to emotional cues (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998). Retrospective studies of home videotapes of first birthday parties of infants who were later diagnosed with autism reveal an early failure to actively attend to other people's faces (Osterling

& Dawson, 1994; Werner, Dawson, Osterling, & Dinno, 2000). Impairments in face processing are well documented in autism (Dawson, Meltzoff, Osterling, & Rinaldi, 1998; Dawson et al., 2002; Dawson et al., 2004; Dawson, Webb, Carver, Panagiotides, & McPartland, 2004), and are likely to present early in the development of the disease. An understanding of the typical infant development of face expertise is therefore necessary to study these effects in cases of ASD.

Faces are common, yet unique and highly complex visual stimuli in our environment from birth, producing both verbal and nonverbal communication, as well as information about emotion and identity. Human adults can rapidly classify a variety of facial features (Bruce & Young, 1986; Bruce, 1986; Valentine & Bruce, 1986), and slight changes in features related to emotional intent are universally recognized (Ekman, 1993). The human development of expertise in face processing appears to occur rapidly over the first few months of life, with several studies supporting the theory that infants as young as 3 months of age have a face schema (Morton & Johnson, 1991). For example, Mondloch et al. (2002) and Carey and Diamond (1994), demonstrated the presence of "face inversion effects" in early infancy. Turati et al. (2004) demonstrated that 4-month-olds process faces differently when the faces are upright as opposed to when they are inverted. Event-related potential (ERP) studies in 3, 6, and 12 month old infants have shown that cortical activation differs between upright and inverted faces (de Haan & Nelson, 1997; de Haan & Nelson, 1999; de Haan, Pascalis, & Johnson, 2002; de Haan, Johnson, & Halit, 2003), suggesting the development of a face schema by 3 months of age.

The neural mechanisms by which infants learn to attend to faces is a topic of much research and debate. It has been hypothesized that infants are "innately" attracted to stimuli that resemble faces, and that subcortical signals cause newborns to begin to orient towards face-like patterns (Johnson, 2005; Morton & Johnson, 1991). Alternatively, it has been theorized that faces contain optimal low-level features for visual development, and face expertise results from a tuning of the visual system to such stimuli (Kleiner & Banks, 1987). Finally, it has been suggested that the rise of face expertise results from an "experience-expectant" model (Nelson, 2001), similar to language development, in which cortical regions have the potential to become specialized for face processing, and are tuned and refined during specific developmental windows.

2.3.2. Infant Processing of Face Identity

Face perception involves the integration of many pieces of visual information, including the features of a face that constitute individual identity. This element of face processing appears to be present as early as four days of age, when infants have shown signs of discriminating their mothers' faces from strangers' (Pascalis, de Haan, Nelson, & de Schonen, 1998). By three months of age, facial recognition of the mother extends to multiple viewpoints – a perceptual ability that challenges complex computer algorithms (B. J. Balas & Sinha, 2007; Pascalis et al., 1998), and infants begin to discriminate between features of face that signify both race (B. Balas, Westerlund, Hung, & Nelson Iii, 2011) and gender (Quinn, Yahr, Kuhn, Slater, & Pascalils, 2002; Quinn et al.,

2008). Soon after these abilities are gained, it has been suggested that face processing follows a trajectory of "perceptual narrowing" (Nelson, 2001). Perceptual narrowing refers to the fact that by about six months of age, infants are able to distinguish among a variety of facial categories, however by nine months, this skill is only retained within categories in which the infant has significant experience. For example, six-month-old infants can successfully discriminate identities of monkey and human faces (Pascalis, de Haan, & Nelson, 2002). By nine months of age, however, infants cannot differentiate between the monkey faces, while retaining the ability to discriminate among the human face category with which they have significant experience (Pascalis et al., 1998; Pascalis et al., 2002). During the process of perceptual narrowing, and perhaps contributing to it, is a shift in the overall mechanism of face perception. Face perception appears to begin as a "featural" process, in which individual elements of a face are separately analyzed, and develops over infancy into relational face processing, which utilizes relations between core features of faces (Carey & Diamond, 1977; Carey & Diamond, 1994; Gathers, Bhatt, Corbly, Farley, & Joseph, 2004; Joseph et al., 2006). The perception of face identity thus becomes a complex discrimination among featural relationships, rather than comparisons between individual components of a face.

2.3.3. Infant Processing of Facial Emotions

In addition to the development of face recognition, infants also learn to discriminate between specific emotions in faces (de Haan & Nelson, 1997; de

Haan, Belsky, Reid, Volein, & Johnson, 2004; Nelson & Dolgin, 1985; Nelson & Salapatek, 1986). Newborns show evidence of being able to discriminate among happy, sad and surprised faces (Field, Woodson, Greenberg, & Cohen, 1982). Happy emotional expressions appear to be the most consistently differentiated from neutral faces within the first six months of life (de Haan et al., 2004; Farroni, Menon, Rigato, & Johnson, 2007; Leppanen, Moulson, Vogel-Farley, & Nelson, 2007). It has been hypothesized that during the first few months of life, infants may learn to discriminate among the expressions that they are frequently exposed to, such as smiling, and cannot yet discriminate among unfamiliar expressions (Farroni, Menon et al., 2007). By 7 months of age, however, infants show the ability to consistently discriminate fearful faces (Nelson & Dolgin, 1985) and demonstrate longer fixations towards this emotional expression (Leppanen et al., 2007; Leppanen, Richmond, Vogel-Farley, Moulson, & Nelson, 2009; Peltola, Leppanen, Vogel-Farley, Hietanen, & Nelson, 2009).

2.3.4. Neuroimaging and the Study of Infant Face Perception

Event-related potentials (ERPs) have provided a relatively easy and noninvasive means of imaging the neural signatures of face processing in infants. Two components, the N290 and the P400, are infant responses thought to be related to the adult N170 in terms of face-responsiveness. The N290 and P400 have been linked to the featural analysis of faces, and both show an adult-like effect of inversion at about 6 months of age (de Haan et al., 2003; Halit, de Haan, & Johnson, 2003), while the P400 shows latency differences specific to

faces in infancy. The Nc component appears to be involved in facial recognition, suggesting a difference between the mother and stranger faces at six months of age (de Haan & Nelson, 1997). At the same time, the Nc component also appears to be involved in the discrimination of fearful and happy faces (T. Grossmann & Johnson, 2007; Leppanen et al., 2007; Leppanen et al., 2009). While ERPs reveal differences in neuronal activity that correlate with cognitive function, they are limited in their ability to localize that activity to specific regions of the brain. It can therefore be difficult to determine whether the differences seen between infants and adults are related to local change in neural responsiveness and connectivity, or developmental shifts in the location of face processing.

Near-infrared spectroscopy (NIRS) is a relatively new neuroimaging technique that is particularly well-suited to the study of infants (for a full explanation of NIRS methods, refer to **Appendix A**; Lloyd-Fox, 2010 for review). The NIRS technique is based upon measurement of hemodynamic response to neural activity, and can therefore be likened to functional MRI. NIRS was first successfully used to examine face processing in infants by Taga et al. (2003), who demonstrated a response to faces in babies as young as two months of age. Face processing responses as measured by NIRS have tended to show a right-sided laterality (Honda et al., 2010; Nakato et al., 2011; Otsuka, Nakato, Kanazawa, & Yamaguchi, 2007), and have demonstrated responses to inversion consistent with those found in ERP studies (Otsuka et al., 2007). Studies of infants' neural responses to social and non-social stimuli have also included

examination of face responsiveness, and suggest that right postero-lateral regions may show greater responses to social face stimuli as opposed to the non-social stimuli (Lloyd-Fox, Blasi, Volein, & Everdell, 2009). Further studies are required to fully characterize the infant response to faces using NIRS, and the localization and examination of this response are therefore one of the primary goals of this project.

2.4. Atypical Face Processing in Autism

As previously mentioned, deficits in face processing are a common feature of autism (Carver & Dawson, 2002; Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Dawson et al., 2002), suggesting that the developmental processes previously described are perturbed during infancy. Significant effort has been made to understand the nature of these deficits in individuals with ASD, as the specific mechanisms affected may be the earliest indicators of the disorder (Carver & Dawson, 2002; Dawson et al., 2004; Schultz et al., 2000; Schultz, 2005; Zwaigenbaum et al., 2005), and may also signify means for early intervention. The face processing deficits reported in ASD include both difficulties in recognizing facial identity (Boucher & Lewis, 1992; Boucher, Lewis, & Collis, 1998; Klin et al., 1999), as well as developmentally abnormal featural processing of faces (Behrmann et al., 2006; Gauthier, Klaiman, & Schultz, 2009; Schultz et al., 2000). In addition, it has been shown that autistic children have difficulty processing facial emotions (Celani, Battacchi, & Arcidiacono, 1999; Corbett, Carmean, Ravizza, Wendelken, Henry, Carter, & Rivera, 2009a; Gross, 2004;

Monk et al., 2010; Pelphrey et al., 2002). The face processing differences noted in ASD have been linked to neural function and anatomy through a variety of imaging methods. ERP studies have shown that the scalp topography of face sensitive ERP components is less lateralized in individuals with ASD as compared to controls (McCleery et al., 2009). In addition, both children and adults with ASD show shorter latencies for objects compared to faces for the N170 component (Webb & Nelson, 2001). Hemodynamic responses, as measured by fMRI, have shown reduced right fusiform response to facial recognition (Dawson et al., 2002; Hadjikhani et al., 2004; Pierce, Muller, Ambrose, Allen, & Courchesne, 2001; Schultz et al., 2000). In addition, the superior temporal sulcus, associated with the social processing of faces, has been shown to be hypoactive in autistic individuals (Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007; Pelphrey, Morris, & McCarthy, 2005). Whether these findings are the result of the primary disease process, or effects of other neurological abnormalities, remains to be determined, and requires further efforts to understand how these differences begin to emerge in early development.

2.5. Conclusions

Face identification and the recognition of facial expressions are key processes in typical human cognitive development. Evidence suggests that these abilities begin to develop shortly after birth, and are constantly tuned over the course of infancy. Furthermore, it has been shown that impairments in face

processing are common among individuals with ASD, and may be the first sign of abnormal development. As it has been suggested that the abilities to discriminate facial identity and positive emotions are present by 5-7 months of age (de Haan & Nelson, 1997; Nelson, 2001; Leppanen, 2007), and perceptual narrowing is present by nine months of age, the focus of this project will be on the 5-7 month age range. We will characterize the response to faces in this infant population using NIRS, and will then determine whether differences in face recognition and face emotion processing can be seen at this early age in a population at high risk for autism.

Chapter 3: Localization and Characterization of the Infant Hemodynamic Response to Faces Using Near-Infrared Spectroscopy

Abstract:

Abnormal face processing is a cognitive deficit common to many individuals with autism spectrum disorder, and this deficit is likely present from a very early age. In order to examine this effect in infants at high risk for autism, the first aim of this project was to characterize the hemodynamic response to faces in typically developing infants at 5-6 months of age as measured by the Hitachi ETG-4000 functional Near-Infrared Spectroscopy (fNIRS) system. Recent work in early human development has utilized near-infrared spectroscopy (NIRS) to reveal the brain areas involved in face processing. The present work sought to extend this pursuit by examining the face inversion effect in infancy. We hypothesized that specific face-responsive brain regions could be isolated with NIRS in response to the face inversion effect at five months of age. The data from fifteen subjects were analyzed using a paired t-test to measure the hemodynamic response to the face inversion effect at each channel location. As in previous reports, inter-hemispheric differences were observed between the upright and inverted face condition. The group pattern of channel activation differed slightly from reported adult results, and with a novel use of Bayesian inference and random walk state space modeling, we were able to produce the first characterization of the infant hemodynamic response to an upright face condition as both laterally localized in the right hemisphere, near the posterior suprasylvian gyrus, and with a predictable and relatively short (2s) latency, and peak response (2-3s following stimulus removal). These results provide new information distinguishing the way infants may process faces from that of adults. These differences may be due to a developmental shift from feature-specific face representation towards the use of featural relationships in face processing, which is evidenced through distinct patterns of cortical activity.

3.1. Introduction

The perception and recognition of faces is an important part of human cognition, and the development of this faculty is hypothesized to be impaired in children presenting with autism spectrum disorder (ASD) (for review see de Haan, 2008; Righi & Nelson, in press). In order to understand the development of atypical face processing in ASD, it is necessary to first examine the aspects of face recognition and encoding that are present during typical development. One example of an early development in face processing is the "face inversion effect" (FIE), which reflects the decrease in face recognition performance in adults when faces are presented upside-down (Yin, 1969). Over the past decade, several studies have demonstrated the presence of face inversion effects in infancy (Mondloch et al., 2002; Turati et al., 2004). Turati et al. (2004) determined that as early as 4-months of age, faces are processed differently when presented upright as opposed to inverted. This effect allows inverted faces to be used as comparison stimuli that are perfectly matched to upright faces in all of their low-level visual properties. Event-related potential studies in 3, 6, and 12 month old infants have shown that cortical activation differs between upright and inverted faces (de Haan and Nelson, 1997, 1999, & 2002; Halit et al., 2003). Some have suggested that the pronounced effect of face inversion is due to the interference of inversion with the processing of visual relationships among facial features (Carey & Diamond, 1994; Mondloch, 2002; Nakato et al., 2009). Whether the processing of relational information changes over development is still a topic of much debate (Itier & Taylor, 2004; Mondloch, 2002), and one that can currently

be investigated with modern neuroimaging techniques. Gathers et al. (2004) used functional magnetic resonance imaging (fMRI) to show that 5-8 year-old children do not show face-preferential activation in the fusiform gyrus as adults do, but rather activation in a more posterior visual association area in the lateral occipital lobe. It has been suggested that an anterior shift in face-processing regions may reflect a developmental change from featural face processing, in which individual elements of a face are separately analyzed, to relational face processing, which utilizes relations between core features of faces (Carey and Diamond,1977; Gathers et al., 2004; Joseph, 2006).

Near-infrared spectroscopy (NIRS) has been increasingly used to examine face processing in both adults and infants. Csibra et al. (2004) utilized a two-channel NIRS system to detect differences in the occipital processing of face and non-face stimuli. In addition, Taga et al. (2003) demonstrated significant inter-hemispheric differences in the 2-4 month-old response to face-like stimuli. Such effects have been shown to be dominant in the right hemisphere, and have served as a particular focus in studies utilizing NIRS to examine the earliest development of face processing (Otsuka et al. 2007; Honda et al., 2009; Nakato, E., et al., 2009). The FIE can be elucidated with NIRS as the differential hemodynamic response obtained from visual stimuli of upright versus inverted faces. Otsuka et al. (2007) demonstrated significant inter-hemispheric differences on the effect of face inversion, with greater change in total hemoglobin concentration in right lateral regions of the brain. This initial study, however, did not assess the timecourse of the hemodynamic response to upright and inverted

faces at individual channels, and it was noted that a posterior placement of the probes might in fact be more amenable to localizing specific regions involved in upright as compared to inverted face processing. The purpose of the first experiment was therefore to validate the location of the normal infant hemodynamic response to faces using NIRS, and to characterize the timecourse of these responses across individual channels. As future studies involving infants at high risk for ASD would begin at six months of age, we focused on the 5-6 month age range, and hypothesized that we would localize posterior regions specifically responsive to the FIE.

3.2. Research Design and Methods

3.2.1. Participants

Fifteen healthy 5-month-old infants (7 male, 8 female) (mean age = 5.38 months, age range: 5.02 to 6.13 months) participated in the study. An additional 15 infants were excluded from the analysis due to insufficient data (10 could not complete the required number of trials due to fussing, 3 did not maintain sufficient fixation for inclusion of trials, and 2 had data in which significant motion artifacts precluded the use of the required number of trials). This attrition rate is similar to that reported in other studies of infants involving NIRS (Taga, 2003; Otsuka et al., 2007). All infants included in the experiment were: 1) born after 36

weeks gestational age, 2) born weighing more than 2500 grams, and 3) born without a known neurological or uncorrected visual abnormality.

Experiments were conducted under approval by the Institutional Review Board at Children's Hospital Boston and Massachusetts Institute of Technology, and in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents of all infant participants.

3.2.2 Experimental Procedure

3.2.2a. Stimulus Presentation

Face stimuli consisted of 20 chromatic images of female faces presented in a 13cm x 13cm square against a standardized gray background. Ten chromatic images of non-face objects with size, contrast and luminance similar to those of the face images were presented as baseline visual stimuli. Face stimuli were presented in blocks of five different, randomly chosen faces for each condition (upright or inverted). The same faces were presented in both the upright and inverted conditions. Each stimulus presentation lasted approximately 800 milliseconds with a variable inter-stimulus interval of approximately 200ms in which a blank screen was present, yielding a total trial time of five seconds for each test block (**Figure 3.1A**). These blocks were followed by blocks of five, randomly chosen non-face object stimuli, which lasted for approximately two seconds each, ensuring that a minimum of ten seconds elapsed between presentations of face stimuli. Ten of the fifteen participants included in the

analysis viewed a presentation order of 20 face orientation blocks that was pseudo-randomized within every ten blocks to include an equal number of upright and inverted face presentations. An additional five participants viewed a different presentation order that was pseudo-randomized to include at least eight blocks of each face orientation, but was not counterbalanced. The order of face orientation seen by each subject differed due to individual differences in subject looking time, but each subject viewed at least five blocks of each condition.

3.2.2b. NIRS Task Procedures and Equipment

Infants were seated on a parent's lap throughout the experiment. Infants passively observed the stimuli. Visual stimuli were presented on a 17-inch Tobii T-120 monitor at a distance of approximately 60cm from the infant. Video recordings were used to monitor eye gaze throughout the experiment, and to present stimulus blocks only while infants were attending to the display. Trials were excluded if infants viewed fewer than four of the five faces presented.

A Hitachi ETG-4000 NIRS system with 24 simultaneously recording channels was used to collect hemodynamic response during stimulus presentation. Details of the NIRS system can be found in **Appendix A.** A soft cap was designed for infants in order to affix the NIRS optical probes to the head (**Figure 3.1B**). The flexibility of the silicone support, as well as the contour and design of the cap, allowed for a posterior placement of the probes that was not technically feasible in prior studies (e.g., Otsuka et al., 2007).

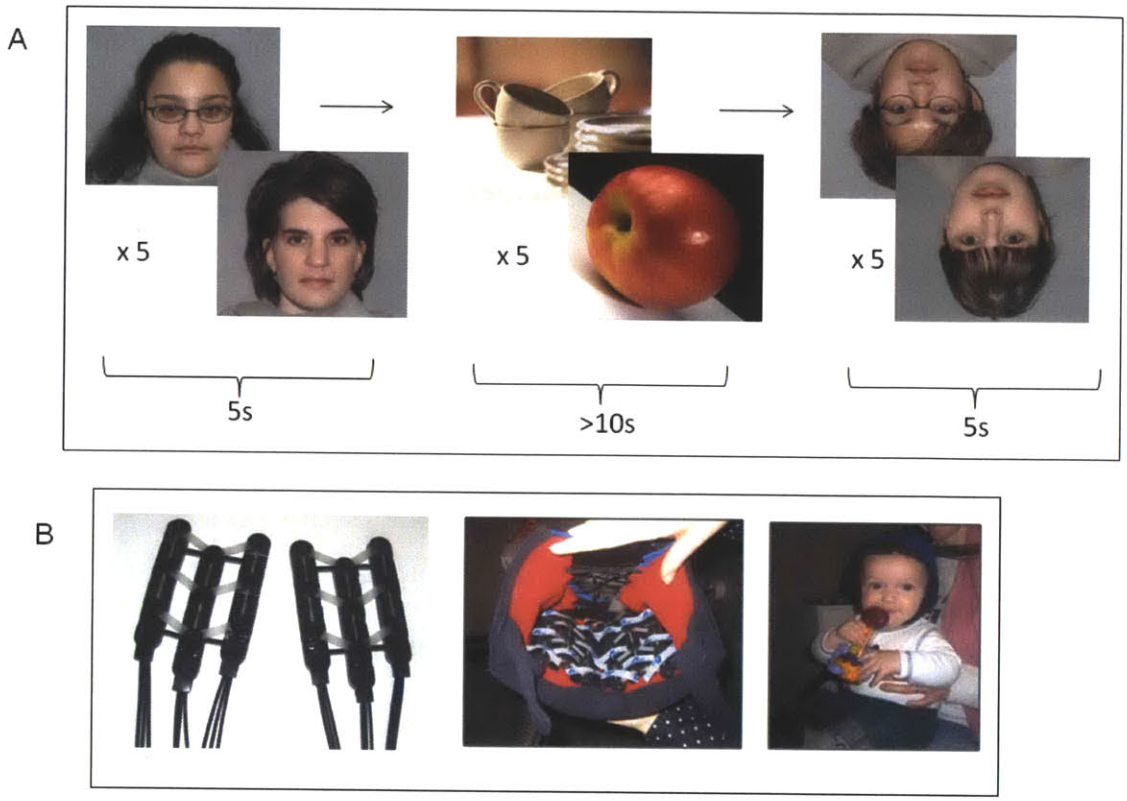

Figure 3.1: Stimuli and Paradigm. A) Blocks of five faces (upright or inverted condition) lasted five seconds each, and were interspersed with blocks of five objects lasting for more than ten seconds. **B)** The chevron array of probes placed into a custom designed infant cap.

Based on the light intensity detected through each channel, relative concentrations of oxygenated and deoxygenated hemoglobin were calculated from absorbance at each wavelength using the modified Beer–Lambert law. This conversion, as well as further data analyses, were implemented through a customized Matlab script (version 7.6, Mathworks Inc., Natick, MA, USA).

3.2.3. NIRS Data Analysis

For each participant, trials were included if at least four of the five faces in a given test block were viewed. Only infants who viewed five blocks of each test condition (upright and inverted) with no significant artifacts after initial filtering were included in subsequent analyses.

Timeseries corresponding to oxy- and deoxy-hemoglobin values were first processed using a 5^{th} order Butterworth filter between 0.01 and 1.0Hz, and additional artifacts were identified and extracted if the raw signal exceeded a value of 4.95, or if total hemoglobin change exceeded 0.3 mM*mm within a 0.7s time window. For each subject, the data were parsed into ten second time windows with 0.1s time resolution beginning at stimulus onset, and ending five seconds after the end of the stimulus presentation. Ten second time windows corresponding to object presentation were also created, and were used to create a visual baseline condition. Face time windows were corrected to a baseline value at the onset of each stimulus. The correction to baseline for individual time windows was based upon the average oxy- and deoxy-hemoglobin values beginning three seconds before each face presentation, and ending at onset of

each trial. This value was subtracted from all values at each timepoint within a trial to produce a timecourse originating from a baseline value. This correction allowed standardization of the response at the onset of each stimulus, and thus allowed for averaging across trials, and an accurate comparison between conditions. Following this correction, trials were grouped by test condition and averaged to obtain a mean value for each timepoint at individual channels. Mean values at each timepoint from each subject were then pooled to produce a group mean value by condition at each channel and timepoint. Channels from individual subjects with low oxy-hemoglobin signal-to-noise (Mean/Standard Deviation < 1.0) were excluded from the group average. An oxy-hemoglobin response was defined as the difference between the maximum value in the latter five seconds of each ten second time window and the minimum value in the first two seconds of a trial. This was based upon methods employed by Otsuka et al. (2007), and measurement of fNIRS responses in infants (Taga et al., 2003). This method accounted for the magnitude of the change in oxy-hemoglobin response if an initial dip were to occur at stimulus onset. Statistical analyses were conducted on the group average to determine the location and timecourse of responses to faces, as well as responses specific to the FIE.

3.3. Results

3.3.1. Analysis of Face Processing by Channel Location

Fifteen infants with data from at least 10 test blocks (5 or more upright blocks and 5 or more inverted blocks) were included in the group average (mean number of upright blocks = 7.8, mean number of inverted blocks = 6.1). An initial set of analyses aimed to identify channels that responded maximally to upright faces, as compared to the baseline. To this end, the responses to upright faces were averaged and compared with the baseline object condition using a paired t-test (**Figure 3.2**, T-value map). Significant face-specific activation was found in occipital areas bilaterally, as well as clustered in a postero-lateral region of the right hemisphere near the approximate location of the posterior suprasylvian gyrus (**Figure 3.2**, channels circled in white). The same seven channels showing significant face-specific activation demonstrated a significant difference in oxy-hemoglobin response for the upright and inverted face conditions (**Figure 3.2**, channels circled in white). Channels significant for face inversion effects were found in both hemispheres (five channels in the right hemisphere, two in the left hemisphere). The average change in oxy-hemoglobin concentration in response to upright faces in these channels was 0.162 mM*mm (SE = 0.025). The mean difference in oxy-hemoglobin response between the upright and inverted face conditions was 0.083 mM*mm (95% CI [0.011, 0.15]; p=0.0245, Bonf. corrected). No significant difference in deoxy-hemoglobin response was found between the two conditions. Subsequent analyses of the FIE focused on these seven channels showing significantly higher responses for upright as compared to inverted faces.

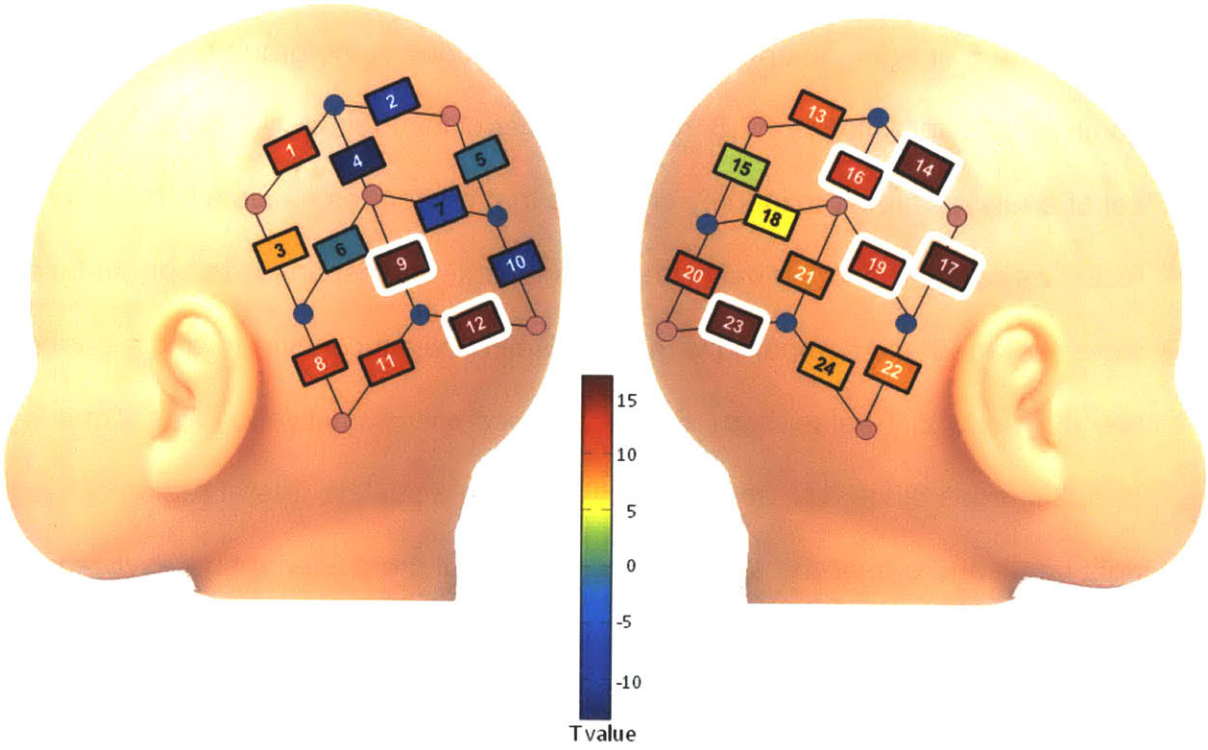

Figure 3.2: A schematic representation of our probe placement is shown on the posterior infant head (red dots = emitter, blue dots = detector). Square boxes between each emitter and detector indicate a channel, and are numbered for each of the 24 channels. Colors within the channels indicate a T-value map across fifteen subjects comparing oxy-hemoglobin response to the upright face condition as compared to the object baseline at each channel location (color bar, $p=0.05$, Bonf. corrected). Channels circled in white indicate those significant for differences in response between the upright condition and object baseline, and are also significant for differences in oxy-hemoglobin response to the upright versus inverted face conditions ($p<0.05$, Bonf. corrected).

3.3.2. Examination of the Face Response using Bayesian Methods

In order to characterize the hemodynamic response over time for these seven channels, the combined oxy-hemoglobin timeseries describing the FIE (upright and inverted conditions) were analysed using Bayesian Markov Chain Monte Carlo (MCMC) methods. Bayesian methods have been employed in neuroscience for a wide range of electrophysiological and behavioral experiments (Smith et al. 2005; Smith et al. 2007; Kaufman et al. 2005; Wood et al. 2006; Cronin et al. 2010), however they have not yet been used in the analysis of NIRS response curves. Bayesian inference using Gibbs sampling (OpenBugs version 3; Lunn et al. 2009) was performed across the timeseries of all subjects, with 20,000 samples drawn after 10,000 burn-in samples. Given the original timeseries, the generation of samples via a random walk state space process yields values at each timepoint, which further allow the approximation of the joint posterior distribution at each timepoint (Metropolis et al. 1953; see Smith et al. 2007 for similar methods). The samples can therefore be used as an approximation of the complete joint distribution of timepoints, and can be referenced to determine the timecourse of differences in response to upright and inverted face conditions. The difference in the posterior distribution of response to upright and inverted face conditions at each timepoint is depicted (**Figure 3.3)**. The global difference in response to upright as compared to inverted faces at each second was first significant at two seconds following the onset of stimulus presentation (mean difference = 0.019 mM*mm; 95% credibility interval [0.0027,

0.036]). The average maximal difference of the posterior distributions occurred between 7 and 8 seconds following stimulus onset, or between two and three seconds following stimulus removal (mean maximal difference = 0.14 mM*mm; 95% credibility interval [0.123, 0.157]). Maximal oxy-hemoglobin responses were noted to occur at varying times within the 5-8 second time period depending upon channel location, indicating that a 10 second time window was sufficient for capturing the infant response to faces as measured by NIRS.

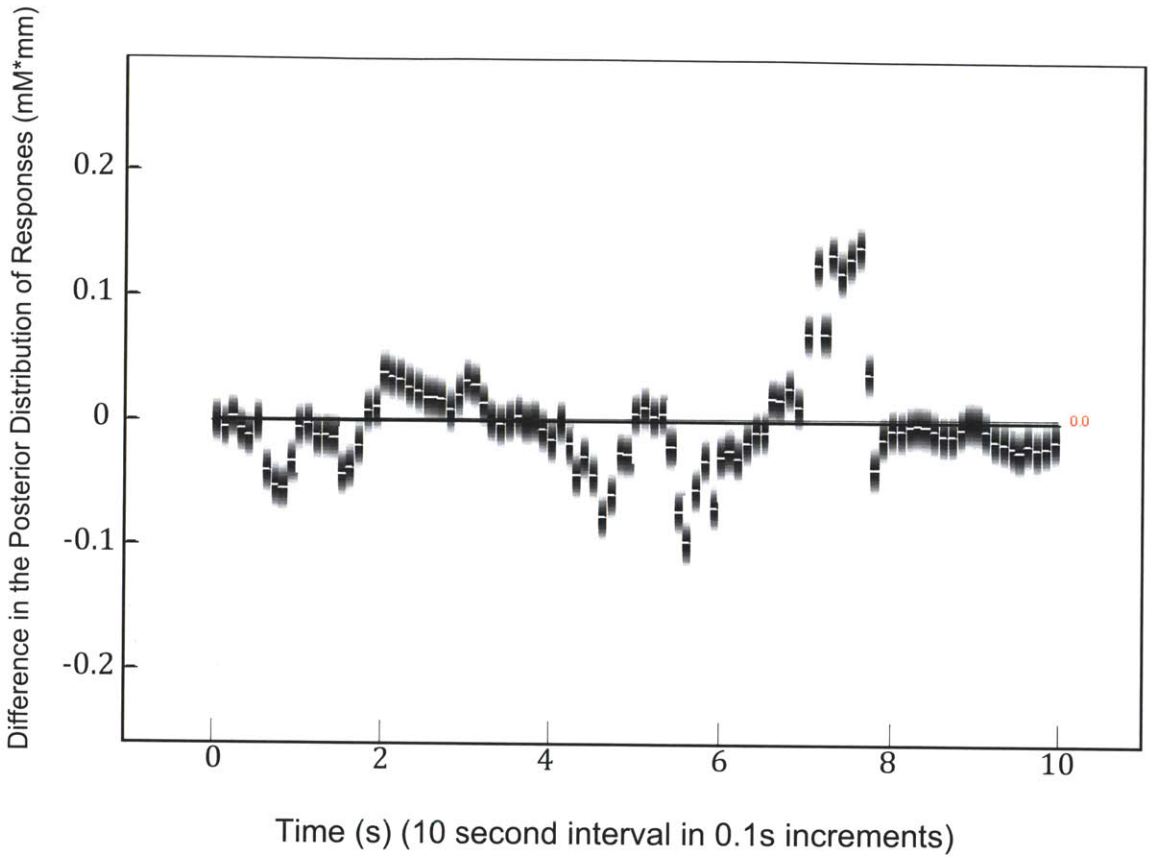

Figure 3.3: The difference in the posterior distribution of response to upright and inverted face conditions at each timepoint. Density strips at each timepoint represent the 95% credibility interval of the difference in the posterior distribution of samples generated by MCMC methods. The difference in response is first significant at two seconds following the onset of stimulus presentation (mean difference = 0.019 mM*mm; 95% credibility interval [0.0027, 0.036]). The maximal difference of the posterior distributions occurs between 7 and 8 seconds following stimulus onset, (mean maximal difference = 0.14 mM*mm; 95% credibility interval [0.123, 0.157]).

3.4. Discussion

The goal of this study was to utilize NIRS to examine the infant hemodynamic response to faces, and to elucidate specific regions of the infant brain in which the FIE could be observed. Our findings are consistent with previous studies of infants showing a larger right as compared to left hemispheric response to face stimuli (Taga et al., 2003; Otsuka et al., 2007). The results, however, are the first to demonstrate channel-specific responses to face inversion over distinct regions of the brain, and reveal that the response to faces in these regions follow a distinct timecourse that can be captured within a ten second time window.

Although an early response to face inversion has been demonstrated, these studies were limited to hemispheric comparisons that demonstrated responses similar to those found in adults (Taga et al., 2003; Otsuka et al., 2007). Our channel-specific findings in infants can now be compared to studies of face perception in young children suggesting that the response may be different from that seen in adults (Gathers et al., 2004; Aylward et al., 2005; Joseph et al., 2006). We were able to demonstrate that the oxy-hemoglobin response is greater in a region localized to the right posterior suprasylvian gyrus. Similarly, Joseph et al. (2006) demonstrated that superior and lateral occipital regions are engaged when 5-8 year-old children process upright faces, and suggested that this may be similar to adult featural processing of inverted faces. Gathers et al. (2004) also noted that 5-8 year-old children did not show a

hemodynamic response as measured by functional MRI in the fusiform face area when viewing faces versus objects, but rather activated lateral occipital cortex. Although NIRS cannot currently be used to assess deep structures such as the fusiform face area, our results suggest that brain regions involved in infant recognition of faces are similar to those activated in the earlier developmental stages of featural face processing as opposed to the adult networks involved in relational face processing. This finding has important implications for the study of typical and atypical development of face processing in infancy, and informs both our choice of face stimuli and probe placement in future studies.

In the present study, the timing of the average infant response to faces suggests a similar onset to adult hemodynamic responses (Taga et al., 2003; Huppert et al., 2004), with a rise lasting until just after the time of stimulus removal. Lloyd-Fox et al. (2009) noted a similar finding in response to faces while examining social versus non-social stimuli. Infants' neural responses generally adapted to stimuli over successive trials, though this was not the case for postero-lateral regions that demonstrated greater response to social face stimuli as opposed to the non-social stimuli (Lloyd-Fox, 2009).

The face inversion effect can be demonstrated as early as five months of age using near-infrared spectroscopy, though localized changes in response to upright versus inverted faces may differ from the patterns seen in adults. The results of this study support previous findings of an inter-hemispheric difference in infant responses to face inversion, and further identify a right suprasylvian oxy-hemoglobin response that is greatest to upright faces. In addition, we were able

to show the channel-specific hemodynamic responses to upright as compared to inverted face conditions in five-month-old infants. These effects demonstrate important characteristics of face perception that are already present at 5 months of age, and also highlight key features of the hemodynamic response to faces in infancy. Both the location and timing of this response will inform the subsequent studies of face perception in infants at high risk for autism.

Chapter 4: The Processing of Facial Identity and Facial Emotions in Infants at High Risk for Autism Spectrum Disorders

Abstract:

The general ability to perceive faces can be divided into many subcomponent processes, including general recognition of a "face-like" object, recognition of facial identity, and perception of social cues and emotions within the face. Diminished face processing and social impairment are characteristics of autism spectrum disorder, but little is known about how or why these effects occur. Having identified regions of the brain responsive to face stimuli in infants using NIRS (see Chapter 3), the second aim of this project was to examine the effects of specific facial characteristics on the hemodynamic response in both typically developing infants and those at high risk for developing autism spectrum disorders. The present work examined ten 6-7 month-old infants at high risk for developing autism (HRA group) and ten typically developing controls (LRC group) using a face perception task designed to differentiate between the effects of face identity and facial emotions. The results of the main effects of face identity and emotion in the LRC group are consistent with prior studies of face and emotion processing in typically developing infants, but reveal significant effects of the deoxyhemoglobin response in regions adjacent to significant oxyhemoglobin responses. In addition, significant differences were observed between the HRA and LRC groups, including complex interactions involving the two factors. These results are consistent with theories of unique interactions between face processing and hyperarousal in individuals with autism. In addition, the HRA group demonstrated significant deoxyhemoglobin responses to face conditions that were not present in controls. This may reflect early differences in local cortical structure and the hemodynamic response, which could in turn increase the risk of developing ASD.

4.1 Introduction

A typically developing human infant acquires the ability to recognize his or her mother's face shortly after birth (Pascalis et al., 1995; de Haan and Nelson, 1997). This processing and categorization of visual information is quite complex, with an accuracy that is superior to that of the most sophisticated facial recognition software available today. In addition to identification of faces, infants learn to recognize basic emotional expressions in the face within the first few months of life. The latter is thought to arise from the experience of mutual interaction between the infant and caregiver (Leppanen et al., 2007; Minagawa-Kawai et al., 2009; Moore, Cohn, & Campbell, 2001).

While deficits in face processing are thought to be characteristic of autism, the nature and development of these deficits has yet to be fully determined. Is attention to faces disrupted from birth, thereby leading to a lack of facial recognition? Is face processing itself intact, but without attention to emotional and social information? These questions require design of a task and measurement method that can yield this level of detailed information, while still remaining suitable for the infant participant.

Several groups have attempted to use neuroimaging methods to better understand the infant processing of facial identity and facial emotion. Using event-related potentials (ERPs), de Haan and Nelson (1997) showed discriminative electrophysiological responses in 6-month-old infants, recorded when the infants were looking at their mother, and at a stranger whose face

either resembled or differed from that of the mother. Their results suggest that functional cortical specialization involved in face identification is already present at the age of 6 months. Minagawa-Kawai et al. (2009) were also able to demonstrate differences in NIRS response patterns between mother and stranger conditions using only four channels placed in frontal locations.

The study by Minagawa-Kawai et al. (2009) focused upon the orbitofrontal cortex (OFC), and is supported by primate studies suggesting that this region is responsible for social behavior and the perception of affect (Babineau et al., 2011). Additional human studies involving NIRS, as well as other imaging modalities, have revealed the OFC's role in processing positive affect (Blasi et al., 2011; Goodkind et al., 2011; T. Grossmann, Oberecker, Koch, & Friederici, 2010; Ito et al., 2011; Volkow et al., 2011). Minagawa-Kawai et al., (2009), found one of four NIRS channels mapped to the OFC of infants to be differentially responsive to smiling in videos of mothers and strangers. Having characterized the infant response to faces (see **Chapter 3**), we next designed a task similar to that described by Minagawa-Kawai et al. in order to simultaneously image responses to facial identity and facial emotions. Accordingly, we placed NIRS probes in right lateral and frontal brain regions of infants who then viewed movie clips of their mothers, and similar strangers, changing from a neutral to smiling expression in order to examine both face responsiveness and affect-related changes. The targeted brain areas for NIRS measurement were the right STS (as described in **Chapter 3**), and the OFC. We then used this paradigm to examine six to seven-month-old infants at high risk for developing autism.

4.2. Research Design and Methods

4.2.1. Participants

Twenty-five 5- to 7-month-old infants (14 female, mean age = 7 months, age range: 6.33 to 7.97 months) participated in the study. As noted by Ozonoff et al. (2011), one in five infants having an older sibling diagnosed with ASD will go on to develop the disorder. This is a much higher risk than the current estimate of one in 110 in the general population (Kim et al., 2011). Ten of these infants (6 female, mean age = 7.06 months, age range: 6.33 to 7.97 months) therefore had an older sibling, designated as the proband, with a diagnosis of ASD, and were thus defined as being at high risk for ASD (HRA group). The other fifteen infants were recruited as low-risk controls (LRC group) for comparison, and were defined as such by having at least one older sibling and no immediate relatives with a diagnosis of ASD. For the purposes of this study, either diagnosis through the Autism Diagnostic Observation Schedule (ADOS) administered by qualified research staff, or a clinical community diagnosis, were sufficient for diagnosis of ASD in the proband after 36 months of age.

Of the low-risk control infants recruited, three were excluded from the analysis due to insufficient data (two could not complete the required number of trials due to fussing, and one had data with significant artifacts). Two were additional control subjects that could not be matched to infants in the HRA group. Ten of the control infants (6 female, mean age = 6.91 months, age range: 6.4 to 7.93 months) were selected for analysis based upon gender and behavior during

the task (**Table 4.1**). All infants included in the experiment were: 1) born after 36 weeks gestational age, 2) born weighing more than 2500 grams, and 3) born without a known neurological or uncorrected visual abnormality.

Experiments were conducted under approval by the Institutional Review Board at Children's Hospital Boston and Massachusetts Institute of Technology, and in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents of all infant participants.

Table 4.1: High Risk Autism (HRA) Group and Low Risk Controls (LRC)

	Age (months)	Gender	Average # of Trials	Average # of Neutral/Smiling	Average # of Mother/Stranger
High Risk Autism	7.06	6 Female; 4 Male	13.4	12.9/11.9	6.1/6.4
Low Risk Control	6.91	6 Female; 4 Male	13.4	13.2/12.8	6.4/6.6

4.2.2. Experimental Procedure

4.2.2a. Stimuli

Prior to NIRS imaging, we created a high-definition digital video recording of the mothers of participants as a movie stimulus. Mothers were asked to stand against a white background, and were draped with a white cloth. They were instructed to answer a series of questions while their faces were being recorded

on video, with the purpose of creating a natural movement of the mouth. Mothers performed this task twice: first with a neutral facial expression, and a second time with a smiling expression (**Figure 4.1A**). Sound was removed from the videos in order to eliminate any multisensory effects of voice. Videos were then edited to obtain 16 second clips under each of the neutral and smiling conditions, and to combine these clips into a continuous 32 second video trial with transition from neutral to smiling expression.

A

B

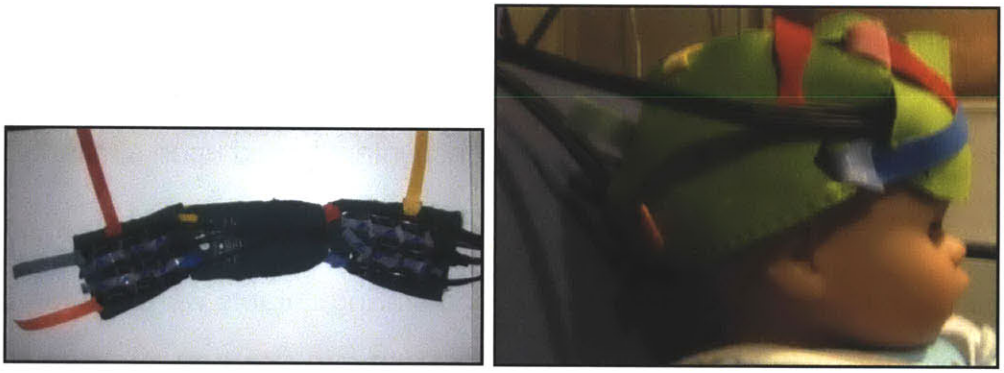

Figure 4.1: Video Stimuli and Probes. A) Mothers of infant participants were instructed to answer a series of questions with both a neutral and smiling expression. Sound was removed from the videos, and 16 video clips of each emotion were combined to achieve a 32 second stimulus with transition from neutral to smiling expression. Stranger videos were selected for similarity in appearance to the mother. **B)** Two chevron arrays of probes placed into a custom designed neoprene cap. Probes were placed in the frontal and right lateral panels, and adjusted to proper fit and location with the colored velcro tabs.

4.2.2b. NIRS Task Procedures and Equipment

Infants were seated on a parent's lap throughout the experiment, and passively observed the stimuli. Visual stimuli were presented on a 17-inch Tobii T-120 eyetracker monitor at a distance of approximately 60cm from the infant. A visual baseline of moving objects was used for at least ten seconds at the beginning of each session to achieve a baseline measurement and to encourage visual attention to the screen. Video recordings and the eyetracker were used to monitor eye gaze throughout the experiment, and to present stimuli only while infants were attending to the display. Infants were presented with seven trials each of the video of their mothers and the video of a stranger. The videos of other participants' mothers and women unknown to the infant were used for the stranger condition, and were selected for each infant based upon similarity to the mother, including hair and eye color, ethnicity, position of facial features, and movement of the mouth during speaking and smiling. Infants were randomized to one of two semi-random orders of video presentation (mother or stranger video), for a total of 14 trials presented to each infant, counterbalanced across participants. Conditions (neutral or smiling) within each trial were excluded if infants viewed fewer than 1.5 seconds of the first five seconds of the condition. This ensured that a ten second time window could be obtained in which the trial stimulus was present on the screen.

The Hitachi ETG-4000 NIRS system with 24 simultaneously recording channels (as described in **Chapter 3** and **Appendix A**) was used to collect

hemodynamic response during stimulus presentation. A new cap was designed for infants in this portion of the study in order to affix the NIRS optical probes to the frontal and right lateral portions of the head (**Figure 4.1B**). The new design consisted of three neoprene panels, each of which could hold one of the two sets of probes, and which could be adjusted with velcro closures to allow for individualized placement of the probes once on the infant's head. The neoprene fabric created support for placing the probes over frontal cortex, while maintaining the level of elasticity necessary for conforming to the infant head, and thus reduced noise due to artifact. The frontal panel was centered in the nasion–inion line, with the inferior frontal probes positioned directly above the eyebrows, in a direction parallel to the T3-Fp1-Fp2-T4 line in the international 10–20 system as described by Minagawa-Kawai (2009). The right lateral panel was positioned so that the anterior portion of the panel was located just superior to the right ear, with the panel extending towards the occiput (**Figure 4.1B**).

Based on the light intensity detected through each channel, relative concentrations of oxygenated and deoxygenated hemoglobin were calculated from absorbance at each wavelength using the modified Beer–Lambert law. This conversion, as well as further data analyses, were implemented through a customized Matlab script (version 7.6, Mathworks Inc., Natick, MA, USA).

4.2.3. NIRS Data Analysis

For each participant, each trial condition was included if 1.5 seconds of video was viewed within the first five seconds of the condition, and no significant

motion occurred. This allowed for use of a ten second observation window within each condition, which would include the peak of the response as demonstrated in lateral face processing regions (**Chapter 3**), and orbitofrontal cortex (Minagawa-Kawai et al., 2009). In their measures of orbitofrontal infant responses to mother and stranger videos, Minagawa-Kawai et al. (2009) found significant group differences between mother and stranger trials with inclusion of 2-3 trials of each, however given our need to compare between groups, in our analyses we included only infants who viewed at least five of the seven videos of each trial and condition pairing with no significant artifacts after initial filtering.

Timeseries corresponding to oxy- and deoxy-hemoglobin values were first processed using a 5^{th} order Butterworth filter between 0.01 and 1.0Hz, and additional artifacts were identified and extracted if the raw signal exceeded a value of 4.95, or if total hemoglobin change exceeded 0.3 mM*mm within a 0.7s time window. These measures of artifact were based upon standard practices in the filtering of infant NIRS data from the ETG optical imaging series. Linear trends were then removed from the data. For each subject, the data were parsed into ten second time windows with 0.1s time resolution beginning at the first instance of 1.5 seconds of looking within the first five seconds of each condition presentation. These time windows were corrected to a baseline value at the onset of each stimulus. Following this correction, trials were grouped by emotion condition (neutral or smiling) as well as by identity (mother or stranger) and averaged to obtain a mean value for oxy- and deoxy-hemoglobin change at individual channels. Mean values at each timepoint from each subject were also

pooled to produce a group mean value by condition at each channel and timepoint. Channels from individual subjects with low oxy-hemoglobin signal-to-noise (Mean/Standard Deviation < 1.0) were excluded from the group average. An oxy- or deoxy-hemoglobin response was defined as the difference between the maximum value in the latter five seconds of each ten second time window and the minimum value in the first two seconds of a window.

4.3. Results

4.3.1. Analysis of Mother versus Stranger Face Responsiveness

The LRC group completed an average of 13.4 total trials (**Table 4.1:** 6.4 mother trials and 6.6 stranger trials). An initial set of analyses aimed to identify channel-specific differences between mother and stranger conditions in the LRC group. Trials of the neutral and smiling conditions were initially separated for analysis in order to examine the difference between mother and stranger conditions without the effect of emotion. The responses to neutral mother and stranger faces were averaged over each trial and subject, and compared using a paired t-test (**Figure 4.2A**, T-value map, Bonferroni corrected). In the low risk control group, oxy-hemoglobin responses to neutral mothers was significantly greater than to neutral strangers in one lateral channel (**Figure 4.2A**, channel 22, mean difference = 0.1472 mM*mm, 95% CI: [0.008, 0.1552], p= 0.0482, Bonf.). This lateral channel corresponded to the postero-lateral region of the right

hemisphere identified as responsive to faces in Chapter 3 (**Figure 4.2A**, channel circled in red). A significant difference in deoxy-hemoglobin was also seen in a neighboring lateral channel, in which the decrease in response to stranger was greater than the decrease in response to mother (Channel 24: mean difference = 0.0888 mM*mm, 95%CI: [0.0419, 0.1357], t(9) = 4.37, p = 0.0024, Bonf.; **Figure 4.2A**, channel circled in blue).

In the smiling condition, the difference in oxy-hemoglobin response between mother and stranger trials was significant in two right-sided central frontal channels for the LRC group (**Figure 4.2B**, channel 6: mean difference = 0.1022 mM*mm, 95% CI: [0.0702, 0.1342], p= 0.0036, Bonf.; channel 7: mean difference = 0.1414 mM*mm, 95% CI: [0.0006, 0.2821], p= 0.0492, Bonf.; channels circled in red). These frontal channels correspond to regions identified by Mingawa-Kawai et al. (2009) and Grossman et al. (2010) as responsive to social and emotional stimuli. This area was estimated to probabilistically cover the OFC in 94.3%-100% of infants when the NIRS channels were registered onto an MNI-compatible canonical brain optimized for NIRS analysis (Minagawa-Kawai et al., 2009; Functional Brain Science Lab at Jichi Medical University: http://www.jichi.ac.jp/brainlab/indexE.html).

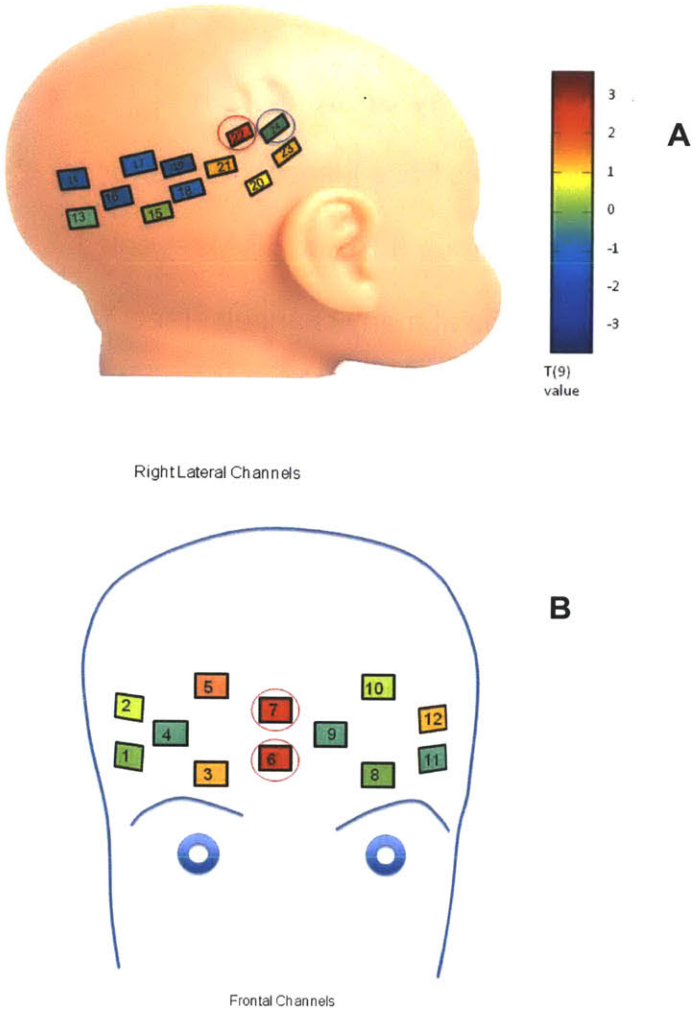

Figure 4.2: A) Comparison of Response to Neutral Mother and Neutral Stranger Faces within the LRC group. Channel placement and numbers are shown on the right lateral portions of the infant head. Colors within the channels indicate a T-value map across ten LRC subjects comparing oxy-hemoglobin response to the neutral mother's face as compared to the neutral stranger's face at each channel location (color bar, p=0.05, Bonf. corrected). The channel circled in red indicates significant differences in oxy-hemoglobin response, and the channel circled in blue indicates significant differences in deoxyhemoglobin response.

B) Comparison of Oxyhemoglobin Response to Smiling Mother and Smiling Stranger Faces within the LRC group. Significant activation occurred in channels placed on the front of the head (numbered as shown). Colors within the channels indicate a T-value map across ten LRC subjects comparing oxy-hemoglobin response to the smiling mother's face as compared to the smiling stranger's face. Channels circled in red indicate significant differences in oxy-hemoglobin response.

4.3.1. Analysis of Face Identity and Emotion by Group

A mixed effects ANOVA involving factors Face Identity (mother or stranger), Emotion (neutral or smiling), and Group (LRC or HRA) revealed significant main effects, as well as interactions at many channels, (see **Table 4.2A-B** for full statistics). A main effect of emotion was seen in three right frontal channels, where oxy-hemoglobin responses to smiling were greater than those to neutral expressions (**Figure 4.3A, Table 4.2A**). Three left-sided frontal channels had deoxy-hemoglobin responses that were greater (more negative) to smiling faces than to neutral faces. A main effect of face identity was noted in centrally located frontal channels, where oxy-hemoglobin responses to mother were greater than to those for stranger (**Figure 4.3A, Table 4.2A**). One channel was significant for a greater deoxy-hemoglobin response to stranger than to mother. There were also seven channels demonstrating a main effect of group, with significantly different changes in oxy-hemoglobin by group in the postero-lateral right hemisphere, and significantly different changes in deoxy-hemoglobin by group in frontal regions. Oxy-hemoglobin responses in three right lateral channels were greater for the LRC group than the HRA group. The opposite pattern was seen in four frontal channels, where deoxy-hemoglobin responses were greater for the HRA group than the LRC group.

Thirteen of the 24 channels were significant, or marginally significant, for interaction effects. These results are summarized in **Table 4.2A**, and depicted in **Figure 4.3A-B**. Oxy-hemoglobin responses across frontal and lateral regions

revealed an interaction in which responses to mother were greater than those to stranger only for the LRC group (no effect of face identity was present for the HRA group). In addition, a three-way effect occurred, in which the interaction of Face Identity and Emotion was significant for the LRC group, but not for the HRA group. An interaction of Group and Face Identity was seen for deoxy-hemoglobin responses in both frontal and lateral channels. For the HRA group, a greater deoxy-hemoglobin response was seen to mother as compared to stranger faces across many channels. Several lateral channels in the LRC group demonstrated a greater deoxy-hemoglobin response to the neutral mother as compared to other conditions, but this interaction effect was not seen in the HRA group. The region-specific effects and interactions are summarized in **Figure 4.3B**.

Table 4.2A: Results of Face Identity x Emotion x Group Anova by Channel. (M = Mother, S = Stranger, E = Emotion, N = Neutral, HRA = High Risk Autism, LRC = Low Risk Control, HbO = oxyhemoglobin, HbD = deoxyhemoglobin, m = difference in population marginal means with corresponding 95% confidence intervals, Tukey's HSD used for correction for multiple comparisons). Note that differences in population marginal mean are described in numeric value, while interactions are described by magnitude of the response regardless of direction.

Ch	Identity (M-S)	Emotion (E-N)	Group (HRA - LRC)	Interactions
1		HbD: m = 0.0235, [0.0001, 0.0468]		HbD: M>S for HRA group only
2			HbD: m = 0.0370 [0.0001, 0.0741]	
3		HbO: m = 0.0865, [0.0001, 0.1779] HbD: m = 0.0811, [-0.0083, 0.1705]		
4	HbD: m = -0.0219, [-0.0431, -0.0007]	HbO: m = 0.0367, [0.0048, 0.0686]		HbD: M>S for HRA group only
5		HbO: m = 0.0491, [-0.012, 0.1103]	HbD: m = -0.0400 [-0.0882, 0.008]	HbO:#see description below HbD: S>M for HRA group only
6	HbO: m = 0.1059, [-0.015, 0.2371]			
7	HbO: m = 0.0547, [0.0004, 0.109]	HbD: m = 0.0437 [0.0014, 0.0859]	HbD: m = -0.0364 [-0.0786, 0.005]	HbO: for LRC & E, M>S HbD: HRA>LRC for M response, S>M for LRC only
8	HbO: m = 0.1615, [-0.0159, 0.3389]			
9		HbD: m = 0.0390, [0.0098, 0.0683]		HbO: M>S for LRC group
10			HbD: m = -0.0518 [-0.0969, -0.0067]	
11		HbD: m = 0.0367, [0.0164, 0.0570]		HbD: +see description below
12				
13			HbO: m = -0.0644 [-0.1285, -0.0002]	
14				
15			HbO: m = -0.0993 [-0.1918, -0.0068]	
16				HbO: M>S for LRC group HbD: M>S for HRA group only
17				
18				
19				HbO: *see description below HbD: S>M for LRC group only
20				
21				HbD: S>M for LRC group only ++see description below
22				HbO: for LRC, M>S
23				HbD: M>S for HRA group only ++see description below
24			HbO: m = -0.0530 [-0.1031, -0.0028]	HbD: S>M for LRC only

#An interaction of Face Identity and Emotion in the LRC group showed greater HbO response to mother smiling than mother in the neutral condition as compared to the difference seen in the stranger condition. This effect was not significant in the HRA group.

*An interaction of Face Identity and Emotion in the LRC group showed greater HbO response to mother smiling than mother in the neutral condition. The opposite effect was true of the HRA group, in which HbO response to mother in the neutral condition was greater than to mother in the smiling condition.

+ Smiling was associated with a reduced HbD response for both mother and stranger in the HRA and LRC groups. There was an interaction effect of Face Identity and Emotion present only in the LRC group, and HbD responses to mother smiling were less in the LRC group than in the HRA group.

++HbD responses to mother in the neutral condition were greater for the HRA group than for the LRC group. An interaction effect in which the effect of the smiling condition reduced the HbD response to mother's face was only seen in the HRA group.

Table 4.2B: Statistical Results of Face Identity x Emotion x Group Anova.

Ch	Identity	Emotion	Group (HRA or LRC)	Interactions
1		HbD: F(1,72) = 4.08, p = 0.047		HbD: Group x Identity (F(1,72) = 10.48, p = 0.0018)
2			HbD: F(1,69) = 3.97, p = 0.05	
3		HbO: F(1,66) = 3.66, p=0.05 HbD(1,66): F= 3.31, p = 0.07		
4	HbD: F(1,68) = 4.28, p = 0.042	HbO: F(1,68) = 5.28, p = 0.0247		HbD: Group x Identity (F(1,68) = 5.88, p = 0.018)
5		HbO: F(1,62) = 2.59, p = 0.1	HbD: F(1,62) = 2.74, p = 0.10	HbO: Identity x Emotion x Group (F(1,62) = 6.96, p = 0.0105) HbD: Group x Identity (F(1,62) = 4.15, p = 0.046)
6	HbO: F(1,56) = 2.65, p= 0.1			
7	HbO: F(1,68) = 4.04, p = 0.048	HbD: F(1,68) = 4.25, p = 0.04	HbD: F(1,68) = 2.95, p = 0.09	HbO: Identity x Emotion x Group* (F(1,68) = 4.37, p = 0.04) HbD: Group x Identity (F(1,68) = 2.55, p = 0.10)
8	HbO: F(1,53) = 3.34, p = 0.07			
9		HbD: F(1,69) = 7.2, p = 0.009		HbO: Group x Emotion (F(1,69) = 5.46, p = 0.022)
10			HbD: F(1,63) = 5.43, p = 0.02	
11		HbD: F(1,71) = 12.97, p = 0.0006		HbD: Identity x Emotion x Group (F(1,71) = 3.69, p = 0.05)
12				
13			HbO: F(1,61) = 4.02, p = 0.04	
14				
15			HbO: F(1,58) = 4.62, p = 0.03	
16				HbO: Group x Identity (F(1,67) = 3.16, p = 0.08) HbD: Group x Identity (F(1,67) = 3.28, p = 0.07)
17				
18				
19				HbO: Identity x Emotion x Group* (F(1,63) = 4.41, p = 0.03) HbD: Group x Identity (F(1,63) = 3.8, p = 0.05)
20				
21				HbD: Group x Identity (F(1,65) = 3.79, p = 0.05) Identity x Emotion x Group* (F(1,65) = 4.61, p = 0.03)
22				HbO: Group x Identity (F(1,66) = 3.62, p = 0.06)
23				HbD: Group x Identity (F(1,67) = 4.14, p = 0.04) Identity x Emotion x Group* (F(1,67) = 3.49, p = 0.06)
24			HbO: F(1,66) = 4.45, p = 0.03	HbD: Group x Identity (F(1,66) = 3.08, p = 0.08)

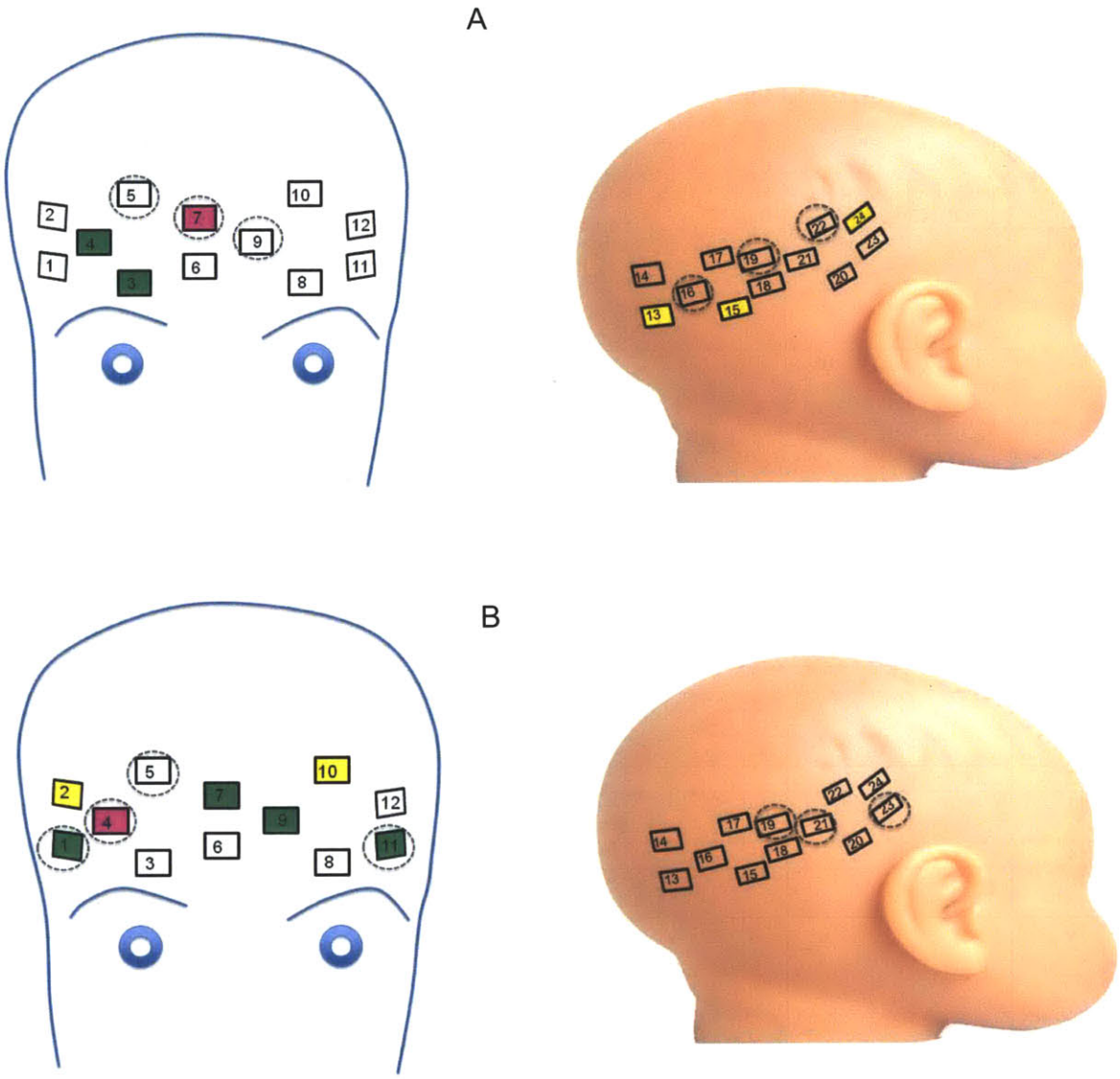

Figure 4.3: A) Channels significant for main effects and interactions on oxyhemoglobin response. Channels with main effects of Face Identity (magenta) and Emotion (green) occurred in frontal regions, while the main effect of Group was seen in right lateral channels (yellow). Significant interactions were seen across frontal and lateral channels (dashed circles).

B) Channels significant for main effects and interactions on deoxyhemoglobin response. Channels with main effects of Face Identity (magenta), Emotion (green), and Group occurred in frontal regions, while significant interactions were seen across frontal and lateral channels neighboring those significant for oxyhemoglobin responses (dashed circles).

4.4. Discussion

In our second study, we built upon our examination of typical infant face processing by using video stimuli that both discriminate between familiar and unfamiliar faces, as well as positive affective and neutral conditions. We were then able to compare the neural correlates of both facial identity and face emotion processing in typically developing infants to those of infants at high risk for autism. The results reveal effects of each variable, as well as complex effects primarily involving the interaction of group with both face identity and facial emotion.

4.4.1. Face Identification

The right suprasylvian region identified as responsive to neutral faces in Chapter 3 was also responsive to neutral face stimuli in the current experiment, and revealed some discriminatory response between mother and stranger conditions in the LRC group. A greater oxy-hemoglobin response to mother as compared to stranger was seen across central orbito-frontal regions, consistent with the results of Minagawa-Kawai et al. (2009). This is also consistent with electrophysiological studies (de Haan and Nelson, 1997 & 1999) showing greater infant ERP responses to a mother's face than to a stranger's face. In these studies, the differences in responses of interest were thought to indicate utilization of attentional memory resources for the processing of familiar and unfamiliar faces. It is possible that the increased orbito-frontal oxy-hemoglobin

responses to mother's faces are a neural correlate of the increased attention to the mother, and particularly the ability to identify the mother's face, which develops in the earliest months of infancy.

Deoxy-hemoglobin responses generally did not discriminate between face identity outside of the effects of group. One right-sided frontal channel was significant for a main effect of face identity on deoxy-hemoglobin response, where the response to mother's face was greater (more negative) than to stranger's face. In the initial analysis of channels responsive to faces within the LRC group, the region associated with a greater oxy-hemoglobin response to neutral mother than to neutral stranger was also associated with a reduced deoxy-hemoglobin change to mother overall. Other interactions of face identity and group status will be discussed in the following sections. Our results for the LRC group are consistent with past reports of typically developing infants, finding no difference in deoxy-hemoglobin concentration in orbitofrontal cortex between neutral mother and stranger faces (Minagawa-Kawai et al., 2009).

4.4.2. Face Emotion Processing

The orbito-frontal cortex is implicated in both attention to social stimuli, the perception of facial emotion (Leppanen & Nelson, 2007) and regulation of autonomic responses to social situations (Dalton et al., 2005; Goodkind et al., 2011; Schultz et al., 2000). Studies involving NIRS have revealed the activation of the OFC in the processing of social cognition and facial emotions (Blasi et al., 2011; T. Grossmann, Parise, & Friederici, 2010b; Minagawa-Kawai et al., 2009).

Our results are consistent with these studies, in that oxy-hemoglobin responses to the effect of smiling were noted in right orbitofrontal cortex. In frontal regions neighboring this area, changes in deoxy-hemoglobin were significantly reduced during smiling conditions as compared to the neutral condition. This reduced deoxy-hemoglobin response surrounding the region of oxy-hemoglobin response to smiling is similar to the deoxy-hemoglobin reduction in face responsive regions during the mother condition, and may reflect the overall hemodynamic and microvascular changes in response to specific stimuli.

In an ERP study of infant social development by Johnson et al. (2005), suprasylvian structures and prefrontal cortex were thought to play a key role in face responses, and the early social brain network. Further, EEG data has demonstrated that high-frequency oscillations in the gamma-band (20–100 Hz) are elicited in the right prefrontal cortex to a greater extent in response to direct gaze from a face than in response to averted gaze (Farroni, Massaccesi, Menon, & Johnson, 2007; T. Grossmann, Johnson, Farroni, & Csibra, 2007). Likewise, Minagawa-Kawai et al. characterize the observed NIRS response to mothers in OFC as the neural correlates of "enhanced emotional valence" in cues given by a known social agent. It is therefore likely that the orbito-frontal responses we have measured through both oxy- and deoxy-hemoglobin changes create a broader picture of an attentive response that is particularly tuned to social positive affect.

4.4.3. The Face-Emotion Task in Infants at High Risk for Autism

The main effect of group revealed significantly greater oxy-hemoglobin responses in lateral regions for LRC as compared to HRA, and significantly greater deoxy-hemoglobin responses in frontal channels for HRA as compared to LRC. The exception to this occurred in channel 2, in which the deoxy-hemoglobin response was greater for the LRC group than the HRA group. There were also numerous interaction effects between Group and Face Identity, generally revealing a greater deoxy-hemoglobin response to mother's face as opposed to stranger face within the HRA group across both frontal and lateral regions. This is in contrast to the LRC group, which demonstrated a lack of effect of face identity upon deoxy-hemoglobin response in frontal regions, and a stranger greater than mother response in lateral regions. The interactive effect of Face Identity upon the greater oxy-hemoglobin response to smiling versus neutral conditions was present only for the LRC group, indicating that while the HRA group demonstrated an oxy-hemoglobin response to smiling in orbitofrontal cortex, the difference in response to the smile of mother and the smile of stranger faces was not significant. Returning to the theory of orbitofrontal cortex as a part of the social brain network, this lack of interaction between identity and emotional state in the HRA group might indicate a lack of selective attention to a known social agent. At the same time, the deoxy-hemoglobin changes seen only in the HRA group indicate that some level of discrimination between mother and stranger must exist.

Oxy-hemoglobin responses were greater in the LRC group for both right lateral occipital channels and the suprasylvian channel identified as differentially responsive to neutral mother versus stranger faces. Several right lateral channels also demonstrated a Group x Face Identity effect, in which oxy-hemoglobin responses were greater for mother than stranger faces only in the LRC group. Deoxy-hemoglobin responses were reduced for stranger as compared to mother in right lateral regions of the LRC group, while the opposite effect was significant in the HRA group. Two channels in the suprasylvian region were significant for a deoxy-hemoglobin response to mother in the neutral condition that was greater for the HRA group than for the LRC group, as well as an effect of the smiling condition that reduced this response to mother's face.

From these data, we can conclude that both HRA and LRC groups demonstrate differential hemodynamic responses to face identity and emotion in orbitofrontal and right postero-lateral cortex. These responses, however, also differ significantly between groups, with a prominent feature of HRA hemodynamics being the deoxy-hemoglobin differential response to mother as compared to stranger. These findings are consistent with functional MRI work by Dalton et al. (2005), in which responses to emotions in static faces, and photographs of familiar and unfamiliar individuals were presented to adolescents with autism and compared to control responses. The study found a region in the right fusiform to occipital cortex associated with greater activation for familiar faces than for unfamiliar faces only for the control group, which they concluded was responsible for facial identification and face processing in general, rather

than emotional processes. Further, the autistic group showed greater activation in the left orbitofrontal cortex specifically in response to the emotional content of the faces and not to faces in general, with corresponding right amygdala activation that was not specific to facial emotions, but a response to faces in general (Dalton et al., 2005). Since deoxy-hemoglobin has been implicated in the early hemodynamic responses as measured by BOLD fMRI, the regions of signal described by Dalton et al. (2005) may indeed correspond to those localized in the present study.

One hypothesis for these findings is that social stimuli cause hyperactivation of affective response pathways in individuals with autism, and this leads to aversion to faces, and thus a reduction in response of face processing regions (Dalton et al., 2005). While this may be true of individuals with autism, similar group differences in hemodynamic response were noted in the present study, in which the design of the stimulus presentation and trial selection eliminated this potential difference due to behavior between groups. An alternative hypothesis is that some of these differences may reflect cortically-related structural differences that affect the hemodynamic response profile in infants at risk for developing ASD. Several studies have noted anatomical differences in the brain morphology of individuals with ASD (Carper et al., 2002; Courchesne et al., 2001; Courchesne et al., 2011; Schumann et al., 2010), as well as their unaffected siblings (Barnea-Goraly et al., 2010). These studies point to early hyperplasia, with increase in head circumference by approximately 6 months of age, and increased size of the prefrontal cortex in toddlers on imaging

(Carper et al. 2002; Courchesne et al., 2001). In addition, recent post-mortem examination of the frontal lobes of young autistic individuals has revealed significantly increased neuronal cell counts in the prefrontal cortex, including the orbitofrontal cortex (Courchesne et al., 2011). As noted by the authors, generation of these neurons must have occurred prenatally, leading to the hypothesis that an abnormality in migration or apoptosis must underlie the pattern of development. It is therefore possible that these structural differences can be inferred at 6-7 months of age through hemodynamic measures, as evidenced by our NIRS study. These differences in neuronal architecture may, in turn, be associated with states of hyperarousal, however further studies are required to determine a causal relationship between structure, function, and behavior.

In sum, the present study showed that NIRS can be used in the examination of emotion and identity as it relates to face processing in infants at six to seven months of age. In addition to the association of right lateral regions with face processing, orbitofrontal regions are implicated in the processing of social information such as emotion and identity. These regions also showed a pattern of response in the HRA group that was distinct from the LRC group, and which was consistent with prior imaging studies of face processing in individuals with autism. As it has been suggested that hyperarousal in social-affective regions may be associated with diminished responses to faces in the right hemisphere (Dalton et al., 2005), the next chapter will further examine group

differences in the integration of these face and emotion responsive regions using methods of connectivity analysis.

Chapter 5: An Analysis of Connectivity of Face and Emotion Processing in Infants at High Risk for Autism Spectrum Disorders

Abstract:

It has been hypothesized that individuals with ASD suffer from the inability to create a coherent cognitive profile from a set of features. In our final analyses, we employed independent component analysis (ICA), as well as a novel method of condition-related component selection and classification to identify waveforms associated with face and emotion processing in 6-7-month-old infants at high risk for autism, and matched low-risk controls. The results indicate similarities of response waveforms, but differences in both the spatial distribution, and timing of oxy-hemoglobin and deoxy-hemoglobin responses between groups. Our findings support a model of altered hemodynamic response in the high risk infant group, and these changes may, in turn, contribute to differences in patterns of functional connectivity.

5.1. Introduction

It has been hypothesized that individuals with autism have a lack of "central coherence," or a lack of ability to create a coherent cognitive percept from a set of features (Happe & Frith, 2006). Recent attempts to address the many cognitive components of ASD have focused on neural connectivity, with some studies suggesting "overconnectivity" (Belmonte et al., 2004; Rubenstein & Merzenich, 2003), and others suggesting "underconnectivity" (Brock et al., 2002; Just et al., 2004; Muller et al., 2011) as a common abnormality. These seemingly different conclusions can be somewhat aligned when considering anatomical studies of autistic brains. In individuals with ASD, high local connectivity may be associated with low long-range connectivity (Courchesne et al., 2011; Herbert et al., 2004; Just et al., 2004), the combination of which may result from differences in neural migration, or the pruning and potentiation of synapses (Herbert, 2011). The picture of functional connectivity is slightly more complicated by the fact that a locally overconnected network may give rise to abnormally large activations, with reduced specificity of those responses. Interestingly, a fMRI study of non-autistic brothers of individuals with ASD performing a visual integration task revealed that they share a pattern of atypical frontal hypoactivation with their affected siblings (Belmonte, Gomot, & Baron-Cohen, 2010). Likewise, similar structural abnormalities in white matter have been reported in both children with autism and their unaffected siblings (Barnea-Goraly et al., 2010). It may therefore be possible that familial patterns of structural and/or functional brain connectivity

predisposes individuals to ASD. Having already examined the magnitude and location of NIRS responses to our paradigm between HRA and LRC groups, our final experiment involved development of a model for understanding the timing and distribution of the waveforms in our data that correspond to functional connectivity.

Several approaches to the analysis of functional connectivity have been taken utilizing fNIRS and fMRI data, and more recent studies have examined the optimal methods for such analyses (Zhang et al., 2010). One method is seed correlation, however this approach has several problems which are discussed in the fMRI literature (Fox & Raichle, 2007; McKeown & Sejnowski, 1998). The results of this method depend upon seed region selection, and evaluate only the relationship between the seed region and other regions. A direct comparison of seed correlation to independent component analysis (ICA), revealed the latter to have superior performance in evaluating the connectivity of regions sampled via NIRS (Zhang et al., 2010). ICA was originally proposed as a powerful blind source separation method (Hyvarinen, 1999), and is now increasingly utilized for neuroimaging data analyses (Katura et al., 2008; Stone & Kotter, 2002; Zhang et al., 2010). Zhang et al. (2010) demonstrated that the intrinsic multivariate approach of ICA allows more information to be utilized than seed correlation by accounting for interactions among multiple brain regions. In addition, as a data-driven approach, ICA requires less a priori knowledge, and allows for blind source separation of neuronal activation signals from noise components (Katura

et al., 2008; Markham, White, Zeff, & Culver, 2009; Morren et al., 2004; Zhang et al., 2010).

One practical problem arises from the ICA approach, however, and that is the assignment of separated components. In fMRI studies, independent components (ICs) can be correlated with known hemodynamic response functions, but these functions are only beginning to be described for fNIRS studies. Moreover, these responses may differ throughout normal infant development, and cannot be assumed to be the same when analyzing neurologically atypical populations. Several studies involving the use of ICA methods with fNIRS data have successfully identified components of interest without the use of hemodynamic models. Zhang et al. (2010) assumed that the signals measured in a resting period contain only systemic or other noises and filtered out noises in the activation period by eigenvector-based filtering. Akgul et al. (2004) utilized FastICA to extract cognitive activity-related waveforms. The correlation between separated components and a Gamma function model was used to show that the correlation coefficient of waveforms extracted was larger during an oddball task than during a resting state. Finally, Katura and Sato et al. (2008) used a block-design finger tapping experiment to evaluate methods of activity-related component selection without the use of hemodynamic response function. We therefore adapted a method of extracting task-related independent components demonstrated by Katura and Sato et al. (2008) to our connectivity analysis in order to better describe neuronal activation signals and their

distribution between emotion and face-processing regions in infants at high risk for ASD as compared to controls.

There were three main steps in our connectivity analysis (**Figure 5.1**, adapted from Katura and Sato et al., 2008). In the first step, ICA was performed (FastICA v.2.5 –www.cis.hut.fi/projects/ica/fastica/) to separate components of NIRS signals obtained from 24 channels. In the second step, ICs were selected based on mean intertrial cross-correlation (MITC), which is based upon the concept that neuronal activation signals should be reproducible at each trial. A high MITC indicates a condition-related independent component (CR-IC). In the case of our experiment, "condition" referred to the emotional state of the face stimuli (neutral or smiling), as we were interested in analyzing the distribution of responses across face-processing and emotion-responsive regions. As noted in the motor experiments conducted by Katura and Sato et al. (2008), systemic hemodynamic changes create global noise components while task-related neuronal activity should localize to specific regions of the brain. Systemic changes may, however, be associated with the task, and will therefore have a high MITC. While the current experiment does not involve motor activity, such systemic changes may also occur due to activation of the autonomic nervous system. These changes are related to trials, but do not necessarily represent localized neuronal activation. A third step using *k*-means clustering methods was therefore employed to categorize CR-ICs into those representing neuronal activity involved in the two cognitive processes of face and emotion perception, as well as a third cluster of condition-related "noise". Finally, we examined the

response functions of these clusters, and classified them as frontal or lateral based upon a weight index.

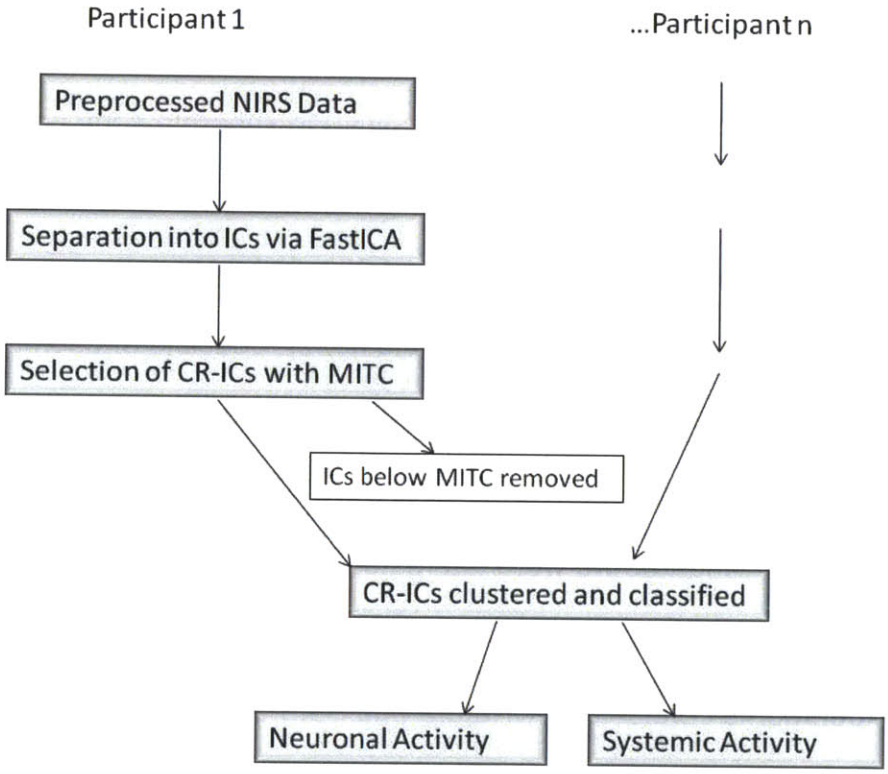

Figure 5.1: ICA Data Analysis Diagram (adapted from Katura & Sato, 2008). IC = independent component. CR-IC = condition-related independent component. MITC = mean intertrial cross-correlation.

5.2. Methods

Participants and experimental procedure were as described in Chapter 4, (**Sections 4.2.1** & **4.2.2**).

5.2.1. NIRS Data Pre-processing

Timeseries corresponding to oxy- and deoxy-hemoglobin values were first processed using a 5^{th} order Butterworth filter between 0.01 and 1.0Hz, and additional artifacts were identified and extracted if the raw signal exceeded a value of 4.95, or if total hemoglobin change exceeded 0.3 mM*mm within a 0.7s time window. The first ten seconds of resting data (recorded prior to the experimental task) were removed to eliminate artifacts from initial stabilization of the signal, and linear trends were then removed from the entire timeseries.

5.2.2. Independent Component Analysis

Our acquired functional NIRS data had a relatively large number of temporal samplings as compared to spatial samplings of signal (10 Hz sampling rate at each of 24 channels), therefore temporal ICA was employed. This method assumes a temporal independence of sources, which can only be a partially valid assumption when considering the connectivity of the brain and condition-dependent responses. Nevertheless, this method has demonstrated convincing results in many task-related fNIRS studies, with care taken in the interpretation of independence among sources (Morren et al., 2004; Katura & Sato et al, 2008;

Markham et al., 2009). Further explanations of temporal ICA involving fNIRS measurements can be found in **Appendix B**.

In this study we utilized FastICA v2.5 (www.cis.hut.fi/projects/ica/fastica/) to conduct the ICA decomposition algorithm (Hyvarinen, 1999). ICA analysis was separately performed on the pre-processed individual oxyhemoglobin and deoxyhemoglobin data sets. Parameter settings included: approach = "deflation", nonlinearity = "skew", stabilization = "on", fine-tune = "on", maximum number of iterations = 10000, epsilon = 0.00001, initial value = "random". These were based upon previous use of FastICA with fNIRS data (Zhang et al., 2010). Prior to running the algorithm, principal component analysis (PCA) reduction was performed on the data from 24 channels to reduce the data dimensionality for each subject. The number of retained principal components was determined according to the minimum number of principal components that retained more than 99% of data variance (van de Ven et al., 2004; Zhang et al., 2010). The reduced data for each individual was put into ICA decomposition with the number of independent components (ICs) equal to the number of the retained principal components. This yielded an average of 14.7 (s.d. = 2.4) oxyhemoglobin components and 13.9 (s.d. = 1.9) deoxyhemoglobin components in the HRA group; 14 (s.d. = 2.4) oxyhemoglobin components and 14 (s.d. = 2.4) deoxyhemoglobin components in the LRC group.

5.2.3. Condition-Related Component Selection

For each participant, each trial condition was included if 1.5 seconds of video was viewed within the first five seconds of the condition, and no significant motion occurred in the original data. This allowed for use of a ten second observation window within each condition, which would include the peak of the response as demonstrated in both orbitofrontal and lateral face processing regions (**Chapters 3** & **4**), and which would be suitable for mean inter-trial cross-correlation (MITC).

For each subject, the ICs were parsed into ten second time windows with 0.1s time resolution beginning at the first instance of 1.5 seconds of looking within the first five seconds of each condition presentation. These time windows were corrected to a baseline value at the onset of each stimulus. Following this correction, trials were grouped by emotion condition (neutral or smiling) at each channel. Smoothing with a Gaussian kernel (FWHM 2s) was applied to each trial in order to calculate MITC.

The selection criterion for CR-ICs was data-based rather than based upon hemodynamic models, and included those components with a MITC greater than the mean value of MITCs within each subject. This allowed for individual differences in inter-trial reproducibility of waveforms, while eliminating components that were likely the result of artifact.

5.2.4. Clustering of Condition-Related Components

In the third step of our analysis, we used *k*-means clustering methods to categorize CR-ICs into three groups based upon waveform. As activation specific to faces had been localized to right lateral cortex (see **Chapters 3** & **4**), and activation associated with the emotion condition appeared to cluster in frontal regions, it was hypothesized that two different hemodynamic response functions could underlie these cognitive processes. This hypothesis was based upon the finding that NIRS hemodynamic responses can differ between brain regions, even in the adult (Jasdzewski et al., 2003). A third cluster was included to account for any task-related systemic activity (Boas, Culver, Stott, & Dunn, 2002; Franceschini, Toronov, Filiaci, Gratton, & Fantini, 2000; Katura et al., 2008). As the sign of individual ICs is randomly determined, CR-ICs were each normalized and assigned a sign so that correlation with the boxcar waveform of trials became positive. The distance minimized in the clustering process was one minus Pearson's correlation coefficient. Each cluster centroid was therefore the mean waveform of the components of the cluster, after centering and normalizing those points to zero mean and unit standard deviation.

The estimated spatial weights (see **Appendix B**) of each component within each subject were then normalized for cluster analysis. The normalized weights of each component at each waveform cluster were averaged in order to examine the distribution of the waveform. This method of analyzing spatial

distribution has been validated in prior work, with comparison to both group-level t-maps and seed correlation (Zhang et al., 2010).

5.3. Results

5.3.1. Centroid Waveforms for Oxygenated and Deoxygenated Hemoglobin

The centroid waveforms associated with oxyhemoglobin and deoxyhemoglobin for each of the neutral and smiling conditions are depicted in **Figure 5.2**. Centroids for the HRA and LRC groups were plotted together for comparison based upon correlation of waveform. The three centroids, generated separately for each group, were very similar in timecourse. As the centroids represent a normalized response, however, this does not yield information about the differences in magnitude of the response discussed in Chapter 4. In the case of oxyhemoglobin, Waveform 1 (**Figure 5.2**) is remarkably similar to the timecourse described for face processing in Chapter 3, with a peak at six to eight seconds following stimulus presentation. This waveform remained relatively unchanged during the neutral and smiling conditions. A second oxyhemoglobin waveform had similar properties, but peaked much earlier, in the 3-4 second range. Finally, a third waveform appeared to peak very late, or even after the ten second time window.

The results of deoxyhemoglobin component clustering paralleled those of oxyhemoglobin, in that the shape of waveforms was notably similar between HRA and LRC groups. In addition, for the LRC group, the timing of the first two deoxyhemoglobin waveforms (**Figure 5.2**) mirrored the timing of oxyhemoglobin

waveforms, with the minimum value for deoxyhemoglobin Waveform 1 and Waveform 2 occurring at the maximum value for the respective oxyhemoglobin waveforms. Notably, there was a time delay of approximately 1.9 seconds between deoxyhemoglobin waveforms for the LRC and HRA groups that was significant for Waveforms 1 and 2 in the smiling condition ($p = 0.026$, 95% CI [0.24s 3.61s]). The deoxyhemoglobin Waveform 3 for the neutral condition contained very few components, and is likely due to systemic noise.

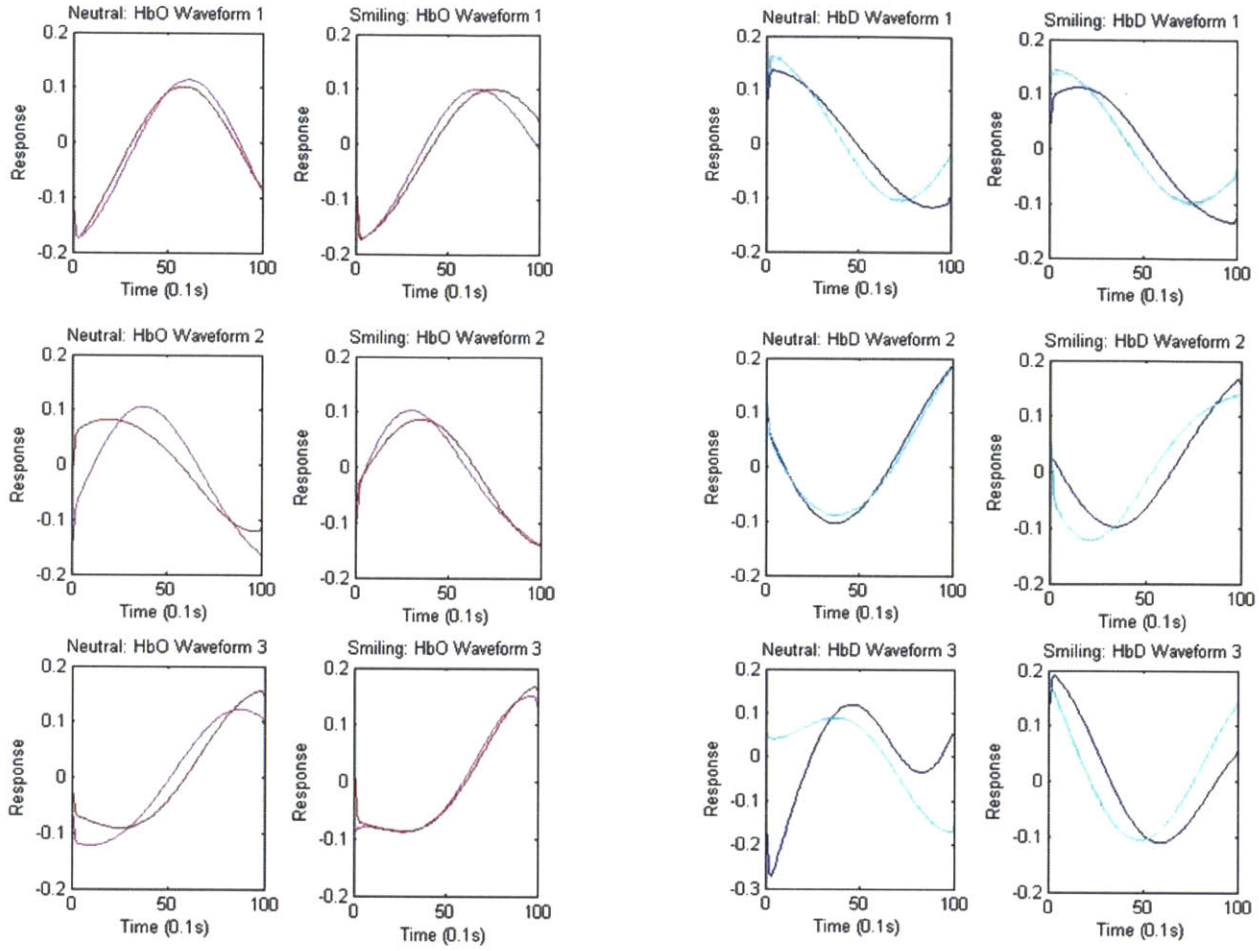

Figure 5.2: Centroid Waveforms of Component Clusters. Each waveform (centroid) is the mean of the components of the cluster, after centering and normalizing points to zero mean and unit standard deviation. Three waveforms were obtained for each of the oxyhemoglobin (HbO: red = HRA, pink = LRC) and deoxyhemoglobin (HbD: blue = HRA, cyan = LRC) components, for both the smiling and neutral conditions. Waveforms from the HRA and LRC groups are shown together based upon correlation.

5.3.2. Analysis of Components within Clusters

The individual components assigned to each cluster are depicted separately for each group and condition in **Figure 5.3**, along with the centroid waveforms. A laterality index (LI) was employed using the normalized spatial weights of each component within the cluster in order to assign the waveform to a frontal or lateral region.

$$LI = \frac{\sum_F \hat{A} - \sum_L \hat{A}}{\sum_F \hat{A} + \sum_L \hat{A}}$$

This assignment is described on each graph in **Figure 5.3**, along with the difference in mean normalized weight across the channels in each region (Frontal – Lateral).

Oxyhemoglobin Cluster 1 (corresponding to Waveform 1) was assigned to right lateral regions, and oxyhemoglobin Cluster 2 (corresponding to Waveform 2) was assigned to frontal regions for the LRC group. The difference between mean weight increased for Cluster 1, and decreased for Cluster 2, in response to smiling. The distance from components to the centroid was significantly greater for the neutral condition as compared to the smiling condition for LRC Cluster 2 ($p=0.0079$, 95% CI = [0.0829, 0.5388]). The third oxyhemoglobin cluster was labeled as "mixed," for having a very low LI and difference of mean weight between regions. A different pattern emerged for the HRA group, with oxyhemoglobin Cluster 1 assigned to frontal regions, and a reduction in LI with smiling. HRA Cluster 2 changed regional assignment from frontal to lateral

between neutral and smiling conditions, respectively, and no significant differences in the distance between components and centroid were present.

Deoxyhemoglobin Cluster 1 was assigned to frontal regions for both LRC and HRA groups. Cluster 2 maintained a constant right lateral index across conditions within the LRC group, but for the HRA group, this cluster changed from a slightly frontal index in the neutral condition to a right lateral index in the smiling condition. This is similar to the pattern of oxyhemoglobin Cluster 2 for the HRA group. Cluster 3 in the neutral condition contains very few components, and due to the unusual shape and non-specific location of both HRA and LRC waveforms, is likely non-neural in origin. In the smiling condition, deoxyhemoglobin Cluster 3 demonstrates a decrease that may represent a widespread pattern of deoxyhemoglobin change across cortical regions. This Cluster is assigned to frontal regions only for the HRA group.

Deoxyhemoglobin Cluster 1 is also notable for the variability in the rise and fall of deoxyhemoglobin component timeseries as compared to the centroid for the HRA group. An analysis of variance in the distance between components and the centroid for this cluster revealed that there is significantly greater variance for the HRA group as compared to the LRC group ($F(1,64) = 1.73$, $p = 0.03$, 95%CI = [1.06, 2.84]).

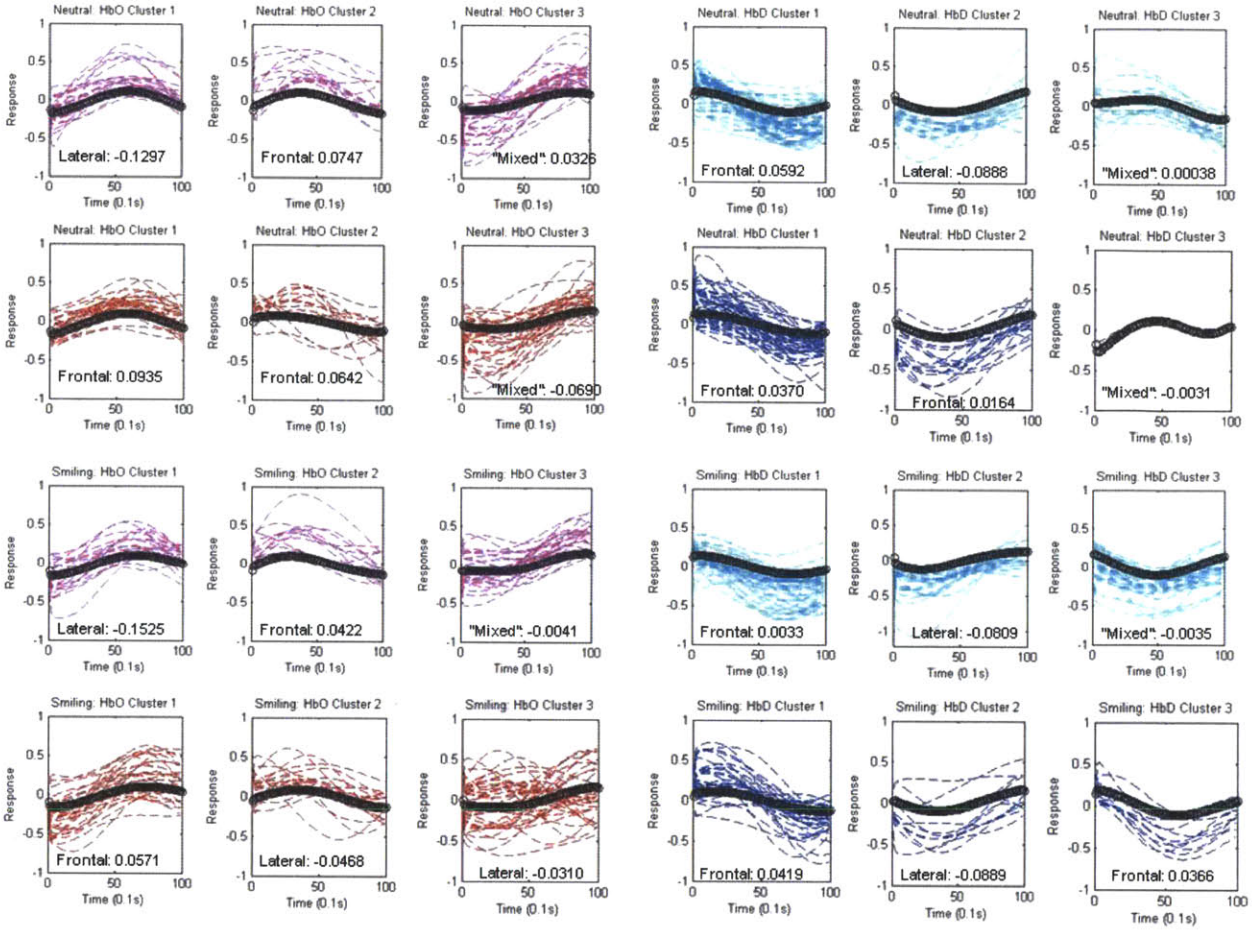

Figure 5.3: Components within Clusters. Individual components assigned to each centroid (black) are depicted separately for each group (HRA and LRC) and condition (neutral and smiling). Oxyhemoglobin (HbO) components are shown in red (HRA) and pink (LRC), while deoxyhemoglobin components (HbD) are shown in blue (HRA) and cyan (LRC). The spatial assignment by laterality index is indicated as "frontal," "lateral," or "mixed" for each cluster, and numeric differences in mean normalized weight across regions are given (Frontal-Lateral).

5.3.3. Spatial Distribution of Specific Oxy-hemoglobin and Deoxy-hemoglobin Waveforms

As mentioned previously, oxyhemoglobin Cluster 2 (corresponding to Waveform 2) was assigned to frontal regions for the LRC group, and the difference between mean weight of this cluster decreased in response to smiling. The spatial distribution of this change is depicted in **Figure 5.4A**. The decrease in difference between the mean weight of frontal and lateral regions can be interpreted as an increase in the right lateral weights of Cluster 2 during the smiling condition, and a focally increased frontal weight. There is also increased right lateral distribution of Cluster 2 for the HRA group **Figure 5.4B**, with a reduction in the spatial distribution of frontal weights between the neutral and smiling conditions.

In the case of LRC deoxyhemoglobin Cluster 1, the waveform moves from a greater frontal weight in the neutral condition, to a distribution between focal frontal and right lateral regions in the smiling condition (**Figure 5.5A**), possibly indicating a connected pattern of response in these two regions. This is not the case for the HRA group, where an increase in frontal weight and channel distribution occurs during the smiling condition, causing a greater mean weight across frontal channels for the smiling as compared to neutral conditions ($p = 0.0002$, 95%CI [0.01437, 0.4609]; **Figure 5.5B**).

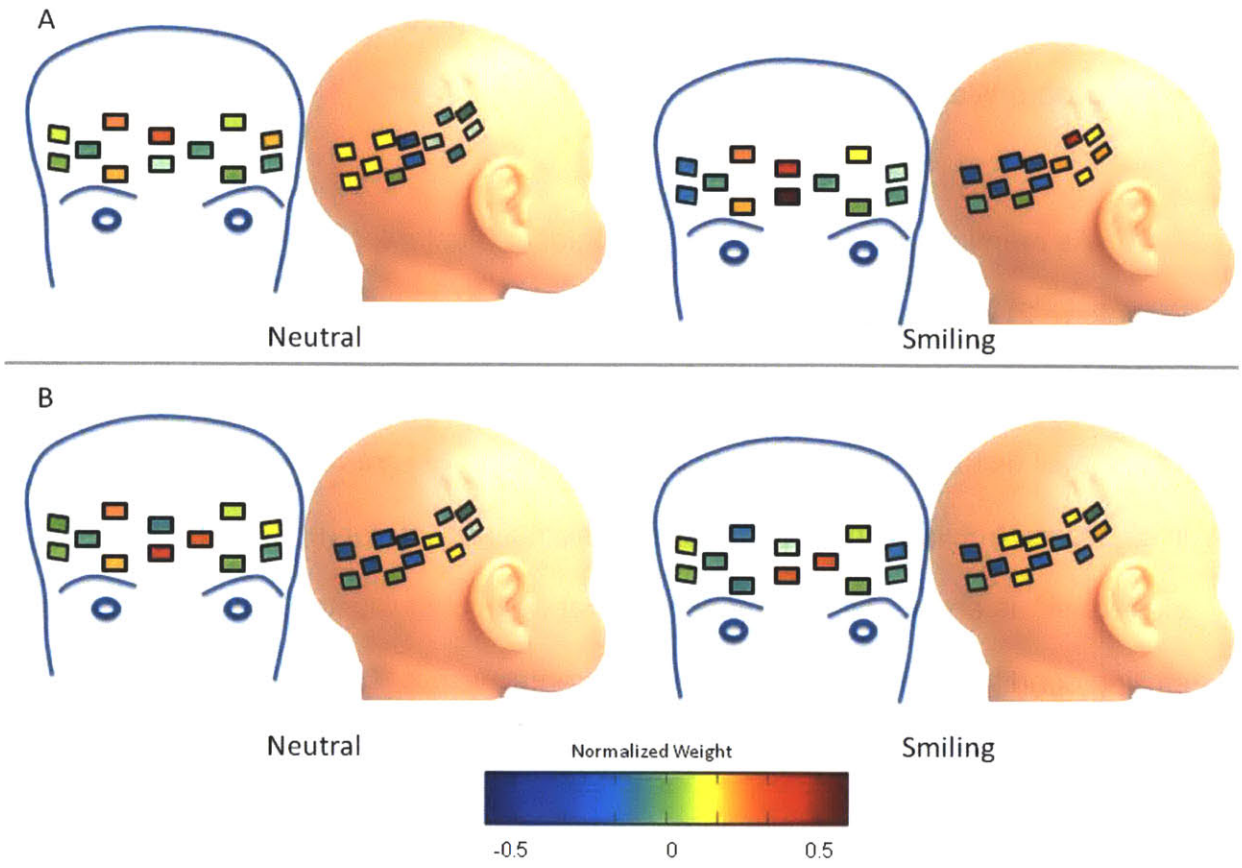

Figure 5.4. Spatial Distribution of Oxy-hemoglobin Waveform 2. Colors within channels indicate normalized weights for this waveform according to the color bar. **A)** LRC Group. Compared to the neutral condition, there is an increase in right lateral distribution of Cluster 2 during the smiling condition, and a focally increased frontal weight. **B**) HRA group. There is increased right lateral distribution of Cluster 2, but without a focal increase of weight. The spatial distribution of frontal weights is reduced between the neutral and smiling conditions.

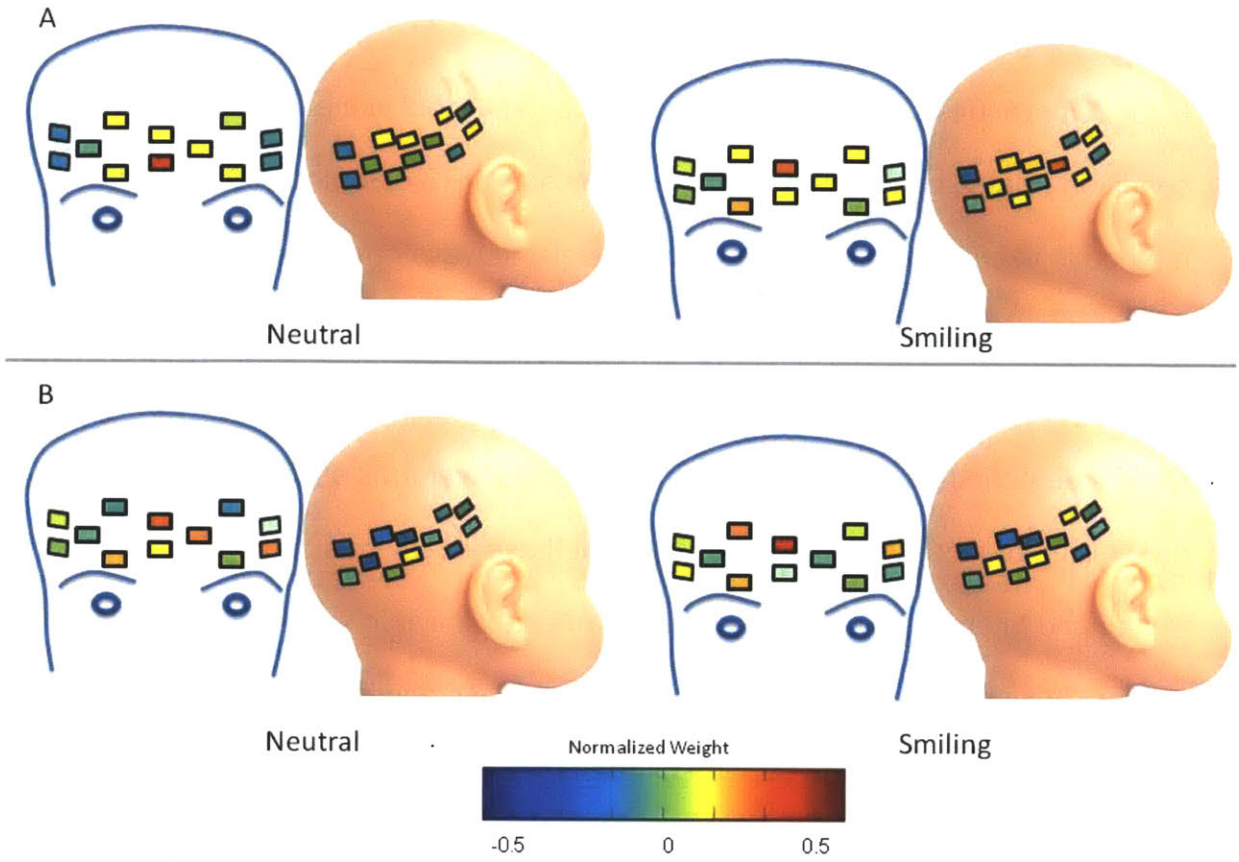

Figure 5.5. Spatial Distribution of Deoxy-hemoglobin Waveform 1. Colors within channels indicate normalized weights for this waveform according to the color bar. **A)** LRC Group. The waveform has greater frontal weight in the neutral condition, and is distributed between focal frontal and right lateral regions in the smiling condition **B**) HRA Group. Frontal weight increases during the smiling condition, resulting in a greater mean weight across frontal channels for the smiling as compared to neutral conditions (p= 0.0002).

5.4. Discussion

Our results suggest that ICA can be a useful tool in the extraction of condition-related components for the analysis of NIRS data. In particular, this method is useful when comparing activity-related waveforms, and can demonstrate both the similarities and differences in response between HRA and LRC groups. From this analysis, we can conclude that both HRA and LRC groups demonstrate at least two hemodynamic waveforms associated with the processing of faces and emotions, and that these waveforms can be localized to frontal and lateral regions. These waveforms are similar in shape between groups, however subtle differences in timing and distribution may in fact relate to an underlying endophenotype. The difference in the timing of deoxyhemoglobin waveforms in the smiling condition between LRC and HRA groups is consistent with the findings of a difference in deoxyhemoglobin response corresponding to the mother smiling condition in **Chapter 4**. In addition, the initial increases in deoxyhemoglobin seen in Waveform 1, as well as its heavily frontal distribution in the HRA group, imply that this element of the hemodynamic response differs between the HRA and LRC groups within frontal regions.

Despite similar oxyhemoglobin waveforms, our results also suggest that long-range connectivity differs between LRC and HRA groups. As shown in **Figure 5.4**, LRC oxyhemoglobin Waveform 2 increases in both frontal and right lateral channels with smiling as compared to the neutral condition, likely representing functional connectivity of these regions. Though the same waveform

is present, the response is diminished in the HRA group. Of note, HRA oxyhemoglobin Waveform 1 is present in frontal regions for both neutral and smiling conditions, while it is more laterally weighted in the LRC group. This waveform is most consistent with the timecourse of the face response described in Chapters 3 and 4, and its right lateral location in the LRC group suggests that these components may be associated with face processing. Taken together, these findings may indicate a difference in frontal lobe hemodynamic responses between the LRC and HRA groups, as well as a difference in how these frontal responses connect to other regions of the brain. In addition, the differences in spatial weight of the waveforms associated with face processing may indicate greater recruitment of frontal regions during the perception of faces in infants at high risk for autism.

It has been suggested that the connectivity patterns seen in autistic brains may be the result of abnormal neuronal migration (Bailey et al., 1998; Courchesne et al., 2011). As these differences in morphometry are most notable in measures taken from early childhood, it is likely that the greatest period for detection of change occurs within the first 6-14 months (Courchesne et al., 2005; Courchesne et al., 2011), coinciding with a period of significant cortical change and remodeling. Our results show significant differences in the timing of deoxyhemoglobin waveforms between LRC and HRA groups at six months of age, as well as differences between the waveforms of oxyhemoglobin responses localized to frontal regions. In anatomical studies, the frontal lobes have shown the greatest degree of enlargement in individuals with autism (Carper et al.,

2002), as well as increased neuronal cell counts (Courchesne et al., 2011). It is likely that any structural differences in this region would affect the hemodynamic response profile in infants at risk for developing ASD, though they might not necessarily cause the ASD phenotype. Our results may therefore represent a complex relationship between structural differences and cognitive function within the HRA group, characterized by changes in frontal lobe hemodynamics, which may in turn result in measures indicative of reduced connectivity between regions. Further discussion of these findings in relation to our study as a whole will continue in Chapter 6.

Chapter 6: General Discussion and Future Directions

6.1. Summary of Experiments

The primary aims of this dissertation were to characterize and quantify the specific neural response of face processing in infants with near-infrared spectroscopy (NIRS), and to then apply these measures at a critical developmental timepoint to the study of infants at high risk for autism. Towards this goal, we first characterized the hemodynamic response to faces in typically developing infants at five months of age as measured by the Hitachi ETG-4000 functional Near-Infrared Spectroscopy (fNIRS) system. In addition to localizing face-specific responses to a right suprasylvian region, we were able to identify key features of the hemodynamic response to faces in infancy that informed our subsequent studies of face perception in infants at high risk for autism.

Our second experiment built upon NIRS measurements of infant face processing to simultaneously monitor responses to face and emotion processing. Face processing deficits that have been reported in ASD include both difficulties in recognizing facial identity (Boucher & Lewis, 1992; Boucher et al., 1998; Gauthier et al., 2009; Kirchner, Hatri, Heekeren, & Dziobek, 2011; Klin et al., 1999; McPartland, Dawson, Webb, Panagiotides, & Carver, 2004), and difficulty processing facial emotions (Celani et al., 1999; Corbett, Carmean, Ravizza, Wendelken, Henry, Carter, & Rivera, 2009b; Dawson et al., 2004; Gross, 2004; Grossman & Tager-Flusberg, 2008; Kliemann, Dziobek, Hatri, Steimke, & Heekeren, 2010; Monk et al., 2010; Pelphrey et al., 2002). Both of these aspects

of face perception have developed to a measureable extent by seven months of age (see **Chapter 2**). Our work therefore examined ten 6-7 month-old infants at high risk for developing autism (HRA group) and ten typically developing controls (LRC group) using a task designed to differentiate between the effects of face recognition and the perception of emotional expressions. This experiment demonstrated that NIRS can be used in the simultaneous study of face recognition and emotion processing in infants, with right lateral regions again associated with face processing, and orbitofrontal regions linked to the processing of social characteristics such as identity and emotion. These regions also revealed a pattern of response in the HRA group that differed from responses of the LRC group. This currently represents the earliest identification of an endophenotypic pattern of functional brain activity in infants at high risk for ASD.

As theories of weak central coherence suggest that individuals with autism may have difficulty with integration of stimulus features, our final analyses further explored group differences in the integration of face and emotion responsive regions with the application of novel methods of connectivity analysis to our NIRS data. We adapted a method first employed by Katura and Sato et al. (2008) to our paradigm, which involved first independent component analysis, followed by selection of components in which mean intertrial correlation suggested reproducibility of the response pattern with stimulus presentation, and finally clustering of those components based upon waveform. We were then able to examine the timecourse of component clusters for both the HRA and LRC

groups, as well as the spatial weighting of those waveforms during the presentation of neutral and smiling facial expressions. These analyses allowed us to develop a clearer picture of the hemodynamic response to emotions in faces in typically developing 6-7-month-old infants, and revealed both the similarities and differences between these responses and those measured from infants at high risk for autism.

6.2. The Infant Response to Faces as Measured by NIRS

In each of our experiments we gained knowledge about the infant hemodynamic response to faces. Our NIRS measurements are consistent with previous studies of infants showing a right-sided response to face stimuli (Taga et al., 2003; Otsuka et al., 2007), and we further localized this response to channels at the occipito-temporal border, at the approximate location of the right posterior suprasylvian gyrus (**Figure 3.2**). While we cannot image the fusiform gyrus using our current NIRS methods, this finding is consistent with the location of face processing in young children (Gathers et al., 2004; Joseph et al., 2006), and may correlate with mechanisms of featural face processing utilized in early development. We were able to replicate this finding from our face inversion paradigm in our second experiment involving pairs of face video stimuli, adding support to the theory that the localized right lateral response is associated with face processing (**Figure 4.2A**).

In addition to identifying regions of the infant brain that were most responsive to faces, we were also able to observe a consistent timecourse of this

response in low risk infants across our experiments. In our first experiment, the oxyhemoglobin response to upright as compared to inverted faces first became significant at two seconds following the onset of stimulus presentation with a maximal difference occurring 7-8 seconds following stimulus onset (**Figure 3.3**). This pattern was again identified as a condition-related component waveform in Chapter 5, and was weighted towards right lateral regions in the low risk control group. It has been noted that the hemodynamic response associated with neural activity can differ between brain regions (Jasdzewski et al., 2003), as well as across development (Taga et al., 2003). The timecourse identified for face responsive oxyhemoglobin waveforms is consistent with that reported for other visual stimuli in infants (Taga et al, 2003), and despite a difference in spatial location, is temporally similar to the waveform of the adult visual response.

Notably, deoxyhemoglobin responses were not significant in our first experiment examining the face inversion effect, nor in our separate analyses of low risk controls in the face and emotion paradigm. This is consistent with previous findings in typically developing infants (Taga et al., 2003; Otsuka et al., 2007; Minagawa-Kawai et al., 2009). Taga et al. (2003) observed that while deoxyhemoglobin responses were not significant on a group level in response to visual stimuli, individual subjects demonstrated a significant decrease in deoxyhemoglobin that accompanied oxyhemoglobin responses. The timing of this response was similar to that of oxyhemoglobin, but reached a minimum value just after the peak of the oxyhemoglobin timecourse. A similar deoxyhemoglobin timecourse was identified for the LRC group in Chapter 5, and

effects of face identity in which the deoxyhemoglobin response to mother was greater than the response to stranger were marginally significant for the LRC group alone. These findings together suggest that deoxyhemoglobin changes occur in the normal infant response to faces, but it is unclear whether this response is modulated with changes in face identity and emotion.

6.3. Face Responsiveness and Recognition in Infants at High Risk for ASD

In Chapters 4 and 5, we extended our observations about the normal infant response to faces to study infants at high risk for ASD. Our paradigm involved the use of both mother and stranger videos of faces, allowing us to explore the effects of face identity upon observed responses. Oxy-hemoglobin responses were greater in the LRC group than in the HRA group for both right lateral occipital channels and the suprasylvian channel demonstrating a discriminatory response between neutral mother and stranger faces. Several right lateral channels also demonstrated a Group x Face Identity effect, in which oxy-hemoglobin responses were greater for mother than stranger faces only in the LRC group.

Despite group differences in the magnitude of the oxyhemoglobin response to face identity, the waveforms identified for both HRA infants and controls were remarkably similar (**Figure 5.2**). The waveform most consistent with the timecourse of face responses was weighted towards a frontal distribution in the HRA group, and a right lateral distribution in controls. This may be due to a difference in the hemodynamic response function of frontal regions in infants at

high risk for autism, where patterns typically seen only in right lateral regions in controls are dominant in frontal areas of the brain. In addition to our oxy-hemoglobin findings, initial increases in deoxyhemoglobin, seen in deoxyhemoglobin Cluster 1 (**Figure 5.3**), as well as its heavily frontal distribution in the HRA group, imply that this element of the hemodynamic response differs between the HRA and LRC groups within frontal regions. The theory that the timing of the response may be different in high risk infants is supported by studies showing that face sensitive ERP components are less lateralized in individuals with ASD as compared to controls (McCleery et al., 2009), and that these components also have shorter latencies for objects as compared to faces (Webb, Dawson, Bernier, & Panagiotides, 2006). It is important to note that the HRA group did demonstrate responses to faces, and no individual outliers caused the difference in spatial distribution of the face responsive waveform. While deoxy-hemoglobin was not significantly associated with face processing or recognition for low-risk infants, the responses in frontal channels were greater for HRA as compared to LRC groups. There were also numerous interaction effects between Group and Face Identity, generally revealing a greater deoxy-hemoglobin response to mother's face as opposed to stranger face within the HRA group across both frontal and lateral regions. These deoxy-hemoglobin changes in the HRA group indicate that some level of discrimination between mother and stranger must exist. Statistically, only 20 percent of the HRA group will go on to develop ASD, so while our observations indicate differences from

low risk controls, they cannot be interpreted as markers of an abnormal cognitive process.

Another possibility is that the difference in spatial weight of the face responsive waveform is due to otherwise normal cognitive activity in frontal regions that is abnormally heightened during the perception of faces in infants at high risk for autism. Greater oxyhemoglobin responses to mother as compared to stranger were seen across central orbito-frontal regions in controls (**Chapter 4**), indicating that this region is involved in some aspects of face recognition and identification. This finding is consistent with the infant responses to mother versus stranger observed by Minagawa-Kawai et al. (2009). As previously mentioned, this response in typically developing infants may be a neural correlate of the increased attention to the mother, and particularly the ability to identify the mother's face, which develops in the earliest months of infancy. Our observations suggest that, as a group, infants at high risk for autism do not lack this response, but rather fail to activate these areas in a discriminatory pattern based upon face identity to the same degree that we observe in low risk controls. Difficulties in recognizing facial identity are common in ASD (Carver & Dawson, 2002; Dawson et al., 2002; Kirchner et al., 2011), and it is possible that this deficit arises from regional differences in the featural processing of faces during early development. The fMRI responses of right lateralized structures associated with face recognition have been shown to be hypoactive in autistic individuals (Corbett, Carmean, Ravizza, Wendelken, Henry, Carter, & Rivera, 2009a; Dalton et al., 2005; Dziobek et al., 2010; Hadjikhani et al., 2007; McPartland et al., 2004;

Monk et al., 2010), with hyperactivity in orbitofrontal regions and amygdala in response to specific facial features (Dalton et al., 2005). It has been suggested that hyperactivation of frontal response pathways in individuals with autism may ultimately lead to aversion to faces, and thus a reduction in response of face processing regions (Dalton et al., 2005). At 6-7 months of age, we observe that there is a connection between frontal and right lateral regions during face processing, and the pattern of frontal as opposed to lateral weighting of the response does exist for high risk infants. This is a key addition to our understanding of both the timing of developmental processes involved in autism, and the broader range of face processing endophenotypes that may be present during infancy. It remains to be determined whether our findings represent a permanent cognitive processing endophenotype associated with high risk of ASD, or a temporary shift in the normal developmental trajectory of face pereption, and further efforts will be made to understand the nature of these differences throughout infancy.

6.4 The Processing of Facial Emotions in Infants at High Risk for ASD

Our observations of the effects of positive facial emotion upon NIRS responses in typically developing infants are consistent with previous studies (Dalton et al., 2005; T. Grossmann, Parise, & Friederici et al., 2010b; Ito et al., 2011; Minagawa-Kawai et al., 2009), with oxyhemoglobin responses to the effect of smiling noted in right orbito-frontal cortex. As previously mentioned, these orbito-frontal responses may represent an attentive response that is particularly

tuned to social stimuli, and differentially responsive to affect. Independent component analysis revealed a frontally weighted oxyhemoglobin waveform with a slightly more rapid timecourse than that of the face response (**Figure 5.2**, oxyhemoglobin Waveform 2), which is likely associated with this attentive response. Both this waveform and a deoxy-hemoglobin waveform became strongly weighted in regions associated with face processing during the smiling condition (**Figures 5.4A & 5.5A**), indicating functional connectivity between orbitofrontal and right lateral face processing regions during attention to emotionally expressive face stimuli in typically developing infants.

Despite the presence of similar oxy- and deoxy-hemoglobin waveforms, our results also suggest that long-range connectivity associated with both face and emotion processing differs between LRC and HRA groups. The HRA oxyhemoglobin waveform corresponding to attention or emotion processing became more focal in frontal locations with diffuse right lateral increases in weight in response to smiling, while deoxy-hemoglobin increased in frontal weight alone. As discussed in Chapter 2, models of reduced connectivity suggest that ASD may result from reduced synchronization between brain regions causing a lack of coherence (Brock et al., 2002; Frith & Happe, 1994; Just et al., 2004). Recently, it has been suggested that deficits in integration may be an outcome of increased local processing (Happe & Frith, 2006), and structural data supports the theory that such differences may be present in undiagnosed siblings of children with autism (Barnea-Goraly et al., 2010). Our findings support this model in infants at high risk for ASD, where increasing attention to a stimulus

may disproportionately increase normal local patterns of response, perhaps at the expense of synchronization with more distant regions.

In addition, there was a notable difference in the timing of deoxyhemoglobin waveforms in the smiling condition between LRC and HRA groups. Deoxyhemoglobin responses were also significant for interactions with the emotion condition for the HRA group, where smiling was observed to reduce deoxyhemoglobin changes. This effect of emotion upon the magnitude and latency of the response may suggest an abnormal pattern of both local and long-range connectivity in the HRA group. It has been suggested that the connectivity patterns seen in autistic brains may be the result of abnormal neuronal migration (Bailey et al., 1998; Courchesne et al., 2011), and that these structural abnormalities may be most evident in the 6-7 month age range, during a period of significant cortical change and remodeling. Anatomical studies frequently implicate the frontal lobes in the pathophysiology of autism (Amaral et al., 2008; Courchesne et al., 2001; Courchesne et al., 2011; N. Schmitz et al., 2007), and it is not surprising that this region is also involved in the integration and long-range connection of many cortical functions. It is possible that structural differences in this region, including an increased neuronal cell count (Courchesne et al., 2011), could affect the hemodynamic response profile associated with neural activity. The mechanisms by which neural activity are coupled to hemodynamic response are still a topic of much research, however experiments comparing electrophysiological measurements with hemodynamic responses have found that the latter correlates better with local mean field potential, rather than local

spiking rates, suggesting that the hemodynamic response is dominantly driven by input synaptic activity rather than output spiking activity (Lauritzen, 2001; Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). A pathological increase in frontal neuronal cell count, in the absence of increased microvasculature, might therefore lead to small but measurable increases in consumption of oxygen in response to the synaptic inputs. This might in turn lead to a reduced measure of the oxy-hemoglobin overshoot in localized frontal channels. The initial increase in deoxy-hemoglobin concentration due to neuronal consumption of oxygen might also lead to the observation of greater change in deoxy-hemoglobin over the course of stimulation, as well as a delay in the decrease of deoxy-hemoglobin concentration with each trial. These changes in hemodynamic activity may reflect a response profile of infants at risk for ASD, though they may not necessarily cause the ASD phenotype. Our results may therefore represent a complex relationship between structural differences and cognitive function within the HRA group, characterized by changes in frontal lobe hemodynamics, which may in turn be correlated with reduced connectivity between regions.

6.5 Conclusions and Future Directions

In sum, the present study showed that NIRS can be used in the study of emotion and identity as it relates to face processing in infants at six to seven months of age. In addition to the association of right lateral regions with face processing, orbito-frontal regions are implicated in the processing of social

information such as emotion and identity. These regions also demonstrate patterns of response in infants at high risk for autism that differ in magnitude, timing and connectivity as compared to low risk controls. While we cannot yet conclude whether structural differences, functional connectivity, or a combination of both account for the observed differences, it is clear that a distinguishing process may be measured at 6-7 months of age.

Due to the diagnostic criteria for ASD, as well as the nature of the disease as a developmental disorder, a limitation of the current study is the inability to determine which individuals within the high risk group will ultimately develop the disease. As a part of the greater Infant Sibling Project, future studies will identify those individuals who meet criteria for ASD on the ADOS, and examine the distribution of our data from 6-7 months of age within both "high risk" and "affected" groups. In addition, measures of the same task at nine and twelve months of age will be included to give a broader picture of the tuning of face and emotion perception throughout later infancy. It is our hope that these measures, in combination with other data collected through the Infant Sibling Project, will allow us to better understand the endophenotypes present in the high risk group, and possibly to assess risk for ASD during infancy.

Finally, future studies should be directed towards the understanding of how anatomical variations may affect hemodynamic response in individuals with ASD. This task is particularly challenging, as many of the structural differences are hypothesized to be greatest in infancy, when ASD cannot yet be diagnosed.

The addition of NIRS studies of orbito-frontal response to a variety of stimulus paradigms may shed light upon the effects observed in this dissertation, and suggest whether the response pattern observed is specific to faces, or represents distinct changes in frontal regions that affect both localized responses, and long-range connections to many other brain regions in infants at high risk for ASD.

Appendix A

Near-Infrared Spectroscopy (NIRS)

Near Infrared Spectroscopy (NIRS) is a FDA approved technology that has been used for the past twenty years with adults and for the past ten years with infants (Aslin and Mehler, 2005; Lloyd-Fox, 2010; Taga et al., 2003). NIRS is a non-invasive technique that is similar to functional magnetic resonance imaging (fMRI) in that it measures localized blood flow and concentrations of oxygen in the human brain. It is widely accepted that there is a strong correlation between blood oxygen concentrations in the brain and neural activity (Lauritzen, 2001). Previous positron emission topography (PET) and fMRI studies have shown that the ratio of oxyhemoglobin to deoxyhemoglobin concentration, or blood oxygen level dependence (BOLD) signal, is a reliable index of neural activity in animals and human adults (Culver et al., 2005; Huppert et al., 2005; Born et al., 1996; Altman et al., 2001). Additionally Robinson et al. (2006) established a strong correlation between the BOLD response, or the change in the BOLD signal as a response to a stimulus, and continuing evoked neuronal activity. Yamada et al. (1997 and 2000) also reported an age-dependent reversal in BOLD signal; infants older than 8 weeks showed a rapid inversion of response to visual stimuli as compared to infants less than 7 weeks of age, suggesting increased neural metabolism. These studies establish a direct relationship between blood oxygen levels and neural activation resulting from a stimulus, and also suggest difference in hemodynamic response function across development.

As hemodynamic responses are generally robust, and easy to monitor with NIRS as compared to fMRI or PET, it is an ideal method for inferring functional neural activity when studying differences between normal and abnormal development.

The NIRS imaging technique is able to measure oxygen levels based on the principle that hemoglobin absorbs near-infrared wavelengths of light in proportion to the concentration levels of oxygen in the blood. The NIRS device works by shining a near-infrared light source onto the exterior of the skull by means of fiberoptic cables, and uses a photodetector to sample the variations in light reflected back from the cortical surface. The device generates two wavelengths in the range of 690nm and 830nm in order to measure the levels of oxy-, deoxy-, and total hemoglobin in the blood. The absorbance of these wavelengths is measured at multiple channels using light-detecting sensors, or NIRS probes. Each pair of adjacent incident and detection fibers define a single measurement channel, allowing the measurement of oxy-, deoxy- and total hemoglobin changes in the brain. Furthermore, NIRS can be performed on awake and non-sedated neonates and infants as well as adults, thus differing from fMRI studies, which may require the sedation of infants in order to acquire motion-free images (Born et al., 2002; Altman et al., 2001). The sensitivity and accuracy of the NIRS imaging technique can be estimated from numerous studies with adults as well as infants. Previous NIRS studies with infants have successfully observed activation of single sites of the cerebral cortex (Meek et al., 1998; Taga et al. 2003; Sakatani et al., 1999; Bartocci et al., 2000).

The Hitachi ETG-4000 NIRS System

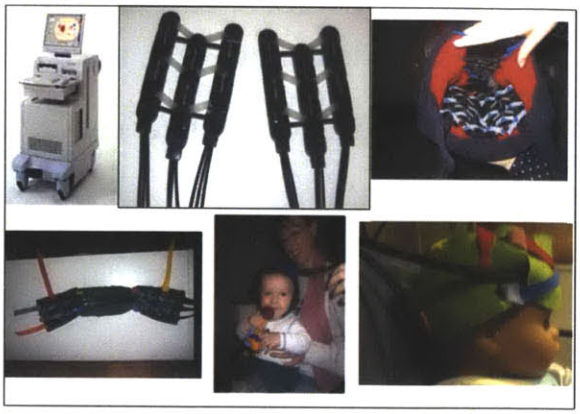

Figure A1: NIRS Probes and Detectors

The Hitachi ETG-4000 NIRS system (Figure A1) is ideal for the study of infant brain function because the light source(s) are not intense (less than 1 mW/cm^2), the light source(s) are largely transparent to biological tissue (i.e., there is insufficient absorption to cause heating), and the light source(s) and photodetectors can be placed on the surface of the head. Two wavelengths of light (695nm and 830nm) were used in our studies to measure cortical levels of oxy-, and deoxy-hemoglobin. The near infrared light was guided by optical fiber bundles that were 1mm in diameter. On the ETG-4000 device, each pair of adjacent incident and detection fibers defined a single measurement channel. The NIRS probes consist of two 3 x 3 chevron arrays, each with five emitting and four detecting fibers held in place by a silicone support with 3cm spacing.

Appendix B

Temporal Independent Component Analysis of fNIRS Data

Temporal independent component analysis (ICA) is classically explained in terms of the "cocktail party" problem (Cherry, 1953; Hyvarinen et al., 2001). In a crowded cocktail party, many people are talking at the same time. If several microphones are present, then their outputs are different mixtures of voices. Given such mixtures, ICA identifies the original voices from the mixtures by assuming that the original voice signals are independent from each other.

In the case of fNIRS measurements, measurements of T time points from N channels $\mathbf{x}(t) = [\mathbf{x}_1(t),\mathbf{x}_2(t),...,\mathbf{x}_N(t)]^T:(t = 1, 2,..., T)$ are comparable to the mixtures recorded by microphones, and the N "true" sources $\mathbf{s}(t) = [\mathbf{s}_1(t), \mathbf{s}_2(t),...,\mathbf{s}_N(t)]^T:(t = 1, 2,..., T)$ can be viewed as the understandable voice signals from each individual. The fNIRS measurements at each channel can thus be expressed as a mixing procedure by multiplying matrix **A**:

$$\mathbf{x}(t) = \mathbf{A}\mathbf{s}(t).$$

ICA decompositions can be viewed as an inversion of this:

$$\hat{\mathbf{s}}(t) = \mathbf{W}\mathbf{x}(t),$$

where $\hat{\mathbf{s}}(t)$ is an estimation of the true sources, and **W** is an unmixing matrix. The pseudoinverse of **W** is an estimation of **A**:

$$\mathbf{A} = \mathbf{W}^{-}.$$

$\hat{\mathbf{A}}$ therefore represents a set of the spatial weights, which can be used to localize sources to brain regions (Zhang et al., 2010), and $\hat{\mathbf{s}}(t)$ represents a set of the temporal activities related to those regions.

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