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Using fNIRS to Identify Brain Regions Involved in Emotional Face Processing in Infants at High Risk for Autism Spectrum Disorder

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THE CITY UNIVERSITY OF NEW YORK

Abstract

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Faces provide an abundance of salient information, and within a few hours of being born, infants already show preferential attention to faces and face-like stimuli. Autism spectrum disorder (ASD) is a developmental disorder consisting of social communication and interaction difficulties, and individuals with ASD show differences in the behavioral and neural processing of faces. Prospective studies with infants at high risk for ASD (HRA; by virtue of an older sibling with ASD) have begun to look at whether responses to faces could be an early marker of later ASD. Using functional near-infrared spectroscopy (fNIRS), the current study measured oxygenated hemoglobin (oxyHb) levels in both the frontal and right lateral regions of the brain in 6- to 14- month-old HRA infants and low-risk control (LRC) infants (with no family history of ASD). Infants viewed videos of their mother and a stranger speaking with a neutral expression and then a happy expression. Results provided evidence that the right lateral region was more involved in face processing than frontal regions. However, there was minimal evidence of group-related effects on oxyHb responding during face processing. Future research would benefit from

a larger sample size as well as incorporating ASD outcomes in order to ask whether fNIRS responses in infancy could provide a marker for later ASD.

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Introduction

Humans find that features of the human face are highly salient. Very soon after birth, newborns show an attentional bias towards human faces compared to other stimuli (Valenza, Simion, Macchi, & Umilta, 1996). For example, in work by Valenza et al. (1996), 20 full-term newborn infants (as young as 58 hours old) were simultaneously presented with two stimuli. The facelike stimulus was a white, head-like form, with three black squares representing human features (i.e., two eyes and a nose/mouth), and the non-facelike stimulus was the same as the facelike stimulus, except inverted. The results showed that even at this young age, infants preferred facelike stimuli over non-facelike stimuli. Similar findings were seen by Simion and colleagues (Simion, Valenza, Umilta, & Barbara, 1998). In this follow-up study, 26 full-term newborns (average age of 74 hours) were shown the same stimuli as in Valenza et al. (1996) while infant's eye movements were recorded. As in Valenza et al. (1996), newborn infants preferred facelike patterns oriented upright as compared to those oriented upside down. Recent research has even suggested that there might be an attentional bias towards face-like configurations beginning as early as the third trimester during pregnancy (Reid et al., 2017).

In addition to studies showing early differentiation between faces and non-faces, a large body of research has looked at infant differentiation between familiar and unfamiliar faces. For example, there is evidence that infants are able to differentiate between familiar and unfamiliar faces (Brooks-Gunn & Lewis, 1981). The familiar face portion of the study was two pictures, one of the infant's mother and one of their father, while the stranger portion was two pictures, one of a female stranger and the other a male stranger. In all, 70 infants, between 9 to 24 months old, were shown the familiar and unfamiliar stimuli (in random order), with an observer recording the infant's fixations of the stimuli. The results showed that infants are able to use

familiarity to differentiate between people. Later research provided evidence that newborn infants are able to differentiate between familiar and unfamiliar faces (Bushnell, Sai, & Mullin 1989). Using a visual preference task with a paired comparison of stimuli, Bushnell et al. (1989) tested 40 neonates between the ages of 12.5 and 101 hours to see if they were able to differentiate between their mother's face and the face of a female stranger. The results showed that newborn babies, like older infants, were capable of discriminating between the face of their mother and the face of a stranger.

More recently, eye-tracking studies have provided further support showing that infants can discriminate between familiar and unfamiliar faces. For example, in Wagner et al. (2013), eye gaze was recorded as 6-month-olds (n = 36), 9-month-olds (n = 42), and 12-month-olds (n = 39) viewed side-by-side color photographs of neutral female faces, one being the infant's mother and the other a stranger with similar features. The results showed evidence that infants preferred to look at their mother's faces as compared to a stranger's face across age. Additionally, the parents of each infant were asked to fill out a measure of their infant's social communication at 18 months; researchers found that the amount of time spent on the eyes at 6 months positively predicted social abilities at 18 months, linking infant eye-gaze to later social outcomes (Wagner et al., 2013).

Infants show development in their selective attention to core features of faces, with two main core features being the eyes and the mouth. Within the first year of life, Lewkowicz and Hansen-Tift (2012) found a major transition between time spent on the eyes and time spent on the mouth when looking at faces. Using an eye-tracker, infants at 4 months (n = 19), 6 months (n = 16), 8 months (n = 17), 10 months (n = 16), and 12 months (n = 20) were shown videos of females speaking in English. The results showed a major transition to more attention being spent

on the mouth rather than the eyes at around 8 months, around the same time infants are increasing their repertoire of babbled speech sounds to more "word-like" sequences. This finding suggests that the ability to selectively pay attention to specific core features of speaking faces could be crucial for language acquisition. Preference for looking at the mouth was shown to persist into the second year of life in a follow up study with a total of 91 infants (47 14-month-olds, 44 18-month-olds) using a similar procedure; results showed that both age groups looked longer to the mouth of the female speaker as compared to the eyes (de Boisferon, Hansen-Tift, Minar, & Lewkowicz, 2018).

Studies with static faces also show a shift in attention between the eyes and mouth. Wagner et al. (2013) looked at attention to eyes vs. mouth across the 6-, 9-, and 12-month-old infants, in addition to face familiarity. Findings showed that all three ages preferred to look at the eyes over the mouth; however, findings showed that between 6 and 12 months, infants increased their attention to the mouth and decreased their attention to the eyes. This is consistent with work by Lewkowicz and Hansen-Tift (2012). These studies provide evidence that infants gain not only the ability to selectively attend to stimuli but are able to deploy this selective attention differently at different ages depending on what salient information they need to gather from the person.

The ability to differentiate between core facial features is thought to be important for processing of emotional faces in infants. As early as 3 months, Barrera and Maurer (1981) found that infants can discriminate between smiling faces and frowning expressions. In a sample of 24 infants, the researchers habituated half of the infants to a picture of their mother smiling and the other half to a picture of their mother frowning. When the infants were presented with the opposite facial expression, they spent more time looking at the novel expression as compared to the familiar expression. Interestingly, when this same task was performed with the stimuli being

a stranger instead of their mother, the same result occurred; infants spent more time looking at the novel expression of a stranger. Older infants also respond differently to emotional faces. For example, work by LaBarbera, Izard, Vietze, and Parisi (1976) found that when infants between the ages of 4 and 6 months (n = 24) were shown pictures of different emotional faces, including joy, anger, and neutral expressions, infants looked longer at the pictures displaying joy as compared to both anger and neutral expressions. This finding suggests that not only do infants differentiate between emotional expressions, but they also show a preference for non-neutral expressions.

In addition to these behavioral studies, researchers have also sought to understand the neural basis of infant face processing. Classic studies have focused on electroencephalography (EEG) and event-related potentials (ERP). EEG and ERP both measure electrical activity in the brain, with ERP being the time-locked EEG activity in response to stimuli. Early work found that infants' neural responses to familiar and unfamiliar faces differed as early as 6 months (de Haan & Nelson, 1997). Using ERP, de Haan and Nelson (1997) showed 22 6-month-old infants videos of their mothers, and either a video of an adult female that looked similar to the infant's mother or an adult female who looked different from the infant's mother. In both situations, infants elicited different ERP activity between the videos of their mothers and the videos of the strangers, providing evidence that infants showed a neural index of discrimination between familiar and unfamiliar faces. More recent work by Safar and Moulson (2020) has found that at 3 months of age, infants also show differences in neural activity between happy and fearful facial expressions. Using ERPs, 36 infants were shown fearful and happy faces in a random order. The ERP results showed that there was a difference in activity between happy and fearful face processing.

Adult studies that incorporate functional magnetic resonance imaging (fMRI) brain imaging techniques have pinpointed several important regions involved in face processing. One fMRI study done by Kanwisher and colleagues (Kanwisher, McDermott, & Chun, 1997) measured participants' (n = 15, adults under the age of 40) occipitotemporal activation when shown pictures of faces or of other stimuli. They found an increase in activity in the fusiform gyrus, a region in the temporal and occipital lobe, when participants were looking at the pictures of faces as compared to the other stimuli. Additionally, subjects also showed activation in response to faces as compared to objects in the right temporal region, around the superior temporal gyrus (STG), specifically the superior temporal sulcus (STS). Infant work has also suggested responses using EEG/ERP that could relate to similar brain regions, and researchers posit that the social abilities seen as early as 6 months in infants could be a result of of development of this "social" brain network (Mori et al., 2015), consisting of the brain structures such as the fusiform gyrus, amygdala, and superior temporal suclus and gyrus.

Neuroscientists must carefully consider a method's strengths and weaknesses in relation to the goals of a study, and these considerations become even more complex when studying infants. The two most well-known brain imaging techniques that are used with adults are EEG and fMRI, with each having their strengths and weaknesses. EEG has been a great technique in order to study temporal information about neural responses, but it is unable to provide precise spatial information even with advanced statistical analysis. In contrast, fMRI is a great technique for providing high spatial resolution of neural activity, but it is unable to provide detailed temporal information. Furthermore, despite the success seen in fMRI studies in adults, it remains limited as a technique to use with infants. This is because absence of movement is required while inside the fMRI apparatus, which can be challenging in awake infants. There have been studies

of infant brains using fMRI when infants are sleeping (e.g., Mitra et al., 2017), but this takes advantage of fMRI's high spatial resolution in a limited situation, particularly regarding cognition. Therefore, researchers who are interested in studying brain sources to cognitive processes in awake and freely-moving infants have explored additional methodologies to achieve these goals.

A newer technique in the brain imaging field, functional near-infrared spectroscopy (fNIRS), shows promise as a more appropriate method for analyzing localized information about the infant brain. In a recent review of fNIRS by Pinti et al. (2018), the technique was described as having three essential characteristics that make it suitable for infant work: high portability, non-invasive technology, and resistance to noise from bodily movements. First, similar to EEG, fNIRS is a multichannel, wearable instrument meant to be placed on top of the subject's head. This method does not require heavy equipment that cannot be moved, such as an MRI machine, serving as both a more cost-effective and dynamic apparatus.

Secondly, unlike PET scans, but like EEG and fMRI, fNIRS is a non-invasive procedure. fNIRS measures changes in the concentration of oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) triggered by changes in localized neural activation (Pinti et al., 2018), similar to the response source of fMRI. fNIRS works by shining near-infrared light into the skull (in the range of 650 nm and 950 nm) and based on differential light absorption patterns for oxyHb and deoxyHb, the attenuation of the light can be used to identify concentration changes in both oxyHb and deoxyHb. This procedure allows for a safe examination of region-specific hemodynamic activity in infants.

The third important characteristic of fNIRS is that it is resistant to noise from bodily movements. fNIRS instruments are lightweight, and the newer models rely less on fiber optics,

making them less sensitive to artifacts. With less sensitivity to movement, fNIRS measurements with awake and freely moving infants can still be a reliable measure of brain activity. These three attributes together support the increased interest in using fNIRS in early infant research.

A review of fNIRS face-processing papers in infancy by Otsuka (2014) indicates that this method has been successful in measuring face-processing biases, including the bias to facelike stimuli. The consensus of the review paper is that studies of facial processing in infants have found differentiation between face and non-face images in cortical responses in particular areas of the brain. The first paper to measure the hemodynamic response in infants using fNIRS came from Csibra et al. (2004). With the fNIRS apparatus set up to measure responses in both the frontal and occipital lobes in 4-month-old infants (n = 11), Csibra et al. (2004) found a larger decrease in oxyHb and an larger increase in deoxyHb in occipital regions in response to the facial images than the noise images. An important note to consider is that adults were also tested in this study and displayed results that were identical to the infant subjects. Together, this shows that facial discrimination is present in infant brains and is cortically similar to the neural processing of facial discrimination in adults.

In studies of the temporal brain region, studies have also shown that there is an increase in the hemodynamic response with regard to facial discrimination (Otsuka, 2014). This finding is important, showing that the infant brain, like the adult brain, has several face processing areas that can be measured using fNIRS. Otsuka et al. (2007) was among the first fNIRS studies to record the temporal hemodynamic response in infants. Infants between the ages of 5 and 8 months (n = 10) were shown pictures of female faces and vegetables. The results showed that there was an increase in both oxygenated and total hemoglobin levels when infants were shown faces versus vegetables.

As discussed earlier, infants at a young age were shown to differentiate between their mother's face and a stranger's face using both ERP and eye-tracking data. The mother-child relationship is one of the most important and dynamic relationships in a child's life and has also been studied using fNIRS. Minagawa-Kawai et al. (2009) showed 12-month-olds (n = 15) a video of either their mother or a stranger, starting with a neutral expression and shifting into a smiling expression, with the fNIRS apparatus placed over the forehead and measuring the frontal lobe. Results showed that there was an increase in the hemodynamic response in the orbital frontal cortex (OFC) when infants were shown their mother smiling as compared to a stranger smiling.

fNIRS work has also looked at infant processing of emotions. One study found that happier face stimuli elicited significant changes in both oxyHb and deoxyHb in the prefrontal cortex (Ravicz, Perdue, Westerlund, Vanderwert, & Nelson, 2015). Ravicz and colleagues (2015) tested 7-month-old infants (n = 24) who participated in a maximum of 30 trials, with each trial including 5 images from one emotional category (happy, fearful, angry, or neutral). While the specific changes between oxyHb and deoxyHb were opposite of what the researchers predicted, there was still a hemodynamic response that differentiated between happy and neutral. The researchers hypothesized that the opposite of the predicted effect could be due to the fact that the infant brain has immature neural connections, but more work is needed to better understand changes in oxyHb and deoxyHb over development.

Research has also found a difference in the overall cortical response to fearful faces versus happy and neutral faces, displayed in sensors over the STS (Nakato, Otsuka, Kanazawa, Yamaguchi, & Kakigi, 2011). Color images of neutral, happy, and angry faces were shown to 6- and 7-month-old infants (n = 12). This study utilized total hemoglobin (the sum of oxyHb and

deoxyHb) to look at change in the STS in response to happy, neutral, and fearful faces. Results showed distinct differences in hemodynamic activation between the two emotional faces from neutral faces. Both emotions elicited a difference in hemodynamic response compared to the neutral faces and differences between each other. Happy faces showed a more sustained response on brain activity compared to the angry faces.

The fNIRS work examining differential responses to emotional faces is consistent with past work using ERPs (e.g., Safar & Moulson, 2020), again supporting the use of fNIRS as a brain imaging technique for the study of face processing in infancy. Importantly, fNIRS has been able to go beyond EEG/ERP findings, allowing researchers to localize where in the brain the differentiation is occurring.

In summary, behavioral work studying infants responding to faces has helped researchers learn about preferences for face stimuli versus non-face stimuli, preferences based on familiarity and emotional expression, and gaze patterns to core facial features. Many studies have also examined the neural responses reflecting facial processing in infancy. Studies using fNIRS display results consistent with other neuroimaging techniques used in adults, such as fMRI, confirming that areas that align with adult face processing regions, such as STS, are activated during facial processing in infancy. The consistency of fNIRS with other neuroimaging techniques, combined with its specific characteristics, including high portability and resistance to noise from movement, make it a good choice for studying regional brain responses during facial processing in infants.

Autism and Face Processing

Work studying individuals with autism spectrum disorder (ASD) has found differences in how faces are processed. ASD consists of both social communication and interaction difficulties,

as well as restricted interests and repetitive behaviors. ASD affects about 1 in 54 children (Center for Disease Control and Prevention, 2021), and common signs and symptoms of ASD are avoiding eye contact and difficulty understanding the emotions of other people (Center for Disease Control and Prevention, 2021). Researchers have looked at face processing in ASD in hopes of better understanding how it might relate to social communication and interaction abilities in ASD.

Early eye-tracking work with adults has found that people with ASD exhibit differences in looking patterns to faces. Pelphrey et al. (2002) used an eye tracking experiment to view the gaze patterns of 5 adult males with ASD (average age of 25 years old) and 5 adult males without ASD (average age of 28 years old). The results showed that the ASD group spent more time inspecting non-feature areas of the face (i.e., cheeks, forehead) while also spending less time examining core features, in particular the eyes, as compared to the non-ASD group. The authors posit that this difference in looking may play a role in both the communication and social interaction difficulties seen in individuals with ASD.

Work examining social attention in children and adolescents with ASD suggest that differences in looking patterns in people diagnosed with ASD arise before adulthood. For example, using an eye-tracker, Klin, Jones, Schultz, Volkmar, and Cohen (2002) recorded attention in 15 ASD adolescents (average age of 15 years old) and 15 non-ASD adolescents (average age of 18 years old) as they viewed movie clips from the 1967 film "Who's Afraid of Virginia Woolf?" The results showed that the adolescents with ASD focused twice as much on the mouth, half as much on the eyes, twice as much on the body, and twice as much on objects than the non-ASD adolescents. Additionally, results showed that for the ASD group, an increase in mouth fixation predicted greater social adaptation and lower autistic social impairment, while

an increased object focus related to the opposite pattern. These results suggest that while looking at the mouth and objects were both strong indicators of having ASD, increased attention to the mouth in ASD may have benefits for overall social functioning. In a study with younger children, Chawarska and Shic (2009) examined face processing using a visual paired comparison paradigm and color images of neutral female faces. The study had both non-ASD (n = 30) and ASD (n = 44) groups of 2- and 4-year-olds. The researchers found that the ASD group spent overall more time looking at the outer features (hair, cheeks, and forehead) than the group with typical development. Additionally, the 4-year-old ASD group spent significantly less time attending to the eyes, nose, and mouth, and spent their time looking away from the face more than the 2-year-old children with ASD. This piece of research is interesting, suggesting that visual scanning patterns of faces in children with ASD may be becoming more characteristically different from the patterns seen in non-ASD as children get older.

One study suggests that ASD may affect an attentional mechanism in relation to facial processing. Chawarska, Volkmar, and Klin (2010) wanted to see if toddlers with ASD (n = 42, average age 32 months old) were able to disengage, or shift attention, from faces similarly to non-ASD children (n = 46, average age 29 months old). Using several cued tasks that utilized central fixation points, the toddlers were required to move their attention between face and non-face stimuli, with their saccadic reaction time measured. The results showed that toddlers with ASD were able to disengage easier from faces than non-ASD toddlers; that this disengagement difference between the two groups was not seen in response to non-face stimuli.

More recently, using a semi-naturalistic eye tracking setup, Chawarska et al. (2012) examined the looking behaviors of children with ASD (n = 54), non-ASD children (n = 48), and children with developmental delays (n = 22), between the ages of 13 and 25 months old. The

authors used four different video segments, each a different instance of a woman performing different actions. The authors found that the children with ASD looked less at the woman's face and more at the hand/object area in comparison to the other two groups.

Studies have also found that individuals with autism have differential recognition and scanning patterns of faces based on emotion. A study done by Ashwin and colleagues (Ashwin, Chapman, Colle, & Baron-Cohen, 2006) tested to see if individuals with autism showed differences in recognizing emotional faces. Participants were 26 male adults with ASD (average age of 32 years old) and 26 non-ASD adult males (average age of 31 years old). Researchers presented pictures of different emotional faces one at a time, and the participants had to match the emotion displayed on the face with one of several choices of emotions. The results showed that the adults with ASD were not able to identify negative emotions as successfully as those without ASD. This provides evidence that the specific emotion displayed by a face may interact with facial processing in ASD.

de Wit, Falck-Ytter, and von Hofsten (2008) used an eye-tracker to test how young children viewed positive and negative emotional faces. The experiment consisted of 13 children with ASD and 14 non-ASD children (average age of 5 years old in both groups). Each child was shown pictures of both male and female faces, with the model expressing a positive or negative emotion. The researchers found that total looking time was higher in the non-ASD group than in the ASD group and that the ASD children looked less at the core areas as well, matching previously mentioned eye-tracking results. Both non-ASD and ASD groups exhibited different scanning patterns based on the emotion, looking more at the eyes in negative emotions than positive emotions. In line with Klin et al. (2002), de Wit et al. (2008) also found that more time

looking at the mouth was associated with less social and communicative impairments in the ASD group, further providing evidence that focusing on the mouth in ASD may be advantageous.

In work by Dalton et al. (2005), 14 male ASD and 12 non-ASD adolescents (average age of 16 years old for both groups) performed a facial emotion discrimination task, where they looked at a human face and answered if it was emotional or neutral by pressing one of two buttons. The researchers found that no matter what emotional face was shown, adolescents with ASD looked less at the eyes than did the non-ASD group. Subjects with ASD also looked longer at faces in general, had less correct discrimination responses, and took significantly longer than the non-ASD group in deciding if the face was emotional or not. These results show that part of the reason people with ASD have difficulty with emotional facial processing may be due to differences in gaze fixation.

Emotion understanding in ASD has also been examined in the context of the Reading the Mind in the Eyes Test (RMET), which has participants look at photos of the eye-region of faces and choose which of several words given would best describe what the person in the photo is thinking or feeling (Baron-Cohen, Wheelwright, Hill, Raste, and Plumb, 2001). Baron-Cohon and colleagues have used this test to examine how autistic adults recognize emotions. The researchers studied adults with ASD (n = 15) and without (n = 122) between the ages of 20 and 47 years old, with each participant completing the RMET. The results showed that the ASD group performed worse than the non-ASD group. Additionally, an individual's accuracy on the RMET was inversely related to their level of autistic traits, suggesting that individuals higher on autistic traits have a more difficult time processing emotional information from the eyes.

Brain imaging techniques have also been utilized in autism studies, including identifying the timing of neural responses and brain regions affected by ASD when processing social

information. Using ERP, Webb and colleagues (Webb, Dawson, Bernier, & Panagiotides, 2006) studied 63 children with ASD and 28 non-ASD children (all between 33 and 54 months), showing participants photos of three different stimuli: their mother, a female stranger, and their favorite toy. The results showed that children with ASD showed faster ERP responses to objects versus faces, opposite of what was seen in the non-ASD children. Work done by Vandewouw et al. (2020) used fMRI to compare the neuronal activity of 54 non-ASD children and 128 children with ASD (all children between 5 and 19 years old) when looking at dynamic happy and angry faces. The researchers found that the hemodynamic response difference between the angry and happy stimuli in the non-ASD group was significantly greater than the difference between the two stimuli in the ASD group in the occipital and temporal region.

The work by Dalton et al. (2005) described above also studied the relationship between gaze fixation and neural responding in adolescents with and without ASD, using both an eye-tracker and fMRI. The brain imaging data revealed that there was more activation in both the bilateral fusiform gyrus and occipital gyrus in the non-ASD group when looking at faces as compared to the autistic group. Brain activation in the fusiform gyrus was strongly and positively associated with eye fixation in the autistic group as well. This shows neuronal differences in the occipital region between ASD and non-ASD groups, similarly to Vandewouw et al. (2020), but also highlights how differential attention to faces could result in these neural differences.

Recently, fNIRS has also been used in several studies as a neuroimaging technique to examine face processing in ASD. These studies have found evidence that face processing is different between children with ASD and controls in temporal-occipital brain regions (Jung, Strother, Feil-Seifer & Hutsler, 2016), as well as frontal regions (Kita et al., 2010). For example, Research by Jung and colleagues (Jung et al., 2016) used fNIRS to measure the hemispheric

activity in response to faces in both ASD (n = 8) and non-ASD (n = 12) males (both groups between 7 and 36 years old). Each participant was shown a series of male and female faces, all with neutral expressions to avoid involving emotional processing. A 14-channel fNIRS system was used to measure both oxyHb and deoxyHb levels over the temporal-occipital regions of each hemisphere. The results showed that the ASD group did not show right hemispheric lateralization in response to the faces, in contrast to the non-ASD group. This provides evidence that there is less lateralized facial processing in people with ASD.

Using fNIRS to measure the frontal area of the brain, Kita et al. (2010) examined self-recognition in 10 children with ASD (average age of 10 years old), 11 healthy adults (average age of 22 years old), and 13 non-ASD children (average age of 11 years old). The level of ASD severity was also measured in the children with ASD. The visual stimuli consisted of two different morphing videos, self-face to unfamiliar face and familiar face to unfamiliar face, with each original face morphing into the opposite sex. Once the original face had morphed into the opposite sex, the participant pressed a button, and the passive hemodynamic response to the initial facial image, measuring oxyHb, was recorded. Results showed that the activity in the inferior frontal gyrus was dependent on the severity of ASD, with the more severely affected children displaying less activity. This suggests that face processing in the inferior frontal gyrus is different in individuals with ASD.

Very little research has been done so far using fNIRS to measure emotion recognition in ASD. Mori et al. (2015) showed 10 boys with ASD (average age of 11 years old) and 10 non-ASD boys (average age of 12 years old) pictures of emotional faces consisting of happiness, sadness, surprise, anger, disgust, and fear, and were told to imitate the emotional face. Using a 34-channel fNIRS system, the results showed that oxyHb concentrations in the inferior frontal

gyrus were significantly lower in the ASD group than in the non-ASD group. This study also featured a training component, where the ASD participants were trained to imitate the emotional faces used in the experiment for 30 minutes, twice a week for a total of 5 training sessions and brought back in to do the same experiment. Results from this training portion of the experiment showed a significant increase in oxyHb concentrations in the inferior frontal gyrus when compared to the initial experiment. Interestingly, this study shows not only a neural difference for ASD during emotion imitation, but also that people with ASD might benefit from interventions aimed at improving emotional mimicry.

Prospective Studies with Infants at High Risk for Autism

Infants who have an older sibling that has been diagnosed with ASD have an elevated genetic risk for being diagnosed with ASD, and have been studied as a group at high risk for autism (HRA). With about 20% of HRA infants later receiving an ASD diagnosis (Landa & Garrett-Mayer, 2006), scientists have conducted prospective research with both HRA and low risk control infants (LRC) to see whether there are any behavioral or brain-related differences in infancy, and if these could be predictive of a later ASD outcome or other developmental difficulty. This can be done by dividing the HRA group into two outcome groups, HRA+ for infants who were HRA and then were diagnosed with ASD, and HRA- for infants who were in the HRA group but were not later diagnosed with ASD.

Prospective work by Chawarska, Macari, and Shic (2013) used eye-tracking data to see if spontaneous social monitoring patterns at 6 months were predictive of ASD diagnosis at 24 months in HRA (n = 67) and LRC infants (n = 50). The infants in both groups were shown a video of a woman engaging in four different activities, with the eye-tracker monitoring where the infants were looking in regard to each activity the woman was participating in. The results

showed that the infants who were later diagnosed with ASD (HRA+) spent less time on the social scene, less time monitoring the actress, and less time on her face compared to the infants from both the HRA- and LRC groups. These results suggest that infants who later develop ASD show different scanning patterns at 6 months, showing that eye-tracking measures have potential for use as early markers for ASD. A meta-analysis conducted by Falck-Ytter, Bölte, and Gredebäck (2013) looked at eye-tracking studies in early ASD, and consistent with the previously-described study, they concluded that behavioral markers of ASD can be found as early as 6 months using eye-tracking data.

A recent prospective study showed different patterns of looking to faces for children at risk for ASD who later receive a diagnosis of ASD compared to those HRA- and LRC. In this study, Wagner, Luyster, Moustapha, Tager-Flusberg, and Nelson (2018) used a visual paired comparison paradigm to look at attention to mother and stranger in 37 HRA and 40 LRC 6-month-old infants. The study also measured cognitive abilities and social communication in these infants at 18 months, as well as their diagnostic outcome of ASD or non-ASD. As in prior infant work, both groups showed a greater attention to eyes than mouth while viewing static faces. The HRA+ and LRC did pay the greatest attention to faces in comparison to the HRA-. However, the HRA+ group spent more time on the eyes than both the HRA- and the LRC groups. Additionally, while greater attention to the eyes at 6 months correlated with better social ability at 18 months in LRC, this was not the case in HRA infants. Instead, HRA infants overall showed a negative correlation between attention to faces at 6 months and expressive language ability at 18 months. This work provides evidence that not only are there facial scanning differences between the three groups, but that scanning patterns at 6 months might be differentially associated with later development depending on family risk for ASD.

Prospective studies with HRA infants regarding emotional face processing have also been conducted. For example, Wagner, Keehn, Tager-Flusberg, and Nelson (2020) used eye-tracking to look at disengagement of attention from emotional faces. The researchers performed a longitudinal eye-tracking study at 6, 9, and 12 months on LRC (n = 50) and HRA (n = 61) infants, splitting the HRA group into HRA+ and HRA- once a diagnosis was reached at 24 to 36 months. Each infant was shown a female face displaying a fearful, happy, or neutral emotion in the center of the screen, followed by the same face with a peripheral distractor on the left or right. The emotions were presented in a semi-randomized order for a total of up to 75 trials (25 for each emotion). Contradicting what the researchers predicted (which were derived from emotion recognition differences in older children and adults with ASD), all three groups had similar attentional biases for fearful faces when compared to both the happy and neutral emotions. However, HRA+ infants at 12 months showed some evidence of slower overall disengagement latency, suggesting more general attentional differences might happen within the first year of life for HRA+ infants.

Brain imaging techniques have also been used to examine neural differences in HRA infants in relation to facial processing. Pairing eye-tracking, ERP, and cognitive measures, Key and Stone (2012b) looked at how 9-month-old HRA (n = 15) and LRC (n = 20) infants process facial features. Using an oddball paradigm, infants were shown their mother's face 70% of the time, and were shown their mother's face with either different eyes or a different mouth 30% of the time. The eye-tracking results showed that there were no differences between the two groups in how they scanned the images. ERP data showed that electrodes over both the occipitotemporal and the frontocentral brain regions differentiated the two groups, with the LRC infants having a faster N290 response to changes in both the eyes and the mouth as compared to HRA. For both

groups, a negative correlation was found between the duration and number of fixations on the mouth during the mouth-changing stimuli and receptive communication skills, and a positive correlation was found between fixations on the eyes during the eye-changing stimuli and scores of interpersonal skills. This work suggests neural differences in facial processing between LRC and HRA at 9 months, and also shows relationships between gaze patterns and social communication.

Key and Stone (2012a) conducted a similar study with HRA (n = 15) and LRC (n = 20) infants at 9 months, this time focusing on eye-tracking and ERP responses to familiar and unfamiliar faces. Using an oddball paradigm with 100 trials, the infants were shown two different faces, the infant's mother's face (standard stimulus), and the stranger's face (deviant stimulus). Again, the researchers found that there were no group differences in the eye-tracking data between the HRA and LRC infants. The results also found that both groups showed ERP differences between seeing their mother's face or a stranger's face, but that there was a delayed response in LRC as compared to HRA when viewing the stranger's face. Additionally, the results showed that there was a relationship between ERP activity and interpersonal skills in both groups. While neither study done by Key and Stone were able to use future diagnostic outcomes to separate the HRA group into HRA+ and HRA-, the researchers did provide evidence that there are neural differences in processing familiar and unfamiliar faces between infants with and without a family history of ASD.

There have been studies with HRA infants that have used fNIRS as a brain imaging technique. For example, Keehn, Wagner, Tager-Flusberg, and Nelson (2013) used fNIRS to measure brain connectivity as infants listened to auditory syllables. HRA (n = 27) and LRC (n = 37) infants were studied at 3-, 6-, 9-, and 12-months-old. Each infant was presented with

trisyllabic sequences, with each auditory block followed by a 15s silent pause. fNIRS was used to measure neural responding in both anterior and posterior lateral locations in both hemispheres. Results showed differences in connectivity across the brain regions between HRA and LRC at both 3 months and 12 months. At 3 months, there was an increase in functional connectivity in HRA compared to LRC, while at 12 months there was decreased connectivity in HRA compared to LRC. This work used fNIRS to identify neural connectivity differences between LRC and HRA groups within the first year of life.

Work done by Lloyd-Fox et al. (2013) looked at the difference between temporal lobe specialization in visual processing in 4- to 6-month-old HRA (n = 18) and LRC (n = 16) infants as they were shown social videos of female actors either moving their eyes right or left or performing hand games such as 'peek-a-boo'. The fNIRS probes measured responses in the temporal lobes, and results showed that there was a stronger response to the visual stimuli in LRC infants than HRA in the STS. Importantly, the group differences observed were not due to differences in overall looking time to the stimuli.

One previous study by our group looked at both facial identity and emotion processing in 6- to 7-month-old HRA (n = 10) and LRC (n = 10) infants (Fox, Wagner, Shrock, Tager-Flusberg, & Nelson, 2013), with infants viewing videos of a female speaker (mother and stranger) expressing a neutral expression first and then transitioning to a smiling expression based on the paradigm of Minagawa-Kawai et al. (2009). Findings showed an increase in oxyHb in the OFC in response to the mother's face, as in Minagawa-Kawai et al. (2009). There was also a greater oxyHb response to the smiling emotion versus the neutral emotion in right frontal channels. A main effect of group in the posterior-lateral right hemisphere, showing a greater hemodynamic response in the LRC group than the HRA group, was also observed. Lastly, when

all 24 channels were averaged, there was a greater hemodynamic response to mother than stranger in the LRC group but not the HRA group. In summary, this work suggests that differential hemodynamic responses can be seen across several brain regions between HRA and LRC infants when viewing familiar and unfamiliar emotional faces.

The Current Study

The present study extends the work of Fox et al. (2013) to include a larger sample of HRA and LRC infants across a wider age range, again measuring fNIRS responses in frontal and right lateral regions as infants viewed videos of their mother and a stranger expressing neutral and smiling expressions (based on the paradigm of Minagawa-Kawai et al., 2009). The current study had two main research goals. The first was to identify and confirm the brain regions involved in familiar vs. unfamiliar and emotional face processing in infancy. Based on past work by Minagawa-Kawai et al. (2009) that found a greater response to the mother than the stranger in the OFC, I hypothesized that there would be a main effect of familiarity in the frontal lobe, specifically driven by responses in the middle frontal region. Additionally, Fox et al. (2013) found a greater response to the smiling emotion as compared to the neutral emotion in the right frontal region; I therefore hypothesized an emotion-related response in the frontal lobe, driven by the response in the right frontal region. Further, past research using fNIRS has found that the STS is involved in emotional face processing (e.g., Nakato et al., 2011). I hypothesized emotion-related responses would also be found in the right lateral posterior region.

The second research goal was to examine the effects of risk group on oxyHb responding. Based on both Lloyd-Fox et al. (2013) and Fox et al. (2013), which both found a greater response for the LRC group than the HRA group in the STS, I hypothesized that there would be a main

effect of group in the right lateral region, particularly in the posterior region, thought to be reflect activityin the STS region.

Method

Participants

The final sample included 98 infants from 6- to 14-months-old (50 female). Out of the 98 infants, 32 had an older sibling that had already been diagnosed with ASD (17 female) and were placed in the HRA group. The older siblings of each HRA infant received community diagnoses of ASD, which were confirmed using the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003). The LRC infants who participated in the study also had at least one older sibling, but no family history of ASD. All infants in both groups were full-term, born after 36 weeks gestational age, and had no known neurological or uncorrected visual abnormality. The experiment was conducted under approval from the Institutional Review Boards at Boston Children's Hospital and Boston University. Written informed consent was obtained from the parent(s) of all infant participants.

Stimuli

As described in further detail in Fox et al. (2013), a high-definition digital video recording of each infant's mother was created prior to the start of the experiment. Each mother stood against a neutral background draped in a white cloth and answered several questions. Two videos of each mother were recorded, the first video displaying a neutral facial expression, the second a smiling expression. Sound was removed from each video to eliminate the multisensory effects that voices would contribute. Finally, both the neutral and smiling videos were edited into 16-second clips. Using the edited clips, a continuous 32-second video was created, where each mother transitioned from a neutral expression to a smile halfway through.

Task Procedures and Equipment

A Hitachi ETG-4000 fNIRS system with 24 simultaneously recording channels was used during the experiment to collect hemodynamic responding during the presentation of the mother/stranger videos. A special cap was created for the infants, with the fNIRS optical probes spanning both the frontal and right lateral portion of the head. To accommodate each infant, the positioning of the probes on the cap was adjustable using Velcro. The frontal panel was centered using the nasion-inion line. On the right lateral panel, the anterior portion of the panel was just superior to the right ear, extending toward the occipital lobe.

After all procedures were explained, the testing session began with infants seated on their parents' lap 60 cm in front of a 17-inch, Tobii T-120 eye tracker monitor where stimuli were displayed. Each session began with a visual baseline of moving objects for at least 10 seconds in order to gain baseline measurements as well as encourage the infants to look at the screen. Each infant saw up to 14 trials, seven of their mother and seven of a stranger, in semi-randomized order. The strangers were other participants' mothers, matched based on similarity to the participant's mother, related to hair and eye color and race/ethnicity. Infants were shown a fixation cross between trials, and videos were presented until infants had seen all 14 trials or until they became too fussy to continue.

fNIRS Pre-Processing

Similar to Percukonis, Perdue, Wong, Tager-Flusberg, and Nelson, (2021), the fNIRS data was processed using the Homer3 MATLAB application (Huppert et al., 2009). The first step in the pre-processing was to exclude channels, a process called pruning, based on the range of intensity of the raw signal from the Hitachi system (Di Lorenzo et al., 2019). Once the appropriate channels were pruned, the raw signal was converted into optical density, with a

subsequent performance of a wavelet motion correction in order to remove motion artifacts (Behrendt et al., 2018). Motion artifacts that were not removed from the wavelet motion correction were identified and removed based on a signal greater than 20 standard deviations within a 0.5s window. Once all motion artifacts were removed, both slow drift noise and cardiac noise were removed using a high pass and a low pass filter, respectively. With all artifacts removed, optical density was converted into concentrations of oxyHb and deoxyHb. As in Minagawa-Kawai et al. (2009), average oxyHb concentrations were calculated, with the use of concentration values from 6-16s post-stimulus onset. All infants in the final sample had usable data from one or more channels in the frontal region and one or more channels in the right lateral region. Additionally, all infants in the final sample had at least 2 usable trials for each of the 4 conditions, where they were looking at the video for 2 seconds out of the first 5 seconds. On average, HRA had 4.33 trials per condition (*SD* = 1.56) and LRC had 4.61 trials per condition (*SD* = 1.86).

Statistical Analysis

Following the completion of the pre-processing of the fNIRS data, two primary regions of interest were defined (see Figure 1). Channels 1-12 were defined as the frontal region, and channels 13-24 were defined as the right lateral region. Each of the two primary regions was then divided into three subregions, with each subregion having a minimum of three channels. For the frontal region, the three subregions included: right (channels 1, 2, 3, 4, and 5), middle (channels 6, 7, and 9), and left (channels 8, 10, 11, and 12). For the right lateral region, the three subregions included: posterior (channels 13, 14, 15, 16, and 17), middle (channels 18, 19, and 21), and anterior (channels 20, 22, 23, and 24). Although a subset of participants contributed data

at multiple time points, the present results focused on cross-sectional analyses. All analyses were conducted using SPSS statistical software.

Results

Effects of Region, Familiarity, and Emotion

The first research question aimed to examine differences in oxyHb activation in different brain regions. To look at this, a series of ANOVAs were conducted, with region, familiarity, and emotion as within-subjects variables.

Frontal vs. Right Lateral

In order to identify and confirm brain regions involved in emotional face processing, we looked at general differences between the frontal region and the right lateral region using a 2 (Region: frontal, right lateral) x 2 (Familiarity: stranger, mother) x 2 (Emotion: neutral, happy) repeated-measures ANOVA. There was a main effect of region on oxyHb, $F(1,97) = 5.497$, $p = .02$, with a significant increase in oxyHb activity in the right lateral region (M = 5.62E-6, SD = 1.85E-5) as compared to the frontal region (M = 1.43E-6 , SD = 1.23E-5). No other significant main effects or interactions were seen.

Frontal Subregions

The frontal region was separated into three subregions in order to identify differences in oxyHb across the frontal area. A 3 (Region: right, middle, left) x 2 (Familiarity: stranger, mother) x 2 (Emotion: neutral, happy) repeated- measures ANOVA was conducted. A main effect of emotion on oxyHb was found, $F(1,90) = 5.429$, $p = .022$, with a significantly greater response for the neutral emotion (M = 6.77E-6, SD = 2.49E-6) than for the happy emotion (M =-4.21E-6 , SD = 2.75E-6). Additionally, there was an interaction between emotion and familiarity, $F(1,90) = 7.124$, $p = 0.009$. A closer look at this interaction with follow-up t-tests revealed that

there was a significant increase in oxyHb for mother between the neutral emotion (M = 1.13E-5, SD = 3.51E-5) and the happy emotion (M = -1.10E-5, SD = 4.64E-5; t(97) = 3.279, p = 0.001), but this did not differ for stranger (p = 0.941). No other significant main effects or interactions were found.

Right Lateral Subregions

The right lateral region was separated into three subregions to identify differences in oxyHb in the right lateral area. A 3 (Region: right, middle, left) x 2 (Familiarity: stranger, mother) x 2 (Emotion: neutral, happy) repeated- measures ANOVA was conducted, and no significant main effects or interactions were found.

Effects of Group

The second research question aimed to see whether infant risk group influenced oxyHb responding. To examine this, a parallel series of ANOVAs was conducted, adding the between-subjects variable of group (LRC, HRA).

Frontal vs. Right Lateral

To examine the differences in oxyHb between the frontal and right lateral regions, a 2 (Group: LRC, HRA) x 2 (Region: frontal, right lateral) x 2 (Familiarity: stranger, mother) x 2 (Emotion: neutral, happy) repeated-measures ANOVA was conducted. Similar to reported above, this analysis again found a main effect of region on oxyHb, F(1,96) = 4.496, p = 0.037, with more responding in the right lateral region than the frontal region (see above for means and standard deviations). Infants also showed an interaction between region, familiarity, and emotion, F(1,96) = 5.461, p = 0.022 (Figure 2). Follow-up analyses examined the frontal and right lateral regions separately. For the frontal region, paired t-tests revealed that for mother, there was a significant increase in oxyHb for the neutral emotion (M = 1.12E-5, SD = 3.51E-5)

as compared to the happy emotion (M = -1.00E-5, SD = 4.56E-5; $t(97) = 3.101$, $p = 0.003$), but no difference was seen for stranger between emotions in the frontal region ($p = 0.815$). In the right lateral region, there were no differences between responses to neutral and happy for mother or for stranger (ps > 0.528). No other significant main effects or interactions were found.

Frontal Subregions

In order to see the effect of group on oxyHb differences in the three frontal regions, a 2 (Group: LRC, HRA) x 3 (Region: right, middle, left) x 2 (Familiarity: stranger, mother) x 2 (Emotion: neutral, happy) repeated- measures ANOVA, was conducted. Similar to reported above, a main effect of emotion on oxyHb was again found, $F(1,89) = 5.789$, $p = 0.018$, with greater responding to the neutral emotion than the happy emotion, and an interaction between emotion and familiarity $F(1,89) = 7.371$, $p = 0.008$ (see above for means, standard deviations, and follow-up t-tests for the interaction),

There was also a significant interaction between region, familiarity, and group on oxyHb, $F(2,173) = 5.027$, $p = .008$ (Figure 3). Follow-up analyses looked at each of the three frontal subregions separately. In the left frontal subregion, there was a significant increase in oxyHb for the mother (M = 5.66E-6, SD = 2.40E-5) as compared to the stranger (M = -5.45E-6, SD = 2.78E-5; $t(64) = -2.090$, $p = .041$) for LRC, but there was no difference in this left frontal subregion for HRA ($p = .195$). No additional follow-up comparisons were significant, and no other main effects or interactions were found.

Right Lateral Regions

To examine the effect of group on differences in oxyHb between the three right lateral regions, a 2 (Group: LRC, HRA) x 3 (Region: posterior, middle, anterior) x 2 (Familiarity: stranger, mother) x 2 (Emotion: neutral, happy) repeated- measures ANOVA was conducted. No

significant main effects or interactions were found; however, there was a marginal interaction between region, emotion, and group on oxyHb, $F(2,176) = 2.586$, $p = 0.078$ (Figure 4).

Discussion

In the present study, we used fNIRS to examine oxyHb responses in infants at high and low risk for ASD as they viewed videos of their mother and a female stranger, with each video showing the individual speaking first with a neutral expression and then a happy expression. We first looked at which brain regions were activated during the task, and then whether there were any differences in oxyHb responses relating to risk group (HRA vs. LRC). Results revealed that overall, the right lateral region exhibited increased oxyHb response as compared to the frontal region, and there was little effect of group on oxyHb responding.

Brain Regions Involved in Emotional Face Processing

The first research question aimed to identify the brain regions involved in emotional face processing of familiar and unfamiliar faces in the current task. Analyses first compared the frontal and right lateral region, and findings showed a significant increase in oxyHb in the right lateral region as compared to the frontal region.

Past infant fNIRS work has also identified right lateral regions as responding to social stimuli (e.g., Lloyd-Fox et al., 2009; Otsuka et al., 2007). For example, work by Lloyd-Fox et al. (2009) found that there was an increase in hemodynamic response in the posterior temporal regions in 5-month-old infants when shown social stimuli. The authors argued that this activity is from the STS, which has been shown to respond to social stimuli in past work in both adults and infants. In the current study, it is thought that the right lateral region fNIRS channels were covering related areas, including the STS/STG regions that have been confirmed to be involved

in face processing through fMRI (e.g., Kanwisher et al., 1997) and in past fNIRS studies with infants (e.g., Otsuka et al., 2014).

In contrast with past work (e.g., Nakato et al., 2011), this initial analysis showed no difference in overall responding in the right lateral regions as a function of familiarity or emotion. This data provides no support for my hypothesis of emotion-related responses in the right lateral area. These findings suggest that the current videos did not elicit differences in emotional or familiarity-based face processing in the right lateral region. This lack of difference remained even when looking separately at the subregions of the right lateral area. This is especially surprising given previous research that had found that the STS was involved in differentiating emotional faces (e.g., Nakato et. al 2011). Our lack of an effect indicates that more work is needed to understand the role of this region in processing emotion and familiarity.

An additional analysis looked separately at subregions of the frontal area to further explore region-specific effects and found a main effect of emotion. The neutral emotion, as opposed to the happy emotion, showed the greater hemodynamic response. This result provides partial support for my hypothesis of an emotion-related response in the frontal lobe. However, the finding is not fully consistent with my hypothesis because I suggested it would be driven by the right frontal regions, as in Fox et al. (2009). In addition, the finding was opposite to that of Fox et al. (2009), as they observed a significant increase in oxyHb for the smiling emotion, while this study found a significant increase in oxyHb for the neutral emotion. This difference might be due to the difference in age groups tested between the two studies.

The significant interaction between emotion and familiarity for the frontal subregions also revealed that for the mother's image, there was a significant increase in oxyHb for the neutral emotion in comparison to the happy emotion. I hypothesized that, like Minagawa-Kawai

et al. (2009), there would be a main effect of familiarity in the frontal lobe, specifically driven by responses in the middle region. Even with the results from the interaction between emotion and familiarity, the evidence is weak to support this hypothesis. As in this study, a significant increase for the neutral emotion versus the happy emotion was also seen in a study by Ravicz et al. (2015) with 7-month-old infants. The researchers specifically mentioned that this was the opposite of what they expected, as is the case for this experiment. Ravicz and colleagues (2015) hypothesized that this opposite effect might be due to immature neural connections. While this is one potential explanation for the current results, limitations in the current task design could also be the reason for this counterintuitive result. Specifically, the neutral emotion was always shown before the happy emotion, with infants already having looked at the same face for 16s before seeing the happy emotion, and this could have lessened the response to the happy emotion throughout.

Effects of Group on fNIRS Responses

The second research goal was to see how the hemodynamic response differed across the various brain regions based on infant family history of ASD. In the analyses of the frontal vs. right lateral regions, there were no significant main effects or interactions involving group. This is in contrast to some previous findings. For example, Lloyd-Fox et al. (2013) found differences between the HRA and LRC groups regarding the temporal hemodynamic response to social stimuli. Specifically, the results indicated an increase in the hemodynamic response in the LRC group compared to the HRA group, which the researchers suggest is a function of the STS. The participants in Lloyd-Fox et al. (2013) were younger than the participants in the current study, with their population ranging from 4-6 months while this study's population ranged from 6-14 months. This age difference may account for differential findings related to group. Additionally,

the fact that the prior research stimuli consisted of hand-games such as 'peek-a-boo' may also be a reason why group differences were seen. Our stimuli were silent talking videos, and thus may not have been as interactive as other studies' stimuli. More work is needed to better understand differences from past work.

Analysis of the frontal subregions revealed a significant interaction between region, familiarity, and group on oxyHb, and follow-up analyses revealed that there was a significant increase in oxyHb for the mother stimulus as compared to the stranger stimulus for the LRC group in the left frontal subregion. This finding in LRC coincides with findings from Minagawa-Kawai et al. (2009) with typically developing 12-month-olds. Their study showed an increase in the hemodynamic response to mother as compared to stranger images in the prefrontal cortex, specifically the OFC. Importantly, Minagawa-Kawai et al. (2009) measured responses only from central areas of the prefrontal cortex, while the current study found results in the left frontal subregion. This difference might be explained by the inclusion of a wider age group of infants. Eye-tracking studies of infants have shown changes in gaze patterns within the first year (e.g., Lewkowicz & Hansen-Tift, 2012), and that ASD does influence gaze patterns (e.g., Pelphrey et al, 2002; Klin et al., 2002). Follow-up work is needed to better understand which regions of the prefrontal cortex are responding to familiarity.

When examining the right lateral subregions, no main effects or interactions with group were found, though there was a marginally significant interaction between region, emotion, and group. In the posterior channels, the HRA and LRC groups showed opposite responses to the happy and neutral stimuli, as Figure 4 displays. While this was only a trend, this does coincide with some previous research. Results from Fox et al. (2013) showed a main effect of group in the postero-lateral right hemisphere, with a greater oxyHb response in the LRC group than in the

HRA group. Additionally, Lloyd-Fox et al. (2013) showed an increase in oxyHb in the STS in the LRC group versus the HRA group. While I hypothesized that there would be a main effect of group in the right lateral region, particularly in the posterior region, the results did not support this. Taken together, the current study provided weak evidence for group differences in the hemodynamic response to familiar and unfamiliar faces expressing neutral and happy emotions.

Future Research and Limitations

The current study was based on prior work with HRA and LRC by Fox et al. (2013); the current study, however, included a larger group of infants and a wider age range (6 to 14 months). One major limitation of the current work has to do with the task itself. The paradigm is identical to Minagawa-Kawai et al. (2013), where the videos always began with the neutral emotion. However, Minagawa-Kawai et al. (2013) used the neutral emotion as a baseline, not focusing on the difference in emotional processing, while the current study analyzed the neutral and happy emotions separately. This major difference may affect how comparable the results of the current experiment are with their study, especially with regards to greater responding to neutral faces than happy faces. Since the neutral emotion was always shown before the happy emotion, there is a chance that the infants may have been overall less stimulated once the happy emotion was displayed, since the face would have already been on the screen for 16 seconds. Moving forward, future research should also create videos beginning with a happy face and transition to a neutral face, thus counterbalancing the order of the emotions presented. This counterbalancing will eliminate any order bias.

An important future direction for the current work relates to longitudinal analyses. The current set of analyses was cross sectional, looking at all infants together, with some, but not all, infants participating at multiple age groups. Future analyses will benefit from analyzing the

differences in fNIRS responses that may relate to age, since this was a longitudinal study. Keehn et al. (2013) used a longitudinal design and found connectivity differences between the HRA and LRC groups at both 3 and 12 months using fNIRS. I would like to see if there are some antinational differences between the two groups at different ages. Future analyses will take advantage of the longitudinal design and separate the HRA group into HRA- and HRA+ using diagnostic assessments at 24 and 36 months. In addition to looking at fNIRS responses in relation to group outcome, we can also look at how early infant neural activity may relate to more general cognitive development based on additional outcome measures assessed in the current sample.

Conclusion

With ASD affecting 1 in 54 children, studies on infants at high risk for ASD within the first year of life hold great promise to provide insight into the beginning stages of ASD. With the power of brain imaging tools such as fNIRS, scientists are able to obtain strong spatial resolution in awake and freely moving infants. fNIRS is an important method in understanding early differences in brain activation that might be predictive of ASD. Prospective studies such as this can elucidate the early development of ASD in infants at familial risk, and hopefully provide insight that can be utilized for early intervention techniques and treatments in order to help alleviate some of the difficulties that come with ASD.

Figure 1. *The fNIRS Probe Setup for the Frontal and Right Lateral Regions*

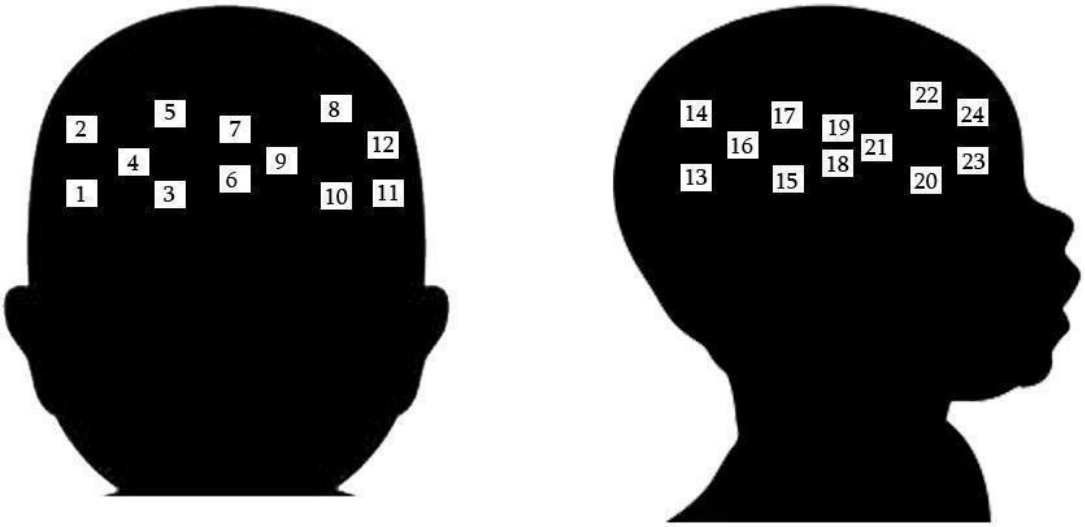


Figure 2. *Significant Interaction between Region, Familiarity, and Emotion for the Frontal and Right Lateral Regions*

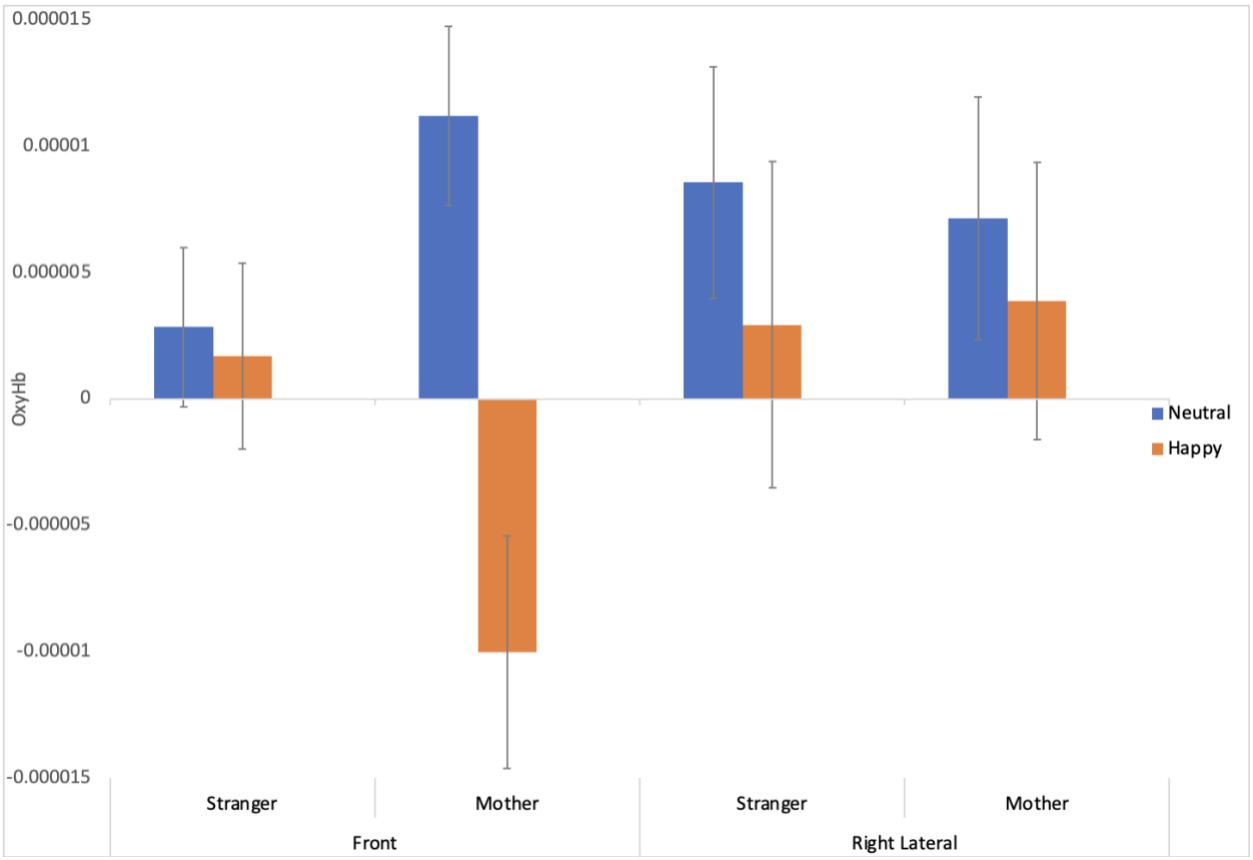


Figure 3. *Significant Interaction between Region, Familiarity, and Group in the Three Frontal Subregions*

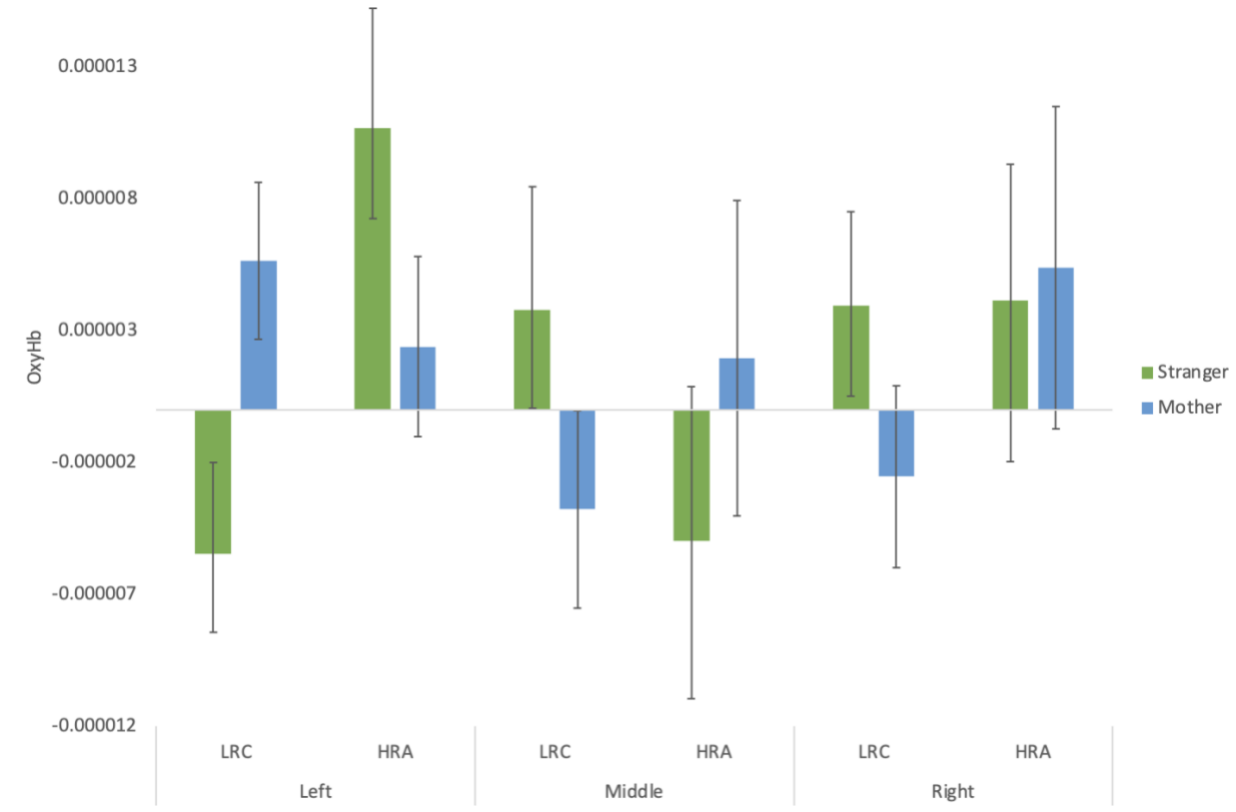
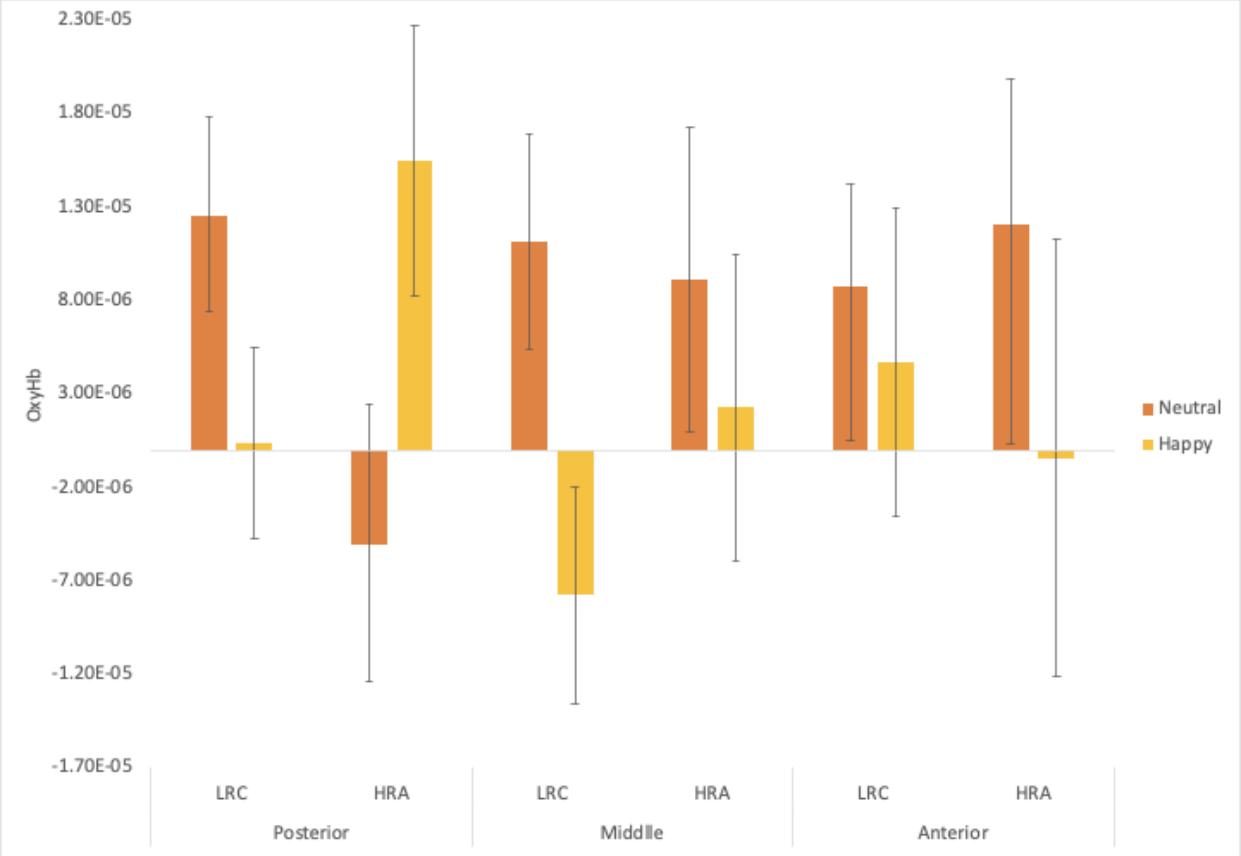


Figure 4. *Marginal Interaction Between Region, Emotion, and Group in the Three Right Lateral Subregions*



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