Neural Precursors of Language in Infants at High Risk for Autism Spectrum Disorder

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Abstract

Autism spectrum disorder (ASD) is a developmental disorder characterized by difficulties in social interaction and communication. Abnormal language development is a pervasive symptom of this disorder, though research has repeatedly shown that children with ASD who develop stronger language abilities have more positive outcomes.

One strategy for improving the language, and thus life experiences, of children with ASD, is to get children at risk for the disorder into effective and appropriately targeted educational interventions in the very earliest stages of life, when precursors of language and other social behaviors are developing. However, ASD is currently not diagnosed until children have reached 2 or 3 years.

In this dissertation, I investigate neural predictors of later language abilities, which may be measurable before behavioral precursors to language and ASD risk emerge. In my first study, I identify neural correlates of early language development in 3-month-old infant siblings of children with ASD, who are thus at high risk of developing the disorder. I find that whereas low-risk infants showed initial neural activation that decreased over exposure to repetition-based language stimuli, potentially indicating a habituation response to repetition in speech, infants at high risk for autism (HRA) showed no changes in their neural activity to these stimuli over exposure. These results suggest that putative precursors of

language acquisition are disrupted in children at high risk for ASD as young as 3 months old.

In my second study, I examine whether neural correlates of language development in 3-month-old infant siblings of children with ASD predict their 18-month social and communicative outcomes. These analyses revealed that neural activity to language stimuli in 3 month olds predicted expressive and receptive language, early gestures, sentence complexity, sentence length, and autism symptomatology in 18 month olds. In many cases, these associations differed for males and females, and for high and low risk children.

The current research thus identifies early putative markers of language disability and ASD symptomatology, which, with future research and educational application, may aid in determining which children are most likely to benefit from placement into language-based educational intervention programs from the very first months of life.

Chapter 1

General Introduction

Autism spectrum disorder (ASD) is a developmental disorder characterized by difficulties in social interaction and communication. The most recent estimates from the Centers for Disease Control and Prevention (CDC) put the rate of ASD at 1 in 68 children in the United States (Baio, 2014). ASD occurs in all racial, ethnic and socioeconomic groups, though it is almost 5 times more prevalent in boys (in which the rate is 1 in 42) than in girls (who show a rate of 1 in 189; Baio, 2014)[1]. Children with ASD may have trouble reading social cues, understanding their own or others' emotions, or adapting well to changes in their activities or routines. They have particular trouble learning from and about socially based information.

Although ASD is, by definition, a group or spectrum of disorders that is both genetically heterogeneous and manifests very differently across individuals behaviorally, a pervasive symptom of ASD is abnormal language development. In fact, in past editions of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), deficits in language acquisition were one of the key criteria for conferral of an ASD diagnosis (APA, 1994). The most common first concerns that parents of children with ASD cite are also around speech and language development; in particular, many children with ASD do not speak their first words and phrases by the expected chronological ages (around the first year of life;

[1] The association between sex and ASD differs with cognitive ability. In children who are cognitively high functioning, the sex ratio may be more than 5.5:1 (M:F), while in children with intellectual disability (ID), the ASD sex ratio is closer to 2:1 (Newschaffer et al., 2006).

DeGiacomo & Fombonne, 1998; Iverson, 2010; Wetherby et al., 2004). Furthermore, children with ASD who have stronger language tend to be higher functioning in cognitive domains as well as social tasks (or less severely affected by their ASD), and are repeatedly shown to have more positive future outcomes—such as higher education levels attained, higher rates of paid employment, and lower rates of psychiatric hospitalizations—than their peers at lower language levels (Rutter, 1970; Lord & Paul, 1997; Kobayashi et al., 1992; Venter et al., 1992; Billstedt et al., 2007). Given these trends, language development is fairly well researched and frequently targeted within current evidence-based ASD interventions; however, there is wide variability in how children with ASD respond to interventions, and what their post-intervention outcomes look like (Schreibman, 2000). As many as 30 percent of children with ASD may still never develop functional language skills, including speech comprehension or production (FNLM, 2013).

One strategy for improving the language outcomes of children with ASD is to provide children at risk for the disorder with educational interventions earlier, as interventions are known to be most effective when started in the earliest years of life (Fenske et al., 1985; Harris & Handleman, 2000; Lovaas, 1987). However, early intervention is predicated on early detection and diagnosis, and current estimates put the median age of diagnosis of ASD at 5.7 years in the general U.S. population, with around 27% of children remaining undiagnosed at 8 years (Baio, 2014). By this age however, children may have already passed through sensitive

periods for certain aspects of language acquisition (Ruben, 1997). Early detection of ASD is thus a primary goal of public education, community based practice, and policy in the U.S. The CDC's "Learn the Signs. Act Early" program is designed to promote awareness around typical healthy child development milestones, and to coordinate care at the State level to improve screening for developmental disorders such as ASD. More recently, the U.S. Department of Health and Human Services also launched the "Birth to Five: Watch Me Thrive!" initiative to promote awareness of typical developmental milestones and to encourage integration of services among early childhood experts in child welfare, mental health, primary care and education (USDHHS, 2014).

Research in the neural and cognitive sciences may hold promise in enabling early diagnosis and intervention, and thus ensuring better outcomes for children with ASD. Although ASD is a disorder of neural development that is highly hereditary and thus has a strong genetic component (of monozygotic (identical) twin pairs in which one has ASD, 60% of second twins also has a diagnosis on the spectrum, and 18.7% of infants with an older sibling with ASD develop the disorder; Bailey et al., 1995; Constantino et al., 2010; Folstein & Rutter, 1977; Ozonoff et al., 2011), the genetic underpinnings of ASD are complex and not well understood. ASD is thus currently diagnosed on the basis of behavioral observations. Even in situations in which families have access to the most advanced services, skilled developmental clinicians can only reliably diagnose this disorder when a typical child's behavioral repertoire—such as receptive and

expressive language skills or motor coordination—would be large enough (usually around 2 years of age). Yet precursors to language and other social behaviors begin to develop from the very first months of life.

Pre-Linguistic Development in the First Years of Life

Typically developing infants across cultures develop speech production quite rapidly and seemingly effortlessly in the first years of life. They begin babbling at around 6 months of age, and are usually able to speak in complete sentences by 3 years. It is widely accepted that the mechanism by which children acquire language involves basic perceptual abilities, which are present even before any speech-like behavior and make infants sensitive to certain language-specific features of auditory stimuli, in combination with specific statistical learning processes, which enable infants to extract and acquire rules from the language stimuli to which they are exposed (Kuhl, 2004). Saffran et al. (1996), for example, showed that 8-month-old infants who were exposed to 2-minute strings of computer-synthesized speech (with no pauses), showed novelty preferences for new speech strings that violated the structural regularities embedded in the 2-minute strings. Recent work by Gervain et al. (2008) has shown that newborns also show evidence of possessing an automatic perceptual system for detecting and learning about structural regularities in speech. Newborns in Gervain's study showed larger neural responses (measured via brain oxyhemoglobin increases) to sequences of syllables containing consecutive repetitions (e.g. ba-lo-lo) than to

random sequences (e.g. pe-na-ku); moreover, newborns exhibited increasing neural activity upon increased exposure to the repetitive speech-like stimuli, while neural responses to random sequences did not change over exposure.

Before infants begin to develop speech then, they are sensitive to linguistic signals. This early sensitivity likely lays the foundation for future language acquisition. A few studies have been conducted that give evidence for this link. In retrospective studies, for example, researchers found that infants' performance on a series of speech segmentation tasks (being able to recognize distinct words from continuous speech streams) between 7 and 12 months of age predicted the sizes of their vocabularies at 24 months (Newman et al., 2006). In a recent prospective study, the phonetic perception abilities of 6-month-old infants significantly predicted their word understanding, word production and phrase understanding at two years (Tsao et al., 2004). Early perceptual abilities might also prove to be important indicators of risk for later developmental and learning difficulties; Molfese (2000) found significant associations between 8 year olds who were normal, poor, or dyslexic readers, and their brain responses to speech and non-speech auditory stimuli as newborns.

Language Development in ASD

Cognitive or neuroscience research into early perceptual abilities and neural patterns of activity that comprise, correlate with or predict specific behaviors or behavioral abnormalities may thus be of particular importance in predicting later

language abilities. Specifically, such research may aid in identifying children at risk for ASD who are also at highest risk for associated language delays and deficits, before a child's speech or other telltale behaviors emerge. One of the most consistent findings of past neuroimaging research on language in individuals with ASD is a trend toward atypical lateralization in these individuals' brain responses to language. Functional neuroimaging studies show that typically, speech perception and language processing involves two neural streams – a bilateral ventral stream that processes speech signals for comprehension, and a left-hemisphere-dominant dorsal stream involved in mapping acoustic signals to frontal articulatory and sensory networks (that is, primarily speech production (Hickok & Poeppel, 2007). However, a positron emission tomography (PET) study on adult males found that those with ASD showed right-lateralized brain responses in a speech comprehension task in which they were required to listen to prerecorded simple sentences spoken by adult females; typically developing adults showed reverse-lateralized activation patterns (Muller et al., 1999). Adults with ASD also showed similar bilateral activation to typical subjects in the superior temporal gyrus while passively listening to synthetic speech-like sounds, but simultaneously greater activation in the right middle frontal gyrus and lower activation in the left temporal regions than typically developing individuals (Boddaert et al., 2003). There is still much to be learned about the developmental trajectories of such atypical lateralization in language processing in individuals with ASD, however. For example, it is not known whether individuals who go on

to develop ASD show lateralization reversals prenatally or at birth, or whether such irregularities instead develop over the first years of life. Such information would be useful for determining the feasibility of intervening in the language development of children at risk for ASD.

Infant Sibling Studies of ASD

One of the challenges of studying developmental changes in brain responses to language-relevant stimuli in individuals with ASD is, of course, the difficulty in predicting which newborns and infants will go on to develop this disorder before behavioral indicators such as language difficulties emerge. In order to address this issue, researchers have turned to studies of infant siblings of children with ASD. Infant siblings of ASD, due to their associated genetic liability, are at higher risk of developing ASD than their peers with typically developing older siblings; they are also a particularly important research population, as they likely share endophenotypes of ASD risk with their affected siblings. Endophenotypes, described by Gottesman and Gould (2003) as "measurable components unseen by the unaided eye along the pathway between disease and distal genotype," have been proposed as having the potential to yield clarifying insight into the etiology of ASD. An endophenotype of ASD at the neural systems level for example, may reflect the decomposition of complex behaviors into quantifiable neural activity. These neural endophenotypes, if associated with a particular gene or gene region, may point to key genes or gene

regions in which risk for ASD lie; they may also help elucidate those components of social behavior that are most relevant for further study in understanding the expression of ASD and avenues for treatment of this disorder. Endophenotypes of ASD are therefore important biomarkers indicating risk of developing ASD, or distinguishing subpopulations within the autism spectrum.

By studying the infant siblings of children with ASD during their development in the first few years of life, we may thus be able to identify early predictors or biomarkers of a later ASD diagnosis, as well as the unique developmental trajectories and challenges that a genetic predisposition for ASD confers (regardless of children's eventual diagnostic outcome). Given the prevalence of language abnormalities in individuals with ASD, infant siblings are a particularly important population to study in order to understand the development of language perception and production, and identify those children who might benefit most from language-based educational interventions.

Functional Near-Infrared Spectroscopy (fNIRS)

Another challenge in studying the development of language over the lifespan – particularly at the neural level – is the methodological challenge of conducting research on very young populations. Much previous research has made use of behavioral methods such as sucking frequency, head turning, and gaze, to infer the auditory processing and discrimination abilities of infants and even newborns. More recently however, technologies that allow us to measure neural

responses in neonates and infants have become prevalent. These technologies enable us to detect brain activity that is occurring before associated behavioral responses emerge, and thus enable us to detect relevant developmental processes earlier in development than was previously possible. One relatively new neuroimaging tool that the studies included herein utilize is functional near-infrared spectroscopy (fNIRS), which measures localized changes in the oxygen content of the blood in the brain (that is, the concentrations of oxy- [HbO] and deoxy-hemoglobin [HbR] in the blood). These changes have been shown in animal and human adult studies to be strongly correlated with neural activity; hemodynamic responses are likely responses to changes in the metabolic demands of firing neurons.

fNIRS is thus an indirect measure of brain activity. It is very similar to functional magnetic resonance imaging (fMRI) in that respect, and both technologies produce significantly correlated results when used for cognitive tasks (Cui et al., 2011). It does not have the spatial resolution or ability to detect neural activity at the same depths as fMRI (fNIRS cannot be used to measure cortical activity more than 1-2mm deep), and its temporal resolution is inferior to that of electroencephalography (EEG); as such, neural correlates of language development in deeper cortical or subcortical brain structures, or which occur on a millisecond timescale cannot be detected by this technology. However, as infants have relatively thin skin and skulls and responses to speech occur in cortical brain areas, fNIRS is particularly useful for studies with this population. Additionally,

fNIRS is more robust towards head movement than either fMRI or EEG, has better spatial resolution than EEG/ERP, is less expensive than fMRI, and has better temporal resolution than fMRI. fNIRS has also been successfully used to elucidate neural regions of interest in several infant studies of linguistically pertinent auditory perception (e.g., Pena et al., 2003; Homae et al., 2006; Saito et al., 2007; Gervain et al., 2008; Telkemeyer et al., 2009).

The Current Research

In the chapters that follow, I take steps to develop a research base upon which successful earlier identification of children at risk for ASD and associated language difficulties may be built. In Chapter 2, I identify and investigate neural correlates of early language development in 3-month-old infant siblings of ASD and their low risk peers. Specifically, using fNIRS technology, I examine infants' neural responses to speech-like auditory stimuli containing structural regularities. The differences that I detect between the neural responses of infants at high and low risk for ASD reveal an endophenotype of ASD risk that may represent divergent neural learning mechanisms crucial to language development.

In Chapter 3, I explore possible functional consequences of the neural endophenotypes identified in Chapter 2, by examining associations between these signals and children's later social and communication behaviors. The results of this study suggest that the neural markers identified in Chapter 2 represent not only endophenotypes of ASD risk, but useful predictors of later social and

language development. It is my hope that the current research thus represents a substantial step toward the early identification of children who are likely to develop ASD and associated language difficulties, so that they may be provided with earlier and more targeted programs of education and intervention.

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Chapter 2

Neural Correlates of Speech Perception in 3 Month Olds at Risk for ASD

Abstract

Autism spectrum disorder (ASD) is a disorder of neural development that is characterized by deficits in social and communication abilities. One in 5 infants with an older sibling with ASD will develop this disorder, compared to the rate of 1 in 68 in the general population. Siblings who do not develop ASD also exhibit a variety of associated developmental problems, including language impairments. Although clinicians are able to give reliable ASD diagnoses to children around their second birthdays, social and language developmental processes are occurring in infants from the very beginning of life, and the atypical development characteristic of children at risk for ASD begin long before specific diagnoses are possible.

In the current study, I investigate neural precursors of language acquisition as potential biomarkers of atypical development in infants at high risk for ASD. Three-month-olds with high (HRA) and low (LRC) familial ASD risk were imaged using functional near-infrared spectroscopy while they listened to speech-like stimuli containing syllable repetitions or control syllable sequences. Whereas LRC infants showed initial neural activation that decreased over exposure to repetition-based stimuli, potentially indicating a habituation response to repetition in speech, HRA infants showed no changes in their neural activity to these stimuli

over exposure. These results suggest that putative precursors of language acquisition are disrupted in children at high risk for ASD as young as 3 months old; furthermore, they give evidence that modern neuroimaging technologies can be used to detect these atypical developmental processes far earlier than behavioral diagnoses can currently be made.

Background

Autism spectrum disorder (ASD) is a disorder of neural development that is characterized by persistent deficits in social communication and interaction abilities and restricted, repetitive patterns of behavior. It is has a strong genetic component—in 60% of monozygotic twin pairs, both have an ASD diagnosis; and 1 in 5 infants with an older sibling with ASD will develop this disorder, compared to the rate of 1 in 68 in the general population (Folstein & Rutter, 1977; Bailey et al., 1995; Ozonoff et al., 2011). The siblings of children with ASD who do not develop this disorder often still exhibit a variety of associated developmental problems, including speech and language impairments (Toth et al., 2007).

In order to address the developmental needs and improve long term outcomes of children at high risk for developing ASD, it is most helpful to ensure that they are enrolled in educational interventions as early as possible in life (Fenske et al., 1985; Harris & Handleman, 2000; Lovaas, 1987). Ideally, through early intervention, one could positively shape children's social communication behaviors as they emerge, or prevent them from developing maladaptive behaviors.

Early intervention assumes early detection of an ASD diagnosis, or of specific developmental problems associated with ASD genetic liability. However, the genetic underpinnings of ASD are complex, and no unequivocal risk genes for the disorder have yet been identified. ASD can thus only be diagnosed when a child's behavioral repertoire is large enough that abnormalities in social

development may be detected through observation. In the most ideal cases, stable ASD diagnoses can be determined as early as at 2 years of age (Kleinman et al., 2008). Social and language developmental processes are occurring in infants from the very first months of life, however, and there is much evidence that the atypical development characteristic of children at risk for ASD begins long before specific diagnoses are possible (Bedford et al., 2012; Cornew et al., 2012; Guiraud et al., 2012; Keehn et al., 2013; Toth et al., 2007).

In order to determine which children are in most need of educational interventions, it may be particularly beneficial to investigate the neural activity that precedes and gives rise to certain social behaviors or patterns of development. Much research has been conducted, for example, on the putative neural precursors to language learning in typically developing children. Infants are not only sensitive to language-like auditory stimuli, but are able to extract structural regularities in speech to which they are exposed, and apply them to novel speech streams (Marcus et al., 1999). In fact, a recent study using functional near infrared spectroscopy (fNIRS) neuroimaging techniques showed that humans may actually be born with perceptual biases toward certain types of speech structures (Gervain et al., 2008).

Gervain and colleagues exposed newborns to syllable sequences containing immediate repetitions (referred to here as an ABB syllable pattern, e.g. “ba-lo-lo” or “pe-na-na”) or control non-repeating syllable sequences (ABC syllable patterns, such as “ba-lo-ti” or “pe-na-ku”). They found that neonates exhibited greater

neural activation (rises in the concentration of oxyhemoglobin) to ABB versus ABC patterns over temporal brain regions, particularly in the left hemisphere. Moreover, over subsequent trials, neonates' neural activation to repeated syllables (ABB structures) increased, while activation to non-repeating syllables (ABC structures) did not change. These results suggest that newborns possess an experience-expectant perceptual mechanism for extracting regularities in speech-like auditory streams, which is enhanced by exposure. Gervain et al. posit that this sensitivity to specific auditory input, present from birth, may facilitate later language development.

In the current study, I replicate Gervain's methods in order to examine the neural responses of 3-month-old infants at high and low familial risk for ASD (having an older sibling with a confirmed ASD diagnosis or not) to speech-like auditory streams containing repetition or non-repetition structures. Given the ability of low risk (for ASD) newborns to distinguish and temporally respond to these speech structures, I aim to determine whether these potential precursors of language acquisition are intact or disrupted in children at high risk for ASD. Atypical neural responses to speech-like auditory stimuli and their repeated exposure might indicate important biomarkers of ASD and ASD-related language or learning deficits. Such neural activity may also shed light on the nature of learning, speech, and social developmental impairments in children at high risk for ASD; this information may be invaluable in determining which children are most likely to need interventions, which intervention and educational programs are best

aligned with children's specific profiles of functioning, and in justifying enrollment of children at high risk for ASD and language difficulties into educational programs from before their first birthdays, when interventions are likely to be most effective (Fenske et al., 1985; Harris & Handleman, 2000; Lovaas, 1987).

Materials and Methods

Participants

Participants were drawn from a larger sample of infants enrolled in an ongoing, longitudinal, prospective study of early development in siblings of children with ASD. Two populations were included in this sample: (1) infant siblings of children with a confirmed clinician's diagnosis of ASD (high risk for autism, HRA), and (2) infant siblings of children who are typically developing, or confirmed to have no behavioral and developmental disorders, and who have no first-degree relatives with known ASD or other neurodevelopmental disorders (low risk for ASD controls, LRC). ASD symptomatology in older siblings (or a lack thereof) was confirmed using the *Social Communication Questionnaire* (SCQ; Rutter, Bailey & Lord, 2003). Infants who were born prematurely (earlier than 36 weeks gestational age), who had low birth weights (under 2500g) or who have known neurological or genetic abnormalities were excluded from the study. Infants who grew up in language environments in which English was spoken less than 80% of the time were also excluded, as bilingual (or multilingual) children may show differential developmental trajectories of language development (Byers-Heinlein & Werker, 2013).

Forty-seven infants participated in the current study (29 HRA, 18 LRC); of this sample, 37 (20 HRA, 17 LRC) provided usable data for analysis. Two infants were excluded for excessive fussiness that resulted in failure to hearing at least 16

stimulus blocks (8 of each syllable sequence type); a further four infants were excluded due to poor signal-to-noise ratios in at least 50% of their measurement channels (i.e. 12 or more channels), caused by excessive or dark hair, or improper headgear fit; four additional infants were excluded for having insufficient trials after motion correction (see details of data processing below). Demographic characteristics for the subjects included in the final analysis are shown in Table 2.1. Infants in the two groups did not differ on their age at testing, weight at birth, socioeconomic status (household income, parents' levels of education), or parents' ages at birth.

Table 2.1. Demographics and family characteristics for subjects included in the final analysis

	Low Risk Controls	High Risk for ASD	P Value
N	17	21	
Age (months)	3.62 (0.35)	3.58 (0.39)	0.40
Household Income[a]	7.29 (2.02)	7.6 (1.35)	0.59
Mother's Level of Education[b]	6.35 (1.32)	5.75 (1.48)	0.20
Father's Level of Education[b]	5.35 (2.40)	5.5 (1.50)	0.82
Mother's Age at Birth	33.07 (2.94)	34.10 (3.64)	0.43
Father's Age at Birth	34.94 (3.78)	35.66 (3.72)	0.76
Infant's Birth Weight (lbs)	7.66 (0.91)	7.62 (0.79)	0.25

Data are reported as group means with standard deviations in parentheses. [a]Income was reported on an 8 point scale: (1) less than $15,000, (2) $15,000-$25,000, (3) $25,000-$35,000, (4) $35,000-$45,000, (5) $45,000-$55,000, (6) $55,000-$65,000, (7) $65,000-$75,000, (8) more than $75,000. [b]Education was reported as highest level attained on a 9 point scale: (1) some high school, (2) high school graduate, (3) some college, (4) community college/two-year degree, (5) four-year college degree, (6) some graduate school, (7) master's degree, (8) doctoral degree, (9) professional degree. Independent two-tailed t-tests were used to determine p values for group differences.

Stimuli

Auditory stimuli were identical to those used in Gervain et al. (2008); they comprised blocks of either repeating or non-repeating trisyllabic sequences. In repeating sequences, the second and third syllables were identical (e.g. ba-lo-lo; ABB pattern) while in the non-repeating sequences, all syllables were different (e.g. pe-na-ku; ABC pattern). The syllable sequences were computer-generated using a female voice from the MBROLA diphone database. They were 270ms long, had a monotonous pitch of 200Hz, and were matched on syllabic repertoire, frequency of syllables, and the auditory transitional properties between syllables (see Gervain et al., 2008 for further details of the ABB and ABC grammar construction).

Trisyllabic sequences of the same type were assembled into blocks of 10 syllable sequences separated by randomly varying intervals (between 500–1500ms) of silence, to avoid inducing phase-locked brain responses. Blocks of both syllable types were 16 seconds long on average. Blocks were presented in one of two pseudo-randomized orders, and these orders were counterbalanced across participants based on their risk status and gender. Syllable blocks were separated by a minimum of 15 seconds of silence to allow brain activation generated during test blocks to return to baseline. Blocks were under manual control of an experimenter, who only started new blocks once infants were relatively calm. Each child heard a maximum of 28 blocks (14 ABB, 14 ABC).

Apparatus

Three-month-old infants were examined using functional Near-Infrared Spectroscopy (fNIRS). fNIRS measures localized changes in the oxygen content of the blood in the brain (that is, the concentrations of oxy- [HbO] and deoxy-hemoglobin [HbR] in the blood). In the current study, fNIRS was carried out using a Hitachi ETG-4000 system with near-infrared light at 690nm and 830nm transmitted to source optodes via 1mm optical fiber bundles. Samples were collected at 10Hz. On this recording device, each pair of adjacent source and detector optodes defines a single measurement channel, allowing the measurement of hemodynamic changes occurring in the brain region(s) directly underlying the channel space. Ten source and 8 detector optodes were arranged into two 3x3 chevron arrays (each containing 5 sources and 4 detectors separated by a distance of 3cm) to produce 24 simultaneously recording channels. The optodes were fitted into a soft cap designed for infants; this hat was adjustable to allow for placement of each optode array over a region spanning anterior to posterior temporal cortices of each hemisphere based on scalp measurements to obtain landmarks from the International 10-20 EEG system (Perani et al., 2011; Okamoto et al., 2004). Specifically, channel 3 in the left hemisphere chevron was centered on 10-20 point T3, and channel 20 in the right hemisphere chevron array was centered on 10-20 point T4. Figure 2.1 illustrates the headgear geometry and its placement on a prototypical infant head.

Procedures

Infants were seated on a caregiver's lap in a soundproof testing room, where they passively listened to auditory stimuli. Stimuli were presented through two speakers, hidden behind a curtain in front of the infants. Infants who became fussy were permitted to nurse, feed from a bottle, or eat finger foods, in order to expose them to as many auditory blocks as possible. Auditory blocks were also under experimenter control, so that they were only played when the infant is relatively calm. Past studies using similar techniques to test infants have shown that it is possible to obtain sufficiently artifact-free data under these circumstances (Thomas & Lykins, 1995; Little et al., 1999). Most infants were able to continue the study until they hear all 28 blocks. Those who become too fussy to continue were allowed to stop prematurely.

Data Processing

For participants who heard at least 16 auditory blocks (8 blocks of each stimulus type), fNIRS data (raw light intensity values) obtained at each measurement channel were first screened to ensure adequate signal-to-noise ratios. For each channel, the root mean square (RMS) of the first temporal derivative was calculated for the HbO signal; channels were excluded if the RMS exceeded a

threshold of 0.25[2]; remaining channels were then excluded if raw light intensity signals exceeded 4.95 or fell below 0.1(indicating saturation signals specific to the Hitachi ETG-4000 system) for more than 5 cumulated seconds during a stimulus block[3]. After these screening steps, participants who failed to retain at least 12 of a total of 24 measurement channels were eliminated from further analysis. The high and low risk groups were indistinguishable in terms of the number of channels that they contributed to the final analysis ($p = 0.77$). Data from remaining participants were processed by conversion to optical density units, motion artifact detection using a threshold of 20 standard deviations change in a 500 ms moving window, motion correction using a 0.80 principal component analysis (PCA) filter, band-pass filtration ($0.01 < f < 1.0$) to remove signal changes due to non-experimental factors (such as heart rate, breathing or instrumental noise), and conversion to relative concentrations of HbO and HbR using the modified Beer-Lambert law (ppf = 5.0). Changes in [HbO] and [HbR] were examined from the 2 seconds preceding auditory block onset, the 16-second auditory block duration, and the 4 seconds following test blocks. The mean optical signal from -2 to 0 (baseline) was subtracted from the other test epochs at each measurement channel, in order to allow for standardized comparisons across channels and stimulus conditions.

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[2] RMS calculations provide an index of signal variability. This criterion was used to exclude channels containing excessive variation in measurement signals, which would have been due to noise or artifact, rather than physiological changes.

[3] Saturation thresholds were used to exclude channels in which the signals were either at ceiling or floor levels. These non-physiological values would exert undue influence on indices of neural activity (mean hemoglobin concentration changes) obtained to each stimulus block.

Optical signals were averaged across trials and then infants for each auditory block type. Blocks occurring within 2 seconds of a motion artifact were eliminated from the means. Both high and low risk groups contributed similar numbers of blocks of each stimulus type to the final analyses ($p_{ABB} = 0.75$; $p_{ABC} = 0.77$), and the number of stimulus blocks of each type was similar within risk groups ($p_{LRC} = 1$; $p_{HRA} = 0.76$).

The data processing and analyses steps above were carried out using the Homer2 NIRS processing package (Huppert et al., 2009) and additional in-house customized Matlab scripts (Mathworks Inc., Natick, MA).

Data Analysis

Channels were grouped into four regions for analysis (left posterior, left anterior, right posterior, right anterior) by simple averaging (Keehn et al., 2013; see Figure 2.1). Average [HbO] values to the two trisyllabic sequence types (ABB or ABC blocks) at each of the four regions of interest, along with infants' group membership (HRA vs. LRC; between-subjects factor) and gender (male vs. female; between-subjects factor) were entered into a 2x4x2x2 mixed-design ANOVA to determine if and where there is a significant hemodynamic response to the experimental speech-like stimuli in 3-month-olds, whether the magnitude and location of infants' hemodynamic responses are different across the two syllable conditions, whether these responses differ between infants at high and low risk for

ASD, whether these responses differ between males and females, and whether there are any interactions between these variables.

Based on the work of Gervain et al. (2008), a second set of analyses investigated the effects of repeated exposure on neural responses to the speech-like stimuli. These analyses may determine whether infants show any short-term neural "learning" or "priming" effects during the experiment; past work has indicated that seven month olds are able to discriminate new speech patterns as a result of only two minutes of exposure to novel stimuli (Marcus et al., 1999). For these analyses, the hemodynamic response to the first four blocks of each condition and the last blocks of each condition (ABB-first, ABB-last, ABC-first, ABC-last) were added as a fourth within-subjects factor for a 2x4x2x2x2 ANOVA, to investigate the effect of repeated exposure to ABB or ABC sequence on hemodynamic neural activity.

[HbR] changes are not as well documented or understood in the infant literature as indices of neural activity (e.g. Devor et al., 2005; Watanabe and Kato, 2004; Kameyama et al., 2004), and have a lower signal-to-noise ratio than [HbO] (Tong and Frederick, 2010, as cited by Keehn et al., 2013). The above ANOVAs were thus repeated for [HbR] change for completeness of reporting, but only the [HbO] results will be analyzed and interpreted in light of the study's aims. Results of the [HbR] analyses are presented in Appendix 2.1.

All analyses were carried out using IBM SPSS Statistics (version 21).

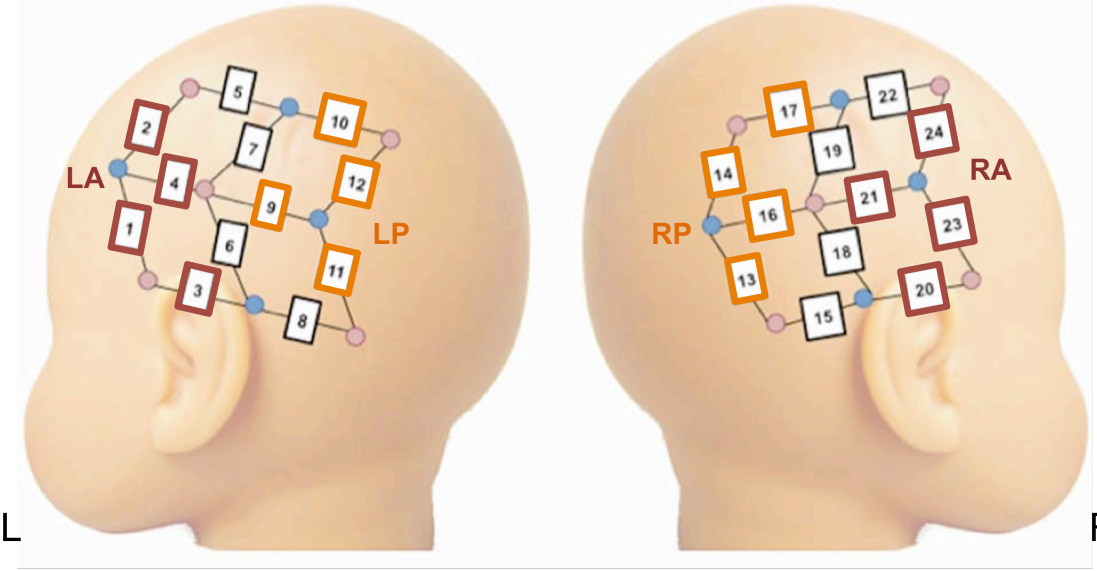

Figure 2.1. *Diagram of fNIRS headgear orientation and placement. Measurement channels are numbered 1-24 (white squares). Hemodynamic responses will be averaged over several channels to form 4 regions of interest (ROIs). Those channels comprising the anterior ROIs are outlined in orange, and those comprising the posterior ROIs are in green. This diagram is an approximate mapping of the placement of the fNIRS channels, but does not represent precisely where they are placed on the infant's head. Adjacent optodes are spaced 3cm apart. LA = left anterior region; LP = left posterior region; RA = right anterior region. RP = right posterior region.*

Results

Neural Responses to Speech-Like Stimuli Across All Trials

All effects are reported as significant at $p<0.05$ (two-tailed) unless otherwise stated. The 2 (syllabic sequence type: ABB, ABC) x 4 (region: left posterior, left anterior, right posterior, right anterior) x 2 (risk group: HRA, LRC) x 2 (gender: male, female) mixed-design ANOVA revealed a main effect of region ($F(1.92, 63.22) = 4.33, p = 0.019$)[4], whereby [HbO] changes to language stimuli of both syllable sequence types across both genders and risk groups were significantly higher in the left anterior region than in the left posterior region ($F(1,33) = 7.53, p = 0.010$), the right anterior region ($F(1,33) = 4.17, p = 0.049$), and the right posterior region ($F(1,33) = 5.79, p = 0.022$).

There was also a significant three-way interaction between region, gender, and risk group ($F(3,99) = 3.74, p = 0.031$). This indicates that the aforementioned differences in [HbO] responses to language-like stimuli in different brain regions were dependent on infants' risk status and gender. Specifically, the results of follow-up simple effects analyses (which break down interaction effects by examining the effect of each independent variable at individual levels of the other independent variable) revealed that in the left anterior region, LRC males and

[4] Mauchly's test of sphericity indicated that the assumption of sphericity (that is, that the variances of the differences between measurement levels are equal; an assumption of repeated-measures and mixed-design ANOVAs) had been violated for the main effect of region, $\chi^2(5)=27.40, p<0.001$. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.64$ for the main effect of region).

females, as well as LRC and HRA males, showed similar [HbO] responses to auditory stimuli, while HRA females exhibited significantly higher [HbO] responses (compared to LRC females: $F(1,33) = 6.35$, $p = 0.017$; compared to HRA males: ($F(1,33) = 4.57$, $p = 0.040$); similarly, in the right anterior region, LRC males and females did not differ from each other, and HRA males did not differ from LRC males, but HRA females exhibited significantly higher [HbO] responses to auditory stimuli than HRA males ($F(1,33) = 4.23$, $p = 0.048$), and LRC females ($F(1,33) = 6.57$, $p = 0.015$); there were no significant simple effects between males and females at either risk group level, or between HRA and LRC risk groups at either gender level in the left or right posterior regions. Figure 2.2 shows average [HbO] responses to auditory stimuli at each region of interest, broken down by gender and risk group.

There were also no significant main or interaction effects of the syllable sequence type (ABB vs. ABC) in this analysis (all *p*s >0.150), indicating that [HbO] responses to both these auditory stimuli were similar across brain region, gender and risk group.

Analyses of [HbR] revealed a main effect of risk status, such that [HbR] changes to language stimuli of both syllable sequence types across all regions and both genders were significantly higher in LRC than in HRA 3 month olds (see Appendix 2.1).

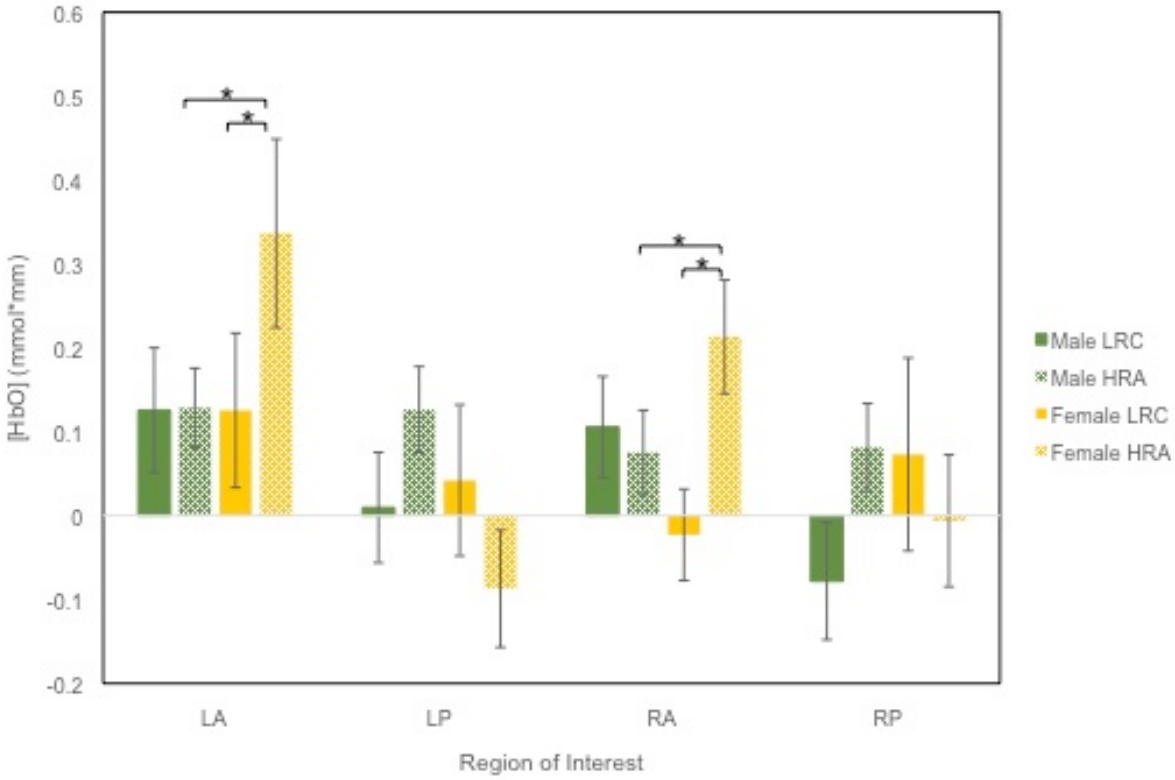

Figure 2.2. Region x gender x risk group interaction. Bars indicate average [HbO] (in mmol*mm) over the course of a stimulus block in the designated regions of interest. Error bars are ±1SE. LA = left anterior region; LP = left posterior region; RA = right anterior region; RP = right posterior region; * $p < 0.05$ in simple effects analysis.

Neural Responses to Speech-Like Stimuli Across Exposure

The 2 (syllabic sequence type: ABB, ABC) x 4 (region: left posterior, left anterior, right posterior, right anterior) x 2 (risk group: HRA, LRC) x 2 (gender: male, female) x 2 (exposure: first vs. last stimuli blocks) mixed-design ANOVA also revealed a main effect of region ($F(2.12, 69.82) = 3.59$, $p = 0.030$)[5], whereby [HbO] changes to language stimuli were significantly higher in the left anterior region than in the left posterior region ($F(1,33) = 7.66$, $p = 0.009$), and marginally higher in the left anterior region than in the right posterior region ($F(1,33) = 3.89$, $p = 0.057$; see Figure 2.3). This ANOVA also revealed a three-way interaction between exposure, syllabic sequence type and risk group ($F(1,33) = 4.95$, $p = 0.033$) and a four-way interaction between exposure, syllabic sequence type, risk group and gender ($F(1,33) = 6.77$, $p = 0.014$). These results indicate that [HbO] responses to the two syllabic sequence types differed over the course of infants' exposure to these stimuli, depending on infants' genders and risk status. Specifically, simple effects analyses collapsing across all brain regions revealed that LRC females showed significantly lower [HbO] responses to the last 4 ABB blocks compared to the first 4 ABB blocks ($F(1,136) = 6.10$, $p = 0.015$), and that their [HbO] responses to the last 4 ABB blocks were also significantly lower than those of HRA females to the last 4 ABB blocks ($F(1,136) = 7.72$, $p = 0.006$). A

[5] Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for the main effect of region, $\chi^2(5)=18.33$, $p=0.003$. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.705$ for the main effect of region).

similar pattern of results was observed between LRC and HRA males across the first and last 4 ABB stimulus blocks, although these differences did not reach statistical significance (see Figure 2.5a). Figure 2.5b depicts the exposure x risk group x gender interaction for the ABC stimulus blocks; none of the [HbO] response patterns to this condition were statistically significant.

Analyses of [HbR] revealed an interaction between exposure and gender, such that female infants' [HbR] responses increased, whereas males' neural responses decreased, over exposure to both syllabic sequence types (see Appendix 2.1).

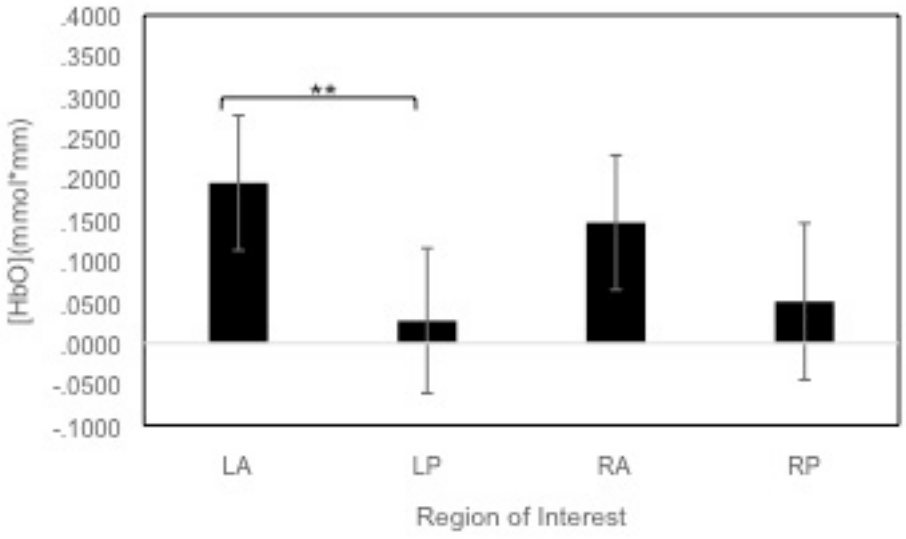

Figure 2.3. Main effect of region in syllable sequence x region x exposure x risk group x gender analysis. Error bars are ±1SE. LA = left anterior region; LP = left posterior region; RA = right anterior region; RP = right posterior region; ** $p <$ 0.01.

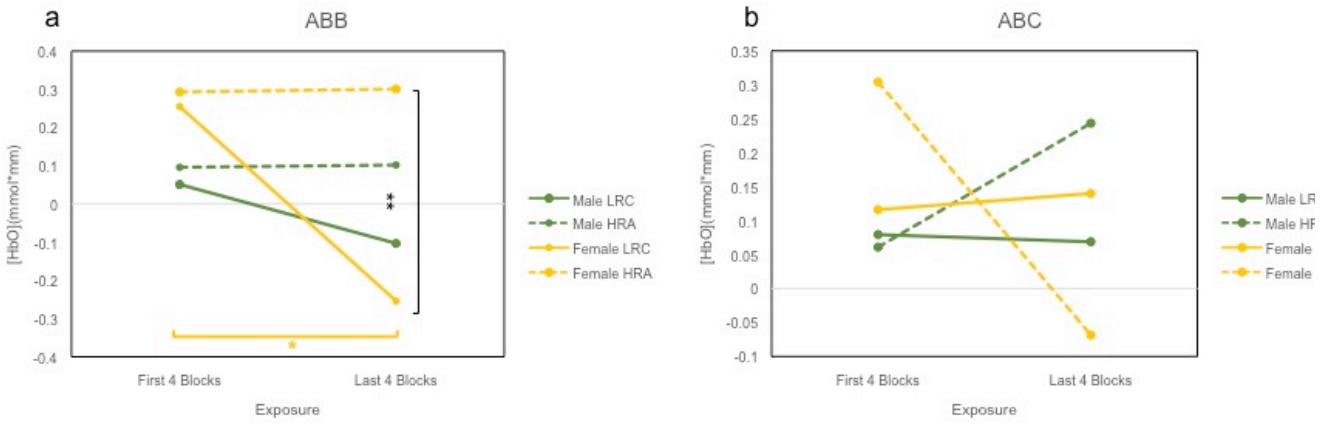

Figure 2.4. Four-way interaction between exposure, syllabic sequence type, risk group and gender ($F(1,33) = 6.77$, $p = 0.014$). (a) Responses to ABB stimuli blocks; (b) Responses to ABC stimuli blocks. * $p < 0.05$; ** $p < 0.01$.

Discussion

Neural Responses to Speech-Like Stimuli Across All Trials

In this study, I used fNIRS to determine the neural correlates of an early speech perception mechanism in a sample of 3-month-old infants at high risk for ASD, and their typically developing peers. This investigation followed up on a study conducted by Gervain et al. (2008), in which healthy newborns were shown to be able to detect certain repetition structures in speech-like auditory stimuli, and to increase their neural responses to these speech patterns over exposure.

In my first set of analyses, an ANOVA examining hemodynamic responses to the experimental speech-like stimuli in 3-month-olds of both genders and ASD risk groups produced no evidence that children from either risk group (or gender) differentiated the repetition from the non-repetition auditory stimuli. This finding might seem particularly surprising since Gervain et al. (2008) established that neonates do discriminate ABB from ABC speech stimuli at the neural level; it stands to reason, based on the newborn findings, that 3-month-olds in the LRC group, due to their increased experience, might have retained their early sensitivity to repetitions and other structural regularities in speech-like auditory stimuli. A number of alternative explanations may account for the current finding however.

First, with increased experience and exposure to speech over the first 3 months of life, infants' responses to auditory stimuli may have matured or become even more finely discriminatory, so that they look different from the neural

responses of newborns. While the neonates tested in Gervain's study presumably only had in utero prior exposure to human language, 3 month olds have a relative abundance and clarity of experience with human speech, particularly that of their primary caregiver. Since the auditory stimuli used in this study were computer-generated speech streams, it is possible that by 3 months of age, infants' responses to the study stimuli may have dampened, and their neural resources instead allocated to human speech, which is the more relevant and prevalent auditory stimulus. The neural mechanism that would produce such changes—synaptic pruning, by which pathways of brain activity that are not utilized or reinforced are pruned away—begins shortly after birth, and in humans, the auditory cortex of the temporal lobe is actually one of the first to mature via the mechanisms of synaptic overproduction and pruning (Huttenlocher, 1999).

Although the study stimuli utilized a female voice to increase relevance and human-like quality, a computer-generated speech stream was chosen instead of a more naturalistic one to ensure that this study could be directly compared to previous studies (Gervain, 2008; Wagner, 2011) utilizing these auditory stimuli on different populations. The computer-generated stimuli also had the advantage of being matched on all non-structural properties of speech (syllabic repertoire, frequency of A, B, and C, syllables, phonological characteristics, flat prosody, and transitional properties between syllables) so that differences in infants' neural responses to the speech streams can be validly inferred as responses to the two different speech structures. Still, the use of human voice stimuli would increase

the ecological validity of the study in terms of its ability to predict how infants at high and low risk for ASD are responding to the speech they hear around them every day, and a recommended future direction of the current research is thus a replication of this study using naturalistic speech as auditory stimuli. Alternatively, a more labor-intensive (though not altogether impractical in our increasingly digital culture) follow-up study might involve regularly exposing children to computer-generated speech in the first few months of life, in order to determine whether 3 month olds raised in these conditions might retain the patterns of neural activity that Gervain et al. (2008) observed in newborns. Past research has established that later on in development, infants who would otherwise lose the ability to discriminate among speech sounds from foreign languages can retain this ability until at least 12 months of age, if they are exposed to non-native languages from 9 months of age (Kuhl et al., 2003). In a similar study carried out in the domain of face processing, researchers demonstrated that although infants usually lose the ability to discriminate faces of non-native species (e.g. macaque monkeys) by 9 months old, those 9 month olds who were continually exposed to a range of macaque faces retained this ability (Pascalis et al., 2005).

Another possible explanation for HRA and LRC infants' lack of discrimination of ABB from ABC stimuli does not dismiss the relevancy or prevalence of these stimuli. Instead it is possible that 3 month olds are more attuned to language (or non-language) features that are similar between the two speech structures. On one hand, infants' neural responses to these stimuli observed

in this study may not actually be due to the specifically linguistic features of the auditory input, but they may represent a more general orienting response to relevant auditory input in the environment. On the other hand, as the auditory stimuli were matched on all nonstructural properties (such as syllabic repertoire, frequency of each syllable type, phonological characteristics, transitional properties between syllables), if infants were showing neural responses to any of these non-structural speech characteristics—as they might if non-structural characteristics were more developmentally relevant for speech perception at 3 months—they should have shown similar patterns of activation across ABB and ABC sequences. In fact, the main effect of region produced by the syllable sequence x region x risk x gender ANOVA ($F(1.92, 63.22) = 4.33, p = 0.019$) suggests that both groups of infants were showing significant increases in [HbO] in response to the speech-like streams in the channels on the left anterior measurement region. Much past research has established that brain activity related to mapping auditory speech input onto conceptual representations involves the frontal lobe and largely left-lateralized networks (Hickok & Poeppel, 2000; Hickok & Poeppel, 2007; Perani et al., 2011), and the activity that Gervain detected in neonates occurred in the temporal and left frontal regions (Gervain et al., 2008). This main effect result thus provides evidence for explanations of the results in which the ABB and ABC stimuli are still being processed in ways that are relevant for speech perception at 3 months old.

Additionally, an fMRI study on 2-day-old infants found that they showed similar patterns of activation to natural and hummed speech, but not flattened speech; the authors of this study interpreted the findings as indicating that newborns may be processing phonemic and prosodic information, rather than lexical and syntactical information, from the stimuli to which they are exposed (Perani et al., 2011). These results stand in contrast to Gervain's findings, but may provide an explanation for the lack of differentiation between ABB and ABC stimuli in the current study. It is also possible that the switch from sensitivity to the unique characteristics of ABB stimuli to the characteristics held in common by ABB and ABC stimuli occurs rapidly, within the first two days of life.

However, this analysis also revealed a region x gender x risk group interaction, and further investigation of this interaction effect (illustrated in Figure 2.2) suggests that the main effect of region might have been a result of female HRA infants' significantly higher [HbO] changes in the left anterior region. Although neither HRA nor LRC infants discriminated the ABB and ABC stimuli then, this interaction effect indicates that the two risk groups (and the two genders) were not processing the auditory stimuli identically. Specifically, female HRA infants showed significantly higher average [HbO] responses than both LRC females and HRA males bilaterally, in the anterior measurement channels. Although interpretation of these trends may be premature (see below for a discussion of the findings revealed by examining infants' responses to these stimuli over the course of their exposure to them), they indicate that, particularly

in the realm of speech perception or early language abilities, where differences between males and females have been established (Gleason & Ely, 2002), it may also be important to examine males and females at high risk for ASD separately, and to be sensitive – both in research and in practice – to the different trajectories along which girls and boys are likely to develop.

Neural Responses to Speech-Like Stimuli Across Exposure

The addition of an exposure variable to the analyses—to determine whether the neural responses of 3 month olds changed between the first and last stimulus blocks, perhaps due to neural learning or priming effects—revealed a main effect of region, wherein neural responses to the language stimuli across risk group, genders and exposure were largest in the left anterior measurement region (LA vs. LP, $p = 0.009$; Figure 2.3). This finding aligns with past research, which has established neural centers of language processing as existing bilaterally, but predominantly in the left hemisphere (Hickok & Poeppel, 2000), and specifically aligns with Gervain et al.'s (2008) finding of stronger left hemisphere involvement in [HbO] activation to the ABB grammar. In these analyses, the main effect of region holds since no other significant interactions involved this variable

The syllable type x region x exposure x risk group x gender analyses also revealed a three-way interaction between exposure, syllabic sequence type, and risk group, and a four-way interaction between exposure, syllabic sequence type, risk group, and gender. Figures 2.4a and 2.4b illustrate the four-way interaction,

which will be interpreted in detail here; note that the presence of the four-way interaction precludes meaningful interpretation of the three-way interaction, as it indicates that the three-way interaction operates differently for different genders.

Examination of the four-way interaction graphs reveals that both female and male LRC infants showed a lowering of their [HbO] responses to ABB syllable sequences between the first and last 4 stimulus blocks. Simple effects analyses revealed that only the patterns of neural activity of LRC females reached statistical significance however; specifically, LRC females showed significantly lower [HbO] responses to the last 4 ABB blocks compared to the first 4 ABB blocks ($F(1,136) = 6.10, p = 0.015$).

These results suggest that although LRC infants did not appear to discriminate ABB and ABC syllable sequences in the previous analysis (in which all trials were averaged) their temporal responses discriminated these two syllable sequences. Specifically, whereas LRC infants appeared to show a lowering of [HbO] response to ABB sequences over time, they showed no such temporal changes in their neural responses to ABC sequences. The lowering of [HbO] over exposure might represent a neural habituation response; this interpretation would suggest that LRC infants habituate to syllable sequences containing consecutively repeated syllables, but do not habituate to random, non-repetitive syllable sequences. Although our initial analyses suggested that LRC infants did not discriminate ABB from ABC sequences, this analysis shows that LRC infants' patterns of responses over time indeed do differ between the two syllable

sequences. These results also support above interpretations of the results collapsed over exposure in which infants are processing both ABB and ABC blocks as relevant language-like stimuli, rather than those explanations in which infants may have lost sensitivity to the computer-generated speech since birth.

The exposure x syllabic sequence type x risk group x gender results are also particularly interesting for their developmental implications in light of Gervain's (2008) results. While Gervain found higher initial [HbO] responses to ABB (vs. ABC) stimuli in newborns, the current 3-month-old sample exhibits similar initial [HbO] responses to both ABB and ABC stimuli (potential explanations for which have been discussed above). Differences seem to arise in 3 month olds however, between the initial responses of males and females, such that across both ABB and ABC syllable sequences, females appear to show higher initial responses to the auditory stimuli than males[6]. Additionally, while newborns showed an increase in the [HbO] response to ABB stimuli over exposure, the current results show that at 3 months of age, LRC infants' neural responses to the repetition stimuli decrease over exposure.

There are a number of possible interpretations for the change observed in the course of the neural response between these two studies. One possible explanation for this difference could be the state of consciousness in which infants were tested. In Gervain's study, newborns were tested in a state of quiet rest or

[6] Note that this observation is based mainly on visual examination of Figures 2.4a and 2.4b, and is not borne out in the statistical analyses, perhaps because of low power to detect statistically significant results.

sleep, while in the current study, infants were kept awake whenever possible. Although this study's relatively small sample size precludes analysis of these results dependent on infants' physical states, future research may systematically investigate this variable's effect on the neural indices examined herein.

Alternatively, the change from Gervain's finding of a temporal increase to this study's finding of a decrease in [HbO] over time might indicate a developmental change that takes place between birth and 3 months of age. It is possible, for instance, that newborns require more trials than 3 month olds need to show habituation effects to a stimulus. If this is the case, we would expect a follow-up study using longer and longer numbers of blocks of stimuli on healthy newborns to eventually show that these subjects' neural responses to ABB stimuli decrease between the first and last few trials; perhaps a critical value necessary for habituation to auditory stimuli in newborns could also be determined from such a study. In past developmental psychology research in the domain of vision for example, one and two month olds showed no changes in their levels of visual attention to a stimulus presented to them repeatedly for ten one-minute periods. By two to three months old however, infants decreased their visual attention significantly between the first and last five exposure periods (Fantz, 1964). Other studies utilizing visual habituation paradigms with newborns have found that they habituate after being exposed to an average of 9.6 stimuli, but that some increase markedly in their habituation after as many as 20 trials (Field et al., 1984; Friedman, 1972); some studies of auditory perception place newborn habituation

anywhere between 11 and 60 trials, depending on their state of consciousness, as well as the amplitude of the stimuli (Vander Maelen et al., 1975). Three month olds however, tend to habituate much more quickly; in a study of dynamic visual displays they took between 6 and 14 trials to habituate (Sommerville et al., 2005).

Gervain et al. (2008) interpreted the temporal increase in [HbO] to ABB stimulus as perhaps due to priming of recognition of subsequent stimuli due to a memory-like process. If this is the case, perhaps the increase in [HbO] observed over exposure to ABB stimuli in neonates promotes or reflects the strengthening of the neural pathway that processes repetition stimuli. Over time, such a process might lead to more efficient neural responsiveness to repetition stimuli, so that by 3 months of age, the very first exposures to such auditory input evoke larger neural responses. This explanation might be supported by the magnitude of the average responses to ABB stimuli across trials in Gervain's study, which range from ~0.01-0.04mmol*mm; in the current study, initial [HbO] responses are as high as ~0.3mmol*mm. Many factors (including individual differences in basal blood-oxygen levels, technical specifications of fNIRS measurement equipment, and more) might affect the absolute values of the [HbO] responses measured using fNIRS across sites however, so this observation is made tentatively and should be interpreted with caution.

HRA infants exhibited different temporal neural response patterns than did LRC infants across both ABB and ABC stimuli. Although differences between initial responses to the ABB stimuli might be larger between genders than between

risk groups (Figure 2.4a), HRA males and females did not show the dampening of neural responses over time that LRC infants' exhibited; nor did they show any increases—they remained constant across exposure to ABB stimuli. In fact, LRC females' [HbO] responses to the last 4 ABB blocks were significantly lower than those of HRA females to the last 4 ABB blocks ($F(1,136) = 7.72$, $p = 0.006$). While HRA infants showed initial responses to ABB stimuli that were similar to their LRC peers then, they may have failed to neurally habituate to these stimuli. Since habituation may function to free cognitive resources from processing stimuli that are neither harmful nor beneficial so that they can be dedicated to more novel, relevant or beneficial stimuli, a deficit or delay in habituation responses in HRA infants might negatively impact their ability to process other, more complex auditory or language structures. Indeed, many children with ASD, or with a family history of ASD show significant delays in acquiring vocabulary, and show a tendency to echo or repeat words that they have previously heard out of context, or without functional communicative intent. Further research might investigate whether the lack of [HbO] lowering to ABB stimuli in HRA children is representative of a delay or deficit in habituation, and whether this 3-month neural signature predicts later specific language difficulties.

The neural responses of HRA male and female infants to ABC syllable sequences also deviate from those of LRC infants. However, in contrast to their responses to ABB stimuli, HRA infants appear to show different patterns of neural activity to the first and last blocks of ABC stimuli. Female HRA infants appear to

show higher responses than any other group to the initial 4 ABC blocks, then show lower [HbO] levels to the last 4 ABC blocks. Male HRA infants show the opposite pattern of activity: their [HbO] responses to ABC stimuli increase from the initial to last 4 stimulus blocks, although they show initial response magnitudes similar to both LRC males and females.

Although no clear hypotheses for systematic differences between LRC and HRA infants emerge from examining differences in their patterns of hemodynamic activity to ABC stimuli, it is again apparent that HRA males and females likely follow different language developmental trajectories, or may show distinct patterns of dysregulation or dysfunction. Future research and clinical work should aim to determine which developmental processes differ between HRA males and females so that interventions can be targeted accordingly.

A significant limitation of the current study that merits consideration in the interpretation of the results obtained herein are the power issues related to the relatively low study sample size. Although the sample size for the current study are similar to those of past studies using neuroimaging methods on young infants or developmentally delayed populations, the current sample size only provides us with adequate power ($P = 0.95$) to detect large effect sizes ($d = 0.4$) via the statistical tests used herein. The finding of a lack of discrimination between ABB and ABC stimuli in LRC infants, therefore, might be a result of a developmental effect, but might also be due to a lack of power for achieving statistical significance. Despite this lack of power, the findings presented and discussed

herein indicate that neural correlates of early speech perceptual abilities are likely to be meaningful biomarkers of ASD risk, and thus warrant continued study.

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Appendix 2.1

Deoxyhemoglobin [HbR] Results

Neural responses to speech-like stimuli across all trials. All effects are reported as significant at $p<0.05$ unless otherwise stated. The 2 (syllabic sequence type: ABB, ABC) x 4 (region: left posterior, left anterior, right posterior, right anterior) x 2 (risk group: HRA, LRC) x 2 (gender: male, female) mixed-design ANOVA revealed a main effect of risk group ($F(1, 33) = 11.925$, $p = 0.002$), whereby [HbR] changes to language stimuli of both syllable sequence types across all regions and both genders were significantly higher in LRC than in HRA 3 month olds (Figure 2.1.1). No other significant main or interaction effects were detected.

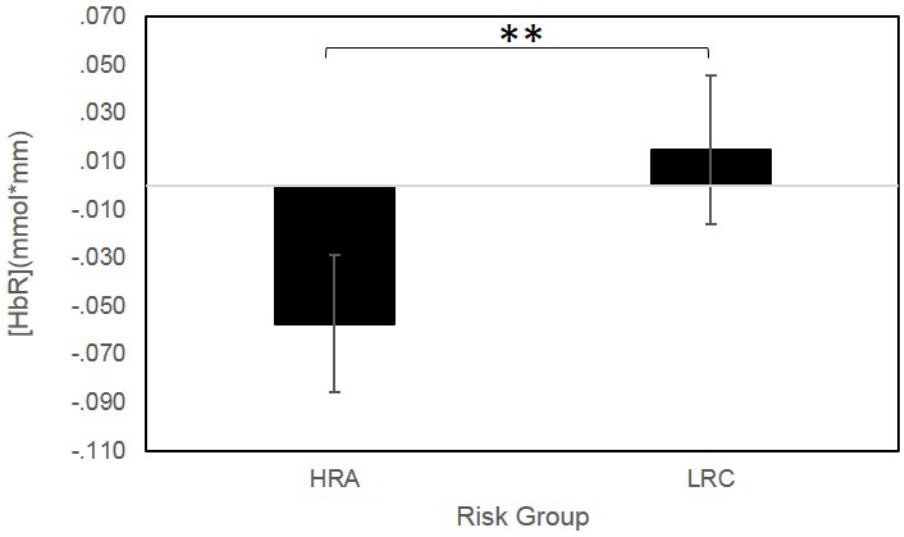

Figure 2.1.1. Main effect of risk group in syllable sequence x region x risk group x gender analysis. Error bars are 95% CI. HRA = high risk for autism, LRC = low risk controls; ** $p < 0.01$.

Neural responses to speech-like stimuli across exposure. The 2 (syllabic sequence type: ABB, ABC) x 4 (region: left posterior, left anterior, right posterior, right anterior) x 2 (risk group: HRA, LRC) x 2 (gender: male, female) x 2 (exposure: first vs. last stimuli blocks) mixed-design ANOVA also revealed a main effect of risk group ($F(1, 33) = 6.755$, $p = 0.014$), whereby [HbR] changes to language stimuli were significantly higher in LRC than in HRA 3 month olds (Figure 2.1.2). This ANOVA also revealed an interaction between exposure and gender ($F(1,33) = 6.096$, $p = 0.019$), which indicates that [HbR] responses differed over the course of infants' exposure to these stimuli, depending on infants' genders, such that female infants' [HbR] responses increased, whereas males' neural responses decreased, over exposure to both syllabic sequence types (Figure 2.1.3). Finally, the ANOVA revealed a marginal interaction between exposure and syllable sequence type ($F(1,33) = 3.998$, $p = 0.054$). These results indicate a trend toward different neural responses across infants of both genders and risk groups, such that [HbR] tended to increase over exposure to ABB sequences and decrease with increased exposure to ABC sequences (Figure 2.1.4).

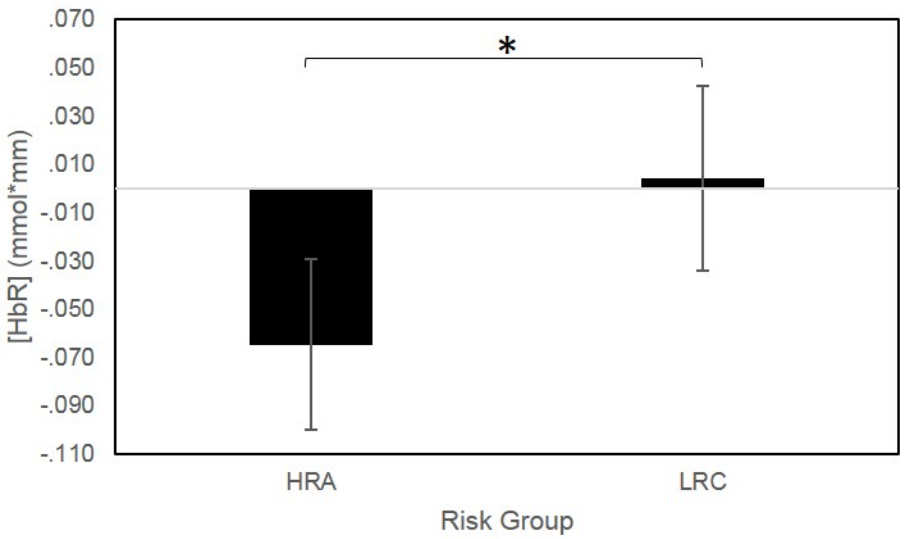

Figure 2.1.2. Main effect of risk group in syllable sequence x region x exposure x risk group x gender analysis. Error bars are 95% CI. HRA = high risk for autism, LRC = low risk controls; * $p < 0.05$.

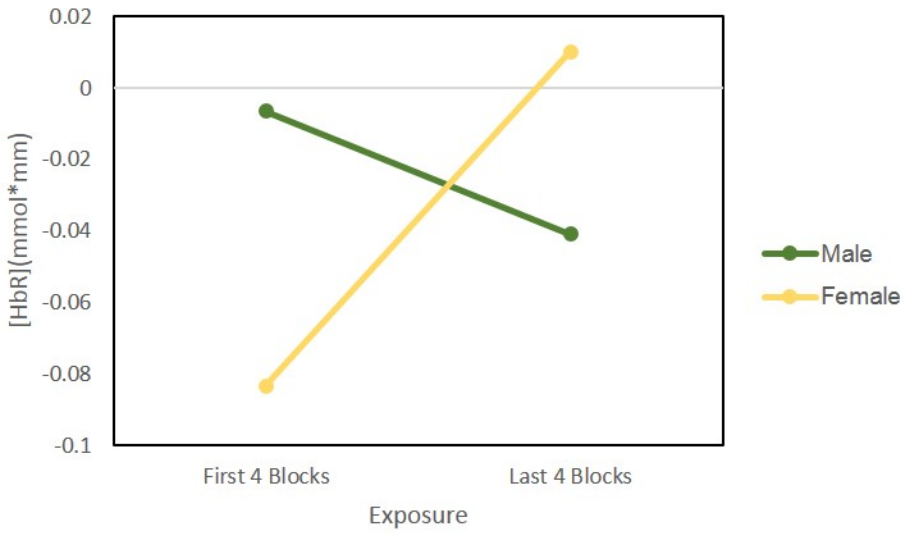

Figure 2.1.3. Interaction between exposure and gender ($F(1,33) = 6.096$, $p = 0.019$).

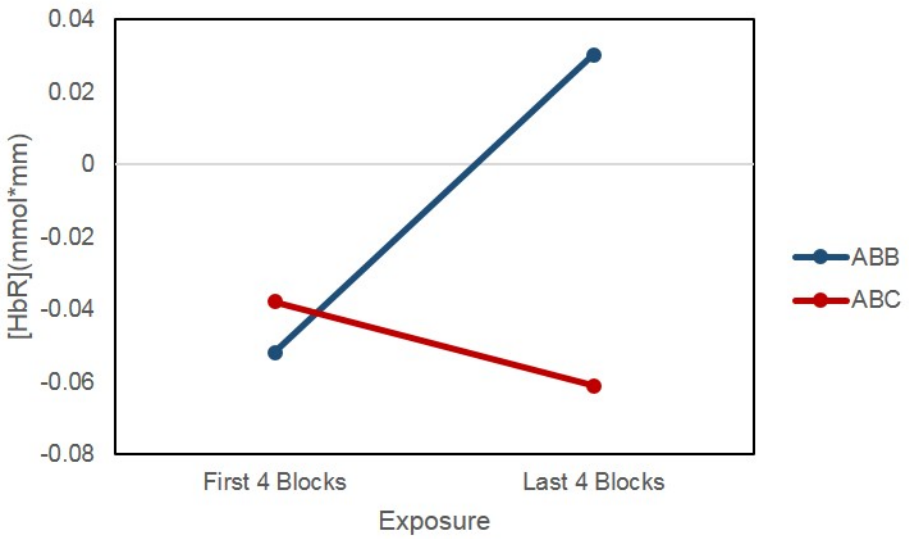

Figure 2.1.4. Trend toward interaction between exposure and gender ($F(1,33) = 3.998, p = 0.054$).

Chapter 3

Neural Markers of Speech Perception in 3 Month Olds Predict Social and Communicative Outcomes at 18 Months

Abstract

Autism spectrum disorder (ASD) is a developmental disorder characterized by difficulties in social interaction and communication. At both the genetic and behavioral levels, ASD is a highly heterogeneous disorder. Although there are a number of high quality evidence-based intervention programs that have been proven successful in remediating aspects of cognitive, language and social functioning in children with ASD, there is often wide variation in the effectiveness of any given intervention program on its participants (Schreibman, 2000). Two factors that contribute to intervention effectiveness are the age at which children enter intervention programs—children who enter earlier in life tend to have better outcomes—and the appropriateness of the specific intervention program for a child's profile of functioning.

In the current study, I investigate whether neural responses to repetition-based speech stimuli in 3-month-old infants at high and low risk for ASD predicts their language and communicative outcomes at 18 months of age. The indices of neural activity examined predicted expressive and receptive language (measured by the Mullen Scales of Early Learning), autism symptomatology (measured by

Autism Diagnostic Observation Schedule severity scores), and early gestures, sentence complexity, and sentence length (measured by the MacArthur-Bates Communicative Development Inventory: Words and Sentences Module). In many cases, the associations between neural indices and behavioral outcomes differed for males and females, and for high and low risk subjects. These results suggest that biomarkers of social and communicative outcomes may be present and identifiable long before children's behaviors emerge.

Background

Autism spectrum disorder (ASD) is a disorder of brain development, which is manifested in difficulties with social interaction, as well as verbal and nonverbal communication, in affected individuals. The term "spectrum" refers to both the genotypic and phenotypic heterogeneity of this population. Although it is known that ASD is a genetic disorder due to the high rates of co-occurrence of this disorder among identical twins (in approximately 60% of monozygotic twins pairs, both have a diagnosis on the spectrum; Bailey et al., 1995) and siblings (approximately 20% of children with an older sibling on the autism spectrum will also receive an ASD diagnosis; Bailey et al., 1995; Ozonoff et al., 2011), hundreds of genes and variants have been implicated in the etiology of ASD (Gupta & State, 2007). Behaviorally, individuals with ASD range from having mild impairments to profound disabilities, and may look qualitatively different from each other. Some individuals with ASD for example, have intact or even advanced verbal abilities, whereas around 25% of individuals with ASD speak few or no words (DeWeerdt, 2013). Relatedly, 50–70% of children with ASD have concurrent diagnoses of intellectual disability (Matson & Shoemaker, 2009), but many individuals with ASD have average or well above average cognitive functioning.

Although there are a number of high quality evidence-based intervention programs that have been proven successful in remediating aspects of cognitive, language and social functioning in children with ASD, there is often wide variation in the effectiveness of any given intervention program on its participants

(Schreibman, 2000). One well established factor that contributes to intervention effectiveness is the age at which children enter intervention programs—children who enter earlier in life tend to have better outcomes (Fenske et al., 1985; Harris & Handleman, 2000; Lovaas, 1987). However, the heterogeneity within the ASD population requires that interventions also be appropriately targeted in order to be effective. For example, children with ASD who have higher cognitive functioning and language skills tend to have better social and educational outcomes, and may even be able to overcome their diagnoses with age and high quality interventions that capitalize on their strengths in order to build social aptitude. Children who have particularly low cognitive or language skills are at elevated risk for poor outcomes however, and should be enrolled into intervention programs that target language or essential precursors to language development from as early as possible. Additionally, children without an ASD diagnosis but who show behavioral similarities to children with ASD across certain domains (in particular, the non-ASD siblings of affected children, who are at elevated risk for language impairments and other socio-emotional difficulties; Cassel et al., 2007; Toth et al., 2007) may also benefit from early access to the interventions that are being funded and developed to remediate specific areas of dysfunction common in ASD.

Even in situations in which families have access to the most advanced services however, skilled developmental clinicians can only reliably diagnose ASD when a child's behavioral repertoire is large enough (around 2 years of age). Yet precursors to language and other social behaviors begin to develop from the

first months of life. For example, newborns show preferences for their mothers' voices over other female voices (Mehler et al., 1978). Infants as young as 2-months can distinguish utterances from their native language from those of foreign languages (Mehler et al., 1988); between 6- and 12-months, infants become less sensitive to non-native speech contrasts (Kuhl et al., 2006). Infants can also detect structural regularities in speech-like auditory streams (Saffran et al., 1996; Teinonen et al., 2009) and generalize these distinctions to novel stimuli (Marcus et al., 1999). These abilities likely lay the foundation for future language acquisition; in some cases they may be directly related to the development of specific linguistic capacities. In research on typically developing populations for example, the phonetic perception abilities of 6-month-old infants significantly predicted their word understanding, word production and phrase understanding at 2 years (Tsao et al., 2004). In studies of pre-verbal 7 to 12 months olds, researchers found that children who were familiarized with isolated words were able to recognize them within streams of continuous speech, sometimes even across changes in the characteristics of the speaker, or in foreign languages with similar rhythmic structures (Houston & Jusczyk, 2000; Houston et al., 2000; Mattys & Jusczyk, 2001). This ability was associated with children's vocabulary when they were 24 months old – those who were better at speech segmentation as infants had larger vocabularies at 2 years of age (Newman et al., 2006). Neural and cognitive studies of early perceptual and pre-linguistic abilities in children at high risk for ASD may therefore be of particular importance in predicting which of these children might

be in most serious need of intervention, and in identifying what the specific targets of such interventions should be.

Typically developing infants rapidly acquire speech through a combination of statistical learning strategies and social interaction (Kuhl, 2004). Past research has shown that aspects of statistical learning—such as the ability to recognize patterns in auditory stimuli—are present from birth (Gervain et al., 2008), but may be disrupted in children at high risk for developing ASD (see Chapter 2).

In the current study, I investigate whether 3-month-old infants' neural responses to repetition-based structural regularities in speech-like auditory stimuli (examined in Chapter 2) predict their later language development. In particular, I will examine language outcomes at 18 months old, as this is one of the earliest time points at which parents of children at risk for developing ASD express concern about their children's development, based on their delay in meeting typical language milestones. Additionally, 18 months is the earliest age at which skilled developmental clinicians confer provisional diagnoses based on children's observed behaviors. I hypothesize that infants' neural responses to repetitions in speech-like stimuli—which may underlie more computational, non-social components of the language acquisition process—might thus be more strongly associated with language outcomes such as word production, word understanding and sentence complexity, than more social aspects of language such as gesture use, or than overall measures of ASD symptom severity. Such findings would provide support for the suggestion that early perceptual abilities, such as those investigated

in Chapter 2, facilitate structural (rather than social) aspects of later language development. They would also validate the neural markers examined in Chapter 2 as potentially useful indicators for determining (from the earliest age possible to date), which children are most likely to need educational interventions that target language development. I will consider children's ASD risk status as well as genders in these predictive models as well, as children at risk for ASD are at elevated risk for language impairments, and show different early patterns of neural activity in response to structural patterns in speech-like stimuli (Gervain et al., 2008; Chapter 2), and past research indicates that males' and females' language development may follow different trajectories (Gleason & Ely, 2002).

Recent studies estimate the costs associated with caring for children with ASD (those without the comorbidity of intellectual disability) at more than an additional $17,000 per child annually (a lifetime cost of $1.4 million), with the majority of this expense being due to non-healthcare-related costs, including special education services (Lavelle et al., 2014)[7]. By identifying those children who are at highest risk for poor developmental outcomes and enrolling them in intensive interventions as early as possible, we might reduce the net financial burden of an ASD diagnosis on families and education systems.

[7] Mean annual costs for children with ASD and ID are considerably higher, and somewhere between 40 and 60 percent of children with ASD have comorbid ID (Buescher et al., 2014).

Materials and Methods

Participants

Subjects are those included in the previous analysis (Chapter 2). They were drawn from a larger sample of infants enrolled in an ongoing, longitudinal, prospective study of early development in siblings of children with ASD. Two populations of children were included in this sample: (1) infant siblings of children with a confirmed clinician's diagnosis of ASD (high risk for autism, HRA), and (2) infant siblings of children who are typically developing, or confirmed to have no known behavioral and developmental disorders, and who have no first-degree relatives with known ASD or other neurodevelopmental disorders (low risk for ASD controls, LRC). ASD symptomatology in older siblings (or a lack thereof) was confirmed using the *Social Communication Questionnaire* (SCQ; Rutter, Bailey & Lord, 2003). Infants who were born prematurely (earlier than 36 weeks gestational age), who had low birth weights (under 2500g) or who have known neurological or genetic abnormalities were excluded from the participant pool. Infants who grew up in language environments in which English was spoken less than 80% of the time were also excluded from the participant pool, as bilingual (or multilingual) children may show differing developmental trajectories of language development (Byers-Heinlein & Werker, 2013).

Thirty-seven (20 HRA, 17 LRC) of the 47 total children enrolled in this study provided usable neuroimaging data for analysis. The details of those infants

who were excluded are found in Chapter 2 (Materials and Methods: Participants). Demographic characteristics for the subjects included in the final analysis are shown in Table 2.1. Subjects in the two risk groups did not differ on their age at testing, weight at birth, socioeconomic status (household income, parents' levels of education), or parents' ages at birth.

Procedures

Neuroimaging Measures. Three-month-old HRA and LRC infants were exposed to speech-like auditory streams containing repetition (ABB) or non-repetition (ABC) structures while they were imaged using functional near-infrared spectroscopy (fNIRS) technology, to determine whether this precursor of language acquisition is intact or disrupted in ASD. Details of the auditory stimuli, fNIRS parameters and study protocol are presented in Chapter 2.

Analysis of the neuroimaging data was carried out by grouping fNIRS measurement channels into four regions for analysis (left posterior, left anterior, right posterior, right anterior) by simple averaging (Keehn et al., 2013; see Figure 2.1). A mixed-design ANOVA was performed to determine if and where there was a significant hemodynamic response to the experimental speech-like stimuli in 3-month-olds, whether the magnitude and location of infants' hemodynamic responses were different across the two syllable conditions, whether these responses differed between infants at high and low risk for ASD, whether these

responses differed between males and females, and whether there were any interactions between these variables.

A second set of analyses investigated the effects of repeated exposure on neural responses to the speech-like stimuli. For these analyses, the hemodynamic response to the first four blocks of each condition and the last blocks of each condition were added to the above ANOVA as a fourth within-subjects factor. All indices of neural activity analyzed in Chapter 2 will be used as predictors in the current study's models.

Behavioral Measures. Subjects (or their parents) were administered additional batteries of behavioral measures at an 18-month follow-up visit to the lab. The behavioral measures that I propose to analyze herein, and which were collected at this follow-up visit are the Autism Diagnostic Observation Schedule (ADOS), Mullen Scales of Early Learning (MSEL), and MacArthur-Bates Communicative Development Inventories (MCDI). They are described in more detail below.

Variables

The variables obtained from the above methods, which I will include in the analyses proposed herein are:

Indices of Hemodynamic Activity at Regions of Interest (ROIs). Hemodynamic responses (in the form of average oxyhemoglobin concentration

([HbO]) changes[8]) over four regions of interest (left posterior, left anterior, right posterior, right anterior) were averaged over all blocks of each of the two trisyllabic sequence types (ABB or ABC blocks) in one set of analyses; they were also separated into those [HbO] responses to the first and last four blocks of each condition (ABB-first, ABB-last, ABC-first, ABC-last) in a second analysis. The indices of brain activity produced are the following continuous variables:

- *Overall [HbO] Change to ABB sequences at (1) Left Posterior, (2) Right Posterior, (3) Left Anterior, and (4) Right Anterior ROIs*
- *Overall [HbO] Change to ABC sequences at (1) Left Posterior, (2) Right Posterior, (3) Left Anterior, and (4) Right Anterior ROIs*
- *[HbO] Change to first four ABB sequences at (1) Left Posterior, (2) Right Posterior, (3) Left Anterior, and (4) Right Anterior ROIs*
- *[HbO] Change to first four ABC sequences at (1) Left Posterior, (2) Right Posterior, (3) Left Anterior, and (4) Right Anterior ROIs*
- *[HbO] Change to last four ABB sequences at (1) Left Posterior, (2) Right Posterior, (3) Left Anterior, and (4) Right Anterior ROIs*
- *[HbO] Change to last four ABC sequences at (1) Left Posterior, (2) Right Posterior, (3) Left Anterior, and (4) Right Anterior ROIs*

[8] Deoxyhemoglobin concentration ([HbR]) changes are also obtained by fNIRS imaging, but are not as well documented or understood in the infant literature as indices of neural activity (e.g. Devor et al., 2005; Watanabe and Kato, 2004; Kameyama et al., 2004), and have a lower signal-to-noise ratio than [HbO] (Tong and Frederick, 2010, as cited by Keehn et al., 2013). [HbR] indices were thus not included in the current analyses.

Autism Diagnostic Observation Schedule (ADOS). This is a semi-structured, play-based standardized assessment. The ADOS is considered the gold-standard instrument for diagnosing ASD, and for research and clinical evaluations of social functioning. It provides a categorical diagnosis (autism, autism spectrum disorder, or non-spectrum), as well as a standard severity score, which is designed to compare individuals across developmental stages and time (Gotham, et al., 2009). Since ADOS classifications (autism, autism spectrum, or non-spectrum) produced by this instrument are meant to be used as one of many factors contributing to a clinical diagnosis of ASD and hide significant heterogeneity within each category, I used each individual's 18-month ADOS severity scores—an integer between 1 and 10 that indicates the child's level of autistic symptoms relative to other children of the same age—as an ordinal measure of overall ASD symptomatology.

Mullen Scales of Early Learning (MSEL). This is a comprehensive, standardized assessment of cognitive and motor function for young children. It comprises five subscales: Gross Motor, Fine Motor, Visual Reception, Receptive Language and Expressive Language. Subjects in the ISP are administered the MSEL at their 6-, 12-, 18-, 24- and 36-month visits. For the analyses herein I used subjects' age-equivalence scores on the following subscales from the 18-month administration of this instrument, as continuous measures of language ability, in keeping with past literature (Akshoomoff, 2006; Weismer et al., 2010):

- *MSEL Receptive Language Subscale*. Includes items such as following commands and identifying body parts.
- *MSEL Expressive Language Subscale*. Includes tasks such as naming pictures and answering questions.

MacArthur-Bates Communicative Development Inventories (MCDI). These are parent report forms for assessing language and communication development in infants and young children. The MCDI: Words and Sentences from the 18-month study visit were used for these analyses. This measure was used in addition to the MSEL measures as it produces standardized scores for more finely separated measures of language ability than the MSEL. Subjects' age-equivalence scores on the following subscales of this questionnaire were used as standardized continuous measures of language ability:

- *MCDI Words Produced*
- *MCDI Early Gestures*
- *MCDI Late Gestures*
- *MCDI Phrases Understood*
- *MCDI Irregular Words*
- *MCDI Sentence Complexity*
- *MCDI 3 Longest Sentences*

Risk Status. This is a dichotomous variable describing children's risk of developing ASD; risk was based on the diagnostic status of the infant's older sibling. HRA infants have an older sibling with a confirmed diagnosis of ASD,

while LRC infants have an older sibling who does not have an ASD diagnosis or other known neurological disorder.

Gender. This is a dichotomous variable to distinguish boys and girls. Gender differences in language development are well documented (Gleason & Ely, 2002), so it is reasonable to expect that the neural correlates of early language abilities might look qualitatively or quantitatively different for males and females. Additionally, ASD is four times more prevalent in males than in females, and may look different between the two genders (Moriuchi et al., 2013).

Data Analysis

In six separate sets of multiple regression analyses, I investigated whether each set of four [HbO] change variables predicted (1) MSEL receptive language age-equivalent scores, (2) MSEL expressive language age-equivalent scores and (3) MCDI age-equivalent scores on each of the seven subscales (separately). I then conducted ordinal regression analyses to determine whether each set of four [HbO] change variables predicted participants' 18-month ADOS severity scores. Each of these analyses was repeated, introducing subjects' risk status and gender as covariates into the regressions, in order to determine the extent to which neural activity at 3-months predicts later language development and ASD symptomatology, controlling for participants' risk status and gender. Models initially contained all four neural markers simultaneously, but were modified and retested until the best fitting solutions for each outcome of interest were obtained.

All analyses were conducted in Stata v11.2 (StataCorp, 2009) using the REGRESS and OLOGIT functions along with the method of full maximum likelihood estimation.

Results

Neural Responses to Speech-like Stimuli at 3-Months

Table 3.1 shows the descriptive statistics on average [HbO] changes to all ABB syllable sequence blocks, all ABC syllable sequence blocks, the first four ABB blocks, the first four ABC blocks, the last four ABB blocks and the last four ABC blocks, of LRC and HRA 3 month olds. Details of intergroup differences in these measures and their implications are explored in Chapter 2.

Behavioral Outcomes at 18 Months

Table 3.2 shows the descriptive statistics on MSEL receptive age equivalent scores, MSEL expressive age equivalent scores and MCDI subscale age equivalent scores at 18 months old, for LRC and HRA subjects. The results of independent sample t-tests to examine the differences in average scores of the two groups of subjects are indicated in column 6, and the number of subjects who contributed data to each measure is indicated in columns 3 and 5. At 18 months, LRC subjects scored significantly higher than HRA subjects on the MSEL expressive language ($p = 0.0320$), CDI words produced ($p = 0.036$), and CDI early gestures ($p = 0.007$) scales, and showed a trend toward lower ADOS severity scores than HRA subjects ($p = 0.0606$).

Table 3.1. Means (and standard deviations) of 3-Month-Olds' neural activity by stimulus and analysis types, measurement regions of interest, and subjects' risk statuses.

Measure	Region of Interest	LRC (n=17)	HRA (n=20)
[HbO] to all ABB blocks	Left Anterior	0.059 (0.361)	0.256 (0.310)
	Left Posterior	0.049 (0.352)	0.078 (0.219)
	Right Anterior	0.023 (0.222)	0.116 (0.257)
	Right Posterior	-0.100 (0.368)	0.044 (0.323)
[HbO] to all ABC blocks	Left Anterior	0.192 (0.296)	0.167 (0.390)
	Left Posterior	-0.00264 (0.266)	0.0128 (0.341)
	Right Anterior	0.0822 (0.278)	0.140 (0.285)
	Right Posterior	0.0687 (0.357)	0.0517 (0.249)
[HbO] to first 4 ABB blocks	Left Anterior	0.188 (0.597)	0.230 (0.580)
	Left Posterior	0.157 (0.557)	0.180 (0.424)
	Right Anterior	0.179 (0.419)	0.248 (0.476)
	Right Posterior	0.016 (0.574)	0.035 (0.668)
[HbO] to first 4 ABB blocks	Left Anterior	0.221 (0.428)	0.210 (0.726)
	Left Posterior	-0.051 (0.546)	0.046 (0.620)
	Right Anterior	0.133 (0.420)	0.348 (0.772)
	Right Posterior	0.075 (0.588)	0.022 (0.602)
[HbO] to last 4 ABB blocks	Left Anterior	-0.020 (0.416)	0.243 (0.280)
	Left Posterior	-0.120 (0.593)	0.027 (0.496)
	Right Anterior	-0.191 (0.507)	0.103 (0.386)
	Right Posterior	-0.331 (0.634)	0.344 (0.449)
[HbO] to last 4 ABC blocks	Left Anterior	0.261 (0.385)	0.210 (0.468)
	Left Posterior	-0.053 (0.555)	0.001 (0.539)
	Right Anterior	0.110 (0.403)	0.179 (0.365)
	Right Posterior	0.075 (0.617)	0.101 (0.390)

Table 3.2. Means (and standard deviations) of low and high risk 18-month-olds' language and autism symptom scores. MSEL = Mullen Scales of Early Learning; ADOS = Autism Diagnostic Observation Schedule; MCDI = MacArthur Communicative Development Inventory.

Measure	LRC	n(LRC)	HRA	n(HRA)	P Value (LRC vs HRA)
MSEL Expressive	19.2 (2.66)	17	16.1 (5.14)	16	0.(
MSEL Receptive	20.2 (6.00)	17	18 (5.53)	16	0.1
ADOS Severity Score	1.2 (0.376)	13	2 (1.51)	15	0.(
CDI Words Produced	15.8^{-+} (9.52)	5	2.25^{-} (4.50)	4	0.(
CDI Early Gestures	19.1^{+} (4.18)	10	15.14 (2.35)	14	0.(
CDI Late Gestures	18.5^{+} (4.58)	10	16.93^{+} (4.84)	14	0.4
CDI Phrases Understood	15.33^{+} (12.04)	6	7.44^{+} (11.28)	9	0.1
CDI Irregular Words	21.3 (2.87)	10	20.3^{-+} (7.01)	12	0.(
CDI Sentence Complexity	22.25^{+} (2.92)	8	22.8^{+} (1.72)	9	0.(
CDI 3 Longest Sentences	22.0^{+} (2.16)	4	17.3^{-} (7.72)	7	0.1

$^{-}$ Denotes subdomains of the CDI for which floor scores (coded as 0) contributed to the means and standard deviations.

$^{+}$ Denotes subdomains of the CDI for which floor scores (coded as 0) contributed to the means and standard deviations.

Predicting 18-month Developmental Outcomes from Neural Activity at 3 Months

Overall [HbO] change to ABB sequences. Table 3.3 presents the final fitted multiple regression models for the prediction of 18-month MSEL Receptive Language age equivalent scores and MCDI Sentence Complexity age equivalent scores by 3-month overall [HbO] responses to ABB sequences, controlling for subjects' risk status and gender. All prediction models for MSEL Expressive Language scores, ADOS Severity Scores and the remaining MCDI subdomains showed poor overall fit to the data, indicating that 3-month [HbO] changes to ABB sequences may not be significant predictors of these 18-month outcomes.

18-Month MSEL Receptive Language. There are significant main effects of [HbO] changes to the left ($p = 0.003$) and right anterior regions ($p < 0.001$) at 3 months old on subjects' 18-month MSEL receptive language scores, indicating that [HbO] to the left anterior region is positively associated with 18-month receptive language scores, while [HbO] to the right anterior region is negatively associated with 18-month MSEL scores.

However, the interaction terms LAxGENDER, RAxGENDER, LAxRISK and RAxRISK indicate that the aforementioned relationships between 3-month neural signals and 18-month receptive language scores at these two regions differ based on subjects' genders and risk statuses. Specifically, in the left anterior measurement region, the relationship between 3-month [HbO] changes and 18-

month MSEL receptive language scores is larger (more positive) by 22.30 units for HRA than for LRC subjects ($p = 0.004$), controlling for all other variables. Additionally, in the same measurement region, the relationship between 3-month [HbO] changes and 18-month MSEL receptive language scores is larger (more positive) by 19.85 units for female than for male subjects ($p = 0.007$). These results indicate that larger [HbO] changes to the left anterior region over all ABB blocks are associated with higher MSEL receptive language scores in males than in females, and in HRA than in LRC subjects. In fact, post-hoc general linear hypothesis (GLH) tests confirm that, based on this model and controlling for all other variables, [HbO] changes to the left anterior region over all ABB blocks are significant predictors of 18-month MSEL receptive language scores for HRA males ($p = 0.003$), HRA females ($p = 0.003$), and LRC males ($p = 0.001$), but not LRC females ($p = 0.832$).

In the right anterior region, the relationship between 3-month [HbO] changes and 18-month MSEL receptive language scores is larger (more negative) by 36.27 units for HRA than for LRC subjects ($p = 0.003$), and in the same measurement region, the relationship between 3-month [HbO] changes and 18-month MSEL receptive language scores is larger (more negative) by 40.23 units for male than for female subjects ($p = 0.001$). In other words, higher [HbO] responses to ABB stimuli blocks in the right anterior region are associated with lower MSEL receptive language scores in males than in females, and in HRA than in LRC subjects. Again, post-hoc GLH tests confirm [HbO] to the right anterior

region over all ABB blocks as statistically significant predictors of 18-month MSEL receptive language scores in HRA males ($p = 0.030$) and females ($p = 0.016$) and LRC males ($p < 0.001$), but not LRC females ($p = 0.200$).

Figure 3.1 displays the fitted relationships between 3-month overall [HbO] and 18-month MSEL receptive language scores to the left and right anterior regions in the study sample, broken down by gender and ASD risk status. The 3-month neural predictors (and covariates) in this model explain 59.8% of the variance in 18-month MSEL receptive language age equivalent scores.

18-Month MCDI Sentence Complexity. There are significant main effects of [HbO] changes to the left anterior ($p = 0.043$) and left posterior regions ($p = 0.003$) at 3 months old on subjects' 18-month MCDI sentence complexity scores, indicating that [HbO] to the left anterior region is positively associated with 18-month sentence complexity, while [HbO] to the left posterior region is negatively associated with 18-month sentence complexity.

However, the interaction terms between 3-month [HbO] changes to the left anterior and posterior regions in response to ABB stimuli, and gender, (LAxGENDER, LPxGENDER) indicate that the aforementioned relationships between 3-month neural signals and 18-month sentence complexity scores differ for males and females ($p = 0.006$, $p = 0.003$, respectively). Specifically, post-hoc tests confirm that in the left anterior measurement region, the relationship between 3-month [HbO] changes and 18-month sentence complexity scores is positive for males ($p = 0.043$), but negative for females ($p = 0.019$). In the left posterior

region, post-hoc tests confirm that the relationship between 3-month [HbO] changes and 18-month sentence complexity scores is negative for males (p = 0.003), but not statistically significant for female subjects (p = 0.174). Figure 3.2 displays the fitted relationships between 3-month overall [HbO] and 18-month MCDI sentence complexity scores to the left anterior and posterior regions, by gender. The 3-month neural predictors (and covariates) in this model explain 70.1% of the variance in 18-month MCDI sentence complexity age equivalent scores.

Table 3.3. Fitted multiple regression models in which 3 month olds' neural responses over all repetition (ABB) sequence blocks predict MSEL receptive language and MCDI sentence complexity age equivalent scores at 18 months of age. N(LRC+HRA) = 37. MSEL = Mullen Scales of Early Learning; MCDI = Macarthur Communicative Development Inventory; LA = left anterior region; LP = left posterior region; RA = right anterior region; W-statistic = Shapiro-Wilks test for residual normality.

Parameter	MSEL Receptive	MCDI Sentence Complexity
Intercept	21.91*** (1.269)	22.62*** (0.590)
LA	20.91* (6.281)	4.940* (2.159)
LP		-11.41** (2.947)
RA	-50.52 (10.39)	
Gender	-2.475 (1.708)	1.296 (0.859)
Risk Status	0.5666 (1.833)	
LAxGender	-19.85** (6.655)	-8.685** (2.554)
LPxGender		15.04** (3.864)
RAxGender	40.23** (10.61)	
LAxRisk	22.30** (6.881)	
RAxRisk	-36.27** (10.85)	
R^2	0.5982	0.7011
W-Statistic	0.962	0.971
F-statistic	4.28**	5.16*

*$p<0.05$, **$p<0.01$, ***$p<0.001$

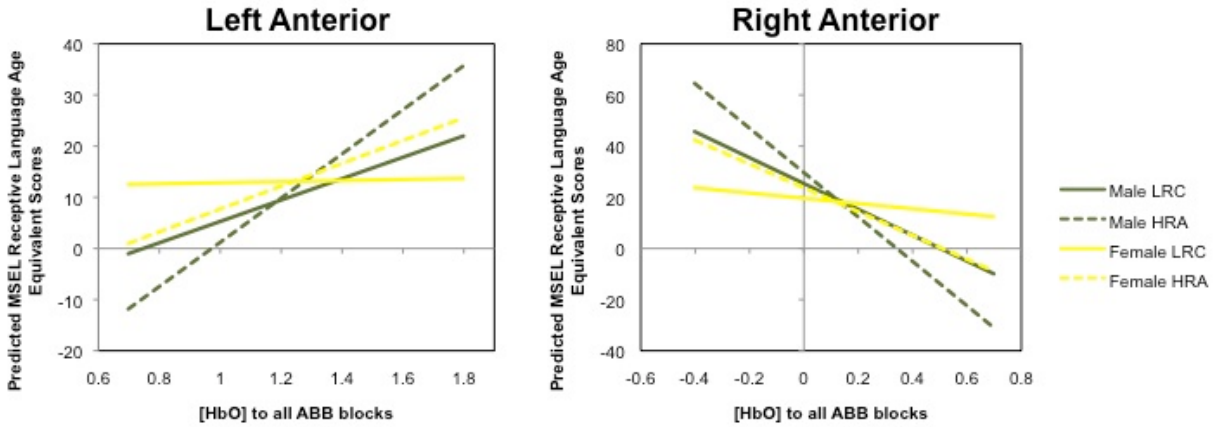

Figure 3.1. *Fitted regression lines for the prediction of 18-month Mullen Scales of Early Learning receptive language age equivalent scores by 3-month [HbO] over all repetition blocks in the left and right anterior regions, broken down by subjects' gender and ASD risk status. LEFT ANTERIOR: [HbO] changes are significant predictors of 18-month MSEL receptive language scores for HRA males ($p = 0.003$), HRA females ($p = 0.003$), and LRC males ($p = 0.001$), but not LRC females ($p = 0.832$). RIGHT ANTERIOR: [HbO] is a significant predictor of 18-month MSEL receptive language scores in HRA males ($p = 0.030$) and females ($p = 0.016$) and LRC males ($p < 0.001$), but not LRC females ($p = 0.200$). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

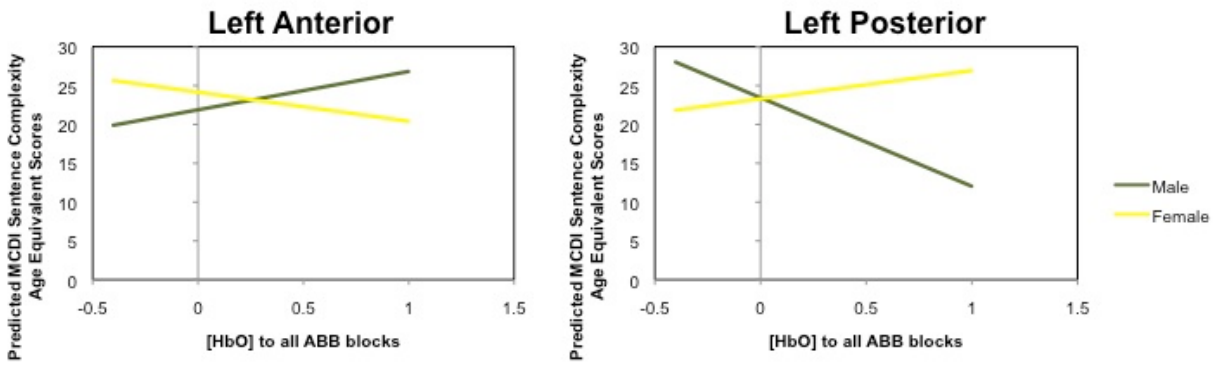

Figure 3.2. *Fitted regression lines for the prediction of 18-month MCDI sentence complexity age equivalent scores by 3-month [HbO] over all repetition blocks in the left anterior and posterior regions, broken down by gender. LEFT ANTERIOR: the association between 3-month [HbO] changes and 18-month sentence complexity scores is positive for males (p = 0.043), and negative for females (p = 0.019). LEFT POSTERIOR: the association between 3-month [HbO] changes and 18-month sentence complexity scores is negative for males (p = 0.003), but not significant for females (p = 0.174). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

Overall [HbO] change to ABC sequences. Table 3.4 presents the final fitted multiple regression model[9] for the prediction of 18-month ADOS severity scores by 3-month overall [HbO] responses to ABC sequences, controlling for subjects' risk status and gender. Prediction models for MSEL Expressive and Receptive Language scores, and all MCDI subdomains showed poor overall fit to the data, indicating that 3-month [HbO] changes to ABC sequences may not be significant predictors of these 18-month outcomes.

[9] Ordinal logistic regression models for this predictor and outcome combination did not converge, so multiple regression analysis was performed.

18-Month ADOS Severity Scores. There are significant main effects of [HbO] changes to the left anterior ($p = 0.044$) and posterior regions ($p = 0.010$) at 3 months old on subjects' 18-month ADOS severity scores, indicating that [HbO] to ABC sequences in the left anterior and posterior regions is associated with 18-month autism symptomatology. Specifically, a 1mmol*mm change in [HbO] to the left anterior region at 3-months of age is associated with a 2.45 point change in ADOS severity scores at 18 months of age, such that infants with higher [HbO] to the left anterior region are predicted to have lower 18-month ADOS severity scores (that is, lower ASD symptomatology). The interaction term LPxGENDER indicates that the relationship between 3-month neural signals and 18-month ADOS severity scores in the left posterior region differs based on subjects' gender, however. Specifically, in the left posterior measurement region, the relationship between 3-month [HbO] changes and 18-month ADOS severity scores is larger (more negative) by 2.80 units for male than for female subjects ($p = 0.038$), such that higher [HbO] in this region is associated with lower ADOS severity scores for males ($p = 0.010$), while post-hoc GLH tests confirm that the relationship between [HbO] and ADOS severity scores in this region is not statistically significant for females ($p = 0.782$). Figure 3.3 displays the fitted relationships between 3-month overall [HbO] to ABC sequences and 18-month ADOS severity scores to the left posterior region for males and females in the study sample. This model explains 42.7% of the variance in 18-month ADOS severity scores.

Table 3.4. Fitted multiple regression model in which 3 month olds' neural responses over all non-repetition (ABC) sequence blocks predicts ADOS severity scores at 18 months of age. N(LRC+HRA) = 37. ADOS = Autism Diagnostic Observation Schedule; W-statistic = Shapiro-Wilks test for residual normality.

Parameter	ADOS Severity Scores
Intercept	2.116*** (0.308)
LA	-2.447* (1.139)
LP	-2.551* (0.905)
Gender	-0.750 (0.467)
LAxGender	2.373 (1.350)
LPxGender	2.799* (1.264)
R^2	0.4273
W-Statistic	0.925
F-statistic	3.13*

*$p<0.05$, **$p<0.01$, ***$p<0.001$

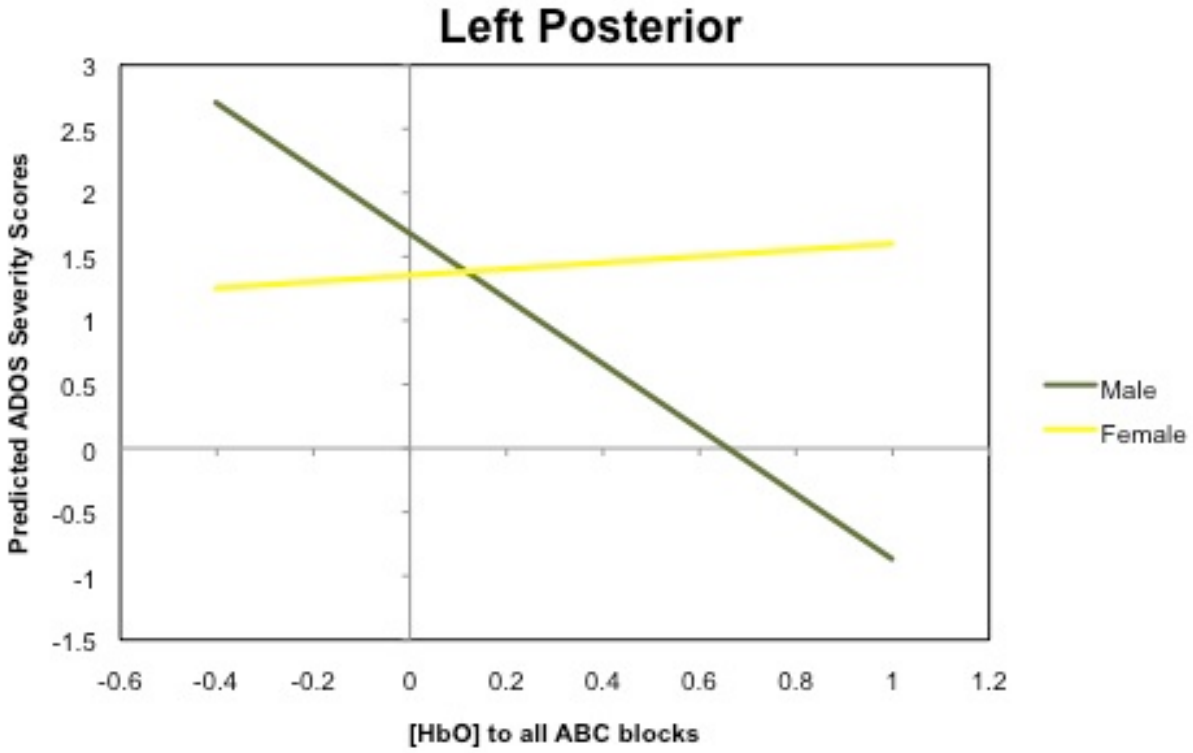

Figure 3.3. *Fitted regression lines for the prediction of 18-month ADOS severity scores by 3-month [HbO] over all non-repetition (ABC) blocks in the left posterior region, broken down by gender. [HbO] is associated with lower ADOS severity scores for males ($p = 0.010$), but the association between [HbO] and ADOS severity scores is not significant for females ($p = 0.782$). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

[HbO] changes to first four ABB sequences. Table 3.5 presents the final fitted multiple regression model for the prediction of 18-month MCDI 3 longest sentence age equivalent scores by [HbO] to the first four ABB sequence blocks. Prediction models for MSEL Expressive and Receptive Language scores, ADOS severity scores, and all other MCDI subdomains showed poor overall fit to the data, indicating that 3-month [HbO] changes to the first four ABB sequences may not be significant predictors of these 18-month outcomes.

18-Month MCDI Early Gestures. There are significant main effects of [HbO] changes to the left and right anterior regions ($p = 0.004$, $p = 0.019$, respectively) at 3 months old, on subjects' 18-month MCDI 3 longest sentences scores, indicating that [HbO] to the first four ABB sequences is associated with this 18-month language outcome. However, there are statistically significant interactions between [HbO] to the left and right anterior regions and left posterior region and risk status, indicating that the relationship between 3-month neural signals and longest sentence scores in these three regions differs for LRC and HRA subjects. Specifically, post-hoc GLH tests confirm that higher [HbO] to the left anterior region is a statistically significant predictor of higher MCDI early gesture scores in LRC subjects ($p = 0.004$), while [HbO] to the right anterior region is a statistically significant negative predictor of MCDI early gesture scores in LRC subjects ($p = 0.019$). [HbO] to the first four ABB blocks in this region does not significantly predict HRA MCDI early gesture scores (LA: $p = 0.580$,

RA: $p = 0.828$). In the left posterior region, although the prediction of MCDI early gesture scores is predicted by [HbO] to the first four ABB blocks in a statistically significantly different manner for HRA and LRC subjects, post-hoc GLH tests confirm that the prediction of HRA and LRC early gesture scores by [HbO] is not statistically significant for either group in this region (HRA: $p = 0.178$, LRC: $p = 0.101$). Figure 3.4 displays the fitted relationships between 3-month [HbO] to the first four ABB sequences and 18-month MCDI early gesture scores in the left anterior, left posterior, and right anterior regions. The 3-month neural predictors (and covariates) in this model explain 61.5% of the variance in 18-month MCDI 3 early gesture age equivalent scores.

18-Month MCDI 3 Longest Sentences. There are significant main effects of [HbO] changes to the left anterior, left posterior, and right anterior regions ($p < 0.001$, $p < 0.001$, $p = 0.001$ respectively) at 3 months old, on subjects' 18-month MCDI 3 longest sentences scores, indicating that [HbO] to the first four ABB sequences is associated with this 18-month language outcome. There is also a statistically significant main effect of gender on this 18-month language measure ($p = 0.002$). However, there are statistically significant interactions between [HbO] to the left and right anterior regions and left posterior region and gender, indicating that the relationship between 3-month neural signals and longest sentence scores in these three regions differs, based on subjects' genders. Specifically, the relationship between 3-month [HbO] changes and 18-month 3 longest sentence scores are larger (more positive) by 13.49, 32.83, and 11.75 units

in the left anterior, left posterior and right anterior regions respectively for male than for female subjects. Post-hoc GLH tests confirm that higher [HbO] in these regions is a statistically significant predictor of higher MCDI longest sentence scores in males ($p < 0.001$ for all), and in the left posterior region, for females ($p = 0.031$), but that [HbO] to the left and right anterior regions is not a statistically significant predictor of this 18-month outcome in females ($p = 0.117$, $p = 0.634$, respectively). Figure 3.5 displays the fitted relationships between 3-month [HbO] to the first four ABB sequences and 18-month MCDI 3 longest sentence scores in the left anterior, left posterior, and right anterior regions. The 3-month neural predictors (and covariates) in this model explain 99.8% of the variance in 18-month MCDI 3 longest sentence age equivalent scores.

Table 3.5. Fitted multiple regression models in which 3 month olds' neural responses to the first four repetition (ABB) sequence blocks predicts MCDI early gesture and 3 longest sentence age equivalent scores at 18 months of age. N(LRC+HRA) = 37. LA = left anterior region; LP = left posterior region; RA = right anterior region; W-statistic = Shapiro-Wilks test for residual normality.

Parameter	Early Gestures	3 Longest Sentences
Intercept	17.32*** (1.185)	13.66*** (0.401)
LA	9.65** (0.004)	12.38*** (0.568)
LP	-3.27 (1.873)	35.28*** (1.997)
RA	-8.50* (3.23)	12.29** (0.802)
Gender		6.18** (0.649)
Risk	-2.16 (1.527)	
LAxRisk	-10.46** (3.222)	
LPxRisk	5.57* (2.479)	
RAxRisk	8.07* (3.767)	
LAxGender		-13.49*** (0.763)
LPxGender		-32.83** (2.096)
RAxGender		-11.75** (1.300)
R^2	0.6149	0.9978
W-Statistic	0.937	0.869
F-statistic	3.42*	193.54***

*p<0.05, **p<0.01, ***p<0.001

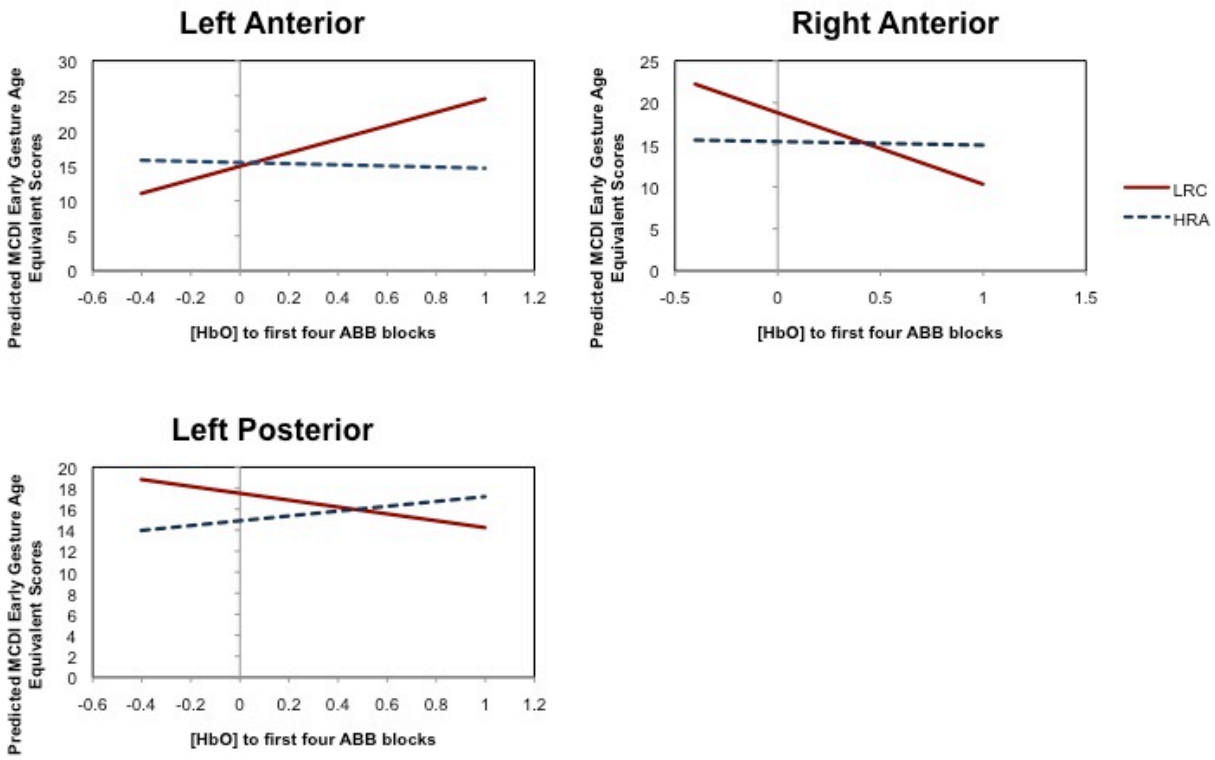

Figure 3.4. Fitted regression lines for the prediction of 18-month MCDI early gesture age equivalent scores by 3-month [HbO] over the first four repetition (ABB) blocks in the left anterior, right anterior and left posterior regions, broken down by risk status. LEFT ANTERIOR: Higher [HbO] is a statistically significant predictor of higher MCDI early gesture scores in LRC subjects (p = 0.004), but not HRA (p = 0.580). RIGHT ANTERIOR: [HbO] is a statistically significant negative predictor of MCDI early gesture scores in LRC subjects (p = 0.019), but not HRA (p = 0.828). LEFT POSTERIOR: the prediction of HRA and LRC early gesture scores by [HbO] is not statistically significant for either group in this region (HRA: p = 0.178, LRC: p = 0.101). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.

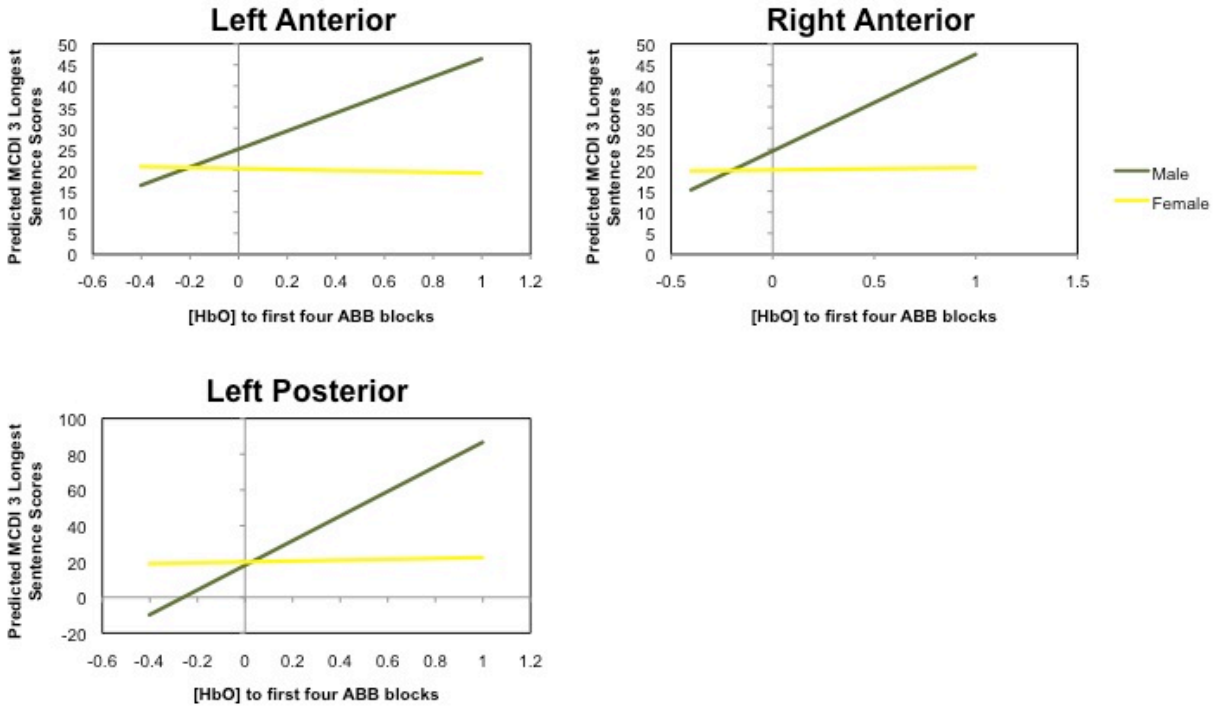

Figure 3.5. Fitted regression lines for the prediction of 18-month MCDI 3 longest sentence age equivalent scores by 3-month [HbO] over the first four repetition (ABB) blocks in the left anterior, right anterior and left posterior regions, broken down by gender. Higher [HbO] in these regions is a statistically significant predictor of higher MCDI longest sentence scores in males ($p < 0.001$ for all), and in the left posterior region, for females ($p = 0.031$); [HbO] to the left and right anterior regions is not a statistically significant predictor of 3 longest sentences in females ($p = 0.117$, $p = 0.634$, respectively). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.

[HbO] Change to first four ABC sequences. Table 3.6 presents the final fitted multiple regression model for the prediction of 18-month MCDI 3 longest sentence age equivalent scores by [HbO] to the first four ABC sequences. Prediction models for MSEL Expressive and Receptive Language scores, ADOS severity scores, and all other MCDI subdomains showed poor overall fit to the data, indicating that 3-month [HbO] changes to the first four ABC sequences may not be significant predictors of these 18-month outcomes.

18-Month MCDI Early Gestures. There are significant main effects of [HbO] changes to the right anterior ($p = 0.011$) and right posterior regions ($p = 0.040$) at 3 months old, on subjects' 18-month MCDI early gesture scores, indicating that [HbO] to the first four ABC sequences is associated with this 18-month language outcome. There is also a statistically significant main effect of ASD risk status on this 18-month language measure ($p = 0.001$). The main effect of [HbO] to the right posterior region indicates that a 1mmol*mm change in [HbO] to the this region at 3-months of age is associated with a 3.12 point change in MCDI early gesture scores at 18 months of age, such that infants with higher [HbO] to the right posterior region are predicted to have higher MCDI scores on this domain. There are statistically significant interactions between [HbO] to the right anterior region and risk status however; this indicates that the relationship between 3-month neural signals and early gesture scores in the right anterior region differs for HRA and LRC subjects. Specifically, post-hoc GLH tests

confirm that in the right anterior measurement region, the relationship between 3-month [HbO] changes and 18-month early gesture scores is statistically significantly negative for LRC subjects, such that higher [HbO] in these regions is associated with lower MCDI early gesture scores in LRC subjects ($p = 0.011$); however, that this relationship is not statistically significant for HRA subjects in the right anterior region ($p = 0.335$)—their MCDI early gesture scores are not predicted by [HbO] to right anterior region in response to the first four ABC blocks. Figure 3.6 displays the fitted relationships between 3-month [HbO] to the first four ABC sequences and 18-month MCDI early gesture scores in the right anterior region. The 3-month neural predictors (and covariates) in this model explain 56.3% of the variance in 18-month MCDI early gesture age equivalent scores.

18-Month MCDI 3 Longest Sentences. There are significant main effects of [HbO] changes to the left posterior ($p = 0.043$), right anterior ($p < 0.001$), and right posterior regions ($p = 0.001$) at 3 months old, on subjects' 18-month MCDI 3 longest sentences scores, indicating that [HbO] to the first four ABC sequences is associated with this 18-month language outcome. There is also a statistically significant main effect of gender on this 18-month language measure ($p = 0.002$). The main effect of [HbO] to the left posterior region indicates that a 1mmol*mm change in [HbO] to this region at 3-months of age is associated with a 6.47 point change in MCDI 3 longest sentence scores at 18 months of age, such that infants with higher [HbO] to the left posterior region are predicted to have higher MCDI

scores on this domain. There are statistically significant interactions between [HbO] to the right anterior and posterior regions and gender however; the interaction terms RAxGENDER and RPxGENDER indicate that the relationship between 3-month neural signals and longest sentence scores in the right hemisphere differs, based on subjects' genders. Specifically, in the right anterior and posterior measurement regions, the relationship between 3-month [HbO] changes and 18-month 3 longest sentence scores is larger (more positive) by 36.57 and 32.09 units respectively for male than for female subjects, such that higher [HbO] in these regions is associated with higher MCDI longest sentence scores in males ($p < 0.001$, $p = 0.001$ respectively); post-hoc GLH tests confirm however, that this relationship is not statistically significant for females in either region ($p = 0.963$, $p = 0.860$ respectively). Figure 3.7 displays the fitted relationships between 3-month [HbO] to the first four ABC sequences and 18-month MCDI 3 longest sentence scores in the right anterior and posterior regions, by gender. The 3-month neural predictors (and covariates) in this model explain 99.6% of the variance in 18-month MCDI 3 longest sentence age equivalent scores. However, the Shapiro-Wilk statistic for residual normality is statistically significant (see Table 3.6), indicating that this model's residuals are not normally distributed; this model thus violates the assumptions of multiple regression, rendering its estimates invalid for statistical inference.

Table 3.6. Fitted multiple regression models in which 3 month olds' neural responses to the first four non-repetition (ABC) sequence blocks predicts MCDI early gestures and 3 longest sentence age equivalent scores at 18 months of age. N(LRC+HRA) = 37. LP = left posterior region; RA = right anterior region; RP = right posterior region; W-statistic = Shapiro-Wilks test for residual normality.

Parameter	Early Gestures	3 Longest Sentences
Intercept	19.53*** (0.892)	35.23*** (1.287)
LP		6.47* (1.919)
RA	-6.28* (2.202)	36.52*** (1.821)
RP	3.12* (1.403)	31.83** (1.996)
Risk	-4.80** (1.233)	
Gender		-15.07** (1.401)
RAxRisk	8.12* (2.877)	
RPxRisk	-4.18 (2.246)	
LPxGender		-5.016 (2.293)
RAxGender		-36.57*** (2.053)
RPxGender		-32.09** (2.408)
R^2	0.5628	0.9965
W-Statistic	0.964	0.758**
F-statistic	4.63**	123.56**

*$p<0.05$, **$p<0.01$, ***$p<0.001$

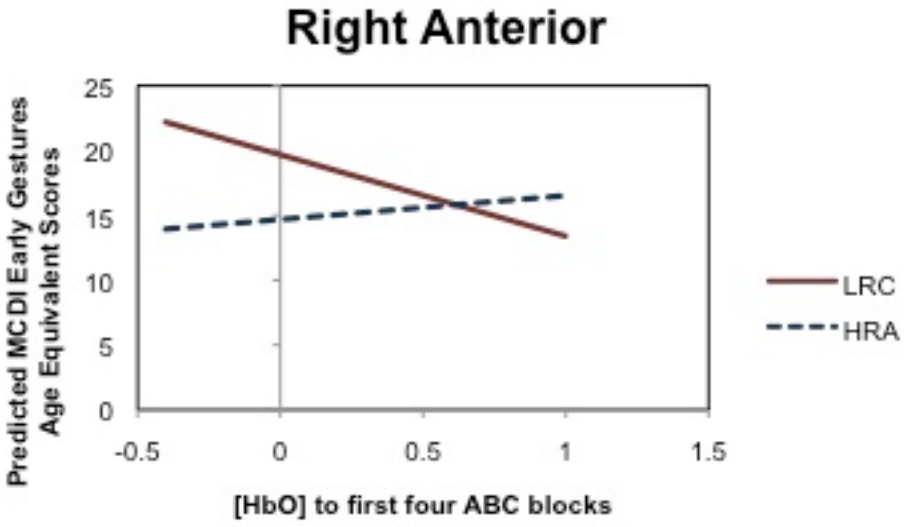

Figure 3.6. *Fitted regression lines for the prediction of 18-month MCDI early gesture age equivalent scores by 3-month [HbO] over the first four non-repetition (ABC) blocks in right anterior region, broken down by risk status. In LRC subjects, higher [HbO] is associated with lower MCDI early gesture scores (p = 0.011); this relationship is not statistically significant for HRA subjects (p = 0.335). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

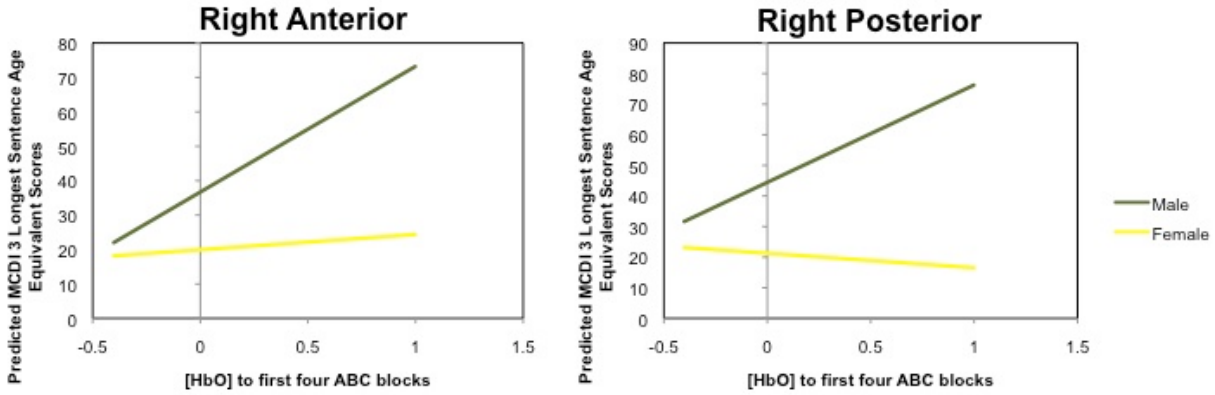

Figure 3.7. *Fitted regression lines for the prediction of 18-month MCDI 3 longest sentence age equivalent scores by 3-month [HbO] over the first four non-repetition (ABC) blocks in right anterior and posterior regions, broken down by gender. Higher [HbO] is associated with higher MCDI longest sentence scores in males (RA: p < 0.001, RP: p = 0.001); this relationship is not statistically significant for females (RA: p = 0.963, RP: p = 0.860). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

[HbO] Change to last four ABB sequences. Table 3.7 presents the final fitted multiple regression model for the prediction of 18-month MSEL Expressive language, MCDI sentence complexity and MCDI 3 longest sentence age equivalent scores by 3-month [HbO] to the last 4 ABB sequence blocks. Prediction models for MSEL Receptive Language scores, ADOS severity scores, and all other MCDI subdomains showed poor fit to the data, indicating that 3-month [HbO] changes to the last four ABB sequences may not be significant predictors of these 18-month outcomes.

***18-Month MSEL Expressive Language Scores*.** This regression reveals a main effect of [HbO] changes to the right posterior region ($p = 0.001$). Additionally, statistically significant interactions exist between [HbO] changes to the left and right posterior regions and gender, indicating that [HbO] to the last four ABB sequences is associated with MSEL expressive language scores at 18 months, but that this relationship is different for males and females. In particular, the relationship between [HbO] to the left posterior region and expressive language is larger (more positive) by 15.14 units for males than females, while the relationship between [HbO] to the right posterior region and expressive language is larger (more positive) by 14.4 units for females than males. Post-hoc GLH tests confirm that in the left posterior region, increases in [HbO] to the last four ABB stimuli are statistically significantly and negatively associated with expressive language at 18 month for females ($p = 0.041$), but are not statistically significant

predictors of 18-month expressive language scores in males ($p = 0.085$). In contrast, in the right posterior region, increases in [HbO] to the last four ABB stimuli are statistically significantly and positively associated with expressive language at 18 months for females ($p = 0.045$), but statistically significantly and negatively associated with expressive language at 18 month for males ($p = 0.001$). Figure 3.6 displays the fitted relationships between 3-month [HbO] to the last four ABB sequences and 18-month MSEL expressive language scores in the left and right posterior regions. This model predicts 50.5% of the variance in 18-month MSEL expressive language age equivalent scores.

18-Month MCDI Early Gestures. There are significant main effects of [HbO] changes to the left posterior ($p = 0.012$), right anterior ($p = 0.005$), and right posterior regions ($p = 0.001$) at 3 months old, on subjects' 18-month MCDI early gesture scores, indicating that [HbO] to the last four ABB sequences is associated with this 18-month language outcome. There is also a statistically significant main effect of gender on this 18-month language measure ($p = 0.021$). The main effect of [HbO] to the right anterior region indicates that a 1mmol*mm change in [HbO] to the this region at 3-months of age is associated with a 5.59 point change in MCDI early gesture scores at 18 months of age, such that infants with higher [HbO] to the right anterior region are predicted to have higher MCDI scores on this domain. There are statistically significant interactions between [HbO] to the left and right posterior regions and gender however; this indicates that the relationship between 3-month neural signals and early gesture scores in

the left and right poster regions differs, by subjects' gender. Specifically, post-hoc GLH tests confirm that in the left posterior measurement region, the relationship between 3-month [HbO] changes and 18-month early gesture scores is statistically significant and positive for males ($p = 0.012$), such that higher [HbO] in these regions is associated with higher MCDI early gesture scores, but negative for females, such that higher [HbO] in these regions is associated with lower MCDI early gesture scores ($p = 0.012$). In the right posterior region, this relationship is reversed for males—higher [HbO] to this region is associated with lower MCDI early gesture scores ($p = 0.005$), and is not statistically significant for females ($p = 0.209$). Figure 3.6 displays the fitted relationships between 3-month [HbO] to the last four ABB sequences and 18-month MCDI early gesture scores in the left and right posterior regions. The 3-month neural predictors (and covariates) in this model explain 70.1% of the variance in 18-month MCDI early gesture age equivalent scores.

MCDI Sentence Complexity. The final model for the prediction of 18-month MCDI sentence complexity scores by 3-month neural responses to the last four ABB stimuli reveals a main effect of [HbO] to the left anterior region, such that [HbO] changes of 1mmol*mm in this region are negatively associated with 4.79 unit changes on 18-month sentence complexity scores, controlling for the [HbO] response in the left posterior region ($p = 0.005$). In contrast, in the left posterior region, [HbO] changes of 1mmol*mm to the last four ABB stimulus blocks are positively associated with changes of 3.54 units in 18-month sentence

complexity scores ($p = 0.004$). This model predicts 50.0% of the variance in 18-month MCDI sentence complexity age equivalent scores.

MCDI 3 Longest Sentences. There are significant main effects of [HbO] changes to the left and right anterior regions ($p < 0.001$ for both) at 3 months old, on subjects' 18-month MCDI 3 longest sentences scores, indicating that [HbO] to the last four ABB sequences is associated with this 18-month language outcome. There is also a statistically significant main effect of gender on this 18-month language measure ($p < 0.001$). However, there are statistically significant interactions between [HbO] to each of the aforementioned regions, and gender ($p < 0.001$ for both), indicating that the relationships between 3-month neural signals and longest sentence scores in the left and right anterior regions differ for males and females. Specifically, post-hoc GLH tests confirm that while MCDI 3 longest sentence scores are positively associated with larger [HbO] changes to the left anterior region in males ($p<0.001$), there is no statistically significant relationship between 3-month [HbO] changes and 18-month 3 longest sentence scores in females ($p = 0.363$). In the right anterior region the relationship between [HbO] changes to the last four ABB stimuli blocks and 18-month 3 longest sentence scores is also not statistically significant for females ($p = 0.782$), while males' 3-month [HbO] changes to the last four ABB stimuli blocks in this region are negatively associated with 18-month MCDI longest sentence scores ($p < 0.001$). Figure 3.7 displays the fitted relationships between 3-month [HbO] to the last four ABB sequences and 18-month MCDI 3 longest sentence scores in the left and

right anterior regions, by subjects' gender. This model predicts 98.4% of the variance in 18-month MCDI 3 longest sentence age equivalent scores.

Table 3.7. Fitted multiple regression models in which 3 month olds' neural responses to the last four repetition (ABB) sequence blocks predict MSEL expressive language age equivalent scores, MCDI early gesture, sentence complexity and 3 longest sentence age equivalent scores at 18 months of age. N(LRC+HRA) = 37. MSEL = Mullen Scales of Early Learning; MCDI – MacArthur Communicative Development Inventory; LA = left anterior region; LP = left posterior region; RA = right anterior region; RP = right posterior region; W-statistic = Shapiro-Wilks test for residual normality.

Parameter	MSEL Expressive Language	MCDI Early Gestures	MCDI Sentence Complexity	MCDI 3 Longest Sentences
Intercept	16.14*** (0.897)	17.49*** (0.725)	23.68*** (0.525)	-15.91*** (1.992)
LA			-4.793** (1.422)	87.27*** (6.020)
LP	4.199 (2.330)	5.08* (1.782)	3.545** (1.033)	
RA		5.59** (1.725)		-97.18*** (6.094)
RP	-6.030** (1.593)	-7.07** (1.718)		
Gender	0.841 (1.687)	-3.964* (1.549)		36.65*** (2.136)
LAxGender				-89.87*** (6.557)
LPxGender	-15.14* (5.558)	-18.56** (5.081)		
RAxGender		-1.07 (3.948)		97.94*** (6.626)
RPxGender	14.40** (4.264)	10.98** (3.447)		
R^2	0.5052	0.7005	0.4999	0.9843
W-statistic	0.958	0.954	0.959	0.972
F-statistic	4.70**	5.35**	7.00**	62.79***

*p<0.05, **p<0.01, ***p<0.001

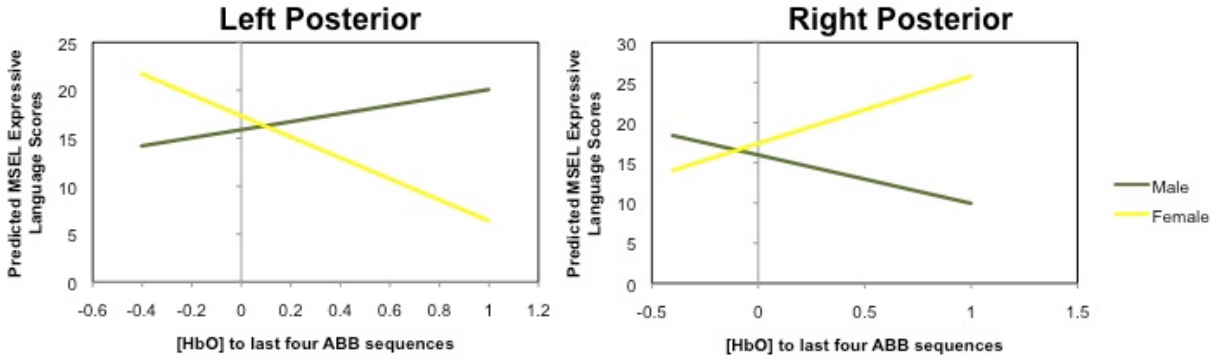

Figure 3.8. *Fitted regression lines for the prediction of 18-month MSEL expressive language age equivalent scores by 3-month [HbO] over the last four repetition (ABB) blocks in left and right posterior regions, broken down by gender. LEFT POSTERIOR: [HbO] is statistically significantly and negatively associated with expressive language at 18 month for females (p = 0.041), but not males (p = 0.085). RIGHT POSTERIOR: [HbO] is statistically significantly and positively associated with expressive language at 18 months for females (p = 0.045), but statistically significantly and negatively associated with expressive language at 18 month for males (p = 0.001). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

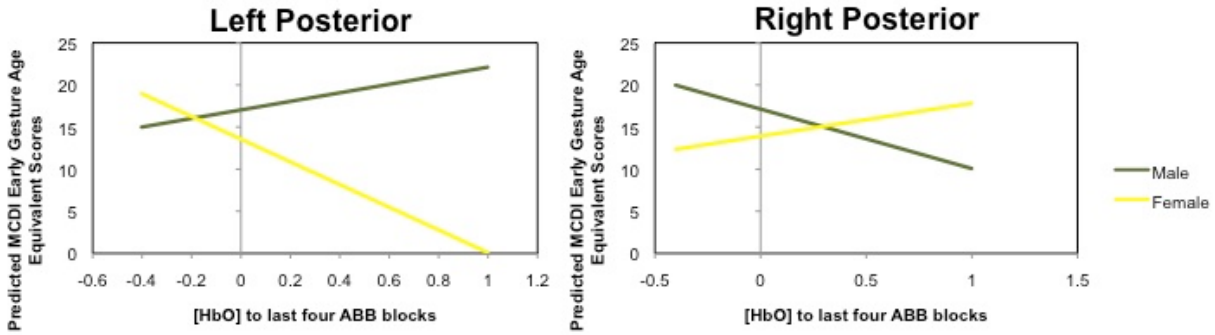

Figure 3.9. *Fitted regression lines for the prediction of 18-month MCDI early gesture age equivalent scores by 3-month [HbO] over the last four repetition (ABB) blocks in left and right posterior regions, broken down by gender. LEFT POSTERIOR: the relationship between 3-month [HbO] changes and 18-month early gesture scores is statistically significant and positive for males (p = 0.012), but negative for females (p = 0.012). RIGHT POSTERIOR: Higher [HbO] is associated with lower MCDI early gesture scores in males (p = 0.005), and is not statistically significant for females (p = 0.209). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

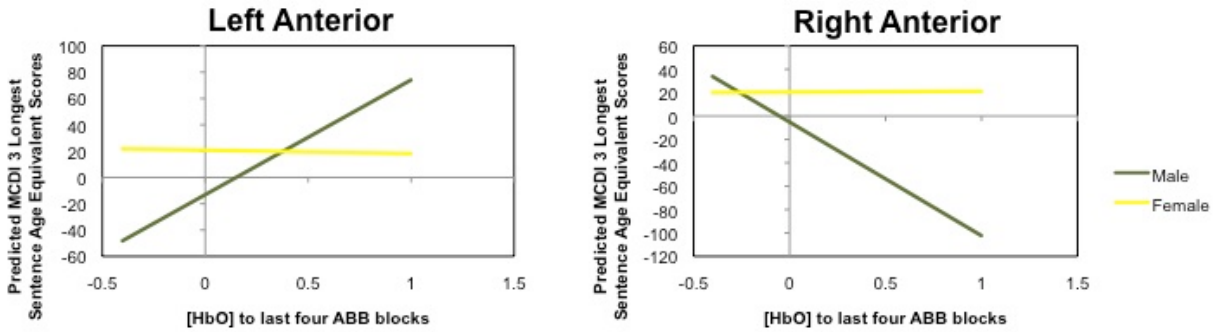

Figure 3.10. *Fitted regression lines for the prediction of 18-month MCDI 3 longest sentence age equivalent scores by 3-month [HbO] over the last four repetition (ABB) blocks in left and right anterior regions, broken down by gender. LEFT ANTERIOR: MCDI 3 longest sentence scores are positively associated with [HbO] changes in males ($p<0.001$), but there is no statistically significant relationship between these variables in females ($p = 0.363$). RIGHT ANTERIOR: the relationship between [HbO] to the last four ABB stimuli blocks and 18-month 3 longest sentence scores is not statistically significant for females ($p = 0.782$), but is negative in males ($p < 0.001$). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

[HbO] Change to last four ABC sequences. Table 3.8 presents the final fitted multiple regression models for the prediction of 18-month MSEL receptive language and MCDI 3 longest sentence age equivalent scores by 3-month [HbO] to the last four ABC stimulus blocks. Prediction models for MSEL Expressive Language scores, ADOS severity scores, and all other MCDI subdomains showed poor overall fit to the data, indicating that 3-month [HbO] changes to the last four ABC sequences may not be significant predictors of these 18-month outcomes.

18-Month MSEL Receptive Language Scores. This regression reveals main effects of [HbO] changes to the left posterior region ($p = 0.013$), and subjects' ASD risk status ($p = 0.035$). Additionally, a statistically significant interaction exists between [HbO] changes to the left posterior regions and risk, indicating that [HbO] to the last four ABC sequences is associated with MSEL receptive language scores at 18 months, but that this relationship is different for high and low risk children. In particular, the relationship between [HbO] to the left posterior region and receptive language is positive for HRA subjects, but negative for LRC subjects. That is, for LRC subjects, higher [HbO] to the left anterior region at 3 months is associated with lower receptive language scores at 18 months ($p = 0.013$), whereas the opposite is true of HRA infants ($p = 0.007$). Figure 3.11 displays the fitted relationships between 3-month [HbO] to the last four ABC sequences and 18-month MSEL receptive language scores in the left

anterior region. This model predicts 47.8% of the variance in 18-month MSEL receptive language age equivalent scores.

MCDI 3 Longest Sentences. This regression reveals significant main effects of [HbO] changes to the left anterior ($p = 0.013$) and posterior ($p = 0.001$) and right posterior regions ($p < 0.001$) at 3 months old, on subjects' 18-month MCDI 3 longest sentences scores, indicating that [HbO] to the last four ABC sequences is associated with this longest sentence length at 18-months. There is also a statistically significant main effect of gender on this 18-month language measure ($p = 0.006$). However, there are statistically significant interactions between [HbO] to each of the aforementioned regions, and gender, indicating that the relationships between 3-month neural signals and longest sentence scores in the left anterior and posterior and right posterior regions differ for males and females. Specifically, in the left anterior and right posterior regions, MCDI 3 longest sentence scores are negatively associated with larger [HbO] changes in males ($p = 0.013$, $p < 0.001$ respectively), while there is no statistically significant relationship between these 3-month [HbO] changes and 18-month 3 longest sentence scores in females ($p = 0.918$, $p = 0.454$ respectively). In the left posterior region the relationship between [HbO] changes to the last four ABC stimuli blocks and 18-month 3 longest sentence scores is also not statistically significant for females ($p = 0.647$), while males' 3-month [HbO] changes to the last four ABC stimuli blocks in this region are positively associated with 18-month MCDI longest sentence scores ($p = 0.001$). Figure 3.12 displays the fitted relationships

between 3-month [HbO] to the last four ABC sequences and 18-month MCDI 3 longest sentence scores in the left anterior and posterior region, and the right posterior region. This model predicts 99.5% of the variance in 18-month MCDI 3 longest sentence age equivalent scores.

Table 3.8. Fitted multiple regression models in which 3 month olds' neural responses to the last four non-repetition (ABC) sequence blocks predict MSEL expressive language age equivalent scores and MCDI 3 longest sentence age equivalent scores at 18 months of age. N(LRC+HRA) = 37. MSEL = Mullen Scales of Early Learning; MCDI = MacArthur Communicative Development Inventory; LA = left anterior region; LP = left posterior region; RA = right anterior region; RP = right posterior region; W-statistic = Shapiro-Wilks test for residual normality.

Parameter	MSEL Receptive Language	MCDI 3 Longest Sentences
Intercept	21.656*** (1.217)	29.91*** (1.206)
LA		-16.07 (3.043)
LP	-5.65* (2.125)	47.98** (3.397)
RA		
RP		-96.15*** (4.587)
ASD Risk	-3.61* (1.623)	
Gender		-9.561** (1.336)
LPxRisk	11.31** (2.873)	
LAxGender		15.77* (4.029)
LPxGender		-46.72** (4.210)
RPxGender		97.61*** (4.893)
R^2	0.4776	0.9952
W-statistic	0.955	0.887
F-statistic	7.62***	89.62**

*p<0.05, **p<0.01, ***p<0.001

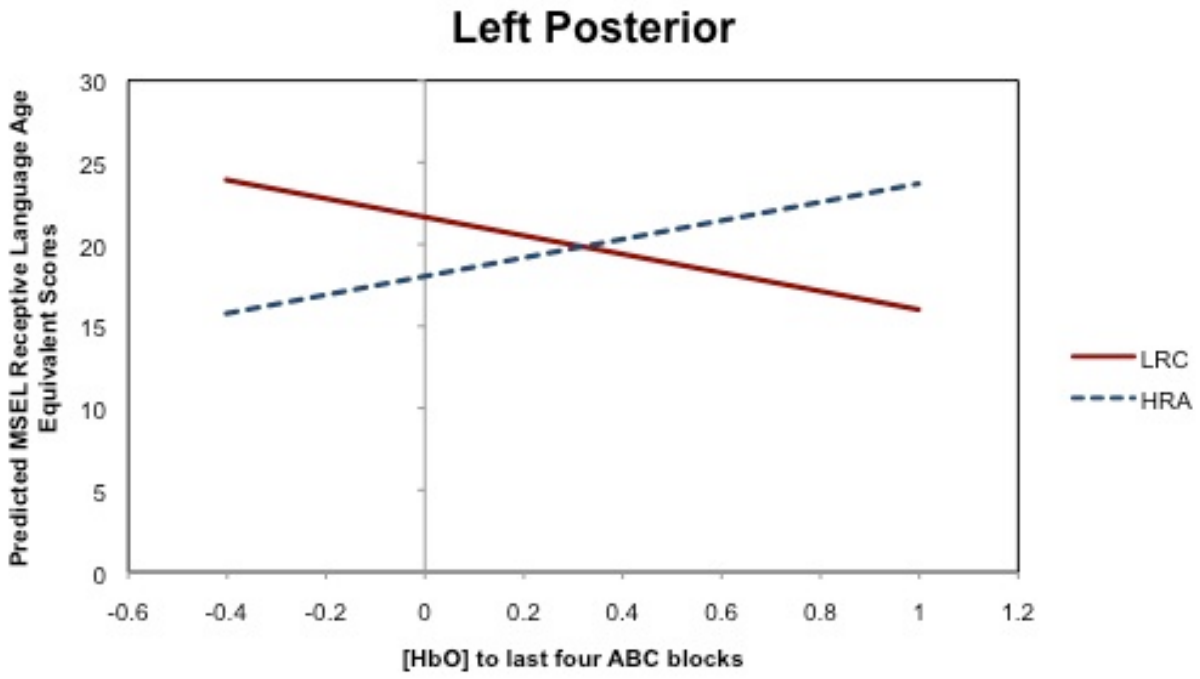

Figure 3.11. Fitted regression lines for the prediction of 18-month MSEL receptive language age equivalent scores by 3-month [HbO] over the last four non-repetition (ABC) blocks in the left posterior region, broken down by risk status. For LRC subjects, higher [HbO] to the left anterior region at 3 months is associated with lower receptive language scores at 18 months ($p = 0.013$), whereas the opposite is true of HRA infants ($p = 0.007$). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.

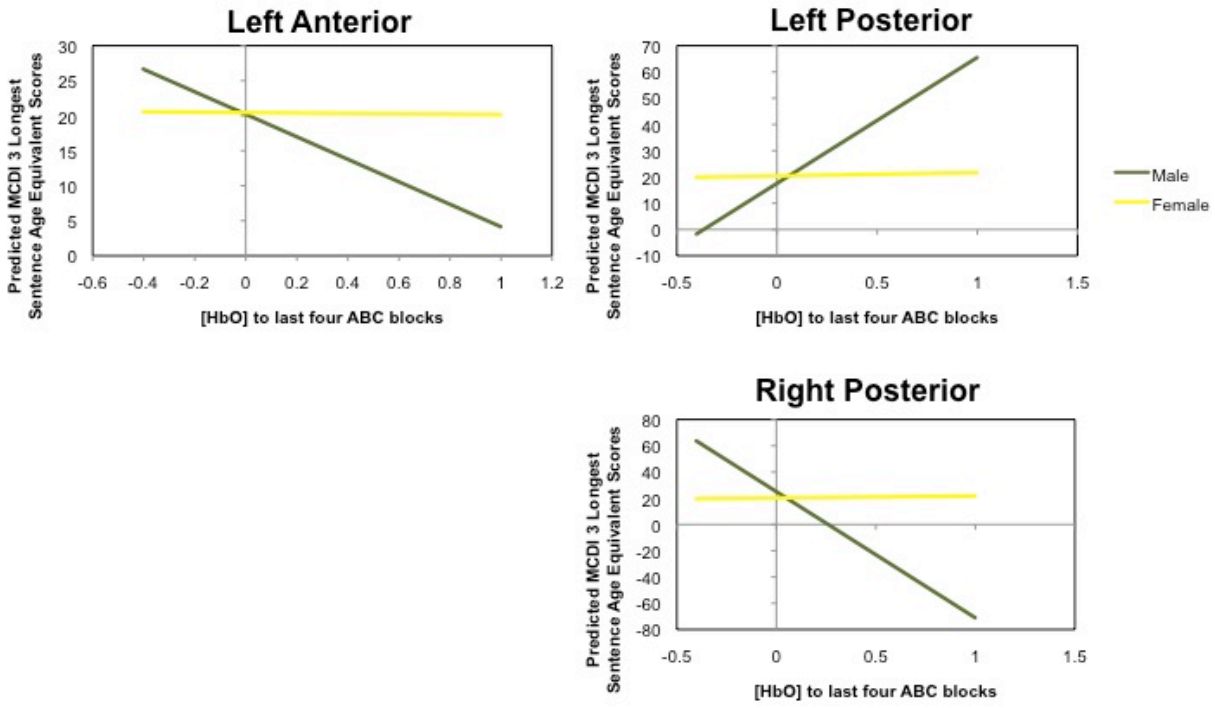

Figure 3.12. *Fitted regression lines for the prediction of 18-month MCDI 3 longest sentence age equivalent scores by 3-month [HbO] over the last four non-repetition (ABC) blocks in left anterior and posterior regions, broken down by gender. LEFT ANTERIOR: MCDI 3 longest sentence scores are negatively associated with larger [HbO] changes in males ($p = 0.013$); there is no statistically significant relationship in females ($p = 0.918$). LEFT POSTERIOR: the relationship between [HbO] changes to the last four ABC stimuli blocks and 18-month 3 longest sentence scores is not statistically significant for females ($p = 0.647$), while males' 3-month [HbO] changes to the last four ABC stimuli blocks in this region are positively associated with 18-month MCDI longest sentence scores ($p = 0.001$). RIGHT ANTERIOR: MCDI 3 longest sentence scores are negatively associated with larger [HbO] changes in males ($p < 0.001$); there is no statistically significant relationship between these 3-month [HbO] changes and 18-month 3 longest sentence scores in females ($p = 0.454$). Note that fitted models in each region are plotted assuming prototypical (average) values at other regions.*

Discussion

The results of these analyses provide much evidence for the association of neural activity to speech-like stimuli at 3 months of age with later (18-month) social and communicative outcomes, in children at high and low risk for developing ASD. In particular, this study shows that group average oxyhemoglobin concentration changes in response to speech-like auditory stimuli in 3-month olds are predictive of expressive and receptive language (as measured by the Mullen Scales of Early Learning), autism symptomatology (as measured by Autism Diagnostic Observation Schedule severity scores), and early gestures, sentence complexity, and sentence length (as measured by the MacArthur-Bates Communicative Development Inventory: Words and Sentences Module).

In interpreting these results it is important to note that these findings do not imply that the early perceptual skills of 3-month-old infants are the building blocks upon which later language acquisition rests, or that delays or deficits in language outcomes may be directly traced to early perceptual delays or deficits. Such a causal connection between the neural markers detected at 3 months and the behaviors of children at 18 months is not possible using the analysis methods employed here. Moreover, these claims are not the aim of this study. Instead, associations between early neural activity and later language and behavioral outcomes are important to establish, as they may help to validate the use of neural activity to indicate, before telltale behaviors emerge, which children—particularly

of those who are members of a high risk population—are likely to require and benefit most from targeted developmental intervention programs.

To this end, the early neural markers examined herein are those that were sensitive to speech-like auditory stimuli containing structural regularities (repetitions) and which differentiated groups of children at high and low risk for ASD in chapter 2. Neural discrimination of repetition from random sequences (which is present from birth in healthy neonates; Gervain et al., 2008) is an indicator of intact statistical learning capabilities, which are likely to be integral to the rapid language acquisition that humans demonstrate (Kuhl, 2004). At the beginning of this report I hypothesized that, if these neural signatures reflect the statistical learning capabilities upon which language acquisition depends, they should be associated particularly with those language outcomes that reflect an individual's ability to construct coherent language structures, rather than those that might be more social or communicative in nature. In fact however, the neural activity investigated in the current study is associated with both language construction abilities (such as measures of sentence length and complexity) and more social or communicative behaviors (such as gestures and general ASD symptomatology). These results likely reflect the fact that no aspect of language can be considered "purely" linguistic or structural in practice, as it is the combination of statistical learning strategies and social proclivities that are thought to underlie language acquisition in humans (Kuhl, 2004).

Conversely, it may also be the case that some of the neural activity examined herein may itself not be purely linguistically based; if this were the case, we would not necessarily have expected to see significant neural activation to random, non-repetition syllable sequences in chapter 2 (recall from Chapter 2 that TD children showed significant and comparable neural activation to both ABB and ABC sequences when these signatures were averaged over the course of the experiment). It is possible that what appears to be similar neural responses to different auditory stimuli actually reflect differing functions or mechanisms. For example, one hypothesis put forward for the observation of neural activation to the non-repetition control sequences in chapter 2 was that this reflected a general neural attentional orienting toward speech-like stimuli. If this is the case, it makes sense that we observed consistent patterns of neural activity to the ABC sequences in LRC infants, but aberrant or inconsistent patterns of neural activity to these stimuli in HRA infants, whose sensitivities toward socially meaningful stimuli may be compromised (see Chapter 2 Results). In the current study, neural responses to all blocks of repetition stimuli at 3 months of age were associated with MSEL receptive language and MCDI sentence complexity scores, whereas neural activity to the random control blocks was associated with 18-month ADOS severity scores. This finding—of associations between neural responses to repetition sequences and language outcomes, but associations between neural responses to random speech-like sequences and social outcomes—provides more support for the hypothesis that the observed neural responses to ABC sequences

may have been due to social orienting to these stimuli, whereas those to ABB sequences may have reflected some aspect of statistical learning that is associated with the ability to successfully parse language structures.

The findings discussed so far are based on neural activity averaged across all syllable sequence blocks of a particular type. However, those findings that were arguably the most interesting and informative in Chapter 2, were those that compared neural activation to syllable sequences over the course of repeated exposure to these stimuli. In Chapter 2, the results indicated that LRC and HRA infants showed similar responses to the first four blocks of repetition-based speech-like stimuli (ABB sequences), but diverged in their neural activity over the course of exposure to these sequences during the experiment. In the current study however, 3-month neural activity to the first four ABB sequences predicted 18-month MCDI early gesture scores in LRC, but not in HRA subjects, even despite the reduced variance in LRC early gestures scores due to some individuals scoring at ceiling levels (see Table 3.2). As above, the relationship of this neural activity to a later social-communicative outcome in LRC children might indicate that the initial neural response to ABB syllable sequences by 3 months of age is in part due to social or attentional orienting toward the stimulus, and that this activity contributes to a foundation of social-communicative sensitivity upon which the capacity to use gestures for communicative intent is built. In fact, the same relationship that exists between neural activity to the first four ABB sequence blocks exists for neural activity to the first four ABC sequence blocks in the right

hemisphere and later early gesture scores in LRC infants. Since ABC sequence blocks were designed as a control for the structural regularities present in ABB blocks, the similarity in LRC responses to both sequence types provides further evidence that this neural activity may perhaps be indicative of some kind of orienting response, rather than a language-specific statistical learning capacity. If this is the case, the presence of significant neural activity to initial ABB syllable sequences without the association of this activity to later social-communicative outcomes in HRA infants might indicate that for this group, the same exposures and sensitivities to early stimuli may not be utilized as they are in LRC infants, for building later social competence.

Another interesting finding regarding associations with neural activation to the first four ABB sequences is that the direction of these associations differ in the left and right hemispheres—in LRC subjects, higher [HbO] to the first four ABB sequences in the left anterior region is associated with higher 18-month early gesture scores, whereas higher [HbO] to the first four ABB sequences in the right anterior region is associated with lower 18-month early gesture scores. Given that a consistent finding of past neuroimaging research on language in individuals with ASD is a trend toward atypical lateralization in these individuals' brain responses to language, follow up studies might aim to determine whether a reversal of neural lateralization may be partially responsible for disrupting processes that might connect early perceptual abilities to later social outcomes in HRA infants.

Subjects' neural responses to the last four ABB sequences were those that most distinctly separated HRA and LRC infants in Chapter 2. Recall that whereas LRC infants showed a decrease in neural activity to the repetition sequences over the course of exposure to them, HRA infants' neural activation to these sequences did not change with repeated exposure. Gender effects were also observed, with LRC males showing a less drastic decrease in neural activity to the last ABB sequences than females, and HRA males showing lower overall activation to these sequences in comparison to females. In the current study, neural activation to the last four ABB sequences was also associated with MCDI early gesture scores, as well as MSEL expressive language scores, MCDI sentence complexity and MCDI 3 longest sentence scores. The association with sentence complexity occurred regardless of subjects' risk statuses or gender; this result supports the previously discussed hypothesis; early neural responses to repetition-based sequences may be indicative of statistical learning capacities in infants, and thus contribute to the development of structural, or "less social," aspects of language development. Along this line of reasoning, it is important to note that no such associations were found between neural activity to ABC sequences and MCDI sentence complexity scores.

Group differences in the relationships between neural activity to the first four ABB sequences and expressive language, early gestures and 3 longest sentence scores however, tended to occur on the basis of gender rather than risk group: in the left hemisphere, the associations between neural activation to the last

four ABB sequences and MSEL expressive language as well as MCDI early gestures was positive in males and negative in females; in the right hemisphere, these relationships were reversed accordingly in both genders (although the association between neural activity and MCDI early gesture scores in the right hemisphere did not reach significance for females); associations between neural activity and 3 longest sentence scores were found only in males (although this specific outcome will be discussed in more detail later). These gender-based lateralization reversals potentially complicate the picture where LRC and HRA infants are concerned; however, as in chapter 2, these results reiterate the importance of examining males and females at high and low risk for ASD separately, and of being sensitive—in research and in practice—to the different trajectories along which these groups' language develops. Past research has established that males and females are behaviorally distinct in certain aspects of their language development; the current results however, demonstrate that the roots of these behavioral differences are likely related to differences in the neural mechanisms used to acquire language and related behaviors in the two genders. It may also stand to reason that the gender-based differences in associations between early neural activity to repetition-based speech-like stimuli and later language outcomes point to the role of early sensitivity to structural regularities in speech for "less social" or structural aspects of language development (where differences between males and females are well established; Gleason & Ely, 2002), rather than socially-oriented aspects of language or communication development (in which

case we might predict that these effects should differ based on children's social sensitivities, as captured by their ASD risk statuses).

A final note about the results of this study concerns the number of associations between neural activity at 3 months and MCDI measures of sentence length at 18 months in males. These findings—and the high amount of variability in 3 longest sentence scores that the neural markers and covariates herein appear to explain—have not been interpreted in detail here because they are likely an artifact of the small sample size (n=4) of males who contributed MCDI 3 longest sentence scores to the analysis. Additionally, one of the four males in this sample has a 3 longest sentences score at floor level, and these models come closest to violating the multiple regression analysis assumption of residual normality (in one case, the model residuals are significantly non-normally distributed; see Table 3.6). These characteristics of this particular dataset make any results to which they contribute potentially too preliminary to interpret meaningfully; they are included here only completeness and transparency of reporting.

A similar—though less severe—caveat exists for all the analyses included herein. In the case of words produced by females for example (n=3) the lack of any significant association between this language outcome and early neural markers cannot be interpreted as meaning that no such relationship exists in the population. In general, sample sizes were much lower at 18 months than at 3 months of age, due to the participant attrition that is an unfortunate characteristic of all longitudinal studies. Importantly, the associations that are represented in this

report are group averages, and are not to be interpreted as developmental trajectories of individuals' development. Although the study from which these data are taken is longitudinal, the relatively small number of participants with data on each of these measures precluded true longitudinal analysis of the subjects' developmental trajectories. Overall, though the results herein suggest some interesting population effects then, further research addressing and probing more deeply into these research questions must be carried out before responsible practical implications can be acted upon. Nonetheless, this study represents a first step in establishing the neural markers investigated in detail in Chapter 2 as potentially useful early indicators of later language development, which may play a role in determining, from a younger age than is currently possible, which children are most likely to need language-based educational interventions, as well as what the specific targets of these intervention programs might be.

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Chapter 4

General Conclusions

Abnormal language development is a pervasive symptom of autism spectrum disorder (ASD). It also affects a number of individuals who are at high risk for ASD, regardless of whether they qualify for official diagnoses. Yet research has repeatedly shown that children with ASD who develop stronger language abilities have more positive future outcomes. The studies in this dissertation sought to inform our ability to improve the language outcomes—and thus life outcomes—of children at risk for ASD, by conducting preliminary investigations of neural markers of language acquisition and language-relevant abilities, which may be used to identify children who might benefit from being enrolled in language-based interventions, from several months earlier than is currently possible in clinical practice.

The first study (Chapter 2) examined a set of neural markers associated with infants' capacities for statistical learning. Three month olds heard blocks of syllable sequences that were matched on all properties except for their structural components—some sequences contained two consecutive syllable repetitions, while control sequences contained no syllabic repetitions. A prior study on healthy newborns found that they are born with the capacity to neurally discriminate and attend to repetitions in auditory speech-like stimuli (Gervain et al., 2008). Here, I showed that by 3 months of age, healthy (LRC) infants seem to show neural

responses to both repetitive and random syllable sequences, but show signs of neural habituation only to repetition-based sequences. Infants at high risk for developing ASD on the other hand, showed aberrant patterns of neural activity to these same stimuli—they showed no evidence of change or neural habituation to repetition grammars over time, and they exhibited neural responses to random syllabic sequences that differed drastically from those of LRC infants.

In Chapter 3 I determined whether the neural markers identified in Chapter 2 were predictive of children's later language and communicative outcomes. I found that neural activity in response to both repetition- and non-repetition-based speech-like auditory stimuli predicted later communicative outcomes, although activity to repetition-based stimuli may be more predictive of structural aspects of language development, while neural responses to random sequences might be more strongly associated with later social outcomes. In both studies, I observed significant differences in the patterns of neural activity exhibited by children at high and low risk for ASD. Additionally, across both studies, I observed many differences in the findings from males and females.

The results of these studies thus give insight into some of the neurodevelopmental mechanisms by which children at high risk for ASD differ from low risk individuals, particularly regarding early perceptual and language abilities. As such, these studies represent a starting point upon which future diagnostic and intervention research and practice might build. Future work should aim to determine whether the neural markers investigated herein at the group level

function similarly in individuals, as well as whether these markers are sensitive and specific to populations of children at risk for ASD (or other disorders). Such work could lay the foundation for employment of the fairly simple, non-invasive technologies employed in these studies in clinical settings, and help to ensure the earliest and most efficacious programs of care and education for children who might otherwise embark upon maladaptive paths of development.

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