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ORIGINAL PAPER

Differences in Neural Correlates of Speech Perception in 3 Month Olds at High and Low Risk for Autism Spectrum Disorder

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Abstract In this study, we investigated neural precursors of language acquisition as potential endophenotypes of autism spectrum disorder (ASD) in 3-month-old infants at high and low familial ASD risk. Infants were imaged using functional near-infrared spectroscopy while they listened to auditory stimuli containing syllable repetitions; their neural responses were analyzed over left and right temporal regions. While female low risk infants showed initial neural activation that decreased over exposure to repetition-based stimuli, potentially indicating a habituation response to repetition in speech, female high risk infants showed no changes in neural activity over exposure. This finding may indicate a potential neural endophenotype of language development or ASD specific to females at risk for the disorder.

Keywords Autism spectrum disorders · Near infrared spectroscopy · Speech processing · Infancy · Endophenotype · Language

Introduction

Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and interaction abilities and restricted, repetitive patterns of behavior (APA 2013). In order to understand the etiology and development of ASD, researchers have turned their attention to the study of infant siblings of children with ASD, who are at substantially higher risk for developing the disorder or associated developmental problems (Messinger et al. 2013; Ozonoff et al. 2011). Prospective studies of infant siblings of children with ASD have the potential to reveal early predictors or biomarkers of ASD that may be detectable before overt signs of the disorder are revealed through behavior (which is how the disorder is currently diagnosed). Moreover, these

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studies may also reveal "endophenotypes" of risk that are shared between individuals with ASD and their first-degree relatives. Endophenotypes are subtle traits or markers, such as neural activity, that are intermediate between genes and clinical phenotypes, and which are present in affected and unaffected individuals who are at genetic risk for a disorder (Gottesman and Gould 2003; Viding and Blakemore 2007). Endophenotypes of ASD risk do not themselves distinguish individuals who will go on to develop ASD from those who will not, but the presence of several endophenotypes of ASD risk within an individual may cumulatively contribute to development of ASD (Tager-Flusberg 2010; Zwaigenbaum et al. 2005).

Several studies have already uncovered promising potential endophenotypes of ASD risk that are present in infant siblings within the first year of life. For example, in several ERP studies differences have been observed in brain responses to faces, objects, and other visual stimuli, between 6- and 10-month-old infants at high risk for ASD and their low risk peers (Elsabbagh et al. 2009; Elsabbagh and Johnson 2010; Guiraud et al. 2011; Keehn et al. 2015; Key and Stone 2012; McCleery et al. 2009; Seery et al. 2014). EEG and ERP studies also reveal differences between these groups, with 6 month olds at high risk for ASD showing less negative alpha asymmetry (Gabard-Durnam et al. 2015) and lower power spectral densities (Tierney et al. 2012), 12 month olds showing reduced functional connectivity (Righi et al. 2014), 9–12 month olds showing reduced entropy (Bosl et al. 2011), and 6, 9, 12, 18, 24, and 36 month olds showing different patterns of P1, N290, P400, and Nc component amplitude and lateralization over development (Luyster et al. 2014), compared to their low risk peers. Functional near-infrared spectroscopy (fNIRS) was employed in a face perception study revealing that 7 month olds at high risk for ASD show lower levels of neural activation to faces in lateral brain regions, compared to their low risk peers (Fox et al. 2013). In a structural neuroimaging study (using diffusion tensor imaging), 6-month-old infants at familial risk for developing ASD also had smaller corpus callosa than their low risk peers (Wolff et al. 2015).

The neural foundations of certain social and communicative behaviors are likely present even earlier in development, however. Studies of healthy newborns, for example, have shown that they are able to differentiate normal speech from backward speech (Peña et al. 2003) and are able to differentiate speech-like stimuli with structural repetitions from random sequences (Gervain et al. 2008). Yet, few studies have investigated neural endophenotypes in infants at risk for ASD within the first 6 months of life. Using fNIRS, Lloyd-Fox et al. (2013) found some of the first evidence of differences in brain function between 4 and 6-month-old children at high and low risk of developing ASD, in a study of auditory and visual processing of social stimuli. In another fNIRS study published later that same year, Keehn et al. (2013) also found that 3-month-old infant siblings of children with ASD appeared to exhibit increased functional connectivity in temporal regions of the brain, compared to children without genetic risk of developing ASD.

In the current study, we aim to add to the small body of literature investigating potential endophenotypes of ASD risk present in the first 6 months of life. In particular, we focus on biomarkers relevant to language development, as delays in language and communication abilities emerge behaviorally from as early as 12 months of age (Zwaigenbaum et al. 2005), and the most common first concerns that parents of children with ASD cite are around speech and language development (DeGiacomo and Fombonne 1998; Iverson 2010; Wetherby et al. 2004). Past neuroimaging work also reveals atypical lateralization of brain responses to language in adults and children with ASD (Boddaert et al. 2003; Eyler et al. 2012; Kleinhans et al. 2008) as well as in infants at familial risk for the disorder (Seery et al. 2013).

In typically developing populations, 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). A study by Gervain et al. (2008) revealed that infants show evidence of possessing an automatic perceptual system for detecting and learning about structural regularities in speech from as early as a few hours after birth. 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.

In the current study, we utilize Gervain's paradigm to follow up and extend her findings. We aim to determine whether the perceptual system for detecting and learning about structural regularities in speech is intact in 3 month olds who are at high risk for developing ASD (HRA) due to the presence of an affected older sibling. Specifically, we hypothesize that 3-month-old HRA infants may not discriminate or show exposure-based changes to repetitive speech-like stimuli compared to random speech-like stimuli as LRC 3 month olds do—or even as LRC newborns do (Gervain et al. 2008)—if such patterns of neural activity

represent early language-relevant capacities upon which later language development is built. Furthermore, we seek to explore the effects of gender on these putative precursors to language acquisition, given established gender-based differences in language performance and language acquisition that are present in typical development (Bauer et al. 2002; Bornstein et al. 2004; Gleason and Ely 2002; Parke and Gauvain 2008), and even across language communities (Eriksson et al. 2012). Additionally, given that ASD is almost five times more prevalent in males (in which the rate is 1 in 42) than in females (who show a rate of 1 in 189; Baio et al. 2014),[1] and that gender-based differences are present across various dimensions of social and cognitive development in ASD (Halladay et al. 2015), including language (Lai et al. 2011), we seek to determine whether any interactions between gender and familial ASD risk may exist from this early developmental time point. Differences in neural activity between HRA infants and a control group of low risk infants (LRC) might reveal an endophenotype of ASD risk, which is detectable in the first 6 months of life, and which might eventually allow us to make predictions about individual infants' risk for developing autism or related language disorders.

Methods

This IRB-approved study is part of a larger longitudinal investigation conducted at Boston Children's Hospital and Boston University. At 3 months, infants participated in the NIRS paradigm detailed below in addition to a battery of electroencephalography/event-related potential (EEG/ERP) and behavioral measures. Institutional Review Board approval was obtained from Boston Children's Hospital. Informed consent was obtained from all parents or legal guardians of individual participants included in the study.

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 community diagnosis of ASD (high risk for ASD, 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). Infants who were born prematurely (earlier than 36 weeks gestational age), who had low birth weights (under 2500 g) or who had known neurological or genetic abnormalities were excluded from the study. Infants who grew up in language environments in which English was spoken less than 75% of the time were also excluded, as bilingual (or multilingual) children may show differential developmental trajectories of language development (Byers-Heinlein and Werker 2013).

Forty-seven infants participated in the current study (29 HRA, 18 LRC); of this sample, 38 (21 HRA, 17 LRC) provided usable data for analysis. Two infants were excluded for excessive fussiness, which resulted in their failure to hear at least 8 stimulus trials for each syllable sequence (out of the 14 for each syllable sequence type presented); 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 of the 24 total channels), caused by excessive or dark hair,[2] or improper headgear fit; three 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 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.

Stimuli

Auditory stimuli were identical to those used in Gervain et al. (2008); they comprised 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 270 ms long, had a monotonous pitch of 200 Hz, 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 trials of 10 syllable sequences separated by randomly

[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. 2007).

[2] Hair, particularly that which is highly pigmented, interferes with NIRS measurements by absorbing near-infrared (NIR) light from source optodes before it penetrates the skull and arrives at the brain tissue. NIRS headgear is thus designed to keep hair out of the path of NIR light as much as possible. Many adaptations for reducing interference by hair may cause discomfort however, making them inappropriate for studies on infant populations.

Table 1 Demographics and family characteristics for subjects included in the final analysis

	Low risk controls	High risk for ASD	p value
Measure	$n=17$	$n=21$	
Gender (M/F)	10/7	13/8	
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 (except in the case of Gender, which is reported as frequencies)

[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

varying intervals (between 500 and 1500 ms) of silence, to avoid inducing phase-locked brain responses. Trials of both syllable types were 16 s long on average. Trials were presented in one of two pseudo-randomized orders, and these orders were counterbalanced across participants based on their risk status and gender. Individual trials were separated by a minimum of 15 s of silence to allow brain activation generated during test trials to return to baseline. Each child heard a maximum of 28 trials (14 ABB, 14 ABC). On average, the protocol took approximately 20 min.

Apparatus

Three-month-old infants were examined using functional Near-Infrared Spectroscopy (fNIRS). fNIRS measures localized changes in oxy- (oxyHb) and deoxy-hemoglobin (deoxyHb) in the blood. In the current study, fNIRS was carried out using a Hitachi ETG-4000 system with near-infrared light at 690 and 830 nm transmitted to source optodes via 1 mm optical fiber bundles. Samples were collected at 10 Hz. 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 eight detector optodes were arranged into two 3×3 chevron arrays (each containing five sources and four detectors separated by a distance of 3 cm) 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 (Okamoto et al. 2004; Perani et al. 2011). 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 1 illustrates the headgear geometry and its placement on a prototypical infant head.

Procedure

Three-month-old infants were seated on a caregiver's lap in a soundproof testing room, where they passively listened to auditory stimuli while wearing the fNIRS cap. 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 trials as possible. Auditory trials were also under experimenter control, so that they were only played when the infant was 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 (Little et al. 1999; Thomas and Lykins 1995). Most infants were able to continue the study until they heard all 28 trials. Those who become too fussy to continue were allowed to stop before the end.

Data Processing

For participants who heard at least 16 auditory trials (8 of each stimulus type), fNIRS data (raw light intensity values)

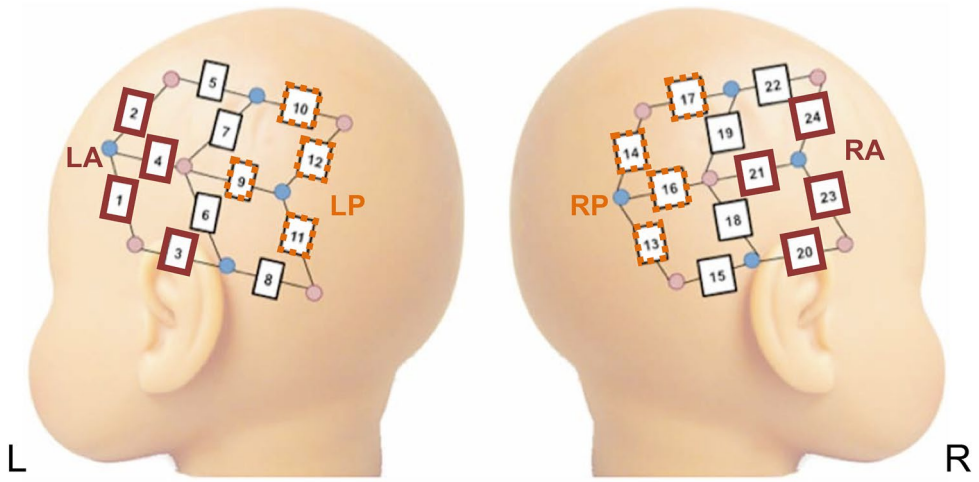

Fig. 1 Diagram of fNIRS headgear orientation and placement. Measurement channels are numbered 1–24 (*squares*). Hemodynamic responses were averaged over several channels to form four regions of interest (ROIs). Those channels comprising the anterior ROIs are outlined in *solid lines*, and those comprising the posterior ROIs are in *dashed lines*. 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 3 cm apart. *LA* left anterior region, *LP* left posterior region, *RA* right anterior region, *RP* right posterior region

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 OxyHb signal; channels were excluded if the RMS exceeded a threshold of 0.25; 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 trial. After these screening steps, participants who failed to retain at least 12 of the 24 total measurement channels were eliminated from further analysis. The HRA and LRC 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 oxyHb and deoxyHb using the modified Beer–Lambert law (ppf = 5.0). Changes in oxyHb and deoxyHb over each trial were examined from the 2 s preceding auditory trial onset, the 16-second auditory trial duration, and the 4 s following test trials. 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. Optical signals were averaged across trials and then infants for each trisyllabic sequence type. Trials occurring within 2 s of a motion artifact (detected as described above) were eliminated from the means. The total number of trials, the number of trials of each stimulus type, and the proportion of stimulus types heard were similar across risk groups (see Table 2). 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).

Table 2 Stimulus trials included in the final analysis

Trial type	Low risk controls	High risk for ASD	p value
Overall	26.88 (2.42)	27.76 (1.09)	0.18
ABB	13.41 (1.33)	13.90 (0.44)	0.16
ABC	13.47 (1.12)	13.86 (0.65)	0.22

Data are reported as group means with standard deviations in parentheses. ABB = trisyllabic sequence trials containing repetitions; ABC = trisyllabic sequences without repeating sequences

Data Analysis

Channels were grouped into four regions of interest (ROIs) for analysis by simple averaging (Keehn et al. 2013): left anterior (channels 1, 2, 3, 4), left posterior (channels 9, 10, 11, 12), right posterior (channels 13, 14, 16, 17), and right

anterior (channels 20, 21, 23, 24; see Fig. 1).[3] Average oxyHb values to the two trisyllabic sequence types (ABB or ABC blocks; within-subjects factor) at each of the four regions of interest (within-subjects factor), along with infants' group membership (between-subjects factor) and gender (between-subjects factor) were entered into a 2×4×2×2 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, or 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 7 month olds are able to discriminate new speech patterns as a result of only 2 min 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 an additional within-subjects factor for a 2×4×2×2×2 ANOVA, to investigate the effect of repeated exposure to the ABB and ABC sequences on hemodynamic neural activity.

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

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

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)×4 (region: left posterior, left anterior, right posterior, right anterior)×2 (risk group: HRA, LRC)×2 (gender: male, female) mixed-design ANOVA revealed a main effect of region ($F(1.92, 63.22)=4.33$, $p=0.019$, partial $\eta^2=0.116$),[4] whereby oxyHb 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 ($F(3,99)=3.74$, $p=0.031$, partial $\eta^2=0.102$), indicating that the aforementioned differences in oxyHb 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 revealed that in the left anterior region, LRC males and females, as well as HRA males, showed similar oxyHb responses to auditory stimuli, while HRA females exhibited significantly higher oxyHb responses than all three other groups (LRC males: $F(1,33)=5.44$, $p=0.033$; LRC females: $F(1,33)=6.35$, $p=0.017$; HRA males: $F(1,33)=4.57$, $p=0.040$, p values are uncorrected); 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 oxyHb responses to auditory stimuli than HRA males ($F(1,33)=4.23$, $p=0.048$, p value uncorrected), and LRC females ($F(1,33)=6.57$, $p=0.015$, p value uncorrected). Parallel analyses examined responses in the left and right posterior regions, but no significant differences were found between males and females at either risk group level, or between HRA and LRC risk groups at either gender level, in either region. Figure 2 shows average oxyHb responses to auditory stimuli at each region of interest, broken down by gender and risk group. There were no other significant main effects or interactions in this analysis (all $ps>0.150$).

[3] These regions of interest (ROIs) cover roughly identical topographies as those used by Gervain et al. (2008). We include fewer channels in each ROI average herein, in order to distinguish regional brain activity more clearly than was perhaps possible in Gervain's study of newborns (who have smaller average head sizes than 3 month olds) and to maintain consistency with other studies conducted in our lab, using the same NIRS headgear (i.e., Keehn et al. 2013; Wagner et al. 2011).

[4] Mauchly's test of sphericity indicated that the assumption of sphericity 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).

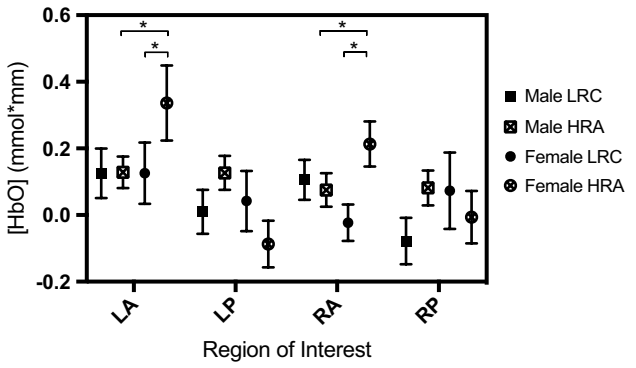

Fig. 2 Region × gender × risk group interaction. Points indicate average oxyHb (in mmol mm) over the course of a stimulus block in the designated regions of interest. *Error bars* are ±1 SE. *LA* left anterior region, *LP* left posterior region, *RA* right anterior region, *RP* right posterior region; * $p < 0.05$ in simple effects analysis

Neural Responses to Speech-Like Stimuli Across Exposure

The 2 (syllabic sequence type: ABB, ABC) × 4 (region: left posterior, left anterior, right posterior, right anterior) × 2 (risk group: HRA, LRC) × 2 (gender: male, female) × 2 (exposure: first vs. last stimuli blocks) mixed-design ANOVA also revealed a main effect of region ($F(2.13, 70.14) = 3.45$, $p = 0.035$, partial $\eta^2 = 0.095$),[5] whereby oxyHb 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 ($F(1,33) = 3.89$, $p = 0.057$). This main effect was not modified by any interactions.

The ANOVA also revealed a three-way interaction between exposure, syllabic sequence type and risk group ($F(1,33) = 4.19$, $p = 0.049$, partial $\eta^2 = 0.113$) and a four-way interaction between exposure, syllabic sequence type, risk group and gender ($F(1,33) = 5.85$, $p = 0.021$, partial $\eta^2 = 0.151$). These results indicate that oxyHb 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 oxyHb responses to the last 4 ABB trials compared to the first 4 ABB trials ($F(1,136) = 6.10$, $p = 0.015$, p value uncorrected), and that their oxyHb responses to the last 4 ABB trials were also significantly lower than those of HRA females to the last 4 ABB blocks ($F(1,136) = 7.72$, $p = 0.006$, p value uncorrected). A similar pattern of results was observed between LRC and HRA males across the first and last 4 ABB stimulus trials, although these differences did not reach statistical significance (see Fig. 3a). Figure 3b depicts the exposure x risk group x gender interaction for the ABC stimulus trials; none of the oxyHb response patterns to this condition were statistically significant.[6] Figure 4 depicts gender- and risk group-based oxyHb time courses averaged across the first and last 4 ABB and ABC trials heard by participants.

Discussion

OxyHb Increases to the Left Anterior Region of Interest

In this study, we used fNIRS to examine the neural correlates of early speech perception in a sample of 3-month-old infants at high familial risk for ASD, and a sample of low risk infants. This investigation served as an extension of Gervain et al. (2008), who reported that healthy newborns were able to detect differences in the structures of speech-like auditory stimuli (as inferred from changes in cortical oxyHb), and that this neural discrimination occurred predominantly in the temporal and left frontal regions of the brain.

Analyses of ABB and ABC syllable sequence discrimination revealed main effects of region, such that neural responses to the language stimuli (across all trials and over exposure) were largest over the left anterior measurement region. In the current study however, these region effects appeared to be driven primarily by the HRA females: across all trials, a three-way interaction revealed that HRA females exhibited significantly higher oxyHb responses than HRA males, LRC females or LRC males in both left and right anterior regions; follow-up analyses investigating

[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).

[6] The exposure effects herein are likely not an artifact of the number of trials that participants heard, as the majority ($n = 33$; 87%) of participants listened to all 28 trials, 2 participants (5.3%) stopped after 25 trials, 1 (2.6%) stopped after 24 trials, 1 (2.6%) stopped after 23 trials, and 1 (2.6%) stopped after 19 trials. Additionally, the number of trials heard did not differ significantly between risk groups ($p = 0.18$), genders ($p = 0.65$), or syllabic sequence type ($p = 1.00$). Finally, the four-way interaction between exposure, syllabic sequence type, risk group and gender was robust to the inclusion of number of trials heard as a covariate in the mixed-design (2 × 4 × 2 × 2x2) ANOVA: exposure × syllabic sequence type × risk group × gender ($F(1,32) = 5.46$, $p = 0.026$, partial $\eta^2 = 0.146$); all interactions with number of trials ($p > 0.05$).

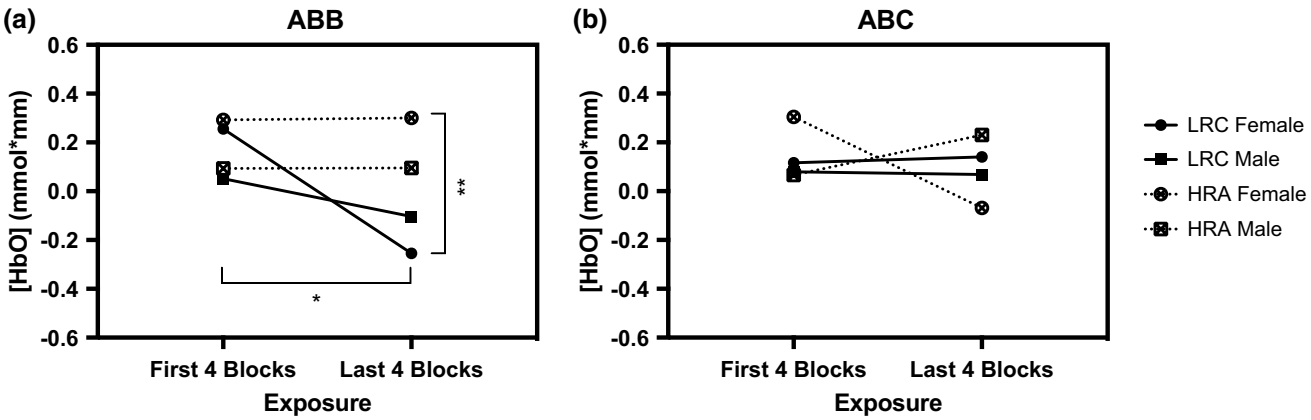

Fig. 3 Four-way interaction between exposure, syllabic sequence type, risk group and gender (F (1,33) = 6.77, p = 0.014). **a** Responses to ABB stimuli blocks (collapsed across all ROIs): LRC females showed significantly lower oxyHb responses to the last 4 ABB trials compared to the first 4 ABB trials (p = 0.015), and to the last 4 ABB blocks compared to HRA females (p = 0.006) **b** responses to ABC stimuli blocks (collapsed across all ROIs). *p < 0.05; **p < 0.01

the main effect of region across exposure to the experimental stimuli also revealed that this group may have been primarily driving the significant increase in oxyHb observed in the left anterior region.[7]

These findings align and extend those of Gervain et al. (2008) discussed earlier who found bilateral activation to these stimuli in temporal regions, in healthy neonates. Our sample of 3-month-old LRC males and females (and even HRA males) show weak or no evidence of lateralized neural activation to language-relevant stimuli in the measured temporal regions, though they do show evidence of bilateral temporal activation to trisyllabic auditory sequences. However, the HRA females in our sample show evidence of left- or even hyper-lateralization of neural activity to these same language-relevant stimuli. This result—though preliminary, given our relatively small sample size of HRA females (n = 8 of 21 HRA subjects)—is worth further consideration in future research, as it may indicate a potential neural endophenotype and/or mechanism of language development or ASD specific to females at risk for the disorder. A small body of research literature, primarily focused on males, suggests that individuals with ASD who have impaired language may show atypical patterns of structural and functional asymmetry (particularly reduced activation of the left hemisphere) in language relevant regions of the brain (Eyler et al. 2012; Flagg et al. 2005; Lombardo et al. 2015; Knaus et al. 2010; Wan et al. 2012). The data herein add nuance to that picture, by suggesting that females at risk for ASD or associated language impairments may exhibit qualitatively distinct language-relevant neural endophenotypes from males. This study's findings suggest that in light of recent efforts to better understand sex differences in ASD, and given the understudied and potentially unique manifestation of this disorder in females (Halladay et al. 2015), future studies aimed at systematically investigating sex-specific ASD phenotypes may consider beginning with a focus on precursors to speech and language development.

Neural Responses to Speech-Like Stimuli Across All Trials

Our first set of analyses produced no evidence that children from either risk group (or gender) differentiated the repetition from the non-repetition auditory stimuli. These findings were unexpected in light of those from Gervain et al. (2008), who established that neonates do discriminate ABB from ABC speech stimuli at the neural level. However, it is possible that 3 month olds are more attuned than newborns, to language (or non-language) features that are similar between the two speech structures, and that this represents a developmental shift that has occurred in the first 3 months of life. For example, the auditory stimuli were matched on all nonstructural properties (such as syllabic repertoire, frequency of each syllable type, phonological characteristics, transitional properties between syllables), so if infants were showing neural responses to any of these non-structural speech characteristics—as they might

[7] At the request of a reviewer, we conducted a 2 (syllabic sequence type) × 4 (region) × 2 (risk group) × 2 (exposure) mixed-design ANOVA, to examine exposure results collapsing across gender. This analysis revealed that the main effect of region was significant when gender was not included as a between-subjects factor (F(2.09, 75.15, corrected using Greenhouse–Geisser estimate of sphericity (ε = 0.696)) = 3.45, p = 0.046, partial η^2 = 0.081). Additionally, elimination of HRA females from the sample extinguished the main effect of region over exposure (F(3,24) = 0.591, p = 0.627).

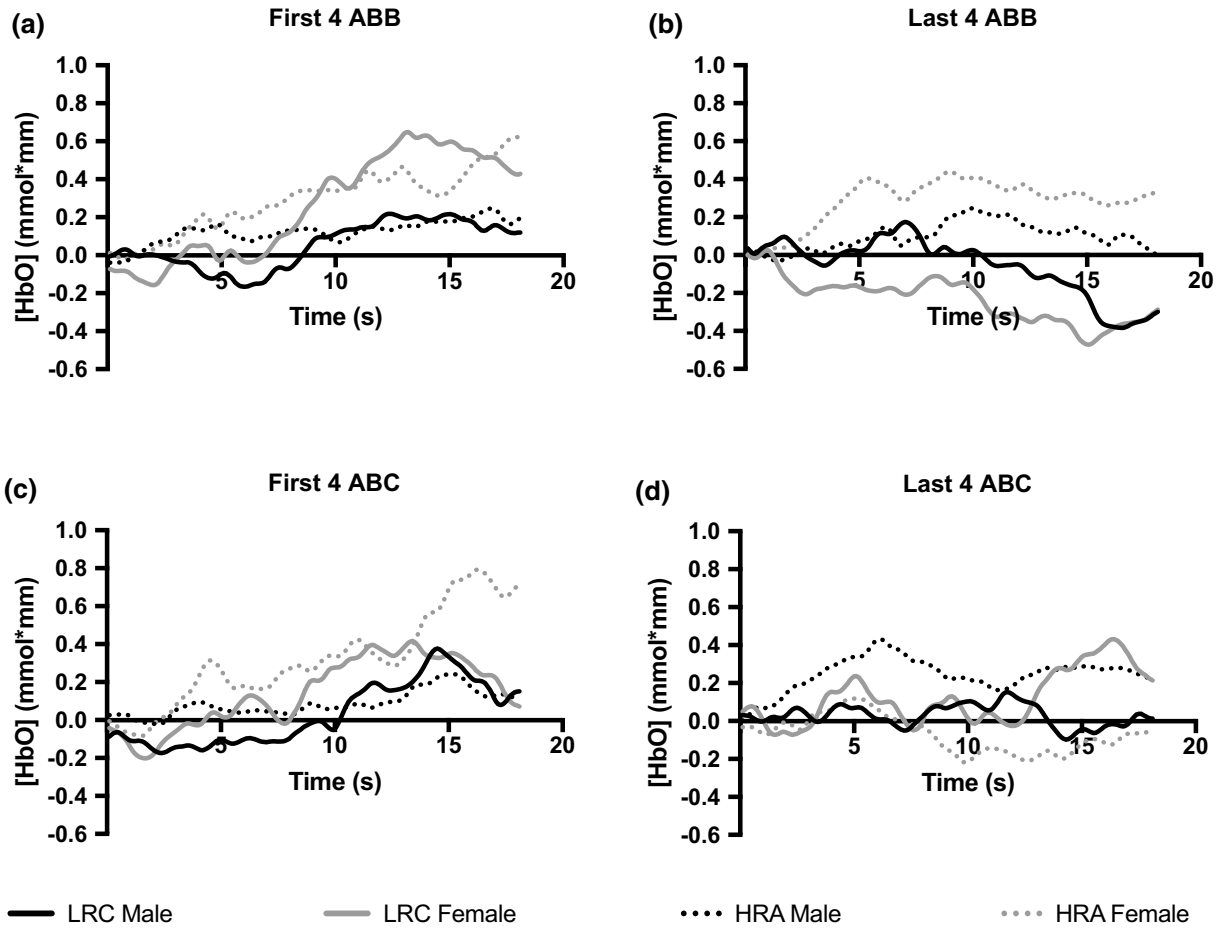

Fig. 4 Average oxyHb concentration at each time point across the 16 s time window for **a** first four ABB, **b** last four ABB, **c** first four ABC, and **d** last four ABC stimulus trials heard, broken down by gender and risk group, and collapsed across all ROIs

if non-structural characteristics were more developmentally relevant for speech perception at and after 3 months of age—they should have shown similar patterns of activation across ABB and ABC sequences. In fact, evidence for a developmental shift from the patterns of neural activation observed in neonates (Gervain et al. 2008) was presented in Wagner et al. (2011), who found a lack of neural discrimination between ABB and ABC syllable sequences in 7- and 9-month-old infants at low familial risk for ASD. The results herein therefore represent a downward extension of Wagner's findings, and suggest that this developmental shift may take place within the first 3 months of life.

Neural Responses to Speech-Like Stimuli Over Exposure

The syllable type × region × exposure × risk group × 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 (Figs. 3, 4). Examination of the four-way interaction graphs reveals that both female and male LRC infants showed a lowering of their oxyHb responses to ABB syllable sequences between the first and last 4 stimulus blocks. These results suggest that although LRC infants did not appear to discriminate ABB and ABC syllable sequences in the analysis in which all trials were averaged, their temporal responses discriminated these two syllable sequences. Specifically, whereas LRC infants appeared to show a diminution of oxyHb response to ABB sequences over time, they showed no such temporal changes in their neural responses to ABC sequences.

Although both LRC males and females showed similar patterns of change in neural activation over time to ABB stimuli (or a lack of change over time to ABC stimuli), the patterns of neural activity of LRC males did not reach statistical significance. This may be due to a combination of statistical factors, including the relatively small number of participants in this study (10 LRC males), and the smaller magnitude of the average oxyHb increase to initial

ABB stimuli in males; that is, we cannot rule out that a significant—albeit smaller—effect exists in LRC males, but that the sample herein is underpowered to detect it. Alternatively, if males and females show distinct profiles of regional neural activation to ABB stimuli at 3 months (for example, if females show fairly consistent levels of initial neural activation followed by neural suppression over time across brain regions, but males show more heterogeneous neural profiles), the analyses utilized in this study, and the aforementioned power issues, might obscure the detection of LRC males' neural activity to ABB stimuli. On the other hand, the LRC female effect we report may represent a neural precursor of language development that develops earlier on average in females than males, and which may lay the groundwork for the observed advanced early language development in females relative to males (e.g., Parke and Gauvain 2008). Longitudinal studies replicating this experimental paradigm on larger sample sizes should be conducted in order to differentiate between these potential explanations.

The lowering of oxyHb over exposure is also particularly interesting for its developmental implications in light of Gervain's finding that in newborns, oxyHb to ABB stimuli increases over exposure. One possible explanation for this difference between studies 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 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 oxyHb to ABB stimuli (in LRC females) over time might indicate a developmental change that takes place between birth and 3 months of age. Turk-Browne et al. (2008) have proposed that repeated stimuli likely receive increased neural resources and enhanced processing until they have been fully represented, after which time the brain may redirect processing efforts (manifested as neural activation) to novel stimuli. It may therefore be the case that newborns require more than 14 trials to fully represent the repetition stimuli, whereas fewer trials are necessary for a complete representation by 3 months of age, and these infants thus show neural suppression—a hallmark response of the perceptual cortex to repeated stimuli or habituation effects (Emberson et al. 2017; Grill-Spector et al. 2006; Turk-Browne et al. 2008)—to the repetition stimulus over time. If this the switch from neural enhancement to suppression is a reflection of the number of trials necessary to adequately represent a stimulus over time, 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. In past developmental psychology research in the domain of vision for example, 1 and 2 month olds showed no changes in their levels of visual attention to a stimulus presented to them repeatedly for ten 1-min periods. By 2–3 months old however, infants decreased their visual attention significantly between the first and last five exposure periods (Fantz 1964). 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). The interpretation of the findings herein as representing a neural habituation response also implies that LRC female infants habituate to syllable sequences containing consecutively repeated syllables, but do not habituate to random, non-repetitive syllable sequences.

HRA females exhibited different temporal neural response patterns than did LRC females across both ABB and ABC stimuli. Specifically, HRA females did not show the dampening of neural responses over time that LRC females exhibited; nor did they show any increases—they remained constant across exposure to ABB stimuli. While HRA females showed initial responses to ABB stimuli that were similar to their LRC peers then, they may have failed to habituate to these stimuli. These findings align with and downwardly extend those of Guiraud et al. (2011) and Seery et al. (2014), who found reduced evidence of neural habituation to auditory stimuli in 9-month-old HRA infants, using an ERP paradigm. 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 (females) 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. One unifying theory of autism even posits that the disorder may be due to impairments in predictive abilities (Sinha et al. 2014), which in turn might be manifested by, or a consequence of, impaired habituation processes, and which may give rise to functional impairments such as the aforementioned language deficits or delays.

The current findings confirm our hypothesis that 3-month-old HRA infants do not show the patterns of

exposure-based neural discrimination between repetition-based and random stimuli that LRC infants do, in the sample of females examined herein. However, our samples of LRC and HRA 3-month-old males are statistically indistinguishable from each other in terms of exposure-based neural discrimination of repetition-based and random speech-like stimuli. Additionally, our results do not replicate those of Gervain et al. (2008), who observed neural discrimination of repetition-based and random speech-like stimuli across all trials, and increasing neural activity to repetition-based stimuli with exposure, in healthy newborns. Our results therefore suggest both a developmental shift in neural activity to such stimuli between birth and 3 months of age in LRC infants, and provide preliminary evidence for potential habituation deficits or delays in precursors to language processing in HRA females. Importantly, our results suggest that such impairments, which may be an endophenotype of ASD risk, are present from much earlier than previously detected. Additionally, females and males (at both high and low risk for ASD) may show differential patterns of neural activity to language-relevant stimuli from as young as 3 months of age. Further research might investigate whether males and females exhibit qualitatively or quantitatively different patterns of neural activity to other language-relevant stimuli over development, whether the lack of oxyHb lowering to ABB stimuli in HRA children might be representative of a more generalized delay or deficit in habituation, and whether the 3-month neural signatures found in this study predict later specific language difficulties in either or both sexes.[8]

Limitations

In addition to the differences in infants' state of consciousness across Gervain et al. (2008) and the present study, another factor that confounds cross-study comparisons is the language environment of the study populations tested. Specifically, while Gervain used a French speaker's voice on a sample of Italian newborns, the current study uses stimuli matched to the language environments of infants being tested. Perceptual narrowing to acoustic features of English may therefore have affected the neural activity of participants in the current study, whereas such a phenomenon would have been less likely to affect Gervain's newborns (see Vannasing et al. 2016, for evidence suggesting that 1 day old infants show different patterns of neural activity to native and non-native speech streams).

Another limitation of the current study pertains to 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, it only provides us with adequate power ($p = 0.95$) to detect large effect sizes (partial $\eta^2 = 0.1379$; Cohen 1969) via the statistical tests used herein. The finding of a lack of discrimination between ABB and ABC stimuli in LRC infants, or the lack of statistical significance of the finding of neural habituation in LRC males, therefore, might be results of developmental effects, but might also be due to a lack of power for achieving statistical significance.[9] Despite this lack of power, several of the effect sizes found herein were in the 'medium' to 'large' range, and the findings presented and discussed indicate that neural correlates of early speech perceptual abilities are likely to be meaningful biomarkers of familial ASD risk, and thus warrant continued study.

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Author Contribution LAE, JBW, HTF, and CAN conceived of the study and its design. LAE processed, analyzed and interpreted the data, and drafted the manuscript. All authors contributed intellectual content and critical revisions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964

[8] Preliminary analyses of this nature—based on a subset of the present sample—suggest that the magnitude of the fNIRS neural response to the last four ABB stimuli predicts MacArthur-Bates Communicative Development Inventory-III (Fenson et al. 2007) gesture production ($p < 0.001$, $R^2 = 0.7005$) and sentence complexity ($p < 0.001$, $R^2 = 0.4999$), and Mullen Scales of Early Learning (Mullen 1995) expressive language scores ($p < 0.001$, $R^2 = 0.5052$) for both LRC and HRA subjects at 18 months, across gender.

[9] It is also possible that the gender effects found herein are confounding results that are instead attributable to participants' eventual ASD outcomes (since more males than females will go on to develop ASD). Unfortunately, we did not have the statistical power to test this hypothesis directly, as only 19 of the 38 infants (11 LRC, 8 HRA) analyzed herein returned for clinical evaluation and diagnosis confirmation (or lack thereof) at 36 months of age.

Helsinki declaration and its later amendments or comparable ethical standards. All study procedures were monitored and approved by the Boston Children's Hospital Institutional Review Board under IRB protocol X10-02-0083.

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