RESEARCH STRATEGY

SIGNIFICANCE

Language abilities vary among children with Autism Spectrum Disorder (ASD).[5] While ~25% of children with ASD go on to develop language abilities that are similar to their typically developing (TD) peers, the majority continue to have significant deficits in expressive and/or receptive language as they enter school-age.[6,7] Given the high prevalence of language deficits in ASD and the predictive power that early language has on various developmental outcomes,[8] it is critical that we understand what is causing these deficits. Various studies have used functional magnetic resonance imaging (fMRI) to investigate the neural mechanisms underlying language deficits in ASD. The majority of these studies have shown that compared to TD controls, individuals with ASD have reduced brain response in left-lateralized regions of the frontal and temporal lobes when listening to language.[31,32] Studies assessing functional connectivity (FC), a measure of synchronization between brain regions, in the context of language processing have found evidence of reduced inter- and intra-hemispheric FC in children with ASD.[33] While researchers have suggested that these differences in brain function may be responsible for the development of language deficits in ASD, few studies have actually explored this brain-behavior relation. Furthermore, only a handful of these fMRI studies investigated the neural bases of language processing in young, preschool-aged children with ASD.[9-11] To better understand the emergence of language deficits in ASD, we must study the brain during early, sensitive periods of language development. **The scarcity of functional neuroimaging research on preschoolers with ASD has limited our understanding of the neural bases of emerging language deficits in autism.**

Another limitation of existing fMRI studies on language processing in preschool-aged children with ASD is that findings may not be generalizable outside of the controlled lab environment. All of these studies presented language stimuli within a non-interactive context – children listened to recordings of stories while they were asleep and socially-isolated in the fMRI scanner. This is *not* how children are exposed to language in everyday life. Some fMRI studies conducted with older, TD children and adults have made neuroimaging tasks more naturalistic by presenting language with semantically matched pictures or videos.[34] Others have found creative ways to study the neural bases of language processing within simulated social interactions.[35] For example, studies that measured brain response to live versus recorded language in TD adults and school-aged children found that brain response to live language was stronger within the temporal parietal junction, superior temporal sulcus, and prefrontal cortex.[14-17] These findings demonstrate that language presented by a live partner within an interactive context modulates brain response in TD individuals. Function of these brain regions, which are involved in real-world social interactions, may be impaired in those with ASD, and may drive the development of language and communication deficits.[36] **However, *no* studies to date have investigated whether live social interaction modulates brain response to language in preschoolers with ASD.** This gap in the literature is likely due to the methodological limitations (e.g., sensitivity to motion) of using fMRI with young, atypical populations.

The proposed research project will fill this gap by using functional near-infrared spectroscopy (fNIRS), a non-invasive neuroimaging tool that affords us the opportunity to study brain function while children are awake and interacting with a live social partner.[37] It is exceptionally well suited for measuring the brains of young, atypical samples.[38] fNIRS uses near-infrared light to measure changes in concentration of oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) on the cortical surface of the brain. Increases in oxyHb and corresponding decreases in deoxyHb reflect increases in blood volume to brain regions that are experiencing increased neuronal activity/brain response.[39] Previous work has shown that the hemodynamic response measured by fNIRS is highly correlated with the BOLD signal measured by fMRI, supporting fNIRS as an alternative neuroimaging method to fMRI.[40] fNIRS has many advantages over fMRI that make it well-suited for studying language processing in preschool-aged children with ASD. fNIRS is robust to motion artifacts, quiet, and most importantly, it allows us to measure activation within and functional connectivity between relevant cortical regions of the brain while children are awake and interacting with a social partner. **Despite these advantages, no studies to date have used fNIRS to study the neural bases of language processing in preschool-aged children with ASD.** fNIRS has been successfully used to study language processing in high risk infants,[41] older children with ASD,[42,43] and TD preschoolers,[27] and has been used to measure brain function of infants during live social interactions,[44] underscoring its promise for successful use in the proposed research project. **Findings from the proposed project will elucidate the neural bases of language processing during real-world social interactions using fNIRS, a neuroimaging tool with great promise but limited application to date in autism research.**

Clinically, results from this study may provide insights into how the brains of children with ASD function during different types of language interventions. This could provide a neurobiological explanation

for why some interventions lead to language gains, and ultimately guide our selection of which interventions to use when treating children with ASD. For instance, if the brains of children with ASD respond more strongly to language presented during a live social context compared to a recorded context, this may explain why children with ASD respond so positively to interventions that target the improvement of language outcomes through naturalistic, play-based social interactions (e.g., JASPER).[45] In contrast, if the brains of children with ASD respond similarly to language presented during a recorded context and a live context, this may suggest that technology-based interventions that do not involve live social interaction may be a practical yet effective intervention option for some children with ASD.

INNOVATION

The proposed research project is innovative in three ways. First, we will **recruit a novel age range** of children with ASD. This will be the *first* study to use fNIRS to investigate the neural bases of language processing in 3- to 5-years-old children with ASD, which is a sensitive period in language development. Second, we will go beyond traditional group level analyses to investigate the relation between measures of brain function (i.e., brain response/oxyHb concentration and FC) and language abilities and communication skills. **Exploring individual differences** in brain function will help us to gain further insights into the neural mechanisms underlying the development of language deficits and heterogeneous language outcomes in ASD. Third, we have **created an original neuroimaging task** that simulates activities that children experience in everyday life (i.e., book reading and television watching). Indeed, this will be the *first* study to measure brain function in preschool-aged TD children *and* children with ASD during a live social interaction. The proposed research project will expand on previous neuroimaging work[9-11] that measured brain response to language via recorded stories by presenting stories within a live social interaction.

APPROACH

Participants

We aim to recruit two groups of children between the ages of 36- and 60-months (3- to 5-years) – $N = 50$ children with ASD and $N = 50$ typically developing (TD) age- and sex-matched controls. Children in the ASD group will have a community diagnosis of ASD, which will be confirmed using the Autism Diagnostic Observation Schedule (ADOS-2).[25] To be included in the TD control group, children must not have any developmental, behavioral, speech, or language disorders/delays, and must not have a family history of autism in any first-degree relatives. Because previous work has shown that ASD is more commonly diagnosed in males than females,[46] we will make a special effort to ensure that each group includes ~33% ($N = 16$) females. All children will be from predominately English-speaking households (i.e., English spoken >50% of the time), as stimuli and behavioral assessments will be presented in English.

Power Analyses

To justify our sample size, we conducted power analyses using the easypower[47] and pwr[48] packages in R. Our power analysis showed that with a sample size of $N = 100$ ($N = 50$ ASD and $N = 50$ TD controls), running a 2 x 2 x 5 x 2 mixed factorial ANOVA with 0.05 significance level and .80 power would allow us to detect medium ($\eta^2 = .115$) to large ($\eta^2 = .165$) main effect sizes and interaction effect sizes. Assuming the most extreme case of data loss (estimated 30% for fMRI studies on children with ASD[49]), we would be able to detect large effect sizes ($\eta^2 = .230$). Additionally, running within group correlations with 0.05 significance level and .80 power would allow us to detect medium effect sizes ($r = .385$). Sample sizes proposed here are similar to sample sizes in published fNIRS studies that have found significant group differences between those with and without ASD.[50,51] We acknowledge that the lack of pilot data for our specific fNIRS task is a limitation of this application. However, we have taken proactive steps to ensure that we can begin collecting data as soon as social distancing restrictions have been lifted and we are allowed to bring children in for testing. To date, we have created recruitment materials, stimuli, fNIRS probe and cap, and scripts for stimulus presentation and data analysis for the proposed research project.

Recruitment

Recruitment of participants will be facilitated by my access to two large registries of prior research participants, which are managed by the developmental science program at Boston University and Dr. Tager-Flusberg's Center for Autism Research Excellence (CARE). The CARE registry currently holds over 40 potential participants with ASD who are within our proposed age range. I will recruit additional participants from two larger, ongoing studies at CARE (NIDCD: P50DC018006 and SFARI: 655054).

Procedure

Each child and his/her parent will come to our lab for two to three visits, each lasting approximately two hours. The number of visits and duration of each visit will be tailored to the needs of each child, including ample time

for breaks. During the first visit, we will obtain consent from the parent, on behalf of the child. We will also explain the study to the child using a social story. A series of behavioral assessments will be completed during the first visit and second visit, as needed. At the end of the first visit, the child will undergo a standard desensitization procedure developed by Dr. Tager-Flusberg,[52] which we have successfully implemented at CARE for several years, to make sure that he/she is comfortable wearing the fNIRS cap. The parent will be given a practice fNIRS cap to continue the desensitization procedure at home. During the last visit, the child will complete the fNIRS task described below while wearing the cap.

Behavioral assessments

Children in the ASD group will receive all behavioral assessments listed below. Children in the TD control group will receive all behavioral assessments listed below, except for the ADOS-2.

Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)

The ADOS-2 is a semi-structured, standardized assessment that uses play-based activities to measure communication, social interaction, and restricted and repetitive behaviors associated with ASD.[25] We will use the ADOS-2 to confirm that participants in the ASD group meet diagnostic criteria for Autism Spectrum Disorder, and to characterize symptoms within the ASD group. ADOS calibrated severity scores will be used for Aim 3 analyses.[26]

Preschool Language Scales, Fifth Edition (PLS-5)

The PLS-5 is a measure used to evaluate two language domains – expressive communication and auditory comprehension.[23] PLS-5 total language standard scores (sum of language domains) will be used for Aim 3 analyses.

Vineland Adaptive Behavior Scales, Third Edition (Vineland-3)

The Vineland-3 is a parent-report measure used to assess children's adaptive behavior.[24] While the Vineland-3 measures various domains of behavior, we will use standard scores from the communication domain of the parent questionnaire for Aim 3 analyses.

Differential Ability Scales, Second Edition (DAS-II)

The DAS-II is a measure used to assess children's cognitive functioning.[53] We will use standard scores from the nonverbal reasoning cluster to control for children's nonverbal intelligence in all analyses.

fNIRS cap

Children will be fitted with a fNIRS cap that holds 14 light sources and 18 detectors (Figure 2a). This probe was designed in AtlasViewer[3] to ensure that channels bilaterally cover the following brain regions of interest (ROIs) – superior temporal gyrus (STG), middle temporal gyrus (MTG), inferior frontal gyrus (IFG), middle frontal gyrus (MFG), and temporal parietal junction (TPJ). ROIs were selected based on previous studies that showed activation in these regions while listening to stories and/or engaging in social interactions.[2,9-13,16,17,27,54] Average fNIRS signal across 3-4 channels within each ROI (Figure 2b) will be used for analyses.

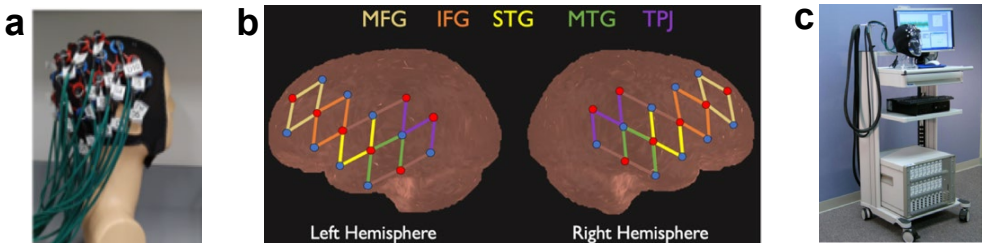

Figure 2. (a) fNIRS cap with optical fibers.[55] (b) fNIRS probe design with sources (red) and detectors (blue) projected onto 4-year-old brain template. 3-4 channels per ROI symmetrically cover both hemispheres of the brain. (c) TechEn CW6 fNIRS system.

fNIRS system

Data will be collected using the TechEn CW6 fNIRS system (Figure 2c), which emits light at 690nm and 830nm, and collects data at a 10 Hz sampling rate. Optical fibers will carry light from the fNIRS system to the sources on the fNIRS cap, and from the detectors on the fNIRS cap back to the fNIRS system. We will also use a Polhemus 3-D digitizer to approximate the MNI coordinates for sources, detectors, and reference points (Cz, Nz, Iz, Lm, Rm) on the child's head, which we will later be used to create activation maps.

fNIRS Task

Once the cap is placed on the child's head, we will begin the fNIRS task. For this task, the child will listen to two different stories. The stories, which are called "Winter" and "Summer," were created by the study PI/F31 applicant and reviewed by speech language pathologists to be matched on number of words, syntax, vocabulary, content/sequence of events, and number of characters (Figure 3d). One story will be presented in the live condition and the other story will be presented in the recorded condition (Figure 3a). In the **live condition**, the live experimenter will initiate interaction with the child by reading a scripted story to him/her from

a book. While listening to the story, the child will be seated next to the experimenter so that he/she can see each page of the book. Each page of the book, which will serve as one trial, will include one illustration and text that matches what the child is hearing. In the **recorded condition**, the child will listen to an audio recording of the same experimenter reading a different scripted story. Each trial will include one illustration and text that matches what the child is hearing, which will be presented through a computer screen instead of a book. In this condition, the experimenter will remain seated next to the child, but will *not* initiate interaction with the child. Each story has been divided into segments to create 18 trials that last 10 seconds each. In between each trial, we will present a jittered fixation cross for 10 to 15 seconds to ensure that the hemodynamic response returns to baseline before the next trial begins (Figure 3b). 18 trials of the same condition will be presented during the first "run," followed by a short break, and then 18 trials of the other condition will be presented during the second "run" (Figure 3c). The order of conditions, as well as the story used in each condition, will be randomized and counterbalanced across participants so that each child receives both conditions and hears both stories. Data collected from both conditions will be used to address Aim 1, while data collected from the live condition will be used to address Aims 2 and 3.

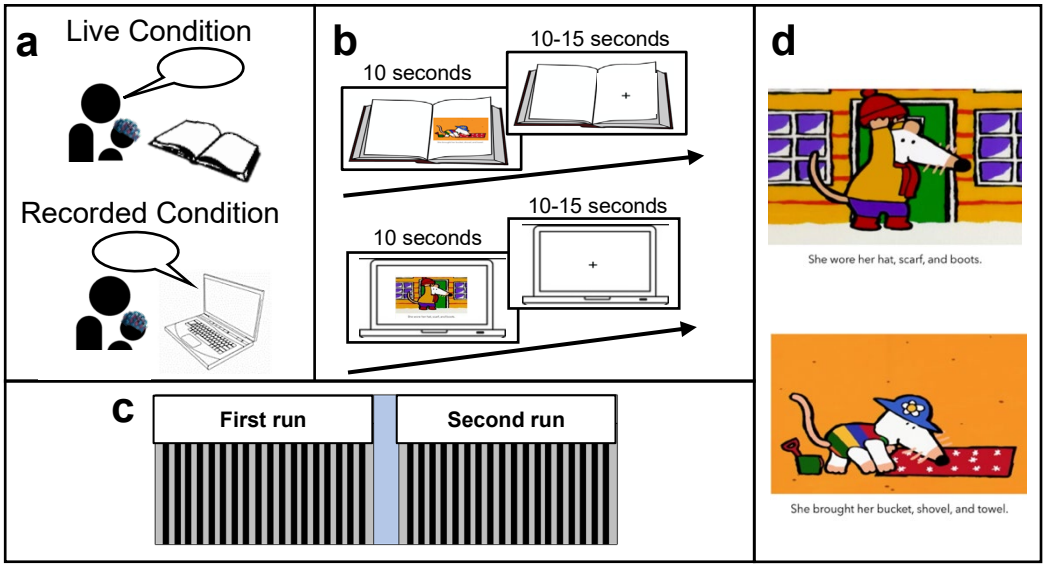

Figure 3. (a) fNIRS task has two conditions – live and recorded. (b) Example of stimulus presentation for each condition. (c) During the first run, 18 trials of the same condition will be presented. During the second run, 18 trials of the other condition will be presented. Participants will be given a short break in between runs (blue bar). Each trial (black bars) will be proceeded and followed by a fixation cross (grey bars). (d) Example of matched trials from the Winter and Summer stories.

The entire task will take 6 to 8 minutes to complete, not including set-up time or breaks. We have found that incorporating breaks and rewards throughout testing keeps young children engaged and motivated. Nevertheless, we acknowledge that young children may have difficulty maintaining attention during our fNIRS task. To account for this, we plan to manually trigger the beginning of each trial in both conditions so that we can ensure that the child is attending to the book or screen before the trial begins. To account for any experimenter error in manual triggers, we will video-record the child's behavior during the fNIRS task and later use behavioral coding to exclude trials during which the child was not visually attending to the book/computer screen at the start of the trial or for >50% of the total trial time.

fNIRS data processing

fNIRS data will be processed using Homer3, a MATLAB Guided User Interface.[4] We will first exclude data from "noisy" channels in which the raw signal exceeds our threshold of 0.1 to 4.95 for more than 5 seconds.[41] With remaining usable data, we will transform the raw signal to optical density (OD). Next, we will apply a low-pass filter of 0.5 Hz and a high-pass filter of 0.01 Hz to exclude biological noise (i.e., heart rate, respiration) and slow drift from the fNIRS system. We will use the gold-standard hybrid spline interpolation Savitzky-Golay method to correct for motion artifacts (i.e., baseline shifts and high frequency spikes) in the fNIRS signal.[56] After correcting for motion artifacts, we will convert OD to oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) concentration values using the modified Beer-Lambert law. Finally, the hemodynamic response function will be estimated using general linear modeling (GLM). For each participant, condition, and ROI, we will calculate average oxyHb concentration values to use for Aim 1 and Aim 3 statistical analyses, as previous literature has shown that oxyHb has a better signal to noise ratio than deoxyHb.[57] We will be able to visualize the precise location of brain response using AtlasViewer and the MNI coordinates obtained from the Polhemus 3-D digitizer.[3] For functional connectivity (FC) data processing, we will use the average hemodynamic response time courses from 10 different "seeds" – left STG, right STG, left MTG, right MTG, left IFG, right IFG, left MFG, right MFG, left TPJ, and right TPJ. To quantify FC between seeds, we will calculate Pearson's correlation coefficients between each seed's time course and the time course of all other seeds.[19,20,22,58] Ultimately, this will provide information about *inter-hemispheric FC* between ROIs on different sides of brain and *intra-hemispheric FC*

between ROIs on same side of brain. Correlation coefficients will be normalized using Fisher's z transformation; z-transformed FC values will be used for Aim 2 and Aim 3 statistical analyses. To ensure that FC data contain only spontaneous cortical activity, we will regress out stimulus structure using GLM.[22]

Planned analyses and expected results

***AIM 1*: Determine how live social interaction modulates brain response to language in preschool-aged children with and without ASD.**

Planned analyses: We plan to conduct a 2 x 2 x 5 x 2 mixed factorial ANOVA to examine the effects of group (ASD, TD control), condition (live, recorded), brain ROI (STG, MTG, IFG, MFG, TPJ), and hemisphere (left, right) on oxyHb concentration values (i.e., brain response). We will control for chronological age and nonverbal intelligence using DAS-II standard scores. Significant main and interaction effects will be explored post-hoc using the False Discovery Rate method for multichannel fNIRS data.[59] Before conducting this mixed factorial ANOVA, we will conduct a preliminary ANOVA to make sure that the order in which participants received conditions (live condition first, recorded condition first) and stories (Summer story first, Winter story first) does not influence brain response. If this preliminary ANOVA shows that order of conditions and/or stories does influence brain response, then we will control for these factors in our mixed factorial ANOVA.

Expected results: We hypothesize that within the TD control group, brain response will be greater during the live condition compared to the recorded condition in all ROIs.[13,15-17] This finding would suggest that the presence of a live social partner *does* modulate brain response to language in TD children. Within the ASD group, we hypothesize that brain response will be similar across both conditions in all ROIs (Figure 4a),[12,14] suggesting that language presented by a live social partner *does not* modulate brain response in children with ASD. Alternatively, brain response in the ASD group may be greater in the live condition compared to the recorded condition, but lower overall in the ASD group compared to the TD control group (Figure 4b).[9-11] This alternative finding would suggest that the language presented during a live social interaction does modulate brain response in children with ASD, but that brain response to language more generally is reduced. Based on previous work, it's likely that this reduced brain response in the ASD group will be found in left-lateralized ROIs.[9-11] Yet another possibility is that effects will vary based on ROI and/or hemisphere. For example, within the ASD group, some ROIs may show similar activation during both conditions, while other ROIs, such as the right STG and TPJ, may show greater activation during the live condition compared to the recorded condition.[14] Such findings would suggest that live social interaction does modulate brain response to language in children with ASD, but that "liveness" is processed in different regions of the brain.

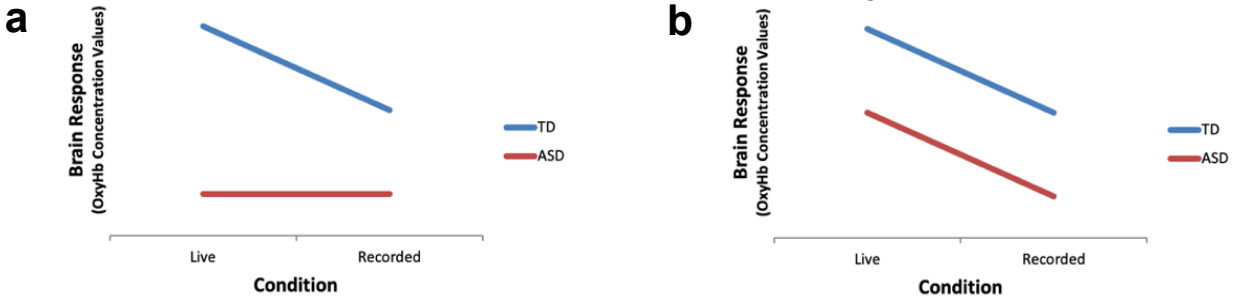

Figure 4. (a) Expected results and (b) alternative results for aim 1.

***AIM 2:* Examine functional connectivity (FC) in preschool-aged children with and without ASD during a live social interaction.**

Planned analyses: Using calculated inter- and intra-hemispheric FC values (see "fNIRS data processing" section above), we will assess between-group differences (ASD versus TD control) in FC during the live condition of the fNIRS task using independent samples *t*-tests.[19,21,22]

Expected results: We hypothesize that the ASD group will demonstrate reduced inter- and intra-hemispheric FC relative to the TD control group.[18-22] Based on findings of previous FC studies, we expect that the ASD group will exhibit reduced inter-hemispheric FC between the left and right STG and the left and right IFG,[22] and reduce intra-hemispheric FC between the left IFG and left MTG.[20]

***AIM 3:* Investigate the relation between brain function during a live social interaction and language abilities, communication skills, and autism severity in preschool-aged children with and without ASD.**

Planned analyses: We will conduct within-group partial correlations to examine the relation between two measures of brain function (brain response and FC) during the live condition and total language standard scores on the PLS-5 (i.e., language abilities) and standard scores on the communication

domain of the Vineland-3 (i.e., communication abilities). Within the ASD group, we will also explore partial correlations between these measures of brain function and ADOS-2 calibrated severity scores (i.e., autism severity). For partial correlation analyses, we will control for chronological age and DAS-II standard scores (i.e., nonverbal intelligence) and correct for multiple comparisons.

Expected results: We hypothesize that within both groups, greater brain response and FC will be related to better language abilities and communication skills.[10,11,22,27] In the ASD group, greater brain response and FC will be related to lower autism severity.[17,28-30] While we do not have any *a priori* hypotheses which seeds or ROIs will show this effect, we expect that language and communication abilities will be correlated with right-lateralized brain response in the ASD group[11] and left-lateralized brain response in the TD control group.[10,27] These findings would suggest that individual differences in language and communication are linked to brain function in both typical and atypical development.

Potential pitfalls and alternative strategies

How will you account for individual differences in social behaviors exhibited by children during the fNIRS task?

It is possible that some children will try to initiate interactions with the experimenter during the task. To account for this variability in children's social behaviors, we will video-record both conditions, and later use behavioral coding to separate trials where the child initiated interaction with the experimenter (e.g., visual attention, pointing gestures, verbal engagement)[60] from trials where the child did not initiate interaction with the experimenter. As needed, we will then conduct exploratory analyses to determine whether brain response differs during these trials. These exploratory analyses will help us to draw a direct relation between the brain response and social behavior of participants.

How will you maintain experimental control during these naturalistic social contexts?

We have taken steps to ensure that our task is naturalistic while still maintaining experimental control. If the child initiates interaction, the experimenter will respond to the child with a scripted response (e.g., "mhm" or "yes, I see" or "let's see what's next"). Also, while we acknowledge that including visual stimuli (illustrations and text) in our trials could be perceived as a confounding variable, we decided to include visual stimuli along with auditory language stimuli to make the task comparable to activities that children experience during everyday life (e.g., shared book reading and television watching). We have taken careful consideration to ensure that auditory and visual stimuli are matched across conditions.

Why aren't you measuring activity on the entire cortical surface of the brain?

Previous work has shown that language activates regions of the parietal and occipital lobes in older individuals with ASD.[24] Unfortunately, the fNIRS system we are using cannot accommodate high density probes that cover all cortical brain ROIs. Increasing the number of ROIs in statistical analyses would also necessitate a larger sample size, which may not be feasible for the timeline of the proposed project. For these reasons, we selected ROIs in the frontal and temporal lobes that are typically involved in language processing. We hope that future studies will extend our findings by measuring activation in other ROIs using denser fNIRS probes.

Proposed Research Project and the NIDCD Strategic Plan

The proposed research project supports the National Institute on Deafness and Other Communication Disorders (NIDCD) mission of funding biomedical and behavioral research on the normal and disordered processes of language, as we will be using a multimethod approach (fNIRS and behavioral assessment) to study language processing in TD children and children with ASD. This project will investigate how the brains of children with ASD function and respond to language presented during a live social interaction (Aims 1 and 2), which directly aligns with the Voice, Speech, and Language Program's extramural research on "understanding of the neural bases of language disorders" and developmental communication disorders. The proposed research also targets several goals outlined in the 2017-2021 NIDCD Strategic Plan, particularly related to Priority Area 2: Understanding Diseases and Disorders. The NIDCD lists the following under this priority area:

- *Pathophysiology: Identify the pathophysiologic and cognitive mechanisms underlying both common and rare voice, speech, and language impairments.*
- *Developmental and Neural Plasticity: Examine changes in brain structure and functioning in response to behavioral, pathologic, or environmental insult as a basis for voice, speech, and language impairments with an emphasis on developmental timing.*

By exploring the relation between brain function and behaviorally assessed language abilities (Aim 3), we will be able to draw conclusions about the neural mechanisms underlying language impairments in ASD. Our project will focus on preschool-aged children (3- to 5-year-olds) specifically to improve our understanding of brain and language development during this sensitive period.