2016

Changes In Intrinsic Local Functional Connectivity In Children With Autism Followed By A Visualizing/Verbalizing Reading Intervention

Jose Omar Maximo
University of Alabama at Birmingham

CHANGES IN INTRINSIC LOCAL FUNCTIONAL CONNECTIVITY IN CHILDREN WITH AUTISM FOLLOWED BY A VISUALIZING/VERBALIZING READING INTERVENTION

by

JOSE O. MAXIMO

RAJESH K. KANA, PH.D., COMMITTEE CHAIR
FRED J. BIASINI, PH.D.
KRISTINA M. VISSCHER, PH.D.

A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Master of Art

BIRMINGHAM, ALABAMA

2016

CHANGES IN INTRINSIC LOCAL FUNCTIONAL CONNECTIVITY IN CHILDREN WITH AUTISM FOLLOWED BY A VISUALIZING/VERBALIZING READING INTERVENTION

JOSE O. MAXIMO

LIFESPAN DEVELOPMENTAL PSYCHOLOGY

ABSTRACT

Deficits in language are a major clinical feature of autism spectrum disorders (ASD). Previous studies suggest that individuals with ASD may rely on visuospatial skills to compensate for these deficits. Existing intervention programs in autism have not been tested at the neural level, thus falling short of quantifiable neurobiological changes underlying behavioral improvement. The current study takes a translational neuroimaging approach to test the impact of a structured visual imagery-based reading intervention on improving reading comprehension and its underlying neural circuitry. Changes in connectivity of a set of brain regions commonly known as the “reading network” were examined in children with ASD who are good readers, but poor comprehenders. Behavioral and resting state functional MRI (rs-fMRI) data were collected from participants with ASD who were randomly assigned to an Experimental group (ASD-EXP; n = 15) and a Wait-list control group (ASD-WLC; n = 14). Both groups were scanned pre- and post-intervention, with the ASD-EXP receiving the intervention before their second scan and the ASD-WLC after their second scan. Participants went through an established reading intervention training program (Visualizing and Verbalizing for language comprehension and thinking or V/V; 4-hours per day, 10-weeks, 200 hours of face-to-face instruction) created by the Lindamood-Bell Learning Processes. For the resting state scans, the participants were asked to look at a fixation cross, rest and relax in the MRI scanner. Local functional connectivity was examined using graph theory. The main results are as follows: I) the ASD-EXP group showed significant improvement in their reading comprehension ability evidenced from comprehension scores; whereas, this effect was absent in the ASD-WLC group; II) the ASD-EXP group showed increased local brain connectivity in reading network regions compared to the ASD-WLC group post-intervention; III) intervention-related changes in local brain connectivity were observed in the ASD-EXP group in reading network regions; and IV) improvement in language comprehension significantly predicted changes in local connectivity in the reading network. The findings of this study will provide insights into understanding brain plasticity in children with developmental disorders using targeted intervention programs.

Keywords: *ASD, intervention, local connectivity, translational neuroscience, brain plasticity*

ACKNOWLEDGMENTS

First and foremost, I would like to express my appreciation for the participants and their families who took part in this study. Without the support of the community that it serves, research cannot continue. I am particularly grateful to my mentor, Dr. Rajesh Kana, for providing me with guidance throughout my thesis and with research training that will be useful in future endeavors. I would also like to thank my committee members, Drs. Fred Biasini and Kristina Visscher, for their valuable input and insight into this project. I would also like to acknowledge the people who assisted in collecting the data and conducting the study. Finally, I am incredibly grateful to my family and friends for being supportive of me throughout this experience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Development of reading skills (both decoding and comprehension) is an important predictor of individual success in literate societies. Reading is a uniquely human skill that has generated considerable interest from evolutionary as well as from scientific perspectives. Comprehension is the ultimate goal of reading, which is crucial in learning and acquiring knowledge. Many of these skills contribute to a child's reading comprehension abilities and are often categorized as higher and lower in language processing (Cain, Oakhill, Barnes, & Bryant, 2001). For example, word recognition is considered a lower-level processing skill, and although the ability to decode and identify words accurately allows resources to be devoted to comprehension, it is often not sufficient for adequately understanding text. In contrast, inference and integration are considered higher-level processing skills as they aid the construction of a meaning-based representation of the text (Hannon & Daneman, 2001). These skills are important for comprehension because they help the reader to construct an integrated and coherent model of text meaning.

Impairment in verbal and non-verbal communication is one of the major characteristics of autism spectrum disorders (ASD), and is part of the DSM-5 diagnostic criteria (American Psychiatric Association, 2013). With a current prevalence rate of 1 in 45 children being diagnosed with ASD (Zablotsky, Black, Maenner, Schieve, & Blumberg, 2015), autism is a pediatric health issue of growing urgency. Thus, there is a growing need for a better understanding of the pathobiology of ASD in order to enhance the pace of diagnosis and to develop and apply interventions targeting core impairments.

Language and communication deficits are a core feature of ASD, which usually extend to impairments in reading comprehension (Nation, Clarke, Wright, & Williams, 2006). These deficits in reading comprehension are of great importance, particularly to the school setting because academic achievement varies widely in students with ASD and also because of a demonstrated discrepancy between expected achievement (based on intellectual functioning) and actual achievement in at least one of spelling, word reading or basic number skills in children ASD (Brown, Oram-Cardy, & Johnson, 2013; Estes, Rivera, Bryan, Cali, & Dawson, 2011). Behavioral studies have reported high-functioning children with ASD having problems with different aspects in higher level processing skills (reading comprehension) including pragmatics, semantics, and phonological processes (Groen et al., 2010; Williams, Botting, & Boucher, 2008), while their lower level processing skills (decoding and word identification) remain relatively intact (Norbury & Nation, 2011). Simultaneously, neuroimaging research has demonstrated alterations in the synchronization of brain activity underlying different aspects of language comprehension, including semantics and integration of social information (Groen et al., 2010), lexical over thematic processing (Just, Cherkassky, Keller, & Minshew, 2004), and pragmatics and syntax (Groen, Zwiers, van der Gaag, & Buitelaar, 2008). In typically developing (TD) individuals, the brain regions that are crucial for reading comprehension show primarily greater left than right hemisphere activation. For example, the left inferior frontal gyrus (LIFG) or Broca’s area has generally been implicated in semantics, and the left posterior superior temporal gyrus (LpSTG) or Wernicke's area in lexical access (Turkeltaub, Gareau, Flowers, Zeffiro, & Eden, 2003). Other regions such as the left posterior middle temporal gyrus (LMTG), superior temporal sulcus (STS), and middle frontal gyrus (MFG) are also implicated in reading comprehension (Tomasi & Volkow, 2012). Individuals with ASD, on the other hand, seem to recruit right hemisphere homologues of language regions (Eyler, Pierce, & Courchesne, 2012; Mason, Williams, Kana, Minshew, & Just, 2008; Redcay & Courchesne, 2008) such as right frontal and temporal cortex, and brain regions primarily involved in visuospatial processing such as fusiform gyrus (BA19, 37), medial parietal cortex (BA7), and posterior MTG (BA21) (Samson, Mottron, Soulieres, & Zeffiro, 2012). Thus, the neural response to language comprehension in ASD is atypical with compensatory recruitment of regions from right hemisphere and posterior areas (Herringshaw, Ammons, DeRamus, & Kana, 2016).

Early behavioral intervention studies targeting reading comprehension deficits in ASD have been sparse and have only examined behavioral performance with no neurobiological measures (El Zein, Solis, Vaughn, & McCulley, 2014). To better understand the observed language comprehension deficits in ASD and to address them at the neurobiological level, research examining behavioral interventions in combination with neural level inferences are needed (Calderoni et al., 2016). Particularly in ASD, two recent neuroimaging studies (using functional MRI) have shown increased brain activation and functional connectivity (the temporal correlation of time series between different brain regions) in regions associated with language processing as the result of behavioral intervention (Murdaugh, Deshpande, & Kana, 2016; Murdaugh, Maximo, &

Kana, 2015). In addition, such changes in neural indices were found to be correlated with improvement in language comprehension in a group of children with ASD. Although these are the first two studies attempting to address this gap, more such studies are needed in order to uncover the effect of behavioral remediation on the brain in ASD.

While task-based fMRI studies are important in addressing the recruitment of brain regions in relation to computational demand (cognitive/linguistic), they are also constrained by factors such as subject compliance, response time, and variations in task-performance (Maximo, Cadena, & Kana, 2014). The advent of resting state functional MRI (rs-fMRI) has marked a paradigm shift in the field of neuroimaging (Raichle, 2009) and has opened new doors for understanding the neurobiology of complex disorders. More recently, studies have used rs-fMRI to assess brain functioning underlying reading comprehension in TD individuals (Koyama et al., 2011; 2010; Tomasi & Volkow, 2012). Previous fMRI studies have identified a set of brain regions that activate more during reading comprehension (Lohmann et al., 2010; Turkeltaub, Eden, Jones, & Zeffiro, 2002). Koyama and colleagues expanded upon this work to identify the extent of this activation across children and adults (Koyama et al., 2011; 2010). These regions were described as the "reading network", which encompasses inferior occipital gyrus, fusiform gyrus, superior temporal gyrus (STG), pre/postcentral gyrus, intraparietal sulcus (IPS), supplementary motor area (SMA), inferior frontal and middle frontal gyrus (IFG, MFG), and thalamus. Only one study so far has examined the reading network exclusively in ASD in the context of an intervention study (Murdaugh et al., 2015).

Despite significant progress in examining brain network connectivity in ASD over the past few years (Kana, Uddin, Kenet, Chugani, & Muller, 2014; Maximo et al., 2014), the majority of findings of atypical brain connectivity in ASD have focused on long-distance connectivity (Nair et al., 2014). While these findings are important, local connectivity (examining the time series correlations in fMRI between parts of cortex that are spatially near each other) may also provide significant information about the specialization and integration of brain functions. Local connectivity is a relatively ill-defined concept, which can encompass a spatial scale from a few microns to millimeters and even to centimeters. For the proposed study, local connectivity will refer to the BOLD time-series correlations between a reference voxel and its nearest neighbors within a 14mm radius (See Methods). Functional differences in local connectivity in individuals with ASD may underlie anatomical and microstructural differences. Evidence from postmortem studies suggests that cytoarchitectonic abnormalities exist in cerebral cortex (Amaral, Schumann, & Nordahl, 2008) in cases with ASD. In particular, tighter packing of cortical minicolumns with reduced lateral inhibition (Casanova & Trippe, 2009) could likely affect local connections in ASD. More numerous and thinner minicolumns may result in a "hyper-specific brain" in individuals with ASD (Casanova et al., 2006). Theoretical accounts of atypical local connectivity in ASD (Belmonte et al., 2004; Courchesne & Pierce, 2005; Rippon, Brock, Brown, & Boucher, 2007) may be consistent with some empirical findings indicating increased cortical excitation/inhibition ratios (Rubenstein & Merzenich, 2003).

In the present study, we examined rs-fMRI data before and after a reading intervention program to assess changes in local connectivity of the reading network in a group of language-impaired high-functioning children with ASD. The intervention used in this study (V/V: Visualizing and Verbalizing for language comprehension and thinking) has been found to be successful in children with reading disorders, but has never been applied to study children with ASD. This intervention is a practical application of the principles of dual coding theory, which posits that cognition involves the activity of two distinct subsystems, a verbal system specialized for dealing directly with language, and a non-verbal (imagery) system specialized for dealing with non-linguistic objects and events (Pavio, 2007; Sadoski & Paivio, 2001). The intervention relies primarily on visual imagery, which has important implications considering that visual imagery is an area of strength in individuals with ASD (Joseph, Keehn, Connolly, Wolfe, & Horowitz, 2009). In addition, visual imagery can aid in developing both oral and reading comprehension (Bell, 1991a, 1991b). We hypothesize that children with ASD who participate in the reading intervention would show stronger local functional connectivity in relatively posterior regions of the reading network. This study is novel in its focus on translational neuroimaging and the findings will have a significant impact on understanding and in applying targeted behavioral interventions to children with ASD.

OBJECTIVES

The overarching goal of the proposed project is to examine the effects of a rigorous reading intervention program on reading comprehension abilities and local functional connectivity in a group of language-impaired high-functioning children with ASD.

Aim 1

Examine the impact of the Visualizing/Verbalizing (V/V) reading intervention on reading comprehension abilities in a group of language-impaired high-functioning children with ASD.

Hypothesis 1

The experimental group (ASD-EXP), which receives the intervention prior to their second scan, will show increased reading comprehension abilities (assessed by the Gray Oral Reading Test [GORT-4]) compared to the waitlist control (ASD-WLC) group. Specifically, the ASD-EXP group will show a significant increase in reading comprehension abilities from Phase1 to Phase2, whereas the ASD-WLC will not show such increase.

Aim 2

Examine the impact of the Visualizing/Verbalizing (V/V) reading intervention on local functional connectivity in regions of the reading network using rs-fMRI in a group of language-impaired high-functioning children with ASD.

Hypothesis 2a

The ASD-EXP group will show an increase local functional connectivity in regions associated with visuospatial processing, such as superior parietal and occipital areas within the reading network (Koyama et al., 2011), compared to the ASD-WLC group (while controlling for Phase1 local connectivity in both groups). This is based on our previous study that examined long-distance rs-fMRI on a sample of children with ASD that were exposed to the V/V reading intervention (Murdaugh et al., 2015).

Hypothesis 2b

Intervention-related effects will be observed in the ASD-EXP group in occipital and superior parietal regions within the reading network, such that Phase2 local functional connectivity will increase relative to that in Phase1. This is also based on our previous study.

Aim 3

To examine the relationship between potential improvement in reading comprehension skills and changes in local functional connectivity of the reading network in a group of language-impaired high-functioning children with ASD.

Hypothesis 3

Improvement in reading comprehension (assessed by percent change in GORT-4 comprehension subtest score from Phase1 to Phase2 as an intervention outcome) will significantly predict a positive relationship with changes (subtracting Phase2-Phase1) in local connectivity in the ASD-EXP group (while controlling for verbal IQ) in regions associated within the reading network.

METHODS

Participants

Twenty-nine children with ASD (mean age = 10.6 years), who underwent two fMRI sessions, 10 weeks apart, were randomly assigned to participate in the V/V intervention either between their first and second imaging sessions (ASD-EXP; n = 15) or after completing both imaging sessions (ASD-WLC; n = 14). Children were determined to have an ASD diagnosis by either a licensed clinical psychologist using the Autism Diagnostic Observation Schedule (ADOS: Lord et al., 2000) and/or the Autism Diagnostic Interview-Revised (ADI-R: Lord, Rutter, & Le Couteur, 1994). The ASD-EXP and ASD-WLC groups did not differ on age ($t[27] = -1.68$, $p = 0.10$), verbal IQ ($t[27] = 0.05$, $p = 0.96$), and reading comprehension skills measured by GORT-4 ($t[27] = -1.59$, $p = 0.12$) prior to the first fMRI session. Among the 29 children with ASD, 4 were female (2 in the ASD-EXP group and 2 in the ASD-WLC group), and all were right-handed **(Table 1)**.

Table 1. Participants' Characteristics at Phase1

	Total ASD participants (N = 29)		
	ASD-WLC (n = 14)	**ASD-EXP (n = 15)**	***p*-value**
Gender	12M, 2F	13M, 2F	-
Age	11.0 ±1.2 (9-13)	10.1 ±1.6 (8-14)	0.10
Full IQ	96.4 ±15.8 (84-112)	94.7 ±14.3 (77-109)	0.76
Verbal IQ	88.2 ±7.5 (77-100)	91.9 ±10.2 (72-108)	0.96
GORT-4 (comprehension)	85.4 ±11.7 (65-105)	78.0 ±13.1 (70-95)	0.12
SORT-R (decoding)	106.8 ±7.4 (95-116)	107.6 ±8.8 (98-127)	0.81

Note: Value ±standard deviation (range).

Participants with ASD were recruited through multiple sites, such as the Civitan-Sparks Clinic at UAB, Mitchell's Place for Autism in Birmingham, the Autism Spectrum Disorders Clinic at the University of Alabama, through the Alabama Autism Society, from the greater Birmingham area, and from nearby cities, such as Montgomery, Mobile, Huntsville, and Tuscaloosa. In addition, the Lindamood-Bell Learning centers across the country recruited potential participants from their centers. All participants with ASD met the following inclusion criteria: ages 8 to 13 years, current diagnosis of ASD as specified above, right-handed, and be recommended for the V/V intervention, indexed by being a native English speaker, having a Slosson Oral Reading Test - Revised (SORT-R) reading score of at least 37^{th} percentile and/or a Gray Oral Reading Test – Fourth Edition (GORT-4) accuracy score of at least 25^{th} percentile, a GORT-4 comprehension score below 37^{th} percentile, and a Verbal IQ score of at least 75, as measured by the Wechsler Abbreviated Scale of Intelligence (WASI). Participants failing to meet any of the inclusion criteria and participants currently taking beta-blockers or vasodilators, a history of ferromagnetic material or neurostimulators in the body, claustrophobia, history of kidney disease, seizure disorders, diabetes, hypertension, anemia, or sickle cell were excluded from the study. All participants were medication naive at the time of their imaging session. All participants' legal guardians gave written informed consent and all participants gave written informed assent, approved by the UAB Institutional Review Board, to participate in the study and were compensated for their participation. For a more detailed description of how the V/V intervention was administered, please refer to Murdaugh et al. (2015).

Data Acquisition

The MRI data were collected using a Siemens 3 Tesla Allegra head-only Scanner (Siemens Medical Inc., Erlangen, Germany) located at the UAB Civitan Functional Neuroimaging Laboratory (CFNL). Functional MR images were acquired using a single-shot T2*-weighted gradient-echo EPI pulse sequence. We used TR= 1000 ms, TE =

30ms, and a 60° flip angle for 17 oblique axial slices 5 mm slice thickness with a 1 mm slice gap, a 24 X 24 cm FOV, and a 64 X 64 matrix, resulting in an in-plane resolution of 3.75 X 3.75 X 5 mm^3. For the eyes open resting state scan, a total of 419 volumes were acquired for a total scan time of 6 min 59 s, with a TR of 1000 ms

Data Preprocessing

Functional images were processed using the Statistical Parametric Mapping software (SPM12: Wellcome Department of Cognitive Neurology, London, UK) and Analysis of Functional NeuroImages software (AFNI: Cox, 1996). Images were corrected for head motion by registering each functional volume to the first time point of the scan, coregistered and normalized to the MNI152 template, and resampled to 3mm isotropic voxels.

Six rigid-body motion parameters (translation: x, y, z directions; and rotation: pitch, roll, yaw angles) acquired from motion correction and their derivatives were regressed from the images. To account for the signal from cerebral white matter and lateral ventricles, masks were defined using the WFU PickAtlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). Masks were trimmed to avoid partial-volume effects, and an average time-series for each region was extracted. Derivatives for head motion parameters, white matter and ventricular time series were also computed. Sources of noise (head motion, white matter, and lateral ventricles plus derivatives for a total of 16 nuisance regressors) were modeled and removed using a general linear model, and residuals were low bandpass filtered ($0.008 < f < 0.08$ Hz) to isolate spontaneous, low-frequency BOLD signal fluctuations (Cordes et al., 2001) and smoothed using a 8mm Gaussian kernel.

Because head motion can impact functional connectivity analyses (Satterthwaite et al., 2013; Van Dijk, Sabuncu, & Buckner, 2012), the following precautions were taken. Head motion was quantified as the Euclidean distance calculated from the six rigid-body motion parameters for two consecutive time points. For any instance >1.5mm, considered excessive motion, the time point as well as the immediately preceding and subsequent time points were censored, or "scrubbed" before low bandpass filtering (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). If two censored time points occurred within ten time points of each other, all time points between them were also censored. Average head motion over each participant's session was defined as the root mean square of displacement (RMSD) and did not significantly differ between all groups [ASD-WLC vs. ASD-EXP Phase1 (all *p's* > 0.1); ASD-WLC vs. ASD-EXP Phase2 (all *p's* > 0.2); ASD-WLC Pre vs. Post (all *p's* > 0.4); and ASD-EXP Pre vs. Post (all *p's* > 0.3)].

Local Connection Density and Reading Network

Local functional connectivity was assessed using local connection density, as previously reported in healthy adults (Sepulcre et al., 2010). In graph theory, connection "density" is defined as the number of "edges" (connections) of a "node" (voxel) in proportion to the total number of possible edges (Bullmore & Sporns, 2009). This measure calculates the "degree" of each voxel, i.e., the number of neighboring voxels with significant BOLD time series correlations at $r \geq 0.25$ ($p < 0.001$) within a 14 mm radius from the reference voxel **(Figure 1A-B)**, based on the Euclidean distance between the centers of the voxel. Then, the reference voxel is assigned a number based on the sum of connected degrees, and this process is completed in every other voxel within the whole brain. To generate connectivity maps and to run statistical analyses, local degree maps were converted to z-scores **(Figure 1D)**.

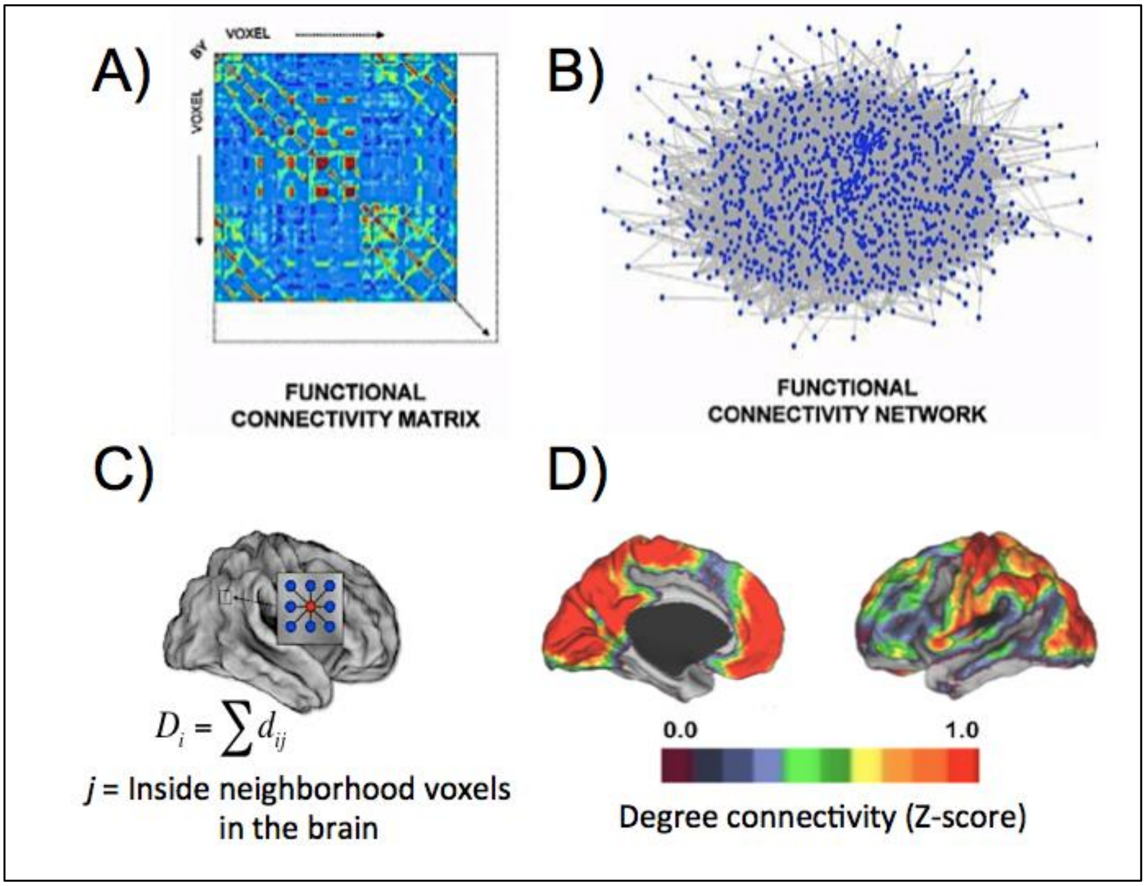

Figure 1. Visual representation of local connection density.

Using this approach, Sepulcre et al. (2010) proposed that the human brain exhibits distinct connectivity profiles with regions displaying increased preferential local connectivity while others have reduced local connectivity **(Figure 2)**.

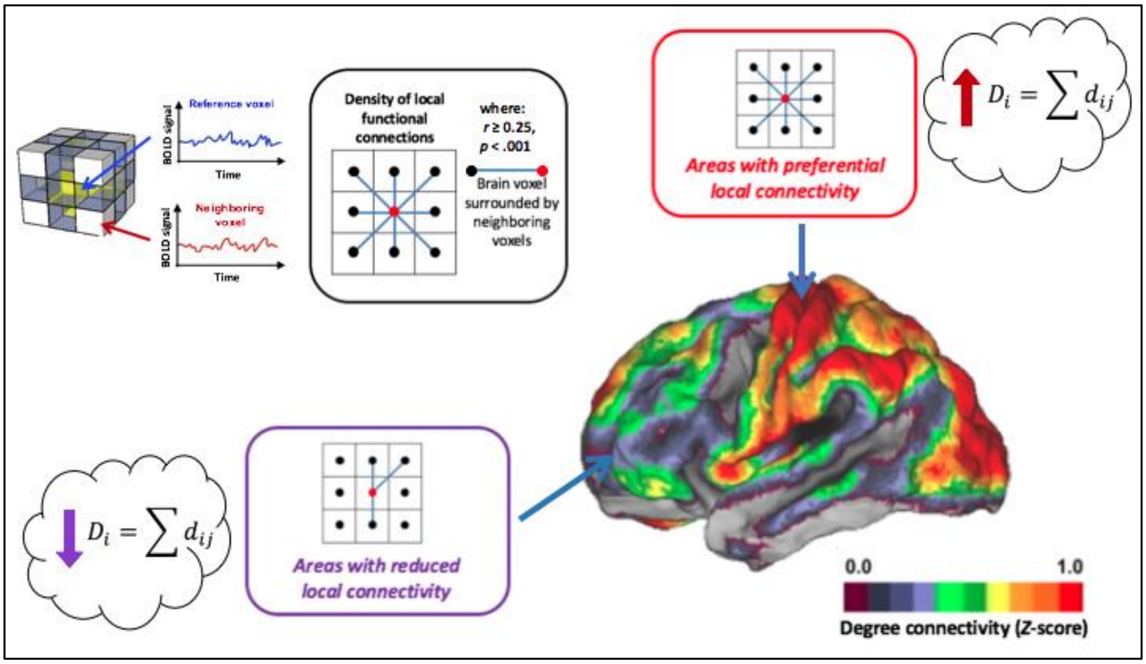

Figure 2. Graphical representation of local connection density in the human brain

Local connection density was first applied to a priori regions of interest (ROI) based on Koyama's reading network (Koyama et al., 2011) to examine within-network connectivity **(Figure 3)**, and then to the whole brain to examine out-of-network local functional connectivity. The regions that are part of the reading network are: inferior occipital gyrus **(LIOG)**, fusiform gyrus **(LFFG)**, superior temporal gyrus **(LSTG)**, precentral gyrus **(LPCG)**, superior parietal lobule **(LSPL)**, supplementary motor area **(LSMA)**, inferior frontal gyrus **(LIFG)**, middle frontal gyrus **(LMFG)**, and thalamus **(LTHAL)**. Anatomical masks from FSL using the Harvard-Oxford atlas were used and binarized, and resampled. Local connectivity was calculated for each ROI separately. To correct for multiple comparisons, 10,000 Monte Carlo simulations were applied to each ROI of the reading network and to the whole brain using AFNI's *3dClustSim* to obtain a corrected significance level of $p < 0.05$.

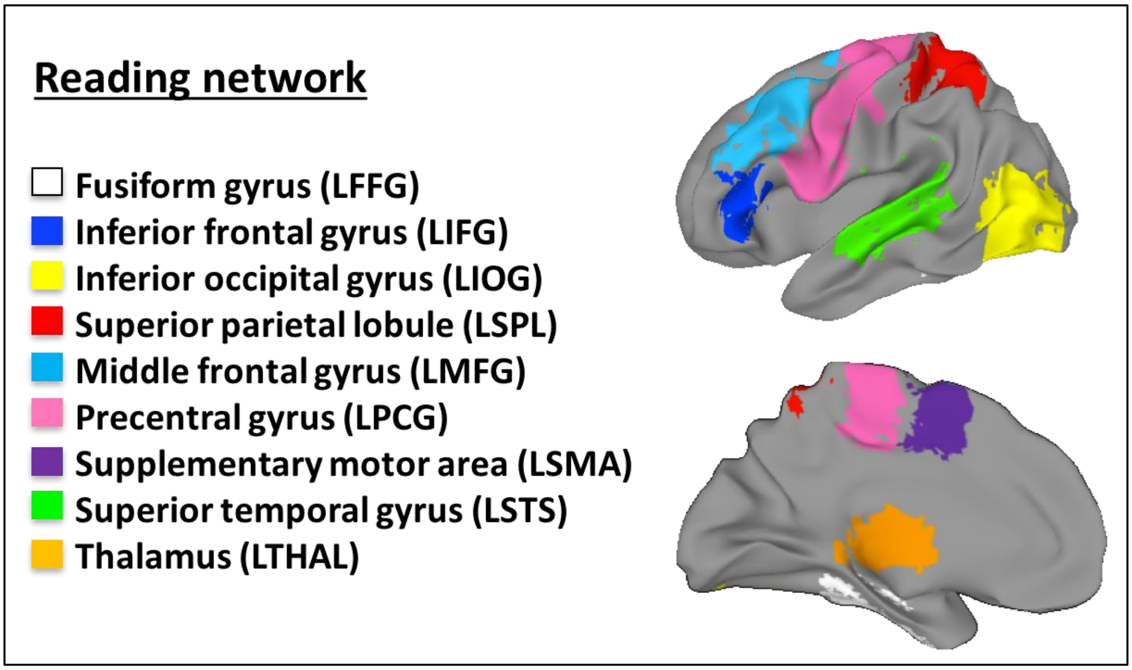

Figure 3. SUMA rendering for each ROI from the Reading network

Statistical Analyses

First, behavioral data were subjected to a series of 2 Phase (Phase1, Phase2) x 2 Groups (ASD-EXP, ASD-WLC) repeated measures ANOVAs. In case of any significant interactions, these were followed-up with post-hoc analyses using a series of two-sample and paired sample *t*-tests. Then, rs-fMRI group comparisons were performed in the following way: I) using ANCOVAs to compare the ASD-WLC vs. the ASD-EXP group differences in connectivity at Phase2 (covarying for Phase1 connectivity); and II) using paired-sample *t*-tests to compare ASD-EXP Phase1 vs. Phase2 connectivity. Both the ANCOVAs and paired-sample *t*-tests were performed on each ROI and to the whole brain using AFNI's *3dttest*$^{++}$.

We further examined the relationship between changes in local functional connectivity in the ASD-EXP group and changes in reading comprehension skills measured by GORT-4. The relationship of the percent change in GORT scores with 1) changes in local connectivity for each ROI and 2) changes in local connectivity for the whole-brain were examined through multiple linear regression analyses. Voxel-wise linear regressions were performed on each ROI and on the whole brain, generating correlation map showing which regions in the brain were correlated with GORT scores, and cluster correction was applied as described above. For regression analyses, verbal IQ was included as a covariate.

RESULTS

Behavioral Results

First, reading (decoding) abilities were assessed by the Slosson Oral Reading Test - Revised (SORT-R) did not show any significant interaction between Phase and Group (F[1, 27] = 1.66, p = .208, η^2 = .066; **Figures 4A** and **4B**).

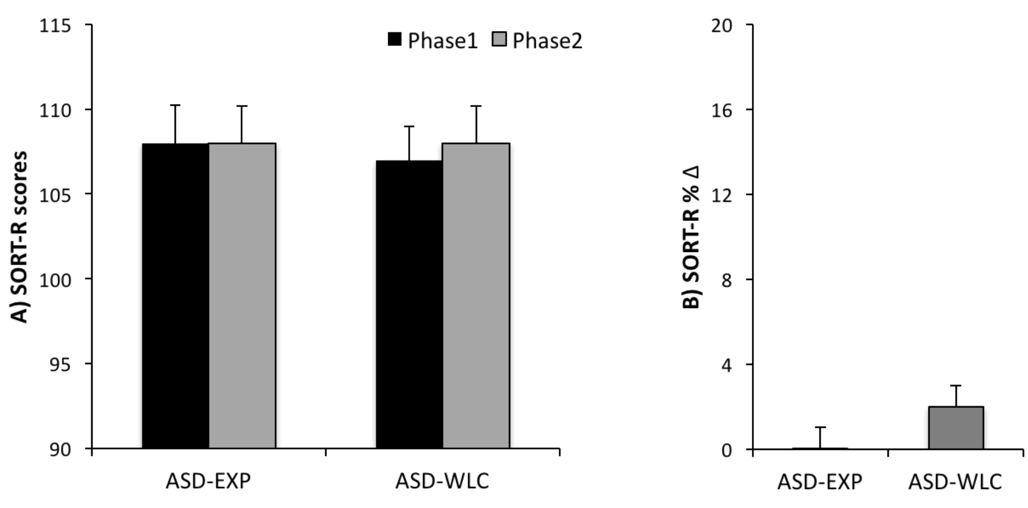

Figure 4. Mean scores for A) reading decoding abilities and B) percent change in SORT-R for ASD-EXP and ASD-WLC. Error bars represent standard error of the mean.

Secondly, a 2 Phase (Phase1, Phase2) x 2 Group (ASD-EXP, ASD-WLC) repeated measures ANOVA revealed a marginal Phase x Group interaction ($F[1, 27] = 3.50$, $p = .07$, $\eta^2 = .11$). Follow-up post-hoc analysis revealed that all participants from the ASD-EXP group showed statistically significant improvement in reading comprehension skills measured by GORT-4 Comprehension subtest from the first to second imaging session (paired-$t[14] = 3.28$, $p < 0.01$; **Figure 5A**), whereas the ASD-WLC group did not show a statistically significant change in reading comprehension (paired-$t[13] = 0.56$, $p = 0.56$; **Figure 5A**). The ASD-EXP group significantly improved their reading comprehension scores (percent change), compared with the ASD-WLC group from the first to second imaging session (ASD-WLC = 3%; ASD-EXP = 15%; $t[27] = 2.0$, $p = 0.03$; **Figure 5B**).

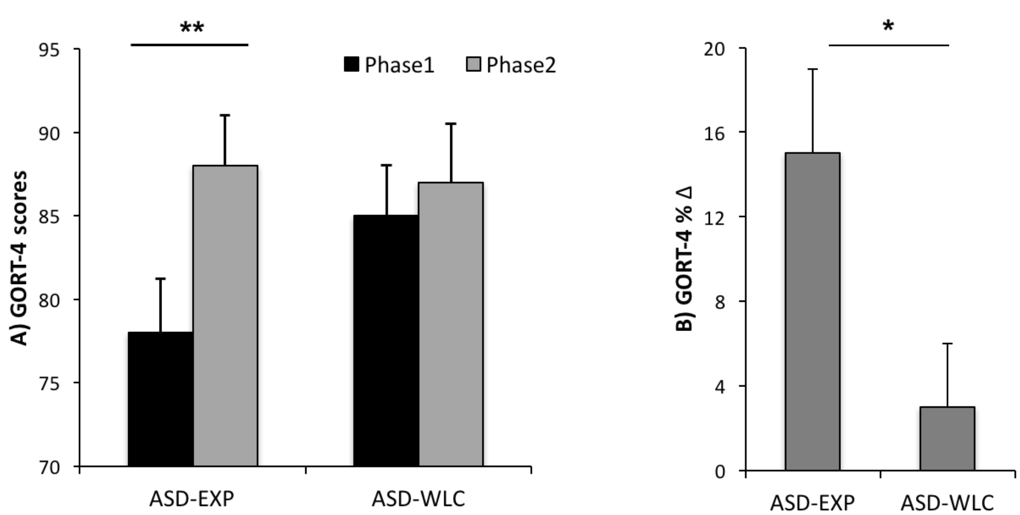

Figure 5. Mean scores for A) comprehension abilities and B) percent change in GORT-4 for ASD-EXP and ASD-WLC. $^{**}p < .01$, $^{*}p < .05$. Error bars represent standard

Local Connectivity (ASD-EXP vs. ASD-WLC at Phase2)

For the reading network analysis, significant differences (ASD-EXP > ASD-WLC at Phase2 while covarying for Phase1) were found in the following ROIs: LFFG, LIOG, LSPL, and two clusters within LPCG (**Table 2 and Figures 6A-D**). For whole-brain analysis, closer examination between the two groups revealed significant clusters where the ASD-EXP group showed an increase in local connectivity compared to the ASD-WLC in bilateral angular gyrus and right MPFC (**Table 2 and Figure 6E**).

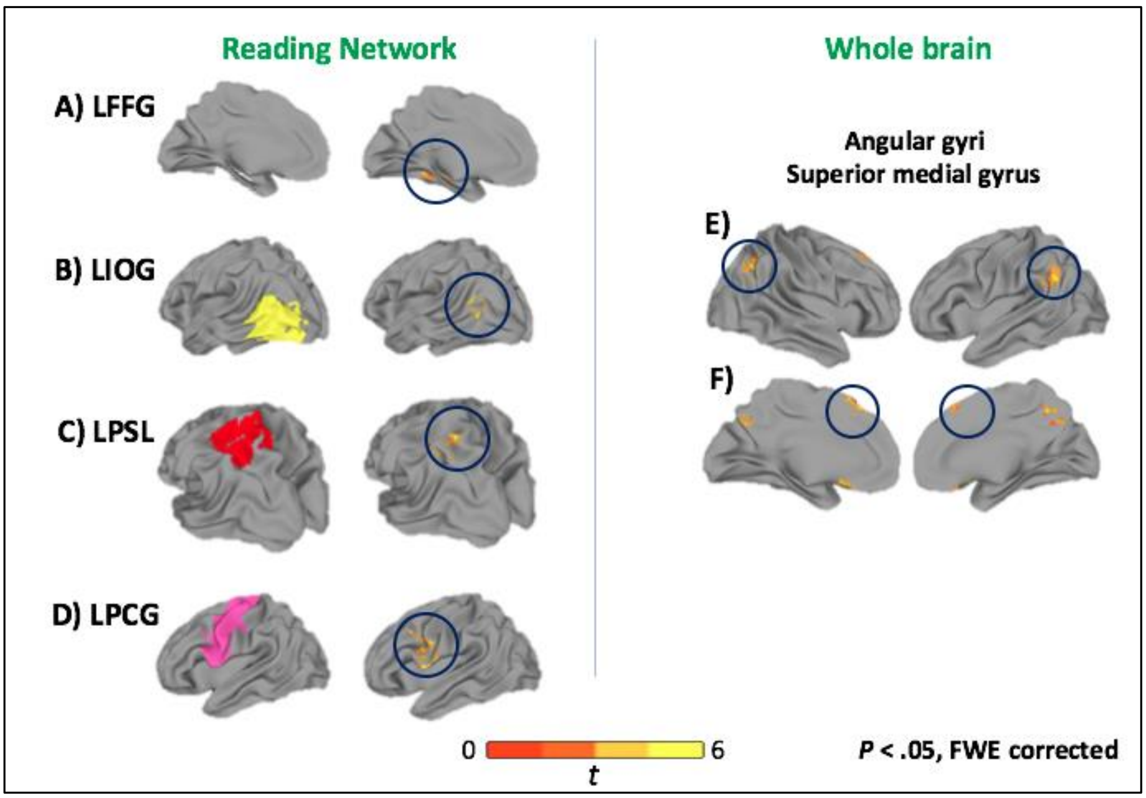

Figure 6. SUMA renderings for ROIs and significant differences (ASD-EXP > ASD-WLC) for A) LFFG, B) LIOG, C) LSPL, D) LPCG; and E) whole-brain analysis ($p < .05$, FWE corrected).

Table 2. Local Connectivity ASD-EXP > ASD-WLC Post-Intervention

	Region	Cluster vol. (in µl)	Peak coordinates MNI x	y	z	Peak *t*
Reading Network	LFFG	1674	-34	-24	18	3.9
	LIOG	1188	-46	-68	18	2.5
	LSPL	1701	-34	-60	60	3.9
	LPCG	4860	-54	4	4	4.2
		837	-10	-14	42	3.0
Whole brain						
	R. Angular gyrus	5670	44	-56	39	5.3
	R. Superior medial gyrus	3456	2	28	60	3.9
	L. Angular gyrus	2268	-48	-54	30	4.6

Abbreviations: L, left; R, right; vol, volume

Local Connectivity (ASD-EXP Phase1 vs. Phase2)

For the reading network analysis, significant differences (ASD-EXP Phase2 > Phase1) were found in the following ROIs: LIOG, LSPL, and LPCG (**Table 3 and Figures 7A-C**). For whole-brain analysis, the ASD-EXP group revealed two clusters (right precentral gyrus and right insula) of significant increase in local connectivity from Phase1 to Phase2 (**Table 3 and Figure 7D**).

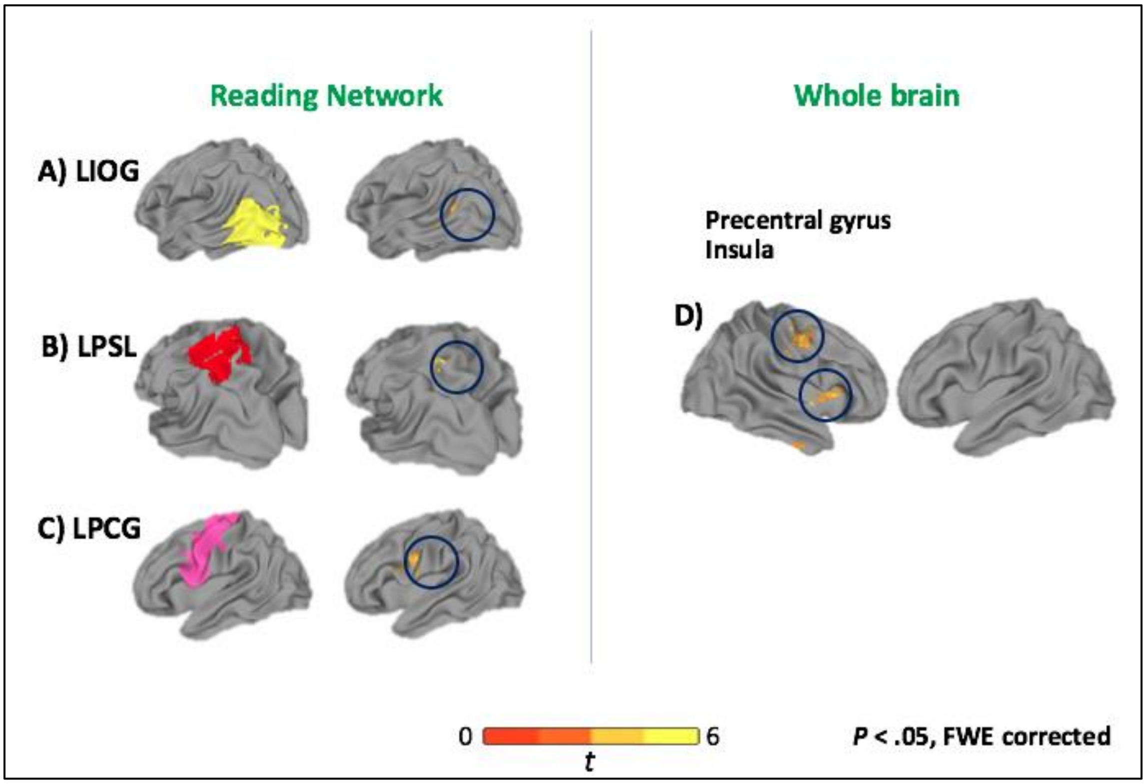

Figure 7. SUMA renderings for ROIs and significant differences (ASD-EXP Phase1 > Phase2) for A) LIOG, B) LSPL, C) LPCG, and D) whole-brain analysis ($p < .05$, FWE corrected).

Table 3. Local Connectivity ASD-EXP Phase2 > Phase1

	Region	Cluster vol. (in µl)	Peak coordinates MNI x	y	z	Peak *t*
Reading Network	LIOG	729	-58	-60	10	3.1
	LSPL	594	-34	-58	66	3.3
	LPCG	1836	-52	4	28	3.5
Whole brain						
	R. Precentral gyrus	3294	54	-2	36	4.9
	R. Insula	2079	36	18	4	3.9

Abbreviations: L, left; R, right; vol, volume

Brain-Behavior Correlations

Multiple regression analyses were performed to determine whether improvement in reading comprehension skills (% increase in GORT-4) could predict changes in local functional connectivity, while controlling for VIQ. Significant positive relationships were found between improvement in reading comprehension skills with changes in local connectivity within the following ROIs: LFFG (b = 2.28, R^2 = .32, p < .05; **Figure 8A**) and LPCG (b = 3.74, R^2 = .29, p < .05; **Figure 8B**). At the whole-brain level, one significant positive relationship was found between improvement in reading comprehension skills and functional connectivity of right supramarginal gyrus of the IPL (b = 1.56, R^2 = .51, p < .05; **Figure 8C**).

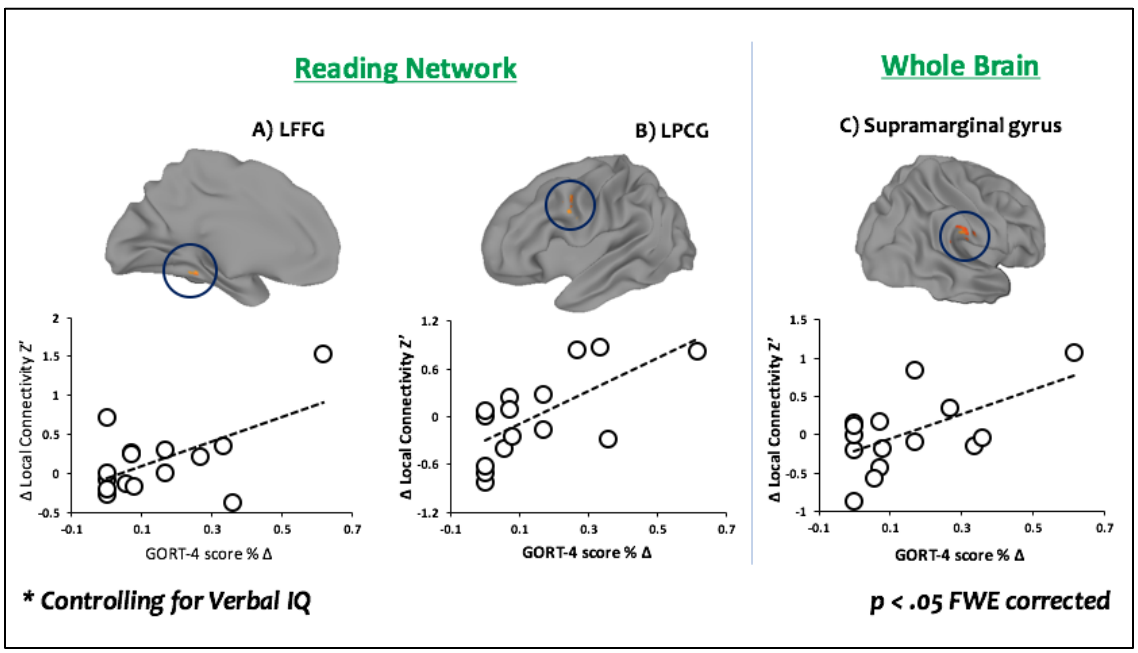

Figure 8. SUMA renderings displaying clusters of significant relationship between changes in local connectivity with changes in reading comprehension scores in A) LFFG, B) LPCG, and C) whole brain analysis (p < .05, FWE corrected).

DISCUSSION

This study examined the impact of an intensive visualizing/verbalizing reading intervention on both behavioral and brain functioning in a group of children with ASD who have difficulties in reading comprehension. Our findings of improved reading comprehension in the children who underwent the intervention, compared to the children that did not, corroborated Hypothesis 1. Secondly, our findings of increased local connectivity within the ASD-EXP group itself (both within and outside the reading network) and also compared to the ASD-WLC group supported Hypotheses 2a and 2b. Finally, Hypothesis 3 was supported since improvement in reading comprehension abilities significantly predicted changes in local functional connectivity in regions within and outside of the reading network.

Changes in Reading Comprehension Abilities

Our findings of improved reading comprehension in the ASD-EXP group compared to the ASD-WLC group as a result of V/V intervention emphasizes the importance of behavioral interventions on populations with developmental delays, such as ASD. For example, a previous study that used the same intervention on a population of children with dyslexia found a significant intervention-related improvement in their reading comprehension abilities as measured by GORT-3 (Eden et al., 2004). Our set of studies are the first of its kind in the ASD literature to examine the effects of V/V intervention. Nevertheless, previous studies have already examined the impact of other reading interventions (El Zein et al., 2014; Reutebuch, El Zein, Kim, Weinberg, & Vaughn, 2015). These studies revealed improvements in reading comprehension, although they used relatively smaller sample sizes and case-studies. Moreover, the neural correlates of such changes were not investigated. Overall, the effect of the V/V intervention on reading comprehension skills adds significance to the already well-established potential of behavioral remediation on neural correlates leading to a better outcome for children with ASD (Ventola, Oosting, Anderson, & Pelphrey, 2013).

The Impact of Reading Intervention on Neural Correlates in ASD

While neurobiological based intervention studies have been limited in children with ASD, the potential of interventions in changing the brain in ASD has been documented in some studies (Ventola et al., 2013). For example, interventions targeting social skills, response to multiple cues, and self-management have revealed changes in both functional (Gordon et al., 2013; Voos et al., 2013) and EEG activity (Dawson et al., 2012), however, our study is one of the first to address the impact of a reading intervention on another hallmark feature of this disorder: deficits in language. Although two previous studies from our group showed that the V/V intervention changed connectivity, they used a traditional ROI to ROI functional connectivity method (Murdaugh et al., 2016; 2015). These two studies focused on long-distance functional connectivity of the reading network in children with ASD, and found that improvement in reading comprehension abilities were significantly correlated with improvement in functional connectivity.

Increase in Local Connectivity Within the Reading Network following V/V Intervention

For our first analysis, we focused on a priori regions that are heavily involved in reading comprehension (Koyama et al., 2011; Koyama et al., 2010). Increased local connectivity was found in the following regions of the reading network in our study: LSPL, LFFG, LPCG, and LIOG. An increase in local connectivity in LSPL may be indicative of the potential plasticity in this region as a result of learning experiences. Interestingly, in one of our previous studies (Murdaugh et al., 2015) we found increased long-distance functional connectivity between Broca's area with left supramarginal gyrus, which is located in the parietal lobe. The changes in local connectivity in IPL and other regions in the ASD-EXP group due to V/V intervention (Murdaugh et al., 2015) underscores the plasticity of this region and its role in language processing. Thus, the functional significance of this region may be taken into consideration while designing reading intervention in future. For example, previous studies of direct stimulation of SPL via transcranial magnetic stimulation (TMS) have helped in phonological memory and decision making in TD individuals, critical skills for language processing (Kirschen, Davis-Ratner, Jerde, Schraedley-Desmond, & Desmond, 2006; Romero, Walsh, & Papagno, 2006). Also in a different study, TD children who were considered poor readers (5^{th} graders) received remedial reading instruction for 100 hours and were scanned post-intervention (Meyler, Keller, Cherkassky, Gabrieli, & Just, 2008). These children were followed up immediately after the remediation and a year after. This study showed an increase in activation in LSPL and other regions within the reading network such as thalamus, middle frontal, precentral and postcentral gyrus, suggesting the functional malleability of these brain areas in response to behavioral interventions.

On the other hand, LFFG, LIOG, and LPCG showed an increase in local connectivity post-intervention as well. Functional MRI studies of LFFG have been shown that this region is heavily involved in reading, particularly in successful priming for both orthographically and semantically related words, but not for pseudo words or unrelated words (Devlin, Jamison, Gonnerman, & Matthews, 2006). On the other hand, LIOG, although not typically associated with language or reading abilities, may mediate visual perception of letters and words (Koyama et al., 2010). Finally, LPCG was found to be active during different aspects of language processing, such as processing abstract sentences, mental rotation and mental imagery, imagining concrete words, and lexical decision making (Murdaugh et al., 2015). This finding is congruent with the motor theory of speech perception, which posits the integrative functioning of speech and motor systems (Liberman, Cooper, Shankweiler, & Studdert-Kennedy, 1967) and with the dual coding theory of cognition (Pavio, 2007; Sadoski & Paivio, 2001), where mental representations to create an imaged gestalt derive from sensory experience (language and thought) and can be classified as verbal (language) and nonverbal (motor). This is especially pertinent given that the V/V intervention heavily relies on imagery skills to ameliorate oral, written, and language comprehension impairments. At the network level, brains of individuals with ASD have been linked to having reduced within-network connectivity, but increased between-network connectivity in brain networks such as salience, executive control, and default mode network (Abbott et al., 2015). Therefore, in our study, increases in local connectivity within the reading network can be translated as increased within-network connectivity post-intervention and a strengthening of brain network specialization within this network.

Increase of Local Connectivity Outside of the Reading Network following V/V Intervention

Estimates of local connectivity outside of the reading network using whole brain analysis revealed increased local functional connectivity in bilateral angular gyrus, right precentral, right superior medial, and right insula. Although not considered part of the reading network by the Koyama study, bilateral angular gyrus has been deemed crucial in mapping the visual percepts of print onto the phonological structures of language, and in translating text into inner speech (Pugh et al., 2001). This finding is noteworthy given the V/V intervention seeks to improve the imagery-language relationship in order to improve oral and reading comprehension. On the other hand, the insula has been described in other studies as integral to an anterior circuit for reading that is highly sensitive to regularity, and shows greater activity for pseudo-words than for real words (Mechelli, Gorno-Tempini, & Price, 2003). Finally, precentral gyrus has been linked to the motor aspect of speech perception (Pulvermuller & Fadiga, 2010) and reading (Longcamp, Anton, Roth, & Velay, 2005). In the Longcamp and collages study, precentral gyrus was responsive to visually presented letters, but not to pseudo letters (which cannot be sounded out). In the present study, an increase in local connectivity in these right hemisphere regions might reflect a compensatory mechanism (Herringshaw et al., 2016). A functional cooperation among these right-hemisphere regions, in addition to the ones from the reading network, might be the result of the V/V intervention.

Relationship Between Changes in Local Connectivity with Changes in Reading Comprehension Abilities

Perhaps the most important finding of this study is the changes in reading comprehension abilities significantly predicting local connectivity changes in the ASD-EXP group. Significant local connectivity changes in LFFG and LPCG from the reading network showed positive correlations with changes in reading comprehension abilities, suggesting that individuals with greater improvement in their reading abilities had a greater change in local connectivity. Previously, positive correlations between LFFG and LPCG with other regions within the reading network and reading competence have been reported in TD adults and children (Koyama et al., 2011). The authors described this relationship as experience-dependent functional development of the FFG. This might translate to an accelerated change in functional development in the LFFG in the ASD-EXP group as a result of the intervention. Similarly, a significant positive correlation between LPCG with changes in reading comprehension abilities might translate to late accounts for motor activity in language comprehension (Tomasino & Rumiati, 2013). This account postulates that mental imagery is used to enhance reading comprehension across different linguistic tasks. This finding is of particular interest to this study given that the V/V intervention heavily relies on mental imagery for enhancing oral and written language comprehension.

On the other hand, a positive relationship between change in local connectivity in the right supramarginal gyrus (which falls outside of the reading network) with changes in reading comprehension abilities was found as well. The right supramarginal gyrus has previously been linked to object-naming, and in one study that used repetitive transcranial magnetic stimulation (rTMS), it was found that perturbation to this area was associated with higher error rates in naming objects (Sollmann et al., 2014). Additionally, this area is crucial for cognitive strategy for reading since orthographic-to-phonological transformations take place in this region (Sliwinska, Khadilkar, Campbell-Ratcliffe, Quevenco, & Devlin, 2012). For this study, perhaps the intervention had an effect on this area, as part of the intervention trained the children to visualize objects and correctly identify and name them.

Implications

Previous studies have revealed that many high functioning children and adolescents with ASD have intact reading fluency, but impaired reading comprehension skills (Nation et al., 2006). There has also been some evidence of poor social and communication skills to be inversely correlated with reading comprehension skills (Jones et al., 2009). This can have a detrimental impact on the school environment for children with ASD as this profile of reading ability could result in a mislabeling as a learning disability, go unnoticed by the teachers, and thus lower academic achievement. The discrepancy between good reading abilities, but poor comprehension skills, suggests the need for vigilance in developing more effective ways of identifying such difficulties, and in designing effective interventions that could provide a better outcome to these individuals.

Behavioral studies have consistently found intact, sometimes superior visuospatial skills in ASD in tasks that require a visual or visuospatial processing (Joseph et al., 2009; Manjaly et al., 2007). Interestingly, some studies have found that individuals with ASD perform similarly to TD individuals on language tasks that require a visual component (Kana, Keller, Cherkassky, Minshew, & Just, 2006; Sahyoun, Belliveau, Soulieres, Schwartz, & Mody, 2010). Similarly, these enhanced visuospatial skills have also been found in studies using fMRI and functional connectivity during visual search tasks (Keehn, Brenner, Palmer, Lincoln, & Muller, 2008; Keehn, Shih, Brenner, Townsend, & Muller, 2013), and even in language tasks, where people with ASD tend to rely on brain regions that are primarily implicated in visual imagery, visual attention, and spatial transformation (e.g., inferior parietal lobule) (Kana et al., 2006), which may suggest that people with ASD might routinely recruit visual imagery for comprehending language rather than comprehending them on a purely linguistic basis. The intervention used in this study was developed specifically to tap into the visual imagery skills of children with language disabilities to help them develop critical thinking skills to improve both oral and reading comprehension, with the ultimate goal of improving the relation between imagery and language (Bell, 1991a, 1991b). Although, not all children with ASD show these superior visuospatial abilities, this intervention or similar ones that heavily rely on visual imagery techniques could be applied to those who indeed show these visuospatial skills for improving the link between imagery and language.

Finally, the benefits of behavioral remediation without biological measures are not being questioned here. Issues such as funding, sample size, methodological, and interpretative reasons affect the feasibility of neurobiological and behavioral intervention research (Ylinen & Kujala, 2015). Particularly in ASD, neurobiological and behavioral intervention research is beginning to experience a shift from purely basic research to translational neuroscience (Calderoni et al., 2016). Over the past 10 years, six fMRI studies have examined rehabilitative interventions and brain plasticity in ASD, and these have revealed very important links between brain function and behavior that were previously unknown in the social and language domains (Calderoni et al., 2016). Also, while some of these studies succumb to weaknesses in their designs, future studies can benefit from them by adjusting their protocols/designs in order to further enhance their studies. Overall, it seems that a combination of neurobiological and behavioral measures can help uncover how and why interventions change the deficient neural networks in neurodevelopmental disorders.

Limitations

For this study, there are some limitations to be considered. Firstly, local connection density was originally designed to measure local connectivity at the whole brain level and not at an ROI level. Several control analyses in the original study went into this method and none involved examining individual brain regions in the original study (Sepulcre et al., 2010). Therefore, these results should be interpreted cautiously. Secondly, although motion was strictly controlled, our motion threshold for censoring and removing outlier functional volumes was quite liberal (1.5mm), and it has been reported that even micromovement (<1mm) can have an overall impact in functional connectivity studies (Power et al., 2012; Van Dijk et al., 2012). Although we attempted to use more stringent criteria for our analyses, our sample size was significantly reduced to around 5 individuals per group. Finally, the vast heterogeneity in ASD, even in high-functioning populations, may explain why some children in the ASD-EXP group experienced little change in their reading comprehension skills.

CONCLUSIONS

Our study revealed an improvement in reading comprehension due to the V/V intervention. Also, there was an increase in local connectivity in regions underlying reading comprehension, and changes in reading comprehension skills were correlated with changes with local connectivity. The findings of this study emphasize the importance of targeted interventions for children with ASD, and the neuroplasticity in ASD is encouraging for future studies to continue to assess intervention-related changes in brain networks.

LIST OF REFERENCES

Abbott, A. E., Nair, A., Keown, C. L., Datko, M., Jahedi, A., Fishman, I., & Muller, R. A. (2015). Patterns of Atypical Functional Connectivity and Behavioral Links in Autism Differ Between Default, Salience, and Executive Networks. *Cerebral Cortex*. doi:10.1093/cercor/bhv191

Amaral, D. G., Schumann, C. M., & Nordahl, C. W. (2008). Neuroanatomy of autism. *Trends in Neurosciences, 31*(3), 137-145. doi:10.1016/j.tins.2007.12.005

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Arlington, VA: American Psychiatric Publishing.

Bell, N. (1991a). Gestalt imagery: A critical factor in language comprehension. *Ann Dyslexia, 41*(1), 246-260. doi:10.1007/BF02648089

Bell, N. (1991b). *Visualizing and Verbalizing for Language Comprehension and Thinking*. Paso Robles, CA: Academy of Reading Publications.

Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., & Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *Journal of Neuroscience, 24*(42), 9228-9231. doi:10.1523/JNEUROSCI.3340-04.2004

Brown, H. M., Oram-Cardy, J., & Johnson, A. (2013). A meta-analysis of the reading comprehension skills of individuals on the autism spectrum. *Journal of Autism and Developmental Disorders, 43*(4), 932-955. doi:10.1007/s10803-012-1638-1

Bullmore, E., & Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature Reviews: Neuroscience, 10*(3), 186-198. doi:10.1038/nrn2575

Cain, K., Oakhill, J. V., Barnes, M. A., & Bryant, P. E. (2001). Comprehension skill, inference-making ability, and their relation to knowledge. *Memory and Cognition, 29*(6), 850-859.

Calderoni, S., Billeci, L., Narzisi, A., Brambilla, P., Retico, A., & Muratori, F. (2016). Rehabilitative Interventions and Brain Plasticity in Autism Spectrum Disorders: Focus on MRI-Based Studies. *Frontiers in Neuroscience, 10,* 139. doi:10.3389/fnins.2016.00139

Casanova, M., & Trippe, J. (2009). Radial cytoarchitecture and patterns of cortical connectivity in autism. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 364*(1522), 1433-1436. doi:10.1098/rstb.2008.0331

Casanova, M., van Kooten, I. A., Switala, A. E., van Engeland, H., Heinsen, H., Steinbusch, H. W., . . . Schmitz, C. (2006). Minicolumnar abnormalities in autism. *Acta Neuropathologica, 112*(3), 287-303. doi:10.1007/s00401-006-0085-5

Cordes, D., Haughton, V. M., Arfanakis, K., Carew, J. D., Turski, P. A., Moritz, C. H., . . . Meyerand, M. E. (2001). Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. *AJNR: American Journal of Neuroradiology, 22*(7), 1326-1333.

Courchesne, E., & Pierce, K. (2005). Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. *Current Opinion in Neurobiology, 15*(2), 225-230. doi:10.1016/j.conb.2005.03.001

Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research, 29*(3), 162-173.

Dawson, G., Jones, E. J., Merkle, K., Venema, K., Lowy, R., Faja, S., . . . Webb, S. J. (2012). Early behavioral intervention is associated with normalized brain activity in young children with autism. *Journal of the American Academy of Child and Adolescent Psychiatry, 51*(11), 1150-1159. doi:10.1016/j.jaac.2012.08.018

Devlin, J. T., Jamison, H. L., Gonnerman, L. M., & Matthews, P. M. (2006). The role of the posterior fusiform gyrus in reading. *Journal of Cognitive Neuroscience, 18*(6), 911-922. doi:10.1162/jocn.2006.18.6.911

Eden, G. F., Jones, K. M., Cappell, K., Gareau, L., Wood, F. B., Zeffiro, T. A., . . . Flowers, D. L. (2004). Neural changes following remediation in adult developmental dyslexia. *Neuron, 44*(3), 411-422. doi:10.1016/j.neuron.2004.10.019

El Zein, F., Solis, M., Vaughn, S., & McCulley, L. (2014). Reading comprehension interventions for students with autism spectrum disorders: a synthesis of research. *Journal of Autism and Developmental Disorders, 44*(6), 1303-1322. doi:10.1007/s10803-013-1989-2

Estes, A., Rivera, V., Bryan, M., Cali, P., & Dawson, G. (2011). Discrepancies between academic achievement and intellectual ability in higher-functioning school-aged children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 41*(8), 1044-1052. doi:10.1007/s10803-010-1127-3

Eyler, L. T., Pierce, K., & Courchesne, E. (2012). A failure of left temporal cortex to specialize for language is an early emerging and fundamental property of autism. *Brain, 135*(Pt 3), 949-960. doi:10.1093/brain/awr364

Gordon, I., Vander Wyk, B. C., Bennett, R. H., Cordeaux, C., Lucas, M. V., Eilbott, J. A., . . . Pelphrey, K. A. (2013). Oxytocin enhances brain function in children with autism. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(52), 20953-20958. doi:10.1073/pnas.1312857110

Groen, W. B., Tesink, C., Petersson, K. M., van Berkum, J., van der Gaag, R. J., Hagoort, P., & Buitelaar, J. K. (2010). Semantic, factual, and social language comprehension in adolescents with autism: an FMRI study. *Cerebral Cortex*, *20*(8), 1937-1945. doi:10.1093/cercor/bhp264

Groen, W. B., Zwiers, M. P., van der Gaag, R. J., & Buitelaar, J. K. (2008). The phenotype and neural correlates of language in autism: an integrative review. *Neuroscience and Biobehavioral Reviews*, *32*(8), 1416-1425. doi:10.1016/j.neubiorev.2008.05.008

Hannon, B., & Daneman, M. (2001). A new tool for measuring and understanding individual differences in the component processes of reading comprehension. *Journal of Educational Psychology*, *93*, 103-128.

Herringshaw, A. J., Ammons, C. J., DeRamus, T. P., & Kana, R. K. (2016). Hemispheric differences in language processing in autism spectrum disorders: A meta-analysis of neuroimaging studies. *Autism Research*. doi:10.1002/aur.1599

Jones, C. R., Happe, F., Golden, H., Marsden, A. J., Tregay, J., Simonoff, E., . . . Charman, T. (2009). Reading and arithmetic in adolescents with autism spectrum disorders: peaks and dips in attainment. *Neuropsychology*, *23*(6), 718-728. doi:10.1037/a0016360

Joseph, R. M., Keehn, B., Connolly, C., Wolfe, J. M., & Horowitz, T. S. (2009). Why is visual search superior in autism spectrum disorder? *Dev Sci, 12*(6), 1083-1096. doi:10.1111/j.1467-7687.2009.00855.x

Just, M. A., Cherkassky, V. L., Keller, T. A., & Minshew, N. J. (2004). Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain, 127*(Pt 8), 1811-1821. doi:10.1093/brain/awh199

Kana, R. K., Keller, T. A., Cherkassky, V. L., Minshew, N. J., & Just, M. A. (2006). Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. *Brain, 129*(Pt 9), 2484-2493. doi:10.1093/brain/awl164

Kana, R. K., Uddin, L. Q., Kenet, T., Chugani, D., & Muller, R. A. (2014). Brain connectivity in autism. *Frontiers in Human Neuroscience, 8,* 349. doi:10.3389/fnhum.2014.00349

Keehn, B., Brenner, L., Palmer, E., Lincoln, A. J., & Muller, R. A. (2008). Functional brain organization for visual search in ASD. *Journal of the International Neuropsychological Society, 14*(6), 990-1003. doi:10.1017/S1355617708081356

Keehn, B., Shih, P., Brenner, L. A., Townsend, J., & Muller, R. A. (2013). Functional connectivity for an "island of sparing" in autism spectrum disorder: an fMRI study of visual search. *Human Brain Mapping, 34*(10), 2524-2537. doi:10.1002/hbm.22084

Kirschen, M. P., Davis-Ratner, M. S., Jerde, T. E., Schraedley-Desmond, P., & Desmond, J. E. (2006). Enhancement of phonological memory following transcranial magnetic stimulation (TMS). *Behavioural Neurology, 17*(3-4), 187-194.

Koyama, M. S., Di Martino, A., Zuo, X. N., Kelly, C., Mennes, M., Jutagir, D. R., . . . Milham, M. P. (2011). Resting-state functional connectivity indexes reading competence in children and adults. *Journal of Neuroscience, 31*(23), 8617-8624. doi:10.1523/JNEUROSCI.4865-10.2011

Koyama, M. S., Kelly, C., Shehzad, Z., Penesetti, D., Castellanos, F. X., & Milham, M. P. (2010). Reading networks at rest. *Cerebral Cortex, 20*(11), 2549-2559. doi:10.1093/cercor/bhq005

Liberman, A. M., Cooper, F. S., Shankweiler, D. P., & Studdert-Kennedy, M. (1967). Perception of the speech code. *Psychological Review, 74*(6), 431-461.

Lohmann, G., Hoehl, S., Brauer, J., Danielmeier, C., Bornkessel-Schlesewsky, I., Bahlmann, J., . . . Friederici, A. (2010). Setting the frame: the human brain activates a basic low-frequency network for language processing. *Cerebral Cortex, 20*(6), 1286-1292. doi:10.1093/cercor/bhp190

Longcamp, M., Anton, J. L., Roth, M., & Velay, J. L. (2005). Premotor activations in response to visually presented single letters depend on the hand used to write: a study on left-handers. *Neuropsychologia, 43*(12), 1801-1809. doi:10.1016/j.neuropsychologia.2005.01.020

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Jr., Leventhal, B. L., DiLavore, P. C., . . . Rutter, M. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30*(3), 205-223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*(5), 659-685.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage, 19*(3), 1233-1239.

Manjaly, Z. M., Bruning, N., Neufang, S., Stephan, K. E., Brieber, S., Marshall, J. C., . . . Fink, G. R. (2007). Neurophysiological correlates of relatively enhanced local visual search in autistic adolescents. *Neuroimage, 35*(1), 283-291. doi:10.1016/j.neuroimage.2006.11.036

Mason, R. A., Williams, D. L., Kana, R. K., Minshew, N., & Just, M. A. (2008). Theory of Mind disruption and recruitment of the right hemisphere during narrative comprehension in autism. *Neuropsychologia, 46*(1), 269-280. doi:10.1016/j.neuropsychologia.2007.07.018

Maximo, J. O., Cadena, E. J., & Kana, R. K. (2014). The implications of brain connectivity in the neuropsychology of autism. *Neuropsychology Review, 24*(1), 16-31. doi:10.1007/s11065-014-9250-0

Mechelli, A., Gorno-Tempini, M. L., & Price, C. J. (2003). Neuroimaging studies of word and pseudoword reading: consistencies, inconsistencies, and limitations. *Journal of Cognitive Neuroscience, 15*(2), 260-271. doi:10.1162/089892903321208196

Meyler, A., Keller, T. A., Cherkassky, V. L., Gabrieli, J. D., & Just, M. A. (2008). Modifying the brain activation of poor readers during sentence comprehension with extended remedial instruction: a longitudinal study of neuroplasticity.

Neuropsychologia, 46(10), 2580-2592. doi:10.1016/j.neuropsychologia.2008.03.012

Murdaugh, D. L., Deshpande, H. D., & Kana, R. K. (2016). The Impact of Reading Intervention on Brain Responses Underlying Language in Children With Autism. *Autism Research, 9*(1), 141-154. doi:10.1002/aur.1503

Murdaugh, D. L., Maximo, J. O., & Kana, R. K. (2015). Changes in intrinsic connectivity of the brain's reading network following intervention in children with autism. *Human Brain Mapping, 36*(8), 2965-2979. doi:10.1002/hbm.22821

Nair, A., Keown, C. L., Datko, M., Shih, P., Keehn, B., & Muller, R. A. (2014). Impact of methodological variables on functional connectivity findings in autism spectrum disorders. *Human Brain Mapping, 35*(8), 4035-4048. doi:10.1002/hbm.22456

Nation, K., Clarke, P., Wright, B., & Williams, C. (2006). Patterns of reading ability in children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 36*(7), 911-919. doi:10.1007/s10803-006-0130-1

Norbury, C., & Nation, K. (2011). Understanding variability in reading comprehension in adolescents with autism spectrum disorders: Interactions with language status and decoding skill. *Scientific Studies of Reading, 15,* 191-210.

Pavio, A. (2007). *Mind and its evolution: A dual coding theoretical approach*. Lawrence Mahwah, NJ: Erlbaum Associates.

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage, 59*(3), 2142-2154. doi:10.1016/j.neuroimage.2011.10.018

Pugh, K. R., Mencl, W. E., Jenner, A. R., Katz, L., Frost, S. J., Lee, J. R., . . . Shaywitz, B. A. (2001). Neurobiological studies of reading and reading disability. *Journal of Communication Disorders, 34*(6), 479-492.

Pulvermuller, F., & Fadiga, L. (2010). Active perception: sensorimotor circuits as a cortical basis for language. *Nature Reviews: Neuroscience, 11*(5), 351-360. doi:10.1038/nrn2811

Raichle, M. E. (2009). A paradigm shift in functional brain imaging. *Journal of Neuroscience, 29*(41), 12729-12734. doi:10.1523/JNEUROSCI.4366-09.2009

Redcay, E., & Courchesne, E. (2008). Deviant functional magnetic resonance imaging patterns of brain activity to speech in 2-3-year-old children with autism spectrum disorder. *Biological Psychiatry, 64*(7), 589-598. doi:10.1016/j.biopsych.2008.05.020

Reutebuch, C. K., El Zein, F., Kim, M. K., Weinberg, A. N., & Vaughn, S. (2015). Investigating a reading comprehension intervention for high school students with autism spectrum disorder: A pilot study. *Research in Autism Spectrum Disorders, 9*, 96-111.

Rippon, G., Brock, J., Brown, C., & Boucher, J. (2007). Disordered connectivity in the autistic brain: challenges for the "new psychophysiology". *International Journal of Psychophysiology, 63*(2), 164-172. doi:10.1016/j.ijpsycho.2006.03.012

Romero, L., Walsh, V., & Papagno, C. (2006). The neural correlates of phonological short-term memory: a repetitive transcranial magnetic stimulation study. *Journal of Cognitive Neuroscience, 18*(7), 1147-1155. doi:10.1162/jocn.2006.18.7.1147

Rubenstein, J. L., & Merzenich, M. M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav, 2*(5), 255-267.

Sadoski, M., & Paivio, A. (2001). *Imagery and text: A dual coding theory of reading and writing*. Mahwah, NJ: Lawrence Erlbaum Associates.

Sahyoun, C. P., Belliveau, J. W., Soulieres, I., Schwartz, S., & Mody, M. (2010). Neuroimaging of the functional and structural networks underlying visuospatial vs. linguistic reasoning in high-functioning autism. *Neuropsychologia, 48*(1), 86-95. doi:10.1016/j.neuropsychologia.2009.08.013

Samson, F., Mottron, L., Soulieres, I., & Zeffiro, T. A. (2012). Enhanced visual functioning in autism: an ALE meta-analysis. *Human Brain Mapping, 33*(7), 1553-1581. doi:10.1002/hbm.21307

Satterthwaite, T. D., Elliott, M. A., Gerraty, R. T., Ruparel, K., Loughead, J., Calkins, M. E., . . . Wolf, D. H. (2013). An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. *Neuroimage, 64*, 240-256. doi:10.1016/j.neuroimage.2012.08.052

Sepulcre, J., Liu, H., Talukdar, T., Martincorena, I., Yeo, B. T., & Buckner, R. L. (2010). The organization of local and distant functional connectivity in the human brain. *PLoS Computational Biology, 6*(6), e1000808. doi:10.1371/journal.pcbi.1000808

Sliwinska, M. W., Khadilkar, M., Campbell-Ratcliffe, J., Quevenco, F., & Devlin, J. T. (2012). Early and sustained supramarginal gyrus contributions to phonological processing. *Frontiers in Psychology, 3*, 161. doi:10.3389/fpsyg.2012.00161

Sollmann, N., Tanigawa, N., Ringel, F., Zimmer, C., Meyer, B., & Krieg, S. M. (2014). Language and its right-hemispheric distribution in healthy brains: an investigation by repetitive transcranial magnetic stimulation. *Neuroimage, 102 Pt 2*, 776-788. doi:10.1016/j.neuroimage.2014.09.002

Tomasi, D., & Volkow, N. D. (2012). Resting functional connectivity of language networks: characterization and reproducibility. *Molecular Psychiatry, 17*(8), 841-854. doi:10.1038/mp.2011.177

Tomasino, B., & Rumiati, R. I. (2013). At the mercy of strategies: the role of motor representations in language understanding. *Frontiers in Psychology, 4*, 27. doi:10.3389/fpsyg.2013.00027

Turkeltaub, P. E., Eden, G. F., Jones, K. M., & Zeffiro, T. A. (2002). Meta-analysis of the functional neuroanatomy of single-word reading: method and validation. *Neuroimage, 16*(3 Pt 1), 765-780.

Turkeltaub, P. E., Gareau, L., Flowers, D. L., Zeffiro, T. A., & Eden, G. F. (2003). Development of neural mechanisms for reading. *Nature Neuroscience, 6*(7), 767-773. doi:10.1038/nn1065

Van Dijk, K. R., Sabuncu, M. R., & Buckner, R. L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage, 59*(1), 431-438. doi:10.1016/j.neuroimage.2011.07.044

Ventola, P. E., Oosting, D., Anderson, L. C., & Pelphrey, K. A. (2013). Brain mechanisms of plasticity in response to treatments for core deficits in autism. *Progress in Brain Research, 207*, 255-272. doi:10.1016/B978-0-444-63327-9.00007-2

Voos, A. C., Pelphrey, K. A., Tirrell, J., Bolling, D. Z., Vander Wyk, B., Kaiser, M. D., . . . Ventola, P. (2013). Neural mechanisms of improvements in social motivation after pivotal response treatment: two case studies. *Journal of Autism and Developmental Disorders, 43*(1), 1-10. doi:10.1007/s10803-012-1683-9

Williams, D., Botting, N., & Boucher, J. (2008). Language in autism and specific language impairment: where are the links? *Psychological Bulletin, 134*(6), 944-963. doi:10.1037/a0013743

Ylinen, S., & Kujala, T. (2015). Neuroscience illuminating the influence of auditory or phonological intervention on language-related deficits. *Frontiers in Psychology, 6*, 137. doi:10.3389/fpsyg.2015.00137

Zablotsky, B., Black, L. I., Maenner, M. J., Schieve, L. A., & Blumberg, S. J. (2015). Estimated Prevalence of Autism and Other Developmental Disabilities Following Questionnaire Changes in the 2014 National Health Interview Survey. *Natl Health Stat Report*(87), 1-20.

APPENDIX A

IRB approval

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Exemption Designation
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator:	MAXIMO, JOSE O
Co-Investigator(s):	KANA, RAJESH KUMAR
Protocol Number:	**E151202003**
Protocol Title:	*Changes in Intrinsic Local Connectivity of the Brain's Reading Network in Children with Autism Followed by Reading Intervention*

The above project was reviewed on 4/25/16. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This project qualifies as an exemption as defined in 45CFR46.101(b), paragraph 4.

This project received EXEMPT review.

Date IRB Designation Issued: 4/25/16

Cari Oliver, CIP
Assistant Director, Office of the
Institutional Review Board for Human
Use (IRB)

Investigators please note:

Any modifications in the study methodology, protocol and/or consent form/information sheet must be submitted for review to the IRB prior to implementation.

470 Administration Building
701 20th Street South
205.934.3789
Fax 205.934.1301
irb@uab.edu

The University of
Alabama at Birmingham
Mailing Address:
AB 470
1720 2ND AVE S
BIRMINGHAM AL 35294-0104