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Intrinsic And Extrinsic Brain Activity In Children With Autism Before And After A Visualization Language Intervention

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INTRINSIC AND EXTRINSIC BRAIN ACTIVITY IN CHILDREN WITH AUTISM BEFORE AND AFTER A VISUALIZATION LANGUAGE INTERVENTION

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ABSTRACT

Deficits in language comprehension have been widely reported in children with autism spectrum disorders (ASD). Recent evidence from neuroimaging research has found that individuals with ASD tend to recruit visuospatial imagery to comprehend language (Kana et al., 2006). The theory of cortical underconnectivity in autism suggests that in tasks that can be interpreted either visually or verbally, brain regions associated with language and visuospatial processing will be substantially less functionally integrated (especially between frontal and more posterior regions) than healthy-matched controls (Just et al., 2004). Indeed, cortical underconnectivity in individuals with ASD has been documented, not only in extrinsic brain activation, but have also been seen during intrinsic brain activation (Cherkassky et al., 2006; Kennedy & Courchesne, 2008a,b). Evidence from these studies suggests that despite relying on visuospatial imagery to interpret language, individuals with ASD may have problems in the coordinated functioning of brain regions. The proposed project used an imagery-based intervention program to improve the brain circuitry underlying language processing and its integrated functioning in children with ASD by tapping into visuospatial processing to improve language comprehension. We use resting state as well as task-based functional MRI to study the neurobiological mechanisms of language impairments in children with ASD, and to test whether a language remediation program can change the brain circuitry in ASD. Our first study investigates the link between visuospatial abilities and language comprehension in children with ASD and found increased activation post-intervention of visual and posterior language regions, as well as right-hemisphere language homologous regions, precentral gyrus (PrCG), postcentral gyrus (PoCG), putamen, and thalamus, as well as, strengthened functional connectivity between left hemisphere language areas, the middle temporal gyrus and inferior frontal gyrus. Our second study assesses the reading network using intrinsic resting state functional connectivity. We found neural changes specific to intervention of stronger functional connectivity of both Broca's and Wernicke's areas in the children with ASD post-intervention. Overall, these novel findings provide valuable insights into the plasticity of brain's language networks and have strong implications for individualization of treatment for children with ASD.

Keywords: Autism spectrum disorder (ASD); Translational neuroimaging; Reading intervention; Independent component analysis; Resting state; Functional connectivity

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INTRODUCTION

Autism spectrum disorders (ASD) are a group of pervasive developmental disorders characterized by impairments in social communication and presence of repetitive behavior and restricted interests (American Psychological Association, 2013). While a biological etiology for ASD was proposed even in the first scientific paper in the field (Kanner, 1943), a firm biological marker has been rather elusive. Seventy years since Kanner's first article, neuroscientists have made significant strides in understanding the neurobiology of ASD. Advanced neuroimaging techniques, which permit in-vivo human studies, have been at the center stage of neurobiological investigations of ASD, especially in the last decade. Neuroimaging research has provided evidence for widespread anatomical, functional and connectional abnormalities in the brains of individuals with ASD (Libero & Kana, 2013). Nevertheless, the limited convergence of such findings into a single coherent neurobiological account has been a major limitation towards characterizing the neurobiology of ASD. Thus, currently neuroimaging research in ASD is at a crossroads and faces two important directions of study: 1) identifying a reliable biomarker and testing the diagnostic utility of such marker; and 2) capturing the clinical and translational potential of neuroimaging in targeting brain plasticity and using cognitive, behavioral, and pharmacological intervention to rewire brain circuitry in children and adults with ASD. This current translational neuroimaging project attempts to address both of these potential directions. We used resting state as well as task-based functional MRI to study the neurobiological mechanisms of language impairments in children with ASD, and to test whether a language remediation program can change the brain circuitry in ASD. In other words, the research project described in this document uses a novel translational neuroimaging approach to assess the impact of a strength-based reading intervention in changing both intrinsic and extrinsic brain activity in children with ASD. Our first study investigates the link between visual-spatial abilities and language comprehension in children with ASD using a sentence comprehension task, and the effect of visualization in aiding language comprehension through intervention. The second study assesses the reading network using intrinsic resting state functional connectivity (synchronization of activity across brain areas) data in order to determine if neural changes based on intervention can be observed independent of task. Overall, our project lends support to specialized intervention for children with ASD to increase higher-order learning skills, and the plasticity of the young brain in ASD to respond positively to targeted treatment.

I. Reading Profile in ASD

A number of behavioral studies have assessed language comprehension, and specifically reading comprehension in children with ASD. Collectively, these studies have found that children with ASD have overall higher reading fluency compared to level of comprehension (e.g., Jones et al., 2009; Lindgren, Folstein, Tomblin, & Tager-Flusberg, 2009; Nation, Clarke, Wright, & Williams, 2006; Newman et al., 2007; Norbury & Nation, 2011). Interestingly, Jones et al. (2009) found that the majority of children with ASD in their sample (n=100) exhibited basic reading skills that were consistent with their full scale IQ, both of which were in the low average range; however this was coupled with statistically poorer reading comprehension compared to their basic reading skills. In addition, a negative correlation was found between reading comprehension and more severe social and communication deficits. Another study, assessing a younger age group of children with ASD (4 to 7 year olds), found that when read short stories and asked questions about them, the children with ASD had specific difficulties in making inferences based on the events of the stories, compared to typically developing (TD) children. However, questions about the stories that only required deductive logical reasoning was intact in children with ASD (Nuske & Bavin, 2011).

II. Interaction of Language and Visuospatial Regions in ASD

Neuroimaging provides a unique framework for assessing integration of functional brain regions mediating different aspects of language processing. Neuroimaging studies assessing language comprehension in adults with ASD have found that they rely heavily on the recruitment of additional brain regions or alternative neural routes outside the language network in order to process higher-order language tasks (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Mason, Williams, Kana, Minshew, & Just, 2008; Kana & Wadsworth, 2012; Tesink et al., 2011; Wang, Lee, Sigman, & Dapretto, 2006). In TD individuals, the language comprehension network is left-hemisphere dominant, and includes the left posterior middle temporal gyrus, superior temporal gyrus, superior temporal sulcus, inferior frontal gyrus, and middle frontal gyrus (Tomasi & Volkow, 2012; Turken & Dronkers, 2011). On the other hand, during language comprehension tasks, individuals with ASD tend to recruit additional right-hemisphere brain regions, as well as posterior parietal and occipital brain regions (Kana, Keller, Cherkassky, Minshew, & Just, 2006; Kana & Wadsworth, 2012; Mason et al., 2008). Just et al. (2004) found that high-functioning adults with ASD, when compared to TD controls, exhibited less activation in inferior frontal gyrus (Broca's area) and greater activation in posterior superior temporal gyrus (Wernicke's area) during sentence comprehension. The authors suggested that individuals with ASD may rely more heavily on lexical processing to comprehend sentences and less on thematic processing. Kana et al. (2006) assessed brain activation in response to high- and low-imagery sentences, and results revealed that individuals with ASD are more reliant on visualization to support language comprehension. The functional activation results revealed that the participants with ASD showed similar activation patterns regardless of imagery type, such that the participants with ASD tended to activate visual-spatial regions, specifically the intraparietal sulcus and the inferior temporal gyrus, regardless of whether the sentence involved high- or low-imagery. TD participants, on the other hand, recruited visual-spatial regions only during the high-imagery sentences. These results tend to fit with the Enhanced Perceptual Functioning (EPF) model, which emphasizes increased reliance on local details and visual processing in ASD. According to the EPF model, perceptual mechanisms are predominantly used in supporting social cognition and language in autism, leading to behavioral deficits in these areas (Mottron, Dawson, Soulières, Hubert, & Burack, 2006; Samson, Mottron, Soulières, & Zeffiro, 2012).

Such increased reliance on visuospatial regions may underlie weaker coordination between primary visual and association areas in ASD, especially while processing complex tasks. The theory of cortical underconnecivity posits that many cognitive functions, such as language, are dependent on the synchronization of brain regions and such coordination between brain areas may be compromised in individuals with ASD (Just et al., 2004; Kana et al., 2006). Collaborative with the activation findings reported above, task-related connectivity findings in ASD have collectively found both decreased connectivity between anterior and posterior brain regions (e.g., Just et al., 2007; Kana et al., 2009) and enhanced local connectivity between occipital and parietal regions (e.g., Sahyoun, Belliveau, Soulières, Schwartz, Mody, 2010; see reviews Minshew & Keller, 2010; Maximo, Cadena, & Kana, 2014). Specific to processing language, Sahyoun et al. (2010) found intact connectivity of relatively posterior networks in individuals with ASD, namely the occipitoparietal and ventral temporal regions, when processing both verbal and visual information. On the other hand, this study found decreased connectivity of frontal and temporal language areas in ASD compared to TD control participants. Functional underconnectivity has also been found between inferior frontal gyrus and posterior STG/Angular gyrus, anterior and posterior language regions, in individuals with ASD when interpreting a visual imagery language task (Kana et al., 2006).

While neural deficits in language comprehension in adults with ASD are well documented, far fewer neuroimaging studies have assessed language comprehension in children with ASD. Nevertheless, it appears that early in development, toddlers with ASD fail to activate left hemisphere brain regions in response to auditory language, and instead have abnormally high activation response in right-lateralized temporal regions (Eyler, Pierce, & Courchesne, 2012). This suggests that language functioning may be disrupted fairly early in development. Groen et al. (2010) found that children and adolescents with ASD (age range 12 to 18 years old) when compared to controls had significantly less activation of the left inferior frontal region when reading sentences that required integration of social information in order to comprehend. In examining remediation effects, Keller and Just (2009) assessed white matter changes using diffusion tensor imaging (DTI) in children (ages 8 to 10) who were classified as poor readers. The results revealed an increase in fractional anisotropy (FA) in relatively anterior brain regions, specifically frontal regions innervated by the left anterior centrum semiovale, after reading remediation, suggestive of increased white matter integrity. Overall, there is a need for more studies assessing the neural correlates underlying language comprehension, especially specific to children with ASD, in order to better understand the developmental trajectory of higher order language skills in ASD.

III. Utility of Task-Independent Measures

One of the inherent difficulties of task-related brain activity measures is that it is constrained by participant performance and the level of comprehension of the task. This makes it difficult, and at times limiting, in characterizing the neural mechanisms underlying complex tasks like language processing as one does not know whether the neural activity is driven by task performance or lack of task performance. One alternate way to assess these underlying neural processes is to investigate activation and connectivity during “resting state”, which requires no task performance. Recently, neuroimaging studies of ASD have begun investigating intrinsic brain activation, or spontaneous brain fluctuations that occur when the participant is not actively engaged in a task. In TD individuals, a distinct network of cortical midline structures are consistently activated when an individual is not engaged in active cognition, or when he/she is “at rest”, and deactivated when the individual is engaged in a cognitively demanding, goaloriented task (Raichle & Snyder, 2007; Raichle et al., 2001). These regions include the medial prefrontal cortex, ventral anterior cingulate cortex, posterior cingulate cortex, precuneus, angular gyrus, and bilateral inferior parietal lobules, among others, and are collectively referred to as the default mode network (DMN; e.g., Broyd et al., 2009; Buckner, Andrews-Hanna, & Schacter, 2008; Fox et al., 2005; Greicius, Krasnow, Reiss, & Menon, 2003; Raichle et al., 2001; Shulman et al., 1997). There is strong evidence that the DMN changes with age during typical development (Fair et al., 2007; Fair et al., 2008; Fair et al., 2009; Stevens, Pearlson, & Calhoun, 2009; Supekar, Musen, & Menon, 2009). Specifically, research has found that as a child's brain develops there is less "cross-talk" between networks, and a strengthening of within-network communication, suggesting a pattern of specialization and increased efficiency of networks during development (Stevens et al., 2009). Research on the DMN in ASD suggests that children with ASD experience prolonged neural development, resulting in an immature DMN that resembles a much younger developmental age, as well as, aberrant functional connectivity, characterized by recruitment of brain regions outside of the DMN. Consistent findings in children with ASD include overall weaker functional connectivity of the DMN compared to controls (Weng et al., 2010) and aberrant functional connectivity during rest in such regions as insular and right superior temporal gyrus that is not seen in the DMN in TD individuals (DiMartino et al., 2011).

While the majority of resting state neuroimaging research has focused on the DMN, a few studies have assessed other functional brain networks using intrinsic low-frequency brain activity (e.g., Toro, Fox, & Paus, 2008). One of the major benefits, especially for the ASD population, of using intrinsic brain activity to assess functional networks is that it can be done without task constraints. Of particular interest in our studies is the ability to assess the language and reading comprehension network using intrinsic brain activity. Indeed, by using intrinsic activity to study language comprehension, a basic network can be identified that acts as a framework for language processing regardless of task (Lohmann et al., 2010). This is especially important for children with ASD, in which developing language tasks that span the spectrum of comprehension ability is challenging. The language network in TD children has been successfully mapped out using intrinsic brain activity (Koyama et al., 2011). The intrinsic reading network in children has been identified to include core language regions, such as superior temporal gyrus, inferior frontal gyrus (pars triangularis), middle frontal gyrus, and intraparietal sulcus, as well as motor regions (supplementary motor area and precentral gyrus), visual regions (inferior occipital gyrus and fusiform gyrus), and the thalamus (Koyama et al., 2011). Additionally, Koyama et al. (2011) found that resting state connectivity between superior temporal and inferior frontal cortical areas was positively correlated with children's reading performance. To our knowledge, only one study has assessed intrinsic brain activity of the language network in children with ASD (Verly et al., 2014). They found functional underconnectivity between Broca's area and supplemental motor area and dorsolateral prefrontal cortex, as well as, between frontal and cerebellar regions in children with ASD (Verly et al., 2014). Our two studies are the first to assess both extrinsic and intrinsic brain activity in children with ASD in response to a language intervention.

IV. Specific Aims

The primary aim of this project was to examine the impact of a language remediation program on brain activity in children with ASD. The Visualizing and Verbalizing for Language Comprehension and Thinking (V/V) Intervention Program (Bell, 1991a,b; Torgesen et al., 2001) 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. Despite the visualizing aspects of the program having significant implications for ASD, this intervention has never been studied in this population. This project will use functional magnetic resonance imaging (fMRI) in a longitudinal design before and after the V/V intervention to compare language task-related, and task-independent brain activity patterns between high-functioning children with ASD who receive the V/V intervention and those with ASD who receive no language-based intervention. The children with autism will also be compared to healthy-matched controls.

Specific Aim #1: To investigate the neurocognitive changes from pre- to post-intervention in response to a visual imagery language task in children with ASD who participate in the language-based V/V intervention (Bell, 1991a,b), compared to children with ASD who do not participate in the intervention between imaging sessions (wait-list control group). In addition, we sought to investigate differences in response to our task between children with ASD and TD children. We developed the following hypotheses: (1) Children with ASD would show atypical patterns of activation in classic language areas, left inferior frontal gyrus and left posterior superior temporal gyrus, and increased recruitment of parietal and right hemisphere areas compared to TD children. Connectivity between LIFG and IPS will be weaker in participants with ASD, relative to TD participants. (2) Children with ASD participating in the invention as compared to the wait-list controls children with ASD would show greater improvement (as measured by reaction time and accuracy) on the visual imagery language task from the first to second scanning session. Additionally, children with ASD would show behavioral improvements in reading comprehension that would not be seen in the wait-list controls. This improvement would be marked by increased brain activation of core language areas, such as superior temporal and inferior frontal cortical areas, from first to second scanning session. (3) We expected find that those children with ASD who participated the intervention would also develop compensatory mechanisms to improve reading comprehension, manifesting itself as increased brain connectivity between language and visual-spatial brain regions, as well as, increased local connectivity between posterior language regions and visual regions (e.g., Wernicke's area and primary visual cortex). We did not expect to find a strengthening of these connections in the wait-list controls.

Specific Aim #2: To use a task-independent method to identify low frequency (< 0.1 Hz) fluctuation of spontaneous brain activity that represent structured and organized reading network in order to investigate changes in brain connectivity before and after the V/V intervention in children with ASD. Additionally, we investigated differences in task-independent activity between children with ASD and TD children. We developed the following hypotheses: (1) Children with ASD would show aberrant resting state activation and connectivity when compared to typical controls; this would be characterized by recruitment of regions and networks that are outside the well-established reading network, such as visual and somatosensory areas, as well as, decreased connectivity between anterior and posterior language regions (such as between Broca's and Wernicke's areas) compared to TD children. (2) Children with autism who participated in the V/V intervention would show changes in their intrinsic brain activation of the reading network, characterized by an increase in activation and connectivity of the core language areas, such as superior temporal and inferior frontal cortical areas. In addition, we expected a positive correlation with activity of the language-related brain regions at the second scanning session and behavioral improvement in reading comprehension. (3) Lastly, we also predicted that for those children with ASD who participated in the intervention, as compared to the wait-list controls, may develop compensatory mechanisms by recruiting regions outside of the reading network, such as increased connectivity between language and visual-spatial brain regions, as well as, increased connectivity between language regions and pre-motor/motor and somatosensory regions.

The main objective of this project was to determine if dysfunctional brain networks in ASD can be improved as a result of an intensive remediation program. Given the translational nature of this project, our goal is to increase the understanding of neural systems in children with ASD in order to apply this knowledge to developing targeted interventions aimed at addressing the symptoms of ASD at the neural level.

THE IMPACT OF READING INTERVENTION ON BRAIN ACTIVITY UNDERLYING LANGUAGE IN CHILREN WITH AUTISM SPECTRUM DISORDERS

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Abstract

Deficits in language comprehension have been widely reported in children with autism spectrum disorders (ASD). Behavioral and neuroimaging studies have consistently found that individuals with ASD tend to rely heavily on visuospatial processing to aid in language comprehension. However, no study to date, has addressed using this advantage to help improve language comprehension difficulties in ASD. This study used a translational neuroimaging approach to examine the impact of an imagery-based reading intervention program to improve the brain circuitry underlying language processing and its integrated functioning in children with ASD. We used functional magnetic resonance imaging (fMRI) in a longitudinal design to investigate change in reading comprehension, brain activation, and functional connectivity (in a sentence comprehension task) as a result of reading intervention in three groups of participants: an experimental group of ASD children (ASD-EXP), a wait-list control group of ASD children (ASD-WLC), and a group of typically developing (TD) control children. After intervention, the ASD-EXP group increased activation of posterior visual and language regions, as well as right-hemisphere language homologous regions, precentral gyrus (PrCG), postcentral gyrus (PoCG), putamen, and thalamus, suggestive of compensatory mechanisms to increase proficiency in reading comprehension. Additionally, children with ASD who showed the most improvement in reading comprehension after intervention showed greater activation post-intervention in response to sentences involving high-imagery, in posterior visual and language regions, as well as strengthened functional connectivity between left hemisphere language areas, the middle temporal gyrus and inferior frontal gyrus. Thus, the findings of this study suggest the potential of a strength-based reading intervention in

changing the brain’s response and facilitating better reading comprehension in children with ASD. Overall, this study has implications for individualizing treatment for children with ASD, and provides support for the growing body of literature on the plasticity of the young brain in ASD.

Introduction

Impairment in language and communication is a major clinical feature of autism spectrum disorders (ASD). The current diagnostic markers include a deficit in language comprehension, behind speech production, in language-able individuals with ASD (American Psychiatric Association, 2013). In addition, reliance on perceptual processes, such as sensory or motor processing, to support higher-order cognitive skills has been identified as a phenotype of ASD (Belmonte, 2004). Behavioral studies have consistently found that children and adolescents with ASD have intact, sometimes enhanced, visuospatial skills (especially in high-functioning children with ASD) in tasks such as the embedded figures test (de Jonge et al., 2006; van Lang et al., 2006; White & Saldaña, 2011) and visual search (Joseph et al., 2009; Manjaly et al., 2007). Despite general deficits, some studies have found that individuals with ASD perform similarly to typically-developing (TD) individuals on linguistic tasks that have a visual component (Kamio & Toichi, 2000; Moseley et al., 2013; Sahyoun et al., 2009; Toichi & Kamio, 2001). However, such increased reliance on visuospatial processing in individuals with ASD may lead to a mode of thinking that relies heavily on primary processing at the expense of higher-order cognitive processes (Belmonte et al., 2004; Goldstein, 1994; Minshew & Goldstein, 1998). For example, Goldstein (1994) found that the primary breakdown in language processing in individuals with ASD occurred when more complex skills were needed. More recent behavioral studies assessing higher-order language skills in children with ASD, such as reading and oral language comprehension, support this account (Jones et al., 2009; Norbury & Nation, 2011).

Another interesting facet of language and communication in ASD is that many children with ASD have better reading fluency accompanied by poor comprehension (e.g., Nation et al., 2006; Newman et al., 2007; Lindgren et al., 2009; Jones et al., 2009; Norbury & Nation, 2011). Interestingly, Jones et al. (2009) found that the majority of children with ASD in their sample exhibited basic reading skills that were equivalent to their full scale IQ, both of which were in the low average range; however, this was coupled with statistically poorer reading comprehension. In addition, reading comprehension was found to be inversely correlated with social and communication deficits. Several earlier accounts also, including Kanner's first report (Kanner, 1943), indicate that age equivalent scores for children with ASD are typically lower for reading comprehension than for reading accuracy (Bartak & Rutter, 1973; Lockyer & Rutter, 1969; Nation et al., 2006; Snowling & Frith, 1986). Thus, the discrepancy between reading ability and comprehension in children with ASD is an important issue that needs to be better understood in order for interventions to be more effective.

Neuroimaging provides a unique framework for investigating the brain mechanisms underlying this complex behavioral profile of language. Functional MRI studies assessing language comprehension in adults with ASD have found an increased reliance on additional brain regions or alternative neural routes outside the typical language network in order to process higher-order language tasks (Baron-Cohen, et al., 2001; Kana & Wadsworth, 2012; Mason et al., 2008; Tesink et al., 2011; Wang et al., 2006). These studies find that adults with ASD tend to recruit additional right-hemisphere brain regions, as well as posterior parietal and occipital brain regions when engaged in a language comprehension task. For example, Kana et al.'s (2006) study of brain activation in response to high and low-imagery sentences revealed greater reliance on visualization in individuals with ASD to support language comprehension. While neural deficits in language comprehension in adults with ASD are well documented, far fewer neuroimaging studies have addressed this in children with ASD. Nevertheless, it appears that early in development, toddlers with ASD fail to activate left hemisphere brain regions in response to auditory language, and instead have abnormally high activation response in right-lateralized temporal regions (Eyler et al., 2012; Redcay & Courchesne, 2008). This suggests that brain response to language may be altered in ASD fairly early in development. Reduced activation in core language areas like the left inferior frontal gyrus (IFG), when reading sentences that required higher-order integration, has also been reported in children with ASD (Groen et al., 2010). Furthermore, Sahyoun et al. (2010) found that children with ASD had increased reliance of visuospatial brain regions, including occipital, parietal, and ventral temporal areas, and reduced activation in frontal language areas when linguistic mediation was needed during a pictorial problem solving task.

Thus, similar to the findings in adults, atypical recruitment of visual and right hemisphere regions in processing language may also be seen in children with ASD. Indeed, a recent meta-analysis (Samson et al., 2012) found greater reliance on mental imagery and visualization to process words and sentences, specifically the fusiform gyrus and medial parietal cortex. These results were coupled with stronger right-hemisphere activity for reading tasks in individuals with ASD. While the previous neuroimaging studies have examined language and visuospatial functioning in conjunction in adults with ASD, with a few studies in children with ASD, to our knowledge, there have been no studies assessing whether increased reliance on visuospatial processing to comprehend written language can be utilized as an appropriate avenue for interventions targeting language comprehension deficits in children with ASD. This would be especially pertinent given the plasticity of the developing brain on higher-order language skills (Mody et al., 2013). Neuroimaging studies of children with reading comprehension difficulties have not only found increased activity after reading intervention in left-lateralized language regions, but also in right hemisphere compensatory regions, such as right IFG (for review see meta-analysis Barquero, Davis, & Cutting, 2014). However, very little research has attempted examining neurological changes in response to targeted reading intervention in children with ASD. Behavioral findings from reading interventions in children with ASD are also limited, with a recent meta-analysis (El Zein et al., 2014) reporting results from only 12 studies, of which the majority were single-subject case studies. This suggests a dearth of translational studies and a greater need for assessing the efficacy of potential reading interventions for children with ASD. Even in very young children with ASD (age 2.5 years), reading proficiency, in the form of alphabet knowledge, was predictive of better cognitive and verbal outcomes at 5.5 years of age (Davidson & Ellis-Weismer, 2014), suggesting the importance of interventions targeted at language comprehension.

The current study is a novel attempt in translational neuroimaging to address some of these gaps in the literature by examining the impact of language remediation training on brain responses in children with ASD. The intervention used in this study (Bell, 1991a,b; Torgesen et al., 2001) 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. Despite its significant implications for children with ASD, this intervention has never been applied to study ASD specifically. The present study used functional magnetic resonance imaging (fMRI) in a longitudinal design before and after a reading intervention to compare brain activity and connectivity between high-functioning children with ASD who receive the intervention and those with ASD who receive no language-based intervention. In addition, we compared the children with ASD to matched healthy control participants. Brain activity and functional connectivity were examined in response to a sentence comprehension task that requires the integration of imagery and language in order to comprehend the sentences. We hypothesized that children with ASD would show differences in language-related activation compared to TD controls, such as recruitment of more right-hemisphere brain regions and relatively more posterior, visual coding, brain regions while comprehending language. Second, children with ASD who participated in the reading intervention were expected to show improvement in performance in our sentence comprehension task and show both increased brain activity in regions underlying language comprehension, such as superior temporal and inferior frontal cortices; they may also develop compensatory strategies for comprehending sentences such as recruitment of additional right-hemisphere regions or visual regions. The unique aspect of this study is the focus on translational neuroimaging with the goal of increasing our understanding of established neural networks in children with ASD and translating this knowledge to develop targeted behavioral interventions.

Materials and Methods

Participants

Participants were 26 children with ASD (mean age = 10.9 ± 1.34) and 19 age and IQ-matched typically-developing (TD) children (mean age = 10.6 ± 1.59; see Table 1). All children with ASD underwent two fMRI sessions, 10 weeks apart. Thirteen randomly selected children with ASD participated in Visualizing and Verbalizing for Language Comprehension and Thinking (V/V) Intervention Program between imaging sessions (experimental group; ASD-EXP). The other 13 children with ASD received the same V/V intervention program after the two imaging sessions were completed (wait-list control group; ASD-WLC). The TD control group underwent one fMRI session and did not participate in any aspect of the V/V program. Children were determined to have an ASD diagnosis by either being diagnosed by a licensed clinical psychologist using the Autism Diagnostic Observation Schedule (ADOS; Lord et al, 2000) and/or the Autism Diagnostic Interview-Revised (ADI; Lord et al., 1994) or being diagnosed by a licensed clinical psychologist and a research diagnosis using the ADOS by trained research-reliable personnel. No statistically significant differences between the ASD group and the TD group were observed for age [$t(43) = 0.70$, $p = 0.487$], verbal IQ [mean ASD = 91.6 ± 11.35; mean TD = 96.1 ± 12.31; $t(43) = 1.26$, $p = 0.264$], or Full-Scale IQ [mean ASD = 96.0 ± 13.67 ; mean TD = 96.2 ± 10.89; $t(43) = 0.044$, $p = 0.965$]. In addition, the ASD-EXP and ASD-WLC groups did not differ on reading comprehension level prior to the first fMRI session, as measured by the Gray Oral Reading Test (GORT-4): Comprehension Score [mean ASD-EXP = 76.43 ± 12.0; mean WLC = 86.07 ± 11.27; $t(43) = 1.99$, $p = 0.06$]. Among the 26 children with ASD, 5 were female (2 in the ASD-

EXP group and 3 in the ASD-WLC group), and all were right-handed. In the TD group, 5 were female, and all were right-handed. Data from 5 children with ASD and 3 TD children were discarded due to artifacts from excessive head motion that were unable to be corrected (see method section below on motion correction).

All participants with ASD were recruited through multiple sources, 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, and the Alabama Autism Society, from the greater Birmingham area. In addition, the Lindamood-Bell Learning centers recruited potential participants at their centers, and sent those families to UAB for eligibility testing. The TD participants and their families were recruited by advertisements in local newspapers and by flyers posted on UAB campus. All participants with ASD met the following inclusion criteria: ages from 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 37th percentile and/or a Gray Oral Reading Test – Forth Edition (GORT-4) accuracy score of at least 25th percentile, a GORT-4 comprehension score below 37th percentile, and a Verbal IQ score of at least 75, as measured by the Wechsler Abbreviated Scale of Intelligence (WASI). The inclusion criteria of TD participants included: ages from 8 to 13, no diagnosis of an ASD or a language disorder, and an average (greater than the 25th percentile) oral reading and reading comprehension, as measured by the SORT-R and GORT-4. Participants failing to meet any of the inclusion criteria, and participants with a history of ferromagnetic material in the body, or neurostimulators, being claustrophobic, or history of seizure disorder were excluded from the study. All participants were off medication 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 University of Alabama at Birmingham (UAB) Institutional Review Board, to participate in the study and were compensated for their participation.

Table 1. Participant Demographics

Characteristic	***ASD-EXP Group (n = 13)***	***ASD-WLC Group (n =13)***	***TD Group (n = 19)***
Age[a]	10.9 ± 1.53	11.0 ± 1.19	10.6 ± 1.59
Gender			
Male	11	10	14
Female	2	3	5
Self-Identify			
Caucasian	10	6	10
Black		1	9
Asian	3	5	
Hispanic		1	
Social Communication Questionnaire (SCQ)[f]	17.28 ± 8.32	20.43 ± 4.85	5.94 ± 3.74
WASI FSIQ[b]	94.1 ± 11.23	97.9 ± 15.91	96.2 ± 10.89
WASI VIQ[c]	91.0 ± 8.91	92.1 ± 13.70	96.1 ± 12.31
WASI PIQ[d]	100.2 ± 11.30	104.4 ± 17.06	98.9 ± 12.41
GORT-4 Comprehension S1[d]	76.4 ± 12.0	86.1 ± 11.27	105.3 ± 20.68
GORT-4 Comprehension S2 [d]	86.1 ± 10.41	87.3 ± 12.84	N/A
SORT-R Reading Score[e]	105.2 ± 5.73	105.7 ± 8.43	107.1 ± 8.24

Note. value ± standard deviation
[a]Age in decimal years at first imaging session
[b]Wechsler Abbreviated Scale of Intelligence, Full Scale Intelligence Quotient
[c]Wechsler Abbreviated Scale of Intelligence, Verbal Intelligence Quotient
[d]Wechsler Abbreviated Scale of Intelligence, Verbal Intelligence Quotient
[d]Gray Oral Reading Test – Forth Edition Comprehension subtest at the first session (S1) and second session (S2) in standard scores
[e]Slosson Oral Reading Test - Revised (SORT-R) reading score at the first session in standard scores
[f] Social Communication Questionnaire – Lifetime Form (SCQ): measures communication skills and social functioning throughout the child's entire developmental history to screen for autism symptoms. Higher score indicated greater autism symptomotology.

Reading Intervention: Visualizing and Verbalizing for Language Comprehension and Thinking (V/V) Intervention Program

The V/V intervention program is a relatively well-established and tested language remediation program designed by Nanci Bell, and developed by Lindamood-Bell Learning Processes (http://www.lindamoodbell.com) (Bell, 1991b). The V/V intervention is based on the use of nonverbal sensory input, in the form of imaged gestalts, in order to develop oral and written language comprehension, establish vocabulary, and develop higher order thinking skills (Bell, 1991a,b). The V/V program is designed to teach children to form imaged gestalts, or concept imagery, as they read and hear language. Through the sequential teaching methods of the program, the imaged gestalt helps develop the imagery-language connection and improve oral and reading comprehension. The student progresses in the program by beginning with word imagery and then extending their understanding to sentence, paragraph, and page imagery. The ultimate goal of this intervention is to apply nonverbal imagery to language comprehension to improve children's reading and listening comprehension, communication skills, and critical thinking skills.

This intervention the children with ASD received was intensive, taking place in 4-hour sessions, 5 days a week for 10 weeks. The intervention was conducted at the Lindamood-Bell Learning Processes center closest to the family. This allowed participants to choose to go to one of the centers closest to their home. Trained clinicians administered the program in a standardized manner, and were monitored by an experienced supervisor who gave constant feedback to the clinicians. Implementation of V/V to the participants was done one-on-one in a distraction-free setting, with clinicians rotating every hour. The clinical teaching method is a guided approach, as opposed to just telling the participant he/she is right or wrong, and is what the program describes as "responding to the response", as opposed to immediately correcting the participant when he/she makes a mistake.

Experimental Paradigm

This experiment assessed brain activation during sentences involving mental imagery. This task was adapted from a previous design (Kana, et al., 2006), and included sentences involving high imagery (e.g., *An H on top of an H on top of another H looks like a ladder*) and low imagery (e.g., *Addition, subtraction, and multiplication are all math skills*). The experiment included 24 high-imagery sentences and 24 low-imagery sentences; these sentences were further divided into true or false categories, such that 12 of the high-imagery sentence and 12 of the low-imagery sentences were true statements, and the other 12 sentences from each imagery group were false statements. Prior to the scan, each participant practiced the task on a laptop; the practice trials consisted of three high-imagery and three low-imagery sentences. During the scan, participants determined, by button press, whether each sentence was true or false. Each sentence was displayed for 8000 ms with an inter-stimulus gap of 2000 ms between each sentence. Each block consisted of three high-imagery or three low-imagery sentences in rotating order, for a total of 16 blocks, 8 low-imagery blocks and 8 high-imagery blocks, with a 6000 ms rest period between each block of 3 sentences. In addition, a 24-s fixation condition was presented before the start of the task, and after every 4 blocks, for a total of five, to provide a baseline measure of brain activation with which to compare each experimental condition. Two sentences, one high-imagery and one low-imagery, were presented at the beginning of the experiment as practice trials to re-familiarize the participant with the task. The experiment was presented in the scanner through the stimulus presentation software E-Prime 1.2 (Psychology Software Tools, Pittsburgh, PA). The sentence stimuli were rear-projected onto a translucent plastic screen and participants viewed the screen through a mirror attached to the head coil. Given the difficulties associated with imaging children with disabilities in the MRI scanner (Kana et al., 2011), the children with ASD in this study were given a social story outlining what they would be doing prior to arrival for the study. Additionally, children were given exposure, via a mock-scanning session, the day before the MRI scan, so that the children were familiar and comfortable with the MRI machine and staff prior to the scan.

Since the Kana et al. (2006) study imaged adults with ASD, the stimuli for this experiment was piloted on 50 neurotypical children between the ages of 8 and 13 with a paper and pencil version of the task. We used an ideal difficulty level for each sentence as 85% of participants being able to correctly identify the sentence as true or false and 50% or less as at chance or too difficult. The sentences from the Kana et al. (2006) study were adapted to the appropriate reading level such that all newly generated sentences fell in the category of 85% or more children were able to correctly identify the sentence as true or false. Additionally, items in which 50% or less children identified correctly were discarded. A total of 96 sentences were developed and then randomly divided into two sets of 48 sentences, 24 high-imagery and 24 low-imagery sentences, for each of the first and second scanning sessions. These 96 sentences were all matched on word length and level of difficulty. In order to minimize the possible task-dependent effects, half of the participants received one version of the task and the other half the second version of the task both at the first and at the second scanning session, such that one version of the task was not used entirely at one scanning session.

MRI Data Acquisition

Structural and functional MRI data were acquired at each of the two scanning sessions. The MRI data were collected using a Siemens 3.0 Tesla Allegra head-only Scanner (Siemens Medical Inc., Erlangen, Germany) located at the UAB Civitan Functional Neuroimaging Laboratory (CFNL). For the high resolution anatomical scan, T1- weighted scans were acquired using a 160-slice 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) volume scan with TR = 200 ms, TE = 3.34 ms, flip angle = 7, FOV = 25.6 cm, 256 X 256 matrix size, and 1 mm slice thickness. 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 degree flip angle for 17 oblique axial slices 5 mm slice thickness with a 1 mm slice gap, a 24 X 24 cm field of view (FOV), and a 64 X 64 matrix, resulting in an in-plane resolution of 3.75 X 3.75 X 5 mm^3.

fMRI Data Analyses

Data were preprocessed using the SPM8 software package (Wellcome Department of Imaging Neuroscience, London, UK) run within Matlab (7.10; Mathworks, Inc). Functional images were slice time-corrected to the onset of the middle slice and spatially realigned using *INRIAlign,* a motion correction algorithm unbiased by local signal changes (Freire et al., 2002). During realignment, a mean functional image was computed for each run and then matched to the EPI template provided within SPM8. Data were then spatially normalized to standard Montreal Neurological Institute (MNI) brain space and spatially smoothed using a three-dimensional Gaussian kernel of 8 mm full-width at half-maximum (FWHM), resulting in a resampled in-plane resolution of $2 \times 2 \times 2$ mm^3 voxels. The functional data were then passed through a 1/128 Hz high-pass filter in order to remove low-frequency artifacts (e.g., respiratory artifact). In addition, at any time point for which estimates of head motion exceed 2 mm in length in any x, y, z dimension, these specific images were censored from the model by inclusion of a separate regressor that accounted for the variance related to that image only using the Art Detection Tool (ART) within Matlab (http://web.mit.edu/swg/art/art.pdf). If over one-third of the images had to be censored due to movement, that participant was excluded from the analyses.

When analyzing the unique brain activation in response to the visual imagery language task, single-subject contrast maps were generated within the context of the General Linear Model (GLM) on a voxel by voxel basis as implemented in SPM8. Separate regressors were created for low-imagery sentences, high-imagery sentences, and fixation by convolving the time course of activation, using a boxcar function, with the canonical hemodynamic response function (HRF). Fixed-effects analyses were performed for each individual participant for both imaging sessions, examining the variance in regional BOLD response to low-imagery sentences vs. fixation, high-imagery sentences vs. fixation, and high-imagery sentences vs. low-imagery sentences. Group analyses were performed using a random effects model of the beta images from the single-subject contrast maps. Additional between-group comparisons were run to determine whether the contrast estimates for both the scanning sessions separately and the first vs. second scanning sessions were significantly different between the children with ASD who received the intervention between scanning sessions (ASD-EXP group) and the ASD-WLC group. Additional multiple regression analyses were performed to assess the relationship between participant's individual activation and their change in GORT-4 comprehension subtest score from first to second session as an outcome measure of improvement in reading comprehension due to the intervention. For all analyses, an uncorrected height threshold of $p < 0.01$ and an extent threshold of 200 voxels were used.

Functional Connectivity Analyses

Functional connectivity (the synchronization of brain activation between regions) was computed separately for each participant as a correlation between the average time course of all the activated voxels in each member of a pair of regions of interest (ROIs) for the visual imagery task. FCA was carried out on anatomical ROIs, defined to encompass the main clusters of activation in the group activation map for each group in both the high imagery vs. fixation and the low imagery vs. fixation contrasts. ROIs were defined structurally using templates from the WFU Pickatlas toolbox within SPM8 using the AAL or Talairach Daemon atlases (Lancaster et al., 2000; Maldjian et al., 2004). The eleven ROIs included in the FCA were: bilateral anterior cingulate cortex (ACC), bilateral lingual gyrus (LG), left middle occipital gyrus (LMOG), left inferior frontal gyrus (LIFG; including Broca's area), left inferior parietal lobule (LIPL; including Wernicke's area, BA39/BA40), left middle frontal gyrus (LMFG), left medial prefrontal cortex (LMPFC), left middle temporal gyrus (LMTG), left parahippocampal gyrus

(LPHG), left precuneus (LPrC), and left fusiform gyrus (LFG; including the visual word form area (VWFA)). The activation time course was extracted for each participant over the activated voxels within each ROI originated from the normalized and smoothed images, which were high-pass filtered and had the linear trend removed. To help control for multiple comparisons, these ROIs were further grouped into larger regions/networks based on location (frontal, parietal, temporal, occipital), with MTG being its own node representing the main language region in the temporal lobe. Functional connectivity was measured for each participant in each group for both the high imagery and low imagery conditions. Fisher's r to z transformation was applied to the correlation coefficients for each participant prior to averaging and performing statistical comparison.

Results

Behavioral Results

The ASD-EXP group showed a significant improvement in reading comprehension (from pre- to post-intervention), as measured by the GORT-4 Comprehension subtest, [paired t-test, $t(12) = 3.79$, $p = 0.002$ (see Table 1 for means)]. On the other hand, the ASD-WLC group did not have a significant change in reading comprehension from the first to second imaging session [paired t-test, $t(12) = 1.12$, $p = 0.247$]. In addition the ASD-EXP group significantly improved their reading comprehension scores, compared to the ASD-WLC group [ASD-EXP mean change in standard score (SS): 9.64, ASD-WLC mean change in SS: 1.92, $t(24) = 2.05$, $p = 0.050$]. The TD group had significantly higher reading comprehension scores than the children with ASD at the first session [ASD-EXP + ASD-WLC groups versus TD; $t(43) = 4.92$, p

< 0.0001]. After completing the intervention, the ASD-EXP group continued to have significantly lower reading comprehension scores than the TD group ($t(30) = 4.61$, $p < 0.0001$).

The behavioral results from the task performance in the MRI scanner demonstrated similar performance between the ASD groups and the TD group at the first imaging session. A 3 Group (ASD-EXP, ASD-WLC, TD) × 2 Sentence Type (high-imagery, low-imagery) ANOVA indicated no significant differences between groups either in reaction time or in performance accuracy; nor were there any significant interactions between group and sentence type for reaction time or accuracy. The high-imagery sentences had reliably longer reaction times across groups [mean low-imagery = 4590 ms, mean high-imagery = 5170 ms, $t(43) = 3.26$, $p = 0.002$]. There were no differences in accuracy between sentence types. The ASD-EXP group showed significant improvements in accuracy in comprehending both high-imagery and low-imagery sentences from pre- to post-intervention [high imagery: paired t-test, $t(12) = 2.99$, $p = 0.011$; low imagery: paired t-test, $t(12) = 2.35$, $p = 0.035$]. There were no differences pre- to post-intervention in reaction times for the ASD-EXP group; nor were there any differences in reaction time or accuracy for the ASD-WLC group between imaging sessions.

Brain Activation Results

Results: Pre-Intervention Neuroimaging

As there were no differences between the ASD-EXP and ASD-WLC groups at the first imaging session for either high-imagery or low-imagery conditions contrasted with fixation, within-group results of the first imaging session reported here include all children with ASD. The ASD group (ASD-WLC + ASD-EXP groups) and the TD group showed strong activation in visual and language regions while comprehending high-imagery sentences (see Table 2). In particular, the children with ASD revealed activation in visual areas, including left middle occipital gyrus (MOG), left lingual gyrus (LG) and right cuneus, and left lateralized language regions, including LIFG, LMTG, and posterior superior temporal gyrus (pSTS). The TD group also activated similar language regions as in the ASD groups, with additional activation in the left visual word form area (VWFA), left parahippocampal gyrus, and left PrCG (see Table 2). In addition, in response to high-imagery sentences, both children with ASD and TD children activated additional right hemisphere regions, including visual regions, such as right LG and right cuneus, as well as right hemisphere frontal-temporal areas including the IFG, MFG, SFG, and superior temporal gyrus (STG). In the low imagery condition, the ASD groups showed primarily left lateralized activation in the MOG, the IFG, as well as the supplementary motor area (SMA; see Table 3). The TD group also showed activation in the left MOG and left IFG, but also showed recruitment of a number of right hemisphere regions, such as right LG, right MFG, bilateral MPFC, and right thalamus (see Table 3).

Between-group comparison results (ASD-EXP + ASD-WLC groups versus TD group) in the high-imagery condition revealed that the ASD groups had less activation than the TD group in a number of posterior brain regions, including the left MOG/LG, left fusiform gyrus, and right cuneus (see Table 2). Between-group results in the low-imagery condition showed a similar pattern, with the ASD groups having less activation than the TD group in posterior brain regions, including bilateral MOG, left fusiform gyrus, and left cerebellum (see Table 3). For both the low-imagery and high-imagery conditions, there were no brain regions that showed less activation in the TD group compared to the ASD groups.

TABLE 2. Brain regions of activation for the high imagery sentence condition both within-group and between-group differences in the ASD group (ASD-EXP + ASD-WLC groups; n = 26) at the first imaging session and the TD group (n = 19) (p <0.005, 200 voxels extent threshold).

Location of Peak Activation	***High Imagery Condition***			***MNI coordinates***		
ASD Group	*BA*	*Cluster*	*t*	*x*	*y*	*z*
L Dorsolateral Prefrontal Cortex	9	4408[a]	6.65	-46	2	34
L Inferior Frontal Gyrus	44	4408[a]	4.47	-58	12	14
R Inferior Frontal – Opercular		436	3.58	46	10	34
L Middle Frontal Gyrus	46	4408[a]	4.88	-24	-94	-2
R Middle Frontal Gyrus		215	3.76	34	-4	66
L Superior Frontal Gyrus	8	1361[b]	4.72	-6	16	50
L Middle Temporal Gyrus	22	571	4.50	-54	-42	-2
L Inferior Parietal Lobule		1375[c]	4.33	-30	-50	44
L Superior Parietal Lobule		1375[c]	4.23	-28	-58	46
R Superior Parietal Lobule		321	4.42	32	-58	46
L Supplementary Motor Area	6	1361[b]	4.77	-2	8	58
L Middle Occipital Gyrus	18	3728[d]	10.29	-24	-102	4
L Lingual Gyrus	18	3728[d]	8.77	-24	-94	-6
R Cuneus	17	3728[d]	7.90	12	-94	-2
TD Group	*BA*	*Cluster*	*t*	*x*	*y*	*z*
L Inferior Frontal Gyrus	45	3554[e]	5.43	-50	22	20
L Middle Frontal Gyrus	9	3554[e]	6.18	-44	8	34
R Middle Frontal Gyrus	9	1698[f]	6.13	44	32	30
R Superior Frontal Gyrus		1698[f]	4.64	30	10	56
R Superior Temporal Gyrus		539	5.05	48	-32	2
L Supramarginal Gyrus	40	302[g]	3.63	-40	-40	34
L Precuneus	7	302[g]	3.87	-24	-62	38
R Precuneus	19	355	4.68	30	-72	30
R Supplementary Motor Area	6	1831	5.06	2	18	58
L Thalamus		795	3.17	-2	-32	2
L Visual Word Form Area	37	232	3.49	-48	-56	-6
L Parahippocampal gyrus	35	795	5.86	-18	-30	-8

R Lingual Gyrus	18	8730[h]	11.56	22	-92	-8
L Inferior Occipital Gyrus	18	8730[h]	8.71	-26	-92	-12
TD > ASD	*BA*	*Cluster*	*t*	*x*	*y*	*z*
L Fusiform Gyrus		2612[i]	3.51	-30	-64	-10
L Lingual/Middle Occipital Gyrus	17	2612[i]	4.43	-26	-92	10
R Cuneus/Superior Occipital	18	1128	3.40	24	-81	32
There were no areas in which the ASD group showed more activation than TD Group						

Note. L = left hemisphere, R = right hemisphere, BA = Brodmann's area
[a,b,c,d,e,f,g,h,i] Regions of activation encompassed within the same cluster

TABLE 3. Brain regions of activation for the low imagery sentence condition both within-group and between-group differences in the ASD group (ASD-EXP + ASD-WLC groups; n = 26) at the first imaging session and the TD group (n = 19) (p <0.005, 200 voxels extent threshold).

Location of Peak Activation	***Low Imagery Condition***			***MNI coordinates***		
ASD Group	*BA*	*Cluster*	*t*	*x*	*y*	*z*
L Dorsolateral Prefrontal Cortex	9	1817[a]	4.45	-50	6	34
L Superior Frontal Gyrus	8	576[b]	4.08	-4	14	54
L Insula	13	1817[a]	4.00	-54	10	4
L Supplementary Motor Area	6	576[b]	3.49	-8	20	68
L Middle Occipital Gyrus	18	3233[c]	8.61	-22	-102	2
R Middle Occipital Gyrus	18	3233[c]	6.52	20	-102	6
R Cuneus	17	3233[c]	7.93	12	-98	-2
TD Group	*BA*	*Cluster*	*t*	*x*	*y*	*z*
L Medial Prefrontal Cortex		1744[d]	5.01	-6	22	50
R Medial Prefrontal Cortex		1744[d]	5.19	6	24	52
L Inferior Frontal Gyrus	45	2784[e]	5.31	-52	22	18
R Middle Frontal Gyrus		391	4.09	44	32	30
L Superior Frontal Gyrus	6	1744	4.74	-4	14	58
L Insula	13	2784[e]	4.02	-32	22	-4
R Middle Temporal Gyrus	39	787[f]	4.40	34	-58	36
R Thalamus		787[f]	5.44	24	-30	2
L Inferior/Middle Occipital Gyrus	18	9991[g]	11.54	-22	-92	-8
R Lingual/Middle Occipital Gyrus	17	9991[g]	11.31	22	-92	-2
TD > ASD	*BA*	*Cluster*	*t*	*x*	*y*	*z*
L Middle Occipital Gyrus	18,19	1279	4.35	-26	-94	8
R Middle Occipital Gyrus/ Precuneus	19,31	610	3.20	30	-72	30
L Fusiform Gyrus	19	506[h]	3.31	-30	-64	-10
L Cerebellum		506[h]	3.22	-8	-70	-10
There were no areas in which the ASD group showed more activation than TD Group						

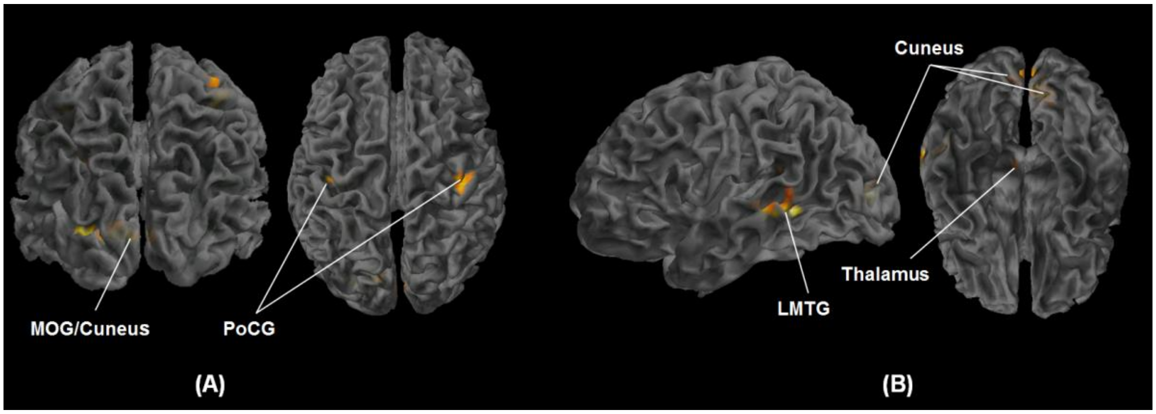
Note. L = left hemisphere, R = right hemisphere, BA = Brodmann's area
[a,b,c,d,e,f,g,h] Regions of activation encompassed within the same cluster

Results: Intervention-related changes in fMRI activation

In order to assess pre-to post-intervention changes in the ASD-EXP group we assessed for differences in activation specific to this group of children with ASD who participated in the intervention between imaging sessions. Of particular interest, were assessing changes in activation related to high-imagery sentences, given the imagery focus of the intervention, but we were also interested in whether the intervention also translated to changes in activation for other types of sentence comprehension (i.e., our low-imagery sentence condition). For the ASD-EXP group, greater activation was seen at the post-intervention session, compared to the pre-intervention session, in the high-imagery condition in the left cuneus, left MOG, bilateral postcentral gyrus (PoCG), and right PrCG (see Figure 1). For the low imagery condition, the ASD-EXP group showed significantly greater activation at the post-intervention session, compared to the pre-intervention session, in the bilateral cuneus, LMTG, left posterior cingulate cortex (PCC), right PoCG, as well as subcortical structures, including the left thalamus and left putamen (see Figure 1). There were no regions that showed greater activation at the pre-intervention session compared to the post-intervention session for the high-imagery or low-imagery condition. Within the ASD-WLC group, there were no significant differences in activation between the first and second imaging session for either the high imagery or low imagery conditions.

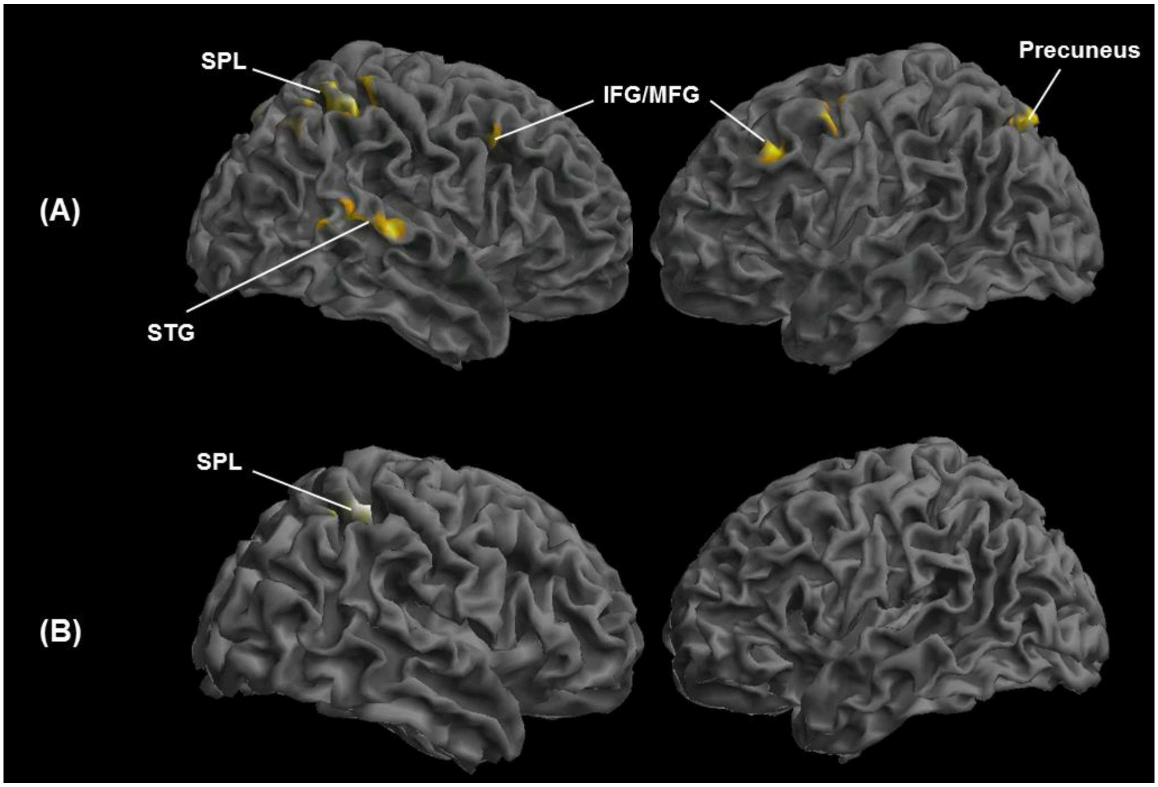
On order to better assess whether the activation related changes in the ASD-EXP group were specific to the intervention, between-group comparisons were conducted in order to assess differences in activation post-intervention between the ASD-EXP group and the ASD-WLC group. In the high imagery condition, between-group comparison revealed significantly greater activation in the ASD-EXP group compared to the ASD-WLC group, at the second imaging session (while covarying activation at the first imaging session), in the left precuneus, left dorsolateral prefrontal cortex (dlPFC), LMFG, as well as a number of right hemisphere regions, including MTG, IPL, SPL, and IFG (see Figure 2). In the low imagery condition, the ASD-EXP group had greater brain activation than the ASD-WLC group, at second imaging session, in the LPCC and right SPL (see Figure 2). The ASD-WLC group showed no areas of more activation than the ASD-EXP group at second imaging session either in the high-imagery or in the low-imagery conditions.

Figure 1. Areas of greater activation post-intervention than pre-intervention in the ASD-EXP group for both the high imagery and low imagery conditions ($p < 0.01$, 200 voxel extent threshold).



MNI coordinates of peak activation: (A) High Imagery: L Cuneus: x = -14, y = -88, z = 8; L MOG: x = -28, y = -84, z = 8; bilateral PoCG: x = -36, y = -34, z = 32 and x = 40, y = -24, z = 52; **R PrCG: x = 40, y = -20, z = 66; (B) Low Imagery: bilateral Cuneus: x = 12, y = -92, z = 8 and x = 12, y = -72, z = 4; L MTG: x = -48, y = -46, z = -2; **L PCC: x = -18, y = -40, z = 32; **R PoCG: x = 52, y = -14, z = 50; **L Putamen: x = -26, x = -20, x = -2; L Thalamus: x = -14, y = -34, z = 4.
**note: Not shown in figure.

Figure 2. Areas of greater activation in the ASD-EXP group > ASD-WLC group at the second imaging session for both the high imagery and low imagery conditions ($p < 0.01$, 200 voxel extent threshold).



MNI coordinated of peak activation: (A) High Imagery: R STG: x = 60, y = -32, z = 4; **R IPL: x = 42, y = -44, z = 56; R SPL: x = 34, y = -58, z = 56; L Precuneus: x = -18, y = -74, z = 50; L IFG: x = -48, y = 20, z = 40; L MFG: x = -34, y = 4, z = 62; R IFG: x = 46, y = 10, z = 40. (B) Low Imagery: **L PCC: x = -18, y = -40, z = 32; R IPL: x = 42, y = -44, z = 56.
**note: Not shown in figure.

Relationship between fMRI activation and improvement in reading comprehension scores

Multiple regression analysis was performed to determine whether behavioral improvement on reading comprehension, as measured by the GORT-4 Comprehension subtest, was related to brain activation in the ASD-EXP group, while controlling for verbal IQ. Brain activation at the post-intervention session for the high-imagery sentences revealed a significant positive correlation between change in GORT-4 scores and activation in visual processing regions [left LG, left cuneus, left MOG], regions involved in language comprehension [LIFG (BA47), LMFG, and LdlPFC], as well as RMPFC and RIFG (BA47; see Figure 3; Table 4). Brain activation at the post-intervention session for the low-imagery sentences also revealed a significant positive correlation between change in GORT-4 scores (controlling for verbal IQ) and activation in the right cingulate cortex (x = 18, y = -10, z = 38) and right insula (x = 36, y = 20, z = 10). These correlations indicate that greater improvement in reading comprehension was associated with greater activation in these brain regions post-intervention. There were no significant negative correlations for either high-imagery or low-imagery sentences.

Brain activation at the pre-intervention session in the ASD-EXP group for the high-imagery sentences revealed a significant negative correlation with change in GORT-4 scores and activation in bilateral premotor cortex, left somatosensory association cortex, and left middle frontal gyrus (BA 6, 7, and 8), as well as right precuneus and right angular gyrus (Table 5). Brain activation at the pre-intervention session for the low-imagery sentences showed a similar negative correlation with change in GORT-4 scores and activation in right precuneus (BA 7; x = 6, y = -66, z = 44) and left premotor cortex

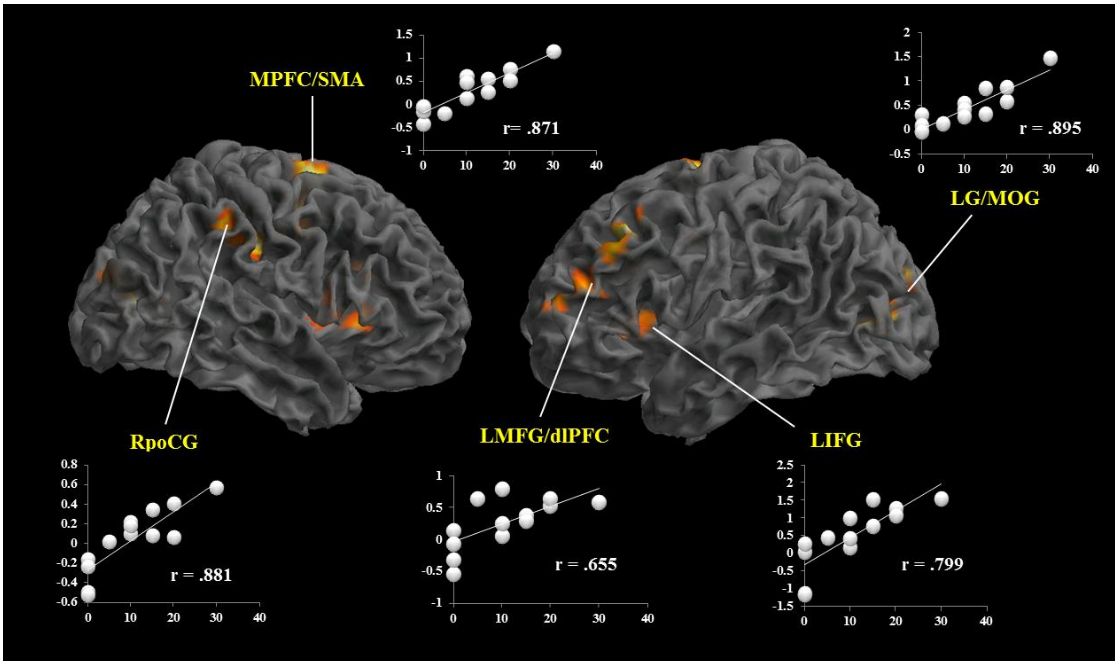
(BA 6; x = -6, y = 20, z = 66). These correlation patterns indicate that less improvement in reading comprehension after intervention was associated with greater activation in these brain regions pre-intervention. There were no significant positive correlations for either high-imagery or low-imagery sentences.

Table 4. Brain regions at the post-intervention session for the high-imagery sentences with a significant positive correlation between change in GORT-4 scores and activation in the ASD-EXP group (n = 13; p < 0.01, 200 voxels extent threshold).

Location of Peak Activation	***High-Imagery Condition***			***MNI coordinates***		
ASD-EXP Group post-intervention	*BA*	*Cluster*	*t*	*x*	*y*	*z*
L Dorsolateral Prefrontal Cortex	9	260[a]	6.00	-40	24	40
L Middle Frontal Gyrus		260[a]	4.47	-34	26	34
R Medial Frontal Gyrus	6	1584[b]	5.74	14	0	64
L Superior Frontal Gyurs	10	281[c]	3.76	-32	56	12
R Postcentral Gyrus	2	1584[b]	5.59	40	-22	34
L Insula/Inferior Frontal-Orbital	47	281[c]	3.84	-32	18	4
R Insula/Inferior Frontal-Orbital	47	451	3.93	34	24	2
L Posterior Cingulate Cortex	30	220[d]	3.69	-22	-64	6
L Cuneus	18	251	4.44	-26	-82	10
L Lingual Gyrus	19	220[d]	6.88	-22	-68	-2
L Middle Occipital Gyrus	18	220[d]	3.81	-34	-68	0

Note. L = left hemisphere, R = right hemisphere, BA = Brodmann's area
[a,b,c,d] Regions of activation encompassed within the same cluster

Figure 3. Brain areas showing significant positive correlations between change in reading comprehension scores (GORT-4) and activation post-intervention to high imagery sentences in the ASD-EXP group (p < 0.01, 200 voxel extent threshold). For graphs, x-axis is the fMRI activation and y-axis is change in GORT-4 reading comprehension scores.

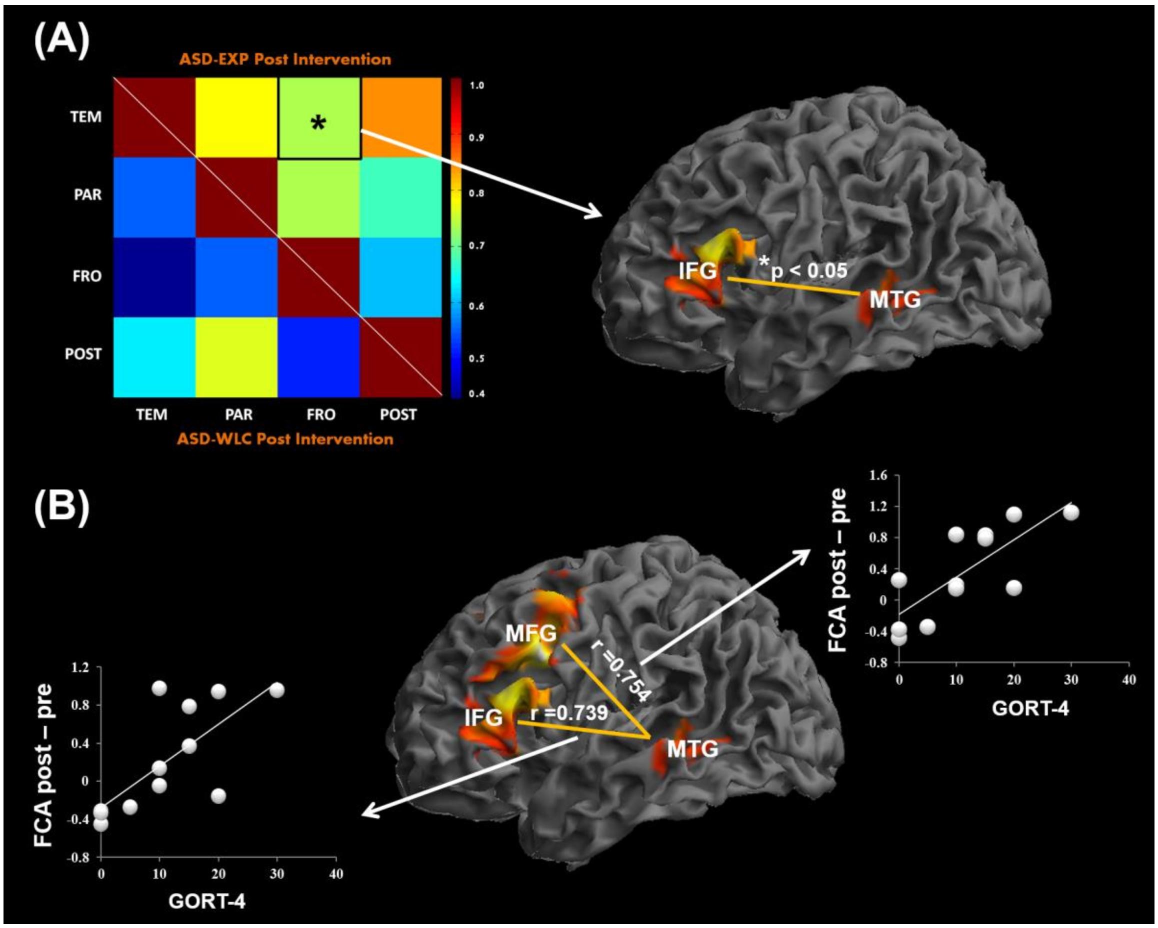


Results: Intervention-related Changes in Functional Connectivity

To assess intervention-related changes in functional connectivity, we conducted a series of univariate ANOVAs to compare the mean connectivities between the ASD-EXP and ASD-WLC groups post-intervention using our inter-lobe groupings of ROIs across six network pairings (frontal–parietal, frontal–LMTG, frontal–occipital, LMTG–parietal, LMTG–occipital and parietal–occipital), while controlling for connectivity strength at the first imaging session. Results revealed significantly stronger functional connectivity for high-imagery sentences in the ASD-EXP group post-intervention specific to the LMTG-frontal network ($F(1,25) = 4.530$, $p < 0.05$; see Figure 4). The LMTG-frontal network also showed a trend towards greater functional connectivity in the ASD-EXP group for low-imagery sentences ($p = 0.033$), but this result was no longer significant when we controlled for connectivity strength at the first imaging session ($p = 0.170$). Given that the only significant network pair was between LMTG and frontal regions for the high-imagery sentences, we further assessed differences in connectivity strength between the ASD-EXP and ASD-WLC post-intervention specific to each of the four frontal ROIs (LMTG-ACC, LMTG-MPFC, LMTG-IFG, and LMTG-MFG). We found that greater connectivity post-intervention (controlling for pre-intervention connectivity strength) in the ASD-EXP group was specific to the LMTG-LIFG pair [$F(1,25) = 4.49$, $p < 0.05$].

Multiple regression analysis was also performed to determine whether behavioral improvement in reading comprehension was related to functional connectivity in any of our six network pairs in the ASD-EXP group, while controlling for verbal IQ. Once again, only the LMTG-frontal network was shown to be significantly correlated with improvement in reading comprehension after intervention. A significant positive correlation was found in the ASD-EXP group between change in connectivity strength from pre- to post-intervention in the LMTG-frontal network and change in GORT-4 comprehension scores for both high imagery [$r(11) = 0.751$, $p = 0.003$] and low imagery [$r(11) = 0.764$, $p = 0.003$] sentences. Given that the only statistically significant network connection was between LMTG and frontal regions, we further examined the correlations of GORT-4 scores to each of the four frontal ROIs paired with LMTG. We found significant positive correlations between change in connectivity strength and change in GORT-4 scores in the LMTG-LIFG pair [high-imagery: $r(11) = 0.754$, $p = 0.003$; low-imagery: $r(11) = 0.687$, $p = 0.01$] and LMTG-LMFG pair [high-imagery: $r(11) = 0.739$, $p = 0.005$; low-imagery: $r(11) = 0.757$, $p = 0.003$]. No differences were found in connectivity patterns between the TD group and ASD groups (ASD-EXP + ASD-WLC groups) at the first imaging session.

Figure 4. (A) Functional connectivities between intra-lobe networks by group (ASD-EXP and ASD-WLC), with the greater connectivity seen in the ASD-EXP group as compared to the ASD-WLC group in the MTG:Frontal network for high-imagery sentences. Significant group differences indicated by an asterisk. (B) Differences in functional connectivities between MTG and frontal regions (IFG and MFG) from pre- to post-intervention in the ASD-EXP group that showed significant positive correlation with improvement in reading comprehension scores (GORT-4) in high-imagery sentences. All rendered brain views are for illustrative purposes only.



Discussion

This study investigated the brain responses to comprehending sentences of varying visualization and tested the potential of an intensive and novel reading intervention in impacting the brain circuitry underlying it in children with ASD. The results revealed significant changes in brain activity and functional connectivity, in comprehending both low-imagery and high-imagery sentences that correlated with improvement in language comprehension ability in children with ASD who received the V/V reading intervention. Greater post-intervention activation in visual brain regions and posterior language regions were correlated with more successful outcomes of the intervention. Furthermore, we found strengthening of connectivity of the frontal language regions, LIFG and LMFG, with LMTG in the children with ASD who participated in the intervention. We also found distinct differences between children with ASD and TD children when comprehending sentences that require mental imagery. Specifically, we found activation of parietal and occipital regions in children with ASD regardless of sentence type, suggesting the use of visualization in children with ASD in aiding sentence comprehension. Additionally, we found unique recruitment of the VWFA in TD children that was not seen in ASD.

Children with ASD who received reading intervention (ASD-EXP group) showed greater post-intervention activation to high imagery sentences in left visual regions and bilateral PrCG and PoCG. Interestingly, increased activation of both the PoCG (BA 3) and PrCG (BA 4) in the ASD-EXP group post-intervention was unique to processing high-imagery sentences. While a number of studies have examined the role of PrCG and PoCG in comprehending action verbs or embodied motor simulation in language (e.g., Pulvermuller, 2005; Papeo et al., 2011; Pulvermuller, Harle, & Hummel, 2001), other studies have found these regions to be activated during different aspects of sentence processing, including abstract sentences (Sakreida et al., 2013) and mental rotation and mental imagery (Kosslyn et al., 2001; Tomasino & Rumiati, 2013). It is likely that as overall comprehension increased from pre- to post-intervention, evident from an increase in task accuracy post-intervention for only the EXP group, so did the participants increase in their mentalization of the high imagery sentences, which includes the ability to mentally rotate letters, numbers, and shapes to form mental images in order to comprehend the sentences (e.g., *The number eight turned sideways looks like a pair of eyeglasses*). Additionally, greater connectivity between Broca's and Wernicke's areas and PrCG and PoCG were correlated with increased reading competence in TD children, suggestive of automatized articulation (Koyama et al., 2011).

The changes observed in activation post-intervention for the ASD-EXP group is also suggestive of compensatory mechanisms for comprehending language, specifically recruitment of right hemisphere regions and subcortical regions. While left IFG is involved in phonological processing, cognitive control, and semantic integration (Rogalsky & Hickok, 2011), and left posterior STS involved in phonological processing (Graves et al., 2014), the ASD-EXP group increased activation post-intervention in right IFG and right IPL. This was also seen in the low imagery condition, with the ASD-EXP group increasing activation of right posterior STS post-intervention when compared to the ASD-WLC group. This suggests the use of right hemisphere language-analogous regions in order to aid in reading comprehension. Another potential compensatory mechanism in the ASD-EXP group is increased post-intervention activation in the left putamen (lentiform nucleus) and the left thalamus. Koyama et al. (2011) mapped the reading network in children and adults, and found that unique to children, but not adults, was an increased reliance on the thalamus during reading. While the thalamus has been considered integral in relaying sensory information to cortical regions (Jones, 1985), it also has a role in language and verbal memory (Hebb & Ojemann, 2013) and in visual attention (Grieve et al., 2000; Fan et al., 2005) suggestive of serial visual scanning during the initial steps of reading (Koyama et al., 2011). Indeed, a meta-analysis of reading interventions for children with reading disorders found the left thalamus to consistently increase in activity after intervention (Barquero et al., 2014). This may be a good predictor of a compensatory mechanism to aid in comprehension via alternative pathways relayed through the thalamus, and increased visual attention and scanning to aid in more effortful reading. The left putamen has also been implicated in reading and language comprehension, especially in sublexical and lexical processing during reading (Oberhuber et al., 2013). In addition, a study assessing reading in skilled readers found that reading increases functional connectivity of the occipito-temporal – PrCG pathway via the putamen (Seghier & Price, 2010). Interestingly, Meyler et al. (2008) found that in children who were classified as poor readers, when compared to good readers, showed increased activation of the putamen after intervention, and that increase in putamen activity was still stable at one-year follow-up. Overall, our results indicate that the children with ASD in the present study seem to employ compensatory mechanisms by increasing activation in posterior language and visual brain regions, right-hemisphere homologous regions (right IFG and right IPL), as well as more unconventional language-related regions, such as PrCG, PoCG, putamen, and thalamus to aid in language comprehension.

It should be noted that, qualitatively, performance on the high-imagery sentences seemed to be more predictive of behavioral success than low-imagery sentences, which is relevant given the visualizing nature of the intervention in improving reading comprehension. As would be predicted, increased activity post-intervention in high-imagery sentences in visual and posterior language regions, bilateral insula (BA47, including orbital part of IFG), and right PoCG showed greater improvements in reading comprehension scores for the ASD- EXP group. Furthermore, increases in functional connectivity between LMTG and left frontal regions, specifically LIFG and LMFG, in the ASD-EXP group was correlated with greater improvement in reading comprehension. The LMTG has been found to be a key region in the reading network, with direct functional connections with LIFG (BA47) and LMFG during language comprehension, and are specifically necessary when processing demands are high (Turken & Dronkers, 2011). Moreover, increased functional connectivity has been found between LMTG and LIFG when more active sentence comprehension is required, such as having to make a judgment based on the sentence, as compared to passively listening or reading a sentence where no response is required (Yue et al., 2013). The anterior insula/IFG (BA47) has been shown to be activated with increasing processing demands of semantic information (Bookheimer, 2002; Friederici, 2002). The left insula/IFG has been proposed to support the integration of individual semantic features to foster reading, and aid in memory retrieval of thematic structure when reading (Friederici, 2002; Poldrack et al., 1999). In addition, reading intervention studies have found increases in both left and right insula in children after intervention (Barquero et al., 2014), suggestive of the role of the insula in increased coordination of relayed information (Menon & Uddin, 2010) to increase reading proficiency.

Conversely, decreased activity pre-intervention in the ASD-EXP group during high imagery sentences in left BA6, BA7, and BA8, left IPL (BA40; Wernicke's area), and bilateral precuneus/PCC and right angular gyrus (AG), were predictive of more success during intervention with greater increases in reading comprehension scores. Somatosensory and motor areas (BA6 and BA7) have been linked, along with the AG and IPL, in reading aloud and sounding out words (Boukrina & Graves, 2013; Segal & Petrides, 2013), which may suggest that less reliance on reading words aloud, or greater skills in silent reading, may be a predictor of better gains in reading comprehension after intervention in children with ASD. Alternatively, the AG, along with the precuneus/PCC have been shown to be part of the default mode network (e.g., Assaf et al., 2010), which is usually deactivated during active cognition (Buckner et al., 2008). One possibility could be that the children with ASD who have increased activity of the default mode during a cognitive task, such as reading, are more prone to distractibility or decreased effort in performing the task, predictive of poorer outcomes after the intervention. Additionally, BA8 has been implicated in decision-making, with increasing activity in BA8 as uncertainty increases (Volz, Schubotz, & von Cramon, 2005). We can therefore posit that for those children with ASD who have more difficulty with reading high imagery sentences, such as by having to read aloud, being more distracted (or perhaps giving up because the task is too difficult), or being more uncertain as to whether the sentence is true or false, have more difficulty in making gains in reading comprehension after the intervention. This has implications for identifying these children prior to intervention in order to better individualize instruction for these children to improve reading comprehension.

Lastly, some differences observed in our children with ASD compared to TD children are noteworthy. Unique to the TD group was the recruitment of left parahippocampal gyrus, and the visual word form area (VWFA). While the parahippocampal and hippocampal regions have been implicated in integration of semantic and syntactic information (Meyer et al., 2005), and in semantic retrieval (Burianova et al., 2010), the VWFA has been consistently shown to activate in response to reading-specific stimuli (Baker et al., 2007; Vinckier et al., 2007; Dehaene & Cohen, 2011). More recently, VWFA has been shown to be involved in processing visual characters, such as Amharic characters, and is responsible for letter-to-letter identification (Vogel, Petersen, & Schlaggar, 2014). This is especially relevant given the mental rotation and visualization required in some of our high-imagery sentences, in which an individual letter character has to be interpreted independently in order to comprehend the high-imagery sentence. Indeed, we did not see the VWFA activated for either the TD or ASD groups for the low imagery condition. Interestingly, when we directly compared activation between the ASD groups and TD group, for both the high-imagery and low-imagery sentences, we found greater activation in posterior visual regions, including left LG, MOG, and fusiform gyrus, in the TD group compared to the two ASD groups. Groen et al. (2010) found that TD adolescents had greater activation of MOG than adolescents with ASD when having to determine congruency of world-knowledge sentences, similar to our low-imagery condition where the participant had to decide if the factual sentence was true or false. Another study assessed functional brain activity in children with poor reading comprehension, but intact word reading skills, and found specific deficits primarily in posterior brain regions, such as LG and cuneus, compared to TD children, during a lexical decision task (Cutting et al., 2013). Given that the children with ASD in our study had the same reading profile (i.e., intact basic reading skills, but poor comprehension), our results fit with this pattern of differential posterior activation. This may underlie the greater visual activity seen in the TD group; it may also be the case that the TD group has increased visual attention to the tasks (Bradley et al, 2003), whereas the ASD groups had less directed attention given the difficulty of the task for them.

In sum, we found that application of a novel reading intervention aimed at using a relatively intact skill, visual-spatial abilities, to aid in boosting a relative deficit, language comprehension in children with ASD was successful in increasing behavioral measures of reading comprehension and mediating brain changes. We found that participation in the intervention increased activation in posterior language and visual brain regions, as well as increased compensatory mechanisms for language comprehension in right-hemisphere homologous regions (right IFG and right IPL), as well as, subcortical regions, such as the putamen and thalamus. Additionally, increased brain activation and functional connectivity of core language areas, left MTG and left IFG was predictive of greater success in the intervention as measured by increased reading comprehension proficiency. Overall, our study lends support of specialized intervention for children with ASD to increase high-order learning skills, and support the growing literature of the plasticity of the young brain in ASD.

References

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

Assaf, M., Jagannathan, K., Calhoun, V.D., Miller, L., Stevens, M.C., Sahl, R., O'Boyle, J.G., . . . Pearlson, G.D. (2010). Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *Neuroimage, 15,* 247–256.

Baker, C.I., Liu, J., Wald, L.L., Kwong, K.K., Benner, T., & Kanwisher, N. (2007). Visual word processing and experiential origins of functional selectivity in human extrastriate cortex. *Proc Natl Acad Sci U.S.A., 104,* 9087–9092.

Barquero, L.A., Davis, N., Cutting, L.E. (2014). Neuroimaging of reading intervention: a systematic review and activation likelihood estimate meta-analysis. *PLoS One. 9,* e83668.

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E., (2001). The autism spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *J. Autism Dev. Disord. 31,* 5–17.

Bartak, L., & Rutter M. (1973). Special educational treatment of autistic children: a comparative study. 1. Design of study and characteristics of units. *J Child Psychol Psychiatry, 14*, 161-79.

Bell, N. (1991a). Gestalt imagery: A critical factor in language comprehension. *Annals of Dyslexia, 41,* 246–260.

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

Belmonte, M.K., Cook, E.H., Jr., Anderson, G.M., Rubenstein, J.L., Greenough, W.T., Beckel-Mitchener, A, Courchesne, E., . . . Tierney, E. (2004). Autism as a disorder of neural information processing: Directions for research and targets for therapy. *Mol Psychiatry, 9*, 646–663.

Bradley, M.M., Sabatinelli, D., Lang, P.J., Fitzsimmons, W.K., & Desai, P. (2003). Activation of the visual cortex in motivated attention. *Behav Neurosci, 117,* 369–380.

Bookheimer, S. (2002). Functional MRI of language: new approaches to understanding the cortical organization of semantic processing. *Annu Rev Neurosci*, *25,* 151–188.

Boukrina, O., & Graves, W.W. (2013). Neural networks underlying contributions from semantics in reading aloud. *Front Hum Neurosci*, *2,* 507-518.

Buckner, R.L., Andrews-Hanna, J.R., & Schacter, D.L. (2008. The brain's default network: anatomy, function, and relevance to disease. *Ann N. Y. Acad Sci*, *1124*, 1–38.

Burianova, H., McIntosh, A.R., & Grady, C.L. (2010). A common functional brain network for autobiographical, episodic, and semantic memory retrieval. *Neuroimage*, *49,* 865–874.

Constantino, J. N., & Gruber, C. P. (2005*). The social responsiveness scale manual*. Los Angeles, CA: Western Psychological Services.

Cutting, L.E., Clements-Stephens, A., Pugh, K.R., Burns, S., Cao, A., Pekar, J.J., Davis, N., & Rimrodt, S.L. (2013). Not all reading disabilities are dyslexia: distinct neurobiology of specific comprehension deficits. *Brain Connect, 3*, 199-211.

Davidson, M.M., & Ellis Weismer, S. (2014). Characterization and prediction of early reading abilities in children on the autism spectrum. *J Autism Dev Disord. 44,* 828-845.

Dehaene, S. & Cohen, L. (2011). The unique role of the visual word form area in reading *Trends in Cognitive Sciences, 15*, 254-262.

de Jonge, M. V., Kemner, C., & van Engeland, H. (2006). Superior disembedding performance of high-functioning individuals with autism spectrum disorders and their parents: the need for subtle measures. *Journal of Autism and Developmental Disorders, 36,* 677–683.

El Zein, F., Solis, M., Vaughn, S., & McCulley, L. (2014). Reading comprehension interventions for students with autism spectrum disorders: a synthesis of research. *J Autism Dev Disord, 44,* 1303-22.

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,* 949-60.

Fan, J., McCandliss, B.D., Fossella, J., Flombaum, J.I., & Posner, M.I. (2005). The activation of attentional networks. *Neuroimage*, *26,* 471– 479.

Freire, L., Roche, A., & Mangin, J.F., (2002). What is the best similarity measure for motion correction in fMRI time series? *IEEE Trans. Med. Imaging, 21,* 470–484.

Friederici, A. D. (2002). Towards a neural basis of auditory sentence processing. *Trends Cogn. Sci, 6*, 78–84.

Goldstein, G., Minshew, N.J., & Siegel, D.J. (1994). Age differences in academic achievement in high-functioning autistic individuals. *Journal of Clinical Experimental Neuropsycholoy, 16*, 671–680.

Graves, W.W., Binder, J.R., Desai, R.H., Humphries, C., Stengel, B.C., & Seidenberg, M.S. (2014). Anatomy is strategy: Skilled reading differences associated with structural connectivity differences in the reading network. *Brain Lang, 133,* 1-13.

Grieve, K.L., Acun˜a, C., & Cudeiro, J. (2000) The primate pulvinar nuclei: vision and action. *Trends Neurosci, 23,* 35–39.

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. *Cereb Cortex, 20,* 1937-45.

Hebb, A.O., & Ojemann, G.A. (2013). The thalamus and language revisited. *Brain Lang, 126,* 99-108.

Johnson-Glenberg, MC. (2000). Training reading comprehension in adequate decoders/poor comprehenders: Verbal versus visual strategies. *Journal of Educational Psychology, 92,* 772-782.

Jones, E.G. (1985) *The thalamus*. New York: Plenum.

Jones, C.R.G., Happe´, F., Golden, H., Marsden, A.J.S., Tregay, J., Simonoff, E., Pickles, G., … Charman, T. (2009). Reading and arithmetic in adolescents with autism spectrum disorders: Peaks and dips in attainment. *Neuropsychology, 23,* 718–728.

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,* 1083–1096.

Kamio, Y., & Toichi, M. (2000). Dual access to semantic in autism: is pictorial access superior to verbal access? *J Child Psychol Psychiatry,41*, 859–67.

Kana, R.K., Keller, T.A., Cherkassky, V.A., Minshew, N.J., & Just, M.A. (2006). Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. *Brain: a journal of neurology, 129,* 2484–2493.

Kana, R.K, & Wadsworth, H.M. (2012). "The archeologist's career ended in ruins": hemispheric differences in pun comprehension in autism. *Neuroimage*, *62,* 77-86.

Kana, R.K., Murdaugh, D.L., Libero, L.E., Pennick, M.R., Wadsworth, H.M., Deshpande, R., & Hu, C.P. (2011). Probing the brain in autism using fMRI and diffusion tensor imaging. *Journal of Visualized Experiments: JoVE, 55,* http://www.jove.com/ details.php?id=3178, doi: 10.3791/3178.

Kanner L. (1943). Autistic disturbances of affective contact. *Nervous Child, 2*, 217-250.

Kosslyn, S.M., Thompson, W.L., Wraga, M., and Alpert, N.M. (2001). Imagining rotation by endogenous versus exogenous forces: distinct neural mechanisms. *Neuroreport 12,* 2519–2525.

Koyama, M.S., Di Martino, A., Zuo, X.N., Kelly, C., Mennes, M., Jutagir, D.R., Castellanos, F.X., & Milham, M.P. (2011). Resting-state functional connectivity indexes reading competence in children and adults. *J Neurosci. 31*, 8617-24.

Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., Rainey, L., Kochunov, P.V., Nickerson, D., . . . Fox, P.T. (2000). Automated Talairach atlas labels for functional brain mapping. *Hum. Brain Mapp. 10*, 120–131.

Lindamood, P., & Bell, N. (1997). Sensory-cognitive factors in the controversy over reading instruction. The Journal of Developmental and Learning Disorders, 1, 143-182.

Lindgren, K.A., Folstein, S.E., Tomblin, J.B., & Tager-Flusberg, H. (2009). Language and reading abilities of children with autism spectrum disorders and specific language impairment and their first-degree relatives. *Autism Research, 2,* 22–38.

Lockyer L, & Rutter M. (1969) A five- to fifteen-year follow-up study of infantile psychosis. *Br J Psychiatry. 115,* 865-82.

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. *J Autism Dev Disord, 24,* 659–85.

Lord, C., Risi, S., Lambrecht, L., Cook, Jr., E.H., Leventhal, B.L., DiLavore, P.C., Pickles, A., & Rutter, M. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord, 30,* 205–23.

Maidjian, J.A., Laurienti, P.J., & Burdette, J.H., (2004). Precentral gyrus discrepancy in electronic versions of the Talairach Atlas. *Neuroimage, 21,* 450–455.

Manjaly, Z.M., Bruning, N., Neufang, S., Stephan, K.E., Brieber, S., Marshall, J.C., Kamp-Becker, I., . . . Fink, G.R. (2007). Neurophysiological correlates of relatively enhanced local visual search in autistic adolescents. *Neuroimage. 35,* 283–291.

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

Menon, V., & Uddin, L.Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct, 214,* 655–667.

Meyer, P., Mecklinger, A., Grunwald, T., Fell, J., Elger, C.E., & Friederici, A.D. (2005). Language processing within the human medial temporal lobe. *Hippocampus, 15,* 451-9.

Meyler, A., Keller, T.A., Cherkassky, V.L., Gabrieli, J.D.E, & 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,* 2580–2592.

Minshew, N.J., & Goldstein, G. (1998). Autism as a disorder of complex information processing. *Mental Retardation and Developmental Disabilities Research Reviews, 4,* 129-36.

Moseley, R.L., Mohr, B., Lombardo, M.V., Baron-Cohen, S., Hauk, O., & Pulvermüller, F. (2013). Brain and behavioral correlates of action semantic deficits in autism. *Front Hum Neurosci, 8,* 717-725.

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,* 911–919.

Newman, T.M., Macomber, D., Naples, A.J., Babitz, T., Volkmar, F., & Grigorenko, E.L. (2007). Hyperlexia in children with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 37*, 760–774.

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.

Oberhuber, M., Parker, J., Hope, T.M., Prejawa, S., Seghier, M.L., Green, D.W., & Price, C.J. (2013). Functionally distinct contributions of the anterior and posterior putamen during sublexical and lexical reading. *Front Hum Neurosci. 7,* 787.

Papeo, L., Corradi-Dell' Acqua, C., & Rumiati, R.I. (2011). "She" is not like "I": the tie between language and action is in our imagination. *J Cogn Neurosci, 23,* 3939–3948.

Poldrack, R. A., Wagner, A. D., Prull, M. W., Desmond, J. E., Glover, G. H., & Gabrieli, J. D. (1999). Functional specialization for semantic and phonological processing in the left inferior prefrontal cortex. *Neuroimage, 10,* 15–35.

Pulvermuller, F. (2005). Brain mechanisms linking language and action. *Nat. Rev. Neurosci, 6,* 576–582.

Pulvermuller, F., Harle, M., & Hummel, F. (2001). Walking or talking? Behavioral and neurophysiological correlates of action verb processing. *Brain Lang, 78,* 143–168.

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. *Biol Psychiatry, 64,* 589-98.

Rogalsky C, & Hickok G. (2011) The role of Broca's area in sentence comprehension. *J Cogn Neurosci, 23,* 1664-80.

Rutter, M., Bailey, A., & Lord, C. (2003). *Manual for the Social Communication Questionnaire.* Los Angeles, CA: Western Psychological Services.

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,* 86–95.

Sahyoun, C.P., Soulieres, I., Belliveau, J.W., Mottron, L., & Mody, M. (2009). Cognitive differences in pictorial reasoning between high-functioning autism and Asperger's syndrome. *J Autism Dev Disord, 39*, 1014–1023.

Sakreida, K., Scorolli, C., Menz. M.M., Heim, S., Borghi, A.M., & Binkofski, F. (2013). Are abstract action words embodied? An fMRI investigation at the interface between language and motor cognition. *Front Hum Neurosci, 9,* 125.

Samson, F., Mottron, L., Soulières, I., & Zeffiro, T.A. (2012). Enhanced visual functioning in autism: an ALE meta-analysis. *Hum Brain Mapp, 33,* 1553-81.

Segal, E., & Petrides, M. (2013) Functional activation during reading in relation to the sulci of the angular gyrus region. *Eur J Neurosci, 38,* 2793-801.

Seghier, M.L., & Price, C.J. (2010). Reading aloud boosts connectivity through the putamen. *Cereb Cortex, 20,* 570-82.

Snowling, M., & Frith, U. (1986). Comprehension in "hyperlexic" readers. *J Exp Child Psychol, 42,* 392-415.

Stoeckel, C., Gough, P.M., Watkins, K.E., & Devlin, J.T. (2009). Supramarginal gyrus involvement in visual word recognition. *Cortex, 45,* 1091–1096.

Tesink, C.M., Buitelaar, J.K., Petersson, K.M., van der Gaag, R.J., Teunisse, J.P., & Hagoort, P. (2011). Neural correlates of language comprehension in autism spectrum disorders: when language conflicts with world knowledge. *Neuropsychologia, 49,* 1095-104.

Toichi, M., & Kamio, Y.(2001). Verbal association for simple common words in high-functioning autism. *J. Autism Dev. Disord. 31,* 483–490.

Tomasino, B., & Rumiati, R.I. (2013). At the mercy of strategies: the role of motor representations in language understanding. *Front Psychol, 4,* 4:27.

Torgesen, J.K., Alexander, A.W., Wagner, R.K., Rashotte, C.A., Voeller, K.K., & Conway, T. (2001). Intensive remedial instruction for children with severe reading disabilities: Immediate and long-term outcomes from two instructional approaches. *Journal of Learning Disorders, 34,* 33-58.

Turken AU, & Dronkers NF. (2011). The neural architecture of the language comprehension network: converging evidence from lesion and connectivity analyses. *Front Syst Neurosci, 10,* 5.

van Lang, N. D. J., Bouma, A., Sytema, S., Kraijer, D. W., & Minderaa, R. B. (2006). A comparison of central coherence skills between adolescents with an intellectual disability with and without comorbid autism spectrum disorder. *Research in Developmental Disabilities, 27,* 217–226.

Vinckier,F., Dehaene,S., Jobert,A., Dubus,J.P., Sigman,M., & Cohen,L. (2007). Hierarchical coding of letter strings in the ventral stream: dissecting the inner organization of the visual word-form system. *Neuron, 55,* 143–156.

Vogel, A.C., Petersen, S.E., & Schlaggar, B.L. (2014). The VWFA: it's not just for words anymore. *Front Hum Neurosci, 20,* 88.

Volz, K.G., Schubotz, R.I., & von Cramon, D.Y. (2005). Variants of uncertainty in decision-making and their neural correlates. *Brain Res Bull, 67,* 403-12.

Wang, A.T., Lee, S.S., Sigman, M., & Dapretto, M., (2006). Neural basis of irony comprehension in children with autism: the role of prosody and context. *Brain, 129*, 932–943.

White, S.J. & Saldaña, D. (2011). Performance of children with autism on the Embedded Figures Test: a closer look at a popular task. *J Autism Dev Disord, 41,* 1565-72.

Yue, Q., Zhang, L., Xu, G., Shu, H., & Li, P. (2013). Task-modulated activation and functional connectivity of the temporal and frontal areas during speech comprehension. *Neuroscience, 237,* 87-95.

CHANGES IN INTRINSIC CONNECTIVITY OF THE BRAIN'S READING NETWORK AFTER INTERVENTION IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

by

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Abstract

Task-based neuroimaging studies have consistently identified alterations in neural circuitry underlying language processing in children with autism spectrum disorders (ASD). While these findings are important, resting state functional magnetic resonance imaging (rsfMRI) is a promising alternative to some of the constraints posed by task-based fMRI. This study used rsfMRI, in a longitudinal design, to study the impact of a reading intervention on the connectivity of the brain's reading network in children with ASD. Functional connectivity was examined using different modalities, group independent component analysis (GICA) and seed-based correlation analysis of Broca's and Wernicke's areas, in three groups of participants: an experimental group of ASD children (ASD-EXP), a wait list control group of ASD children (ASD-WLC), and a group of typically developing (TD) control children. The GICA results revealed stronger functional connectivity of both Broca's and Wernicke's areas, in the ASD-EXP group post-intervention. Our seed-based functional connectivity results showed increased connectivity post-intervention between Broca's area and motor regions, Broca's area and supramarginal gyrus, and between Wernicke's area and left-lateralized language regions (e.g., left IFG and left MTG). Additionally, correlation of functional connectivity and improvement in reading comprehension in the ASD-EXP group revealed greater connectivity in both Broca's and Wernicke's area within our GICA identified network. Also, increased connectivity between the Broca's area seed and right postcentral gyrus (PoCG) and right STG, and the Wernicke's area seed and left IFG and right cerebellum, were also correlated with greater improvement in reading comprehension. Overall, this study revealed widespread changes in reading network functional connectivity as a result

of a strength-based intervention in children with ASD. These novel findings provide valuable insights into the plasticity of brain's language networks and the potential of intensive intervention in changing them in children with ASD.

Introduction

Autism spectrum disorders (ASD) are characterized by profound impairments in verbal and non-verbal communication (American Psychiatric Association, 2013). With the rising prevalence in ASD (currently 1 in 68 children; Center for Disease Control, 2014), there is a growing need for improved understanding of the neurobiology of this disorder in order to develop targeted interventions. Both behavioral and neuroimaging studies have reported that high-functioning children with ASD struggle with many different aspects of oral and reading comprehension, including pragmatics, semantics, and phonological processes (Groen et al., 2008, 2010; Williams et al., 2008), while their decoding and word identification skills remain intact (Nation et al., 2006; Newman et al., 2007; Norbury & Nation, 2011). Concurrently, neuroimaging research has consistently delineated alterations in synchronization of the brain across many aspects of language comprehension, including semantics and integration of social information (Groen et al., 2010), lexical over thematic processing (Just et al., 2004), and pragmatics and syntax (Groen et al., 2008). In typically developing (TD) individuals, the language comprehension network is left-hemisphere dominant, and includes the left posterior middle temporal gyrus, superior temporal gyrus, superior temporal sulcus, inferior frontal gyrus, and middle frontal gyrus (Turken & Dronkers, 2011; Tomasi & Volkow, 2012). Individuals with ASD, on the other hand, tend to recruit additional right-hemisphere regions, as well as favor local connectivity between parietal and occipital brain regions during language comprehension (Kana et al., 2006; Kana & Wadsworth, 2012; Mason et al., 2008), at the expense of more long-range frontal to posterior connections (Courchesne et al., 2007; Minshew & Keller, 2010; Sahyoun et al., 2010). These results are consistent with the theory of cortical underconnectivity which posits that many cognitive functions, such as language, are dependent on the synchronization of brain regions which may be compromised in individuals with ASD (Just et al., 2004; Kana et al., 2006).

While task-based neuroimaging studies (e.g., sentence comprehension, theory-of-mind, problem-solving) are important in understanding the neural mechanisms of cognitive processes, they are also constrained by factors such as the level of difficulty, response time, and participant performance. Such demands make it especially difficult in pediatric neuroimaging as well as in imaging individuals with neurodevelopmental disorders. The advent of resting state functional MRI (rsfMRI) has marked a paradigm shift in the field of neuroimaging (Raichle, 2009), and has opened new doors for understanding the neurobiology of disorders like autism. Neuroimaging studies have applied rsfMRI as a task-independent method to identify low frequency (< 0.1 Hz) fluctuations of spontaneous brain activity that represent structured and organized brain networks that closely relate to functional patterns of connectivity seen during task performance (Deco & Corbetta, 2011; Smith et al., 2009; Toro et al., 2009). More recently, studies have used rsfMRI to assess the neural networks underlying language comprehension, specifically reading comprehension in TD individuals (Hampson et al., 2006; Koyama et al., 2010, 2011; Tomasi et al., 2012).

By using rsfMRI brain activity to study language comprehension, a basic network can be identified that acts as a framework for language processing regardless of task (Lohmann et al., 2010). This is especially important for children with ASD, in which developing language tasks that can address the spectrum of comprehension ability is challenging. The language network in TD children has been successfully mapped in previous studies using rsfMRI brain activity; in addition, reading performance of TD children was positively correlated with resting state connectivity between posterior superior temporal (Wernicke's area) and inferior frontal (Broca's area) cortical areas (Koyama et al., 2011). To our knowledge, only one study has assessed the language network using rsfMRI in children with ASD (Verly et al, 2014), finding intact connectivity between Wernicke's and Broca's area, when compared to the control group. However, underconnectivity was identified between Broca's area and supplemental motor area (SMA) and dorsolateral prefrontal cortex (DLPFC), as well as between frontal and cerebellar regions (Verly et al., 2014). Thus, recent research indicates the potential of rsfMRI to characterize language comprehension networks, including the reading network, during task-free resting state. While such characterization is vital in understanding ASD, an equally or more important next step, which is translational, is to determine the plasticity of this intrinsically organized language network, and to test whether intervention can effect changes in rsfMRI brain connectivity in children with ASD. The main goal of the present study is to address this question in children with ASD with the use of an intense, behaviorally tested, and comprehensive reading intervention.

While all previous studies have assessed the reading network connectivity during rest using a seed-based approach (Hampson et al., 2006; Koyama et al., 2010, 2011; Tomasi et al., 2012; Verly et al., 2014), this study uses a purely data-driven independent component analysis (ICA) approach to identify spatially distinct, temporally correlated brain networks (Calhoun et al., 2001), in addition to, using a seed-based approach to assess the connectivity of both Broca's and Wernicke's area with the rest of the brain

(Tomasi & Volkow, 2012). Seed-based approaches are advantageous in analyzing correlations in rsfMRI between specific regions of interest (ROI), or seeds, with other brain regions in networks that have been previously well defined; whereas, the benefit of the ICA approach is that all the significantly weighted voxels within an independent component are highly correlated, and can be considered a functionally connected network. In ICA, the time series of all of the significant voxels can be assumed to share commonality, and can be considered to be functionally connected with one another within the network (Erhardt et al., 2011). Given the relatively novel nature of using rsfMRI to better understand the language network, both ICA and seed-based approaches can provide valuable information to identify specific regions within the reading comprehension network that are altered in children with ASD, and additionally increase the specificity to determine intervention-related changes in functional connectivity of this network.

In the present study, we used rsfMRI in a longitudinal design before and after a reading remediation program to assess changes in brain connectivity of the reading comprehension network in children with ASD. In addition, we also assessed differences in rsfMRI connectivity between children with ASD and TD children. The intervention used in this study [Visualizing and Verbalizing for language comprehension and thinking (V/V)] has been found to be successful in children with reading disorders like dyslexia, but has never been applied to study children with ASD (Torgesen et al., 2001). The V/V intervention has significant implications for autism, as it relies primarily on visual non-verbal methods, a relative strength in individuals with ASD, to aid in developing both oral and reading comprehension (Bell, 1991a,b; Torgesen et al., 2001). We applied two connectivity analysis approaches in this study, data-driven ICA and seed-based correlation analysis, in order to evaluate the functional connectivity of the reading network during resting state in children with ASD and to delineate changes in functional connectivity due to targeted intervention in children with ASD. We hypothesized that children with ASD who participated in the reading intervention would show strengthened functional connectivity within the reading network after intervention, as well as, recruitment of additional brain regions as compensatory aids for reading comprehension. This study is novel in its translational neuroimaging focus and the findings will have a significant impact on understanding and in applying targeted behavioral interventions to children with ASD.

Materials and Methods

Participants

Participants were 31 children with ASD (mean age = 10.6) and 22 age and IQ-matched TD children (mean age = 10.4; see Table 1). The children with ASD underwent two fMRI sessions, 10 weeks apart; the TD children only participated in one fMRI session. The children with ASD were randomly assigned to participate in the V/V Intervention Program either between imaging sessions (experimental group; ASD-EXP; n = 16) or after completing both imaging sessions (wait-list control group; ASD-WLC; n = 15). The TD control group did not participate in the V/V intervention program. Children were determined to have an ASD diagnosis by either being diagnosed by a licensed clinical psychologist using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and/or the Autism Diagnostic Interview-Revised (ADI; Lord et al., 1994) or being diagnosed by a licensed clinical psychologist and a research diagnosis using the ADOS by trained research-reliable personnel. No statistically significant differences between the ASD group and the TD group were observed for age [t(51) = 0.427, p = 0.671] or Full-Scale IQ [mean ASD = 95.8; mean TD = 97.6; t(51)= 0.497, p = 0.621], although there was a significant difference in verbal IQ [mean ASD = 91.6 ; mean TD = 98.9; t(51) = 2.04, p = 0.048]. In addition, the ASD-EXP and ASD-WLC ASD groups did not differ on reading comprehension level prior to the first fMRI session, as measured by the Gray Oral Reading Test (GORT-4): Comprehension Score [mean ASD-EXP = 76.5; mean ASD-WLC = 84.2; t(29) = 1.59, p = 0.062]. Among the 31 children with ASD, 7 were female (4 in the ASD-EXP group and 3 in the ASD-WLC group), and all were right-handed. In the TD group, 6 were female, and all were right-handed.

Participants with ASD were recruited through multiple sources, 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 recruited potential participants at their centers, and sent those families to UAB for eligibility testing. The TD participants and their families were recruited by advertisements in local newspapers and in *UAB Reporter*, and by flyers posted on UAB campus.

All participants with ASD met the following inclusion criteria: ages from 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 37th percentile and/or a Gray Oral Reading Test – Forth Edition (GORT-4) accuracy score of at least 25th percentile, a GORT-4 comprehension score below 37th percentile, and a Verbal IQ score of at least 75, as measured by the Wechsler Abbreviated Scale of Intelligence (WASI). The inclusion criteria of TD participants included: ages from 8 to 13, no diagnosis of an autism spectrum disorder or a language disorder, and an average (greater than the 25th percentile) oral reading and reading comprehension, as measured by the SORT-R and GORT-4. Participants failing to meet any of the inclusion criteria and participants currently taking beta-blockers or vasodilators, having cochlear implants, history of ferromagnetic material in the body, or neurostimulators, being claustrophobic, or history of seizure disorder, diabetes, hypertension, anemia, or sickle cell disease were excluded from the study. All participants were off medication 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.

Table 1. Participant Demographics

Characteristic	***ASD-EXP Group (n = 16)***	***ASD-WLC Group (n =15)***	***TD Group (n = 22)***
Age[a]	10.3 ± 1.49	11.0 ± 1.14	10.4 ± 1.56
Gender			
Male	12	12	16
Female	4	3	6
Self-Identify			
Caucasian	10	9	10
Black	1	1	9
Asian	3	4	
Hispanic		1	
Social Communication Questionnaire (SCQ)[f]	18.53 ± 8.09	19.93 ± 5.05	5.06 ± 4.54
WASI FSIQ[b]	94.7 ± 12.98	97.2 ± 15.53	97.6 ± 12.08
WASI VIQ[c]	91.3 ± 9.19	92.1 ± 13.21	98.9 ± 15.50
WASI PIQ[d]	99.7 ± 17.20	103.3 ± 16.98	97.3 ± 15.50
GORT-4 Comprehension S1[d]	76.7 ± 11.56	84.2 ± 9.76	105.7 ± 19.18
GORT-4 Comprehension S2 [d]	86.3 ± 11.04	87.6 ± 11.44	N/A
SORT-R Reading Score[e]	107.5 ± 7.78	105.5 ± 8.18	109.2 ± 9.08

Note. value ± standard deviation
[a]Age in decimal years at first imaging session
[b]Wechsler Abbreviated Scale of Intelligence, Full Scale Intelligence Quotient
[c]Wechsler Abbreviated Scale of Intelligence, Verbal Intelligence Quotient
[d]Wechsler Abbreviated Scale of Intelligence, Verbal Intelligence Quotient
[d]Gray Oral Reading Test – Forth Edition Comprehension subtest at the first session (S1) and second session (S2) in standard scores
[e]Slosson Oral Reading Test - Revised (SORT-R) reading score at the first session in standard scores
[f] Social Communication Questionnaire – Lifetime Form (SCQ): measures communication skills and social functioning throughout the child's entire developmental history to screen for autism symptoms. Higher score indicated greater autism symptomotology.

Reading Intervention Program

The Visualizing and Verbalizing for Language Comprehension and Thinking (V/V) Intervention Program is based on the use of nonverbal sensory input, in the form of imaged gestalts, in order to develop oral and written language comprehension, establish vocabulary, and develop higher order thinking skills (Bell, 1991a,b; Lindamood & Bell, 1997; Johnson-Glenberg, 2000). The V/V program is designed to teach children to form imaged gestalts, or concept imagery, as they read and hear language. Through the sequential teaching methods of the program, the imaged gestalt helps develop the imagery-language connection and improve oral and reading comprehension. The student progresses in the program by beginning with word imagery and then extending their understanding to sentence, paragraph, and page imagery. The ultimate goal of this intervention is to apply nonverbal imagery to language comprehension to improve children's reading and listening comprehension, communication skills, and critical thinking skills. The V/V intervention the children with ASD received in the current study was intensive, taking place in 4-hour sessions per day, 5 days a week for 10 weeks. The intervention was conducted at the Lindamood-Bell Learning Processes center closest and/or convenient to where each participant's family lives. Trained clinicians administered the intervention in a standardized manner, and were monitored by an experienced supervisor who gives constant feedback to the clinicians. Implementation of V/V instruction to the participants was done one-on-one in a distraction-free setting, with clinicians rotating every hour.

fMRI Data Acquisition

The MRI data were collected using a Siemens 3.0 Tesla Allegra head-only Scanner (Siemens Medical Inc., Erlangen, Germany) located at the UAB Civitan Functional Neuroimaging Laboratory (CFNL). Each session consisted of the following scans: 1) 3-plane localizer scan; 2) SENSE calibration scan; 3) 3-D high-resolution anatomical scan; 4) fMRI scans of activation to the resting state scan. For the high resolution anatomical scan, T1- weighted scans were acquired using a 160-slice 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) volume scan with TR = 200 ms, TE = 3.34 ms, flip angle = 7, FOV = 25.6 cm, 256 X 256 matrix size, and 1 mm slice thickness. Functional MR images were acquired using a single-shot T2*-weighted gradient-echo EPI pulse sequence. We will use TR= 1000 ms, TE = 30ms, and a 60 degree flip angle for 17 oblique axial slices 5 mm slice thickness with a 1 mm slice gap, a 24 X 24 cm field of view (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 resting state scan, children were instructed to relax, but not fall asleep, and look at a white fixation cross on a black screen. To decrease anxiety associated with the MRI scanner for children with ASD (Kana et al., 2011), the children with ASD in this study were given a social story outlining what they would be doing prior to arrival for the study. Additionally, all participants were given exposure, via a mock-scanning session, the day before the MRI scan, so that the children were familiar and comfortable with the MRI machine and staff prior to the scan.

Data preprocessing

Functional images were processed using the Statistical Parametric Mapping (SPM8) software (Wellcome Department of Cognitive Neurology, London, UK) and Analysis of Functional NeuroImages AFNI software (Cox et al., 1996). Functional images were corrected for slice acquisition timing, motion-correction by registering each functional volume to the first time point of the scan, normalized to the MNI space, resampled (3mm isotropic) and smoothed with an 8mm Gaussian kernel. The normalized and smoothed images were then low bandpass filtered ($0.008 < f < 0.08$ Hz).

Accounting for Head Motion

Because head motion can impact functional connectivity analyses, the following precautions were taken (Satterthwaite et al., 2013; Van Dijk et al., 2012). Head motion was quantified as the Euclidean distance calculated from the six rigid-body motion parameters for two consecutive time points. For any instance >2mm, considered excessive motion, the time point as well as the immediately preceding and subsequent time points were censored, or "scrubbed" (Power et al., 2012). If two censored time points occurred within ten time points of each other, all time points between them were also censored. All participants retained >90% of their time points after censoring. 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 (all *p values* not significant from two- and paired-sample *t*-tests). We additionally computed correlations between RMSD and functional connectivity values and found no significant correlations in the TD and ASD groups. Three subjects with ASD (2 in the ASD-EXP group and 1 on the ASD-WLC group) and three TD subjects had to be excluded because of excessive head motion in which too many time points would have had to be censored.

Group independent component analysis

Group independent component analysis (GICA) was conducted on all 53 participants using the fMRI Group ICA Toolbox (GIFT; icatb.sourceforge.net, version 1.3e). The GICA was carried out twice, with all subjects at the first imaging session (n = 53), and the ASD groups (ASD-EXP and ASD-WLC) at the second imaging session (n = 31). For both GICA, independent components were estimated using the minimum description length criteria, modified to account for spatial correlation, (Li et al., 2007), with 34 independent components being estimated at the first session and 21 independent components being estimated for the second session. The GIFT toolbox organizes the data into three main batch steps: data reduction using principal component analysis (PCA), ICA and back reconstruction. Two data reduction steps are used in which each subject's data is reduced by PCA, which helped to reduce the impact of noise and made the estimation computationally tractable. ICA was then applied to the data set, and then back reconstruction of subjects' time courses and spatial maps are generated (Calhoun et al., 2001; Calhoun et al., 2008). Each component was visually inspected for artifacts, such as activation primarily in white matter or ventricles, or components suggestive of eye movements, head motion or cardiac=-inducted pulsatile artifact at the base of the brain. This resulted in 23 components at the first session and 17 components at the second session that were selected for further analysis.

Based on our specific hypotheses of intervention related changes to the reading language network, we performed a spatial correlation analyses with the surviving components to determine which components had a high spatial overlap with this network. For the reading language network we created a spatial map based of the anatomical regions identified during rest from a meta-analysis of core language regions in children conducted by Koyama et al. (2011). These bilateral seeds included: inferior occipital gyrus (IOG), Fusiform gyrus (FFG), superior temporal gyrus (STG), precentral gyrus (PrCG), Intraparietal sulcus (IPS), supplementary motor area (SMA), inferior frontal gyrus triangularis (IFGtr), middle frontal gyrus (MFG), and thalamus (THAL). All regions were defined structurally using templates from the WFU Pickatlas toolbox within SPM8 using the AAL or Talairach Daemon atlases (Lancaster et al., 2000; Maldjian et al., 2004). The exception was the intraparietal sulcus, for which we used a 6 mm predefined sphere identified by Koyama et al. (2011). Five components were identified as having a correlation value over 0.1, and visual inspection of these components confirmed that they included regions from our spatial map of the reading network. The component with the highest correlational value (0.272), which we will refer to as the reading network component, was selected for the between-group analyses (described below). It should be noted that this corresponding network had the highest spatial correlation with our reading network mask at both the first and second imaging session.

GICA component identification

Of the five components with relatively moderate correlation (range = 0.117 to 0.272) with our spatial map of the reading network, only one component had a significant correlation value with our reading network spatial map. This component we identified as the *reading network component*, which included bilateral IFG – triangularis (IFGtr ; BA45), right IFG – opercularis (IFGop), left IFG – orbital, bilateral middle temporal gyrus (MTG), bilateral FFG, left IPS, bilateral medial prefrontal cortex (MPFC), and right cuneus (see Figure 1a; Table S1). One-sample t-tests of the regions included in this component for each group for each imaging session demonstrated the same regions were represented within this component across groups and time points.

Statistical analysis of reading network independent component

For each subject, the reading network component was transformed to z-scores and were entered into SPM8 for a series of two-sample tests, with the TD children contrasted with the children with ASD (ASD-EXP + ASD-WLC groups) at the first imaging session, and the ASD-EXP group contrasted with the ASD-WLC group at the second imaging session. In addition, a paired t-test was conducted to assess differences pre- to post-intervention in the ASD-EXP group. All results were masked with all subjects' (TD, ASD-EXP and ASD-WLC groups) averaged independent component thresholded at FWE corrected $p < 0.05$ at either the first or second imaging session in order to assess only within network differences. In addition, for the ASD-EXP group, we also assessed for differences outside the masked average component at both the first and second imaging sessions in order to better identify differences based on the intervention. For regions outside the masked average component, we corrected for multiple comparisons using a FDR cluster correction of $p < 0.05$.

Seed-based whole-brain analysis

The MNI coordinates for Broca's area (-51, 27, 18), and Wernicke's area (-51, -51, 30) were selected based on a previous study involving TD population (Tomasi & Volkow, 2012). Seeds were created using spherical binary masks (6mm-radius) and residual time-series were extracted for each seed and correlated with every other voxel in the brain for every participant. A Fisher's *r* to *z* transformation was applied to the correlation maps for each participant before averaging and computing statistical maps for each seed. Group comparisons for each seed connectivity map included two-sample *t*-tests to compare: 1) TD vs. all ASD participants (ASD-EXP + ASD-WLC) at first imaging session, 2) ASD-EXP vs. ASD-WLC at second imaging session, and 3) ASD-EXP vs. ASD-WLC at second imaging session while using the first imaging session connectivity maps as covariates; and paired-sample *t*-tests to compare: 4) ASD-WLC first imaging session vs. second imaging session, and 5) ASD-EXP pre-intervention vs. post-intervention. To correct for multiple comparisons, 10,000 Monte Carlo simulations were computed to obtain a cluster-size-corrected FWE threshold of $p < 0.05$. To account for the signal from cerebral white matter and lateral ventricles, masks were defined by anatomical masks using the WFU PickAtlas (Maldjian et al., 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 computed. Sources of noise (head motion, white matter, and lateral ventricles plus derivatives) were low bandpass filtered, modeled, and removed using a general linear model, and residuals were used for functional connectivity analysis.

Correlating improvement in reading comprehension with connectivity

We further examined the relationship between changes in functional connectivity in the ASD-EXP group and changes in reading performance measured by the Gray Oral Reading Test (GORT, 4th edition). The change in GORT scores from pre- to post-intervention were correlated in a voxel-wise manner using z-score maps of each whole-brain analysis (Wernicke's and Broca's area) using AFNI's 3dTcorr1D. This generated a whole-brain correlation map showing which regions in the brain were correlated with GORT scores, and cluster correction was applied as described above. In addition, changes in GORT scores were correlated with the change in connectivity pre-to post-intervention in the reading network component identified by GICA for the ASD-EXP group. For all analyses, verbal IQ was included as a covariate.

Results

Overview

The key findings of this study include: 1) The ASD-EXP group showed a significant increase in their reading comprehension abilities that were not seen in the ASD-WLC group; 2) ICA-based connectivity analysis revealed a strengthening of within-network functional connectivity of both Broca's (left IFG) and Wernicke's (left STG) areas in the ASD-EXP group post-intervention; 3) Seed-based functional connectivity results revealed the ASD-EXP group showing increased connectivity post-intervention between Broca's area and motor regions (e.g., SMA and precentral gyrus) and supramarginal gyrus, and between Wernicke's area and left-lateralized language regions (e.g., left IFG and left MTG); and 4) Lastly, greater connectivity in both Broca's and

Wernicke's area was correlated with greater improvement in reading comprehension in the ASD-EXP group.

Behavioral Results

The ASD-EXP group showed significant improvement in reading comprehension (from pre- to post-intervention), as measured by the GORT-4 Comprehension subtest, [paired t-test, $t(15) = 5.01$, $p < 0.0001$ (see Table 1 for means)]. On the other hand, the WLC group did not have a significant change in reading comprehension from the first to second imaging session [paired t-test, $t(14) = 1.29$, $p = 0.218$]. In addition, the ASD-EXP group significantly improved their reading comprehension scores, indexed by change from first to second session in GORT-4 scores, compared to the WLC group [ASD-WLC mean change in standard score (SS): 2.00, ASD-EXP mean change in SS: 11.31, $t(29) = 2.70$, $p = 0.01$]. The TD group had significantly higher reading comprehension scores than the children with ASD [ASD-EXP + ASD-WLC groups versus TD; $t(51) = 5.91$, $p < 0.0001$]. After the intervention, the ASD-EXP group continued to have significantly lower reading comprehension scores than the TD group [$t(36) = 3.74$, $p = 0.0004$].

Reading network connectivity: independent component analysis

When the independent component with highest spatial correlation to the reading network was compared between the TD children and the children with ASD (ASD-EXP + ASD-WLC) at the first imaging session, there was significantly reduced functional connectivity in the ASD group compared to the TD group (ASD < TD) in the left insula (x= -32, y = 12, z=10) and left IFG (Broca's area; x=-52, y=20, z=12), and overconnectivity (ASD > TD) in the right IFGop (see Figure 1b). There were no differences between the EXP and WLC group at the first imaging session. In a second set of comparisons, we assessed intervention effects in two different analyses: 1) The ASD-EXP group revealed an increase in functional connectivity from pre-to post-intervention within the reading network component in Wernicke's area (pSTS; BA39/40; x =-58, y=-58, z=44), and a decrease in connectivity from pre- to post intervention within the right IFG - orbital (IFGor; BA 47; see Figure 2). In addition, outside the reading network, we found additional recruitment of left SFG (x=-30, y=-62, z=-4) and right MFG (x = 52, y = 50, z = -4) at post-intervention that was not seen in the reading network component at pre-intervention; 2) The ASD-EXP group vs. ASD-WLC group at the second imaging session revealed greater functional connectivity in the ASD-EXP group after intervention within the reading network component in bilateral IFG (x=-38, y=50, z=4; x=52, y=50,z=-4), left MFG (x=-44,x=30,x=22), left MTG (x=-58, y=-50, z = -8), and Wernicke's area (BA40, x=-46, y=-52, z=44; see Figure 2). There were no regions with greater connectivity in the ASD-WLC group compared to the ASD-EXP group at the second imaging session.

Figure 1. (A) The independent component with the highest spatial correlation with our reading network mask derived from all subjects combined (TD + ASD-EXP + ASD-WLC groups; n = 53) at the first imaging session (FWE corrected at $p < 0.05$). (B) Between-group differences in within-network connectivity for all ASD combined at the first imaging session (ASD-EXP + ASD-WLC groups) versus TD group ($p < 0.001$, FDR corrected).

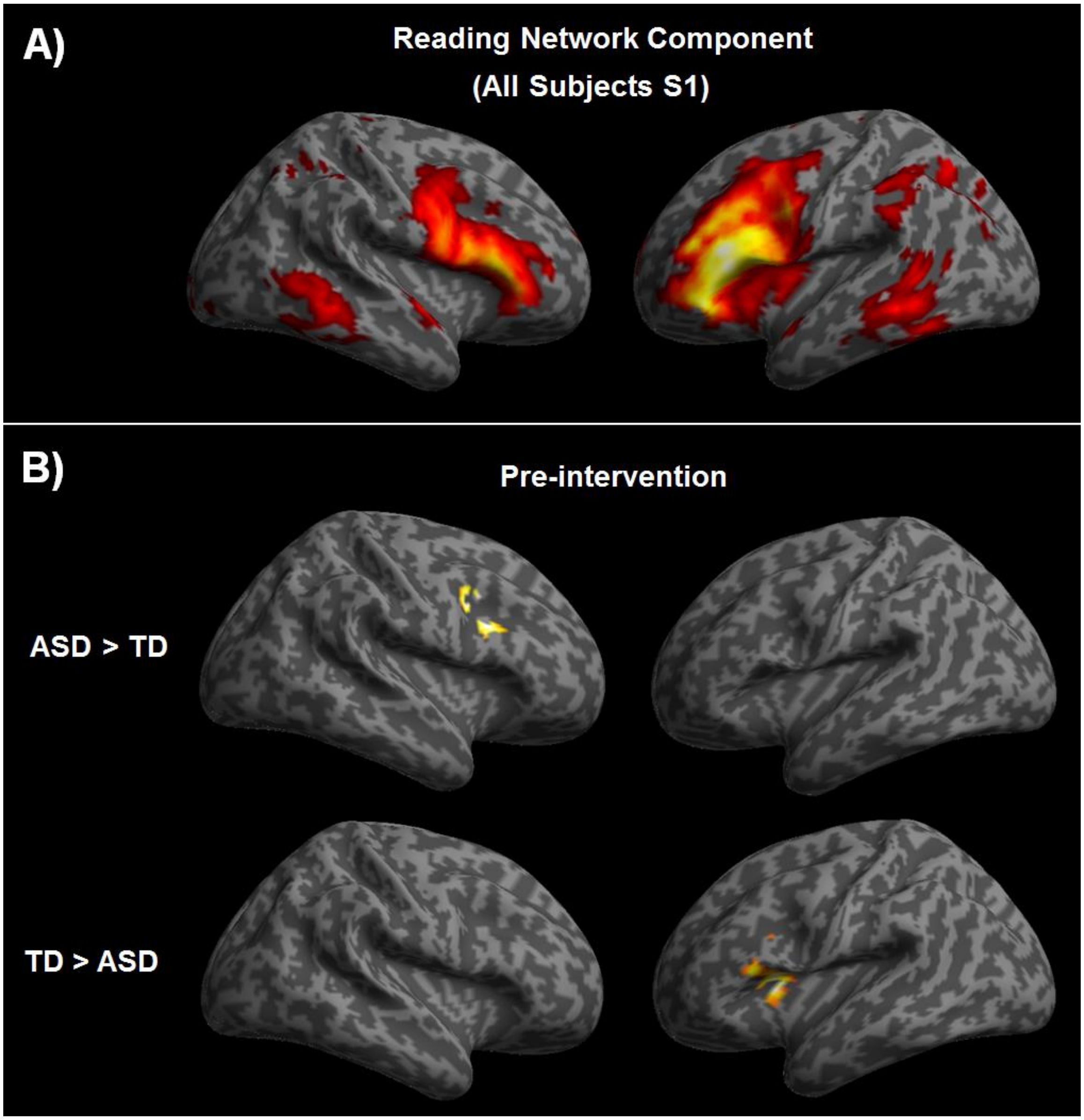
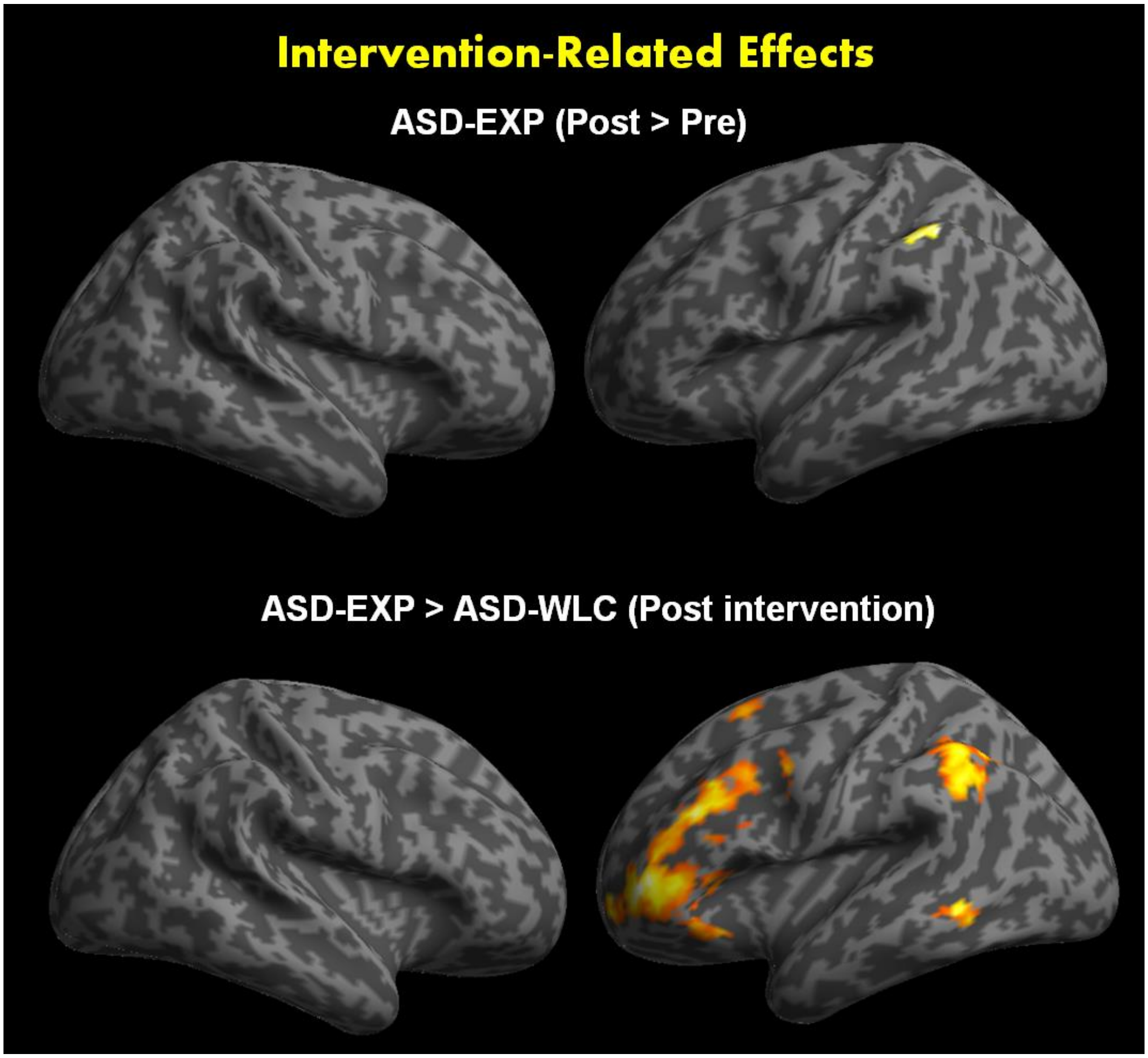


Figure 2. Intervention related effects: top panel: Between-group analysis showing greater network connectivity at post-intervention than pre-intervention for the ASD-EXP group; bottom panel: Between group analysis showing greater network connectivity in the ASD-EXP group compared to the WLC-EXP group at the second imaging session. Maps are thresholded at $p < 0.001$, FDR corrected.



Functional connectivity of Broca's area

An examination of all within-group results in this study revealed strong functional connectivity between Broca's area with bilateral IFG and IPL, with some additional connectivity, which varied across analyses in regions such as SMA, subcortical regions, and temporal lobe regions (see Table S2). Analysis of group differences comparing TD children with all children with ASD (ASD-EXP + ASD-WLC groups) at the first imaging session showed overconnectivity (ASD > TD) between Broca's area with left IFG, bilateral MFG, and left MOG, along with underconnectivity in one cluster (TD > ASD) with left FFG (see Table 2). The second set of analyses was specifically aimed at assessing intervention-related changes by comparing 1) ASD-WLC and ASD-EXP group at the second imaging session, while controlling for connectivity at the first imaging session, which revealed an overall shift in connectivity such that the ASD-EXP group showed enhanced connectivity of Broca's area with right middle cingulate, right SMA, right precentral gyrus (PrCG) and right supramarginal gyrus (SMG), and weaker connectivity with the right SPL and right precuneus (see Figure 3; Table 3); and 2) Additionally, the ASD-EXP group pre- to post-intervention showed an overall shift in connectivity such that stronger connections of Broca's area with right MFG, right STG, left supramarginal gyrus, and right caudate were detected post-intervention (see Figure 4; Table 3). This was not seen in the ASD-WLC group, who showed an overall reduction from first to second imaging session in connectivity between Broca's area with right SMA, and bilateral occipital, temporal, and subcortical regions. (see Table S3).

Functional connectivity of Wernicke's area

Functional connectivity of Wernicke's area with the rest of the brain in all of our groups showed strong time-series correlations between this seed and IFG, temporal lobe regions, and with midline cortical structures, such as middle cingulate and precuneus depending on the type of analysis (see Table S2). Group difference analysis involving TD children and all children with ASD, at the first imaging session, revealed overconnectivity in the ASD group between Wernicke's area and calcarine sulcus (see Table 2). Our second analyses specific to intervention-related changes compared 1) the ASD-EXP and ASD-WLC group at the second imaging session, while controlling for connectivity at the first imaging session, and revealed that the ASD-EXP group showed enhanced connections post-intervention with left IFG and left MTG and bilateral cerebellum when compared the ASD-WLC group (see Figure 3; Table 3); 2) the ASD-EXP group pre- to post-intervention showed an overall shift in connectivity such that stronger connections of Wernicke's area with bilateral IFG, bilateral MFG, right PrCG, and cingulate cortex were detected post-intervention (see Figure 4; Table 3). Lastly, we again found that the ASD-WLC group from first to second session showed an overall reduction in connectivity between Wernicke's area and left FFG and right IPL (see Table S3).

Table 2. Between-group differences in connectivity with Broca's and Wernicke's seeds in the ASD group (ASD-EXP + ASD-WLC groups; n = 31) at the first imaging session and the TD group (n = 22) ($p < 0.05$, FWE corrected).

Seed	Direction	Region	Hem	Volume (# of voxels)	Peak coordinates (RAI) x	y	z	Peak *t*
Broca	ASD > TD	Middle frontal gyrus	R	1049	52	-28	13	3.5
		Middle frontal gyrus	L	612	-54	-34	10	3.8
		Inferior frontal gyrus	L	221	67	42	-6	4.5
		Middle occipital gyrus	L	127	52	42	49	4.0
	TD > ASD	Fusiform gyrus	L	112.0	-63	33	10	-3.6
Wernicke	ASD > TD	Calcarine sulcus	L	103	13	78	13	2.9

Figure 3. Areas of greater connectivity with Broca's and Wernicke's seeds in the ASD-EXP group than the ASD-WLC group post-intervention, while controlling for within-group connectivity at the first imaging session ($p < 0.05$, FWE corrected).

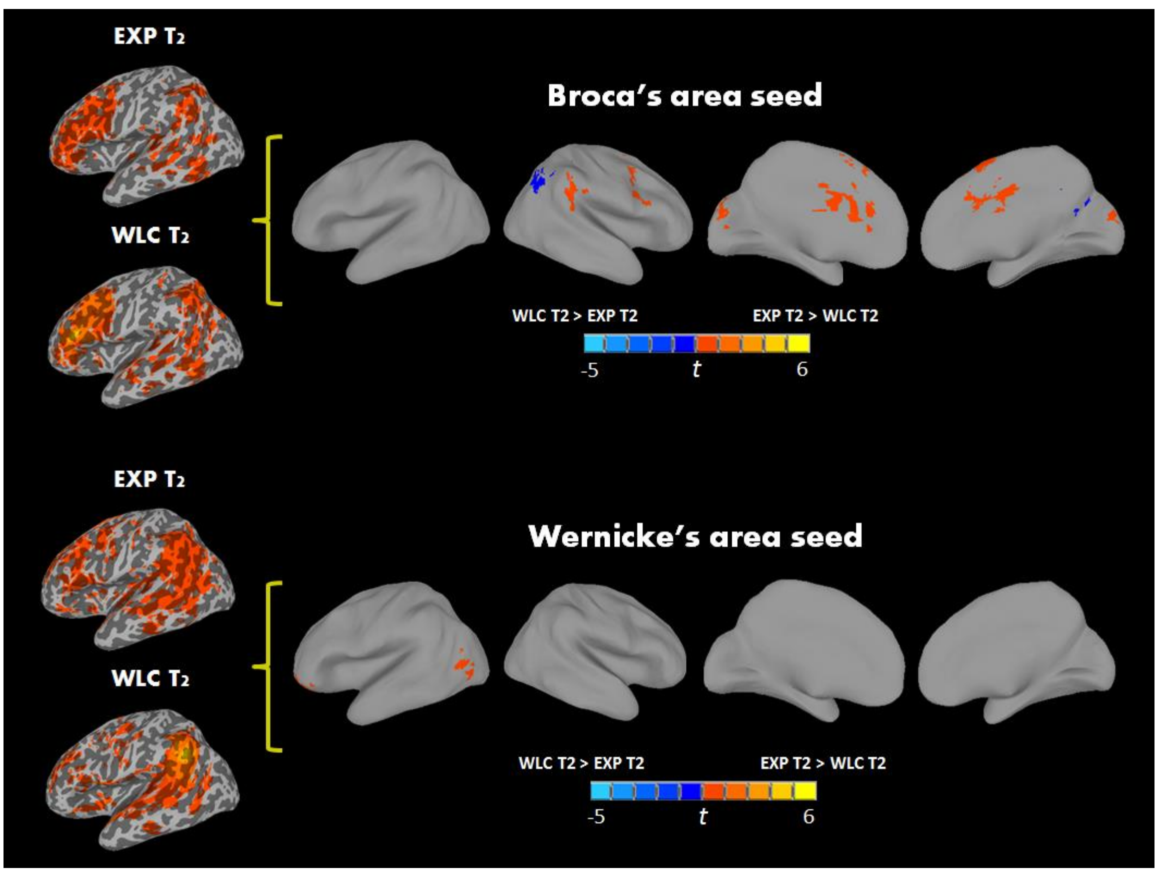


Figure 4. Areas of greater connectivity with Broca's and Wernicke's seeds post-intervention than pre-intervention in the ASD- EXP group ($p < 0.05$, FWE corrected).

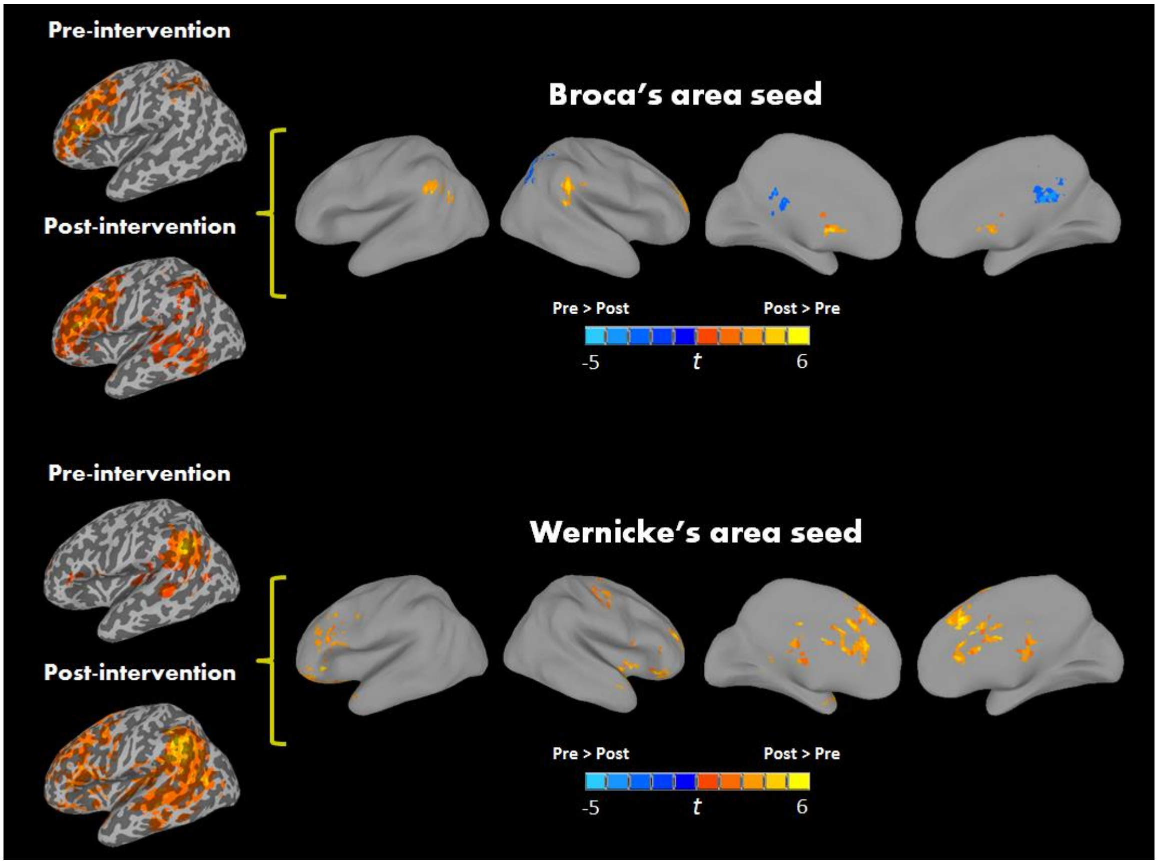


Table 3. Intervention related effects in whole-brain functional connectivity with both Broca's and Wernicke's seed ($p < 0.05$, FWE corrected).

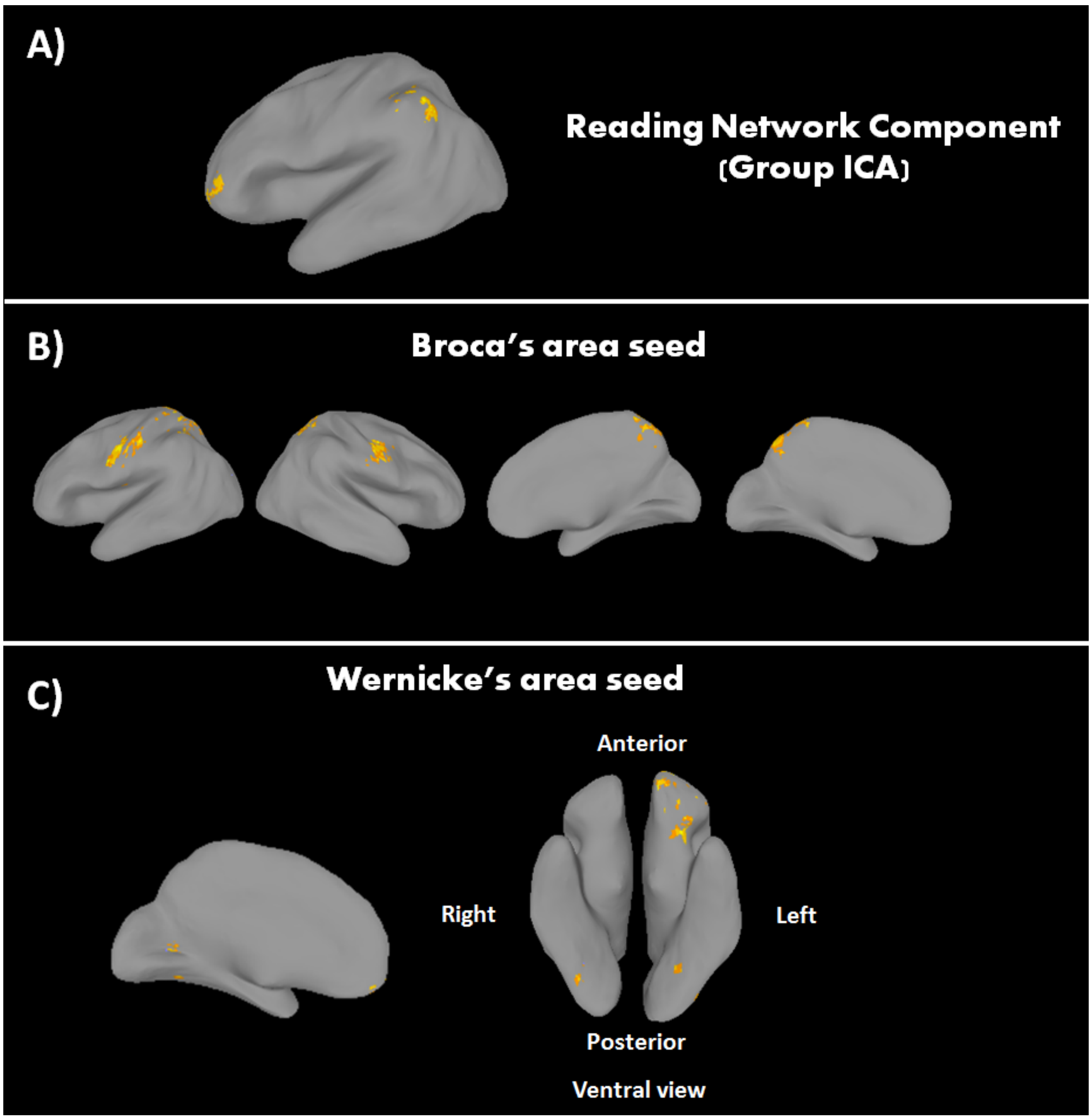
Seed	Effect	Region	Hem	Volume (# of voxels)	Peak coordinates (RAI) x	y	z	Peak *t*
ASD-EXP vs. ASD-WLC at second session								
Broca	WLC > Exp	Superior parietal lobule	R	166	-36	75	55	5.3
	WLC > Exp	Precuneus	R	116	-18	42	37	5.6
	Exp > WLC	Middle cingulate	R	464	-3	6	37	-5.2
	Exp > WLC	SMA	R	162	-12	-10	70	-4.5
	Exp > WLC	Precentral gyrus	R	112	-48	-4	22	-4.7
	Exp > WLC	Supramarginal gyrus	R	111	-57	42	31	-4.1
Wernicke	Exp > WLC	Cerebellum	R/L	313	7	60	-9	-4.1
	Exp > WLC	Inferior frontal gyrus	L	187	19	-64	-15	-2.1
	Exp > WLC	Middle temporal gyrus	L	109	58	78	4	-3.5
ASD-EXP Pre- vs. Post-intervention								
Broca	Pre > Post	Middle occipital gyrus	R	152	-42	75	34	-4.7
		Posterior cingulate cortex	R	113	-9	48	28	-4.6
	Post > Pre	Middle frontal gyrus	R	150	-33	-49	25	5.0
		Supramarginal gyrus	L	105	58	48	25	5.0
		Caudate nucleus	R	100	-21	-16	16	4.9
		Superior temporal gyrus	R	100	-69	45	13	5.4
Wernicke	Post > Pre	Anterior cingulate	R	505	-3	-31	10	4.8
		Middle orbital gyrus	L	318	34	-61	-9	4.6
		Inferior frontal gyrus	R	211	-51	-19	-12	4.5
		Inferior frontal gyrus	L	205	34	-16	22	4.9
		Middle frontal gyrus	R	149	-33	-52	37	4.7
		Middle cingulate	R	131	-9	24	25	5.8
		Precentral gyrus	R	103	-36	24	55	4.2

Relationship between fcMRI and change in reading comprehension scores

Multiple regression analyses were performed to determine whether improvement in reading comprehension due to intervention, as measured by the GORT-4 Comprehension subtest (controlling for verbal IQ), was correlated with functional connectivity in either our GICA or seed-based analyses in the ASD-EXP group. A significant positive correlation was found between change in GORT-4 comprehension scores and change in connectivity of the reading network component pre-to post-intervention for Wernicke's area (BA40) and left IFGor. There were no significant negative correlations with the reading network component (see Figure 5a).

For the seed-based whole brain analyses involving Broca's area seed, a significant positive correlation between change in GORT-4 scores and increase in connectivity from pre- to post- intervention was found in the bilateral postcentral gyrus (PoCG) and right SPL (see Figure 5b). From Wernicke's area seed, a significant positive correlation between change in GORT-4 scores and increase in connectivity from pre-to post-intervention was found in left IFGor, right cerebellum, and left MOG/SOG (see Figure 5c). There were no significant negative correlations with the Broca's or Wernicke's seed.

Figure 5. Positive correlations with change in reading comprehension scores (GORT-4), controlling for verbal IQ, and functional connectivity from pre- to post-intervention in the ASD-EXP group: (A) Greater within-network connectivity for our GICA identified reading component post-intervention was correlated with greater reading improvement; (B) Greater connectivity in brain regions with Broca's seed was correlated with greater reading improvement; and (C) Greater connectivity in brain regions with Wernicke's seed was correlated with greater reading improvement. Maps are thresholded at $p < 0.05$, FWE corrected.



Discussion

This study identified low frequency fluctuations of spontaneous brain activity using rsfMRI to characterize the functional integrity of the reading comprehension network in children with ASD. More importantly, we were able to demonstrate the plasticity of functional connectivity across brain regions in children with ASD who received an intensive language intervention, resulting in stronger connectivity of the reading network post-intervention independent of task. it should also be noted that the improvement in reading comprehension in children with ASD was correlated with increase in functional connectivity in the reading network identified by GICA (left IFG and left STG), as well as between Broca's and right STG and Wernicke's and left IFG in our seed-based analyses. Moreover, children with ASD who received the intervention showed additional recruitment of regions outside of the reading network post-intervention.

Both the GICA and seed-based approaches were sensitive enough to show intervention-related changes in our ASD-EXP group at the second imaging session. In addition to both types of analyses showing increased strengthening of functional connectivity between Broca's and Wernicke's area as a result of intervention, strengthening of connectivity of these regions with motor regions (SMA and PrCG) was also seen. Moreover, greater improvement in reading comprehension was correlated with increased connectivity between Broca's area and PoCG. Both the SMA and PrCG/PoCG have been shown to be activated during different aspects of language processing, including abstract sentences (Sakreida et al., 2013), mental rotation and mental imagery

(Kosslyn et al., 2001; Tomasino & Rumiati, 2013), imagining concrete words (Postle et al., 2008; Pulvermuller & Hank, 2006), and lexical decision making (Carreiras et al., 2006; Tomasino et al., 2010). Additionally, resting state studies of the language network have consistently found connectivity between Broca's and Wernicke's areas and premotor and motor regions (Koyama et al., 2010, 2011; Tomasi et al., 2012; Verly et al., 2014). For example, more effortful lexical processing (differentiating between words and pseudo-words) elicited greater recruitment of motor regions, specifically SMA (Carreiras et al., 2006). Similarly, Koyama et al., (2011) found that increased functional connectivity between Broca's and Wernicke's and PrCG and PoCG, in children, but not adults, was correlated with increased reading competence, and may reflect more effortful sight reading. Specific to children with ASD, loss of connectivity between Broca's area and SMA were correlated with lower level of language skills, including word and sentence comprehension (Verly et al., 2014). The increased connectivity between language and motor regions in our ASD-EXP group is of especial interest in the context of, Tomasino and Rumiati's (2013) proposed theory of motor activation utilization in language comprehension. They proposed that, given the wide number of language studies that find sensorimotor activity independent of action related stimuli, activation of these areas are related to a selective cognitive strategy (whether explicitly or automatically) of utilizing mental imagery in order to enhance reading comprehension across linguistic tasks (Tomasino & Rumiati, 2013). This is especially pertinent given the V/V intervention children with ASD received in our study focused on imagery to enhance oral and written language comprehension.

Another specific difference we observed in intrinsic connectivity was an increase in functional connectivity between Broca's area and SMG bilaterally unique to the ASD-EXP group. The SMG has been consistently found to be a key region in language comprehension, specific to phonological processes (Harwigsen et al., 2010; Sliwinska et al., 2012; Turkeltaub et al. 2003). The SMG has also been found to be involved in not just overt phonological processes, but also when mentally sounding out words (Stoeckel et al., 2009), during visual word recognition (Carreiras et al., 2009; Stoeckel et al., 2009), lexical processing (Osipowicz et al., 2011), and verbal working memory (Acheson et al., 2011; Deschamps et al., 2014). Additionally, greater activation of bilateral SMG has been correlated with greater accuracy and efficiency on phonological decision making (Harwigesn et al., 2010). Interestingly, a diffusion tensor imaging (DTI) study by Li et al. (2014) found that greater white matter connectivity associated with the SMG was correlated with increased reading comprehension (using the same GORT-4 measure as our study), but only in the ASD group, not in the TD group. Interestingly, it was a strengthening of right SMG connectivity with motor regions (PrCG and PoCG; Li et al., 2014), which our study also found increased connectivity with both Broca's area and PrCG and PoCG, as well as SMG. This suggests compensatory mechanisms in increasing proficiency in reading comprehension that involves both the SMG and motor regions that may underlie unique strategies in children with ASD.

Intervention-related effects were also seen in brain regions that have only recently begun to be identified as having a role in language comprehension, with strengthening of connectivity post-intervention in the caudate and cerebellum. Additionally, improvement in reading comprehension after intervention was correlated with increased connectivity between Wernicke's area and right cerebellum. The caudate has been shown to be functionally connected to Broca's and Wernicke's areas in typical adults (Tomasi et al., 2012). Recent studies have found caudate to be involved in accuracy of phonological processing (Tettamanti et al., 2005) and suppressing word interference (Ali et al., 2010). Indeed the frontal-caudate loop returns back to Broca's area (Middleton & Strick, 1994), and lesions of the caudate show similar cognitive deficits as lesions to Broca's area including semantic and phonological processing and memory retrieval for fluent reading (Abdullaey et al., 1998). The cerebellum has been shown to be involved in conjunction with inferior frontal regions in processes associated with semantic word association (Frings et al., 2006; Xiang et al., 2003). Moreover, cerebellar lesion studies have found specific deficits in higher-order language processes, such as verbal fluency, syntax, word retrieval, and reading and writing (Marien et al., 2001; Schmahmann and Sherman, 1998; Silveri et al., 1994). Specific to ASD, abnormal function of the right cerebellum has shown to be related to deficits in language functioning (Hodge et al., 2010; Verly et al., 2014).

Interestingly, there was a distinct hemispheric difference in the improvement in connectivity in the Broca's and Wernicke's seed regions when the ASD-EXP group was compared to the ASD-WLC group post-intervention. The children with ASD who received intervention (ASD-EXP group) showed increased connectivity between Broca's area and right hemisphere language-analogous regions, including the SMA, PrCG, and SMG. Conversely, Wernicke's area showed strengthening of connectivity with left hemisphere language regions, including left IFG and MTG. It has been well documented that individuals with ASD tend to rely heavily on posterior parietal and occipital brain regions when engaged in a language task, at the expense of left frontal regions (Kana et al., 2006; Kana & Wadsworth, 2012; Mason et al., 2008). In addition, recent studies have demonstrated that even young children with ASD fail to activate left hemisphere brain regions in response to both auditory and verbal language tasks (Eyler et al., 2012; Groen et al., 2010; Sahyoun et al. 2010). This suggests that the children with ASD who received the intervention may have difficulties modulating Broca's area with other left lateralized regions, and are compensating by increasing functional connectivity between Broca's area and right lateralized regions. Conversely, the more intact posterior parietal regions in our children with ASD help in increasing connectivity between Wernicke's area and more traditional left hemisphere language regions. Indeed, in the resting network component, identified by GICA, the ASD-EXP group strengthened the connectivity of left pSTG while decreasing reliance on right lateralized inferior frontal regions. This is further supported by the fact that increased connectivity across different regions was correlated with greater improvement in reading comprehension. Additionally, while failure to activate left hemisphere regions in response to language has been a characteristic feature of ASD (Herbert et al., 2005), recent studies assessing the language network in TD individuals have found right hemisphere involvement specific to the right MTG and right SMG when interpreting higher-order language comprehension, such as idiomatic language (Proverbio et al., 2009) and phonological interpretation (Hartwigsen et al., 2010). Lexical decoding also has been shown to be associated with activation of right hemisphere regions, specifically right MFG, right SMG, and bilateral cerebellar, as a function of skilled reading ability (Osipowicz et al., 2011). This suggests that it could also be the case that the ASD-EXP group may be strengthening a left-right connection between Broca's and MFG, SMG and MTG that already exists in unimpaired adult readers (Tomasi et al., 2012).

One interesting result that deserves mention is a significantly greater connectivity in ASD children, relative to TD children, between Broca's and left frontal regions, and Wernicke's area and calcarine sulcus. While initial literature in intrinsic connectivity had focused on adults with ASD (e.g., Assaf et al., 2010; Kennedy and Courchesne, 2008; Monk et al.,2009; von dem Hagen et al., 2012), studies investigating intrinsic connectivity in children with ASD have consistently shown hyperconnecitivy compared to TD children (Supekar et al., 2013; Di Martino et al., 2011), suggesting a developmental shift from hyperconnectivity to hypoconnectivity as individuals with ASD mature into adulthood. In addition, the underconnectivity account emphasizes weaker connectivity of long range cortical connections in favor of local connectivity (e.g., Just et al., 2004; Muller er al., 2011; Courchesne et al., 2007). This is consistent with what we observed in our study, with hyperconnectivity between Broca's and spatially adjacent frontal regions, and between Wernicke's and calcarine regions, when compared to TD controls. Indeed, greater connectivity in the TD group was seen only for more long range connections, between Broca's area and FFG. Interestingly, Li et al. (2014), found hyperconnectivity differences specific to local connectivity in children with ASD compared to TD children, and that greater local connectivity in the ASD group, but not the TD group, was positively correlated with reading ability. However, it is important to note that through intervention our ASD-EXP group was able to strengthen both long distance functional connections and frontal to posterior connections in reading related brain regions, which has larger implications for the necessity of targeted intervention for children with ASD.

While we have carefully controlled and matched the different groups of participants, a potential limitation pertains to the selection of children with ASD with a specific reading profile that would be most likely to benefit from the intervention, namely adequate decoding skills coupled with statistically poorer reading comprehension. As such, the results of this study may not be generalized to different subtypes of ASD. Future studies should assess whether reading interventions targeting other deficits in ASD show similar patterns of change in functional connectivity. Additionally, it is unclear whether the changes in functional connectivity seen in this study may be contributed by anatomical changes in the regions involved. Future work should assess structural changes of the reading network in children with ASD after intervention and its relation with functional connectivity. To our knowledge, there have been no translational studies involving intervention that have assessed structural changes of the reading network in children, which would be the next logical step.

In summary, we found that improvement in reading comprehension due to reading intervention in children with ASD was associated with strengthening of functional connectivity in Wernicke’s area and left IFGor and other premotor and language regions. We also found increased connectivity specific to the children with ASD who participated in the reading intervention between Wernicke’s area and posterior language and visual brain regions, increased compensatory mechanisms for language comprehension in right-hemisphere connections with Broca’s area, strengthening of connections with SMG, and strengthening of connectivity of regions outside to conventional language network, including caudate and cerebellum. Furthermore, our study utilized intrinsic resting state data to accurately identify the reading comprehension network and determine functional connectivity differences independent of the constraints of task. 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 circuitry.

References

Assaf, M., Jagannathan, K., Calhoun, V.D., Miller, L., Stevens, M.C., Sahl, R., O'Boyle, J.G., . . . Pearlson, G.D. (2010). Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *Neuroimage, 15,* 247–256.

Abdullaev, Y.G., Bechtereva, N.P., & Melnichuk, K.V. (1998). Neuronal activity of human caudate nucleus and prefrontal cortex in cognitive tasks. *Behav Brain Res, 97,* 159-77.

Acheson, D.J., Hamidi, M., Binder, J.R., & Postle, B.R. (2011). A common neural substrate for language production and verbal working memory. *J Cogn Neurosci, 23,* 1358-67.

Ali, N., Green, D.W., Kherif, F., Devlin, J.T., & Price, C.J. (2010). The role of the left head of caudate in suppressing irrelevant words. *J Cogn Neurosci, 22,* 2369-86.

Barquero, L.A., Davis, N., & Cutting, L.E. (2014). Neuroimaging of reading intervention: a systematic review and activation likelihood estimate meta-analysis. *PLoS One, 9,* e83668.

Bell, N. (1991a). Gestalt imagery: A critical factor in language comprehension. *Annals of Dyslexia*, *41*, 246–260.

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

Carreiras, M., Mechelli, A., & Price, C.J. (2006) Effect of word and syllable frequency on activation during lexical decision and reading aloud. *Hum Brain Mapp, 27,* 963-72.

Carreiras, M., Seghier, M., Baquero, S., Este´vez, A., Lozano, A., Devlin, J., & Price, C.J. (2009). An anatomical signature for literacy. *Nature, 461,* 983–986.

Centers for Disease Control and Prevention (CDC). (2014). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2010. *MMWR Surveillance Summary, 63,* 1–21.

Calhoun, V.D., Adali, T., Pearlson, G.D., & Pekar, J.J. (2001). Spatial and temporal independent component analysis of functional MRI data containing a pair of task-related waveforms. *Hum. Brain Mapp. 13,* 43–53.

Calhoun, V.D., Kiehl, K.A., & Pearlson, G.D. (2008) Modulation of temporally coherent brain networks estimated using ICA at rest and during cognitive tasks, *Hum. Brain Mapp. 29,* 828–838.

Courchesne, E., Pierce, K., Schumann, C.M., Redcay, E., Buckwalter, J.A., Kennedy, D.P., & Morgan, J. (2007). Mapping early brain development in autism. *Neuron, 56,* 399–413.

Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res, 29,* 162-173.

Deco, G., & Corbetta, M., (2011). The dynamical balance of the brain at rest. *Neuroscientist 17,* 107–123.

Deschamps, I., Baum, S.R., & Gracco, V.L. (2014) On the role of the supramarginal gyrus in phonological processing and verbal working memory: evidence from rTMS studies. *Neuropsychologia, 53,* 39-46.

Di Martino, A., Kelly, C., Grzadzinski, R., Zuo, X.N., Mennes, M., Mairena, M.A., Lord, C., . . . Milham, M.P. (2011). Aberrant striatal functional connectivity in children with autism. *Biol. Psychiatry, 69,* 847–856.

Erhardt, E.B., Allen, E.A., Damaraju, E., & Calhoun, V.D., (2011). On network derivation, 753 classification, and visualization: a response to Habeck and Moeller. *Brain Connect., 754*, 1–19.

Eyler LT, 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,* 949-60.

Fan, J., McCandliss, B.D., Fossella, J., Flombaum, J.I., & Posner, M.I. (2005). The activation of attentional networks. *Neuroimage, 26,* 471– 479.

Grieve, K.L., Acun˜a, C., & Cudeiro, J. (2000). The primate pulvinar nuclei: vision and action. *Trends Neurosci, 23,* 35–39.

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. *Neurosci. Biobehav. Rev, 32,* 1416–1425.

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. *Cereb Cortex, 20,* 1937-45.

Hampson, M., Tokoglu, F., Sun, Z., Schafer, R.J., Skudlarski, P., Gore, J.C., & Constable, R.T. (2006). Connectivity-behavior analysis reveals that functional connectivity between left BA39 and Broca's area varies with reading ability. *Neuroimage, 31,* 513–519.

Hartwigsen, G., Baumgaertner, A., Price, C., Koehnke, M., Ulmer, S., & Siebner, H. (2010). Phonological decisions require both the left and right supramarginal gyri. *Proc Natl Acad Sci USA, 107,* 16494–16499.

Hebb, A.O., & Ojemann, G.A. (2013). The thalamus and language revisited. *Brain Lang, 126,* 99-108.

Herbert, M., Ziegler, D., Deutsch, C., O'Brien, L., Kennedy, D., Filipek, P., Bakardjiev, A.I., . . . Caviness, V.S., Jr. (2005). Brain asymmetries in autism and developmental language disorder: a nested whole-brain analysis. *Brain, 128,* 213–226.

Joel, S.E., Caffo, B.S., van Zijl, P.C., & Pekar, J.J. (2011). On the relationship betweenseed-based and ICA-based measures of functional connectivity. *Magnetic Resonance in Medicine, 66,* 644–57.

Johnson-Glenberg, MC. (2000). Training reading comprehension in adequate decoders/poor comprehenders: Verbal versus visual strategies. Journal of *Educational Psychology, 92,* 772-782.

Kana, R.K., Keller, T.A., Cherkassky, V.A., Minshew, N.J., & Just, M.A. (2006). Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. *Brain: a journal of neurology, 129,* 2484–2493.

Kana, R.K., Murdaugh, D.L., Libero, L.E., Pennick, M.R., Wadsworth, H.M., Deshpande, R., & Hu, C.P. (2011). Probing the brain in autism using fMRI and diffusion tensor imaging. *Journal of Visualized Experiments: JoVE, 55,* http://www.jove.com/ details.php?id=3178, doi: 10.3791/3178.

Kana, R.K., & Wadsworth, H.M. (2012). "The archeologist's career ended in ruins": hemispheric differences in pun comprehension in autism. *Neuroimage, 62,* 77-86.

Keller, T.A., & Just, M.A. (2009). Altering cortical connectivity: remediation-induced changes in the white matter of poor readers. *Neuron, 64,* 624–631.

Kennedy, D.P., & Courchesne, E. (2008). The intrinsic functional organization of the brain is altered in autism. *Neuroimage 39,* 1877–1885.

Kosslyn, S.M., Thompson, W.L., Wraga, M., & Alpert, N.M. (2001). Imagining rotation by endogenous versus exogenous forces: distinct neural mechanisms. *Neuroreport 12,* 2519–2525.

Koyama, M.S, Kelly, C., Shehzad, Z., Penesetti, D., Castellanos, F.X., & Milham, M.P. (2010). Reading networks at rest. *Cereb Cortex, 20,* 2549 –2559.

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. *J Neurosci*, *31,* 8617-8624.

Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., Rainey, L., Kochunov, P.V., . . . Fox, P.T. (2000). Automated Talairach atlas labels for functional brain mapping. *Hum. Brain Mapp. 10,* 120–131.

Li, Y.O., Adali, T., & Calhoun, V.D. (2007). Estimating the number of independent components for functional magnetic resonance imaging data. *Human Brain Mapping, 28,* 1251–66.

Li, H., Xue, Z., Ellmore, T.M., Frye, R.E., & Wong, S.T. (2014). Network-based analysis reveals stronger local diffusion-based connectivity and different correlations with oral language skills in brains of children with high functioning autism spectrum disorders. *Hum Brain Mapp, 35,* 396-413.

Lohmann, G., Hoehl, S., Brauer, J., Danielmeier, C., Bornkessel-Schlesewsky, I., Bahlmann, J., Turner, R., & Friederici, A. (2010). Setting the frame: the human brain activates a basic low-frequency network for language processing. C*ereb Cortex*, *20*, 1286-92.

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. *J Autism Dev Disord, 24,* 659–85.

Lord, C., Risi, S., Lambrecht, L., Cook, Jr., E.H., Leventhal, B.L., DiLavore, P.C., Pickles, A., & Rutter, M. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord*, *30*, 205–23.

Lindamood, P., Bell, N. (1997). Sensory-cognitive factors in the controversy over reading instruction. *The Journal of Developmental and Learning Disorders*, *1,* 143-182.

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

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,* 1233-1239.

Maidjian, J.A., Laurienti, P.J., & Burdette, J.H., (2004). Precentral gyrus discrepancy in electronic versions of the Talairach Atlas. *Neuroimage 21,* 450–455.

Marien, P., Engelborghs. S., Fabbro, F., & De Deyn, P.P. (2001). The lateralized linguistic cerebellum: a review and a new hypothesis. *Brain Lang, 79,* 580–600.

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

Monk, C.S., Peltier, S.J., Wiggins, J.L., Weng, S.J., Carrasco, M., Risi, S., & Lord, C. (2009). Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *Neuroimage 47,* 764–772.

Middleton, F.A., & Strick, P.L. (1994). Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science, 266,* 458–461

Frings, M., Dimitrova, A., Schorn, C.F., Elles, H.G., Hein-Kropp, C., Gizewski, E.R., Diener, H.C., & Timmann, D., (2006). Cerebellar involvement in verb generation: an fMRI study. *Neurosci. Lett, 409,* 19–23.

Minshew, N.J., & Keller, T.A. (2010). The nature of brain dysfunction in autism: functional brain imaging studies. *Curr Opin Neurol, 23,* 124–130.

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,* 911–919.

Newman, T.M., Macomber, D., Naples, A.J., Babitz, T., Volkmar, F., & Grigorenko, E.L. (2007). Hyperlexia in children with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 37,* 760–774.

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.

Osipowicz, K., Rickards, T., Shah, A., Sharan, A., Sperling, M., Kahn, W., & Tracy, J. (2011). A test of the role of the medial temporal lobe in single-word decoding. *Neuroimage, 54,* 1455-64.

Postle, N., McMahon, K.L., Ashton, R., Meredith, M., & de Zubicaray,G. I. (2008). Action word meaning representations in cytoarchitectonially defined primary and premotor cortices. *Neuroimage 43,* 634–644.

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*, 2142-2154.

Proverbio, A., Crotti, N., Zani, A., Adorni, R. (2009) The role of left and right hemispheres in the comprehension of idiomatic language: an electrical neuroimaging study. *BMC Neurosci, 10,* 116.

Pulvermuller, F., & Hauk, O. (2006). Category-specific conceptual processing of color and form in left fronto-temporal cortex. *Cereb.Cortex*, *16*, 1193–1201.

Sahyoun, C.P., Belliveau, J.W., Soulières, I., Schwartz, S., & Mody, M. (2010). Neuroimaging of the functional and structural networks underlying visuospatial vs. linguistic reasoning in highfunctioning autism. *Neuropsychologia, 48,* 86–95.

Sakreida, K., Scorolli, C., Menz, M.M., Heim, S., Borghi, A.M., & Binkofski, F. (2013). Are abstract action words embodied? An fMRI investigation at the interface between language and motor cognition. *Front Hum Neurosci, 9,* 125.

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.

Schmahmann, J.D., & Sherman, J.C., (1998). The cerebellar cognitive affective syndrome. *Brain 121 (Pt 4),* 561–579.

Sliwinska, M.W., Khadilkar, M., Campbell-Ratcliffe, J., Quevenco, F., & Devlin, J.T. (2012). Early and sustained supramarginal gyrus contributions to phonological processing. *Front Psychol, 3,* 161.

Silveri, M.C., Leggio, M.G., Molinari, M., (1994). The cerebellum contributes to linguistic production: a case of agrammatic speech following a right cerebellar lesion. *Neurology, 44,* 2047–2050.

Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., Mackay, C.E., Filippini, N., . . . Beckmann, C.F. (2009) Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A, 106,* 13040 –13045.

Stoeckel, C., Gough, P.M., Watkins, K.E., & Devlin, J.T. (2009). Supramarginal gyrus involvement in visual word recognition. *Cortex. 45*, 1091-6.

Supekar, K., Uddin, L.Q., Khouzam, A., Phillips, J., Gaillard, W.D., Kenworthy, L.E., Yerys, B.E., . . . Menon V. (2013). Brain hyperconnectivity in children with autism and its links to social deficits. *Cell Rep, 5,* 738-47.

Tettamanti, M., Moro, A., Messa, C., Moresco, R.M., Rizzo, G., Carpinelli, A., Matarrese, M., . . . Perani, D. (2005). Basal ganglia and language: phonology modulates dopaminergic release. *Neuroreport, 16,* 397-401.

Tomasi, D., & Volkow, N. D. (2012). Resting functional connectivity of language networks: characterization and reproducibility. *Mol Psychiatry, 17,* 841-854.

Tomasino B, & Rumiati RI. (2013). At the mercy of strategies: the role of motor representations in language understanding. *Front Psychol, 4,* 24-27.

Tomasino, B., Weiss, P.H., & Fink, G. R. (2010).To move or not to move: imperatives modulate action-related verb processing in the motor system. *Neuroscience, 169,* 246–258

Torgesen, J.K., Alexander, A.W., Wagner, R.K., Rashotte, C.A., Voeller, K.K., & Conway, T. (2001). Intensive remedial instruction for children with severe reading disabilities: Immediate and long-term outcomes from two instructional approaches. *Journal of Learning Disorders, 34,* 33-58.

Toro, R., Fox, P.T., & Paus, T. (2008). Functional coactivation map of the human brain. *Cereb Cortex, 18,* 2553–2559.

Turkeltaub, P.E., Gareau, L., Flowers, D.L., Zeffiro, T.A., & Eden, G.F. (2003). Development of neural mechanisms for reading. *Nat Neurosci, 6,* 767--773.

Van Dijk, K. R., Sabuncu, M. R., & Buckner, R. L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage, 59*, 431-438.

Verly, M., Verhoeven, J., Zink, I., Mantini, D., Peeters, R., Deprez, S. …, Sunaert S. (2014). Altered functional connectivity of the language network in ASD: Role of classical language areas and cerebellum. *Neuroimage Clin., 4,* 374-82.

von dem Hagen, E.A., Stoyanova, R.S., Baron-Cohen, S., & Calder, A.J. (2012) Reduced functional connectivity within and between 'social' resting state networks in autism spectrum conditions. *Soc Cogn Affect Neurosci, 8,* 694-701

Williams, D., Botting, N., Boucher, J. (2008). Language in autism and specific language impairment: where are the links? *Psychol Bull, 134,* 944–963.

Xiang, H., Lin, C., Ma, X., Zhang, Z., Bower, J.M., Weng, X., & Gao, J.H. (2003). Involvement of the cerebellum in semantic discrimination: an fMRI study. *Hum. Brain Mapp. 18,* 208–214.

SUMMARY

This project investigated the changes in task-dependent and task-independent brain activity following a strength-based reading intervention (Visualizing and Verbalizing for Language Comprehension and Thinking) in children with ASD. A number of themes emerged from both studies that improve our understanding of the neurobiology of ASD and how dysfunctional neural circuitry can be altered through targeted intervention. We found that the ASD children who received intervention showed increased brain activation and strengthened functional connectivity of core language areas, including left IFG, left MFG, left STG, left MTG, and bilateral SMG. Additionally, in our task-based study, we found additional recruitment of visual (e.g., MOG, LG, FFG) and posterior language regions (e.g., pSTS, MTG, and IPL). Moreover, both studies revealed the application of neural compensatory mechanisms in order to aid in language comprehension unique to the ASD-EXP group post-intervention, namely, (1) recruitment of right hemisphere language-analogous regions, (2) increased brain activation and functional connectivity of premotor and motor regions, and (3) increased reliance on subcortical regions (caudate, putamen, and thalamus) and the cerebellum when processing language.

Currently, the evidence from neuroimaging research is mixed as to the specific deficits found in brain activation and functional connectivity of the language network in children with ASD. Studies investigating toddles with ASD, have found a failure to activate left hemisphere language regions, specifically frontal regions (IFG, MFG,

MPFC), in response to language, showing activation responses in right-lateralized temporal regions (Eyler et al., 2012; Redcay & Courchesne, 2008). Additional studies assessing reading in school-aged children with ASD have found reduced activation in frontal language regions, and increased reliance on visuospatial regions and posterior language regions (occipital, parietal, and ventral temporal). However, some studies assessing functional and structural connectivity of the reading network in ASD found intact connectivity between Broca's and Wernicke's areas (Li et al., 2014; Verly et al., 2014). Our study provides clarity in regards to the issue of discrepancy across studies on frontal language activity, and specifically, the ability of children with ASD to modulate activity in frontal language networks. Our findings reveal that through targeted intervention, children with ASD can alter both brain activation and functional connectivity of left-lateralized frontal language regions, specifically IFG and MFG, as well as strengthening of distant functional connections between frontal and temporal language regions. This has significant implications, as some current accounts of brain dysfunction in ASD suggest excess neuronal growth early in development which triggers widespread pruning of neurons, specific to the frontal lobe, leading to favoring of short-distance cortical connections at the expense of more long-distance ones (Courchesne et al., 2008; 2011). In light of this, our study shows the importance of targeted interventions in promoting brain activity in regions showing deficit in children with ASD that correlates with behavioral measures of improvement.

Additionally, the type of intervention utilized in this project was one that used a relative strength in children with ASD, visual and non-verbal processing, to help improve an area of deficit, oral and reading comprehension. The use of visual techniques in teaching novel skills is not new in ASD, with the application of visual based systems in order to increase communication while decreasing reliance on complex language skills (e.g., picture exchange communication system (PECS), and visual organizers utilized in the TEACCH method). The findings of our study show improvements in behavioral performance and in functional integrity of neural circuitry as a result of V/V intervention, and add support to the use of such evidence-based and individualized interventions in children with ASD. Given the visual nature, specifically the concept imagery teaching method, of the intervention, we not only found increased activity of left language regions, but also increased activity of relatively posterior language regions, visual regions, and premotor/motor regions, to support reading comprehension. This fits with Tomasino and Rumiati's (2013) proposed model of visual and motor activity in relation to language comprehension. They suggest that activation in visual and motor regions, and connections between these regions and language regions, are related to a selective cognitive strategy in which mental imagery is used to aid in language comprehension. Additionally, this type of strategy is not seen in every individual and is not necessary for adequate comprehension in TD individuals. This fits with what our project found in terms of increased activity in visual and motor brain regions unique to our ASD-EXP group, given the strategies for visual imagery taught by the V/V intervention.

Increased reliance on subcortical regions, such as the caudate and putamen in our children with ASD who participated in the V/V intervention is not unique to children with ASD. Koyama et al. (2011) mapped the reading network in children and adults, and found that unique to TD children, but not adults, was an increased reliance on the thalamus during reading. This study also found that greater functional connectivity of language regions with the thalamus predicted greater reading skills in TD children but not in adults. Additionally, a study assessing reading in skilled readers found that reading increases functional connectivity of the occipito-temporal – PrCG pathway via the putamen (Seghier & Price, 2010). Studies have also found the caudate to be involved in accuracy of phonological processing (Tettamanti et al., 2005) and suppressing word interference (Ali et al., 2010) in healthy individuals. This suggests that of the recruitment of subcortical regions by our children with ASD post-intervention may reflect a neural process common in TD children when they increase visual attention and scanning to aid in more effortful reading.

Overall, the findings of this project provide a framework for developing new and effective interventions, as well as better tailoring of existing interventions to children with ASD. This study extrapolates the basic research findings of aberrant brain activity and connectivity in ASD to a translational level by examining the impact a targeted intervention can have on improving brain activation and functional connectivity. Participating in the V/V intervention not only increased cortical connectivity between frontal and posterior brain regions, but also increased functional activation in language regions in children with ASD. Of especial clinical significance is to what extent improvement in brain responses due to the intervention translates to improvement in behavior in ASD. We found that increased brain activation and strengthening of functional connectivity of both frontal and posterior language regions was positively correlated with behavioral improvement in reading comprehension. Thus, we found that a strength-based, targeted intervention can tap into a relatively intact domain of cognitive functioning, visuospatial abilities, in children with ASD, and use this cognitive ability to improve an area of deficit. The new knowledge obtained through this study can provide valuable insights into the development of intervention programs that are based on our understanding of the neurobiological processes that cause language and communication deficits in ASD. Given the heterogeneity of ASD and the myriad of deficits that can present from one child to the next, future studies should continue to assess the neural circuitry in children with ASD in order to increase specificity of behavioral based interventions to better target the deficits seen in children with ASD.

GENERAL LIST OF REFERENCES

Ali, N., Green, D.W., Kherif, F., Devlin, J.T., & Price, C.J. (2010). The role of the left head of caudate in suppressing irrelevant words. *J Cogn Neurosci, 22,* 2369-86.

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

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., Clubley, E., (2001). The autism spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *J. Autism Dev. Disord. 31,* 5–17.

Bell, N. (1991a). Gestalt imagery: A critical factor in language comprehension. *Annals of Dyslexia, 41,* 246–260.

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

Broyd, S.J., Demanuele, C., Debener, S., Helps, S.K., James, C.J., & Sonuga Barke, E.J. (2009). Default-mode brain dysfunction in mental disorders: a systematic review. *Neurosci Biobehav Rev, 33,* 279–96.

Buckner, R.L., Andrews-Hanna, J.R., & Schacter, D.L. (2008). The brain's default network: Anatomy, function, and relevance to disease. *Ann NY Acad Sci, 1124,* 1–38.

Courchesne, E., Pierce, K., Schumann, C.M., Redcay, E., Buckwalter, J.A., Kennedy, D.P., & Morgan, J. (2007). Mapping early brain development in autism. *Neuron, 56,* 399–413.

Di Martino, A., Kelly, C., Grzadzinski, R., Zuo, X.N., Mennes, M., Mairena, M.A., Lord, C., . . . Milham, M.P. (2011). Aberrant striatal functional connectivity in children with autism. *Biol. Psychiatry, 69,* 847–856.

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,* 949-60.

Fair, D.A., Cohen, A.L., Dosenbach, N.U., Church, J.A., Miezin, F.M., Barch, D.M., Raichle, M.E., . . . Schlaggar, B.L. (2008). The maturing architecture of the brain's default network. *Proc Natl Acad Sci U S A, 105,* 4028–32.

Fair, D.A., Cohen, A.L., Power, J.D., Dosenbach, N.U., Church, J.A., Miezin, F.M., Schlaggar, B.L., & Petersen, S.E. (2009). Functional brain networks develop from a "local to distributed" organization. *PLoS Comput Biol, 5,* e1000381.

Fair, D.A., Dosenbach, N.U., Church, J.A., Cohen, A.L., Brahmbhatt, S., Miezin, F.M., Barch, DM,. . . Schlaggar BL. (2007). Development of distinct control networks through segregation and integration. *Proc Natl Acad Sci U S A, 104,* 13507–12.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., & Raichl,e M.E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci USA, 102,* 9673–78.

Goldstein, G., Minshew, N.J., & Siegel, D.J. (1994). Age differences in academic achievement in high-functioning autistic individuals. *Journal of Clinical Experimental Neuropsycholoy,16,* 671–680.

Greicius, M.D., Krasnow, B., Reiss, A.L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci USA, 100*, 253-58.

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. *Cereb Cortex, 20,* 1937-45.

Jones, C.R.G., Happe´, F., Golden, H., Marsden, A.J.S., Tregay, J., Simonoff, E., Pickles, G. & Charman, T. (2009). Reading and arithmetic in adolescents with autism spectrum disorders: Peaks and dips in attainment. *Neuropsychology, 23,* 718–728.

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: a journal of neurology, 127,* 1811–1821.

Just, M.A., Cherkassky, V.L., Keller, T.A., Kana, R.K., & Minshew, N.J. (2007). Functional and Anatomical Cortical Underconnectivity in Autism: Evidence from an fMRI Study of an Executive Function Task and Corpus Callosum Morphometry. *Cerebral Cortex, 17,* 951-961.

Kana, R.K., Keller, T.A., Cherkassky, V.A., Minshew, N.J., & Just, MA. (2006). Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. *Brain: a journal of neurology, 129,* 2484–2493.

Kana, R.K., Keller, T.A., Cherkassky, V.L., Minshew, N.J., & Just, M.A. (2009). Atypical frontal-posterior synchronization of Theory of Mind regions in autism during mental state attribution. *Social Neuroscience, 4,* 135–152.

Kana, R.K, & Wadsworth, H.M. (2012). "The archeologist's career ended in ruins": hemispheric differences in pun comprehension in autism. *Neuroimage*, *62,* 77-86.

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child, 2,* 217-250.

Keller, T.A., & Just, M.A. (2009). Altering cortical connectivity: remediation-induced changes in the white matter of poor readers. *Neuron, 64,* 624–631.

Koyama, M.S., Di Martino, A., Zuo, X.N., Kelly, C., Mennes, M., Jutagir, D.R., Castellanos, F.X., & Milham, M.P. (2011). Resting-state functional connectivity indexes reading competence in children and adults. *J Neurosci. 31*, 8617-24.

Libero, L., & Kana, R.K. (2013). Advancing our understanding of the brain in autism: contribution of functional MRI and diffusion tensor imaging. *Imaging in Medicine,* 5, 453-465.

Li, H., Xue, Z., Ellmore, T.M., Frye, R.E., & Wong, S.T. (2014). Network-based analysis reveals stronger local diffusion-based connectivity and different correlations with oral language skills in brains of children with high functioning autism spectrum disorders. *Hum Brain Mapp, 35,* 396-413.

Lindgren, K.A., Folstein, S.E., Tomblin, J.B., & Tager-Flusberg, H. (2009). Language and reading abilities of children with autism spectrum disorders and specific language impairment and their first-degree relatives. *Autism Research, 2*, 22–38.

Lohmann, G., Hoehl, S., Brauer, J., Danielmeier, C., Bornkessel-Schlesewsky, I., Bahlmann, J., Turner, R., & Friederici, A. (2010). Setting the frame: the human brain activates a basic low-frequency network for language processing. *Cereb Cortex, 20,* 1286-92.

Mason, R.A., Williams, D.L., Kana, R.K., Minshew, N.J., & Just, MA. (2008). Theory of Mind disruption and recruitment of the right hemisphere during narrative comprehension in autism. *Neuropsychologia, 46,* 269-280.

Maximo, J.O., Cadena, E.J., & Kana, R.K. (2014). The implications of brain connectivity in the neuropsychology of autism. *Neuropsychol Rev, 24,* 16-31.

Minshew, N.J. & Keller, T.A. (2010). The nature of brain dysfunction in autism: functional brain imaging studies. *Curr Opin Neurol, 23*, 124–130.

Mottron, L., Dawson, M., Soulières, I., Hubert, B., & Burack, J. (2006). Enhanced perceptual functioning in autism: an update, and eight principles of autistic perception. *J Autism Dev Disord, 36,* 27-43.

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*, 911–919.

Nuske, H.J., & Bavin, E.L. (2011). Narrative comprehension in 4-7-year-old children with autism: testing the Weak Central Coherence account. *Int J Lang Commun Disord, 46*, 108-19.

Newman, T.M., Macomber, D., Naples, A.J., Babitz, T., Volkmar, F., & Grigorenko, E.L. (2007). Hyperlexia in children with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 37*, 760–774.

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.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., & Shulman, G.L. (2001). A default mode of brain function. *PNAS, 98,* 676-82.

Raichle, M.E., & Snyder, A.Z. (2007). A default mode of brain function: a brief history of an evolving idea. *NeuroImage, 37,* 1083-90.

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. *Biol Psychiatry, 64,* 589-98.

Samson, F., Mottron, L., Soulières, I., & Zeffirol T;A. (2012). Enhanced visual functioning in autism: an ALE meta-analysis. *Hum Brain Mapp, 33,* 1553-81.

Sahyoun, C.P., Belliveau, J.W., Soulières, I., Schwartz, S., & Mody, M. (2010). Neuroimaging of the functional and structural networks underlying visuospatial vs. linguistic reasoning in highfunctioning autism. *Neuropsychologia, 48,* 86–95.

Seghier, M.L., & Price, C.J. (2010). Reading aloud boosts connectivity through the putamen. *Cereb Cortex, 20,* 570-82.

Shulman, G.L., Fiez, J.A., Corbetta, M., Buckner, R.L., Miezin, F.M,, Raichle, M.E., & Petersen, S.E. (1997). Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. *J Cogn Neurosci, 9,* 648–663.

Stevens, M.C., Pearlson, G.D., & Calhoun, V.D. (2009). Changes in the interaction of resting-state neural networks from adolescence to adulthood. *Hum Brain Mapp, 30,* 2356–66.

Supekar, K., Musen, M., & Menon, V. (2009). Development of large-scale functional brain networks in children. *PLoS Biol, 7,* e1000157.

Tesink, C.M., Buitelaar, J.K., Petersson, K.M., van der Gaag, R.J,, Teunisse, J.P., & Hagoort, P. (2011). Neural correlates of language comprehension in autism spectrum disorders: when language conflicts with world knowledge. *Neuropsychologia, 49,* 1095-104.

Tettamanti, M., Moro, A., Messa, C., Moresco, RM., Rizzo, G., Carpinelli, A., Matarrese, M., . . . Perani, D. (2005). Basal ganglia and language: phonology modulates dopaminergic release. *Neuroreport, 16*, 397-401.

Tomasi, D., & Volkow, N.D. (2012). Resting functional connectivity of language networks: characterization and reproducibility. *Molecular Psychiatry*, 17, 1–14.

Torgesen, J.K., Alexander, A.W., Wagner, R.K., Rashotte, C.A., Voeller, K.K., & Conway, T. (2001). Intensive remedial instruction for children with severe reading disabilities: Immediate and long-term outcomes from two instructional approaches. *Journal of Learning Disorders, 34*, 33-58.

Toro, R., Fox, P.T., & Paus, T. (2008). Functional coactivation map of the human brain. *Cereb Cortex, 18*, 2553–2559.

Turken, A.U., & Dronkers, N.F. (2011). The neural architecture of the language comprehension network: converging evidence from lesion and connectivity analyses. *Front Syst Neurosci, 5*, 1-20.

Verly, M., Verhoeven, J., Zink, I., Mantini, D., Peeters, R., Deprez, S., … Sunaert S. (2014). Altered functional connectivity of the language network in ASD: Role of classical language areas and cerebellum. *Neuroimage Clin., 4,* 374-82.

Wang, A.T., Lee, S.S., Sigman, M., Dapretto, M., (2006). Neural basis of irony comprehension in children with autism: the role of prosody and context. *Brain 129*, 932–943.

Weng, S.J., Wiggins, J.L., Peltier, S.J., Carrasco, M., Risi, S., Lord, C., & Monk, C.S. (2010). Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. *Brain Res, 1313*, 202-14.

APPENDIX A

SUPPLEMENTAL MATERIAL

Table S1. Brain regions encompassing the reading network component for all subjects combined (ASD-EXP + ASD-WLC + TD groups) as identified using GICA ($p < 0.05$, FWE corrected).

Region	Hem	Volume (# of voxels)	Peak coordinates (MNI) x	y	z	Peak *t*
Inferior frontal gyrus – triangularis	L	101890[a]	-48	28	16	24.26
Inferior frontal gyrus – triangularis	R	5595[b]	60	20	10	22.01
Inferior frontal gyrus – orbital	L	101890[a]	-50	28	-8	18.29
Inferior frontal gyrus - opercularis	R	5595[b]	52	10	22	15.25
Medial prefrontal cortex	L	246[c]	-4	66	18	7.48
Medial prefrontal cortex	R	246[c]	6	58	16	6.18
Middle temporal gyrus	L	842[d]	-66	-48	-8	11.89
Middle/Inferior temporal gyrus	R	756[e]	66	-54	-8	10.67
Intraparietal sulcus	L	1166	-30	-68	45	8.86
Fusiform gyrus	L	842[d]	-58	-50	4	7.87
Fusiform gyrus	R	756[e]	58	-36	-16	5.45
Cuneus	R	216	4	-90	6	7.39

[a,b,c,d,e,f,g,h,i] Regions of activation encompassed within the same cluster

Table S2. Within-group results for the TD group and the ASD group at the first imaging session (ASD-EXP + ASD-WLC) of brain regions with significant functional connectivity with both Broca's and Wernicke's area seeds ($p < 0.05$, FWE corrected).

Seed	Group	Region	Hem	Volume (# of voxels)	Peak coordinates (RAI) x	y	z	Peak *t*
Broca	TD	Inferior frontal gyrus	L	1049	52	-28	13	13
		Inferior frontal gyrus	R	612	-54	-34	10	6.11
		Middle temporal gyrus	L	221	67	42	-6	5.83
		Inferior parietal lobule	L	127	52	42	49	5.14
		Superior temporal gyrus	R	112	-63	33	10	5.37
	ASD	Inferior frontal gyrus	L	4362	52	-25	13	13
		Inferior frontal gyrus	R	1585	-54	-22	28	7.71
		Inferior parietal lobule	L	1116	49	45	43	6.5
		Inferior parietal lobule	R	859	-36	51	49	6.66
		Caudate nucleus	R	329	-15	-4	10	6.1
		Superior temporal gyrus	R	144	-66	15	4	5.62
		Middle cingulate	L	107	1	3	31	6
Wernicke	TD	Supramarginal gyrus	L	897	55	48	31	7.93
		Supramarginal gyrus	R	433	-60	48	34	6.43
		Precuneus	L	376	10	51	49	6.03
		Inferior temporal gyrus	L	268	61	24	-18	6
		Inferior frontal gyrus	L	225	52	-16	1	6.03
		Middle cingulate	L	197	7	18	43	6
		Superior medial gyrus	L	143	7	-37	52	5.6
	ASD	Superior temporal gyrus	L	6767	61	48	22	13
		Middle cingulate	L	2708	1	6	43	6.84
		Precentral gyrus	L	759	40	-7	49	6.28

Table S3. Between-group results of brain areas with greater connectivity with Broca's and Wernicke's area seeds at the first imaging session (S1) versus the second imaging session (S2) in the ASD-WLC group (n = 15). There were no areas with greater connectivity at the S2 > S1 (p < 0.05, FWE corrected).

Seed	Direction	Region	Hem	Volume (# of voxels)	Peak coordinates (RAI) x	y	z	Peak *t*
Broca	S1 > S2	Superior occipital gyrus	L	969	16	99	31	-5.0
		Precentral gyrus	R	688	-57	-7	22	-7.1
		Pallidum/Putamen	L	293	22	-4	-3	-5.2
		Precentral gyrus	L	293	43	6	49	-4.5
		Middle occipital gyrus	L	241	40	81	10	-5.2
		SMA	R	187	-6	-13	61	-4.4
		Middle temporal gyrus	R	182	-42	63	1	-3.9
		Pallidum/Putamen	R	125	-15	-4	-6	-5.8
		Superior temporal gyrus	R	119	-51	36	16	-5.8
Wernicke	S1 > S2	Fusiform gyrus	L	131	34	54	-6	-3.5
		Inferior parietal lobule	R	117	-27	57	58	-3.5

APPENDIX B

INSTITUTIONAL REVIEW BOARD APPROVAL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
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:	MURDAUGH, DONNA
Co-Investigator(s):	
Protocol Number:	**X130108003**
Protocol Title:	*Intrinsic and Extrinsic Brain Activity in Children with Autism Before and After a Visualization Language Intervention*

The IRB reviewed and approved the above named project on 3-25-14. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 3-25-14

Date IRB Approval Issued: 3-25-14

IRB Approval No Longer Valid On: 3-25-15

HIPAA Waiver Approved?: Yes

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

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

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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