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Report

Disrupted Neural Synchronization in Toddlers with Autism

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SUMMARY

Autism is often described as a disorder of neural synchronization. However, it is unknown how early in development synchronization abnormalities emerge and whether they are related to the development of early autistic behavioral symptoms. Here, we show that disrupted synchronization is evident in the spontaneous cortical activity of naturally sleeping toddlers with autism, but not in toddlers with language delay or typical development. Toddlers with autism exhibited significantly weaker interhemispheric synchronization (i.e., weak "functional connectivity" across the two hemispheres) in putative language areas. The strength of synchronization was positively correlated with verbal ability and negatively correlated with autism severity, and it enabled identification of the majority of autistic toddlers (72%) with high accuracy (84%). Disrupted cortical synchronization, therefore, appears to be a notable characteristic of autism neurophysiology that is evident at very early stages of autism development.

INTRODUCTION

Autism has been hypothesized to arise from the development of abnormal neural networks that exhibit irregular synaptic connectivity and abnormal neural synchronization (Belmonte et al., 2004; Courchesne et al., 2007; Geschwind and Levitt, 2007; Levy et al., 2009). Disrupted synchronization between neural networks located in particular brain areas may give rise to the specific cognitive, social, and sensory behavioral symptoms exhibited by individuals with autism. Supporting evidence for this hypothesis comes from genetic (Geschwind and Levitt, 2007), anatomical (Courchesne et al., 2007), and neuroimaging (Minshew and Keller, 2010) studies. Several key questions, however, remain unanswered. (1) How early in development does abnormal synchronization appear? (2) Is abnormal synchronization related to the behavioral symptoms exhibited during early autism development? (3) Is abnormal synchronization specific to a particular cortical system or widespread across multiple brain areas? (4) How consistent is the abnormality across different individuals with autism? Obtaining answers to these questions will not only advance our understanding of autism development but will also enhance our understanding regarding the importance of synchronization for typical brain development. Here, we used functional magnetic resonance imaging (fMR) to examine these questions.

In the typical brain, neural activity is synchronized/correlated in time across functionally related cortical areas (e.g., visual cortex) not only during the completion of a task (e.g., watching a movie) but also in the complete absence of a task, during rest and sleep (Raichle, 2010). It has been suggested that the strength of spontaneous activity synchronization between two brain areas may offer a measure for the strength of their functional relationship. Indeed, the strongest synchronization is reliably found between areas belonging to a particular functional system (e.g., visual, auditory, motor, or "default mode") rather than between areas belonging to different functional systems (Damoiseaux et al., 2006; Nir et al., 2008). Since the cortex is functionally organized in a symmetrical manner across the two hemispheres, the strongest synchronization is found between corresponding contralateral locations (e.g., right and left auditory cortex). This form of "interhemispheric" synchronization is evident even in newborn infants (Fransson et al., 2007; Gao et al., 2009). Recent studies in adults have suggested that reduced synchronization between particular cortical areas characterizes particular brain disorders such as Alzheimer's disease (Greicius et al., 2004), schizophrenia (Bluhm et al., 2009), loss of consciousness (Vanhaudenhuyse et al., 2010), and autism (Anderson et al., 2011; Cherkassky et al., 2006; Kennedy and Courchesne, 2008). These studies have suggested that the neural pathologies associated with each disorder may reveal themselves in particular synchronization abnormalities between specific brain areas, thereby offering possible insight into the characteristics of the underlying pathology and/or a possible biological marker that may aid in the diagnosis of the disorder.

It is challenging to measure brain activity in awake toddlers because of their inability to remain still. Several studies, however, have successfully measured brain activity in typically developing toddlers under anesthesia (Kiviniemi et al., 2000), under mild

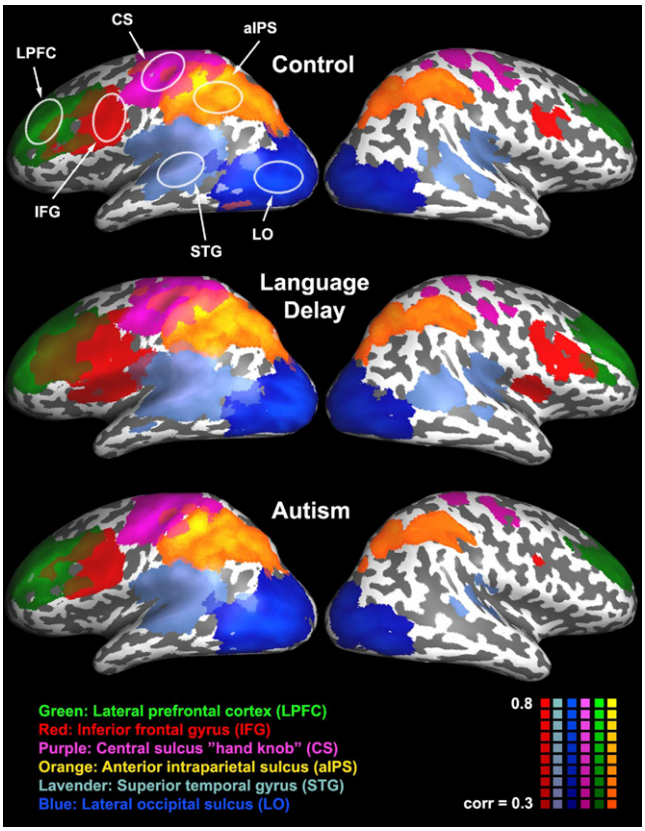

Figure 1. Interhemispheric Synchronization in Each Toddler Group
Correlation maps averaged across toddlers from the typically developing (top), language-delay (middle), and autism (bottom) groups. fMRI activity during natural sleep was sampled in six left-hemisphere "seed" locations outlined by white ellipses: lateral prefrontal cortex (LPLC), inferior frontal gyrus (IFG), "hand knob" area of central sulcus (CS), anterior intraparietal sulcus (aIPS), superior temporal gyrus (STG), and lateral occipital sulcus (LO). Each color represents voxels that exhibited strong correlation (above 0.3) with a particular seed. Note the spatial selectivity of the correlations in all groups. Only voxels located close to the seed's location in the left hemisphere and the corresponding contralateral location in the right hemisphere exhibited strong correlation values.

sedation (Fransson et al., 2007), or during natural sleep (Gao et al., 2009; Liu et al., 2008). Here, we report fMRI data acquired from 72 naturally sleeping toddlers (1–3.5 years old) who were either typically developing, language delayed, or autistic. Compared to both other groups, toddlers with autism exhibited significantly weaker interhemispheric correlations in inferior frontal gyrus (IFG) and superior temporal gyrus (STG), two areas commonly associated with language production and comprehension. Interhemispheric synchronization strength was positively correlated with verbal ability and negatively correlated with autism severity, and it enabled accurate identification of autistic toddlers with high sensitivity (72%) and specificity (84%). These results suggest that poor neural synchronization is a notable neurophysiological characteristic that is evident at the earliest stages of autism development and is related to the severity of behavioral symptoms. Finally, the ability to measure this characteristic during sleep, when task compliance and subject cooperation are not required, suggests its utility as a possible diagnostic measure to aid growing efforts of identifying autism during infancy (Pierce et al., 2009; Zwaigenbaum et al., 2009).

RESULTS

The data presented in this study were gathered from several studies performed at the Autism Center of Excellence (ACE) in San Diego, CA. In all scans, toddlers were presented with blocks of soft auditory stimuli that were interleaved with silence. To ensure that the differences in synchronization between the groups were not due to differences in possible auditory-evoked responses, we first "regressed out" the experiment structure from the data of each subject (see Experimental Procedures). This ensured that there was zero correlation between each voxel's time course and the experiment structure, effectively removing stimulus-evoked responses while leaving spontaneous fMRI fluctuations in the data (see analyses below).

Spatial Selectivity of Interhemispheric Synchronization

Spontaneous fMRI activity during natural sleep exhibited robust and spatially selective correlations between homologous locations across the two hemispheres. To demonstrate this, we sampled activity in six left hemisphere "seed" regions of interest (ROIs) and computed the correlation between each "seed" time course and the time course of every voxel in the cortex. These voxel-by-voxel correlation values were averaged across individuals of each group to generate six maps per group: one for each seed (Figure 1). The six seed ROIs selected for this analysis were defined in the left hemisphere according to anatomical criteria (see Experimental Procedures; see also Figure S1 and Table S1 available online) and included the lateral prefrontal cortex (LPFC), posterior part of inferior frontal gyrus (IFG), "hand knob" area of central sulcus (CS), anterior intraparietal sulcus (aIPS), posterior part of superior temporal gyrus (STG), and lateral occipital sulcus (LO). Selecting right hemisphere ROIs would have yielded a complementary analysis with equivalent findings.

Strong correlations with the seed time course were found in voxels adjacent to the location of the seed (white ellipses, Figure 1) and in voxels located in the homologous area of the contralateral right hemisphere. Note two important points. First, the voxels that exhibited correlation with each seed showed high spatial selectivity with very little overlap across seeds: this means that the spontaneous activity found for each seed and its corresponding contralateral location was relatively unique and different from that found for each of the other seeds and their contralateral locations. Second, the strength and spread of correlation in the contralateral locations are qualitatively similar across groups in all areas except for STG and IFG, which appear abnormally reduced in the autism group.

Poor Interhemispheric Synchronization in Autism

Voxel-by-voxel comparisons showed that toddlers with autism exhibited significantly weaker interhemispheric correlations than both typically developing and language-delayed toddlers in the STG, a cortical area commonly associated with language processing (Figure 2). The comparisons of the autism group to

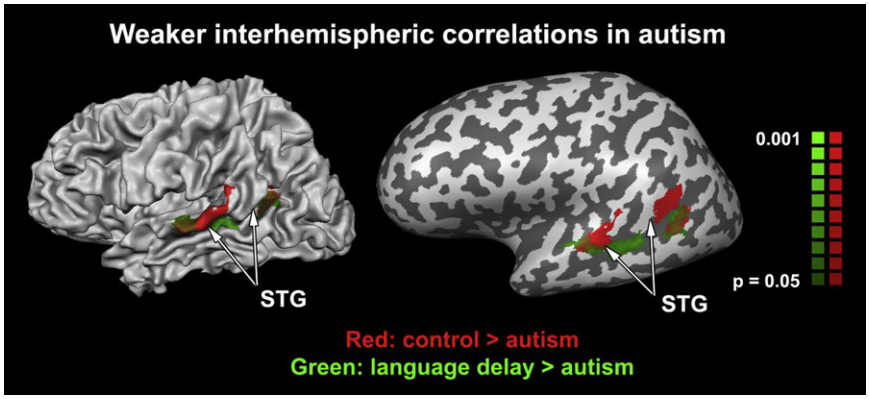

Figure 2. Interhemispheric Synchronization Difference between Groups

Voxels exhibiting weaker interhemispheric correlations in the autism group as compared with the typically developing (red) and language-delay (green) groups. The two independent-comparison maps are overlaid on a folded (left) and inflated (right) left hemisphere of a single individual. Significantly weaker interhemispheric correlation was apparent in STG voxels in both comparisons. No voxels exhibited stronger interhemispheric correlation in children with autism. STG denotes superior temporal gyrus.

each of the other groups were independent of one another, yet both revealed significant synchronization differences only in voxels located within the STG. This analysis was performed by first computing the correlation between the time course of each left-hemisphere voxel and the time course of its corresponding contralateral right-hemisphere voxel in each subject. This gave us an interhemispheric correlation value for each pair of corresponding left/right voxels, which signified their synchronization strength. We then performed a t test for each voxel, contrasting the correlation values across individuals of different groups. This analysis yields symmetrical results across the two hemispheres, hence the presentation of the voxel-wise group differences only on the left hemisphere. Presenting the results on the right hemisphere yields a reciprocal "mirror image."

The results found in STG raised the possibility that poor interhemispheric synchronization may be a characteristic of the language system in toddlers with autism. To evaluate this further, we performed an ROI analysis in six anatomically defined ROIs that included two putative language areas, STG and IFG, and four control areas, LO, aIPS, CS, and LPFC. The ROI analysis was more sensitive than the voxel-wise analysis reported above, since averaging across ROI voxels reduces any spatial noise inherent in the data. The results showed that interhemispheric synchronization was indeed significantly weaker in the autism group not only in STG, but also in IFG ($p < 0.05$, randomization test and t test, see Experimental Procedures). None of the control ROIs exhibited significant differences between groups (Figure 3, top). Toddlers with language delay exhibited a trend for stronger synchronization in LPFC, as compared with autism and control groups ($p < 0.1$, randomization test). Similar results were found when comparing only the youngest toddlers (Figure 3, right panels). Synchronization difference remained significant in STG ($p < 0.05$) and was almost significant in IFG ($p < 0.07$).

The ROIs used in this analysis were selected manually in left and right hemispheres, and the left hemisphere ROIs were identical to those used in the seed analysis described above (Figure 1). The anatomical criteria used for selection were identical in all groups, and there was, therefore, no bias for any of the ROIs to exhibit stronger interhemispheric correlations in one group or another. This lack of bias was evident in the equivalent ROI sizes (Figure S1) and locations (Table S1) across groups.

Synchronization Strength and Clinical Diagnosis

Weak interhemispheric correlations in IFG and STG could be used to accurately identify the majority of toddlers with autism (Figure 3, bottom). We performed sensitivity-specificity and receiver operating characteristics (ROC) curve analyses to determine the usefulness of IFG and STG correlations for autism classification (Figure S2). In these analyses, toddlers who exhibited a below-threshold correlation value in either IFG or STG were classified as autistic, while those exhibiting above-threshold correlation values in both IFG and STG were classified as nonautistic (control or language delay). The accuracy of the correlation-based classification was determined by comparing it with the actual clinical diagnosis performed by experienced psychologists. Selecting a correlation threshold/criterion of 0.38 enabled accurate classification of toddlers with autism, yielding a sensitivity of 72% and specificity of 84%. In other words, 21 out of 29 toddlers in the autism group were correctly identified, while only 7 (5 control and 2 language delay) out of 43 nonautistic toddlers were mistakenly identified as autistic. When considering only the young toddlers, the same threshold yielded a sensitivity of 60% and specificity of 80%. Interestingly, different subsets of toddlers with autism exhibited poor interhemispheric correlation in IFG and in STG.

To ensure that weak interhemispheric correlation was not a consequence of our particular choice of ROI voxels, we examined single subject data in the toddlers with autism who exhibited the weakest interhemispheric correlations in IFG. We present the results for IFG, but equivalent results were found for STG in the autistic toddlers who exhibited the weakest STG correlations. Using a similar analysis to that described in Figure 1, we sampled the activity in left IFG and searched for correlated voxels throughout the brain (Figure S3). The toddlers did not show any correlated voxels, above a threshold of 0.3, in the vicinity of the contralateral right IFG. Weak interhemispheric correlations in these individuals were, therefore, not a consequence of particular IFG ROI location or size.

Synchronization Strength and Autism Severity

There was a significant relationship between synchronization strength and expressive language scores, as assessed using the Mullen test ($r = 0.53$, $p < 0.005$). This association held only in the autism group and was evident only in IFG (Figure 4), not in STG

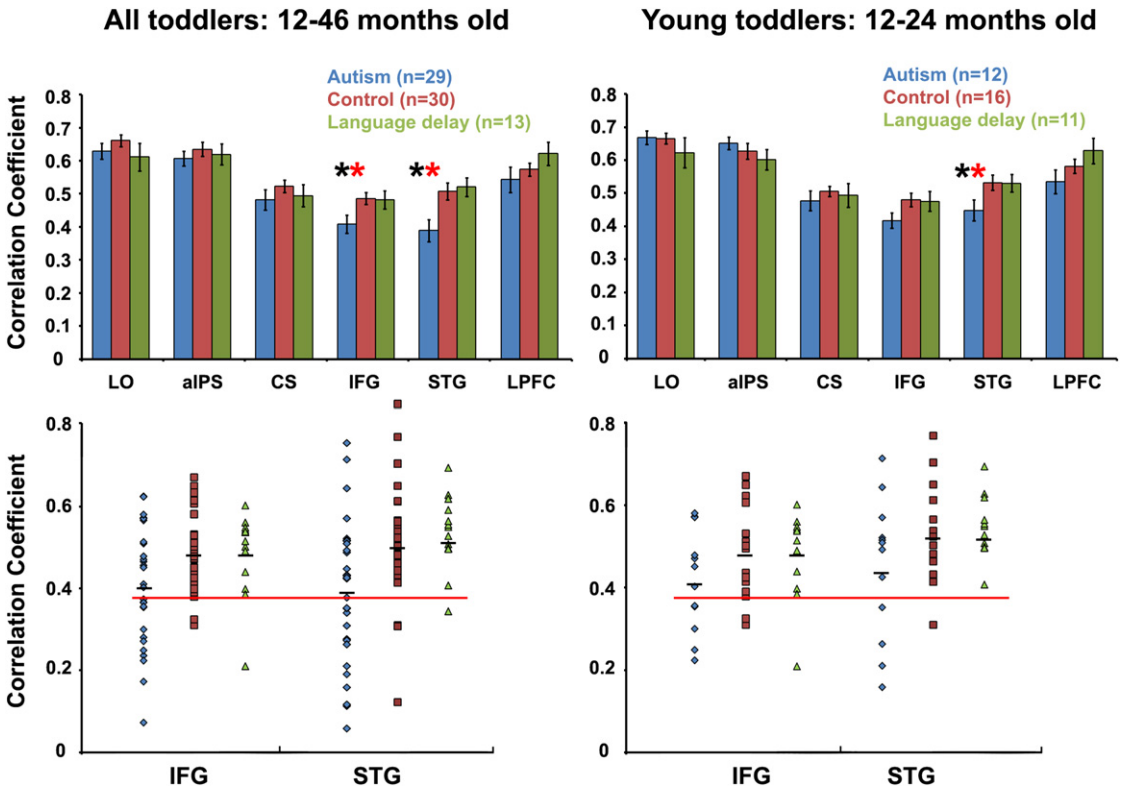

Figure 3. Interhemispheric Synchronization in Specific ROIs

Interhemispheric correlation strength between right and left ROIs in the autism (blue), typically developing (red), and language-delay (green) groups when considering all subjects (left panels) or only the younger toddlers (right panels).

Top panels: average correlation strength in each toddler group for each of the six examined ROIs. The autism group exhibited significantly weaker interhemispheric correlation ($p < 0.05$) only in putative language areas (IFG and STG). When comparing younger toddler groups, IFG correlation difference was almost significant ($p < 0.07$). Error bars denote standard error across subjects. Black asterisk denotes significant difference between autism and control groups; red asterisk denotes significant difference between autism and language-delay groups.

Bottom panels: single subject correlation values in IFG and STG. The majority of toddlers with autism, but only a small minority of control (red) and language-delay (green) toddlers, exhibited IFG or STG correlation values below 0.38 (red line). Black lines denote mean correlation across the group.

or any of the other ROIs. There was also a significant inverse relationship between synchronization strength and autism severity. IFG synchronization was significantly anticorrelated with the ADOS communication scores ($r = -0.4$, $p < 0.05$), and a negative trend was found with the ADOS social scores ($r = -0.26$, $p = 0.1$). The statistical significance of these correlations was assessed using a randomization test (see Experimental Procedures).

Control Analyses

We performed several control analyses to rule out alternative interpretations of the results. First, the strength of interhemispheric synchrony in IFG did not depend on age in any group (Figure S4A). Second, the spectral power of spontaneous fMRI activity was equivalent at all frequencies across all three groups (Figure S4B). Weaker interhemispheric synchrony in IFG of toddlers with autism was, therefore, not a consequence of smaller/weaker spontaneous fluctuations, but was rather a reflection of their disrupted temporal synchronization across the hemispheres. Third, the amount of time between sleep onset and fMRI acquisition was equivalent across groups ($p > 0.2$ for all three between-group comparisons, two-tailed t tests). This suggests that the toddlers of all three groups, on average, were in a similar state of sleep. Also note that the synchronization difference was specific to language areas rather than a general property of the whole cortex, which would be expected from a difference in arousal or vigilance. Furthermore, as mentioned above, the amplitude of spontaneous fMRI fluctuations was equivalent across the groups in all ROIs (Figure S4), indicating that there were no general differences in the amount of cortical activity exhibited by the three groups, as may be expected in different sleep states.

Finally, we assessed whether there were any residual evoked responses evident in any of the analyzed ROIs despite having projected out the stimulus structure from each voxel. We estimated the fMRI responses in each ROI and each subject group for each of the four auditory stimulus types. Residual evoked responses, if present at all, were minimal and did not differ across the six ROIs or across the groups (Figure S5A). Furthermore, the amplitude of any possible residual evoked responses was an order of magnitude smaller than the amplitude of spontaneous activity (Figure S5B). This reassured us that the reported difference in synchronization between the groups was not driven

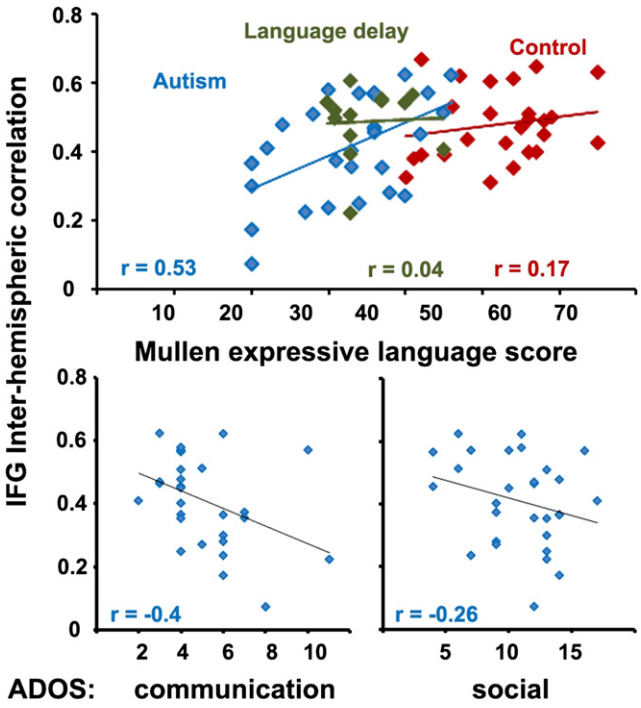

Figure 4. Interhemispheric Synchronization and Behavioral Measures

Interhemispheric correlation in IFG and verbal ability (top) or autism severity (bottom). Toddlers with autism (blue) showed a significant positive correlation between interhemispheric correlation value and their expressive language ability, as measured by the Mullen test (top), while typically developing (red) and language-delayed toddlers (green) did not. Toddlers with autism exhibited a significant negative correlation between interhemispheric correlation and the ADOS communications score (left) while exhibiting a negative trend with the ADOS social scores (right).

by responses to the auditory stimuli but rather was driven by fluctuations in spontaneous activity.

DISCUSSION

Our results suggest that reduced neural synchronization is a notable characteristic of autism, evident at very early stages of autism development. Compared with language-delayed and control toddlers, toddlers with autism exhibited significantly weaker interhemispheric synchronization in IFG and/or STG, two areas commonly associated with language processing (Figures 2 and 3). Furthermore, in the autism group, IFG synchronization strength was correlated with behavioral scores, scaling positively with language abilities and negatively with autism severity (Figure 4). Whether poor interhemispheric synchronization in putative language areas plays a causal role in generating autistic behavioral symptoms cannot be determined by this study. Nevertheless, the fact that poor synchronization was found in the language system of toddlers with autism, and not in toddlers with language delay (both groups exhibited similarly low expressive language scores; Figure S6), suggests that reduced synchronization may reflect the existence of a specific pathophysiological mechanism that is unique to autism.

Poor Synchronization as an Early Diagnostic Tool

It is remarkable that quantifying the synchronization of spontaneous cortical activity during natural sleep holds such valuable information about the developmental state of a toddler. The majority of the toddlers with autism in our sample (72%) could be identified with high accuracy (84%) by the strength of interhemispheric correlation in putative language areas (Figure 3 and Figure S2). These results were obtained when selecting a correlation threshold of 0.38. Raising the threshold would increase the number of identified toddlers with autism (higher sensitivity) at the expense of reduced accuracy (lower specificity). Regardless of the precise threshold chosen, these results suggest that quantifying spontaneous cortical activity during sleep may aid in the early diagnosis of autism and enable earlier intervention (Pierce et al., 2009; Zwaigenbaum et al., 2009). There are many clear advantages to this technique. Scanning during natural sleep does not require subject compliance, eliminating the possibility that group differences in brain activity arise from task differences or behavioral strategies. In fact, in toddlers it is practically the only way of avoiding incessant movement artifacts and random uncontrolled behaviors. Even more importantly, scanning during sleep permits the inclusion of individuals with severe autistic traits who are usually excluded from autism imaging studies. Note that this study is one of a handful of fMRI studies that include individuals with severe autism, a critical requirement for an early diagnostic tool and for thorough evaluation of hypotheses regarding autism neurophysiology.

Poor Synchronization as a Marker of Common Pathology

The disruption of synchronization during sleep may be generated by numerous pathophysiological mechanisms, including abnormal anatomical connectivity, synaptic function, excitation-inhibition balance, local neural network structure/function, and so forth (Belmonte et al., 2004). The assumption is that these underlying pathophysiological mechanisms also disrupt cortical function during wakefulness, alter perception and behavior, and may generate autistic behavioral symptoms. While our study cannot pinpoint the underlying pathophysiological mechanism(s), the results do suggest that such mechanisms may exist in putative language areas at very early stages of autism development.

Our results are compatible with several recent reports of reduced resting-state synchronization in adolescents and adults with autism (Anderson et al., 2011; Cherkassky et al., 2006; Kennedy and Courchesne, 2008; Monk et al., 2009; Weng et al., 2010). Most importantly, one recent study has reported that adults and adolescents with autism exhibit significantly decreased interhemispheric synchronization in multiple cortical areas, including a similar IFG area to the one described here (see Figure 3 in Anderson et al., 2011). One speculative possibility is that reduced interhemispheric synchronization found during early autism development may persist and become even more widespread with age. Further studies exploring other aspects of cortical and subcortical synchronization are warranted for determining the spatial specificity of synchronization abnormalities throughout autism development.

Converging evidence from multiple fields of neurobiology, not just neuroimaging, suggests that autism is a disorder of abnormal neural connectivity and synchronization (Levy et al., 2009).

Genetic studies have reported abnormalities in genes associated with synaptic formation, maturation, and transmission in autism, which are expected to generate abnormally connected neural networks in individuals with autism (Geschwind and Levitt, 2007; Rubenstein and Merzenich, 2003). Electrophysiology studies in mouse models of autism have reported neural network abnormalities, including excitation-inhibition imbalances (Gibson et al., 2008) and abnormal synaptic transmission (Etherton et al., 2009). Anatomical MRI studies have reported increased white matter volumes (Herbert et al., 2004) along with abnormal white matter myelination (Alexander et al., 2007; Ben Bashat et al., 2007). Finally, several fMRI studies in adults and adolescents with autism have reported abnormal synchronization across brain areas under active task conditions (Hasson et al., 2009; Jones et al., 2010) or spontaneously fluctuating during rest/sleep (Anderson et al., 2011; Cherkassky et al., 2006; Kennedy and Courchesne, 2008; Monk et al., 2009; Weng et al., 2010). The emerging hypothesis suggests that the formation of abnormal neural networks exhibiting irregular anatomical connections and/or irregular neural synchronization leads to the development of autistic behavioral symptoms.

Our study supports this hypothesis in several novel ways. It presents evidence showing that synchronization is disrupted during early autism development (when toddlers are only beginning to manifest autistic behavioral symptoms) and that the extent of disruption is related to the severity of existing symptoms (Figure 4). With this in mind, it is tempting to speculate that early abnormal development marked by disrupted synchronization in key brain areas, such as those mediating language, may be at the core of autism pathophysiology.

Poor Synchronization and Cortical Lateralization

Weak interhemispheric synchronization in language areas of toddlers with autism may be a signature of early "abnormal lateralization." Responses to language seem to be lateralized in typically developing infants (Dehaene-Lambertz et al., 2002; Redcay et al., 2008) but tend to exhibit reduced amplitudes and/or different lateralization in children with autism (Boddaert et al., 2004; Redcay and Courchesne, 2008). The significance of language lateralization for proper language development and maintenance is unknown (Hickok and Poeppel, 2007). Furthermore, the relationship between functional lateralization during language processing and interhemispheric synchronization during rest or sleep is also poorly understood. Spontaneous activity tends to correlate across areas that share a particular function (Fox and Raichle, 2007), suggesting that lateralized cortical systems such as language should exhibit less correlation across hemispheres than bilateral systems such as vision. Indeed, our results show weaker interhemispheric correlations in language areas as compared with visual areas across all groups (Figure 3). One might speculate that weaker interhemispheric synchronization in language areas of toddlers with autism suggests early "overlateralization" of language function. Note that the directionality of lateralization to the left or right hemisphere cannot be determined using our data.

Uniqueness to Autism

Delayed and impaired language capabilities are a defining hallmark of both autism and language delay diagnoses (DSM-IV-TR, 2000). While both groups exhibited equivalently reduced expressive language abilities in comparison to control toddlers, only those with autism exhibited the social abnormalities indicative of autism, as measured by the ADOS scale (Figure S6), suggesting that weak interhemispheric synchronization marks a pathological mechanism that is unique to autism. In the current study, we did not include a group of toddlers with developmental delay who exhibit low IQ and lack the social symptoms of autism. It would be important to characterize interhemispheric synchronization in this additional group to determine whether the presented results are indeed unique to autism or not. In addition, it would be useful to perform longitudinal studies to determine the predictive value of poor synchronization by assessing the stability of individual autism diagnosis over time.

Final Note

We would like to emphasize the importance of studying autism physiology specifically in infants and toddlers at the developmental period where autistic symptoms and abnormal physiology begin to emerge (Courchesne et al., 2007). Studying early development is critical for understanding autism pathophysiology, as it is manifested closer to "critical period" windows of development (Hensch, 2005). Such understanding may reveal novel intervention methods that could be applied prior to the closure of critical period windows before possibly irreversible cortical changes have occurred.

EXPERIMENTAL PROCEDURES

Subjects

Seventy-two toddlers participated in this study: 29 with autism (mean age: 29 months; range: 12 to 46), 13 with language delay (mean age: 19 months; range: 13 to 27), and 30 typically developing controls (mean age: 28 months; range: 13 to 46). All parents provided written informed consent and were paid for their participation. The UCSD human subject research protection program approved all experimental procedures. Toddlers were scanned late at night, during natural sleep, without the use of sedation.

Diagnosis

Toddlers were diagnosed by a clinical psychologist with over 10 years of experience in autism using the three initial modules of the Autism Diagnostic Observation Schedule (toddler, 1, or 2) and the Mullen scale for early learning (Mullen, 1995) (Figure S6). Autism diagnosis was based on clinical judgment and ADOS scores, with those meeting the criteria having a composite ADOS score larger than 10. In all toddlers, behavioral exams were performed within 3 months of the fMRI scan (typically they were performed within the same week). The diagnosis of toddlers with autism who were younger than 24 months at the time of the scan was confirmed at later ages (Table S2). Toddlers in the autism group did not include individuals with PDD-NOS or other less-severe forms of autism. Toddlers were diagnosed with language delay if their expressive language score was below 40. On average, the expressive language scores were almost identical across autism and language delay groups, indicating a similar level of language difficulty/delay. However, only toddlers with autism exhibited the social and communication difficulties assessed by the ADOS test.

Data Acquisition and Preprocessing

Functional and anatomical data was acquired using a GE 1.5T Signa scanner located at the UCSD Radiology Imaging Laboratory in Sorrento Valley, CA. Scanning was performed with a standard GE birdcage head coil used for RF transmit and receive. BOLD contrast was obtained using a T2-sensitive echo planar imaging sequence (repetition time of 2000–2500 ms with 150–288 time points in length depending on the precise protocol used, 31

slices, 3 × 3 × 3 mm voxels). Anatomical volumes were acquired with a T1-weighted SPGR pulse sequence (0.94 × 0.94 × 1.2 mm). Data were processed with the Brain Voyager software package (R. Goebel, Brain Innovation). Preprocessing included 3D motion correction and temporal high-pass filtering with a cutoff frequency of six cycles per scan. In 18 cases (ten autism, four control, and four language delay), anecdotal head movements were found, and the corresponding time points were discarded. Functional images were aligned with the anatomical volume and transformed to the Talairach coordinate system. Data were spatially smoothed using a Gaussian kernel with 8 mm width at half height.

Auditory Stimuli

Four different types of stimulus protocols were included in this study. All included blocks of auditory stimulation containing words, pseudo words, sentences, tones, or environmental sounds (e.g., train, phone, plane, and dog bark), which were 20–35 s in length and were interleaved with rest blocks of equal length. Any possible evoked responses to the stimulus were regressed out of the data as described below.

Regressing Out Stimulus Structure and Global Mean

To ensure that the analyzed data contained only spontaneous cortical activity and no auditory evoked responses, we regressed out the relevant stimulus structure from each fMRI scan (Jones et al., 2010). This process included building a general linear model (GLM) of the expected hemodynamic responses to the auditory stimuli throughout the scan. We used linear regression to estimate the response amplitude (beta value) in every voxel to each stimulus condition and extracted the residual time course in each voxel. The analyses described throughout the manuscript were performed on these residuals. In a second step, we also regressed out the "global" (average) fMRI time course across all gray matter voxels. We assumed that this average time course reflected spontaneous "global" fluctuations due to arousal, heart rate, and respiration (Birn et al., 2006). This step was performed in an identical way to that described above except that here the "global" time course was used in place of the GLM with the resulting residuals describing the variability in each voxel that was not explained by the "global" time course. This analysis was performed separately for each subject.

ROI Definition

We defined six anatomical ROIs individually for each subject, manually selecting voxels along the following anatomical landmarks separately in each hemisphere: (1) lateral occipital area: voxels surrounding the lateral occipital sulcus; (2) anterior intraparietal sulcus: voxels surrounding the junction of anterior intraparietal sulcus and postcentral sulcus; (3) motor and somatosensory cortex: voxels surrounding the central sulcus around the "hand knob" landmark; (4) superior temporal gyrus: voxels in the posterior part of the superior temporal gyrus (commonly referred to as "Wernicke's area"); (5) inferior frontal gyrus: voxels in the posterior part of the inferior frontal gyrus (commonly referred to as "Broca's area"); (6) lateral prefrontal cortex: voxels in the anterior part of the middle frontal gyrus. An example of ROI selection is described in Figure S1. Table S1 lists the average Talairach coordinates of each ROI in each group, and Figure S1 shows a comparison of ROI sizes across the groups.

Seed Correlation Maps

Spontaneous fMRI activity was averaged across voxels of each left-hemisphere ROI to compute six seed time courses for each subject separately. The correlation between activity in each seed and the activity of every voxel in the cortex was then computed for each subject separately. Voxel-by-voxel correlation values were averaged across subjects of each group and displayed on the inflated brain of a representative subject (Figure 1). The average correlation values were thresholded at 0.3, with voxels exceeding this threshold displayed in distinct colors corresponding to each of the six seeds. A similar analysis was performed with the seven toddlers exhibiting weakest IFG interhemispheric correlations (Figure S3).

Voxel-by-Voxel Interhemispheric Correlation Difference Maps

To compare interhemispheric correlation strength across the groups, we first computed, separately for each subject, the correlation between the time courses of each left-hemisphere voxel and its corresponding contralateral right-hemisphere voxel (determined by their Talairach X coordinate). This yielded a voxel-by-voxel measure of interhemispheric correlation for each subject, which was compared across groups using a random-effects analysis. Correlation values were normalized using the Fisher Transform, and then two-tailed t tests were used to identify voxels with statistically significant between-group differences in correlation (Figure 2). Only voxel clusters exceeding 50 anatomical voxels are displayed in the statistical map, which was overlaid on the inflated anatomy of an exemplar subject.

ROI Correlation Analysis

Spontaneous activity was averaged across voxels to compute a single time course for each ROI in each hemisphere. The correlation between time courses of right and left ROIs was computed for each subject separately and then averaged across subjects of each group. We used both standard t tests and randomization tests to assess the significance of differences in correlation values across the three groups (Figure 3). Randomization tests were carried out by generating a distribution of correlation differences for each pair of groups, according to the null hypothesis that there was no difference between groups, by randomly assigning individuals to either subject group (i.e., randomly shuffling subject identities). This randomization was repeated 10,000 times separately for each ROI to characterize ROI-specific randomized distributions. For the correlation difference between autism and either comparison group to be considered statistically significant, it had to fall above the 95th percentile of the relevant distribution (analogous to a one-tailed t test). Note that this statistical test does not assume that data are normally distributed and is, therefore, more conservative than a standard t test. This was evident in that significance was always weaker when assessed with the former compared with the latter. The reported weaker interhemispheric correlations in autism (Figure 3) were significant using either statistical test.

The correlation between synchronization strength and behavioral measures (i.e., Mullen or ADOS scores, Figure 4) was computed for each ROI across individuals of each group separately. The statistical significance of these correlations was also determined using both randomization and t test analyses. Here, the behavioral measures were shuffled across subjects to determine a distribution of correlation values expected by chance. For the real correlation to be considered significant, it had to exceed the 95th percentile of this random distribution. The reported significant relationships between synchronization strength and behavioral measures were significant when assessed with either statistical test.

Trigger Average Analysis

To determine whether there were any residual auditory-evoked responses in the analyzed ROIs, we performed a "trigger average analysis." Segments of data corresponding to the different blocks of stimulation were extracted, aligned to stimulus onset, and averaged. There were no visible BOLD increases at stimulus onset, as would be expected from a stimulus-evoked response in any of the ROIs or any of the groups (Figure S5).

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and two tables and can be found with this article online at doi:10.1016/j.neuron.2011.04.018.

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REFERENCES

Alexander, A.L., Lee, J.E., Lazar, M., Boudos, R., DuBray, M.B., Oakes, T.R., Miller, J.N., Lu, J., Jeong, E.K., McMahon, W.M., et al. (2007). Diffusion tensor imaging of the corpus callosum in Autism. Neuroimage *34*, 61–73.

Anderson, J.S., Druzgal, T.J., Froehlich, A., Dubray, M.B., Lange, N., Alexander, A.L., Abildskov, T., Nielsen, J.A., Cariello, A.N., Cooperrider, J.R., et al. (2011). Decreased interhemispheric functional connectivity in autism. Cereb. Cortex *21*, 1134–1146.

Belmonte, M.K., Allen, G., Beckel-Mitchener, A., Boulanger, L.M., Carper, R.A., and Webb, S.J. (2004). Autism and abnormal development of brain connectivity. J. Neurosci. *24*, 9228–9231.

Ben Bashat, D., Kronfeld-Duenias, V., Zachor, D.A., Ekstein, P.M., Hendler, T., Tarrasch, R., Even, A., Levy, Y., and Ben Sira, L. (2007). Accelerated maturation of white matter in young children with autism: a high b value DWI study. Neuroimage *37*, 40–47.

Birn, R.M., Diamond, J.B., Smith, M.A., and Bandettini, P.A. (2006). Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. Neuroimage *31*, 1536–1548.

Bluhm, R.L., Miller, J., Lanius, R.A., Osuch, E.A., Boksman, K., Neufeld, R.W., Théberge, J., Schaefer, B., and Williamson, P.C. (2009). Retrosplenial cortex connectivity in schizophrenia. Psychiatry Res. *174*, 17–23.

Boddaert, N., Chabane, N., Belin, P., Bourgeois, M., Royer, V., Barthelemy, C., Mouren-Simeoni, M.C., Philippe, A., Brunelle, F., Samson, Y., and Zilbovicius, M. (2004). Perception of complex sounds in autism: abnormal auditory cortical processing in children. Am. J. Psychiatry *161*, 2117–2120.

Cherkassky, V.L., Kana, R.K., Keller, T.A., and Just, M.A. (2006). Functional connectivity in a baseline resting-state network in autism. Neuroreport *17*, 1687–1690.

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

Damoiseaux, J.S., Rombouts, S.A., Barkhof, F., Scheltens, P., Stam, C.J., Smith, S.M., and Beckmann, C.F. (2006). Consistent resting-state networks across healthy subjects. Proc. Natl. Acad. Sci. USA *103*, 13848–13853.

Dehaene-Lambertz, G., Dehaene, S., and Hertz-Pannier, L. (2002). Functional neuroimaging of speech perception in infants. Science *298*, 2013–2015.

DSM-IV-TR. (2000). Diagnostic and Statistical Manual of Mental Disorders (Washington, DC: American Psychiatric Press).

Etherton, M.R., Blaiss, C.A., Powell, C.M., and Südhof, T.C. (2009). Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proc. Natl. Acad. Sci. USA *106*, 17998–18003.

Fox, M.D., and Raichle, M.E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. *8*, 700–711.

Fransson, P., Skiöld, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., and Aden, U. (2007). Resting-state networks in the infant brain. Proc. Natl. Acad. Sci. USA *104*, 15531–15536.

Gao, W., Zhu, H., Giovanello, K.S., Smith, J.K., Shen, D., Gilmore, J.H., and Lin, W. (2009). Evidence on the emergence of the brain's default network from 2-week-old to 2-year-old healthy pediatric subjects. Proc. Natl. Acad. Sci. USA *106*, 6790–6795.

Geschwind, D.H., and Levitt, P. (2007). Autism spectrum disorders: developmental disconnection syndromes. Curr. Opin. Neurobiol. *17*, 103–111.

Gibson, J.R., Bartley, A.F., Hays, S.A., and Huber, K.M. (2008). Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. J. Neurophysiol. *100*, 2615–2626.

Greicius, M.D., Srivastava, G., Reiss, A.L., and Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc. Natl. Acad. Sci. USA *101*, 4637–4642.

Hasson, U., Avidan, G., Gelbard, H., Vallines, I., Harel, M., Minshew, N., and Behrmann, M. (2009). Shared and idiosyncratic cortical activation patterns in autism revealed under continuous real-life viewing conditions. Autism Res. *2*, 220–231.

Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. Nat. Rev. Neurosci. *6*, 877–888.

Herbert, M.R., Ziegler, D.A., Makris, N., Filipek, P.A., Kemper, T.L., Normandin, J.J., Sanders, H.A., Kennedy, D.N., and Caviness, V.S., Jr. (2004). Localization of white matter volume increase in autism and developmental language disorder. Ann. Neurol. *55*, 530–540.

Hickok, G., and Poeppel, D. (2007). The cortical organization of speech processing. Nat. Rev. Neurosci. *8*, 393–402.

Jones, T.B., Bandettini, P.A., Kenworthy, L., Case, L.K., Milleville, S.C., Martin, A., and Birn, R.M. (2010). Sources of group differences in functional connectivity: an investigation applied to autism spectrum disorder. Neuroimage *49*, 401–414.

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

Kiviniemi, V., Jauhiainen, J., Tervonen, O., Pääkkö, E., Oikarinen, J., Vainionpää, V., Rantala, H., and Biswal, B. (2000). Slow vasomotor fluctuation in fMRI of anesthetized child brain. Magn. Reson. Med. *44*, 373–378.

Levy, S.E., Mandell, D.S., and Schultz, R.T. (2009). Autism. Lancet *374*, 1627–1638.

Liu, W.C., Flax, J.F., Guise, K.G., Sukul, V., and Benasich, A.A. (2008). Functional connectivity of the sensorimotor area in naturally sleeping infants. Brain Res. *1223*, 42–49.

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

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

Mullen, E. (1995). Mullen Scales of Early Learning (Circle Pines, MN: American Guidance Services).

Nir, Y., Mukamel, R., Dinstein, I., Privman, E., Harel, M., Fisch, L., Gelbard-Sagiv, H., Kipervasser, S., Andelman, F., Neufeld, M.Y., et al. (2008). Interhemispheric correlations of slow spontaneous neuronal fluctuations revealed in human sensory cortex. Nat. Neurosci. *11*, 1100–1108.

Pierce, K., Glatt, S.J., Liptak, G.S., and McIntyre, L.L. (2009). The power and promise of identifying autism early: insights from the search for clinical and biological markers. Ann. Clin. Psychiatry *21*, 132–147.

Raichle, M.E. (2010). Two views of brain function. Trends Cogn. Sci. (Regul. Ed.) *14*, 180–190.

Redcay, E., and 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*, 589–598.

Redcay, E., Haist, F., and Courchesne, E. (2008). Functional neuroimaging of speech perception during a pivotal period in language acquisition. Dev. Sci. *11*, 237–252.

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

Vanhaudenhuyse, A., Noirhomme, Q., Tshibanda, L.J., Bruno, M.A., Boveroux, P., Schnakers, C., Soddu, A., Perlbarg, V., Ledoux, D., Brichant, J.F., et al. (2010). Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. Brain *133*, 161–171.

Weng, S.J., Wiggins, J.L., Peltier, S.J., Carrasco, M., Risi, S., Lord, C., and Monk, C.S. (2010). Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. Brain Res. *1313*, 202–214.

Zwaigenbaum, L., Bryson, S., Lord, C., Rogers, S., Carter, A., Carver, L., Chawarska, K., Constantino, J., Dawson, G., Dobkins, K., et al. (2009). Clinical assessment and management of toddlers with suspected autism spectrum disorder: insights from studies of high-risk infants. Pediatrics *123*, 1383–1391.