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Anatomical and functional connectivity in autism spectrum disorders

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Abstract

There is growing consensus that atypical cognitive and behavioral function in ASD is associated with abnormalities in brain network organization. This article reviews the anatomical and functional evidence on connectivity in ASD from diffusion tensor imaging (DTI) and functional connectivity MRI (fcMRI) studies. DTI studies overwhelmingly present evidence of white matter compromise across virtually all major fiber tracts of the brain in children and adults with ASD (although infants may show evidence of abnormally early white matter development). While many fcMRI studies have found underconnectivity in ASD, others have reported diffuse overconnectivity. Both types of findings may reflect a reduced differentiation of networks in ASD. However, the field has been hampered by lack of methodological awareness, which is necessary to avoid misleading generalizations. Functional and anatomical connectivity have often been studied in isolation in the past, but a combination of imaging techniques will be necessary for a more comprehensive understanding of the impact of abnormal connectivity on cognitive processing in ASD.

Introduction

Background

There has been broad consensus for decades about the neurological nature of autism (e.g., Damasio & Maurer, 1978; Rutter, 1974). Following the emergence of modern imaging techniques, such as positron emission tomography (PET) and magnetic resonance imaging (MRI), the neuroimaging literature of autism spectrum disorder (ASD) has grown exponentially over the past three decades. It is so extensive that comprehensive reviews of all findings have become all but infeasible, although selective reviews of anatomical (Amaral et al., 2008) and functional (Chugani, 2000; Philip et al., 2011) imaging studies are available.

Many conventional anatomical and functional imaging approaches, such as structural MRI, PET, or functional MRI (fMRI), have an intrinsic localizing bias because they are designed to "map" effects, such as group differences in cortical thickness or in task-related activation. Statistics are commonly performed independently for each image element ("voxel") and group differences that reach significance can be pinpointed, for example to a single coordinate in a standard space. While these approaches have overall been enormously successful in identifying numerous sites and types of potential abnormality in the autistic brain, the mapping approach appears to suggest that such anatomical or functional abnormalities are in a specific location. An example from anatomical studies concerns the cerebellar vermis, which was heatedly debated some years ago with respect to a possible ASD-specific abnormality in size (Courchesne et al., 1988; Piven & Arndt, 1995). Another prominent example, this one from functional studies, relates to a possibly deficient fusiform face area (FFA), for which reduced activation in response to faces was found in some studies (Schultz et al., 2000). Pierce and colleagues reported two sets of

apparently contradictory findings, with absent activity in FFA in one (Pierce et al., 2001), but largely intact activity in another (Pierce et al., 2004). The differences in response to faces of strangers in the first and familiar faces in the second study suggested that activity in FFA was modulated by other brain regions (e.g., regions related to attention or emotional response). This exemplifies the insufficiency of localizing approaches in ASD research: The FFA does not fail to respond (in some experimental settings) because it is broken, but because it *connects* and *cooperates* with other parts of the brain in atypical ways.

Other lines of evidence support a connectivity-based network approach to ASD. Early brain overgrowth, which has been found to characterize ASD in several studies (reviewed in Courchesne et al., 2011), affects both gray *and white* matter, suggesting early-onset anomalies in axonal organization and myelination. Indeed, recent diffusion tensor imaging (DTI) studies (Weinstein et al., 2011; Wolff et al., 2012) suggest abnormal profiles of white matter maturation in the first years of life in infants eventually diagnosed with ASD (see detailed discussion below). Apart from such evidence specific to ASD, the network approach is also consistent with a general paradigm change in cognitive neuroscience, away from strict localization and towards an understanding of functional brain organization with respect to distributed systems. While the distributed view has been around in cognitive neuroscience for decades (Mesulam, 1990), it has only recently begun to be fully appreciated in the study of psychiatric disorders (Menon, 2011).

Roadmap

This article will first review the evidence relevant to anatomical and functional connectivity in ASD. As will become apparent, not all the evidence is consistent, and a discussion of the most likely explanations for these inconsistencies in the literature will be necessary. The article will end with a review of promising directions in the study of connectivity, in particular with respect to the use of combined data from complementary techniques and the attempt to reunite approaches examining connectivity with those investigating cortex and its cellular architecture.

At the outset, some terminological clarifications are required. Whereas the term *anatomical connectivity* is relatively straightforward, relating to the organization of axons and synaptic connections between neurons, the term *functional connectivity* (FC) is rather equivocal. FC is conventionally defined with respect to signal correlation in different parts of the brain, or more comprehensively as "the statistical dependence between remote neural processes" (Honey et al., 2007). Note that correlations can exist on widely differing time scales, specifically high frequencies in the gamma band (>25 Hz), as detected in electroencephalography (EEG) and magnetoencephalography (MEG), and low frequencies (<0.1 Hz) in functional connectivity MRI (fcMRI). A related distinction has been further made between FC – defined as "temporal correlations between spatially remote neurophysiological events" – and *effective connectivity*, defined as "influence one neural system exerts over another" (Friston et al., 1993). Most FC studies of ASD have, however, skirted the question of causality invoked by the concept of effective connectivity.

Findings

Anatomical connectivity

As mentioned above, indirect evidence of early-onset disturbances in anatomical connectivity comes from studies showing early white matter overgrowth in infants and toddlers with ASD (reviewed in Courchesne et al., 2011). Carper et al. (2002) found white matter significantly enlarged in frontal and parietal lobes of children with ASD ages 2.7-4 years, compared to matched typically developing (TD) children. White matter growth curves were atypical in all four forebrain lobes, with early overgrowth and subsequent flat growth rate.

There is furthermore limited evidence on white matter in ASD from magnetic resonance spectroscopy (MRS). The most commonly examined metabolite in MRS is N-acetyl-aspartate (NAA), considered a marker of neuronal integrity and function. However, MRS scanning is relatively time-consuming and most available studies did not acquire data for the whole brain. Often large voxels are imaged, including both gray and white matter. A recent meta-analysis of MRS studies in ASD by Ipser and colleagues (2012) indicated a relatively consistent finding of reduced NAA in white matter for children with ASD.

In recent years, DTI has become the method of choice for investigating white matter in ASD. DTI is sensitive to the diffusion of water, which is affected by tissue organization in white matter. High *fractional*

anisotropy (FA) indicates that water diffuses preferentially along tracts of myelinated axons (cf. Fig. 1). FA is therefore considered a positive index of white matter integrity. The very first DTI study in a small sample of male autistic adolescents by Barnea-Goraly (2004) found atypically reduced FA in various white matter regions and in the corpus callosum. The corpus callosum has been the topic of several subsequent DTI studies in larger samples. The rationale for looking at the anatomical substrate for interhemispheric connectivity was in part provided by earlier findings of reduced callosal volume (see meta-analysis by Frazier & Hardan, 2009). Alexander and colleagues (2007) reported decrease in callosal volume and fractional anisotropy for high-functioning children and young adults with ASD. Reduced FA was accompanied by increased *mean diffusion* (MD). This latter DTI index suggest that water moves more freely in white matter, presumably because of impaired tissue organization (Figure 1). MD is therefore considered a negative index of white matter integrity. Consistent DTI findings indicating white matter compromise in the corpus callosum have been reported in several more recent studies of children and adolescents with ASD (Cheon et al., 2011; Jou et al., 2011; Shukla et al., 2010; Shukla et al., 2011). Two other studies failed to detect significant differences for FA in the corpus callosum (Hong et al., 2011; Poustka et al., 2012). While such null findings may be due to low sample size and cohort effects in a highly heterogeneous disorder, it is notable that these two studies included mostly pre-teen children. The question of abnormal early trajectories of white matter maturation in ASD will be raised further below in this section.

With respect to the regional patterns of interhemispheric connectivity, Alexander and colleagues (2007) detected evidence of white matter compromise (either reduced FA or increased MD) in genu, body, and splenium of the callosum. Another negative index of white matter integrity, increased *radial diffusion* (RD; Figure 1), was also found in all three segments. RD reflects water diffusion perpendicular to the main axis of diffusion (presumed to correspond to the main orientation of axons) and can therefore be interpreted as a sign of reduced myelination or axonal membrane defects (Le Bihan, 2003). Similar findings for the corpus callosum were reported by Shukla and colleagues (2010). A follow-up study by the same group (Shukla et al., 2011) used tract-based spatial statistics (TBSS), which is a technique that tests for diffusion differences on the voxel level within parts of white matter most likely to contain major fiber tracts (Figure 2). White matter compromise appeared more robust in the splenium with reduced FA and increased MD and RD, whereas in genu and body of the callosum MD was not significantly increased. A second TBSS study (Jou et al., 2011) observed reduced FA in anterior and posterior segments, as well as the body of the corpus callosum.

A large number of DTI studies in ASD have further examined intrahemispheric cortico-cortical tracts. A few of these have targeted specific fibers or sets of fibers. Fletcher and colleagues (2010) reported atypical asymmetry of MD and RD in arcuate fasciculus, a tract connecting anterior and posterior perisylvian regions, suggesting that the typically more robust development of this tract in the left (compared to the right) hemisphere was absent in high-functioning adolescents and young adults with ASD. The finding was interpreted with respect to a possible lack of left-hemisphere dominance for language in the ASD group. These results were replicated by Lo and colleagues (2011), who also observed loss of leftward asymmetry in FA for two additional tracts, the cingulum (which connects cingulate gyrus with other regions of the limbic system) and the uncinate fasciculus (which connects medial temporal and orbitofrontal regions). These latter findings implicating the limbic system are further supported by studies specifically targeting socio-affective circuits. Conturo et al. (2008) examined pathways important for face perception and found atypical asymmetry of RD for tracts connecting fusiform gyrus with hippocampus. Notably, in the right hemisphere RD was *reduced* in adults with ASD compared to a matched TD group, possibly reflecting tighter axonal packing. This finding was most robust for an ASD subsample of participants who performed poorly on the Benton Facial Recognition task. Using TBSS, Ameis and colleagues (2011) found significant interaction effects for diagnostic group (ASD vs. TD) by age (children vs. adolescents) in several tracts considered important for socio-affective processing, such as the right inferior longitudinal fasciculus, bilateral superior corona radiata, and bilateral uncinate fasciculus. These interactions were driven by atypically increased RD and MD in children (but not adolescents) with ASD.

Sundaram and colleagues (2008) compared children with ASD to matched TD children with focus on frontal lobe connectivity. FA was reduced only in short-range fibers (those connecting regions within the frontal lobe), whereas it was at normal levels for long-range fibers connecting frontal with posterior regions outside the frontal lobes. The finding of impaired short-range connections, which is supported by another DTI study showing compromised short-distance tracts throughout the forebrain (Shukla et al., 2011), is especially interesting in view of

theoretical speculations of a dichotomy between intact or even excessive local connectivity and reduced long-distance connectivity in ASD (Courchesne & Pierce, 2005). The study by Sundaram and colleagues (2008) further detected increased MD for both short and long frontal connections in both hemispheres suggesting that long-range fibers were also compromised. Poustka et al. (2012) found reduced FA for several long-range frontal tracts (right superior longitudinal fasciculus, bilateral uncinate fasciculus) in slightly older children with ASD (ages 6-12 years). Reduced FA was also found to be associated with symptom severity on diagnostic measures in several frontal tracts. Examining the superior longitudinal fasciculus, which connects frontal and parieto-occipital regions, Nagae and colleagues (2012) furthermore detected atypically increased MD in ASD children with language impairment, but not in those without language impairment.

While many DTI studies of ASD have targeted callosal and intrahemispheric cortico-cortical connectivity, the evidence for tracts connecting with deep, subcortical brain regions remains limited. Cheon and colleagues (2011) found reduced FA bilaterally in the anterior thalamic radiation, which connects thalamus with frontal lobes. In the right hemisphere this finding was associated with a measure of sociocommunicative impairment. Evidence of impaired connectivity between thalamus and frontal lobe was also reported by Shukla et al. (2010), who found reduced FA in anterior and posterior limbs and genu of the internal capsule. Compromise in the posterior limb would suggest impairment related to sensorimotor functions. Finally, two studies (Shukla et al., 2010; Sivaswamy et al., 2010) detected white matter defects in the cerebellar peduncles, suggesting impaired connectivity between cerebellum and cerebral cortex.

Overall, the DTI evidence on anatomical connectivity reviewed above suggests relatively consistent patterns of white matter compromise across many brain regions and fiber tracts. However, consideration of limitations and further implications with respect to several demographic variables is called for. First of all, almost the entire DTI literature is limited to ASD participants at the high-functioning end of the spectrum, often defined with respect to a full-scale IQ >70 (i.e., people without mental retardation). The reasons for this limitation are strictly pragmatic and are related to the sensitivity of MRI to motion artifacts. Since low-functioning people with ASD often cannot cooperate with verbal instructions to hold still for the duration of a DTI scan (typically about 10 minutes), they are excluded from participation. As one exception, Pardini et al. (2009) studied young low-functioning men with ASD (with verbal IQs ranging from 38-58), finding reduced FA in a number of white matter regions overall consistent with findings for high-functioning people with ASD reviewed above. However, in order to obtain motion-free DTI data, ASD participants were scanned under general anesthesia, which may raise ethical issues.

A second demographic consideration concerns the typically low number of female participants. While male-to-female ratios are generally high in the ASD population (c. 4:1), they are often found to be even higher at the high-functioning end of the spectrum, presumably because female variants of ASD tend to be more severe. Only one DTI study to date has addressed sex differences. Beacher and colleagues (2012) studied 38 adults with ASD (including 13 women) and matched TD samples. For several regions, including body of the corpus callosum, as well as cingulum and corona radiata bilaterally, sex by diagnosis interactions were found, driven by sex differences in the TD group (higher FA in men compared to women) that were absent in the ASD group. However, similar to the lack of studies on low-functioning ASD, evidence on female variants of ASD is currently too limited for any conclusions.

A third demographic variable worthy of discussion is age. Most studies reviewed above included children and adolescents, typically between the ages of 6 and 18 years, with a few additional studies in adults. DTI studies in infants and toddlers remain rare, but the currently limited findings are intriguing. The very first study including seven toddlers with ASD (ages 1.8-3.3 years) by Ben Bashat and colleagues (2007) reported *increased* FA in several white matter regions (such as corpus callosum and internal capsule) for which *reduced* FA has been reported rather consistently in older children and adolescents. The same group (Weinstein et al., 2011) more recently followed up with a report on a larger sample of children ages 1.5-5.8 years, supporting the earlier findings, with greater FA in children with ASD compared to matched TD children in genu and body of the corpus callosum, the left superior longitudinal fasciculus, and the cingulum bilaterally (Fig. 3A-B). Viewed in the context of the evidence for early anatomical brain overgrowth of gray and white matter in ASD mentioned above (Courchesne et al., 2011), these findings could be interpreted as evidence of possible 'precocious maturation' of white matter in infants with ASD. However, the evidence is far too slim and complicated to warrant this conclusion. In a recent longitudinal multisite study (Wolff et al., 2012), children that were considered "high-risk" because they had an older sibling with ASD

were first scanned at 6 months of age. Comparison between children later diagnosed with ASD and those who were ASD-negative may at first glance support the idea of 'precocious maturation'. However, the age window for which greater FA was found in infants with ASD was about 6-9 months, whereas at 24 months these children showed reduced FA, compared to ASD-negative children (Fig. 3C). For the age range examined in the studies described above (Ben Bashat et al., 2007; Weinstein et al., 2011), the findings are thus not consistent. As mentioned, the comparison group in the study by Wolff and colleagues (2012) also consisted of high-risk infants. Although these did not eventually receive a diagnosis of ASD, they may not be considered to represent the TD population because they were likely to share some genetic risk for ASD.

In summary, there is extensive evidence indicating white matter impairment (reduced FA, increased MD or RD) in numerous tracts connecting the two hemispheres, connecting different cortical regions within one hemisphere, and connecting cerebral cortex and subcortical and cerebellar regions. This evidence is overall quite consistent for children above the age of 6 years, as well as for adolescents and adults. Divergent findings have been reported in several studies of infants and toddlers. While these appear to suggest abnormal trajectories, not only of volumetric brain growth, but also of diffusion indices of white matter maturation (such as myelination), the evidence is limited and uncertainty remains about the exact timing of an atypically early increase in FA in infants with ASD.

Functional connectivity

The investigation of FC in ASD dates back surprisingly far to the late 1980s with a study by Horwitz et al. (1988) that used glucose positron emission tomography (PET) to examine signal correlations between numerous brain regions of interest (ROIs). Correlations were found to be reduced in young men with ASD for ROI pairs in frontal, parietal, subcortical, and cerebellar regions and between these. However, PET – which provided the primary techniques available for mapping functional brain activity back then – is severely hampered by its lack of temporal resolution, and FC studies using this technique have remained rare. One other PET study by Castelli and colleagues (2002) reported reduced activity in right superior temporal sulcus, and medial temporal and medial prefrontal regions for a mentalizing task in ASD, and complemented this finding with a test of connectivity using blood flow correlations. FC between visual cortex and superior temporal sulcus was found to be reduced, possibly related to impaired biological motion perception.

More recently, the FC literature in ASD has grown rapidly, primarily due to developments in functional connectivity MRI. Although the principles of fcMRI were first described in the 1990s (Biswal et al., 1995), it took almost a decade for the technique to become sufficiently known and established for applications in psychiatric and clinical populations. FCMRI uses the same type of signal as conventional functional MRI, i.e., the BOLD signal, which is indirectly tied to local neuronal activity (Logothetis & Pfeuffer, 2004). However, rather than modeling the BOLD signal changes with respect to task designs, as in conventional fMRI activation analysis, fcMRI is based on correlations of the BOLD signal between voxels (Biswal et al., 1995). In TD populations, the fcMRI approach has been implemented in numerous studies, mapping out motor (Biswal et al., 1995), visual (Nir et al., 2006), language (Hampson et al., 2002), working memory (Lowe et al., 2000), task control (Dosenbach et al., 2007), default mode (Greicius et al., 2003), and attention (Fox et al., 2006) networks.

FCMRI investigations of ASD were jumpstarted by Just an colleagues (2004), who reported that BOLD correlations for sentence comprehension were reduced between 10 ROI pairs in adults with ASD (compared to TD participants; Fig. 4). Based on this finding, the authors presented an *underconnectivity theory*, which proposes that ASD is "caused by underfunctioning integrative circuitry" (ibid.: 1817). The theory has often been misunderstood as implying *general* underconnectivity. However, this is misleading in view of the empirical findings reported (176 out of 186 total ROI pairs examined did not show underconnectivity in ASD) as well as the prediction by Just and colleagues (2004) that functional connectivity in ASD would actually be atypically increased in domains of cognitive strength (as recently corroborated in a study on visual search by Keehn et al., 2012). In the years following the milestone paper by Just et al. (2004), numerous fcMRI studies provided strong support for the underconnectivity theory. Among these were studies on verbal working memory (Koshino et al., 2005), visuomotor integration (Villalobos et al., 2005), sentence comprehension (Kana et al., 2006), problem solving (Just et al., 2007), response inhibition (Kana et al., 2007; Lee et al., 2009), face processing (Kleinhans et al., 2008), finger movement (Mostofsky et al., 2009), cognitive control (Solomon et al., 2009), self-representation (Lombardo et al., 2010), and

tasks tapping into theory of mind (Kana et al., 2009; Mason et al., 2008). Some of these studies also detected associations between underconnectivity and neurological, diagnostic, or cognitive measures in ASD. Correlations between fronto-parietal FC and size of the callosal genu have been observed in adults with ASD (but not in matched control groups; (Just et al., 2007; Kana et al., 2006). Kleinhans et al. (2008) found that reduced FC between fusiform face area and amygdala was associated with social symptom severity in adults with ASD. Solomon and colleagues (2009) detected an association between reduced fronto-parietal connectivity and attention deficit in adolescents with ASD. In a recent study in toddlers with ASD scanned during natural sleep (Dinstein et al., 2011), which showed reduced interhemispheric synchronization of activity in language-related brain regions (inferior frontal and superior temporal gyri), inferior frontal synchronization was correlated with an expressive language score.

At first glance, the fcMRI literature on ASD seems to be strong and consistent. However, it is notable that some studies have reported findings that appear at odds with the underconnectivity theory. The first of these was by Welchew et al. (2005), who examined BOLD correlations for all pairings of 90 cortical and subcortical ROIs in high-functioning adults with ASD. Underconnectivity was detected within the medial temporal lobe, but many ROI pairs showed greater FC in the ASD group (compared to TD participants). Two studies in a small sample of adolescents and adults with ASD reported predominant overconnectivity of thalamus (Mizuno et al., 2006) and caudate nucleus (Turner et al., 2006) with cerebral cortex. The latter finding was more recently corroborated in a larger sample of children with ASD by Di Martino et al. (2011), who found atypically increased ("ectopic") cortical connectivity of caudate and putamen. However, any suggestion that such overconnectivity may specifically apply to connections with subcortex is inconsistent with additional evidence. Noonan et al. (2009) found widespread overconnectivity in ASD for cortical networks associated with source memory. Wicker and colleagues (2008) examined networks involved in emotion processing in adults with ASD and found that underconnectivity affecting ventrolateral and dorsomedial prefrontal regions was accompanied by overconnectivity especially with posterior regions in temporal and occipital lobes. Rudie et al. (2012) found that both positive and negative connectivity, as observed for bilateral amygdala seeds in TD adolescents during viewing of emotional faces, was reduced in adolescents with ASD. For a seed in right inferior frontal cortex (pars opercularis), overconnectivity in the ASD group was found with right prefrontal cortex and basal ganglia. Several other studies have reported findings that strongly contradict the underconnectivity theory, showing widespread and robust cortico-cortical overconnectivity in ASD. These include one study on several regions within an imitation circuit (Shih et al., 2010), one on the posterior superior temporal sulcus (Shih et al., 2011), one related to visual search (Keehn et al., 2012), and one examining regions related to lexical semantic processing (Shen et al., 2012). The study by Shen and colleagues (2012) is especially surprising given their finding of extensive overconnectivity in adolescents and adults with ASD for a seed in left inferior frontal gyrus (Broca's area; Fig. 5). The initial study by Just et al. (2004) used an ROI in a similar location and their findings of underconnectivity with dorsolateral prefrontal cortex and primary visual cortex is directly contradicted by those from Shen et al. (2012), who found atypically increased functional connectivity in both of these regions. An attempt to explain these stark inconsistencies will be provided below.

An additional line of fcMRI research uses resting state data. Studies in healthy adults have shown spontaneous BOLD fluctuations (in the absence of a task) with network-specific correlations (e.g., Smith et al., 2009). Cherkassky et al. (2006) were the first to implement this approach in ASD. They used resting blocks from studies applying diverse tasks and examined 12 ROIs attributed to the default mode network (DMN). Most ROI pairs were underconnected in young adults with ASD, compared to TD participants. These findings were supported by Kennedy et al. (2008), who used data from a continuous resting state fMRI scan. Correlations within a task-negative network (equivalent to the DMN) were reduced in ASD. However, Kennedy et al. (2008) also examined a task-positive network (regions that frequently activate across different types of task), for which was a mixture of increased and reduced connectivity was found when ASD and TD groups were compared. Another study by Monk and colleagues (2009) used only a single seed in posterior cingulate cortex (PCC) and observed mixed results of partial under- and overconnectivity within the DMN in adults with ASD, inconsistent with the two earlier studies described above. Monk et al. (2009) also found both positive and negative correlations between FC within the DMN and diagnostic scores. A further study examining default mode networks, as detected by independent component analysis, identified regions in frontal and parietal lobes where reduced connectivity was associated with symptom severity in adolescents and adults with ASD (Assaf et al., 2010).

Resting state fcMRI has also been used to investigate networks other than the DMN. Ebisch et al. (2011) examined connectivity of the insula, considered a crucial area for "emotional awareness", and found several sites with reduced connectivity, such as amygdala and PCC. Note that correlation matrices presented in this study also showed a number of ROI pairings that were overconnected in the ASD group, which were, however, not discussed by the authors. Studying adolescents and young adults with ASD, Anderson et al. (2011) tested BOLD correlations for each cortical voxel with the homotopic voxel in the contralateral hemisphere. The main finding was interhemispheric underconnectivity in several perisylvian and parietal regions.

Inconsistencies

Underconnectivity vs. overconnectivity in fcMRI

Although a majority of fcMRI studies in ASD have reported underconnectivity findings, the contrast with a growing number of studies reporting partial or exclusive overconnectivity is of concern. In the ASD literature, relatively little attention has been paid until recently to methodological issues that might elucidate such inconsistencies. In a first such attempt, Jones et al. (2010) showed that underconnectivity effects in ASD gradually disappeared with the removal of effects for an overt speech task through regression. A survey of fcMRI studies of ASD (Müller et al., 2011) supported this finding, showing that fcMRI studies were most likely to generate underconnectivity findings in ASD when task effects were left intact and BOLD correlations were tested only in task-related ROIs. On the other hand, studies reporting overconnectivity or mixed effects frequently applied different methods, regressing out task effects, applying temporal low-pass filters to BOLD time series, and testing for BOLD correlations across the whole brain. Such methodological minutiae may seem tedious and confusing to the clinical ASD researcher, but they require careful consideration if misinterpretation of empirical results is to be avoided.

As mentioned above, when the fcMRI approach was first developed in healthy adults more than a decade ago, it became clear that network-specific BOLD fluctuations are not driven by a task or stimulus, but are spontaneous (Biswal et al., 1995) and are most robust at very low frequencies (Cordes et al., 2001), which is serendipitous given the modest temporal resolution of fMRI. These observations have resulted in the more recent concept of *intrinsic connectivity* (Van Dijk et al., 2010), which refers to low-frequency (< 0.1 Hz) fluctuations of the BOLD signal that may reflect electrophysiological fluctuations in local field potentials (Schölvinck et al., 2010). Notably, these low-frequency fluctuations can be isolated in BOLD datasets acquired during task performance, by regressing out the modeled task and performance effects and by applying temporal low-pass filters (Cole et al., 2010; Fair et al., 2007; Fox & Raichle, 2007).

These methodological details shed light on the serious inconsistencies in the fcMRI literature on ASD. Remarkably, most of the studies reviewed above that reported underconnectivity in ASD used methods very different from the intrinsic connectivity approach, by focusing on BOLD correlations that were driven by task-related activity. Conversely, most of the studies that adhered to the intrinsic fcMRI approach through task regression and low-pass filtering detected evidence of overconnectivity in ASD or mixed effects (Müller et al., 2011). Note that neither of the two approaches, which could be contrasted as *co-activation* vs. *intrinsic* fcMRI, is 'right' or 'wrong'; however, confusion of the two will inevitably result in misinterpretation of the findings in ASD research. Nevertheless, one may wonder which of the two approaches and sets of findings is more informative with respect to the neurobiological signature of ASD. Arguments can be made either way. For example, Anderson and colleagues (2011) recently used fcMRI to develop a prediction algorithm for diagnostic classification between adolescents and adults with ASD and a group of matched TD participants. They found that mid-range connections (c. 50-100 mm in length) with reduced BOLD correlations in the ASD group were among the most informative. In other words, these underconnectivity effects most heavily contributed to the algorithm's ability to determine whether a dataset belonged to an ASD or a TD participant. This might suggest that co-activation approaches, which are most likely to yield underconnectivity findings, may have greater power to inform us about neurofunctional abnormalities specific to ASD.

On the other hand, it has been argued that co-activation fcMRI may not truly reflect connectivity at all because it detects correlations driven by tasks and stimuli (Jones et al., 2010). As an example, two brain regions may show highly correlated BOLD changes simply because they respond equally to a stimulus, without actually being

interconnected. Other arguments in favor of the intrinsic connectivity approach are empirical. It has been shown that intrinsic fcMRI effects are overall consistent with anatomical connectivity, as detected by DTI tractography (Honey et al., 2009). More interestingly, spontaneous low frequency BOLD fluctuations have been related to Hebbian plasticity and the sculpting of neuronal connections based on long-term experience (Lewis et al., 2009; Sadaghiani et al., 2010). The study of intrinsic functional connectivity in ASD may thus be informative of abnormalities in fundamental mechanisms of network organization.

In view of the divergent findings on functional connectivity, it remains open what exactly these may mean with respect to the neurobiology of ASD. As a tentative conclusion, there is good evidence from numerous co-activation fcMRI studies that online information processing during task performance is less coordinated in ASD within domain-specific networks. For example, cooperation between prefrontal and parietal association areas during executive performance (Just et al., 2007) or between amygdala and fusiform face area during face perception (Kleinhans et al., 2008) is reduced in ASD. On the other hand, there is growing evidence from intrinsic fcMRI studies that the underlying organization of networks includes diffusely excessive connectivity not seen in the TD brain. For example, the caudate nucleus shows connectivity with temporal regions not found in the TD brain (Di Martino et al., 2011) and Broca's area is atypically connected with regions outside language networks, such as the primary visual cortex in the occipital lobe (Shen et al., 2012). Overall, it therefore appears that in the ASD brain functional networks are less distinctly organized, or insufficiently 'sculpted' for efficient information processing.

DTI vs. fcMRI

Once one acknowledges that the fcMRI evidence goes beyond simple underconnectivity, as described above, a new problem arises. A nuanced interpretation of fcMRI appears at odds with the DTI evidence, which overwhelmingly indicates white matter compromise throughout the brain (except for studies in infants). However, this apparent conflict between fcMRI and DTI evidence is not surprising when one considers the very different sources of signals used to identify "connectivity" in the two techniques. As explained above, fcMRI commonly tests for correlations of the BOLD signal. Apart from the fact that the BOLD signal is vascular in origin and reflects neuronal activity only indirectly, it is primarily or exclusively detected in gray matter (because the BOLD signal in white matter is extremely small). FCMRI is thus a doubly indirect measure: It estimates connectivity from the "wrong" signal source (hemodynamic rather than neuronal) extracted from the "wrong" tissue compartment (gray matter, which contains few myelinated axons). In view of these limitations, the enormous success the fcMRI technique has seen in the cognitive and clinical neurosciences of the past decade is astounding, although obviously deserved given the quality of findings overall. However, while fcMRI is generally reliable in determining whether different brain regions "talk to each other" (exchange information), it cannot establish whether these regions are directly (monosynaptically) connected (Honey et al., 2009).

DTI, on the other hand, detects the diffusion of hydrogen protons, which is affected by tissue organization in white matter. It therefore derives its signal from the "right" tissue compartment, the one containing most myelinated axons that make long-distance connections possible, but the signal itself is epiphenomenal because water diffusion plays no direct role in information transfer between neurons. Currently available diffusion indices are therefore ambiguous with respect to the underlying connectivity. For example, the most commonly used index FA can be reduced for a number of reasons: There may be fewer axons within a given voxel, axons may be less coherently organized (running in different directions), axons may be damaged (with membranes more permeable to water diffusion), or myelin sheaths around axons may not have developed well or may have degenerated. It is therefore not possible to pinpoint what type of white matter compromise is at the root of the numerous and overall quite consistent findings of reduced FA in ASD. More specifically, reduced FA may (in some white matter regions) reflect reduced coherence of axonal organization. This could in principle be consistent with the interpretation of reduced 'sculpting' of brain circuits from fcMRI, in the sense that some axons that are lost due to synaptic pruning in the TD brain are retained in ASD (resulting in diffuse overconnectivity seen in intrinsic fcMRI). Based on the current evidence, it cannot be determined whether this is truly the case; however, some ideas on how more comprehensive datasets may address the question will be discussed in the following section.

Challenges and perspectives

Multimodal approaches

An obvious limitation of the current literature on connectivity in ASD is that fcMRI and DTI are two separate lines of research. Studies presenting combined fcMRI and DTI data for the same ASD cohort remain rare. This is hard to understand, given that fMRI and DTI data can be easily acquired together in a single short MRI session. Since many groups are in fact acquiring such multimodal datasets, one can expect corresponding publications in the near future. These will be a first step to addressing the basic questions about apparent differences in functional and anatomical results raised above.

It will be equally important to extend the notion of functional connectivity to higher temporal frequencies that cannot be captured with fcMRI. There is a relatively small functional connectivity literature from EEG and MEG studies. The findings are diverse and partly divergent and are not discussed in this article (see reviews in Vissers et al., 2012 and Müller, in press). While improving methods will probably boost the impact of EEG and MEG coherence studies in the connectivity debate on ASD, even more important will be the combination of electrophysiological and MRI techniques. Ideally, a most promising connectivity study will combine data from EEG or MEG with fcMRI, DTI, anatomical MRI, and MRS to test for abnormalities of connectivity from multiple different angles, for example examining how reduced BOLD correlations tie in with reduced power in high frequency bands (such as gamma or alpha), with impairments of fiber tracts detected with DTI and reduced NAA from MRS, and with white matter volume or cortical thickness from anatomical MRI. This kind of comprehensive multimodal approach is likely to advance the currently incomplete understanding of connectivity ASD.

Reuniting cortex and connectivity

The challenges facing connectivity research in ASD are not exclusively methodological, however. Improvements in technical approaches need to be paired with a refined conceptual understanding. One oddity in neuroimaging is the common notion that gray and white matter are separate entities. This is 'technically correct', given that there are automated softwares that will draw contours around each and neatly separate the two tissue compartments. The many volumetric studies of ASD have therefore always reported separate measurements of gray and white matter. While this is surely desirable, it can easily be forgotten that a cortical pyramidal cell will occupy both compartments, with dendrites and neuron body in gray matter and axon on white matter. Abnormalities of gray and white matter in ASD are therefore unlikely to emerge independently. However, despite the fact that the signal in fcMRI actually originates from gray matter, the links between impaired connectivity and abnormalities of cortical architecture are often not considered. This is conceptually inadequate, given that cortical architecture and connectivity are intimately linked (Mountcastle, 1997), but methodologically plausible because what is known about cortical architecture in ASD comes exclusively from postmortem brains (reviewed in Amaral et al., 2008), which are by definition ineligible for studies of functional connectivity. Technical progress (e.g., anatomical MRI at higher spatial resolution or improved diffusion MRI techniques) may in the future help alleviate the problem. However, there are also conceptual advances that can be help address the missing links between cortex and connectivity.

In a landmark paper presenting findings from animal studies, Passingham and colleagues (2002) defined the functional specialization of cortical regions with respect to 'connectional fingerprints'. These can be optimally illustrated in radar plots. The example in Fig. 6 shows profiles of afferent connectivity distinguishing Walker's areas 9 and 14 in the macaque brain. One result from this approach is that each cortical region has unique connectivity profiles. While this finding is useful in distinguishing regions, there is a much deeper aspect to the concept of connectional fingerprints. Not only are they different, but they actually *determine the functional role* of a given regions or smaller cortical site (Friston & Price, 2001). In simple terms, this concept implies that the functional specialization of a given cortical site is fully determined by what other parts of the brain it "listens to" (afferent connectivity) and "talks to" (efferent connectivity).

The connectional fingerprint approach tightly links local cortical differentiation and specialization with long-distance connectivity. It therefore has the potential to incorporate evidence of abnormalities in cortical architecture and in long-distance connectivity, as observed in ASD, into a single coherent framework. Unfortunately, very little direct evidence is currently available that would be relevant to such a framework. Rudie

and colleagues (2012) examined FC associated with passive viewing of emotional face expressions. FCMRI maps for two seeds in amygdala and right inferior frontal pars opercularis showed distinct regional patterns of positive and negative correlations in TD children. Between-group comparisons showed that children with ASD had reductions both in region-specific positive and negative correlations. In other words, the connectivity profiles in ASD were found to be less segregated, suggesting less distinct connectional fingerprints. A second study by Shih and colleagues (2011) implemented the connectional fingerprint approach more directly. This study focused on the posterior superior temporal sulcus (pSTS), which plays a role in biological motion perception, face processing, joint attention, auditory-visual integration, and language perception (Redcay, 2008), making it an obvious region of interest in ASD. FCMRI analyses for three subregions within pSTS were performed, which in TD participants showed highly distinct connectivity patterns between the mid-section of pSTS (red clusters in Fig. 7A), on the one hand, and rostral and caudal subsections (blue and cyan clusters), on the other. The connectivity patterns for the subregions were much less distinct in the ASD group, with large swaths of cortex showing overlapping connectivity (dark clusters in Fig. 7B). This study showed, not only that connectional fingerprints were less distinct in ASD, but also that this reduced functional differentiation was associated with the diffuse overconnectivity, similar to the results of several other intrinsic fcMRI studies (as described above; Fig. 7C). The finding was further strengthened by a cross-sectional test of age effects, showing that the differentiation between the pSTS subregions steadily increased with age in the TD group, as to be expected as an effect of maturation and the gradual emergence of distinct networks (Johnson, 2011). This increase in differentiation was seen in the temporal domain (with respect to differences in BOLD time series between subregions; Fig. 7D) as well as in the spatial domain (with respect to the whole brain connectivity pattern of each subregion; Fig. 7E). The latter differentiation index directly relates to connectional fingerprints as described by Passingham et al. (2002).

Conclusion

Abnormalities of sociocommunicative, cognitive, and sensorimotor functions in ASD cannot be explained by local brain defects, but require an understanding on the network level. The study of network connectivity has yielded a large and growing body of evidence on functional and anatomical abnormalities. While inconsistencies and questions remain, many of the findings indicate that functional networks may be insufficiently differentiated (or 'sculpted') in ASD and that this may be associated with inefficient or noisy information processing. However, improvements both on the methodological level (e.g., the use of multimodal imaging approaches) and on the conceptual level (e.g., linking cortical architecture and connectivity) will be needed for a more comprehensive understanding of the neurofunctional bases of ASD. Such a comprehensive model of brain markers in ASD will most likely be a prerequisite for identifying biologically based subtypes of the disorder, which may in turn be necessary for pinpointing genetic and environmental causes for those subtypes.

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Key terms

BOLD signal. The blood oxygen level dependent signal in fMRI reflects changes in blood oxygenation linked to local changes in neuronal and synaptic activity. It is the signal used in functional and functional connectivity MRI.

Co-activation fcMRI. A functional connectivity technique that detects signal changes driven by stimulus presentation or task performance.

Connectional fingerprint. The distinct input (afferent) and output (efferent) connectivity pattern of a brain region with other brain regions (see example in Figure 6).

Diffusion tensor imaging (DTI). An MRI technique that characterizes white matter based on its effects on water diffusion.

Fractional anisotropy (FA). The most commonly used DTI index of white matter integrity. It reflects the predominant diffusion of water along the orientation of axons (see Figure 1).

Functional connectivity MRI (fcMRI). A technique that detects correlations of the functional MRI (BOLD) signal between different brain regions. Signal correlation is considered to indicate cooperation between regions.

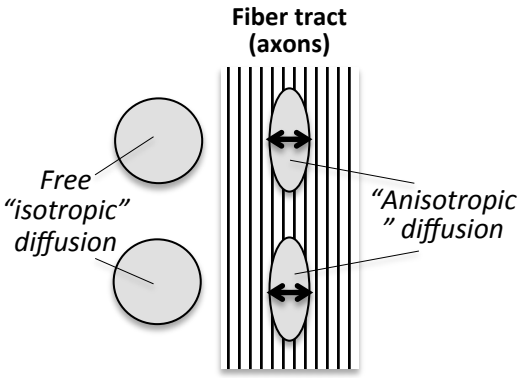
Intrinsic fcMRI. A functional connectivity technique that detects spontaneous low frequency changes in the fMRI signal, which are correlated within networks.

Mean diffusion (MD). A negative DTI index of white matter integrity, reflecting reduced tissue organization or tissue damage (see Figure 1).

Multimodal imaging. The use of several imaging techniques (e.g., fcMRI, DTI, MEG) in the same group of participants, allowing investigators to examine brain connectivity from multiple different perspectives.

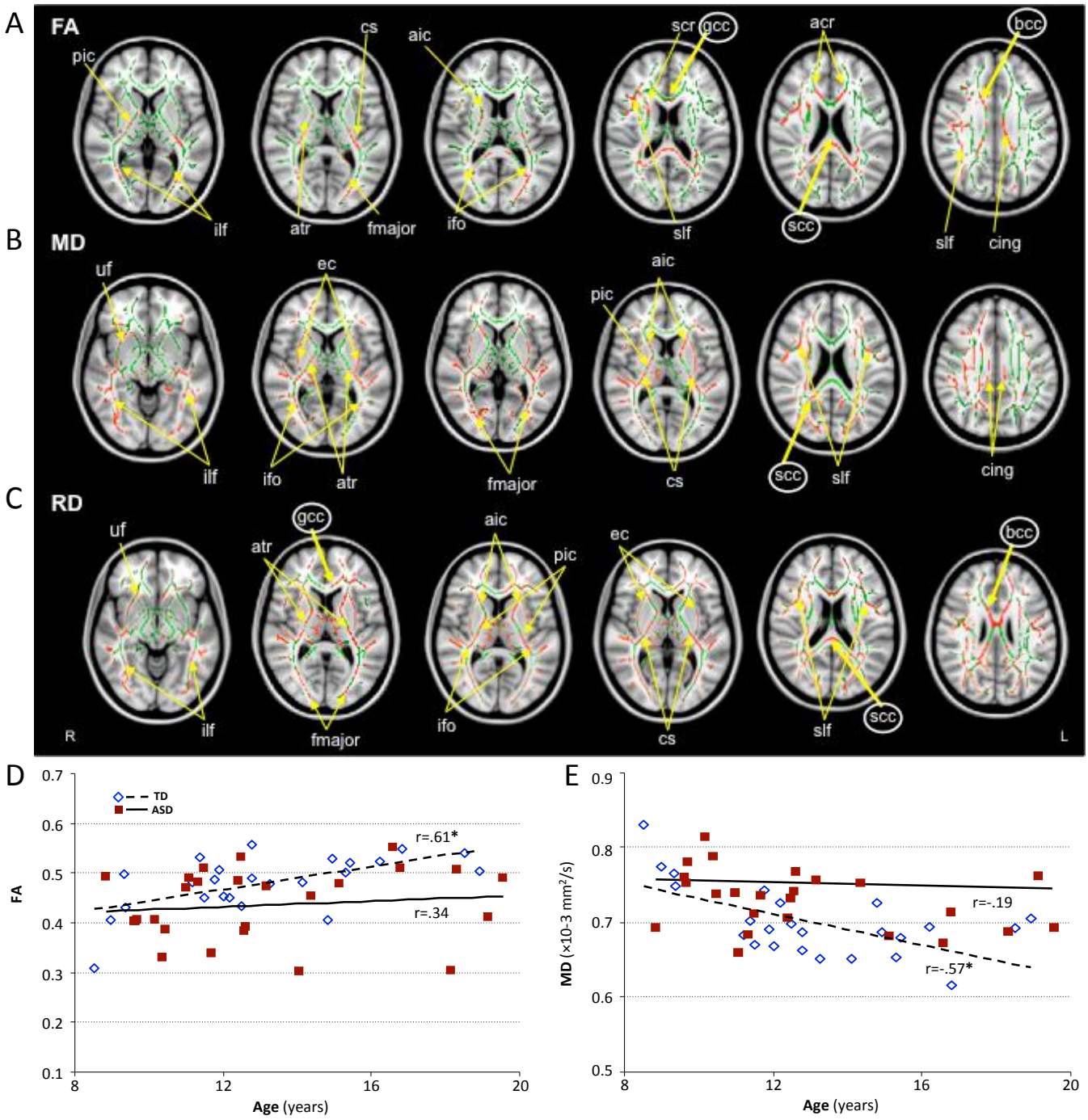
Radial diffusion (RD). A negative DTI index of white matter integrity. Increased diffusion perpendicular to main diffusion direction (along axons) may reflect damage to axons or lack of myelination (see Figure 1).

Figure 1: Water diffusion within and outside fiber tracts



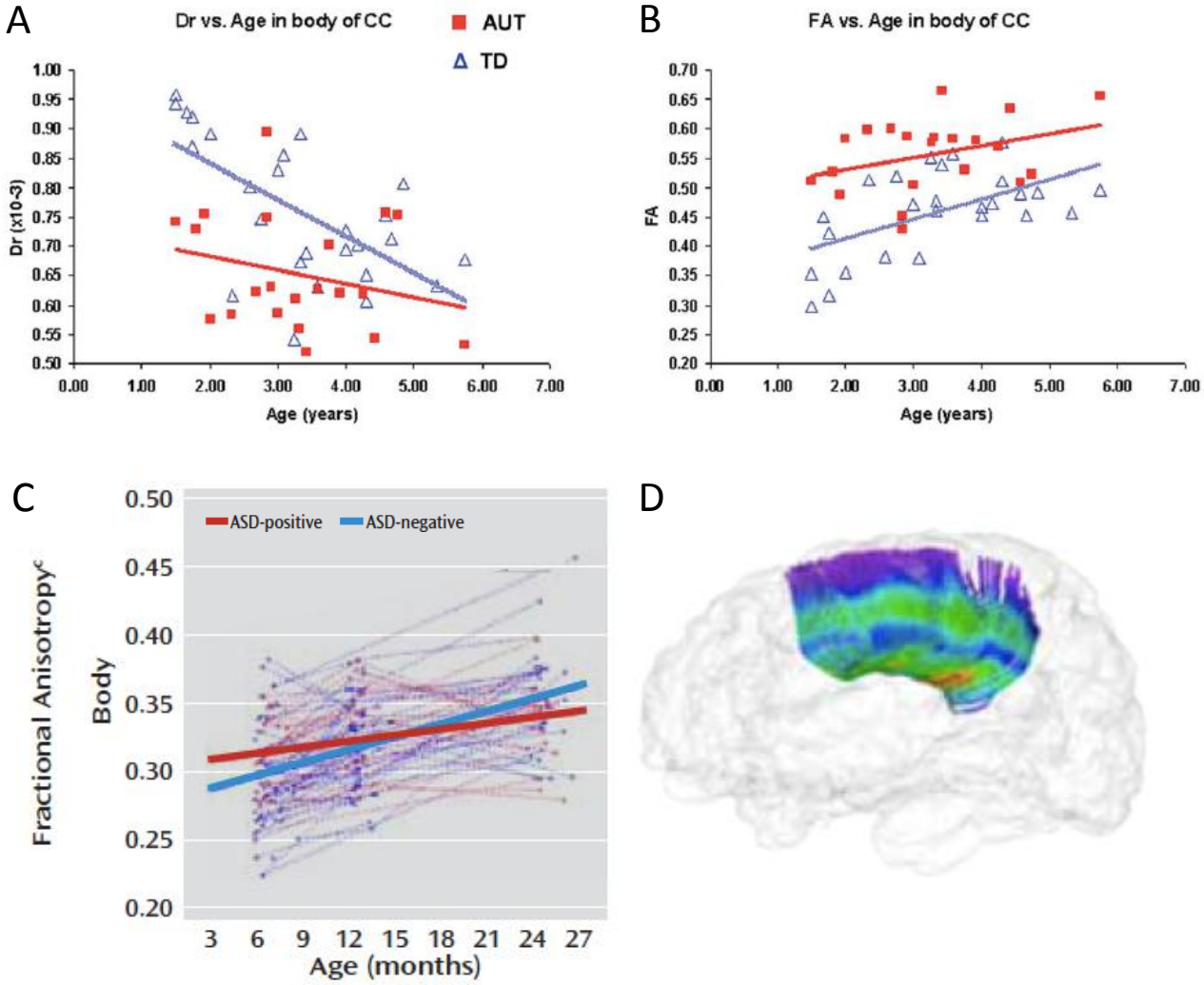
Legend to Figure 1. Water diffusion is *isotropic* (same in all directions) outside fiber tract (indicated by gray circles). Inside fiber tract, diffusion is *anisotropic* (strong in the axial direction, weak in the radial direction) because it is restricted by axon membranes and myelin sheaths. Tissue integrity is therefore reflected in high fractional anisotropy (FA), low radial diffusion (RD; indicated by short arrows inside ellipsoids), and low mean diffusion (MD; illustrated by reduced overall size of ellipsoids compared to circles).

Figure 2: DTI evidence of white matter compromise and reduced age-dependent changes in ASD



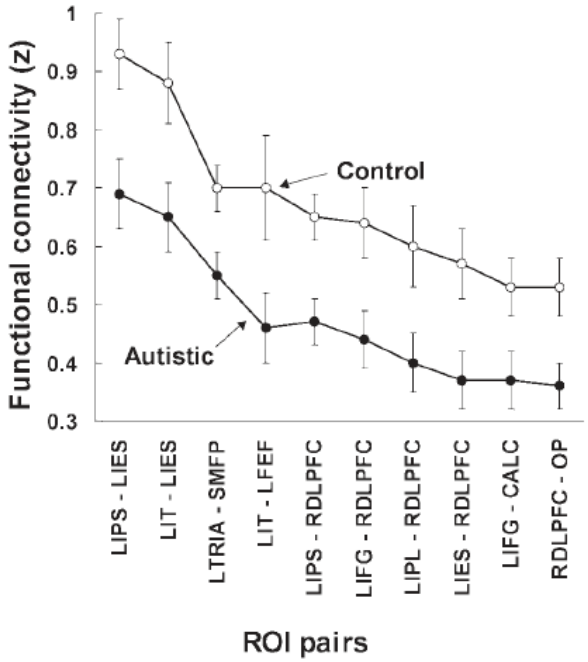
Legend to Figure 2. (A-C) Axial brain slices showing main fiber tracts (in green) and regions of significantly reduced FA (A) and increased MD (B) and RD (C) in children and adolescents with ASD, compared to matched TD children. Findings in corpus callosum are circled and highlighted by thick yellow arrows. Evidence of white matter compromise is found in numerous other tracts as well. Abbreviations are (in alphabetical order): acr: anterior corona radiata, aic: anterior internal capsule, atr: anterior thalamic radiation, bcc: body of corpus callosum, cing: cingulum, cs: corticospinal tract, , ec: external capsule, fmajor: forceps major, gcc: genu of corpus callosum, ifo: inferior fronto-occipital fasciculus, ilf: inferior longitudinal fasciculus, pic: posterior internal capsule, scc: splenium of corpus callosum, scr: superior corona radiate, slf: superior longitudinal fasciculus, uf: uncinate fasciculus. (D) Significant age-related increase in FA and decrease in MD (E) seen in TD group is diminished in ASD. Adapted from Shukla et al. (2011).

Figure 3: DTI evidence for infants and toddlers



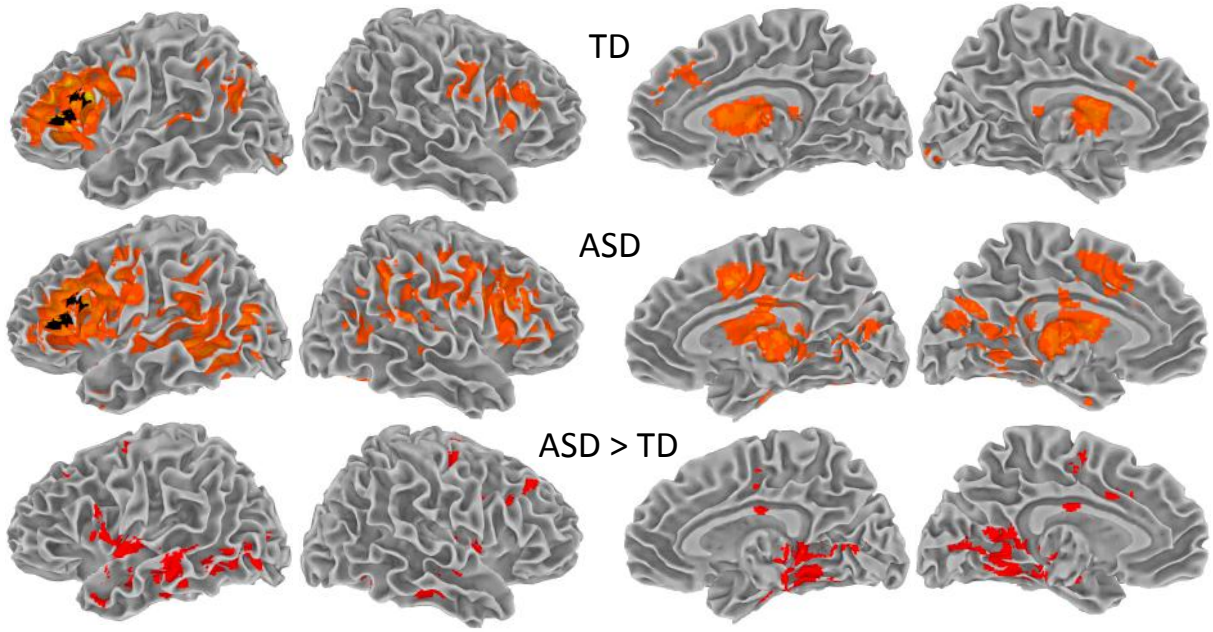
Legend to Figure 3. (A-B) Age-related changes in RD (A) and FA (B) within the body of the corpus callosum from Weinstein et al. (2011). FA is increased and MD reduced in infants and toddlers with ASD compared to TD participants. (C) Age-related changes in FA within the callosal body, from Wolff et al. (2012). FA is increased in high-risk infants later diagnosed with ASD, compared to infants that turned out to be negative for ASD. However, the difference disappears towards the end of the first year and is inversed by 24 months of age. (D) Illustration of the callosal tract examined.

Figure 4: Functional underconnectivity for sentence comprehension in ASD



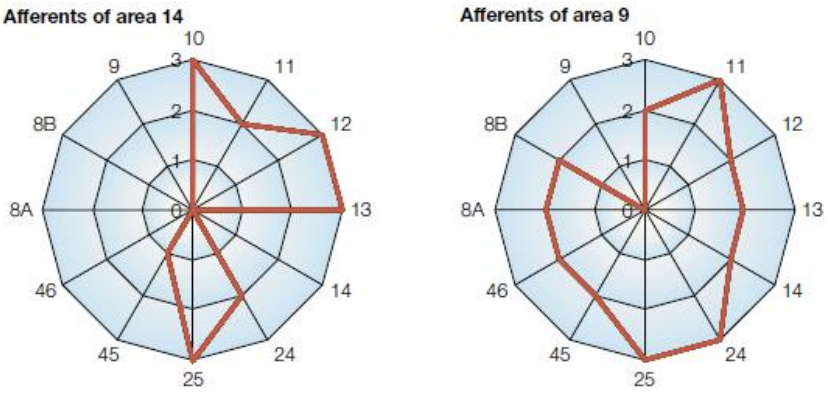
Legend to Figure 4. BOLD time series correlations for ASD and TD control participants in 10 ROI pairs with significant group difference (p <.05). Error bars represent the standard error of the mean. Abbreviations: L: left; R: right; CALC: calcarine fissure; DLPFC: dorsolateral prefrontal cortex; FEF: frontal eye field; IES: inferior extrastriate; IFG: inferior frontal gyrus; IPL: inferior parietal lobe; IPS: intraparietal sulcus; IT: inferior temporal; TRIA: triangularis; OP: occipital pole; SMFP: superior medial frontal paracingulate. From Just et al. (2004).

Figure 5: Functional overconnectivity of Broca's area in ASD from intrinsic fcMRI



Legend to Figure 5. Functional connectivity of seed in left inferior frontal gyrus (black area), identified based on shared activation for lexical semantic decision in TD and ASD groups. Connectivity within each group is shown in the top rows. Results from the group comparison show extensive effects of overconnectivity (ASD > TD). No inverse effects (TD > ASD) were detected. All clusters p < .05, corrected. Adapted from Shen et al. (2012).

Figure 6: Connectional fingerprints from studies in macaque



Legend to Figure 6. Radar plots of connectional fingerprints for Walker's areas 9 and 14 in the macaque prefrontal cortex, based on afferent (input) connections. From Passingham et al. (2002).