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Diffusion tensor imaging in autism spectrum disorders: Preliminary evidence of abnormal neural connectivity

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Abstract

Objective—This study indirectly tested the hypothesis that individuals with autism spectrum disorders (ASDs) have impaired neural connections between the amygdala, fusiform face area, and superior temporal sulcus, key processing nodes of the “social brain.” This would be evidenced by abnormalities in the major fibre tracts known to connect these structures, including the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus.

Method—Magnetic resonance diffusion tensor imaging was performed on 20 right-handed males (ASD = 10, controls = 10) with a mean age 13.5 ± 4.0 years. Subjects were group-matched according to age, full-scale IQ, handedness, and ethnicity. Fractional anisotropy was used to assess structural integrity of major fibre tracts. Voxel-wise comparison of white matter fractional anisotropy was conducted between groups using ANCOVA adjusting for age, full-scale IQ, and brain volume. Volumes of interest were identified using predetermined probability and cluster thresholds. Follow-up tractography was performed to confirm the anatomic location of all volumes of interest.

Results—All volumes of interest were regions of lower FA and were observed primarily in pericallosal regions and temporal lobes. As confirmed by tractography, affected white matter structures included the inferior longitudinal fasciculus/inferior fronto-occipital fasciculus, superior longitudinal fasciculus, and corpus callosum/cingulum. Notably, some volumes of interest were adjacent to the fusiform face area, bilaterally, corresponding to involvement of the inferior longitudinal fasciculus. The largest effect sizes were noted for volumes of interest in the right anterior radiation of the corpus callosum/cingulum and right fusiform face area (inferior longitudinal fasciculus).

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Conclusions—This study provides preliminary evidence of impaired neural connectivity in the corpus callosum/cingulum and temporal lobes involving the inferior longitudinal fasciculus/ inferior fronto-occipital fasciculus and superior longitudinal fasciculus in ASDs. These findings provide preliminary support for aberrant neural connectivity between the amygdala, fusiform face area, and superior temporal sulcus – temporal lobe structures critical for normal social perception and cognition.

Keywords

autism; connectivity; diffusion tensor imaging; social brain; white matter

INTRODUCTION

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by impairments in reciprocal social interaction and verbal/non-verbal communication, and the presence of stereotyped behaviour [1]. When Leo Kanner first described autism in 1943, he noted affected individuals’ “innate inability to form the usual, biologically-provided affective contact with other people” [2]. For over half a century, what exactly was “biologically provided” to typically developing children and missing in those with ASDs remained poorly understood. Concurrent advances in functional/anatomical imaging techniques studying brain-behaviour relationships [3] and the application of these promising technologies in the study of ASDs, has resulted in a substantial body of evidence converging on theories of aberrant brain connectivity [4–6]. It has been argued that the most adverse effects involve temporal lobe structures critical for normal social perception and cognition [7].

Until relatively recently it was impossible to know whether aberrant connectivity existed without post-mortem studies. With the advent of diffusion tensor imaging (DTI), *in vivo* assessment of white matter pathology became possible by measuring fractional anisotropy (FA), a measure of fibre tract coherence derived from water diffusion properties [8]. Moreover, DTI can also be used to examine the 3D structure of major axonal bundles, using a technique called tractography [9]. Use of DTI has become increasingly common in the study of neuropsychiatric disorders, including ASDs [10–12]. However, earlier studies using DTI in ASDs have limited their analyses to voxel-wise comparisons of FA. Although useful for identifying the presence of white matter pathology, this approach is very limited in its ability to identify the underlying white matter structures involved (i.e. specific fibre tracts). The latter can be addressed by taking advantage of the wealth of anatomical information available through tractography, a technique implemented in the current study.

Thus, using the combination of both voxel-wise comparisons of FA and follow-up tractography, the current DTI study was conducted to investigate the integrity of major fibre tracts between key structures critically important for normal social information processing. Based on a large body of converging evidence and a heuristic model of the pathophysiology of ASDs [7], it was hypothesized that individuals with ASD might have abnormalities in fibre tracts connecting the amygdala, fusiform face area, and superior temporal sulcus. This will be evidenced by reduced FA along the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus, two major fibre tracts known to link these major nodes of the “social brain” [13,14]. Given the consistent reports of corpus callosum abnormalities in ASDs, FA reductions are also predicted along this structure.

MATERIALS AND METHODS

Participants

Participants were 20 males with a mean age 13.5 ± 4.0 years (range = 8 to 19 years): 10 with an ASD and 10 typically developing controls (TDCs). Those included in the ASD group had confirmed DSM-IV [1] diagnoses of autistic disorder ($n = 4$), Asperger's syndrome ($n = 4$), or pervasive developmental disorder not otherwise specified (PDD-NOS, $n = 2$); and absence of medical and/or neurologic disease that might be associated with ASDs. Diagnosis of an ASD (autistic disorder, Asperger's syndrome, or PDD-NOS) was based on parental information, clinical history, and expert evaluation. In addition, two standard research diagnostic instruments were used: the Autism Diagnostic Interview - Revised [15] and the Autism Diagnostic Observation Schedule [16]. The TDC group consisted of healthy volunteers recruited from the same community as participants with an ASD. A semi-structured clinical interview was conducted with TDCs (and/or their parents) to rule out any history of neurological problems, neurological insult resulting in loss of consciousness, psychiatric disorders, and history of pervasive developmental disorders in first- or second-degree relatives. An age-appropriate version of the Wechsler Intelligence Scale [17,18] was administered to assess full-scale IQ. The study was approved by the University Human Investigations Committee. After study procedures were thoroughly explained, informed consent was obtained from all subjects or from their parent/guardian as appropriate. Verbal assent was obtained from all participants.

Image acquisition

MRI was performed using a 1.5-Tesla General Electric Signa system (Milwaukee, WI, USA). Single-shot, echo-planar imaging was used to acquire diffusion-weighted data using the following parameters: echo time = 92 ms, repetition time = 11200 ms, field of view = 256 mm, matrix = 128×128 , resolution = $2 \times 2 \times 4$ mm, slices = 36, thickness = 4 mm, and skip = 0. Diffusion sensitizing gradients were applied along six non-collinear directions ($b = 1000$ s/mm²). Two reference T2-weighted ($b = 0$) images were obtained per run, totalling six runs per subject (average = 6). Total DTI scan time was approximately 15 min, including all runs.

In addition, high-resolution T1-weighted images were acquired for each subject: 3D spoiled gradient recalled (SPGR), sagittal acquisition, contiguous images = 124, thickness = 1.2 mm, repetition time = 24 ms, echo time = 5 ms, flip angle = 45° , matrix = 192×256 , number of excitations = 2, field of view = 300 mm, and frequency encoding direction = superior/inferior. All T1 images included were judged as good quality by an experienced rater.

Image processing

Diffusion-weighted images were processed using the BioImage Suite software platform [19] and consisted of a motion correction and averaging process followed by a registration procedure to reslice images to a common space (Figure 1). All registrations used a normalized mutual information similarity metric [20]. Nonlinear registrations used the intensity-only component of an integrated registration method [21]. Applied mathematical approaches related to the analysis of structural MRI data (neuroanatomical MRI and diffusion-weighted imaging) are discussed in detail elsewhere [22].

All six diffusion-weighted runs for each subject underwent motion correction (Figure 1). Briefly, all volumes within each run were registered to the first b0 volume of that run. Next, all six runs of each subject were registered to the first b0 volume of the subject's fourth run. Prior to tensor calculation and generation of FA maps, all six runs for each subject were then

averaged to improve signal-to-noise ratio. The averaging procedure is equivalent to adding all runs together and dividing by the total number of runs.

Tensor analysis was performed on averaged motion-corrected runs to generate a motion-corrected FA map. These maps were subsequently resliced to a common space (a TDC subject), using transformations generated from (i) registrations of the individual b0 to SPGR images, and (ii) individual SPGR images to the common space SPGR image. To the common space image, six brain-limiting points, along with both anterior and posterior commissures were identified to place the image in standard Talairach 3D space. After fitting images to a standard 3D space, voxels representing grey matter, white matter, and cerebrospinal fluid were identified using a segmentation algorithm as described elsewhere [23].

Data analysis

The twenty FA maps were separated into ASD and TDC groups and used to create group-specific average and standard deviation (SD) FA maps. Voxel-wise, two-tailed t tests were performed at a threshold value of $t = 2.878$ ($p < 0.005$, two-tailed) to generate a t map displaying voxel clusters representing significant group differences between mean FA values. Any voxel cluster located within white matter (i.e. grey matter excluded) and surviving a threshold of 40 contiguous voxels (640 mm^3) was identified and labelled as a volume of interest (VOI). For each VOI, FA means and SDs were calculated and used for other statistical analyses. Effect sizes (Cohen's d) were calculated using means and SDs. Finally, ANCOVA of FA data was performed to adjust for (i) age and full-scale IQ, and (ii) age, full-scale IQ, and total brain volume. Covariance analyses were performed using a p-value of 0.005 (two-tailed). Again, any voxel cluster located within white matter and surviving a threshold of 40 contiguous voxels was identified and labelled as a VOI.

Anatomical locations of VOIs were first estimated with the aid of post-mortem, MRI, and DTI brain atlases [24–26]. These locations were then confirmed using tractography [27]. The fibre tracking process was initiated from a seed volume ($4 \times 4 \times 4 \text{ mm}$) placed according to the Talairach coordinates of the most significant p-value corresponding to each VOI. The fibre tracking program used the Runge-Kutta method for integrating the eigenvector field, following the principal direction of diffusion. The step length was set at 0.25 with a maximum angle of 45° allowed between steps. A minimum FA of 0.1 and a maximum FA of 1.0 were used as stopping criteria. Tractography was performed individually on all ASD subjects for each VOI with the resulting tracts sorted by VOI. For illustrative purposes, the authors identified one tractography image for each unique VOI as most representative of the underlying affected fibre tract(s).

RESULTS

Subject characteristics and volumetric measurements are summarized in Table 1. There were no significant differences in age and full-scale IQ between groups; however, full-scale IQ data were not available for three subjects in the TDC group. Total brain volume and total white matter volume were significantly higher in the ASD group; however, total grey matter volume was not significantly different between groups, though there was a trend towards statistical significance. Mean white matter FA did not differ significantly between groups.

Results are summarized in Table 2 and Figure 2. It is notable that there were no VOIs where FA was significantly higher in the ASD group. As illustrated in Figure 3, VOIs with significantly lower FA in the ASD group were located along the following white matter tracts as identified using atlases and confirmed by tractography:

1. Right anterior radiation of the corpus callosum/cingulum
2. Left anterior radiation of the corpus callosum/cingulum
3. Left anterior body of the corpus callosum/cingulum
4. Left posterior body of the corpus callosum/cingulum
5. Right body of the corpus callosum/cingulum
6. Left superior longitudinal fasciculus
7. Left inferior fronto-occipital fasciculus
8. Right inferior longitudinal fasciculus/inferior fronto-occipital fasciculus
9. Left inferior longitudinal fasciculus/inferior fronto-occipital fasciculus, seeded anteriorly
10. Left inferior longitudinal fasciculus/inferior fronto-occipital fasciculus, seeded posteriorly
11. Left inferior longitudinal fasciculus, seeded from the left fusiform face area
12. Right inferior longitudinal fasciculus, seeded from the right fusiform face area

Large effect sizes were noted across all VOI comparisons, ranging from 1.94–3.37. The largest were observed for the right anterior radiation of the corpus callosum/cingulum and right inferior longitudinal fasciculus, seeded from the right fusiform face area. This indicated a 100% non-overlap of TDC and ASD distributions for both VOIs. The peak *p* values for VOIs ranged from 0.001 to 0.000008, with the most significant *p* values observed in the right anterior radiation of the corpus callosum/cingulum and right inferior longitudinal fasciculus, seeded from the right fusiform face area. All VOIs remained statistically significant after co-varying for age and full-scale IQ, using the aforementioned thresholds. After adding total brain volume as a covariate along with age and full-scale IQ there was a loss of statistical significance in the left inferior fronto-occipital fasciculus (no. 7). VOIs where FA was lower in the ASD group were located along major white matter association and commissure tracts (Figure 2). While there was some expected individual variability, diffusion tensor tractography yielded fibre bundles easily identified as those major fibre tracts known to course through the identified VOIs (Figure 3).

DISCUSSION

The central question addressed by the present study was whether individuals with an ASD might have abnormalities in major fibre tracts connecting the amygdala, fusiform face area, and superior temporal sulcus as evidenced by reductions in FA along the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus. This study provides preliminary evidence of impaired connectivity in the temporal lobe involving these structures. The inferior longitudinal fasciculus and inferior fronto-occipital fasciculus have been demonstrated to extend from the amygdala and fusiform face area [13]. As is evident in Table 2, almost half (*n* = 5) of the identified VOIs involve the inferior longitudinal fasciculus and/or inferior fronto-occipital fasciculus and lie within the temporal lobe or near the temporal/occipital junction, all within proximity to the fusiform face area, amygdala, or superior temporal sulcus. In fact, inferior longitudinal fasciculus involvement is not apparent in the two VOIs seeded from the fusiform face area (Figure 2) until tractography is performed (Figure 3). As expected, abnormalities of the corpus callosum are also prominent and nearly half (*n* = 5) of the identified VOIs involve this structure (Table 2).

In addition to involvement of the inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and corpus callosum, there appears to be involvement of the superior longitudinal fasciculus and cingulum, which were not previously predicted. However, the involvement of the superior longitudinal fasciculus is intuitive given its well-known role in language and communication [28], which are core deficits of autism [1]. Moreover, its connection to the “social brain” has been demonstrated by virtue of its bridging the frontal lobe to the superior temporal sulcus [14]. The unexpected cingulum finding does not occur in isolation. That is, VOIs are not located in the cingulum, only. Therefore, it is plausible that this structure is highlighted with tractography because of normal anatomical overlap/crossing of the cingulum with the corpus callosum which cannot be resolved using conventional tractography techniques (see further discussion in limitations section). Moreover, these unexpected findings would not necessarily contradict the hypotheses set forth, and remain consistent with the literature on the neurobiology of ASDs. Abnormal connectivity need not be limited to the inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and corpus callosum. The possibility that other white matter structures are affected is expected, as this is consistent with the widely held belief that ASDs are heterogeneous and distributed disorders [29].

The finding of reduced FA in the current study is consistent with the majority of DTI studies performed in ASDs which also report mainly reductions in FA. It is important to note that despite the between-study differences in subject characteristics, image acquisition, and processing/analysis methodology, FA is consistently reduced, although the distribution of the reduction is variable. Out of six DTI studies in ASDs which examined whole-brain FA, four reported primarily on areas of reduction [11,12,30,31], one reported areas of decrease and increase [32], and one reported increases [33]. The last of these studies is uniquely different from the others because of its focus on young children ages 1.8–3.3 years (compared to older children and adults in the other studies), which is the age range for which accelerated brain growth has been reported [5]. Five studies restricted their analysis to specific areas including the corpus callosum [10], superior temporal gyrus [34], cerebellum [35], temporal lobe [36], and frontal lobe [37]. These regional studies primarily report abnormal FA or other measures of white matter structure, suggesting some degree of impairment. It is notable, however, that the last of these studies specifically examined short-range and long-range connections and did not find evidence of excessive short-range connectivity or reduced long-range connectivity [37]. However, the focus of that study was examining frontal lobe connections which excludes other key regions thought to play vital roles in the pathobiology of autism such as the temporal lobe [7] and possibly the cerebellum [38].

It is worth emphasizing that most of the aforementioned studies identified areas where FA is decreased compared to controls. Although there is some variation in anatomic location, most studies report a reduction in FA. In addition, studies examining the corpus callosum have reported abnormalities, but again there is some variation in exact locations. To date, these abnormalities may be the most consistent DTI findings in ASDs, and support a theory of impaired neural connectivity. Moreover, the corpus callosum consists of long commissural fibres, thus lending support to the possibility of reduced long-range connectivity and impaired inter-hemispheric communication. Evidence for impaired long-range connectivity is also supported by the current study, not only through extensive corpus callosum involvement, but also through the involvement of various association fibres such as the inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and superior longitudinal fasciculus. However, another version of the disconnectivity theory asserts that there is an overabundance of short-range connections [5,39]. This might theoretically result in higher FA in white matter closer to the cortex, which has not been reported in any study to date. However, the lack of findings could be due to methodological limitations (e.g. image

resolution). More specifically, the microscopic information about fibre tracts is averaged over a significantly larger voxel volume. If multiple U-fibres are overlapping with different orientations (i.e. disorganized), their contributions to the signal could be cancelled out [8].

In addition to the growing body of DTI literature examining ASDs, findings of the current study are also consistent with the larger body of behavioural, neuropathological, and neuroimaging data. Taken together, these data provide convergent support for abnormal brain connectivity in ASDs, with clear adverse effects on those temporal lobe structures critical for normal social perception and cognition [7]. Early hints to the neurobiology of ASDs were apparent in Kanner's seminal report when he noted that some affected children had enlarged heads [2]. Although a tendency towards megencephaly was confirmed in earlier studies [40], this observation only gained considerable attention in the 1990s when it was confirmed by neuroimaging studies [41], becoming the most consistent structural MRI finding in ASDs. Later research suggested that megencephaly was driven more by an increase in white matter rather than grey matter [42]. However, it also appeared that white matter was not uniformly enlarged. More recent volumetric studies in ASDs found that temporal lobe enlargement is greater than other lobes, with temporal lobe white matter enlarged about 10% [43]. Another consistently reported structural abnormality is a smaller corpus callosum which consists of long-range fibre tracts [44]. As mentioned previously, investigators have also suggested that the white matter abnormality consists of an overabundance short-range fibres [5,39]. These latter studies are consistent with neuropathological evidence of decreased neuronal cell size in ASDs, suggesting a paucity of long-range fibres since longer fibres require larger cell bodies to support their metabolic needs [45]. Therefore, the structural data seem to suggest a paucity of long-range fibres and relative overabundance of short-range fibres, possibly affecting the temporal lobes greater than other lobes of the brain.

Other than implicating the temporal lobe, which contains key components of the "social brain" [46], the structural findings alone do little to explain the social impairment unique to ASDs. Much behavioural data suggested that individuals with ASDs have deficits in face perception [47] which has been extensively studied using functional MRI. In fact, perhaps the best replicated functional MRI finding in ASDs is hypoactivation of the fusiform face area during face perception tasks [48], which has been replicated by at least nine independent laboratories [7]. In addition to fusiform face area hypoactivation, other studies have reported hypoactivation of the amygdala [46] and superior temporal sulcus [49] in ASDs. Functional MRI has also been used to study the presence of abnormal connectivity in ASDs which was implied by structural MRI findings. Multiple studies have reported reduced synchronization and functional connectivity in ASDs [49,50]. More importantly, there appears to be reduced effective connectivity between the fusiform face area and amygdala in ASDs [51]. Therefore, even without more direct evidence of impaired connectivity from DTI, convergent evidence from behavioural, neuropathological, structural MRI, and functional MRI findings appear to suggest aberrant brain connectivity with profound effects and consequences on the temporal lobe. The white matter abnormalities reported in the current study provide more direct evidence of aberrant connectivity in ASDs. Reductions in FA along the corpus callosum are consistent with abnormal long-range connectivity. FA reductions along the inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and superior longitudinal fasciculus in ASDs support aberrant connectivity between the amygdala, fusiform face area, and superior temporal sulcus, structures critical for normal social perception and cognition. Notably, these data are consistent with one proposed model on the developmental deficits in social perception and cognition in ASDs [7]. According to this model, congenital dysfunction of the amygdala disrupts normal social learning and adversely affects fibre pathways of the occipital

temporal projection system, including connections involving the amygdala, fusiform face area, and superior temporal sulcus.

Findings reported in this study must be interpreted in the context of several methodological limitations. The ASD sample was relatively heterogeneous, consisting of individuals with autistic disorder, Asperger's syndrome, and PDD-NOS. Including these individuals in one ASD group assumes that these disorders have a common neurobiological underpinning, an assertion which currently lacks strong empirical support as ASDs continue to be considered separate disorders according to DSM-IV [1]. Also, DTI remains an indirect method used to study fibre tracts. While 2D visualization (FA maps) has some validation, the 3D reconstruction of white matter tracts (tractography) requires further anatomic validation [52]. Resolution of DTI is limited such that conventional tractography techniques cannot resolve crossing/overlapping fibre tracts [8]. Furthermore, the current study uses only six gradient directions, which is the minimum number required for tensor calculation and generation of FA maps. Finally, tractography could not be performed using group-averaged data because tensors could not be properly averaged using standard registration procedures [53].

In summary, this study provides evidence of impaired neural connectivity in the corpus callosum and temporal lobes involving the inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and superior longitudinal fasciculus in ASDs. These findings partially support a theory of aberrant long-range neural connectivity between the amygdala, fusiform face area, and superior temporal sulcus; temporal lobe structures critical for normal social perception and cognition. The clinical implication of these findings is that individuals with ASDs are not, as Kanner put it, "biologically provided" the proper neural connections between those cortical nodes vital for processing social information, thus leading to the characteristic deficits that define this unique group of disorders. How such a system develops remains to be determined and continues to be an area of vigorous research.

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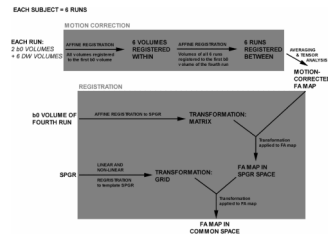


Figure 1. Schematic diagram of image processing procedure: diffusion-weighted images were processed using the BioImage Suite software platform and consisted of a motion correction and averaging process followed by a registration procedure to reslice images to a common space.

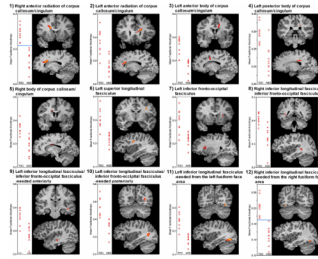


Figure 2. Scatterplots of mean fractional anisotropy for the 12 volumes of interest and anatomical location as seen in coronal and sagittal views; TDC, typically developing control group; ASD, autism spectrum disorder group, the horizontal blue line (#1 and #12) represents complete separation of data between groups.

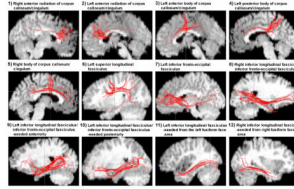


Figure 3. Diffusion tensor tractography: seeding from Talairach coordinates of the most significant p value for each volume of interest reveals fibre bundles readily identified as major association and commissure tracts. Seed locations are identified by green dots.

Table 1

Subject characteristics and volumetric measurements

	ASD		TDC		Student's t-test	
	N = 10		N = 10		df = 18	
	Mean	SD	Mean	SD	t-statistic	p-value
Clinical data [†]						
Age	13.06	3.85	13.94	4.23	0.487	0.632
FSIQ [‡]	91.00	24.79	105.00	17.83	1.276	0.222
Brain volume measurements						
Total volume	1522.51	96.55	1393.86	125.40	-2.570	0.019
Total gray matter volume	787.28	71.66	724.88	72.02	-1.942	0.068
Total white matter volume	476.11	50.07	423.41	60.22	-2.128	0.047

ASD, autism spectrum disorder group; TDC, typically developing control group; SD, standard deviation; FSIQ, full-scale IQ; age in years, volume in ml, italicized print indicates statistical significance ($p < 0.05$, two-tailed).

[†] Study was confined to right-handed Caucasian males.

[‡] FSIQ data missing from three TDC subjects; $df = 15$.

Table 2

Summary of affected fibre tracts corresponding to volumes of interest (VOI) or regions where structural integrity is significantly compromised

VOI/LOCATION SORTED BY MAJOR CEREBRAL REGIONS		ANALYSIS USING STUDENT'S T-TEST					Talairach coordinates: Peak p-value
		Volume	Mean FA ± SD		Cohen's d	Peak p-value	
			TDC	ASD			
Peri-callosal region							
1) Right anterior radiation of corpus callosum/cingulum	721	0.35 ±0.02	0.27 ±0.03	3.37	0.00003	14, 18, 25	
2) Left anterior radiation of corpus callosum/cingulum	553	0.38 ±0.05	0.28 ±0.03	2.26	0.00009	-14, 21, 23	
3) Left anterior body of corpus callosum/cingulum	252	0.36 ±0.04	0.28 ±0.03	2.43	0.0002	-11, -3, 33	
4) Left posterior body of corpus callosum/cingulum	91	0.32 ±0.02	0.26 ±0.03	2.32	0.0002	-18, -26, 30	
5) Right body of corpus callosum/cingulum	110	0.37 ±0.02	0.29 ±0.03	2.54	0.0005	11, -3, 29	
Frontal lobe							
6) Left superior longitudinal fasciculus	146	0.31 ±0.04	0.22 ±0.02	2.60	0.00004	-33, -9, 33	
7) Left inferior fronto-occipital fasciculus *	109	0.30 ±0.05	0.22 ±0.03	1.94	0.001	-23, 18, 14	
Temporal lobe							
8) Right inferior longitudinal fasciculus/ inferior fronto-occipital fasciculus	79	0.41 ±0.02	0.33 ±0.05	2.22	0.0007	35, -42, 5	
9) Left inferior longitudinal fasciculus/ inferior fronto-occipital fasciculus –seeded anteriorly	66	0.36 ±0.03	0.28 ±0.03	2.37	0.0005	-40, -20, -7	
10) Left inferior longitudinal fasciculus/ inferior fronto-occipital fasciculus –seeded posteriorly	290	0.47 ±0.05	0.36 ±0.04	2.33	0.0002	-34, -57, 8	
Temporal/Occipital lobe							
11) Left inferior longitudinal fasciculus –seeded left FFA	319	0.25 ±0.03	0.16 ±0.02	2.90	0.00002	-35, -68, -6	
12) Right inferior longitudinal fasciculus –seeded from right FFA	72	0.22 ±0.02	0.15 ±0.02	3.24	0.000008	35, -64, -8	

FA, fractional anisotropy; SD, standard deviation; ASD, autism spectrum disorder group; TDC, typically developing control group; FFA, fusiform face area, volume in ml.

* Loss of statistical significance after controlling for age, full-scale IQ, and total brain volume.