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The anatomy of extended limbic pathways in Asperger syndrome: A preliminary diffusion tensor imaging tractography study

Luca Pugliese [a,b,*], Marco Catani [a,b], Stephanie Ameis [a], Flavio Dell'Acqua [a,b], Michel Thiebaut de Schotten [a,b], Clodagh Murphy [a], Dene Robertson [a], Quinton Deeley [a], Eileen Daly [a], Declan G.M. Murphy [a]

[a] Section of Brain Maturation, Institute of Psychiatry, King's College London, London SE5 8AF, UK
[b] NatBrainLab, Institute of Psychiatry, King's College London, London SE5 8AF, UK

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ABSTRACT

It has been suggested that people with autistic spectrum disorder (ASD) have altered development (and connectivity) of limbic circuits. However, direct evidence of anatomical differences specific to white matter pathways underlying social behaviour and emotions in ASD is lacking. We used Diffusion Tensor Imaging Tractography to compare, *in vivo*, the microstructural integrity and age-related differences in the extended limbic pathways between subjects with Asperger syndrome and healthy controls. Twenty-four males with Asperger syndrome (mean age 23 ± 12 years, age range: 9–54 years) and 42 age-matched male controls (mean age 25 ± 10 years, age range: 9–54 years) were studied. We quantified tract-specific diffusivity measurements as indirect indexes of microstructural integrity (e.g. fractional anisotropy, FA; mean diffusivity, MD) and tract volume (e.g. number of streamlines) of the main limbic tracts. The dissected limbic pathways included the inferior longitudinal fasciculus, inferior frontal occipital fasciculus, uncinate, cingulum and fornix. There were no significant between-group differences in FA and MD. However, compared to healthy controls, individuals with Asperger syndrome had a significantly higher number of streamlines in the right ($p = .003$) and left ($p = .03$) cingulum, and in the right ($p = .03$) and left ($p = .04$) inferior longitudinal fasciculus. In contrast, people with Asperger syndrome had a significantly lower number of streamlines in the right uncinate ($p = .02$). Within each group there were significant age-related differences in MD and number of streamlines, but not FA. However, the only significant age-related between-group difference was in mean diffusivity of the left uncinate fasciculus ($Z_{obs} = 2.05$) ($p = .02$). Our preliminary findings suggest that people with Asperger syndrome have significant differences in the anatomy, and maturation, of some (but not all) limbic tracts.

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Introduction

Autism spectrum disorder (ASD, including autism and Asperger syndrome) is a relatively common neurodevelopmental disorder characterized by a triad of repetitive and stereotypic behaviour, impaired communication and striking deficits in social reciprocity (WHO, 1992). The biological basis of ASD, however, is poorly understood.

It has been suggested that some of the social and communication abnormalities typically found in people with ASD are secondary (Damasio and Maurer, 1978) to abnormalities in limbic structures, and perhaps also in their connectivity (Courchesne and Pierce, 2005b; Wickelgren, 2005).

The limbic system of the human brain is crucially involved in emotion, motivation and social behaviour. Limbic structures such as the cingulate and orbitofrontal cortex contribute to the development of self-awareness and the capacity to understand the intensions of others (Mega et al., 1997; Mundy, 2003). Other limbic structures, such as amygdala and hippocampus, are important for the storage of memory associated with emotional events, emotional processing of visual cues, and face perception (Cabeza and Nyberg, 2000; Kanwisher et al., 1997). Many of these cognitive/social functions attributed to the limbic system are affected in people with ASD.

Several lines of evidence suggest that people with ASD have differences in the anatomy of limbic regions. For example, early post-mortem investigations of both adults and children with autism reported reduced neuronal size and increased cell packing in the hippocampus, amygdala and to a lesser degree in the enthorinal cortex, mammilary bodies and septal nuclei (Bauman and Kemper, 1985; Bauman and Kemper, 2005; Palmen et al., 2004; Raymond et al., 1996). Moreover, recent *in vivo* voxel-based morphometry (VBM) studies reported significant differences in the anatomy of limbic regions, but with contrasting results with respect to the white matter compartment (Barnea-Goraly et al., 2004; Boddaert et al., 2004;

* Corresponding author. Institute of Psychiatry, PO50, De Crespigny Park, London SE5 8AF, UK. Fax: +44 207 848 0650.
E-mail addresses: luca_pugliese@yahoo.com, lucapugliese@hotmail.it (L. Pugliese).

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Herbert et al., 2003; Kwon et al., 2004; Lee et al., 2007, 2009; McAlonan et al., 2005; Salmond et al., 2005). For example, several groups reported decreased gray and white matter volumes in the inferior temporal regions and fusiform gyrus in both autism and Asperger syndrome in young adults (Boddaert et al., 2004; Kwon et al., 2004; McAlonan et al., 2005; Salmond et al., 2005). Herbert et al. also reported decreased gray matter volume in the same regions in young people with autism but increased white matter volume in regions containing limbic pathways (Herbert et al., 2004). White matter differences have also been reported in a recent voxel-based DTI study, which found that children with autism have significant microstructural differences (e.g. reduced fractional anisotropy) in the anterior cingulum and medial temporal lobe (Barnea-Goraly et al., 2004).

These studies were valuable first steps, however they measured regional differences in the anatomy of gray and/or white matter and did not address the pathology of specific limbic white matter tracts. Hence, evidence for an involvement of *specific* connections within the limbic system is still lacking. A technique that (in part) overcomes the limitations of VBM approaches is tractography applied to diffusion tensor magnetic resonance imaging (DT-MRI) datasets (Catani, 2006; Kanaan et al., 2006). DTI-tractography is the only technique that allows the simultaneous quantification of the white matter volume and microstructural integrity within specific tracts in the living human brain (Le Bihan, 2003). DTI-tractography has been used to perform virtual white matter dissection of the major limbic tracts in healthy brains (Basser et al., 2000; Beckmann et al., 2009; Behrens et al., 2003; Catani et al., 2002; Conturo et al., 1999; Mori et al., 2000) and in different neurological and psychiatric conditions (Ashtari et al., 2007; Ciccarelli et al., 2008; Concha et al., 2005; Gutman et al., 2009; Johansen-Berg et al., 2008; Jones et al., 2006; Kubicki et al., 2008; Price et al., 2008; Schneiderman et al., 2009; Widjaja and Raybaud, 2008).

We have recently applied DTI-tractography to study the anatomy of the cerebellar connections in adults with Asperger syndrome (Catani et al., 2008). However, to the best of our knowledge, tractography has never been applied before to study the anatomy and maturation of white matter pathways of the limbic system in ASD. Hence, in this study we used DTI-tractography to measure the volume and microstructural integrity of the major limbic tracts (uncinate, cingulum and fornix) in adults with Asperger syndrome and healthy controls. We also extended the analysis to other pathways connecting sensory areas to limbic regions (inferior longitudinal fasciculus and inferior frontal occipital fasciculus) for which there is indirect evidence of structural abnormalities in autism (Kleinhans et al., 2008; Pierce et al., 2001; Schultz et al., 2003; Wong et al., 2008). Finally we explored correlations between age and diffusivity properties along the dissected tracts to identify possible differences in maturational trajectories.

Materials and methods

Subject recruitment

We included twenty-four right-handed males with Asperger syndrome (aged 9–54 years; mean age 23 ± 12 years) and forty-two healthy control males (range 9–54 mean age 25 ± 10 years). People with Asperger syndrome were recruited from our clinical research program at the Maudsley Hospital and Institute of Psychiatry — part of the MRC (UK) A.I.M.S. network; whereas controls were recruited locally by advertisement. None had a history of head injury, major psychiatric disorder or medical illness affecting brain function (e.g. psychosis or seizures). All had routine blood tests and a clinical examination to rule out biochemical and hematological abnormalities, or genetic disorders that may be associated with ASD (including fragile × syndrome). Comorbidity was assessed using the Beck Depression Inventory (Beck et al., 1961), the Hamilton Depression and Anxiety Scales (Hamilton, 1960), and the Yale-Brawn Obsessive–Compulsive Scale (Goodman et al., 1989). No participant was taking medication for psychiatric disorder. After complete description of the study, written informed consent was obtained.

Patients were diagnosed by ADI and ADOS trained psychiatrists using International Classification of the Disease (ICD-10) research criteria. All patients fulfilled criteria for autism (F84.0) but without a history of language delay and so were classified as having Asperger syndrome (F84.5) (WHO, 1993). In addition, the Autism Diagnostic Interview (ADI) (Lord et al., 1994) was obtained in 18 people whose parents were available/willing to undergo additional interviewing. The ADI is a semi-structured clinical review that focuses on behaviours within the three domains that characterize the core features of autism. We carried out the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 1989) in people whose parents were unavailable or unwilling to undertake the ADI. The ADOS is a semi-structured evaluation for assessing social and communicative behaviours, through the observation of patients' behaviour.

All subjects underwent assessment of general intellectual functioning using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999), a 4-subtest IQ measure was administered. The subscale's 'similarities', 'vocabulary', 'block design' and 'matrix reasoning' were used to derive verbal, performance and full-scale IQ. These measures are age-standardised.

Image acquisition

A coronal three-dimensional spoiled gradient (SPGR) dataset covering the whole head was acquired. The parameters were: TR = 13.8 ms, TE = 2.8 ms, voxel resolution 256 × 256, field of view 220 mm, 124 slices, 1.5 mm slice thickness for the whole brain volumetric measurements. For the DTI analysis, a multislice echo-planar imaging (EPI) acquisition sequence, fully optimized for DT-MRI of white matter was used, providing isotropic resolution (2.5 mm × 2.5 mm × 2.5 mm) with a field of view 240 mm × 240 mm and coverage of the whole brain (echo time 107 ms, repetition time 15 R–R intervals, b-value 1300 s/mm^2). The acquisition was gated to the cardiac cycle using a peripheral gating device placed on the subjects' forefinger. A more detailed description of the acquisition protocol is reported in Jones et al. (2002).

Whole brain volumetric measurements

Manual tracing of intracranial volume (all brain tissue and cerebrospinal fluid within the dura mater) and total brain volume (all brain tissue but cerebrospinal fluid excluded) was performed on SPGR images using Measure software (Barta et al., 1997). Images were realigned along the anterior and posterior commissure line (AC/PC line) and volumes calculated by multiplying the summed pixel cross-sectional areas by slice thickness. To control for the relationship of total brain size to head size, volumes were normalized as a percentage of traced intracranial volume, and analyses were performed on both normalized and raw volumes. All the analysis was carried out blind to subject status and inter- and intra-rater reliabilities determined on a dataset of 10 images (>.90) (Bartko and Carpenter, 1976; McAlonan et al., 2002).

DTI processing and tractography algorithm

Following correction for the image distortions introduced by the application of the diffusion encoding gradients, the diffusion tensor was determined in each voxel following the method of Basser et al. (1994). Following diagonalization of the diffusion tensor, the fractional anisotropy (FA), which quantifies the directionality of the diffusion on a scale from zero (when the diffusion is totally random) to one (when the water molecule are able to diffuse along one

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direction only) (Basser and Pierpaoli, 1996), was estimated in each voxel.

Diffusion tensor data were processed according to the procedure originally described by Basser et al. (2000). Briefly a continuous description of the diffusion tensor field was derived from the voxel-wise discrete estimates by B-spline fitting a series of basis functions to the elements of the tensor matrices (Basser et al., 2000). This procedure allows rapid evaluation of the diffusion tensor at any arbitrary location within the imaged volume, and permits also smoothing of the tensor field. A set of locations for the initiation of the tracking algorithm, (referred to here as 'seed-points'), was first selected on the fractional anisotropy images. At the time of the analysis the operator was completely blind to subject status and additionally the brain hemispheres were flipped as further precaution. For each seed-point, the diffusion tensor was estimated to determine the principal eigenvector. The tracking algorithm then moved a distance of .5 mm along this direction. The diffusion tensor at this new location was determined from the continuous description of the tensor field and its orientation, of its principal eigenvector, was estimated. The tracking algorithm then moved further .5 mm along this new direction. A pathway was traced out in this manner until the fractional anisotropy of the tensor fell below a fixed arbitrary threshold (set to .2). A three-dimensional representation of the pathways was then generated by a set of stream tubes, to connect up the points, using MATLAB (Catani et al., 2002). Further details can be found in previously published papers (Basser et al., 2000; Catani et al., 2002; Jones et al., 2002).

Virtual dissections of the limbic tracts

We performed virtual dissections of the three major limbic pathways: i) the cingulum; ii) the uncinate fasciculus; iii) the fornix (Catani and Thiebaut de Schotten, 2008). In addition we dissected the inferior longitudinal fasciculus and the inferior frontal occipital fasciculus to extend the analysis to pathways that connect limbic structures to visual and auditory associative areas (Fig. 1). Other limbic tracts (e.g. mammillo-thalamic tract, stria terminalis) were not included in the analysis due to their small size and low degree of myelination.

The *cingulum* is a medial associative bundle that runs within the cingulate gyrus all around the corpus callosum. It contains fibers of different length, the longest of which run from the anterior temporal gyrus to the orbitofrontal cortex. The short U-shaped fibers connect the medial frontal, parietal, occipital, and temporal lobes and different portions of the cingulate cortex. The cingulum was dissected using a one-ROI approach. A single cigar-shaped region was defined on the top three slices. When the cingulum separated into two branches an anterior and posterior region were defined on each slice. It is important to remember that the majority of the fibers of the cingulum are short U-shaped fibers connecting adjacent gyri. Hence, the use of one-ROI approach allows the inclusion of most fibers of the cingulum in the analysis. Artifactual (callosal) fibers were removed using an exclusion ROI defined around the corpus callosum.

The *uncinate fasciculus* is a ventral anterior associative bundle that connects the anterior temporal lobe with the medial and lateral orbitofrontal cortex (Catani et al., 2002). A two-ROI approach was also used to dissect the uncinate fasciculus. The first ROI (temporal) was defined in the anterior temporal lobe, as described for the inferior longitudinal fasciculus. A second ROI was defined around the white matter of the anterior floor of the external/extreme capsule. The insula defined the lateral border of the ROI, and the lenticular nucleus its medial border. Artifactual fibers were removed by applying an *n* exclusion ROI defined around the occipital lobe.

The *fornix* is a projection bundle that connects the medial temporal lobe to the mammillary bodies and hypothalamus. A single ROI was defined around the body of the fornix. To visualize the entire course of the fornix (including its temporal portion), additional regions around the fimbriae of each side were included in the ROI.

The *inferior fronto-occipital fasciculus* is a ventral associative bundle that connects the ventral occipital lobe and the obitofrontal cortex. In its occipital course the inferior fronto-occipital fasciculus runs parallel to the inferior longitudinal fasciculus. On approaching the anterior temporal lobe, the fibers of the inferior fronto-occipital fasciculus gather together and enter the external capsule dorsally to the fibers of the uncinate fasciculus. A two-ROI approach was used to dissect the fibers of the inferior fronto-occipital fasciculus. The first region was delineated around the occipital lobe, and the second around the external/extreme capsule. The criteria used for the delineation of

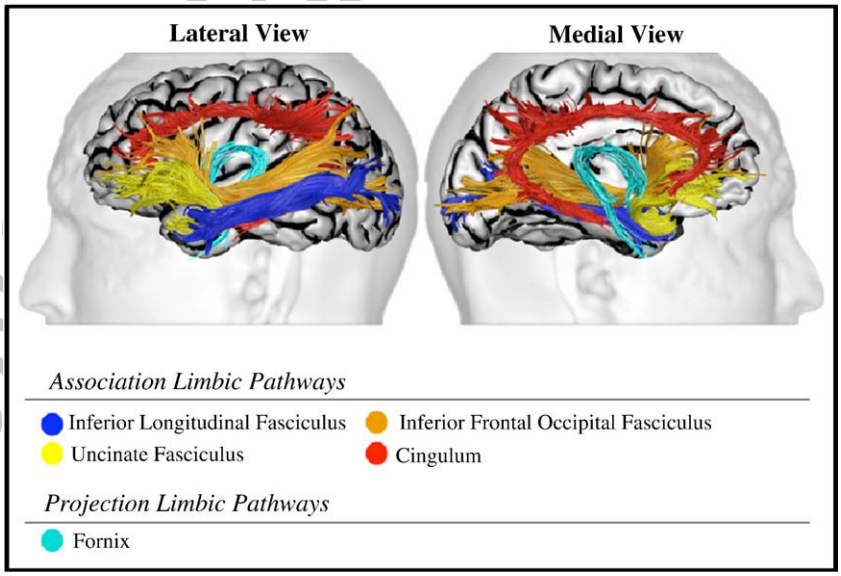

Fig. 1. Limbic association pathways: inferior longitudinal fasciculus (blue), uncinate (yellow), inferior frontal occipital fasciculus (orange) and cingulum (red). The fornix (light blue) belongs to projection system fibers. On the left hand side, lateral view of the limbic pathways, is easily to detect the most lateral tracts: inferior longitudinal fasciculus, uncinate and inferior frontal occipital fasciculus. The right hand side represents the middle view of the brain, where cingulum and fornix are easily to detect. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 1
Subject demographics.

	Subject groups		Significance	
	ASP ($n=24$)	HC ($n=42$)	Statistic	p value
Age, y	23.3 (12.4)	25.3 (10.3)	$t=.7$	.48
FSIQ	104.7 (12.05)	121.2 (16.1)	$t=4.3$	<.001
VIQ	107.6 (13.1)	119.2 (18.7)	$t=2.3$	.03
PIQ	99.2 (10.7)	117.5 (15.7)	$t=4.4$	<.001

Abbreviations: ASP, Asperger syndrome; HC, healthy controls; FSIQ, Full Scale IQ; VIQ, Verbal IQ; PIQ, Performance IQ. Data are expressed for combined sites as mean (SD).

these ROIs have already been described above (please see respectively sections on the inferior longitudinal fasciculus and uncinate fasciculus). Artifactual fibers were again removed by applying an exclusion ROI defined around the temporal pole.

The *inferior longitudinal fasciculus* is a ventral associative bundle with long and short fibers connecting the occipital and temporal lobes. The long fibers are medial to the short fibers and connect visual areas to the amygdala and hippocampus (Catani et al., 2003). A two-ROI approach was used to dissect the inferior longitudinal fasciculus. The first ROI (temporal) was defined around the white matter of the anterior temporal lobe. The second ROI (occipital) was defined around the white matter of the occipital lobe. The lowest region was defined on a slice containing the white matter of the lingual and fusiform gyrus. The most dorsal region was defined on the slice where the fibers of the left and right splenium join at the midsagittal line.

Inter-rater reliability between the operator (LP) and an experienced tractographer (MC) for the volume of the ROIs, the number of streamlines, the length of tracts and the tract-specific indices (FA, MD) was calculated on a sample of 10 datasets and was highly significant (>.90).

Main tractography outcome measures

Number and length of streamlines (SL) were calculated for each tract. Also fractional anisotropy (FA) and mean diffusivity (MD) at regular (.5 mm) intervals along the defined tracts were extracted and the means for each tract computed (Jones et al., 2006).

Statistical analysis

Statistical comparisons of the data were performed using SPSS software (SPSS Inc, Chicago, Ill). An independent sample *t*-test was used to compare age and IQ data and volumetric measurements. For the tractography outcome measurements, general linear model (GLM) analysis for repeated measures was used with side (left and right hemisphere) and tracts (uncinate, cingulum, inferior frontal occipital fasciculus and inferior longitudinal fasciculus) as the within-subject factors and group as between-subjects factor. Then, where significant interactions were detected, post hoc analysis was performed using independent student's *t*-test. The same analysis was repeated after co-varying for IQ. Within each group correlation analysis was performed between age and tractography measures using Pearson's correlation coefficient and between-group differences calculated using *Z*-observation analysis. All results are presented before and after Bonferroni correction for multiple comparisons ($p<.006$).

Results

Demographic and neuropsychological performances

There were no significant age differences between the two groups. However, the Asperger syndrome group had a significantly lower FSIQ as compared to the healthy controls (Table 1).

Tract-specific measurements

Streamlines (Fig. 2, Table 2)

Overall people with Asperger syndrome had a significantly higher number of limbic streamlines (mean = 154 ± 28) than controls (mean 136 ± 20; $p=.004$). Also there was a significant group-by-tract interaction [$F(3,62)=.81$, $p=.004$], which remained significant after co-varying for FSIQ. There was no significant group-by-side [$F(1,64)=.13$, $p=.72$] or group-by-side-by-tract interaction [$F(3,62)=.65$, $p=.58$]. Subsequent post hoc comparison of the individual tracts revealed that people with Asperger syndrome had a significantly higher number of streamlines bilaterally within the cingulum and the inferior longitudinal fasciculus as compared to controls, also after co-varying for brain volume. By contrast the Asperger group had

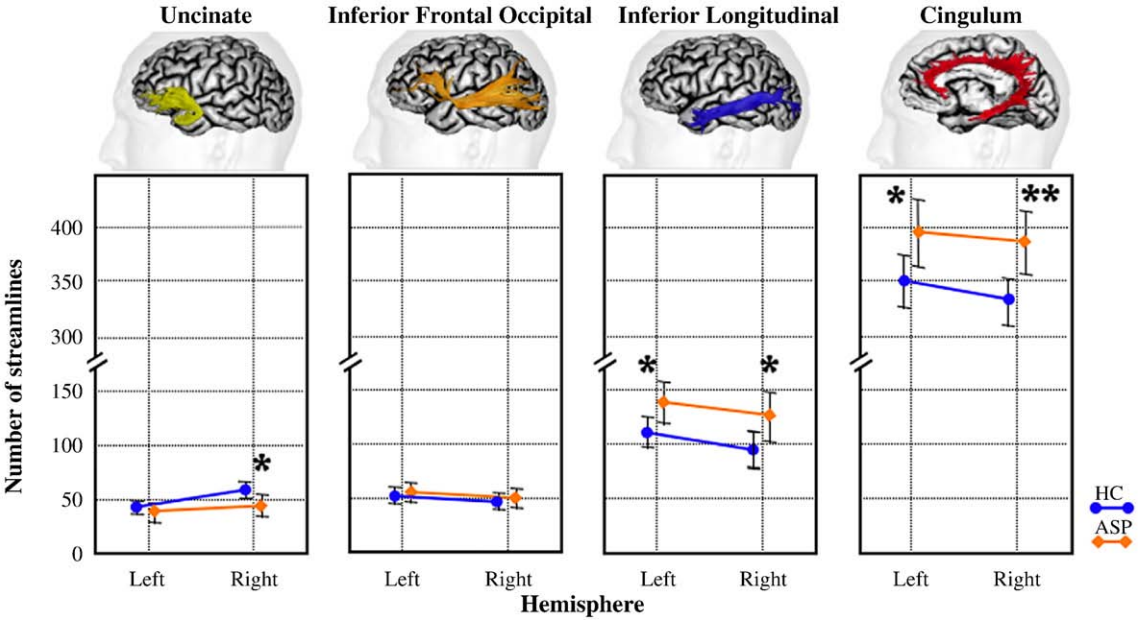

Fig. 2. Differences between autistic spectrum disorder (green) and healthy comparison (purple) group in the number of streamlines for limbic pathways. * Differences are significant at $p<.05$. **Differences are significant at $p<.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 2
Streamlines.

	Subject groups		Significance	
	ASP ($n=24$)	HC ($n=42$)	Statistic	p value
Uncinate left	36.3 (20.5)	40.6 (18.3)	$t=.87$	.40
Uncinate right	43.5 (20.5)	57.7 (25.0)	$t=2.4$	.02
ILF left	138 (46.2)	111 (51.5)	$t=2.1$	.04
ILF right	126.6 (65.7)	95.1 (47.1)	$t=2.2$	.03
IFOF left	56.0 (23.1)	53.4 (21.6)	$t=.47$	.64
IFOF right	50.2 (24.6)	46.4 (22.4)	$t=.64$	.52
Cingulum left	395.7 (93.3)	352.2 (66.4)	$t=2.2$	.03
Cingulum right	387.6 (72.1)	332.7 (67.9)	$t=3.1$	.003*
Fornix	148.3 (24.5)	150.3 (18.7)	$t=.36$	.72

After significant tract-by-group effect interaction [$F(3,62)=.81$, $p=.004$] post hoc test was performed. Abbreviations: ASP, Asperger syndrome; HC, healthy controls; ILF, Inferior longitudinal fasciculus; IFOF, Inferior frontal occipital fasciculus. Data are expressed for combined sites as mean (SD).

* Significance level set at $p<.006$ followed by Bonferroni adjustment.

a significantly lower number of streamlines within the right uncinate. All these differences survived Bonferroni correction (Fig. 2, Table 2).

An independent sample *t*-test analysis showed no statistically significant differences in the streamlines length in people with Asperger syndrome (mean = 84.5 ± 13.8) than controls (mean = 82.9 ± 19.6; $p=.51$).

MD and FA (Table 3, Table 4)

MD in the Asperger group was significantly increased in the inferior longitudinal fasciculus bilaterally, and in the right cingulum and inferior fronto-occipital fasciculus (Table 3). In contrast, individuals with Asperger syndrome had a significant decrease in FA of the inferior frontal occipital fasciculus bilaterally, and in the right uncinate fasciculus (Table 4). However, none of these differences survived Bonferroni correction.

Whole brain volumetric measurements

An independent sample *t*-test analysis showed no statistically significant differences in the whole brain volume between people with Asperger syndrome (mean = 1104.9 ± 116.8) and controls (mean = 1142.5 ± 102.1; $p=.31$). Similarly, no difference was found in the total cranial volume between people with Asperger syndrome (mean = 1442.9 ± 122.7) and controls (mean = 1471.8 ± 116.7; $p=.47$).

Age-related differences

Streamlines

Within people with Asperger syndrome there was no significant correlation between the number of streamlines and age in any of the limbic tracts we examined. In contrast, within controls, there was a significant positive correlation in the right ($r=.364$; $p=.018$) (Fig. 3) and left cingulum ($r=.332$; $p=.031$) (Table 5).

Table 3
Mean diffusivity (MD).

	Subject groups		Significance	
	ASP ($n=24$)	HC ($n=42$)	Statistic	p value
Uncinate left	.81 (.036)	.80 (.032)	$t=1.4$	.16
Uncinate right	.82 (.037)	.80 (.030)	$t=1.4$	.17
ILF left	.80 (.043)	.78 (.031)	$t=2.1$	.04
ILF right	.79 (.043)	.77 (.029)	$t=2.2$	.03
IFOF left	.79 (.040)	.78 (.026)	$t=1.7$	.08
IFOF right	.79 (.035)	.77 (.034)	$t=2.4$	.02
Cingulum left	.77 (.035)	.75 (.030)	$t=2.0$	.05
Cingulum right	.77 (.035)	.76 (.027)	$t=2.3$	.02
Fornix	1.10 (.05)	1.13 (.096)	$t=1.2$	.22

Abbreviations: ASP, Asperger syndrome; HC, healthy controls; ILF, Inferior longitudinal fasciculus; IFOF, Inferior frontal occipital fasciculus. Data are expressed for combined sites as mean (SD).

Table 4
Fractional anisotropy (FA).

	Subject Groups		Significance	
	ASP ($n=24$)	HC ($n=42$)	Statistic	p value
Uncinate left	.42 (.029)	.43 (.023)	$t=1.4$	.14
Uncinate right	.41 (.018)	.43 (.019)	$t=2.7$	.008
ILF left	.45 (.021)	.46 (.021)	$t=1.5$	.13
ILF right	.45 (.024)	.45 (.019)	$t=1.3$	.18
IFOF left	.47 (.021)	.48 (.023)	$t=2.2$	.03
IFOF right	.47 (.023)	.48 (.027)	$t=2.1$	.04
Cingulum left	.46 (.028)	.47 (.027)	$t=1.4$	.16
Cingulum right	.45 (.027)	.45 (.025)	$t=.2$	.87
Fornix	.42 (.016)	.41 (.22)	$t=.5$	.59

Abbreviations: ASP, Asperger syndrome; HC, healthy controls; ILF, Inferior longitudinal fasciculus; IFOF, Inferior frontal occipital fasciculus. Data are expressed for combined sites as mean (SD).

MD and FA

In people with Asperger syndrome MD was negatively correlated with age in all the limbic tracts except the fornix. Similarly, in the control group there was a negative correlation between MD and age in all tracts except for the inferior frontal occipital fasciculus, bilaterally, and the right inferior longitudinal fasciculus (Table 5). The only between-group difference in the correlation with age was in the MD of the left uncinate fasciculus. The age-related decrease in MD was greater for the Asperger group compared with the healthy controls ($Z_{obs}=2.05$) ($p=.02$). There were no statistically significant correlations between age and FA (Table 5).

Brain volume

Within people with Asperger syndrome there was no significant correlation between the brain volume and age. Similarly, within controls, no significant correlation between brain volume and age was found.

Discussion

In this DTI-tractography study we found significant differences in the limbic white matter anatomy of people with Asperger syndrome as compared to healthy controls. The most significant differences were

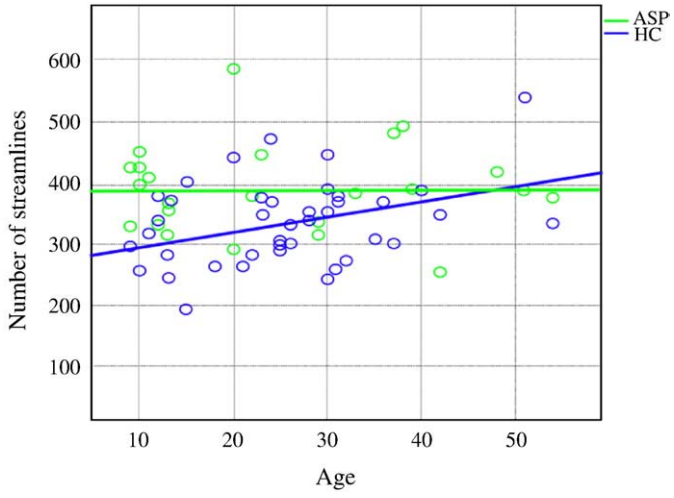

Fig. 3. Correlation between age and number of streamline in the right cingulum for autistic spectrum disorder (green) and healthy comparison group (purple). There is a medium positive correlation $r=.364$ between the two variables wit a statistical significant $p=.018$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 5
Correlation between age and tract-specific measurements.

		Fractional anisotropy				Mean diffusivity				Streamlines			
		HC ($n=42$)		ASP ($n=24$)		HC ($n=42$)		ASP ($n=24$)		HC ($n=42$)		ASP ($n=24$)	
		Statistic	p value	Statistic	p value	Statistic	p value	Statistic	p value	Statistic	p value	Statistic	p value
Left	Uncinate	$r=-.16$	.3	$r=.29$	.2	$r=-.32$	.04	$r=-.71$	.001*	$r=-.10$	.5	$r=.22$	.3
	IFOF	$r=.08$	.6	$r=.29$	.2	$r=-.30$	.05	$r=-.47$	.02	$r=.19$	.2	$r=.11$	.6
	ILF	$r=.06$	.7	$r=.23$	.3	$r=-.32$	.04	$r=-.63$	.04	$r=.11$	.5	$r=.10$	.7
	Cingulum	$r=.12$	.4	$r=.20$	.4	$r=-.49$	.001*	$r=-.52$	.009	$r=.33$	.03	$r=.19$	.4
Right	Uncinate	$r=.04$	.8	$r=.25$	.2	$r=-.47$	.002*	$r=-.66$	.001*	$r=-.22$	.15	$r=.13$	.5
	IFOF	$r=.06$	.7	$r=.25$	.2	$r=-.21$	.20	$r=-.47$	.02*	$r=.11$	.5	$r=-.10$	.6
	ILF	$r=-.14$	.4	$r=.15$	.5	$r=-.24$	.12	$r=-.64$	.001*	$r=.14$	.4	$r=.02$	.9
	Cingulum	$r=-.12$	.4	$r=.21$	.3	$r=-.41$	.004*	$r=-.67$	.001*	$r=.36$	.02	$r=.01$	.9
	Fornix	$r=-.06$	.6	$r=.25$	.2	$r=.16$	.29	$r=-.14$	.5	$r=-.21$	.17	$r=-.48$	.02

Abbreviations: ASP, Asperger syndrome; HC, healthy controls; ILF, Inferior longitudinal fasciculus; IFOF, Inferior frontal occipital fasciculus. Data are expressed for combined sites as mean (SD).

* Statistical significant after Bonferroni correction ($p<.006$).

in those tracts projecting to the anterior temporal lobe and the orbitofrontal cortex of both hemispheres (i.e. the cingulum, uncinate, and ILF). After correction for multiple comparisons, the differences were particularly marked in the right cingulum, which had both an increased number of streamlines (a possible surrogate of tract volume) and reduced MD (an index of microstructural integrity and/or tissue composition).

Our findings converge on previous reports on anatomical, metabolic and functional differences in the limbic regions of people with ASD. Decreased glucose metabolism and reduced FA have been reported, respectively, in the gray and white matter of the anterior cingulate regions across ASD (Buchsbaum et al., 1992; Haznedar et al., 1997, 2000; Rumsey et al., 1985; Siegel et al., 1992). The cingulate cortex is part of a network that engages in tasks associated with empathic cognition, social behaviour and pain perception; and this region has been reported to be significantly less activated during social tasks in people with ASD (Di Martino et al., 2008; Thakkar et al., 2008). Also the anterior and posterior cingulate cortices form part of the *default network,* a network that shows increased activation during "rest" condition or tasks involving individuals envisioning future events or considering other people's perspectives (Buckner et al., 2008), but which deactivates during cognitive or motor tasks (Fransson, 2006; Uddin et al., 2009). It has been hypothesized that a normal pattern of activation and deactivation of the default network is associated with normal identity development, and socio-emotional functioning (Buckner et al., 2008). Recent studies have shown reduced functional connectivity within the default network regions in ASD, suggesting an inability to disengage from internally driven self-reflective thinking (Cherkassky et al., 2006; Kennedy and Courchesne, 2008). Our findings suggest that these differences in the activation and metabolism of the cingulate cortex in ASD reported by others are accompanied by anatomical differences in white matter tracts connecting these medial limbic areas.

However we also found anatomical differences in other limbic tracts such as the right uncinate fasciculus and the ILF, albeit at a lower level of statistical significance (i.e. these additional findings did not survive correction for multiple comparisons). The right uncinate fasciculus, connects the anterior temporal lobe to the orbitofrontal cortex, and is part of a network underpinning episodic memory and autonoetic awareness (awareness of oneself as a continuous entity across time) (Levine et al., 1998). The ILF, connects the fusiform gyrus to amygdala and hippocampus (Catani et al., 2003), and plays an important role in social tasks requiring recognition of face emotion expression (which is altered in some subjects with ASD) (Kleinhans et al., 2008; Pierce et al., 2001; Schultz et al., 2003; Van Kooten et al., 2008; Wong et al., 2008). Hence, these preliminary findings suggest that white matter differences in Asperger syndrome extend beyond the cingulum network to other tracts connecting brain regions involved in visual processing and emotions (Sundaram et al., 2008).

Although the anatomical localization of our findings is consistent with a growing body of literature that implicates white matter abnormalities in people with Asperger syndrome, the biological meaning of the differences we found remains unknown. DTI-tractography does not visualize axons directly but it reconstructs their trajectories by measuring the diffusivity of water along different directions and by tracing a pathway of least hindrance to diffusion (parallel to the dominant fiber orientation) to form continuous streamlines. We, therefore, do not assume that our findings are equivalent to data obtained from post-mortem dissections, although, they are likely to reflect highly reproducible features of human brain anatomy (Catani et al., 2007). Thus, the increased number of streamlines in the cingulum may indicate a larger tract volume in people with Asperger syndrome, possibly due to increased myelination or number of axons/fibers; or alternatively a greater complexity of the connectional architecture (e.g. increased streamlines but normal volume). For some tracts we observed reduced FA and increased MD. In the adult healthy brain, anisotropy and mean diffusivity appear to be independent, i.e., the mean diffusivity is uniform across all brain parenchyma irrespective of the anisotropy (Pierpaoli et al., 1996). This pattern of reduced anisotropy, but elevated mean diffusivity, is commonly seen in DT-MRI studies of brain diseases where the integrity of the axonal membrane, or the myelin sheath, is compromised leading to an increase in the diffusivity across the fibers — and thus a reduction in anisotropy and increase in mean diffusivity. Intense inflammation and gliosis of white matter have been observed by some in post-mortem autistic brains (Vargas et al., 2005); if this is also the case in the population we studied then it may affect both fractional anisotropy and mean diffusivity due to breakdown of neuronal membranes.

Finally we investigated age-related differences in the limbic pathways. There was a significant correlation between age and mean diffusivity in almost all pathways in both the Asperger syndrome and control groups. The only significant difference between the two groups was represented by the age-related decrease in MD of the left uncinate fasciculus, which was greater for the Asperger group as compared to controls. With respect to volumetric measurements we did not observe between-group differences in the age-related differences for the total brain volume. However, within people with Asperger syndrome the number of streamlines is increased at a younger age and does not change in adulthood. In contrast healthy controls have a relatively lower number of streamlines at a younger age but this increases throughout adolescence and early adulthood.

Previous volumetric studies of children with ASD reported significant differences from controls in brain growth trajectory — with ASD children having increased total brain volume during the first

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two years of life in but an apparent growth arrest at a later age (Courchesne, 2003; Courchesne and Pierce, 2005a; Dementieva et al., 2005; Gillberg and de Souza, 2002; Lainhart et al., 1997; Redcay and Courchesne, 2005). There are no studies in children with Asperger syndrome, but previous studies found no differences in the total brain volume between adults with Asperger syndrome and controls. Our volumetric results are in line with previous studies in people with Asperger syndrome. However, the age-related differences in the cingulum may reflect a specific overgrowth with subsequent arrest in early adulthood (Fig. 3). These initial findings also suggest that age should be carefully taken into account as a possible confound in future studies.

Our study has a number of limitations. It was a cross-sectional investigation restricted to older children and adults with Asperger syndrome. Clearly future studies are required including younger populations, and others from across the autistic spectrum. Further, we did not have ADI and ADOS scores for all individuals in the study and this limited our ability to relate biological differences to behaviour. Also there was a significant difference in the IQ between the two groups, which we have taken into account by co-varying for it in all our analyses. Further our findings cannot be explained in differences in physical health as we excluded people with physical disorders affecting brain.

In conclusion this preliminary study found that DTI-tractography can be applied to study the limbic pathways of people with ASD. Our findings suggest that people with Asperger syndrome have anatomical differences in specific limbic white matter tracts and other tracts connecting sensory areas to temporal and orbitofrontal limbic regions. Future studies are needed to replicate our findings, and to relate anatomical differences to abnormal behaviour.

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