

# Abnormal functional connectivity in autism spectrum disorders during face processing

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**Abnormalities in the interactions between functionally linked brain regions have been suggested to be associated with the clinical impairments observed in autism spectrum disorders (ASD). We investigated functional connectivity within the limbic system during face identification; a primary component of social cognition, in 19 high-functioning adults with ASD and 21 age- and IQ-matched control adults. Activation during identification of previously viewed faces and houses using a one-back paradigm was compared. The fusiform face area (FFA) was individually localized in each participant and used as the seed point for functional connectivity analyses. The degree of correlation between FFA and the extended neural circuitry involved in face identification was tested. A whole brain analysis was also conducted in order to determine whether connectivity from the FFA to aberrant brain locations was present in the ASD group. Measures of clinical severity (ADOS social score and ADI-R social score) were included as independent variables into the functional connectivity analyses. Significant FFA-amygdala and FFA-superior temporal sulcus functional connectivity was found in both the ASD and control participants. However, the control group had significantly increased connectivity to the left amygdala and the posterior cingulate compared to ASD. *Post hoc* analyses additionally found increased connectivity to the thalamus in the controls. A significant relationship between abnormal functional connectivity and clinical severity in the ASD group was observed. Specifically, greater social impairment was associated with reduced FFA-amygdala connectivity and increased FFA-right inferior frontal connectivity. These results suggest that abnormal neural connections within the limbic system may contribute to the social impairments observed in ASD.**

**Keywords:** autism; Asperger's disorder; functional connectivity; face processing; brain imaging

**Abbreviations:** ASD = autism spectrum disorders; FFA = fusiform face area; RFFA = right fusiform face area; fMRIB = centre for functional MRI of the brain; ROI = region of interest

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## Introduction

Face processing deficits are an early-emerging characteristic of individuals with autism spectrum disorders (ASD) that may contribute to impairments in eye gaze, joint attention, responses to emotional displays and face recognition (Dawson *et al.*, 2005). Behavioural studies in ASD have identified specific face processing abnormalities including poor memory for faces (Ozonoff *et al.*, 1990; Boucher and Lewis, 1992; Teunisse and De Gelder, 1994; Boucher *et al.*, 1998), impaired perception of trustworthiness (Adolphs *et al.*, 2001), reduced face inversion effect (Hobson *et al.*, 1988) and reduced attention to the eye

region (Klin *et al.*, 2002), as well as abnormalities in perceiving emotions associated with facial expressions. Because of the fundamental role that face processing plays in guiding social interactions, it has been hypothesized that abnormalities in the neural circuitry involved in face processing contribute to social dysfunction in individuals with ASD (Schultz *et al.*, 2003; Dawson *et al.*, 2005).

Typically developing individuals exhibit increased activation to faces compared to other classes of objects in the lateral fusiform gyrus (see e.g. Kanwisher *et al.*, 1997; Haxby *et al.*, 2002), suggesting that face processing is mediated by this brain region, termed the fusiform face

area (FFA). Abnormal development of the FFA was initially proposed as a critical component of face processing abnormalities in high-functioning ASD following several reports of reduced and/or absent activation to faces in this brain region (Schultz *et al.*, 2000; Pierce *et al.*, 2001; Hubl *et al.*, 2003; Dalton *et al.*, 2005). However, subsequent studies have failed to find differences in FFA activation (Hadjikhani *et al.*, 2004, 2006; Bird *et al.*, 2006; Dapretto *et al.*, 2006), or found that FFA activation differences were mediated by task demands (Piggot *et al.*, 2004; Wang *et al.*, 2004), familiarity (Pierce *et al.*, 2004) or amount of time spent fixating on the eyes (Dalton *et al.*, 2005). Thus, current evidence suggests that abnormal fusiform activation to faces may not be driven by a primary neuroanatomical deficit in this brain region. Instead, several investigators have proposed that neural abnormalities in social brain circuitry, particularly the amygdala, contribute to face processing abnormalities in ASD (Pelphrey *et al.*, 2004; Dalton *et al.*, 2005; Schultz, 2005; Hadjikhani *et al.*, 2006).

This study investigated the neural correlates of face processing in autism by acquiring fMRI data during attentive viewing of neutral faces and houses. Three main analyses were conducted. First, we tested whether individuals with ASD have reduced neural sensitivity to faces compared to typically developing individuals. Because our sample consisted of high-functioning ASD individuals with clinical profiles in the milder range, we hypothesized that no significant differences in fusiform activation to faces would be observed. Next, we tested whether abnormalities in the functional integration of the social brain network were present in ASD (i.e. connectivity to aberrant brain regions, reduced connectivity to expected brain regions) and whether functional connectivity abnormalities were associated with the level of clinical impairment in ASD. Functional connectivity techniques are statistical methods applied to fMRI data that are used to identify brain regions that may be exerting an influence over other brain regions. We used the psychophysiological interaction method described by Friston *et al.* (1997) in this study. The strength of this approach is the ability to identify brain regions that demonstrate a change in the strength of functional connections in relation to an experimental manipulation, which goes beyond identifying simple correlations between regional brain activation that may arise from unknown sources (Friston *et al.*, 1997). In the current study, the experimental manipulation involved comparing face perception to house perception. Thus, we identified regions with significantly stronger functional connectivity to the FFA when viewing faces as opposed to viewing houses. We hypothesized that individuals with ASD would show reduced connectivity between the FFA and the extended face processing brain regions (amygdala, inferior frontal gyrus) (Haxby *et al.*, 2000) and that the more severely affected individuals would have the most abnormal functional connectivity between brain regions. In contrast, we did not expect to find significantly reduced connectivity

between the FFA and the superior temporal sulcus (STS), because this brain region is part of the core face processing system involved in visual evaluation of faces, specifically the perception of eye gaze, expression and lip movement (Haxby *et al.*, 2000; Schultz, 2005).

## Methods

### Participants

Twenty-four adults with an ASD and 23 typically developing controls participated in the fMRI study. Three individuals with autism were excluded for excessive head motion during the fMRI scan. Two additional individuals with ASD and two typically developing controls were excluded because the functionally defined right 'fusiform face area' was not identified following fMRI scanning (see fMRI methods section below). Thus, the final sample included 19 individuals with ASD and 21 typically developing controls. All participants had a Full Scale IQ and Verbal IQ  $\geq 80$  as assessed by the Wechsler Adult Intelligence Scales, Third Edition. The ASD group was composed of eight individuals with autistic disorder, nine individuals with Asperger's disorder and two individuals with pervasive developmental disorder-not otherwise specified. Diagnoses were confirmed with the Autism Diagnostic Interview-Revised (ADI-R, Lord *et al.*, 1994), the Autism Diagnostic Observation Schedule (ADOS, Lord *et al.*, 2000) and clinical judgement based on all available information and DSM-IV criteria (American Psychiatric Association, 1994). The ASD and control groups did not significantly differ on age, Verbal IQ, Performance IQ or Full-scale IQ. Clinical and demographic information is reported in Table 1. Control participants were screened for current and past psychiatric disorders, history of a developmental learning disability and contraindications to MR imaging.

All included fMRI study participants (40/40) also participated in a separate study that assessed eye tracking during viewing of faces and other visual stimuli. Sixteen participants (nine ASD and seven typical) were excluded from the analyses for the following reasons: (i) For 11 participants, it was not possible to obtain a clear image of the pupil for the purpose of calibrating (e.g. due to droopy eyelids, watery eyes, dark eye colour that does not allow for enough contrast between pupil and iris or excessive blinks); (ii) four of the participants wore glasses or contact lenses, making it difficult to obtain accurate calibration and resulting in unsatisfactory data quality; and (iii) one participant experienced experimenter or equipment error (e.g. software, or difficulty calibrating/tracking due to problems with eye-tracking equipment, resulting in unsatisfactory data quality). The remaining sample that had usable eye-tracking and fMRI data included 10 individuals with ASD and 14 individuals in the control group ( $n=20$ ). Clinical and demographic information for this subset of participants is reported in Supplementary Table S1.

This study was approved by the University of Washington Human Subjects Institutional Review Board. Informed written consent was obtained from all study participants.

### fMRI data acquisition

Structural and functional MRI were performed on a 1.5T Signa MR imaging system (General Electric, Waukesha). The fMRI scan was collected using an echo-planar pulse sequence (TR/TE 3000/50 msec, 21 slices; 6-mm thick with 1-mm gap,  $64 \times 64$  matrix,

**Table 1** Participant characteristics

	ASD ( <i>n</i> = 19)			Control ( <i>n</i> = 21)			<i>P</i> -value
	Mean	SD	Range	Mean	SD	Range	
Age	23.5	7.8	18–44	25.1	7.6	18–43	0.58
Full-scale IQ	106.7	15.7	85–139	109.1	14.4	83–139	0.53
Verbal IQ	106.8	15.9	76–140	107.5	13.6	84–132	0.69
Performance IQ	105	16.2	83–133	108.5	17.1	78–136	0.51
ADOS subscales							
Communication	3.74	1.19	2–6				
Social	7.84	2.41	4–12				
ADI-R subscales							
Communication	14.68	5.29	8–24				
Social	17.84	6.06	10–29				
Repetitive behaviour	5.63	2.93	1–10				

114 vol total). An SPGR was collected for fMRI registration and anatomical localization (Sagittal 3D SPGR, TR/TE 11.1/2 msec, with voxel dimensions of .97 × 1.1 × 1.2 mm).

### fMRI task

A block-design paradigm was utilized. Eight blocks of 36 s each presented pictures of neutral faces (F) or houses (H). Each stimulus was presented for 3 s with no inter-stimulus interval. Participants were instructed to press a button every time identical stimuli appeared in succession; 30% of the trials were target stimuli. In addition to the eight stimulus blocks, 18-s fixation (+) blocks were inserted at the beginning, middle and end of the experiment. Thus, the experimental design was: +FHFH+FHFH+. Face stimuli were digital, greyscale photos of 24 Caucasian adult men and women without glasses or jewellery. House stimuli were digital photos of houses in the Seattle area edited for extraneous details (e.g. parked cars).

### fMRI processing and statistical analysis

#### Data pre-processing

fMRI data analyses were performed using the Centre for Functional MRI of the Brain (fMRIB) Software Library version 3.3 (FSL; <http://www.fmrib.ox.ac.uk/fsl/>). The following pre-processing steps were applied: the first two volumes were discarded; motion correction was conducted using Motion Correction fMRIB's Linear Registration Tool (MCFLIRT) (Jenkinson *et al.*, 2002); non-brain structures were removed using Brain Extraction Tool (BET) (Smith, 2002); data were filtered spatially using a Gaussian kernel of FWHM 5 mm and temporally using a Gaussian high pass filter of sigma = 72 s. Motion related components, identified using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) (Beckmann and Smith, 2004), were filtered from the data prior to statistical analyses. Condition effects were estimated at each voxel for the contrasts Face > House and House > Face for each participant using fMRIB's Improved Linear Model (FILM) with local autocorrelation correction (Woolrich *et al.*, 2001).

### Face and house identification task

In order to determine whether abnormalities in the fusiform gyrus were present in ASD, we investigated activation to neutral faces and houses during a one-back working memory task. Individual fMRI data were registered to the high-resolution MPRAGE and then warped to the MNI152 standard image using FLIRT (fMRIB's Linear Image Registration Tool) and re-sampled to 2 mm<sup>3</sup> voxels. Analysis of group-wise effects were conducted using FLAME (fMRIB's Local Analysis of Mixed Effects), a method for modelling and estimating the random-effects component of the measured inter-session mixed-effects variance. This method allows inference to be made about the wider population from which the subjects were drawn. Unpaired *t*-tests were conducted for the contrasts Face > House and House > Face. Two *a priori* regions of interest (ROI) were defined on the standard brain MNI152 (right fusiform gyrus and left fusiform gyrus) and tested separately for each statistical analysis.

### Functional connectivity analyses

We used functional connectivity techniques (Friston *et al.*, 1997) to identify face-specific abnormal connectivity between the FFA and the other brain regions involved in socio-emotional processing in individuals with ASD. The seed point for the functional connectivity analysis was the functionally defined FFA for each individual. The FFA was localized in each individual in native space by identifying his or her significant cluster of activation ( $P < 0.01$ , uncorrected) for the Face > House contrast within the right and left fusiform gyrus ROI using FILM. The right FFA (RFFA) was selected for the connectivity analysis because more participants had significant FFA activation in the right fusiform than the left fusiform. Two individuals with ASD and two typically developing controls did not have a significant FFA cluster in the right fusiform gyrus and were excluded from the study (see Participants section above). Following identification of the seed cluster, 19 individuals with ASD and 21 typically developing participants were included in the analyses. The average signal across the time series was extracted from the RFFA seed cluster and used to create individually specific, data-driven statistical models for each participant. The interaction between the time

series and conditions (Face > House) was tested (Friston *et al.*, 1997) using FILM with local autocorrelation correction (Woolrich *et al.*, 2001). The RFFA time series was used for all functional connectivity analyses. Each participant's output data from this procedure was input into the higher-level group analysis tool FLAME and analysed in the same manner detailed above. Statistical corrections for multiple comparisons were conducted using cluster-thresholding based on Markov Chain Monte Carlo sampling. The resulting clusters represent those voxels showing a significantly stronger correlation to the RFFA time series during the face blocks than the house block. Functional connectivity analyses were conducted between the RFFA and six previously defined ROIs: right and left amygdala, right and left temporal lobe and right and left inferior frontal gyrus. These ROIs were selected based on their known involvement in face processing (e.g. Cabeza and Nyberg, 2000; Haxby *et al.*, 2000) and to facilitate comparisons to previously published reports of face processing in ASD (Pierce *et al.*, 2004; Wang *et al.*, 2004; Dalton *et al.*, 2005; Bird *et al.*, 2006; Dapretto *et al.*, 2006; Hadjikhani *et al.*, 2006). In addition, a whole brain analysis was conducted to investigate whether connectivity from the RFFA to aberrant locations were present in the ASD group.

### Relationship between functional connectivity and clinical severity

In order to determine whether functional connectivity abnormalities were related to level of social impairment in the ASD group, we entered the ADOS social score and ADI-R social score, separately as regressor variables into FLAME using the functional connectivity data. The clusters of significant activation identified in this analysis reflect those voxels in which the degree of functional connectivity varies as a function of clinical severity. Analyses were conducted between the RFFA and the six previously defined ROIs noted above. In addition, a whole brain analysis was conducted to investigate whether connectivity to aberrant locations was related to clinical severity in ASD.

### Eye tracking

On a separate research study visit, subjects participated in a study of eye tracking during viewing of photographs of faces and other visual stimuli (Sterling *et al.*, in press). As stated previously, 10 subjects with ASD and 14 subjects from the control group had usable eye-tracking data.

### Testing apparatus

Eye saccades were recorded using the ISCAN Eye Tracking Laboratory (ETL-500) with the Headband Mounted Eye/Line of Sight Scene Imaging System (ISCAN Incorporated, Burlington, MA). The apparatus consists of a baseball cap supporting a solid-state infrared illuminator, two miniature video cameras and a dichroic mirror. These cameras send output directly to the Pupil/Corneal Reflection Tracking System and the Autocalibration system. The Pupil/Corneal Reflection Tracking System tracks the centre of the participant's pupil and a reflection from the surface of the cornea. The Autocalibration System uses the raw eye position data generated by the Pupil/Corneal Reflection System to calculate point of gaze information. Samples were collected at a rate of 240 Hz.

### Recording procedures

Participants were familiarized with the eye-tracking equipment then seated in a chair ~75 cm from a 21-inch computer monitor. Eye movements were recorded during viewing of static face stimuli. In addition, the experimenter monitored the participant's behaviour and eye movements. During calibration, the participant was required to hold his or her head still and look at calibration and fixation points on the monitor in front of them. Once calibration was successfully established, stimuli were presented. For participants requiring additional behavioural support during the eye-tracking session, a staff research assistant sat to the side and out of view of the participant, providing verbal reminders, encouragement and reiteration of the instructions as needed.

### Eye-tracking stimuli

Visual stimuli consisted of digitalized greyscale images of faces. Each image was 640 pixels in height and 480 pixels in width. These photos were matched to the face stimuli in the fMRI study, although different photographs were used in each experiment. Three categories of faces were presented: Familiar (either a parent or significant other); 'Unfamiliar' (photos of middle-aged women), and 'New Friend' (a repeating, randomly chosen unfamiliar face). Only the data from the 'Unfamiliar' condition was analysed for the current experiment.

### Passive viewing of stimuli

Each type of face (Familiar, Unfamiliar and New Friend) was presented 10 times for 8 s duration with a 2-s inter-stimulus (ISI) interval. Participants were given the following instructions: 'Please look at the pictures in any way you want.' No decision or judgement regarding the stimulus was required from the participant during passive viewing. During the ISI, participants were instructed to look at a crosshair in the centre of the screen; this allowed the examiner to verify that calibration remained accurate throughout the experiment and placed the participant's initial fixation between the eyes.

### Eye-tracking data

Fixations were defined as a gaze of at least 100 ms duration within 1° of visual angle. The ISCAN Point of Regard Data Acquisition software was used to define the ROIs within the visual face stimuli. The following uniform ROIs were defined on each stimulus: Head (including hair and ears), face (including the 'internal' features of the face, or the eyes, nose and mouth), right eye, left eye and mouth. The right eye and left eye regions were combined during data analysis to comprise a total 'eye' region. Because regions are mutually exclusive, fixations from all regions were combined to reflect total time on the face stimulus, designated 'total face' region. Raw data (number of recorded fixations and fixation durations for each region) were extracted with the ISCAN Fixation Analysis software and adapted to SQL and SPSS databases in order further explore the data via statistical analyses.

## Results

### fMRI behavioural performance

All participant's behavioural performance was at or near ceiling levels on the fMRI one-back task (Mean number

**Table 2** fMRI activation loci

Faces > Houses	location	Number of voxels <sup>a</sup>	max z	MNI coordinates		
				x (mm)	y (mm)	z (mm)
ASD	L FFA <sup>b</sup>	26	3.08	−44	−48	−22
	R FFA <sup>b</sup>	40	3.42	42	−54	−22
	R FFA <sup>b</sup>	16	3.53	46	−42	−22
Control	L FFA <sup>b</sup>	39	4.09	−42	−50	−24
ASD > Control		None				
Control > ASD		None				
House > Faces						
ASD	L fusiform <sup>b</sup>	839	6.66	−28	−48	−8
	R fusiform <sup>b</sup>	651	5.86	28	−54	−8
Control	L fusiform <sup>b</sup>	848	6.55	−28	−66	−12
	R fusiform <sup>b</sup>	647	5.97	30	−44	−14
ASD > Control		None				
Control > ASD		None				
RFFA connectivity						
ASD	R amygdala <sup>c</sup>	177	3.9	22	−12	−18
	L amygdala <sup>c</sup>	185	4.92	−24	−10	−16
	R mid. temp.(BA 22) <sup>c</sup>	907	3.71	50	−50	6
Control	R cuneus (BA17/18) <sup>d</sup>	2964	5.22	6	−96	2
	<i>R posterior cingulate</i>			6	−56	23
	<i>L thalamus</i>			−2	−12	6
	<i>superior colliculus</i>			4	−32	0
	L amygdala <sup>c</sup>	212	5.4	−22	−12	−16
	R amygdala <sup>c</sup>	183	4.65	20	−10	−18
	R mid temp. (BA 22) <sup>c</sup>	710	3.69	44	−60	10
ASD > Control		None				
Control > ASD						
	R posterior cingulate <sup>d</sup>	980	3.92	6	−62	14
	<i>L cuneus (BA 18/19)</i>			−10	−86	10
	<i>L posterior cingulate</i>			−12	−62	14
	L amygdala <sup>c</sup>	2	2.53	−26	0	−22
	thalamus <sup>e</sup>	35	3.33	2	−18	6
Clinical correlations						
ADOS social positive	R inferior frontal <sup>c</sup>	367	3.37	52	20	6
ADI-R social negative	L amygdala <sup>c</sup>	17	2.53	−32	−6	−22
	L amygdala <sup>c</sup>	1	2.35	−20	−8	−18

<sup>a</sup>voxel size = 2 mm<sup>3</sup>. <sup>b</sup>voxel height = z = 2.58,  $\alpha = 0.05$  (ROI volume corrected). <sup>c</sup>voxel height = z = 2.3,  $\alpha = 0.05$  (ROI volume corrected).

<sup>d</sup>voxel height = z = 2.3,  $\alpha = 0.05$  (whole brain volume corrected). <sup>e</sup>voxel height = z = 2.3,  $\alpha = 0.05$  (uncorrected).

Note: L = left, R = right, FFA = fusiform face area, MNI = Montreal Neurological Institute, mid = middle, temp = temporal.

MNI coordinates for additional brain regions included in the cluster are listed separately and italicized.

of accurate responses for the ASD group was 97.9% for Faces and 96.7% for Houses; and for the Control group was 99.8% for Faces and 98.7% for Houses).

## fMRI results

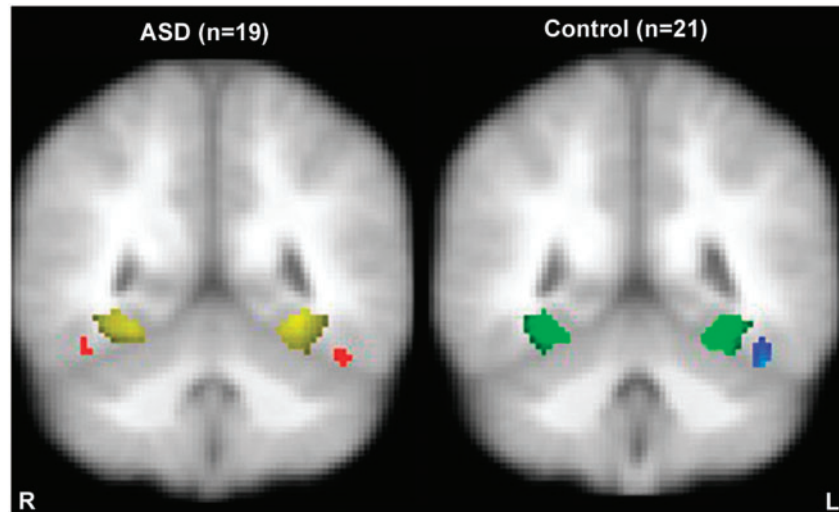
### Face and house perception task

We investigated face-specific (Face > House) and house-specific (House > Face) activation in the fusiform gyrus. Both groups showed significant activation in the lateral fusiform gyrus to faces and in medial fusiform gyrus

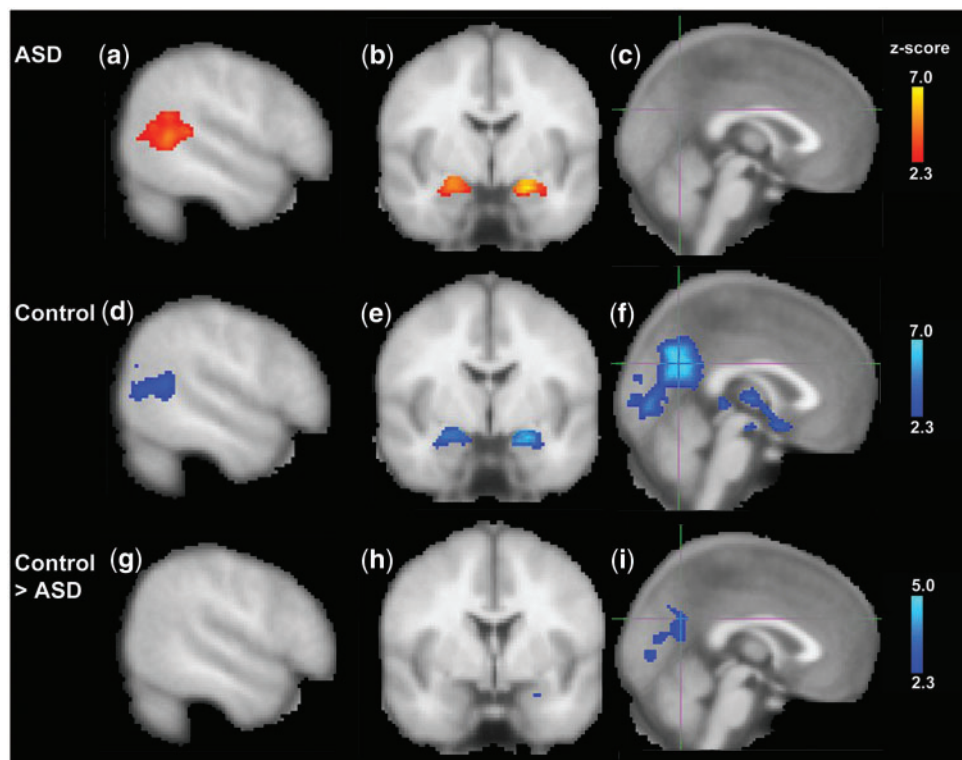
to houses (see Table 2, Fig. 1). No between-group differences in fusiform activation to faces or houses were found (see Table 2).

### Functional connectivity analyses

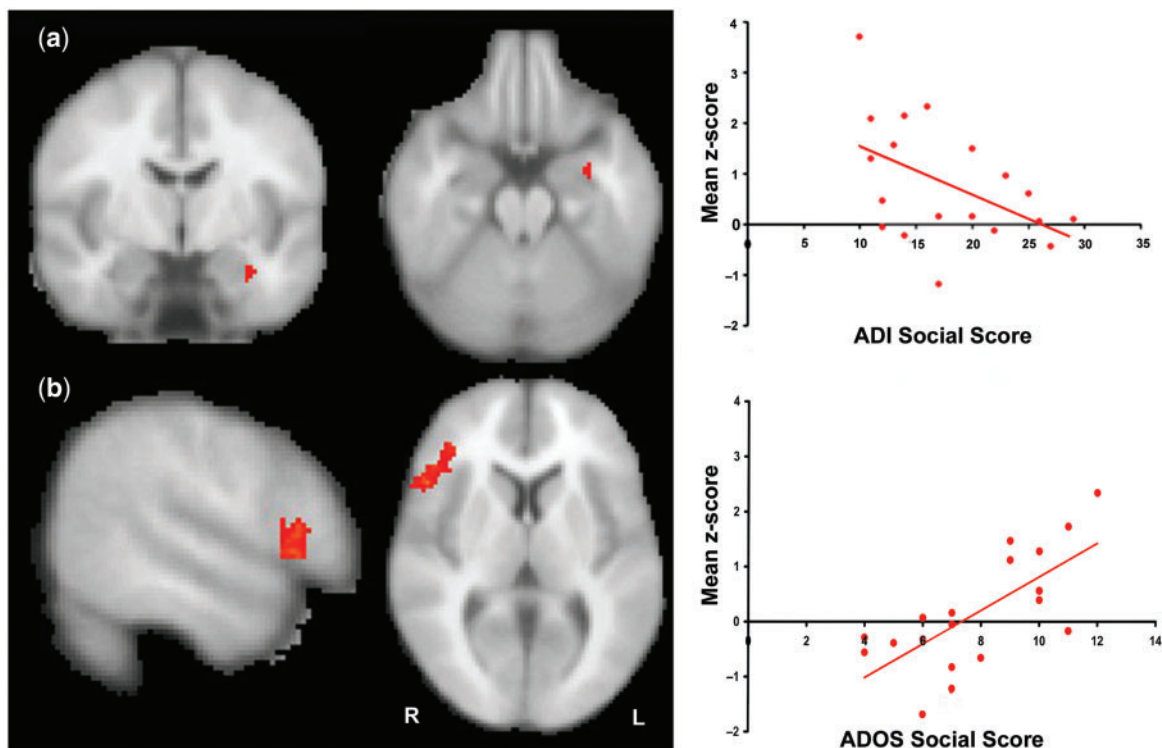
**Within group.** Clusters of activation demonstrating significantly increased connectivity to the right FFA associated with processing faces are reported in Table 2 and depicted in Fig. 2. In the autism group, significant functional connectivity clusters were found in the right amygdala,



**Fig. 1** Functional localization of faces and houses in the fusiform gyrus. Red activation corresponds to Face > House and yellow corresponds to House > Face in the ASD group. Blue activation corresponds to Face > House and green corresponds to House > Face in the control group. fMRI activation is cluster thresholded at  $P < 0.05$ , corrected.



**Fig. 2** Functional connectivity group results. The ASD group (a, b and c) showed significant, face-specific functional connectivity between the RFFA and right middle temporal gyrus/STS and bilateral amygdala (voxel height  $z > 2.3$ , cluster extent  $P < 0.05$ , small volume corrected). The control group (d, e and f) showed significant, face-specific functional connectivity between the RFFA and the bilateral amygdala and right middle temporal gyrus/STS (voxel height  $z > 2.3$ , cluster extent  $P < 0.05$ , small volume corrected); bilateral posterior cingulate, bilateral cuneus and the left thalamus (voxel height  $z > 2.3$ , cluster extent  $P < 0.05$ , whole brain corrected). In the direct group comparison (g, h and i), the control group showed significantly greater connectivity from the RFFA to the posterior cingulate, cuneus, (voxel height  $z > 2.3$ , cluster extent  $P < 0.05$ , whole brain corrected) and the left amygdala (voxel height  $z > 2.3$ , cluster extent  $P < 0.05$ , small volume corrected) than the ASD group. The ASD did not show significantly greater connectivity than the control group in any brain region (not pictured). Since the between-group difference in connectivity to the thalamus was based on *post hoc* analyses, that cluster of activation was not included in image (i). Descriptive information on this cluster is reported in Table 2.



**Fig. 3** Relationship between functional connectivity and clinical severity in the ASD group. **(a)** An inverse correlation between FFA-left amygdala connectivity and the ADI-R social score was found. A scatter plot depicting the relationship between face-specific connectivity and social severity on the ADI-R is shown to the right of the functional activation map. The mean z-score value for the amygdala was based on the average z-score of the cluster showing a significant relationship between connectivity and ADI-R social score (see Table 2). **(b)** A direct relationship between ADOS social score and activation in the right inferior frontal gyrus was found in the ASD group. The individuals with ASD with the most severe level of current functioning as measured by the ADOS showed increased connectivity to the right inferior frontal gyrus during face processing. The scatter plot depicts the relationship between the face-specific functional connectivity activation and the ADOS. The mean z-score values for the right inferior frontal was based on the average z-score of the cluster showing a significant relationship between connectivity and the ADOS social score (see Table 2).

right posterior middle temporal gyrus and right anterior middle temporal gyrus. In the control group, significant clusters spanning both hemispheres were found in several midline brain regions including the posterior cingulate, cuneus, the superior colliculus and thalamus. In addition, significant connectivity to the bilateral amygdala, which extended to the right anterior middle temporal gyrus was found.

**Between group.** There were no brain regions with significantly greater connectivity in the ASD group compared to the control group. However, the control group evidenced significantly greater connectivity between the right FFA and the left amygdala, bilateral posterior cingulate and the left cuneus than the ASD group. (see Table 2, Fig. 2). An exploratory *post hoc* analysis was conducted to follow up the observation that FFA connectivity to the thalamus and superior colliculus was present in the control group but not the ASD group. Using a liberal threshold, we found a cluster of activation in the midline thalamus reflecting significantly reduced connectivity between the right FFA and the thalamus in the ASD group compared to the controls ( $P < 0.05$ , uncorrected; see Table 2). There were

no group differences in the superior colliculus. An additional *post hoc* analysis investigating connectivity between the right and left FFA was conducted to provide additional verification that between-group differences in functional connectivity were unlikely to be related to noisier signal in the ASD group. Both groups evidenced significant connectivity between the right and left FFA. However, no significant between-group differences were found even at the liberal threshold of  $P < 0.05$ , uncorrected.

#### *Relationship between functional connectivity and clinical severity*

The ADI-R social score was significantly correlated to the strength of the connectivity between the right FFA and the left amygdala ( $P < 0.05$ , small volume corrected), indicating that more severely affected individuals have reduced connectivity between these two critical brain regions involved in social perception (see Fig. 3). In addition, increased connectivity between the right FFA and the right inferior frontal gyrus was significantly correlated to increased social severity on the ADOS social score

(see Fig. 3). Statistical corrections for multiple comparisons were conducted using cluster-thresholding based on Markov Chain Monte Carlo sampling.

### Eye-tracking measures

Between-group differences in eye movements while viewing neutral, unfamiliar faces were tested using independent samples *t*-tests. Four measures were evaluated: (i) number of fixations on the eye region (EyeFix); (ii) duration of the fixations on the eye region (EyeDur), (iii) number of fixations on the face (FaceFix); and (iv) duration of the fixations on the face (FaceDur). No significant group differences were found for any of these measures. However, the participants with ASD spent less time fixating on the eyes and had fewer fixations on the eyes and face than the controls. These results are reported in detail in Supplementary Table S1.

### Relationship between functional connectivity and eye tracking

*Post hoc* analyses were performed to test whether viewing patterns were correlated to the functional connectivity measures in the ASD group. To determine whether eye movements were correlated to functional connectivity, each eye-tracking measure (EyeFix, EyeDur, FaceFix, FaceDur) was entered separately, as an independent variable into the group analyses. All correlational analyses between eye-tracking measures and functional connectivity were thresholded at  $P < 0.05$ , uncorrected. This liberal threshold was selected to reduce the possibility of reporting false negatives, particularly in light of the reduced sample size that was included in the eye-tracking analyses. The brain regions included in these analyses were those in which significant between-group differences were found in functional connectivity: the amygdala, the posterior cingulate/cuneus area and the thalamus. Although some of the significant group-differences were lateralized, we tested each region bilaterally to minimize false negatives. No positive correlations ( $P < 0.05$ , uncorrected) were found between any of the eye-tracking measures (EyeFix, EyeDur, FaceFix, FaceDur) and functional connectivity between the FFA and the amygdala or thalamus in the ASD group. A small cluster in the posterior cingulate/cuneus area showed a significant correlation between connectivity to the FFA and the FaceDur measure (see Table S2 and Fig. S1). No other eye-tracking measure showed a correlation to functional connectivity in the posterior cingulate/cuneus.

Lastly, we tested whether the significant group differences in functional connectivity observed in the intact group ( $n = 40$ ) were present in the group with valid eye-tracking data ( $n = 24$ ). These between-group differences were tested using the same stringent methods as detailed in the fMRI processing and statistical analysis section above. All of the results remained with slight variations in cluster size and

peak activation coordinates. These results are reported in Table S2 and Fig. S1.

### Discussion

Consistent with a growing body of recent evidence, we did not find significant hypoactivation in the fusiform gyrus when individuals with ASD view faces (Hadjikhani *et al.*, 2004; 2006; Pierce *et al.*, 2004; Bird *et al.*, 2006). Both the amount of activation and location of activation were similar for the ASD and control groups. These findings provide further evidence that face processing deficits in autism may not stem from functional abnormalities at this level of perceptual processing or from reduced neural sensitivity to faces. Instead, our study points to the hypothesis that abnormalities in the extended social brain network may be associated with the social impairment in individuals with ASD.

### Functional connectivity analyses

Functional connectivity was determined by evaluating the positive inter-regional linear correlation coefficient between the peak activation cluster in the right FFA and six, *a priori* ROIs (right and left amygdala, right and left temporal lobe and right and left inferior frontal gyrus) associated with social processing in both the ASD and control groups. In addition, a whole-brain functional connectivity analysis was conducted testing the interaction between the Face > House contrast and the right FFA time course in all other voxels within the brain. The whole brain analysis was performed in order to determine whether the ASD group would show connectivity to aberrant brain regions not typically associated with social cognition. In addition, this analysis addressed the specificity of functional connectivity deficits to the social brain network in ASD. We found significant face-specific functional connectivity between the right FFA and the right cuneus (BA17/18), posterior cingulate (BA 31), superior colliculus, thalamus, bilateral amygdala and the right middle temporal gyrus (BA 21) in the control group. A more limited network of functionally connected regions was observed in the ASD group that included only the right amygdala and right middle temporal gyrus. In the direct group comparison, we found significantly reduced connectivity between the right FFA and the bilateral posterior cingulate gyri, left cuneus and the left amygdala in ASD group. These results were consistent with our hypothesis that difficulties with face processing may stem specifically from poor integration of brain regions in the extended social brain network. However, because of the limitations inherent in functional connectivity techniques, we are not able to specify the neuroanatomical basis of the reduced temporal correlations between these spatially distinct nodes of face processing circuitry. This analysis also cannot rule out the possibility that reduced connectivity may be mediated by abnormalities through an



indirect neural pathway. Future studies that combine functional connectivity with structural connectivity techniques [e.g. diffusion tensor imaging (DTI) with probabilistic tractography] will be useful in determining the relationship between abnormal functional connectivity and structural abnormalities in ASD.

An unexpected but potentially important finding was the lack of significant connectivity between the FFA and subcortical structures involved in face processing (i.e. thalamus, superior colliculus) in the ASD group. In the subcortical face processing pathway, the retina sends direct projections to the superior colliculus that project to the pulvinar in the thalamus, which in turn project to the amygdala (LeDoux, 1996). This face information is conveyed rapidly, with less than 100-ms latencies (Braeutigam *et al.*, 2001; Eimer and Holmes, 2002; Streit *et al.*, 2003; Pourtois *et al.*, 2005). Whereas the cortical pathway is sensitive to high-spatial frequency images of faces (Livingstone and Hubel, 1988; Merigan and Maunsell, 1993), the subcortical pathway processes low-spatial frequency information and is thought to be involved in social orienting to faces (Hariri *et al.*, 2002; Adolphs and Tranel, 2003; Vuilleumier *et al.*, 2003), a factor with particular relevance to autism. Human eye-tracking studies of typically developing infants through adults show that eyes are tracked more frequently than other facial features (Haith *et al.*, 1977; Janik *et al.*, 1978). However, 9- to 10-year-old children with autism rely more on the lower face or the mouth on a matching task than they do on the eyes (Langdell, 1978). Adults with autism viewing a naturalistic social scene show less attention to eyes and more to mouths, body and objects (Klin *et al.*, 2002); increased focus on the mouth predicted better social competence while greater fixation on objects predicted poorer social functioning. Johnson (2005) recently proposed that a disruption of the subcortical face processing route, through its involvement in social orienting, could account for some of the social deficits present in autism. Our study provides very preliminary support for this theory, and suggests that future work delineating cortical and subcortical face processing deficits in autism may provide valuable insight into the neural basis of social deficits in ASD.

### Relationship between reduced connectivity and clinical severity

We evaluated the relationship between functional connectivity and clinical severity by testing whether the strength of the correlation between brain regions was dependent on level of clinical severity. We found a significant inverse correlation between functional connectivity and the ADI-R social score, indicating that individuals who were more socially impaired showed the weakest functional connectivity between the right FFA and the left amygdala. In addition, we found that more severely affected individuals

demonstrated increased connectivity between the right FFA and right inferior frontal gyrus.

Converging evidence from structural (Aylward *et al.*, 1999; Pierce *et al.*, 2001; Sparks *et al.*, 2002; Schumann *et al.*, 2004; Munson *et al.*, 2006; Nacewicz *et al.*, 2006), biochemical (Otsuka *et al.*, 1999; Page *et al.*, 2006), functional (Baron-Cohen *et al.*, 1999; Critchley *et al.*, 2000; Wang *et al.*, 2004; Dalton *et al.*, 2005; Ashwin *et al.*, 2006; Williams *et al.*, 2006), and post-mortem studies (Schumann and Amaral, 2006) indicate that amygdalar abnormalities are present in ASD. In addition, preliminary evidence suggests that the developmental pattern of amygdala growth may be related to clinical severity. Munson *et al.* (2006) found that the children with autism who had abnormally enlarged right amygdalar volume at ages 3–4 had the poorest social and communication impairments by age 6. In contrast, adolescents and adults with the smallest amygdala volumes exhibited the most severe current deficits in processing facial emotions and the greatest severity on childhood non-verbal social behaviours estimated from the ADI-R (Nacewicz *et al.*, 2006). These studies suggest that the most severely affected individuals undergo excessive amygdalar growth early in childhood and subsequent atrophy in adolescence and adulthood. This pattern has been suggested to be the result of hyperactivity that leads to excitotoxic changes in the amygdala (Nacewicz *et al.*, 2006). Such developmental abnormalities of the amygdala may contribute to abnormal face perception in autism, via abnormalities in neural signalling originating from the amygdala (Schultz, 2005). Thus, it is possible that the relationship between functional connectivity and clinical severity may reflect varying degrees of structural abnormalities in the amygdala secondary to abnormal brain growth patterns. Notably, the relationship between functional connectivity abnormalities to the amygdala and social severity were limited to the ADI-R (not the ADOS). The ADI-R social score rating is based on early social development while the ADOS is a complimentary measure that rates current social functioning. Though speculative, this pattern of correlations may indicate that early developmental history is associated with abnormal functional connectivity between the FFA and the amygdala in adults with ASD to a larger degree than current social functioning.

Consistent with a prior study on face processing in ASD (Hadjikhani *et al.*, 2006), we found a positive correlation between the ADOS social score and connectivity with FFA in the right inferior frontal gyrus. Specifically, less severely affected individuals with ASD demonstrated greater connectivity during the house-processing blocks, while the more severely affected individuals demonstrated greater FFA-frontal connectivity during the face blocks (see Fig. 3). Note that neither group evidenced significant connectivity between the FFA and the frontal lobes in the group-wise analyses nor were between-group differences in connectivity found. Thus, we suggest that these results reflect the increased cognitive demands associated with processing

faces compared to houses in the more severely affected individuals with ASD.

### Lack of relationship between eye movements and abnormal connectivity

As reported by Dalton *et al.* (2005), patterns of activation can be related to where an individual is looking during fMRI face processing studies. Specifically, Dalton *et al.* found that activation in the fusiform gyrus and amygdala was positively correlated to the amount of time spent fixating on the eyes in the autism group. This effect was not observed in the controls. Although we did not find significant group differences in overall activation in the fusiform gyrus and amygdala to faces (see Fig. 1 and Table 2), we addressed whether differences in gaze patterns were related to our findings of reduced functional connectivity by including data obtained during a behavioural eye-tracking study conducted outside of the scanner. A liberal threshold ( $P < 0.05$ , uncorrected) was selected to reduce the probability of reporting false negatives, particularly in light of the reduced sample size that was included in the eye-tracking analyses. The brain regions included in these analyses were those in which significant between-group differences were found in functional connectivity. No correlations ( $P < 0.05$ , uncorrected) were found between any of the eye-tracking measures (EyeFix, EyeDur, FaceFix, FaceDur) and functional connectivity between the FFA and the amygdala in the ASD group. Fourteen voxels (out of 1984 which showed a between-group difference in connectivity) in the posterior cingulate/cuneus area showed a significant correlation between connectivity to the FFA and the FaceDur measure (see Table S2 and Fig. S1). Although it is notable that the duration of time spent looking on the face may be correlated to functional connectivity in ASD, it is unlikely that this relationship would account for the robust group differences observed in this area, given that the groups performed very similarly on this measure (FaceDur: ASD mean = 61.02 s; Cont mean = 61.70 s; see Table S1). Taken together, it is unlikely that different eye movements between the groups produced the different patterns of connectivity observed in this study.

### Evidence for abnormal connectivity in ASD

Investigators now recognize that abnormalities in specific regions of the brain are not likely to explain the deficits observed in individuals with autism; instead, it is expected that abnormalities in neural circuitry are more likely to be involved. Just and colleagues (2007, 2004) have proposed an underconnectivity theory, which states that any component of psychological or neurological function that is dependent on the coordination or integration of distinct brain regions is susceptible to disruption in autism. It has been suggested that altered levels of brain activation and underconnectivity could be secondary to abnormal development of grey matter, white matter or both

(Just *et al.*, 2004, 2007). The primary support for this theory has been obtained from functional imaging studies.

Studies utilizing functional connectivity techniques in ASD have identified abnormal connectivity between brain regions involved in mediating complex language, selective attention, visuomotor coordination, emotion perception and executive functioning tasks (Just *et al.*, 2004, 2007; Koshino *et al.*, 2005, 2007; Villalobos *et al.*, 2005; Welchew *et al.*, 2005; Bird *et al.*, 2006; Cherkassky *et al.*, 2006; Kana *et al.*, 2006; Mizuno *et al.*, 2006; Murias *et al.*, 2007). Reported functional abnormalities in ASD include reduced connectivity between occipital and frontal regions during a visuomotor sequencing task and a sentence comprehension task (Just *et al.*, 2004; Villalobos *et al.*, 2005), fronto-parietal (Koshino *et al.*, 2005; Just *et al.*, 2007) and fusiform-frontal (Koshino *et al.*, 2007) regions during visually mediated executive functioning tasks and amygdala and parahippocampal regions during fearful face processing (Welchew *et al.*, 2005). Reduced connectivity between the occipital lobes and parietal lobes was reported by one group during a language task requiring visual imagery (Kana *et al.*, 2006) but not another which required visuomotor sequencing (Villalobos *et al.*, 2005). Increased connectivity has been reported between the thalamus and fronto-parietal regions (Mizuno *et al.*, 2006) and the caudate nuclei and fronto-parietal regions (Turner *et al.*, 2006) during visuomotor sequencing. A compelling selective attention study of faces and houses by Bird and colleagues (Bird *et al.*, 2006) found that connectivity between V1 and the FFA (the brain region that responded the most strongly to faces) was not modulated by attention in ASD although connectivity between V1 and the parahippocampal place area (the brain region that responded the most strongly to houses) was. As such, the body of evidence including the present study indicates that global 'underconnectivity' may not be ubiquitous in ASD. Instead, factors such as clinical severity, early experiential factors, cognitive strengths and weakness and task demands may play an important role. At this point, it is unclear whether abnormal connectivity (under or over) is linked to structural abnormalities including neuronal, neuroglial or synaptic dysfunction resulting in abnormal specialization, abnormal functional reorganization/compensation or massive disorganization of connections; these questions remain to be tested empirically.

Structural MRI provides evidence of abnormal brain development, with growth of specific brain regions occurring earlier than normal (Kemper and Bauman, 1998; Courchesne *et al.*, 2001; Aylward *et al.*, 2002; Sparks *et al.*, 2002; Hazlett *et al.*, 2005; Redcay and Courchesne, 2005) and out of typical synchrony with other brain regions (Tsatsanis *et al.*, 2003; Hardan *et al.*, 2006; Aylward *et al.*, submitted for publication). Increased brain size in young children is largely driven by white matter, particularly in the superficial/radiate white matter regions of the cerebrum (Herbert *et al.*, 2004) and in the frontal lobes (Carper *et al.*, 2002). These findings suggest that the greatest volume

changes in white matter occur in later-myelinating brain regions, which further suggests a developmental link between white matter abnormalities and rapid brain growth in the first years of life (Herbert, 2005). Although these findings do not directly demonstrate abnormal connectivity among brain structures in autism, the abnormal pattern of growth in white matter is consistent with this possibility.

More direct evidence of abnormal white matter connections is provided by DTI studies. The first published study in autism (Barnea-Goraly *et al.*, 2004) included seven children with autism and nine age- and IQ-matched controls. DTI is a non-invasive, *in vivo* method for characterizing the integrity of anatomical connections and provides a quantitative assessment of the brain's white matter microstructure. Reduced fractional anisotropy [FA; a measure of orientational coherence ranging from 0 (isotropic) to 1 (anisotropic)] values were observed in the autism group in the white matter adjacent to several brain regions involved in social cognition: the ventromedial prefrontal cortices, anterior cingulate gyri, temporoparietal junction, STS, the temporal lobes approaching the amygdala bilaterally and occipitotemporal tracts. Reduced FA was also observed in the corpus callosum. Increased FA was not observed in any brain region in the ASD group. Two subsequent studies investigated white matter structure in the corpus callosum (Alexander *et al.*, 2007; Keller *et al.*, 2007) and found significantly reduced FA in the autism group. Though preliminary due to the small number of studies, these results provide important corroboration that early aberrant brain growth may result in abnormalities in white matter and contribute to the social deficits in ASD.

## Conclusion

In sum, evidence is beginning to accrue that abnormal brain connections, possibly secondary to aberrant early developmental processes, may underlie functional abnormalities and their concomitant behavioural abnormalities observed in ASD. In the current study, we found reduced functional connectivity between the FFA and the extended network involved in face processing in the ASD group. In addition, greater social impairment was correlated with reduced FFA-amygdala connectivity and increased FFA-right inferior frontal connectivity. These results suggest that abnormal neural connections involving the limbic system may contribute to the widespread social impairments observed in ASD.

## Supplementary material

Supplementary material is available at *Brain* online.

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