Functional Disconnectivity of the Medial Temporal Lobe in Asperger's Syndrome

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Background: *Autistic spectrum disorders (ASD) are neurodevelopmental conditions that may be caused by abnormal connectivity between brain regions constituting neurocognitive networks for specific aspects of social cognition.*

Methods: *We used three-way multidimensional scaling of regionally parcellated functional magnetic resonance imaging (fMRI) data to explore the hypothesis of abnormal functional connectivity in people with ASD. Thirteen high-functioning individuals with Asperger's syndrome and 13 healthy volunteers were scanned during incidental processing of fearful facial expressions.*

Results: *Using permutation tests for inference, we found evidence for significant abnormality of functional integration of amygdala and parahippocampal gyrus ($p < .05$, false discovery rate [FDR] corrected) in people with Asperger's syndrome. There were less salient abnormalities in functional connectivity of anterior cingulate, inferior occipital, and inferior frontal cortex, but there was no significant difference between groups in whole brain functional connectivity.*

Conclusions: *We conclude there is evidence that functional connectivity of medial temporal lobe structures specifically is abnormal in people with Asperger's syndrome during fearful face processing.*

Key Words: Autism, functional connectivity, multivariate analysis, neuroimaging, systems, dysmodularity

Autistic spectrum disorders (ASD) range in severity from classic autism, associated with generally reduced intelligence and delayed language development, to Asperger's syndrome, which occurs in "high-functioning" adults with normal or superior intelligence quotient (IQ) and intact language. All autistic spectrum disorders, however, are associated with repetitive behaviors and obsessional interests and abnormalities in socioemotional and communicative skills (American Psychiatric Association 1994). Although the etiology is unknown, autism is highly heritable (Bailey et al 1996), so it seems likely that there is some genetically determined difference in early brain development at its root (Akshoomoff et al 2002).

The brain basis for autistic spectrum disorders is not entirely clear. Structural neuroimaging studies have reported volumetric differences in both global and specific regional measures (Boddaert and Zilbovicius 2002; Cody et al 2002; Mink and McKinstry 2002; Sparks et al 2002; Salmond et al 2003). Abnormal increase in global brain size (megalencephaly) has been reported in children with autism, as has an abnormality of normal age-related reduction in global gray matter volume (Courchesne 2002; Courchesne et al 2001, 2003), perhaps implying an abnormality of synaptic pruning or other normal regressive processes in brain development (Frith 2003). Gray matter volume reductions have also been reported in cerebellum, striatum, amygdala, and various cortical regions (Cody et al 2002). More recently, computationally intensive methods of brain morphometry have allowed a finer-grained and more comprehensive analysis of case-control differences. For example, McAlonan et al (2002) reported gray matter volume deficits in cerebellar and fronto-striatal systems, as well as widespread white matter deficits, in a study of normal IQ adults with Asperger's syndrome.

Functional neuroimaging studies have focused on case-control differences in local brain activation engendered by tasks demanding social cognition, e.g., mentalizing or "theory of mind" tasks, which depend on specific social cognitive processes that are selectively impaired in autistic spectrum disorders (Baron-Cohen 1995; Frith and Frith 1999; Frith 2001). The profile of neurophysiological abnormalities defined by this literature implicates several regions collectively comprising a "social brain" network (Brothers 1990, 1997; Baron-Cohen et al 1999). Frith's (2001) meta-analysis of mentalizing experiments highlighted the consistent involvement of a periamygdalar region in the medial temporal lobe, a paracingulate region in medial frontal cortex, and an area of cortex at the boundary between temporal and parietal lobes coinciding approximately with motion-sensitive visual area V5 (homologous to middle temporal area [MT] in monkey cortex). Reduced activation of these and other social brain regions has been repeatedly demonstrated in people with autistic spectrum disorders during mentalizing tasks (Baron-Cohen et al 1999; Castelli et al 2002; Di Martino and Castellanos 2003; Frith and Frith 1999). The key role of anatomical and functional abnormality of the amygdala in pathogenesis of mentalizing deficits in autism has been articulated as an amygdala theory of autism (Baron-Cohen et al 2000). Perhaps surprisingly, there have been relatively few functional imaging studies addressing the neurophysiological correlates of repetitive stereotypies or other nonsocial aspects of ASD.

One theoretical formulation that draws together much of these data is that autistic spectrum disorders may be characterized by functional disconnectivity of networks important for specific aspects of social cognition and motor control, i.e., ASD may be conceived as functional disconnection syndromes. By functional disconnectivity, we mean any state of abnormally correlated neurophysiological activity in two or more anatomically distributed brain regions (Friston et al 1993). This concept of autism as a functional disconnection syndrome originates in one of its earliest neurobiological accounts (Damasio and Maurer 1978) and is broadly compatible with the evidence for anatomical and functional disconnectivity in other neurodevelopmental

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syndromes such as schizophrenia (Bullmore et al 1997). However, there have previously been only a few attempts to test directly the hypothesis of abnormal brain functional integration in people with autistic spectrum disorders–in short, the disconnection hypothesis–by comparative multivariate analysis of functional neuroimaging data from people with autistic syndromes. Horwitz et al (1988) reported abnormalities of interregional correlation between frontal, parietal, and neostriatal metabolic rates for glucose measured by positron-emission tomography (PET). In a more regionally restricted analysis, Castelli et al (2002) found reduced correlation or functional connectivity between functional magnetic resonance imaging (fMRI) time series recorded in extrastriate visual cortex and superior temporal sulcus.

Here we report the first whole brain analysis of functional connectivity in high-functioning adults with Asperger's syndrome performing a mentalizing task. Based on prior evidence implicating the amygdala and neighboring medial temporal lobe structures in normal social cognition and autism, we hypothetically predicted functional disconnectivity of medial temporal regions in the patient group. We used multidimensional scaling combined with innovative permutation based methods of inference to demonstrate significantly reduced functional integration of amygdala and parahippocampal gyrus, cingulate cortex, and inferior occipital gyrus in data acquired during a task entailing incidental attention to fearful facial expressions.

Methods and Materials

Subjects

Thirteen right-handed male adult volunteers with high-functioning autism or Asperger's syndrome, diagnosed clinically using standard operational criteria (American Psychiatric Association 1994), and 13 healthy right-handed male adult volunteers were recruited. Participants were not currently taking prescribed or illicit psychotropic drugs and had no history of neurological or nonautistic psychiatric disorders. The two groups were not significantly different in terms of age (mean age [SD] = 31.2 years [9.1] for the clinical group and 25.6 years [5.1] for healthy volunteers) or full-scale IQ measured using the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999) (mean IQ [SD] = 108.6 [17.1] for the clinical group and 117.9 [9.6] for healthy volunteers). In addition, all but one of the participants completed the Autism-Spectrum Quotient (AQ), a self-administered questionnaire for measuring the severity of autistic traits (Baron-Cohen et al 2001). Autism-Spectrum Quotient scores for this sample of people with Asperger's syndrome (N = 12, mean AQ [SD] = 35.2 [6.3], 75% scoring 32+) were closely matched to AQ scores in a larger sample of autistic individuals previously reported (Baron-Cohen et al 2001) (N = 58, mean AQ [SD] = 35.8 [6.5], 80% scoring 32+). The study was approved by the Local Research Ethics Committee, Addenbrooke's Hospital NHS Trust, and all participants gave informed consent in writing.

Implicit Facial Affect Processing Paradigm

We used a blocked periodic design in which four classes of visual stimuli were presented in 30-second epochs: faces expressing a high intensity of fear, faces representing a low intensity of fear, faces with an emotionally neutral expression, and randomly scrambled facial images as a low-level control condition. Each face was presented for 3 seconds, followed by a blank screen for 750 milliseconds, giving eight trials per epoch. There were four repetitions of each epoch, in random order, for a total experimental duration of 8 minutes. The participants were asked simply to press a response button with their right index finger whenever they saw a visual stimulus, i.e., participants were not instructed explicitly to attend to the emotional valence or intensity of the stimuli.

Explicit recognition of fearful faces was tested using a set of 12 faces in a separate session after completion of scanning. Healthy volunteers correctly identified 9.62 (SD = 2.22) fearful faces on average and people with Asperger's syndrome correctly identified 6.46 (SD = 1.90) faces on average. The between-group difference in accuracy was statistically significant; independent samples t test, $t = 3.89$, $df = 24$, $p < .001$.

Functional MRI Data Acquisition

Gradient-echo echoplanar images depicting blood oxygenation level dependent (BOLD) contrast in 21 oblique axial planes were acquired at the Wolfson Brain Imaging Centre, Cambridge, United Kingdom, using a Bruker Medspec Advance S300 system (Bruker Medical, Ettlingen, Germany) operating at 3 Tesla with the following parameters: echo time (TE) = 30 milliseconds; repetition time (TR) = 3 seconds; in-plane resolution = 1.95 mm; slice thickness = 4 mm plus 1 mm interslice gap; matrix size = 128^2. One hundred sixty-six images were acquired for each subject; the first 6 were subsequently discarded to avoid T1 equilibration effects, leaving 160 images per subject.

Functional MRI Data Analysis

Activation mapping by mass univariate analysis of these data has been previously reported (Ashwin et al, in press); here we focus on multivariate analysis of whole brain connectivity using methods described more completely by Welchew et al (2002).

Preprocessing. Functional magnetic resonance images from each subject were corrected for differences in acquisition time between slices and head movement during scanning by sinc interpolation. Areas of susceptibility artefact were manually "masked" prior to co-registration of each image with the Montreal Neurological Institute (MNI) echoplanar imaging (EPI) template image. Statistical Parametric Mapping (SPM99) software was used for these operations (http://www.fil.ion.ucl.ac.uk/spm).

Automated Anatomical Labeling and Regional Mean Time Series Extraction. The normalized images were anatomically divided into 45 regions of interest (ROIs) in each cerebral hemisphere using a previously parcellated template image in MNI space (Tzourio-Mazoyer et al 2002). See Figure 1A for a full list of regions, their abbreviations, and color-coded affiliation to major lobes. A regional mean time series was estimated for each of 90 regions in each subject by averaging the fMRI time series over all voxels in each region. We used first-order differencing of each regional mean time series to attenuate low-frequency components in the data due to residual head movement effects or long memory resting state fluctuations in the BOLD signal.

The interregional Pearson's correlation matrix was estimated for each individual and converted to a distance matrix by subtraction from unity before collating over subjects within each group to produce two sets of interregional distance matrices, one for the patients with autism and one for the healthy comparison subjects.

Multidimensional Scaling. We used three-way multidimensional scaling (3-MDS), specifically a method of weighted individual differences scaling derived from prior work by Carroll and Chang (1970), to project the stacks of interregional distance matrices into a low-dimensional space such that the Euclidean distances between regions in the reduced space approximated as closely as possible the distances between their time series. For

A

Region	Abbreviation
Precentral Gyrus	PreCG
Superior Frontal Gyrus, Dorsolateral	SFGd
Superior Frontal Gyrus, Orbital Part	SFGo
Middle Frontal Gyrus	MFG
Middle Frontal Gyrus Orbital Part	MFGo
Inferior Frontal Gyrus, Opercular Part	IFGop
Inferior Frontal Gyrus, Triangular Part	IFGtr
Inferior Frontal Gyrus, Orbital Part	IFGo
Rolandic Operculum	ROL
Supplementary Motor Area	SMA
Olfactory Cortex	OLF
Superior Frontal Gyrus, Medial	SFGm
Superior Frontal Gyrus, Medial Orbital	SFGmo
Gyrus Rectus	REC
Insula	INS
Anterior Cingulate and Paracingulate Gyri	ACC
Median Cingulate and Paracingulate Gyri	MCC
Posterior Cingulate Gyrus	PCC
Hippocampus	HIP
Parahippocampal Gyrus	PHG
Amygdala	AMY
Calcarine Fissure and Surrounding Cortex	CAL
Cuneus	CUN
Lingual Gyrus	LIN
Superior Occipital Gyrus	SOG
Middle Occipital Gyrus	MOG
Inferior Occipital Gyrus	IOG
Fusiform Gyrus	FFG
Postcentral Gyrus	PoCG
Superior Parietal Gyrus	SPG
Inferior Parietal, but Supramarginal and Angular Gyri	IPL
Supramarginal Gyrus	SMG
Angular Gyrus	ANG
Precuneus	PCN
Paracentral Lobule	PCL
Caudate Nucleus	CAU
Lenticular Nucleus, Putamen	PUT
Lenticular Nucleus, Pallidum	PAL
Thalamus	THA
Heschl Gyrus	HES
Superior Temporal Gyrus	STG
Temporal Pole: Superior Temporal Gyrus	TPOs
Middle Temporal Gyrus	MTG
Temporal Pole: Middle Temporal Gyrus	TPOm
Inferior Temporal Gyrus	ITG

B

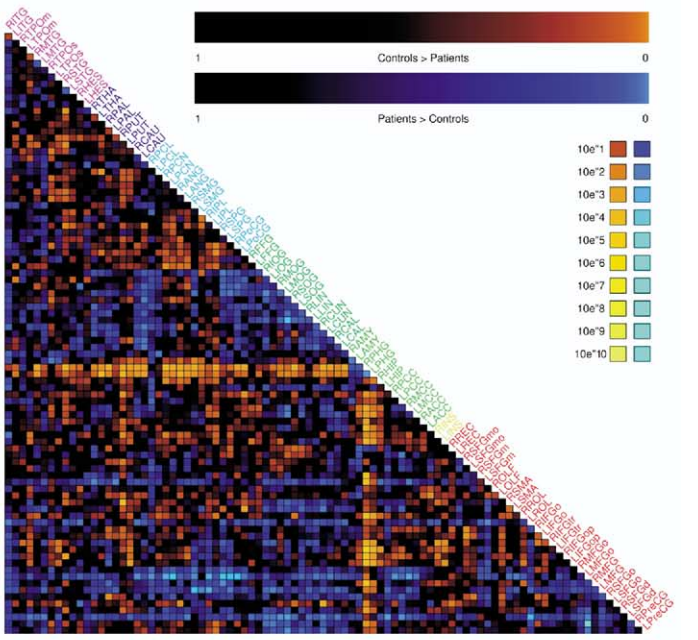

Figure 1. (A) Cortical and subcortical regions (45 in each cerebral hemisphere; 90 in total) as anatomically defined by a prior template image in standard stereotaxic space. The abbreviations listed are those used in this article, which differ slightly from the original abbreviations by Tzourio-Mazoyer et al (2002). Color code is as follows: red = frontal cortex; yellow = insular cortex; green = medial temporal lobe and cingulate cortex; turquoise = occipital cortex; blue = parietal cortex; purple = subcortical nuclei; cyan = temporal cortex. **(B)** Differences in functional distance between brain regions in people with Asperger's syndrome compared with healthy volunteers. Between-group differences in functional distance between each of 4005 pairs of 90 cortical and subcortical regions are color-coded according to their probability under the null hypothesis that the two groups were drawn from the same population. The yellow/orange stripes across the matrix indicate that several distances between medial temporal lobe (MTL) structures (parahippocampal gyrus, amygdale, and hippocampus) and most other brain regions are significantly greater–and so the correlations in activity between MTL and other brain regions are significantly smaller–in healthy volunteers compared with the Asperger's group. This is a preliminary indicator that Asperger's syndrome is associated with abnormal functional connectivity or, more specifically, dysmodularity of MTL in the context of incidental facial affect processing. Anatomical abbreviations and color codes are as listed in **(A)**. Each small square within the matrix represents the significance of the between-group difference in functional distance between the regions corresponding to the column and row intersecting on it.

example, the distance matrices for the group of healthy volunteers have been projected by 3-MDS into a three-dimensional (3-D) space (of which only the first two dimensions are shown in Figure 2) such that a small distance between any two regions in the graphical space of this scatterplot indicates a small functional distance (or a large correlation) between the corresponding time series "on average" over individuals in the group, and conversely, large graphical distances indicate large functional distances (or small correlations) between regions. Individual differences scaling differs from classical multidimensional scaling (MDS) or principal components analysis by explicitly taking into account intersubject variability in computing the optimal low-dimensional representation of interregional distances for each group on average.

The 3-MDS solutions for the two groups were statistically compared by a permutation test described in detail by

Welchew et al (2002) and similar in principle to methods previously described by Scannell et al (1995, 1999) and Young et al (1995) for comparison of anatomical connectivity matrices derived from nonhuman tract tracing experiments and by McIntosh et al (1996) for comparison of functional connectivity matrices derived from human positron emission tomography experiments.

In brief, a symmetric Procrustes rotation with scaling was used to minimize spurious differences between the 3-MDS solutions for the healthy volunteer group and the Asperger's syndrome group. Procrustes analysis finds the translation, rotation, and scaling necessary to minimize discrepancies between the two 3-D configurations of regions due to scaling differences or arbitrary reversal or reflection of dimensions. Following Procrustes analysis, there will remain some residual differences between groups in terms of the locations of any particular region. We estimated the residual between-group difference in location individually for each region, denoted m_i for the ith region, and also a global summary statistic, denoted M^2, which summarized the residual difference between configurations over all regions. These statistics were calculated as the squared Euclidean residual distance between the ith region in the 3-MDS solutions of the two groups (m_i) and the sum of these squared distances M^2, respectively.

We used a permutation method to test the null hypothesis that any residual between-group difference in regional location or overall configuration was not related to the diagnostic status of the participants. Briefly, the participants were randomly reassigned to two groups (each of size $N = 13$) and distance matrices were collated within each permuted group. We then proceeded exactly as for the observed distance matrices, using 3-MDS to estimate a 3-D solution for each group and applying Procrustes analysis to minimize spurious between-group differences in the resulting configurations. Finally, we estimated overall residual differences and region-specific differences between the two permuted groups. This procedure was repeated 24,999 times yielding 24,999 estimates of the test statistics m_i and M^2 under the null hypothesis. These statistics were ordered and combined with the corresponding statistics estimated in the observed data to constitute a permutation distribution comprising 25,000 estimates of each test statistic.

Significant differences in functional connectivity between groups were first identified with one-tailed $p < .05$ simply by comparing the observed statistics to a critical value corresponding to the 95th percentile of the corresponding permutation distribution. Additionally, to protect against false-positive errors in the context of multiple, possibly correlated comparisons of specific regions between groups, we also used the method of Benjamini and Yekutieli (2001) to control the false discovery rate (FDR) for dependent observations at 5%, which naturally entailed a more stringent significance threshold for each test of m_i. Only those regions passing this FDR-controlled test are reported as formally significant, ensuring that the probability of declaring false-positive results across the study is no larger than .05.

Results

Between-Group Differences in Interregional Distance Matrices

Arguably, the simplest approach to identifying group differences in whole brain functional connectivity is direct inspection of the matrix of differences in interregional distances. The 90 cortical and subcortical regions under consideration yield 4005 distinct interregional distances for each group. The difference between groups for each distance, after centering of the distance matrices, is summarized in a triangular matrix (Figure 1B) that provides some preliminary evidence for abnormal connectivity of medial temporal lobe structures in the patients with autism. In particular, note that several medial temporal regions (amygdala, hippocampus, parahippocampal gyrus) have significantly greater functional distance from all other brain regions in the healthy volunteers compared with the people with Asperger's syndrome.

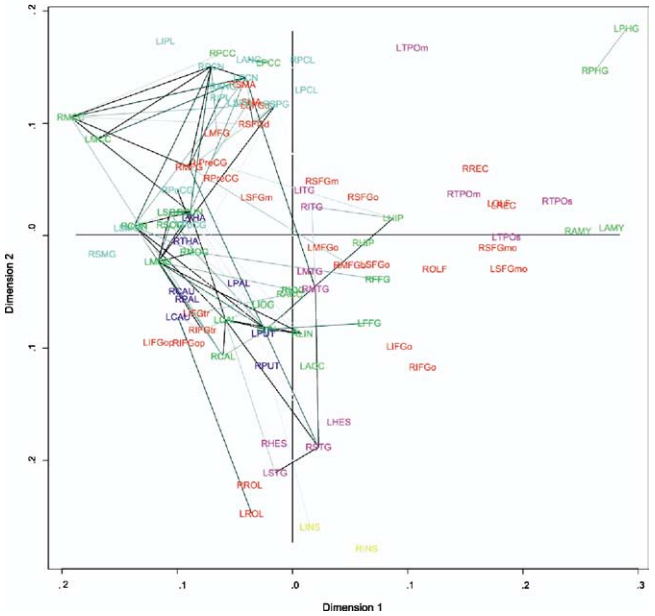

Figure 2. Whole brain functional network by multidimensional scaling of the healthy volunteer group distance matrix. The graphical distance between regions in the space of this plot is the best low-dimensional approximation to the Euclidean distance between their functional MRI time series. The first dimension (*x*-axis) is dominated by the distance between medial temporal structures and other brain regions; the second dimension (*y*-axis) is dominated by the distance between a cluster of regions important for auditory-verbal processing (Heschl's gyrus, superior temporal gyrus, insula) and a large cluster of prefrontal and premotor regions. The functional distance between MTL and other brain regions highlighted by this analysis is compatible with a functionally specialized role for MTL structures in facial affect processing by healthy volunteers. Strong interregional correlations are represented by lines of variable intensity drawn between regions; broken line $p < .01$, solid line $p < .001$. Anatomical abbreviations are as listed in Figure 1A. MRI, magnetic resonance imaging; MTL, medial temporal lobe.

3-D Multidimensional Scaling Solution for the Healthy Volunteer Group

The configuration of all 90 cortical and subcortical regions in the first two dimensions of the 3-D MDS solution for the control group distance matrices is shown in Figure 2. Regions in the same cortical lobe and bilaterally homologous regions of right and left hemispheres tend generally to be located in close proximity to each other, implying that anatomical relations between regions strongly condition their functional connectivity (see Salvador et al 2005, for more detailed discussion of anatomical constraints on normal functional network architecture demonstrated by MDS). The first dimension (*x*-axis) of this plot is dominated by the Euclidean distance between medial temporal, temporal polar, orbitofrontal and olfactory cortex, and all other brain regions. This indicates a large functional distance between medial temporal and other brain regions in the context of this

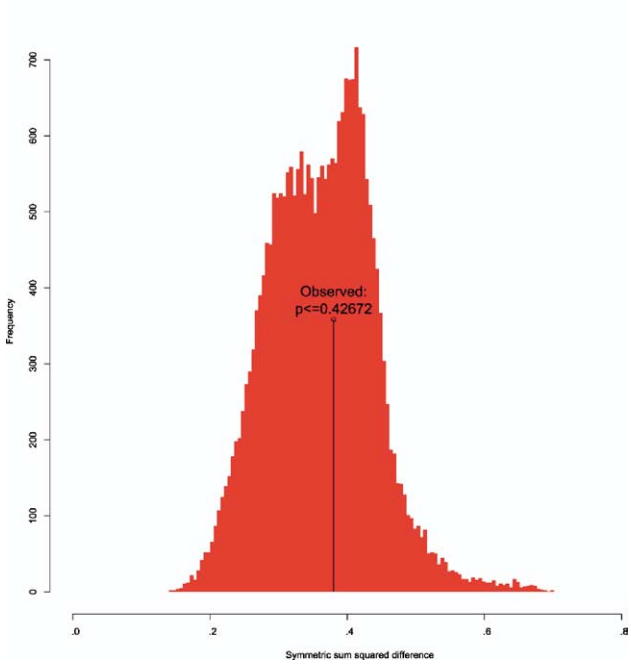

Figure 3. Permutation tests for a regional difference in functional connectivity between people with Asperger's syndrome and healthy volunteers. Each boxplot summarizes the permutation distribution of test statistic m_i for a single region; observed values of the test are indicated by small circles; observed values of the test statistics with $p < .05$ are indicated by intermediate circles; and those which remain significant after controlling the false discovery rate (FDR) at 5% are indicated by large circles and annotated by their *p*-values.

experiment, compatible with the expectation that medial temporal structures may normally play a modular or specialized information-processing role in analysis of affective facial stimuli.

Permutation Tests for Between-Group Differences in Global and Regional Connectivity

As shown in Figure 3, there was no evidence for a significant difference between groups in the overall configuration of their 3-MDS solutions after generalized Procrustes analysis had been used to minimize spurious differences. However, as shown in Figure 4, there was evidence for significant group differences at a regional level of analysis. In particular, right and left parahippocampal gyrus and left amygdala were significantly different between groups in their functional configuration, even after controlling the overall false discovery rate at 5%. There were also marginally significant differences at the uncorrected 5% level for left anterior cingulate gyrus, right median (dorsal) cingulate gyrus, right inferior occipital gyrus, and the triangular part of left inferior frontal gyrus.

Discussion

This is the first study comprehensively to test the hypothesis that autistic spectrum disorders, specifically Asperger's syndrome, may be associated with functional disconnectivity between brain regions. By combining a classical method of exploratory multivariate analysis (multidimensional scaling) with novel permutation methods for inference, we have been able to demonstrate a number of significant case-control differences in functional integration of specific brain regions.

The clearest evidence for functional disconnectivity in autism was found in the medial temporal lobe. The amygdala and parahippocampal gyrus were evidently abnormal in their functional configuration, as assessed both by inspection of the matrix of between-group differences in interregional distance (Figure 1B) and by FDR-controlled permutation tests of the difference in location of these regions in low-dimensional scaling solutions separately estimated by 3-way MDS for each of the two groups (Figure 4). These differences can be taken as evidence for functional disconnectivity, broadly defined earlier as abnormal physiological correlation of anatomically distributed brain regions. However, it is noteworthy that connectivity of medial temporal lobe structures is more specifically abnormal in the sense that the normal pattern of large functional distance (low correlation) between these regions and the rest of the brain functional network (Figure 2) is reduced in patients with Asperger's syndrome, who show smaller functional distance (larger correlation) between medial temporal and other brain structures

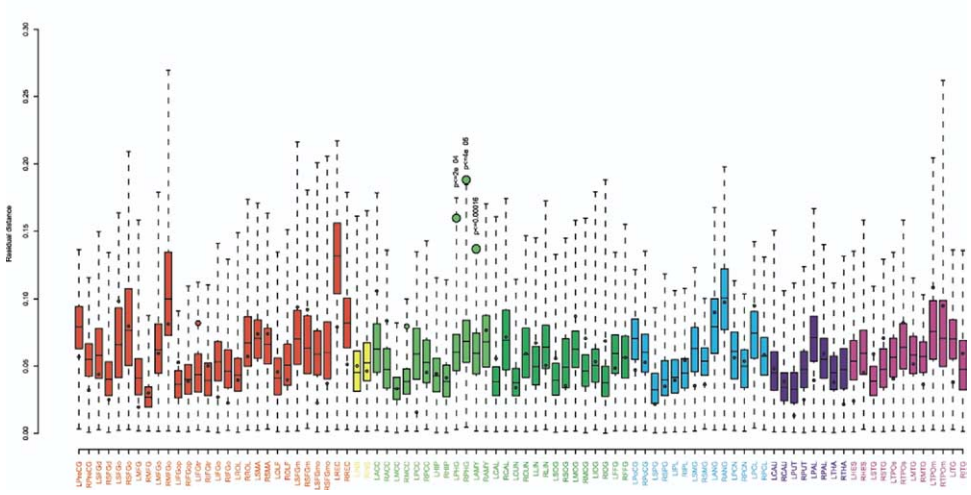

Figure 4. Permutation test for a global difference in functional connectivity between patients with Asperger's syndrome and healthy volunteers. The histogram shows the distribution of the test statistic M^2 sampled by 24,999 random permutations of group membership; the observed value of M^2 lies almost exactly in the middle of the permutation distribution with $p = .43$, indicating that the null hypothesis of zero between-group difference in global brain connectivity cannot be refuted.

compared with healthy volunteers. This pattern of results is consistent with the notion that medial temporal lobe structures, normally specialized for modular processing of affectively valent facial stimuli and therefore physiologically active in a way which distinguishes or distances them from the rest of the functional brain network, are less clearly modular or physiologically specialized for processing affectively valent faces in patients with Asperger's syndrome. In short, we could summarize these results as evidence for dysmodular organization (David 1994) of medial temporal structures in patients with Asperger's syndrome (see also Belmonte et al 2004 for a review of disconnectivity in autism from a developmental perspective).

There is strong prior interest in the role of the amygdala in normal social cognition; in particular, there are numerous functional neuroimaging and neuropathological studies suggesting a key role for amygdala in facial affect recognition and affective conditioning (LeDoux 1996; Aggleton 2000; Adolphs 2001; Adolphs et al 2002). There is a complementary literature documenting abnormalities of amygdala structure and function in patients with autistic spectrum conditions (see Baron-Cohen et al 2000 for review of the amygdala theory of autism). Kluver-Bucy syndrome, which produces autistic-like behaviors in the context of multiple cognitive abnormalities, emerges from damage to the medial temporal lobe (Hetzler and Griffin 1981) and the best currently available animal model of autism is produced by disconnection of the amygdala early in life (Bachevalier 1994, 2000). Autism has also been compared with an amnesic syndrome characterized by disconnection of the hippocampus (DeLong 1992). Many models of the underlying social-emotional deficits that are core to autism emphasize a disconnection between medial temporal lobe structures and other brain areas (Bachevalier 2000; Baron-Cohen et al 2000; Fotheringham 1991; Schultz et al 2000; Waterhouse et al 1996). So, our finding that functional integration of amygdala is abnormal in patients with Asperger's syndrome, though novel, is arguably not surprising. We should note also that the demonstration of abnormal amygdala connectivity in patients with Asperger's syndrome, compared solely with healthy volunteers, does not link this functional abnormality specifically to autistic spectrum disorder. It will be interesting in future studies to test the hypothesis that impaired social cognition arising in the context of diagnostic categories other than ASD, such as schizophrenia, might also be associated with abnormal functional connectivity of amygdala.

There is less prior evidence for autism-related abnormalities in other medial temporal structures, but neuropathological studies have implicated the hippocampus and entorhinal cortex (Kemper and Bauman 1993). More circumstantially, it has been reported that functional connectivity between amygdala and hippocampus is necessary for episodic memory encoding of emotionally salient stimuli and events (Cahill 1997; Cahill et al 2001; Hamann 2001; LeDoux 1996), and a recent neuroimaging study used path analysis to model effective connectivity changes during emotional memory storage (Kilpatrick and Cahill 2003). These authors found that the connection between amygdala and parahippocampal gyrus showed increased connectivity during an emotionally valent memory task compared with an emotionally neutral control task (Kilpatrick and Cahill 2003). In this context, our finding of abnormal functional connectivity of parahippocampal gyrus, although less clearly predicted by prior functional neuroimaging studies of autism, might help to explain the deficits in social cognition associated with the disorder.

Less saliently, we also detected abnormalities in functional integration of anterior and dorsal cingulate cortex. An anterior paracingulate region was identified by Frith's (2001) meta-analysis as critical for normal theory of mind or mentalizing and previous functional neuroimaging studies (Fletcher et al 1995) have indicated reduced activation of an area of medial prefrontal cortex that lies close to the region defined as anterior cingulate cortex by this parcellation scheme. Other regions of marginally significant abnormality included the inferior frontal gyrus and the inferior occipital gyrus, which are consistently activated in normal volunteers by tasks demanding face perception (Haxby et al 2000, 2004). However, bearing in mind the unpredicted nature of these observations and their marginal significance in the context of multiple comparisons, it is probably advisable to withhold detailed interpretation of these findings pending their replication in larger studies.

Some methodological issues deserve comment. First, it is important to recall that the fMRI data were acquired during an experiment that entailed incidental attention to fearful faces, a condition that is known to induce strong activation of amygdala in healthy volunteers and reduced amygdala activation in patients with autism. It seems likely, therefore, that the pattern of functional disconnectivity we have demonstrated may be conditioned by the experimental task or, to put it another way, a different experimental paradigm might yield evidence for disconnectivity involving other brain regions in autism. A second issue is that the sample size was modest ($N = 13$ for each group), meaning that there is a real risk of type 2 error. We suggest that future studies of functional disconnectivity in larger samples might more strongly implicate other brain regions, perhaps including the cingulate and inferior occipital regions that were marginally significant in this study or regions such as fusiform cortex which have been previously implicated in disordered social cognition but were not even marginally abnormal in this study. Also related to the study sample, we recall that our results were obtained from a group with Asperger's syndrome or high-functioning autism and should not immediately be generalized to the population of patients with any degree of autistic disorder. Third, the preprocessing steps applied to the fMRI time series before MDS will have substantially eliminated any head movement-related effects on signal (co)variance that could otherwise substantially confound analysis of interregional connectivity. However, these necessary preprocessing steps will also have attenuated low-frequency components of the time series. Since different frequency bands of electroencephalogram (EEG) and fMRI data may subtend different connected brain systems, it is arguable that the pattern of abnormality demonstrated here should be defined more exactly as a relatively high-frequency functional disconnection syndrome. Fourth, the multivariate analysis of functional connectivity reported here is agnostic about the direction of causal influences between connected regions and incorporates no prior knowledge about which regions might be expected to have abnormal functional associations with the rest of the brain. Future work might profitably apply methods of effective connectivity analysis sensitive to directional effects between regions or take a more hypothesis-driven approach specifically to characterization of between-group differences in amygdala connectivity (Friston et al 1997).

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