# Autism: Reduced Connectivity between Cortical Areas Involved in Face Expression, Theory of Mind, and the Sense of Self

Wei Cheng[1, #] Edmund T. Rolls[2,3, #] Jie Zhang[1] Jianfeng Feng[1, 2, *]

1. Centre for Computational Systems Biology, Fudan University, Shanghai, PR China

2. University of Warwick, Department of Computer Science, Coventry CV4 7AL, UK

3. Oxford Centre for Computational Neuroscience, Oxford, UK

Running head: Functional connectivity in autism

# These authors contributed equally to this work.

* Corresponding author information:

Professor Jianfeng Feng

Centre for Computational Systems Biology,

School of Mathematical Sciences, Fudan University, Shanghai 200433, China, and

Department of Computer Science, University of Warwick, Coventry CV4 7AL, UK

Jianfeng.Feng@warwick.ac.uk

**ABSTRACT**

Whole-brain voxel-based unbiased resting-state functional connectivity was analyzed in 523 people with autism and 452 typically developing individuals. We identified a key system in the middle temporal gyrus / superior temporal sulcus (STS) region which has reduced cortical functional connectivity (and increased with the medial thalamus), which is implicated in face expression processing involved in social behavior. This system has reduced functional connectivity with the ventromedial prefrontal cortex which is implicated in emotion and social communication. The middle temporal gyrus system is also implicated in theory of mind processing. We also identified in autism a second key system in the precuneus / superior parietal lobule / posterior cingulate cortex with reduced functional connectivity which is implicated in spatial functions including of oneself, and of the spatial environment. It is proposed that these two types of functionality, face expression-related, and of one's self and the environment, are important components of the computations involved in theory of mind, whether of oneself or of others, and that reduced connectivity within and between these regions may make a major contribution to the symptoms of autism.

## INTRODUCTION

Autism spectrum disorder (ASD) is a complex developmental disorder that is characterized by difficulties in social communication and social interaction; and restricted and repetitive behavior, interests, or activities (Lai *et al.* , 2014). Recently, a great deal of attention has been focused on the delineation of neural systems for brain–behavior relationships in ASD given that ~1% children are being diagnosed with this disorder (Kim *et al.* , 2011). At the brain circuit level, most of what we understand about autism and its biological abnormalities during the resting state comes from fMRI studies targeting changes in a small number of brain regions, as recently reviewed (Maximo *et al.* , 2014, Minshew and Keller, 2010, Müller *et al.* , 2011). These studies have suggested abnormality in connectivity among distant brain circuit, including the default mode network (Assaf *et al.* , 2010, Lynch *et al.* , 2013), social brain circuits (Gotts *et al.* , 2012, Kennedy and Adolphs, 2012), self-representation circuitry (Lombardo *et al.* , 2010), reward circuitry (Dichter *et al.* , 2012a, Dichter *et al.* , 2012b), the salience network (Uddin *et al.* , 2013), a motor control network (Kenet *et al.* , 2012), and an imitation network (Shih *et al.* , 2010). Despite the large and growing body of reports of abnormal functional connectivity in autism, inconsistencies in findings remain about the altered pattern of connectivity and the localization of the brain areas involved. This may be attributed to individual site-specific studies with relatively small sample sizes, coupled with the analysis performed.

The conclusions drawn from these studies are based on either seed-based analysis, independent component analysis (ICA), or parcellation-based analysis (using for example the 90 regions in the AAL template (Tzourio-Mazoyer *et al.* , 2002)) and these have some limitations. Seed-based analysis is a hypothesis-driven approach which means the foci (seeds) of the disorder must be specified a priori. It is therefore a biased approach lacking a global and independent view. With the ICA approach it is assumed that the human brain is composed of independent components, whereas different parts of the human brain work in a coordinated fashion. Parcellation-based whole brain analysis also is not fully unbiased due to the choice of the parcellation scheme, which directly specifies the nodes (regions) and edges (connections) of a macroscopic brain network (de Reus and Van den Heuvel, 2013). Hence, given the complexity and multiple causes of autism together with variability between individuals, a novel, unbiased approach is urgently called for which identifies pathway changes in a whole-brain voxel-based manner.

In the current paper, we describe the first voxel-based whole brain comparison of resting state functional connectivity differences between people with autism and controls. For this we needed a large number of autistic people and controls, and were able to use for this analysis data in a large resting state fMRI dataset, the autism brain imaging data exchange (ABIDE http://fcon_1000.projects.nitrc.org/indi/abide/), which has already proved useful (Di Martino *et al.* , 2013).

## METHOD

### Overall design

We analyzed resting state fMRI data from 523 autistic people and 452 controls in order to achieve sufficient statistical power for this first voxel-based whole brain comparison of resting state functional connectivity differences between people with autism and controls. A flow chart of the brain-wide association analysis (termed BWAS, in line with genome-wide association studies (GWAS)) is shown in Fig. 1. This

'discovery' approach tests for differences between patients and controls in the connectivity of every pair of brain voxels at a whole brain level. Unlike previous seed-based or independent components-based approaches, this method has the great advantage of being fully unbiased, in that the connectivity of all brain voxels can be compared, not just selected brain regions. Additionally, we investigated clinical associations between the identified abnormal circuitry and symptom severity; and we also investigated the extent to which the analysis can reliably discriminate between patients and controls using a pattern classification approach.

**Participants**

The ABIDE repository is hosted by the 1000 Functional Connectome Project / International Neuroimaging Data-sharing Initiative (INDI) (see http://fcon_1000.p rojects.nitrc.org for more information and other datasets). It consists of 1112 datasets comprised of 539 autism and 573 typically developing individuals. All data are fully anonymized in accordance with HIPAA (Health Insurance Portability and Accountability) guidelines, and research procedures and ethical guidelines were followed in accordance with the Institutional Review Boards (IRB) of the respective participating institution. All data released were visually inspected by members of the ABIDE project. Details of diagnostic criteria, acquisition, informed consent, and site-specific protocols are available at: http://fcon_1000.projects.nitrc.org/indi/abide/. The inclusion criteria for sample selection include: (1) fMRI data were successfully preprocessed with manual visual inspection of normalization to MNI space. (2) Any data affected by head motion of > 3 *mm* or rotation of > $3^{o}$ were excluded. (3) Sites were only included in our analysis if they had a total of at least 20 participants after the above exclusions. (4) Subjects were excluded if the percentage of 'bad' points (framewise displacement>0.5 mm) was over 10% in volume censoring (scrubbing, see below). A total of 975 subjects met all inclusion criteria (523 typically developing subjects and 452 subjects with autism from 16 sites). The demographic and clinical characteristics of participants satisfying the inclusion criteria are summarized in Table S1.

**Image Acquisition and Preprocessing**

In the ABIDE initiative, preexisting data are shared, with all data being collected at a number of different sites with 3 Tesla scanners. Details regarding data acquisition for each sample are provided in the ABIDE website (http://fcon_1000.projects.nitrc.org/indi/abide).

Preprocessing and statistical analysis of functional images were carried out using the Statistical Parametric Mapping package (SPM8, Wellcome Department for Imaging Neuroscience, London, UK). For each individual participant's dataset, the first 10 image volumes were discarded to allow the fMRI signal to reach a steady state. Initial analysis included slice time correction and Motion realignment. The resulting images were then spatially normalized to the Montreal Neurological Institute (MNI) EPI template in SPM8, resampled to $3\times3\times3$ $mm^3$, and subsequently smoothed with an isotropic Gaussian kernel (FWHM 8 mm).

To remove possible sources of spurious correlations present in resting-state BOLD data, all fMRI time-series underwent high-pass temporal filtering (0.01 Hz), nuisance signal removal from the ventricles and deep white matter, global mean signal removal, and motion correction with 6 rigid-body parameters, followed by low-pass temporal filtering (0.08 Hz). In addition, given views that excessive movement can impact between-group differences, we used four procedures to achieve motion correction. In the first step, we carried out three-dimensional motion correction by aligning each functional volume to the mean image of all volumes, and any data affected by head motion of > 3 mm or rotation of > $3^{o}$ was excluded. In the second step, we

implemented additional careful volume censoring ('scrubbing') movement correction (Power *et al.* , 2014) to ensure that head-motion artifacts were not driving observed effects. The mean framewise displacement (FD) was computed with FD threshold for exclusion being a displacement of 0.5 mm. In addition to the frame corresponding to the displaced time point, 1 preceding and 2 succeeding time points were also deleted to reduce the 'spill-over' effect of head movements. Thirdly, subjects with >10% displaced frames flagged were completely excluded from the analysis as it is likely that this level of movement would have had an influence on several volumes. Finally, the mean displacements were computed as the root-mean-square of the translation parameters and rotation parameters (computed as the average of the absolute value of the Euler angle of the rotation of each brain volume as compared to the previous volume) (Van Dijk *et al.* , 2012). We used the root mean square displacement as a covariate when comparing the 2 groups during statistical analysis.

**Voxel-level association study**

In the present study, each resting-state fMRI image included 47,636 voxels. For each pair of voxels, the time series were extracted and their correlation was calculated for each subject followed by *z*-transformation and two-tailed, two-sample t-tests were performed on the 1,134,570,430 (47636×47635/2) Fisher's *z*-transformed correlation coefficients to identify significantly altered functional links in autism patients compared to controls within each imaging centre. The Liptak-Stouffer *z* score method was then used to combine the results from the individual datasets, weighted by sample size, after removing the variance explained by differences in age, gender ratios, handedness and mean head motion. To avoid possible head motion artefacts, the mean displacement and rotation were regressed in again in the meta-analysis. This can be described as a meta-analytic approach performed across data sets from different imaging centres at the individual voxel-level across the whole brain to more precisely identify the localization of altered functional connectivity that typifies autism. A Bonferroni procedure was used to correct for multiple comparisons. A measure for the association (*MA*) between a voxel *i* and the brain disorder was then defined as: $MA(i) = N_\alpha$, where $N_\alpha$ is the number of links between voxel *i* and every other voxel in the brain that have a p-value of less than α (in the present study α=0.05/(47363×47635/2)) in t-tests. A larger value of *MA* implies a more significant alteration in functional connectivity.

The measure of association (MA) value described above shows voxels with significantly different functional connectivities, but not the brain regions to which these voxels have altered connectivity. To facilitate the explanation of our results, we also show the pattern of the altered connectivity in the Results section 2.

**Correlations with symptom severity**

We used the kernel generalized variance (S1) (Bach and Jordan, 2003) to quantify the dependency between differences in functional connectivity and social symptom severity in autism spectrum disorder as assessed by the Autism Diagnostic Observational Schedule (ADOS) total, communication, social and sterotyped behavior scores. The kernel generalized variance gives an estimate of the mutual information between two sets of variables (e.g. functional connectivities and ADOS scores), is exact when the variables are jointly Gaussian, and is an approximation to the second order when the variables in question are from arbitrary distributions and 'nearly independent' (Bach and Jordan, 2003). Statistical inference was based on a permutation procedure.

**Pattern classification based on the altered functional connectivity**

To further test the clinical relevance of the main identified autism links as diagnostic features of autism, we applied a support vector machine (SVM) approach using the alterations in the ROIs as a biomarker to test how well this could discriminate patients with autistic spectrum disorder from the healthy controls, that is, could classify individuals into one of these groups. In particular, features were first extracted from the voxel-based ROI-wise Functional Connectivities ($16 \times 15/2 = 120$ correlation coefficients) exhibiting significant group differences examined by two-sample t-test. We used a leave-one-out cross-validation strategy to estimate the generalization of this classifier and to estimate its accuracy, sensitivity and specificity.

**RESULTS**

**Whole Brain Voxel-Based Functional Networks**

Fig. 2B (and Fig. S1 with coronal slices) shows the locations of all voxels in the brain that had significantly different functional connectivities between the autistic and the control population. These voxels has some functional connectivities that were significantly different across the whole brain after Bonferroni correction. (After Bonferroni correction, the significance level had to be $p < 4.4 \times 10^{-11}$. Many of the functional connectivities had $p < 10^{-13}$, and this was only possible with the large numbers of subjects in the investigation.) Voxels that had functional connectivities in the autism population that were larger than in controls are shown in red, with the measure of association reflecting the number of significant links a particular voxel had. Voxels that had functional connectivities in the autism population that were weaker than in controls are shown in blue. Several main groups of voxels are evident in Fig. 2B, in the middle temporal gyrus (MTG), precuneus (PCUN), the post-central cortex (PoCG), a medial region of the thalamus (THA), the posterior cingulate cortex (DCG), and the ventromedial prefrontal cortex (ORBsupmed). These voxel-based data are shown in coronal slices of the brain in Fig. S1, and on whole brain diagrams in Fig. S2.

To help define the locations of these significant voxels and further analyze the connectivities, the voxels were categorized into Regions of Interest (ROIs) defined by the 90 areas of the AAL atlas (see Table S2 for a list of abbreviations and the corresponding numbers). Fig. 2A shows for each AAL region the p values for each voxel in each AAL region with a significant ($p < 10^{-5}$) functional connectivity link to any of the other 47635 voxels in the brain. Fig. 2A shows that there were many functional connectivity links that were significant after Bonferroni correction. The 16 AAL regions that contained 5 or more significant voxels are named in Fig. 2A. The coordinates of the clusters of 5 or more voxels are shown in Table 1 and the voxel clusters are illustrated in Fig. S2.

One main cluster of voxels was in the left middle temporal gyrus [-54 -24 0] (MTG.L ROI 14), with also a cluster in the right middle temporal gyrus (MTG.R ROI 15 and ITG.R ROI 16). The coordinates of the posteroventral part of the voxels in this MTG.L area were [-56 -39 2], and this has been identified as an area with activations related to face expression, with this and the more anterior part of this cluster implicated in theory of mind processing (see Discussion). (ROI 13 is a small further group of voxels in the temporal cortex.) A second cluster was in the left precuneus [-3 -54 39] (PCUN.L ROI 8), with a corresponding cluster in the right hemisphere. This is part of medial parietal cortex area 7. A third cluster was in the paracentral lobule

(PCL.L ROI 10), which is part of superior parietal cortex area 7 with connections to the precuneus (Margulies *et al.* , 2009). A fourth cluster is in the left post-central cortex in the face somatosensory area (PoCG, ROI 6 and 7, e.g. lower area in Fig. S2 top left). The fifth cluster is in the medial thalamus (THA [-3 -9 3]) (see Table 1 and Figs. S1 and S2). A sixth cluster (DCG) is in the posterior cingulate cortex, a region implicated in visuo-spatial orientation (Vogt, 2009). A seventh cluster is in the ventromedial prefrontal cortex (ORBsupmed, ROI 2) (in or close to cytoarchitectonic area 32 cingulate cortex / 10 medial prefrontal cortex / 11 medial orbitofrontal cortex), a region implicated in emotion (Rolls, 2014a, Rolls, 2014b).

**Altered Functional Connectivity Pattern**

In order to investigate the abnormal connectivity pattern in the functional connectivity networks in autism, all significant voxels (after Bonferroni correction) were parcellated into regions according to the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer *et al.* , 2002). On this basis, 16 regions of interest (ROIs) were identified in which there were 5 or more significant voxels, as shown in Table 1 and Fig. S2. The time series were extracted in each ROI by averaging the BOLD signals of all significant voxels within that region. The functional connectivity was evaluated between each pair of ROIs using a Pearson correlation coefficient. Then ROI-wise functional connectivity analysis on the significant voxels within each ROI was performed to compare patient groups and their respective healthy controls. In this part, we also regressed the effect of IQ. Finally, we obtained a 16×16 symmetric matrix (in Fig. 3A, which only show links with Bonferroni correction with a threshold of $p<4.2\times10^{-4}$), which shows the overall pattern of the different connectivity pattern between these voxel clusters in the autistic group.

The findings in Fig. 3 may be summarized as follows, based on the effects measured using the 5 or more significant voxels in a region. There was increased functional connectivity in the autistic group between the medial thalamus and the left and right middle temporal gyrus (MTG) and superior temporal gyrus (STG); and between the medial thalamus and the post-central gyrus (somatosensory cortex). Most cortico-cortical links that showed a significant change had a decrease in functional connectivity in people with autism. These included links of the middle temporal gyrus (MTG.R and ITG.R) with the ventromedial prefrontal cortex (ORBsupmed.R), precuneus (PCUN) and posterior cingulate cortex (PCG and DCG). Decreased functional connectivity was found between the precuneus (PCUN) and posterior cingulate gyrus (PCG), middle temporal gyrus, and ventromedial prefrontal cortex (ORBsupmed). The postcentral gyrus though had increased connectivity in the autism group with the posterior cingulate cortex (DCG) and paracentral lobule (PCL). Fig 3B provides a schematic brain-based diagram of these altered functional connectivities based on the significant voxels in the 16 ROIs. The altered patterns of functional connections were very consistent across the datasets from the 16 different imaging centers.

**Correlations of the altered functional connectivity links with the autism scores**

The kernel generalized variance was used to give an estimate of the correlation between the two sets of variables, namely the altered functional connectivities (16×15/2 links) and the four ADOS symptom severity scores. This test showed that the functional connectivity changes were significantly associated with the symptom severity scores ($p<0.02$). On this basis, we performed a post hoc analysis to investigate whether

differences in individual functional connectivity links were correlated with the symptoms of autism. In a post hoc illustrative analysis, we calculated the partial correlation between the strength of the functional connectivities between the significant voxels in the 16 ROI AAL-based areas, and the ADOS subscores removing the variance explained by differences in age and sex. As can be seen from Table 2 there were significant correlations ($p<0.05$) between some of the altered functional links and the symptom severity scores. A negative correlation (R) in Table 2 indicates that the weakening of a connectivity link is associated with the severity of a symptom. Of particular interest is that weakened connectivity between the middle temporal gyrus (MTG.L and ITGL) and the posterior cingulate cortex (DCG.L) was associated with the ADOS stereotyped behavior score.

**Classification of autism based on the altered functional connectivity links in autism**

To further test the functional significance of the links identified as related to autism, we employed a support vector machine (SVM) analysis to investigate the utility of the altered network as a biomarker for discriminating autistic people from controls. Fig. 4 shows that the highest discrimination power is achieved in the Stanford dataset (sample size = 20), with an accuracy of 100% for distinguishing autistic people from controls; and that the accuracy from the data from most of the imaging centres reached 80%. Permutation tests revealed that within and across datasets discrimination accuracies were highly significant ($p < 0.01$ in most cases). These results are summarized in Supplemental Table S3.

**DISCUSSION**

This is the first whole-brain voxel-based analysis of the functional connectivity differences between people with autism and controls, and the voxel-based results shown in Figs. 2B, S1 and S2 and Table 1 are therefore of great interest. Clusters of voxels with altered functional connectivity were associated with the middle temporal gyrus; with the ventromedial prefrontal cortex; and with the precuneus and paracentral lobule in the parietal cortex and the posterior cingulate cortex. The functional connectivity links between most of these clusters of voxels were decreased (Fig. 3), while the functional connectivity of some of these areas, especially the connectivity of the medial thalamus with the postcentral gyrus and middle temporal gyrus was increased (Fig. 3). The functional significance and correlation with the symptoms of these changes and of the altered functional connectivity between these areas are considered next.

The middle temporal gyrus (MTG) voxel clusters are in a region that may relate to face processing (in the cortex in the superior temporal sulcus) and to theory of mind impairments in autism. Rolls and colleagues discovered face cells not only in the macaque inferior temporal visual cortex (Perrett *et al.* , 1982, Rolls, 2011), where they provide transform-invariant representations of face identity (Rolls, 2012, Rolls and Treves, 2011), but also in the cortex in the superior temporal sulcus (Baylis *et al.* , 1987, Hasselmo *et al.* , 1989a), where the neurons respond to face expression (Hasselmo *et al.* , 1989a) and to movements and gestures of the head and body and gaze direction used in social communication (Hasselmo *et al.* , 1989b, Perrett *et al.* , 1985a, Perrett *et al.* , 1985b). For example, a neuron might respond to both turning the head away, and to closing the eyes, both of which are signals related to social communication (Hasselmo *et al.* , 1989b). Faces including those with expressions activate a region that corresponds in humans, with coordinates that include [-49 -36 2], [-49 -42 4]

and [52 -42 4] (Critchley *et al.* , 2000). Now the coordinates of the posteroventral part of the MTG voxels with altered functional connectivity in our investigation were [-57 -39 0], and so this is highly likely to be a face expression processing region. The altered functional connectivity in this area in autism is of great interest, for some of the key typical symptoms of autism spectrum disorder (ASD) are face processing and especially face expression processing deficits (Lai *et al.* , 2014), which will impair social and emotional communication (Rolls, 2014a). Further, in a meta-analysis of fMRI studies on the cortex in the STS / along the MTG, a posterior cluster of activations was identified with mean [-50 -55 14] that reflected motion processing, audio-visual correspondence (e.g. the sound and sight of an utterance), and face processing (Hein and Knight, 2008).

The MTG region we identified with altered functional connectivity also has a more antero-dorsal band extending from [-56 -17 -2] anteriorly to [-56 -47 18] posteriorly (Fig. 2). A more anterior cluster of activations in the STS (with mean at [-57 -19 -4]) is related to speech processing in a meta-analysis (Hein and Knight, 2008). In the meta-analysis, activations related to theory of mind (ToM) were equally distributed over the anterior and posterior clusters (Hein and Knight, 2008). (On the right, the MTG/ITG cluster has coordinates [60 -9 -25], and this region is implicated in theory of mind (Hein and Knight, 2008).) Thus the region that we identified with reduced functional cortical connectivity in ASD in the MTG/STS has functions related also to theory of mind and even speech processing. This is fascinating, given that there are major impairments of theory of mind and communication in ASD (Lai *et al.* , 2014).

The details of the changes in the connectivity of the MTG voxels are of interest, and are discussed using the voxel-based voxel connectivity analysis parcellated by AAL region shown in Fig. 3. The functional connectivity between the medial thalamus (the area shown in Fig. S1) and the MTG was increased (Fig. 3). However, in addition to this, it is shown in Fig. 3 that there is reduced functional connectivity of the MTG with the ventromedial prefrontal cortex (ORBsupmed), a region involved in emotion (Rolls, 2014a, Rolls, 2014b), including neurons with firing related to face expression (Rolls *et al.* , 2006). Indeed, the cortex in the macaque superior temporal sulcus that contains face expression neurons projects to the orbitofrontal cortex in which face expression neurons are also found (Rolls *et al.* , 2006). This is of interest, for the orbitofrontal cortex is important in social behavior communication (Rolls, 2014a), when damaged in humans produces face and voice emotional expression identification impairments (Hornak *et al.* , 2003, Hornak *et al.* , 1996), and has connections with the amygdala which also contains face-selective neurons (Leonard *et al.* , 1985), and both are implicated in autism in some other approaches (Baron-Cohen *et al.* , 1999, Lombardo *et al.* , 2010, Nordahl *et al.* , 2012). Further evidence for the importance of the ventromedial prefrontal cortex in autism is that it is a second main region in which voxels showed reduced functional connectivity (Figs. 2, S2 and Table 1,ORBsupmed), and this reduced connectivity was not only with the middle temporal gyrus (MTG and ITG), but also with the precuneus and posterior cingulate cortex (PCG and DCG) (Fig. 3). There is also reduced functional connectivity of the middle temporal gyrus with areas involved in spatial function and the sense of self, including the precuneus and posterior cingulate cortex (PCG and DCG). We interpret this as showing that there is cortical disconnection of the MTG with other cortical areas implicated in the present analysis as being related to autism, and this disconnection of the MTG region, given the contributions it appears to make to face expression processing and theory of mind, from other cortical areas is we hypothesize relevant to how the symptoms of autism arise. In this context, the reduced functional connectivity of the middle temporal gyrus

with areas involved in emotion, the ventromedial prefrontal cortex, and areas involved in the sense of self (the precuneus and its connected areas), appears to be very relevant to autism spectrum disorder, in which disorders of face processing, emotional and social responses, and theory of mind (to which the sense of self contributes) are important.

The third main set of voxels with reduced functional connectivity is in the precuneus (PCUN) region which is part of medial parietal cortex area 7, and regions closely related to it (Vogt, 2009) including the posterior cingulate cortex (PCG and DCG) (Fig. 2). The precuneus is a region with spatial representations not only of the self, but also of the spatial environment, and it may be partly in relation to this type of representation that damage to this region impairs the sense of self and agency (Cavanna and Trimble, 2006). The reduced functional connectivity of this region is therefore of great interest in relation to the symptoms of autism that relate to not having a theory of others' minds, for which a representation (or 'theory') of oneself in the world may be important (Lombardo *et al.* , 2010). The precuneus has associated with it the adjoining paracentral lobule (PCL) which is part of the superior parietal cortex with somatosensory and perhaps visual spatial functions, and which has string anatomical connections with the precuneus (Margulies *et al.* , 2009). Both the paracentral lobule with its body and spatial representation, and the precuneus, operate together to produce a sense of self, in which the representation of the body and how it acts in space is likely to be an important component. We therefore hypothesize that the reduced functional connectivity of these precuneus / superior parietal (PCL), and the related posterior cingulate cortex, regions is related to the altered representation or disconnection of the representation of oneself in the world that may contribute to the reduction in the theory of mind in autism (Lombardo *et al.* , 2010). In this context the reduced functional connectivity of this precuneus region with the MTG/ITG/STS areas (Fig. 3) is of interest, for theory of mind including of oneself and others, and face and voice communication with others, would seem to be a set of functions that should normally be usefully communicating to implement social behavior, which is impaired in autism. The reduced functional connectivity of this paracentral lobule with the somatosensory cortex (PoCG) (Fig. 3) in autism may reflect the fact that somatosensory inputs are likely to be important in the body and self-related functions of the parietal cortex.

The voxel-based analysis described here was especially important because it provided evidence about exactly where the altered functional connectivity was different in autistic people compared to controls. By using voxel-based analysis we were able to show for example that the face expression selective areas in the middle temporal gyrus, and also other MTG areas implicated in theory of mind, are both implicated as having altered functional connectivity in autism. The voxel-based analysis also showed that it is a medial / anterior part of the thalamus that has increased connectivity with the MTG, and this part of the thalamus is probably directly connected to temporal cortical areas as shown by diffusion tractography (Johansen-Berg *et al.* , 2005). Further, because voxel clusters may span AAL regions, incorrect inferences may be drawn by the use of AAL parcellation. For example ROI 15 and 16 in Table 1 are parcellated as MTG and ITG, but are in fact the same cluster of voxels in the right middle temporal gyrus. We suggest that an important way forward, in view of the usefulness of voxel-based analyses that is made evident in this paper, would be to continue with voxel-based analysis, but with even larger samples than the large samples used here, in order to provide more statistical power, to reveal further clusters of voxels, in for example the amygdala, that are linked to the voxel clusters

described in this paper, and to obtain further correlations between functional connectivity and the different symptom scores of people with autism spectrum disorder.

The analysis of the neural basis of autism described here was based on resting state functional connectivity. This complements other approaches such as neuroimaging activation studies by focussing on measures of functional connectivity, and by doing this when no external stimuli are applied, which may enable the inherent functional relations between brain areas to be investigated, as the system may be more largely influenced by statistical fluctuations and noise in the system (Deco *et al.* , 2013a, Deco *et al.* , 2013b). However, it is not always sufficiently acknowledged that during the resting state in the magnet with the eyes closed or when looking at a fixation point with no other assigned task, that one may well be thinking and mentalizing about one's plans for the day, other pending activities etc, which will almost always involve thinking about oneself, and about other people, i.e. other selves. The 'default mode network' may perhaps with this perspective be thought of as an 'inner mentalizing' network. Now of course it may be that this mentalizing process is different in people with autism, who may perform this type of thinking much less, or in a different way (Lai *et al.* , 2014). So we point out that resting state fMRI in autistic people may help to reveal differences in their brains relative to controls, in part because the thinking of people with autism may be different during resting state fMRI. Indeed, this difference of thinking during the imaging may contribute to the usefulness of the type of investigation described here of the brain systems implicated in autism.

In conclusion, we have described the first voxel-level analysis of functional connectivity in autism, made possible by the great efforts put into acquiring the data in the ABIDE database which we fully acknowledge and appreciate. We have identified a key system in the middle temporal gyrus / superior temporal sulcus region which has reduced functional connectivity with other cortical areas (and increased connectivity with the medial thalamus), which is implicated in face expression and motion processing involved in social behaviour, and which has onward connections to the orbitofrontal cortex/ventromedial prefrontal cortex.. The same system is implicated in theory of mind processing, and in audio-visual integration for e.g. speech, and possibly in further aspects of communication using language. We have identified a second main system with reduced cortical connectivity in autism, the ventromedial prefrontal cortex, which itself is implicated in emotion and social communication by virtue of its reward and punishment including face expression processing (Rolls, 2014a). Its reduced connectivity includes it connectivity with both the middle temporal gyrus regions implicated in face expression and theory of mind processing, and the precuneus / posterior cingulate regions involved in the sense of self. We have identified a third key system in the precuneus / superior parietal lobule (PCL) / posterior cingulate cortex with reduced functional connectivity which is implicated in spatial functions including of oneself, and of the spatial environment, and have suggested that this provides an important contribution to theory of mind processing which is impaired in autism. The hypothesis that we have in mind is that these types of functionality, face expression-related, and of one's self and the environment, are important components of the computations involved in theory of mind, whether of oneself or of others, and that reduced functional connectivity within and between the components of this circuitry may make a major contribution to the symptoms of autism. The reduced functional connectivity could reflect reduced connection strengths within and between areas, or impaired functioning of one of more of the areas that leads to reduced functional connectivity.

Acknowledgements.

J Feng is a Royal Society Wolfson Research Merit Award holder. The research was partially supported by the National Centre for Mathematics and Interdisciplinary Sciences (NCMIS) of the Chinese Academy of Sciences and Key Program of National Natural Science Foundation of China (No. 91230201). The research was supported by grants from the Natural Scientific Foundation of China 61104143 and 61104224 to J Zhang.

**Table 1.** Significant regions of interest (ROIs) in the voxel-based whole brain analysis. A ROI was defined as an area in an AAL region with 5 or more significant voxels after Bonferroni correction. The measure of association (MA) is shown as positive if overall the voxel has stronger functional connectivity links in people with autism, and as negative if they are weaker.

| No. | Areas | Cluster size #Voxels | Peak MA value | MNI coordinates (Peak) |
|---|---|---|---|---|
| **ROI 1** | SFGdor.R | 6 | 1 | 29 -26 69 |
| **ROI 2** | ORBsupmed.R | 18 | -4 | 6 47 -4 |
| **ROI 3** | DCG.L | 11 | -6 | -6 -39 36 |
| **ROI 4** | DCG.R | 7 | 8 | 2 -24 34 |
| **ROI 5** | PCG.L | 5 | -4 | 0 -51 33 |
| **ROI 6** | PoCG.L | 23 | -15 | -54 -18 51 |
| **ROI 7** | PoCG.R | 25 | -16 | 39 -27 48 |
| **ROI 8** | PCUN.L | 38 | -12 | -3 -54 39 |
| **ROI 9** | PCUN.R | 36 | -20 | 6 -54 27 |
| **ROI 10** | PCL.L | 7 | -5 | -9 -33 69 |
| **ROI 11** | THA.L | 38 | 57 | -3 -9 3 |
| **ROI 12** | THA.R | 16 | 78 | 3 -9 3 |
| **ROI 13** | STG.L | 26 | 11 | -54 -45 15 |
| **ROI 14** | MTG.L | 110 | 10 | -54 -24 0 |
| **ROI 15** | MTG.R | 11 | -11 | 60 -9 -24 |
| **ROI 16** | ITG.R | 6 | -3 | 60 -9 -27 |

**Table 2.** Correlations between the functional connectivity links and the autism symptom severity scores. The functional connectivity measure was that between the BOLD signals in the significant voxels in the two AAL regions specified.

| Links | | ADOS_TOTAL | | ADOS_COMM | | ADOS_SOCIAL | | ADOS_STEREO_BEHAV | |
|---|---|---|---|---|---|---|---|---|---|
| | | ***R*** | ***P*** | ***R*** | ***p*** | ***R*** | ***P*** | ***R*** | ***p*** |
| **THA.R** | **MTG.L** | **0.1107** | **0.0299** | 0.0842 | 0.0953 | **0.1214** | **0.0282** | 0.0500 | 0.4612 |
| **THA.L** | **MTG.L** | **0.1130** | **0.0466** | 0.0849 | 0.1516 | 0.0949 | 0.0969 | 0.0720 | 0.1246 |
| **DCG.L** | **ITG.R** | -0.0729 | 0.1941 | -0.0848 | 0.1559 | -0.0868 | 0.1509 | **-0.1499** | **0.0174** |
| **DCG.L** | **MTG.R** | -0.0878 | 0.1265 | -0.0894 | 0.1671 | -0.1041 | 0.0828 | **-0.1316** | **0.0426** |
| **PoCG.R** | **THA.R** | **0.1695** | **0.0032** | **0.1317** | **0.0321** | **0.1292** | **0.0317** | 0.0495 | 0.4537 |
| **SFGdor.R** | **DCG.L** | **-0.1106** | **0.0468** | **-0.1354** | **0.0291** | **-0.1306** | **0.0269** | -0.1119 | 0.0812 |
| **PCL.L** | **THA.R** | 0.0886 | 0.1221 | **0.1187** | **0.0493** | 0.0722 | 0.2165 | 0.0359 | 0.4647 |
| **DCG.L** | **PoCG.R** | **0.1277** | **0.0282** | **0.1459** | **0.0156** | 0.1153 | 0.0530 | 0.0735 | 0.2616 |
| **PCG.L** | **PoCG.R** | 0.1099 | 0.0618 | **0.1331** | **0.0319** | 0.0967 | 0.1121 | 0.0412 | 0.5772 |
| **SFGdor.R** | **DCG.R** | **-0.1913** | **0.0006** | **-0.2133** | **0.0002** | **-0.1700** | **0.0037** | **-0.1517** | **0.0315** |
| **DCG.R** | **THA.L** | -0.0737 | 0.2203 | -0.0749 | 0.2501 | -0.0863 | 0.1532 | **-0.1616** | **0.0153** |
| **PCUN.R** | **THA.L** | 0.0471 | 0.3523 | 0.0445 | 0.3862 | 0.0080 | 0.8727 | **0.1378** | **0.0483** |
| **PoCG.R** | **PCUN.L** | **0.1455** | **0.0111** | **0.1276** | **0.0339** | **0.1377** | **0.0202** | 0.0878 | 0.2277 |
| **PCUN.R** | **THA.R** | **0.1322** | **0.0152** | **0.1452** | **0.0091** | **0.1228** | **0.0312** | **0.1795** | **0.0107** |
| **PCUN.L** | **THA.R** | 0.0855 | 0.1585 | **0.1230** | **0.0497** | 0.0777 | 0.2066 | 0.0623 | 0.3877 |

## FIGURE LEGENDS

**Figure 1.** A flow chart of the voxel-wise functional connectivity meta-analysis on the Autism dataset.

**Figure 2.** Voxels with different functional connectivity in autism. **A.** Manhattan plot showing the probability values for each link being different in the autistic group compared to the control group. Each dot is a functional connectivity link between two voxels. Note there are a total of $47636 \times 47635 / 2$ links, and we only plot a dot if $p<10^{-5}$. The red dotted line is the Bonferroni correction threshold $4.4 \times 10^{-11}$. The regions indicate the AAL areas in which the voxels were located, with the numbers for each region specified in Table S2. **B.** Location of the voxels that had significantly different altered functional connectivity with other voxels (using whole brain Bonferroni correction). The color bar represents the measure of association (MA, see text) given by the number of significantly affected links relating to each voxel. Voxels that had functional connectivities in the autism population that were weaker than in controls are shown in blue, and that had stronger functional connectivity in the autism population are shown in red. Five main groups of voxels are evident, in the middle temporal gyrus (MTG), ventromedial prefrontal cortex (ORBsupmed), precuneus (PCUN) and paracentral lobule (PCL), the post- and pre-central cortex (PoCG and PreCG), and a medial region of the thalamus (THA). SFGmed - superior frontal gyrus, medial part.

**Figure 3 A.** The pattern of altered functional connectivity. The functional connectivity matrix calculated from the BOLD signals in the significant voxels in each of the 16 regions of interest based on the 16 AAL regions that contained 5 or more significant voxels. Probability values are shown in the table that are red if they are significantly stronger in the autistic group, and are blue if they are significantly weaker in the autistic group, after Bonferroni correction (see color bar for P values). **B**. A schematic diagram showing these voxel ROI based connectivity differences between the autistic and the control group.

**Figure 4.** Classification of individuals as autistic vs controls using the voxel-based ROI-wise functional connectivity measures identified. A. Receiver operating characteristic (ROC) curves showing the true positive rate plotted against the false positive rate are provided for each of the centres that contributed subjects to the database. B. Accuracy (percentage correct) classification of a subject as autistic or in the control group for the data from each of the centers.

**Figure 1**

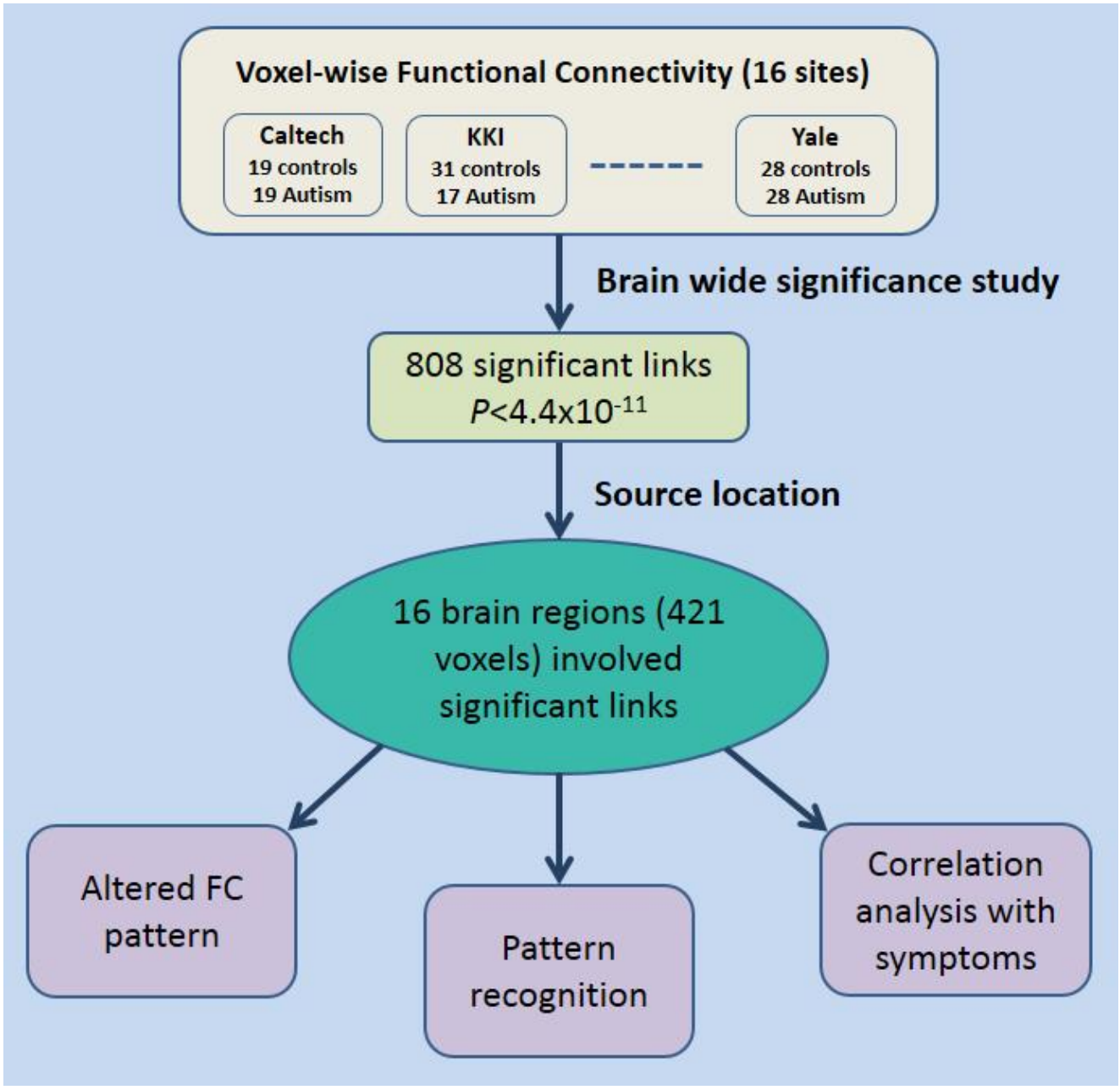

Voxel-wise Functional Connectivity (16 sites)
Caltech
19 controls
19 Autism
KKI
31 controls
17 Autism
Yale
28 controls
28 Autism
Brain wide significance study
808 significant links
P<4.4x10-11
Source location
16 brain regions (421 voxels) involved significant links
Altered FC pattern
Pattern recognition
Correlation analysis with symptoms

**Figure 2**

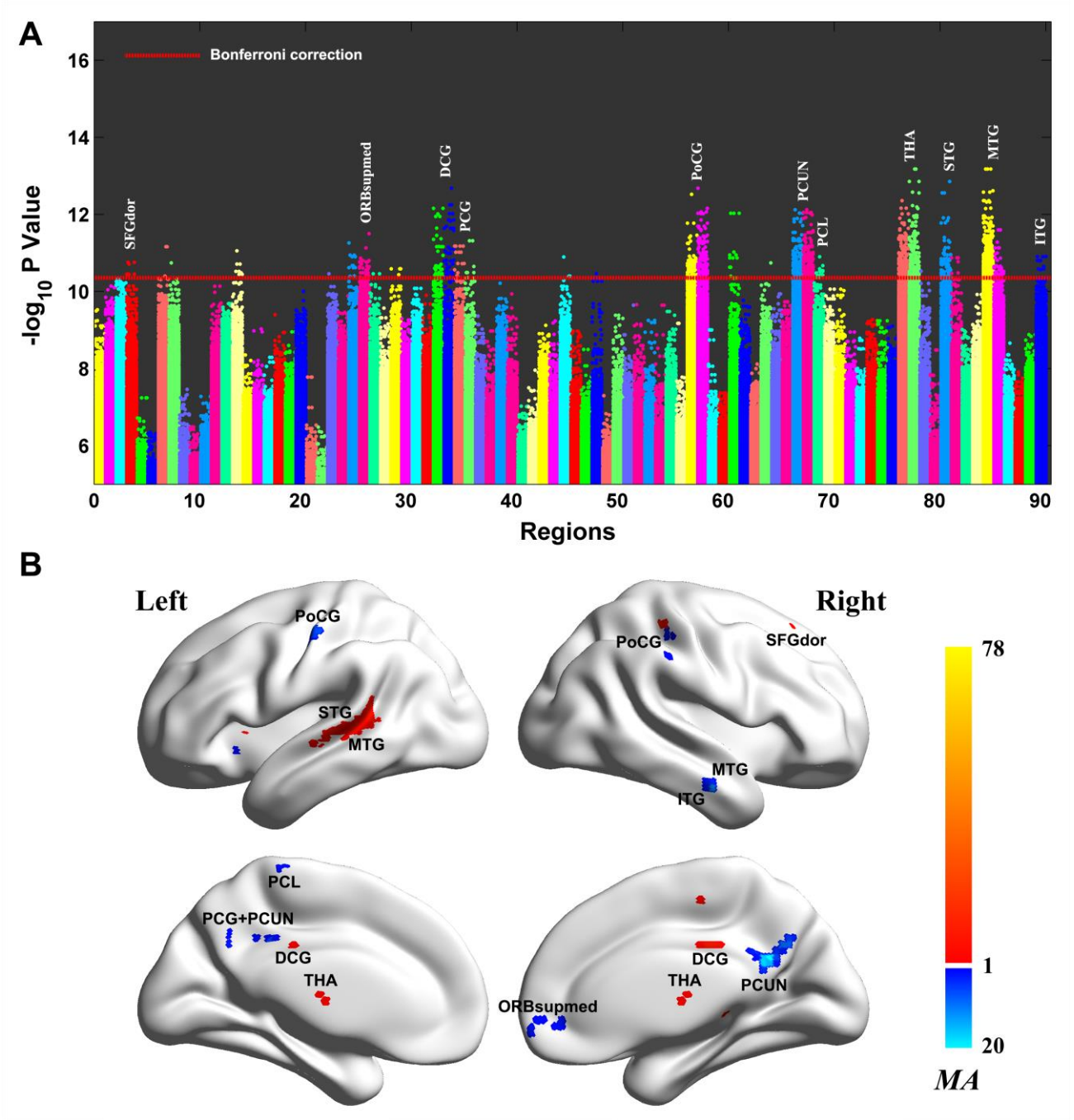

A
Bonferroni correction
-log10 P Value
Regions
SFGdor
ORBsupmed
DCG
PCG
PoCG
PCUN
PCL
THA
STG
MTG
ITG
B
Left
Right
PoCG
STG
MTG
SFGdor
ITG
PCL
PCG+PCUN
DCG
THA
ORBsupmed
PCUN
78
1
20
MA

**Figure 3**

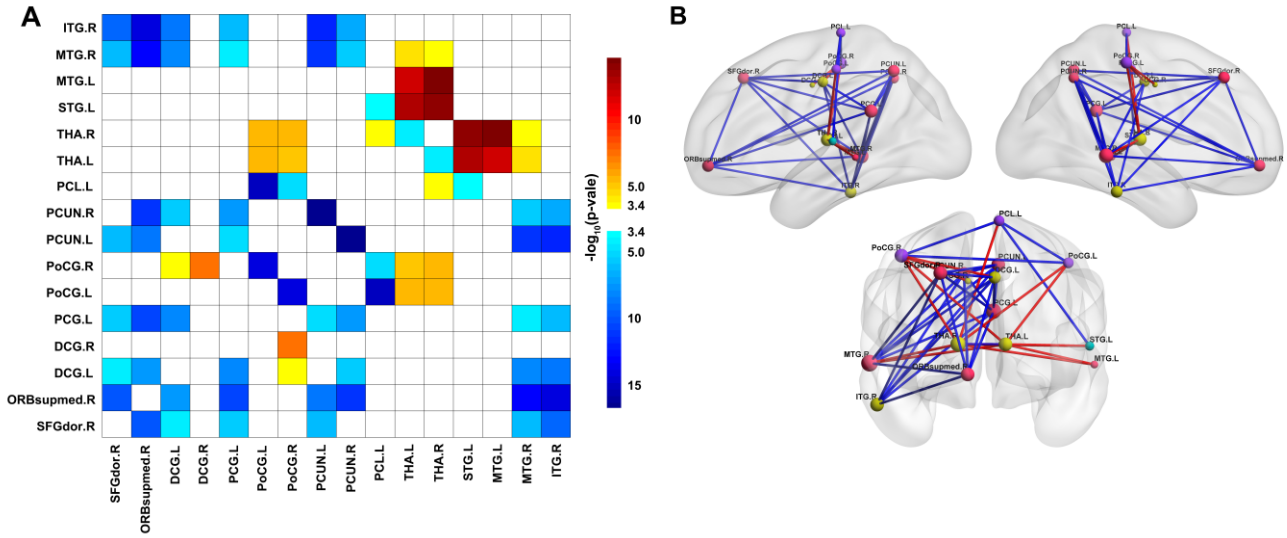
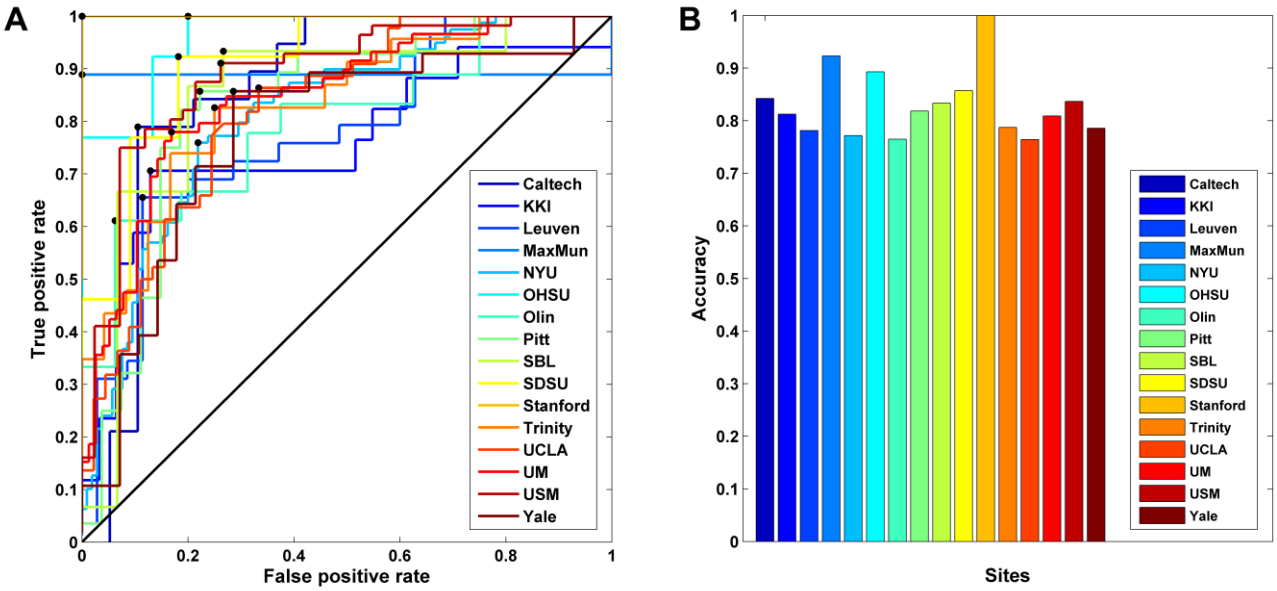

A
ITG.R
MTG.R
MTG.L
STG.L
THA.R
THA.L
PCL.L
PCUN.R
PCUN.L
PoCG.R
PoCG.L
PCG.L
DCG.R
DCG.L
ORBsupmed.R
SFGdor.R
SFGdor.R
ORBsupmed.R
DCG.L
DCG.R
PCG.L
PoCG.L
PoCG.R
PCUN.L
PCUN.R
PCL.L
THA.L
THA.R
STG.L
MTG.L
MTG.R
ITG.R
-log10(p-vale)
10
5.0
3.4
3.4
5.0
10
15
B

**Figure 4**


A
True positive rate
False positive rate
Caltech
KKI
Leuven
MaxMun
NYU
OHSU
Olin
Pitt
SBL
SDSU
Stanford
Trinity
UCLA
UM
USM
Yale
B
Accuracy
Sites

## References


Assaf M, Jagannathan K, Calhoun VD, Miller L, Stevens MC, Sahl R, et al. Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. NeuroImage. 2010;53(1):247-56.

Bach FR, Jordan MI. Kernel independent component analysis. The Journal of Machine Learning Research. 2003;3:1-48.

Baron-Cohen S, Ring HA, Wheelwright S, Bullmore ET, Brammer MJ, Simmons A, et al. Social intelligence in the normal and autistic brain: an fMRI study. The European journal of neuroscience. 1999 Jun;11(6):1891-8.

Baylis GC, Rolls ET, Leonard CM. Functional subdivisions of the temporal lobe neocortex. Journal of Neuroscience. 1987;7:330-42.

Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. Brain. 2006 Mar;129(Pt 3):564-83.

Critchley H, Daly E, Phillips M, Brammer M, Bullmore E, Williams S, et al. Explicit and implicit neural mechanisms for processing of social information from facial expressions: a functional magnetic resonance imaging study. Hum Brain Mapp. 2000 Feb;9(2):93-105.

de Reus MA, Van den Heuvel MP. The parcellation-based connectome: Limitations and extensions. NeuroImage. 2013;80:397-404.

Deco G, Jirsa VK, McIntosh AR. Resting brains never rest: computational insights into potential cognitive architectures. Trends in neurosciences. 2013a May;36(5):268-74.

Deco G, Ponce-Alvarez A, Mantini D, Romani GL, Hagmann P, Corbetta M. Resting-state functional connectivity emerges from structurally and dynamically shaped slow linear fluctuations. J Neurosci. 2013b Jul 3;33(27):11239-52.

Di Martino A, Yan C, Li Q, Denio E, Castellanos F, Alaerts K, et al. The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. Molecular psychiatry. 2013.

Dichter GS, Felder JN, Green SR, Rittenberg AM, Sasson NJ, Bodfish JW. Reward circuitry function in autism spectrum disorders. Social cognitive and affective neuroscience. 2012a;7(2):160-72.

Dichter GS, Richey JA, Rittenberg AM, Sabatino A, Bodfish JW. Reward circuitry function in autism during face anticipation and outcomes. Journal of autism and developmental disorders. 2012b;42(2):147-60.

Gotts SJ, Simmons WK, Milbury LA, Wallace GL, Cox RW, Martin A. Fractionation of social brain circuits in autism spectrum disorders. Brain. 2012;135(9):2711-25.

Hasselmo ME, Rolls ET, Baylis GC. The role of expression and identity in the face-selective responses of neurons in the temporal visual cortex of the monkey. Behavioural brain research. 1989a;32:203-18.

Hasselmo ME, Rolls ET, Baylis GC, Nalwa V. Object-centred encoding by face-selective neurons in the cortex in the superior temporal sulcus of the the monkey. Experimental Brain Research. 1989b;75:417-29.

Hein G, Knight RT. Superior temporal sulcus--It's my area: or is it? Journal of cognitive neuroscience. 2008 Dec;20(12):2125-36.

Hornak J, Bramham J, Rolls ET, Morris RG, O'Doherty J, Bullock PR, et al. Changes in emotion after circumscribed surgical lesions of the orbitofrontal and cingulate cortices. Brain. 2003;126:1691-712.

Hornak J, Rolls ET, Wade D. Face and voice expression identification in patients with emotional and behavioural changes following ventral frontal lobe damage. Neuropsychologia. 1996;34:247-61.

Johansen-Berg H, Behrens TE, Sillery E, Ciccarelli O, Thompson AJ, Smith SM, et al. Functional-anatomical validation and individual variation of diffusion tractography-based segmentation of the human thalamus. Cereb Cortex. 2005 Jan;15(1):31-9.

Kenet T, Orekhova EV, Bharadwaj H, Shetty NR, Israeli E, Lee AK, et al. Disconnectivity of the cortical ocular motor control network in autism spectrum disorders. NeuroImage. 2012;61(4):1226-34.

Kennedy DP, Adolphs R. The social brain in psychiatric and neurological disorders. Trends in cognitive sciences. 2012;16(11):559-72.

Kim YS, Leventhal BL, Koh Y-J, Fombonne E, Laska E, Lim E-C, et al. Prevalence of autism spectrum disorders in a total population sample. American Journal of Psychiatry. 2011;168(9):904-12.

Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet. 2014 Mar 8;383(9920):896-910.
Leonard CM, Rolls ET, Wilson FAW, Baylis GC. Neurons in the amygdala of the monkey with responses selective for faces. Behavioural brain research. 1985;15:159-76.
Lombardo MV, Chakrabarti B, Bullmore ET, Sadek SA, Pasco G, Wheelwright SJ, et al. Atypical neural self-representation in autism. Brain. 2010;133(2):611-24.
Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V. Default mode network in childhood autism: posteromedial cortex heterogeneity and relationship with social deficits. Biological psychiatry. 2013;74(3):212-9.
Margulies DS, Vincent JL, Kelly C, Lohmann G, Uddin LQ, Biswal BB, et al. Precuneus shares intrinsic functional architecture in humans and monkeys. Proceedings of the National Academy of Sciences of the United States of America. 2009 Nov 24;106(47):20069-74.
Maximo JO, Cadena EJ, Kana RK. The Implications of Brain Connectivity in the Neuropsychology of Autism. Neuropsychology review. 2014;24(1):16-31.
Minshew NJ, Keller TA. The nature of brain dysfunction in autism: functional brain imaging studies. Current opinion in neurology. 2010;23(2):124.
Müller R-A, Shih P, Keehn B, Deyoe JR, Leyden KM, Shukla DK. Underconnected, but how? A survey of functional connectivity MRI studies in autism spectrum disorders. Cerebral Cortex. 2011;21(10):2233-43.
Nordahl CW, Scholz R, Yang X, Buonocore MH, Simon T, Rogers S, et al. Increased rate of amygdala growth in children aged 2 to 4 years with autism spectrum disorders: a longitudinal study. Archives of general psychiatry. 2012 Jan;69(1):53-61.
Perrett DI, Rolls ET, Caan W. Visual neurons responsive to faces in the monkey temporal cortex. Experimental Brain Research. 1982;47(3):329-42.
Perrett DI, Smith PA, Potter DD, Mistlin AJ, Head AS, Milner AD, et al. Visual cells in the temporal cortex sensitive to face view and gaze direction. Proceedings of the Royal Society of London B. 1985a;223:293-317.
Perrett DI, Smith PAJ, Mistlin AJ, Chitty AJ, Head AS, Potter DD, et al. Visual analysis of body movements by neurons in the temporal cortex of the macaque monkey: a preliminary report. Behavioural brain research. 1985b;16:153-70.
Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage. 2014 Jan 1;84:320-41.
Rolls ET. Face neurons. In: Calder AJ, Rhodes G, Johnson MH, Haxby JV, editors. The Oxford Handbook of Face Perception. Oxford: Oxford University Press; 2011. p. 51-75.
Rolls ET. Invariant visual object and face recognition: neural and computational bases, and a model, VisNet. Frontiers in computational neuroscience. 2012;6, 35:1-70.
Rolls ET. Emotion and Decision-Making Explained. Oxford: Oxford University Press; 2014a.
Rolls ET. Limbic systems for emotion and for memory, but no single limbic system. Cortex. 2014b;doi: 10.1016/j.cortex.2013.12.005.
Rolls ET, Critchley HD, Browning AS, Inoue K. Face-selective and auditory neurons in the primate orbitofrontal cortex. Experimental Brain Research. 2006;170:74-87.
Rolls ET, Treves A. The neuronal encoding of information in the brain. Progress in neurobiology. 2011;95:448-90.
Shih P, Shen M, Öttl B, Keehn B, Gaffrey MS, Müller R-A. Atypical network connectivity for imitation in autism spectrum disorder. Neuropsychologia. 2010;48(10):2931-9.
Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage. 2002 Jan;15(1):273-89.
Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, et al. Salience Network–Based Classification and Prediction of Symptom Severity in Children With Autism. JAMA psychiatry. 2013;70(8):869-79.
Van Dijk KR, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. NeuroImage. 2012 Jan 2;59(1):431-8.
Vogt BA, editor. Cingulate Neurobiology and Disease. Oxford: Oxford University Press; 2009.