

Functional connectivity in a baseline resting-state network in autism

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Abstract: Brain activity in people with high-functioning autism has been shown to be atypical in a number of ways, including reduced synchronization across areas of fMRI-measured activation. This activation atypicality has been observed mostly during the performance of cognitive tasks. This study compares the resting-state network of 57 participants with autism and 57 control participants matched for age and IQ. The results indicate that both groups have a resting-state network which is very similar both in volume and in organization, but in autism, this network is much more loosely connected. This functional underconnectivity was observed in the anterior-posterior connections. The results expand the theory of cortical underconnectivity in autism to the resting state of the brain.

Keywords: autism; functional connectivity; corpus callosum; fMRI; resting-state network

Converging evidence from fMRI studies has indicated that autism is a system-wide disorder with reduced synchronization of the time course of brain activation found in several tasks, such as language processing [1], working memory [2], executive functioning [3], and visual imagery [4]. All these previous fMRI studies explored brain activation during task performance. Fixation or the “resting state” in fMRI tasks provides a new domain to measure cortical synchronization. The flow of mental events continues even during the resting state activating a set of brain regions that have been associated with a “default mode” of brain function [5]. The brain regions identified as default mode areas have been localized most often to medial structures including the medial frontal gyrus, the anterior cingulate, the posterior cingulate, and the precuneus [5,6,7,8].

Researchers have found that individuals with autism report very different internal thoughts than control participants [9,10]. The main objective of the present study was to investigate the brain activation and synchronization in autism in the most “diluted”,

content-free baseline “resting state”. Resting state fMRI was used in this study to investigate both the volume and location of activation, and functional connectivity in people with autism and control participants. Examining functional connectivity during a baseline resting state provides an opportunity to pool data across several studies, resulting in an unusually large sample size for a functional imaging analysis. To our knowledge, this is the first study to investigate functional connectivity during rest in autism. In addition, we examine the relationship between functional connectivity during resting baseline and the size of the corpus callosum, a major component of anatomical connectivity.

Based on previous findings of regions activated during rest in the typical population, and brain activation findings in autism during task performance, we set out to examine the following hypotheses. First, we predicted that people with autism would show reduced functional connectivity relative to controls during the resting state. The rationale behind this hypothesis is that inward thinking during rest involves subprocesses, such as

representation, monitoring, evaluation, and integration. These processes take effect by the coordinated interaction of the medial cortical structures [11], which demands robust connectivity. Second, we anticipated that functional connectivity in autism would be positively correlated with the corpus callosum size. This hypothesis is based on our findings of such correlation in autism during task performance. In general, autism may affect all types of brain activity, and the resting state may be no exception.

Method

Participants. 57 high-functioning individuals with autism and 57 control participants were included in the analyses. Participants were matched on the basis of age (autism mean \pm SD: 24 ± 10.6 ; control mean \pm SD: 24 ± 9.0), Full Scale IQ (autism: 106 ± 16.2 ; control: 113 ± 11.3), and Verbal IQ (autism: 104 ± 16.1 ; control: 108 ± 10.5). Four participants in the autism group and five in the control group were females; seven participants in the autism group and four control participants were left-handed. The diagnosis of autism was established using the ADI-R [12] and the ADOS-G [13]. The detailed description of participant selection and exclusion is reported elsewhere [3]. Each participant signed an informed consent that had been approved by the University of Pittsburgh and Carnegie Mellon University Institutional Review Boards.

Experimental paradigm. The resting-state network was analyzed using the data from six separate fMRI autism studies performed at the Center for Cognitive Brain Imaging (Carnegie Mellon University). The studies addressed executive function (Tower of London task), various language-related tasks (visual imagery, irony comprehension, understanding discourse), response inhibition, and “theory of mind”.

In addition to the two or three conditions of the cognitive task, each of these studies had a separate Fixation task, when an asterisk was presented at the middle of the computer screen and the participant was instructed to focus on it, think about nothing and “clear his/her mind”. Each study had four or five blocks of fixations distributed throughout the experiment. Each block lasted for 24 seconds. Therefore, it was possible to perform the analysis of brain activation during fixation for a large pool of participants (57 in each group). 16 participants with autism and 15 control participants took part in more than one study.

fMRI parameters. For all the studies but the Tower of London, the imaging was carried out on a 3T Siemens Allegra scanner. The details of the stimulus presentation and data acquisition parameters were reported elsewhere [4]. Functional scans were performed at TR=1000 ms, with 16 oblique-axial 5-mm slices, 1-mm slice gap, and an in-plane resolution of 3.125×3.125 mm. For the Tower of London study, imaging was performed on GE 1.5T and 3T scanners with similar parameters except for a TR=3000 ms and 14 slices. In addition to functional scans, T1-weighted scans were also obtained.

Distribution of activation. The data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, London, UK). Images were corrected for slice timing and motion, normalized to the MNI template, resampled to $2 \times 2 \times 2$ mm voxels, and smoothed with an 8-mm Gaussian kernel. For each participant, a contrast between the fixation and all cognitive tasks was computed, and the group analyses were performed using a random-effects model

Functional connectivity. Functional connectivity was computed for each participant and each pair of regions of interest (ROIs) as a correlation between the average time courses of all the voxels of the first and second ROIs. 12 functional ROIs (spheres with a radius of 6 mm) were defined that best captured the peaks of activation in the combined group activation map. Labels for these 12 ROIs [the posterior cingulate cortex (PCC), ventral anterior cingulate cortex (VACC), precuneus (PREC), paracentral lobule (PCL), plus four bilateral ROIs, namely medial/middle prefrontal cortex (MPFC), inferior parietal cortex (IPC), parahippocampal gyrus (PHG), and inferolateral temporal cortex (insula) (ITC)] were assigned with reference to the parcellation of the MNI template subject [14]. The averaged time courses were high-pass filtered (cut-off value range 120-225 s) and had the linear trend removed. The correlation was computed on the images belonging only to the fixation condition, so it reflects the interaction between the activation in two areas while the participant is at rest. The first 6 seconds of each fixation block were removed to accommodate the hemodynamic delay. Fisher’s r to z' transformation was applied to the correlation coefficients for each participant. For the subjects who participated in multiple studies (i.e. had multiple scans), the appropriate connectivities were averaged. We also performed the analysis using a single random scan for these subjects, and obtained very similar results. In order to summarize the

connectivity differences, the 12 ROIs were grouped into larger regions in separate analyses on the basis of lobes, anterior or posterior locations, and hemispheres. Group differences in connectivity were computed using single averaged (according to an ROI grouping) z-value for each subject.

Corpus callosum morphometry. The cross-sectional area of the midsagittal slice of the corpus callosum was measured using the parcellation scheme described by Witelson [15] (see [4] for details).

Limitations. Since this study used fixation blocks in experiments, the nature of the data limits our capability to estimate very low frequency components of interactions between the brain areas (the fixation block duration was 24 seconds). There is also a possibility of some after-effects of specific tasks on the baseline activity, though all efforts were made to eliminate such effects. There is no reason to assume that such effects might affect the two groups differently.

Results

Functional connectivity. Despite similar levels of activation in autism and control groups, the functional connectivity between many ROIs was lower for the participants with autism. This effect was widespread, such that out of a total 66 pairwise connectivities computed for the set of 12 ROIs, 94% showed lower connectivity for the participants with autism. To characterize these differences statistically, ROIs were grouped together based on a few principles.

First, the connections of the lobe-based groups of ROIs (frontal with other lobes) were compared for the two groups. The reason for this grouping is that the most affected functional connectivity in autism is between frontal areas and other cortical areas [1,3,4]. To compute this functional connectivity, all connectivities of the ROI pairs consisting of any frontal (VACC, LMPFC, RMPFC) and any parietal (PCC, PREC, LIPC, RIPC) ROI were averaged, i.e. we averaged the connectivities of VACC with PCC, VACC with PREC, etc. Frontal-parietal connectivity was lower for the autism group, but the difference did not reach significance (autism mean: 0.30, control mean: 0.36, ns). At the same time, the connectivity of the anterior cingulate region (VACC) with the posterior cingulate and precuneus group (PCC+PREC) was significantly lower for the autism group (autism mean: 0.27, control mean: 0.37, two-tail t-test; $t(112) = 2.05$, $p < 0.05$).

Second, the connections of the left and right parahippocampal areas with the rest of the ROIs were explored separately. The connectivity of the LPHG with the rest of the ROIs was significantly lower for the autism group (autism mean: 0.14, control mean: 0.23; $t(112) = 2.44$, $p < 0.05$), but this was not the case for the RPHG (autism mean: 0.16, control mean: 0.22, ns).

Additionally, the 12 ROIs were grouped based on the hemisphere, resulting in three networks (within left hemisphere, within right hemisphere, and between hemisphere connections). Medial ROIs (VACC, PCL, PCC, and PREC) were excluded from this analysis. Within left hemisphere connectivity was significantly lower for the group with autism (autism mean: 0.16, control mean: 0.24; $t(112) = 2.05$, $p < 0.05$). There were no reliable differences in within right hemisphere connectivity (autism mean: 0.17, control mean: 0.22, ns) or between hemispheres connectivity (autism mean: 0.23, control mean: 0.30, ns).

Corpus callosum size and functional connectivity. The size of the entire corpus callosum as well as some subregions was correlated with the functional connectivity measures in both groups. The size of the rostral body, an anterior subregion of the corpus callosum, was reliably positively correlated with the functional connectivity between left frontal (LMPFC) and posterior (PCC) regions in the autism group [$r = 0.36$, $t(55) = 2.84$, $p < 0.01$], while the control group showed no such correlation ($r = -0.07$, ns). The scatter plots for these data are presented in Figure 1. The same corpus callosum region was also reliably positively correlated with the functional connectivity between left and right parahippocampal areas in the autism group [$r = 0.29$, $t(55) = 2.25$, $p < 0.05$], while the control group showed no such correlation ($r = -0.07$, ns).

The result of positive correlation between the size of the anterior areas of corpus callosum and the functional connectivity of anterior-posterior connections in autism is similar to the findings in our previous studies [3,4] related to functional connectivity during the performance of cognitive tasks. These findings provide insight concerning an anatomical basis of underconnectivity in autism.

Group similarity in brain activation. The resting-state brain activation for the group with autism is presented in Figure 2a. The activation was very similar for both groups. Direct comparison of the two groups' activation showed no differences at $p=0.001$, extent threshold of 100 voxels; when the extent threshold was lowered to 20 voxels,

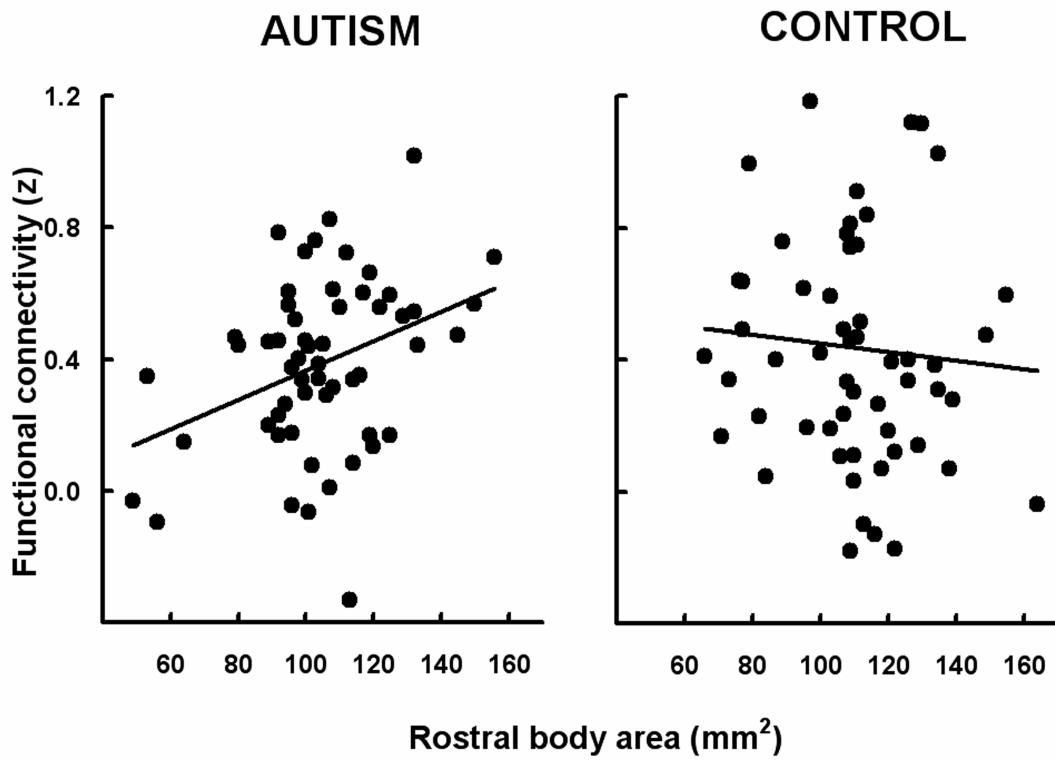


Figure 1: Correlations between functional connectivity and the corpus callosum size. The connectivity of left frontal (LMPFC) and posterior (PCC) regions vs. the size of rostral body of corpus callosum: autism, $r = 0.36$; control, $r = -0.07$

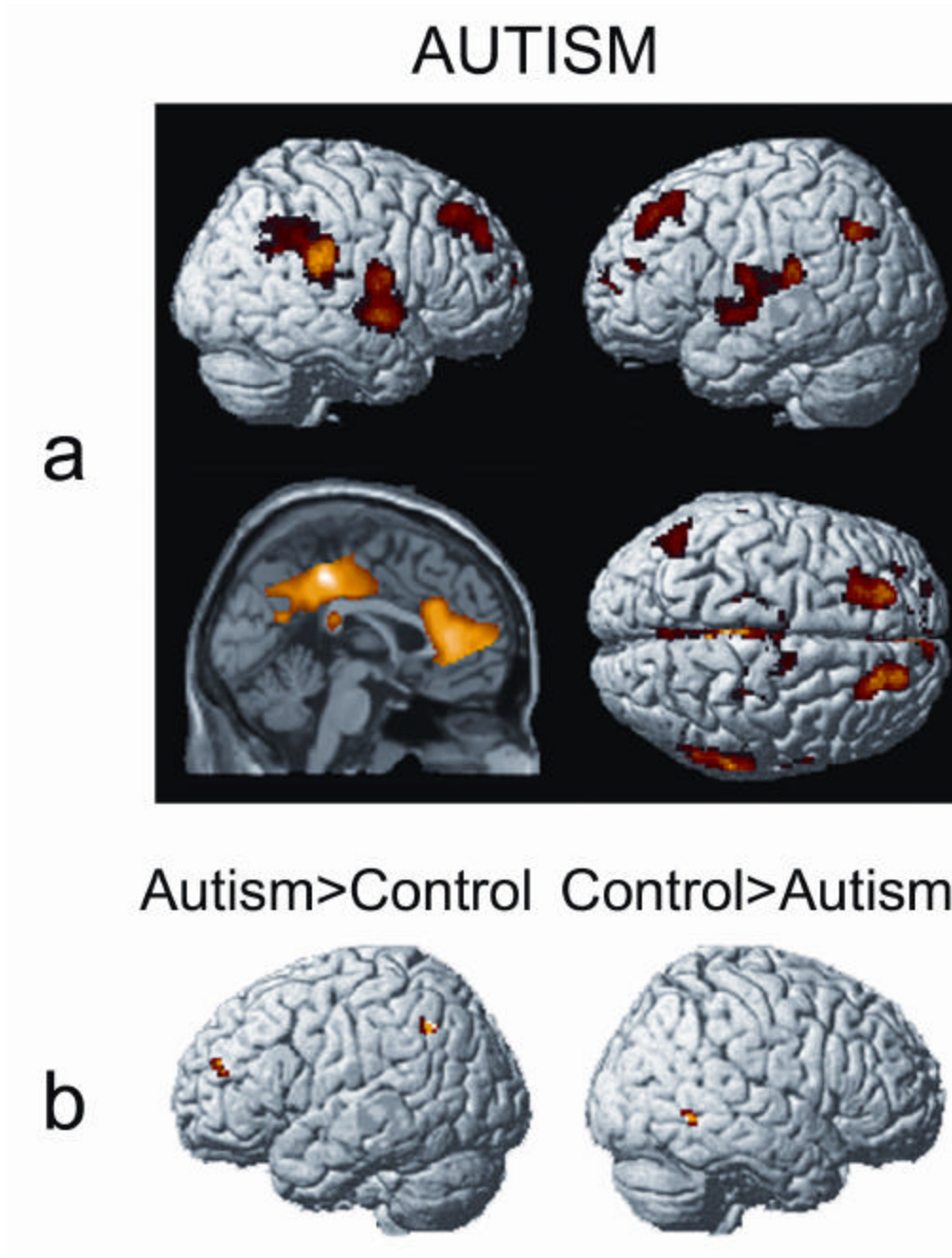


Figure 2: Resting-state network activation.

a. Autism group resting-state activation ($p < 0.001$);

b. Minor group differences in the resting-state activation: autism group $>$ control group (left), control group $>$ autism group (right). Other brain surface renderings (not shown) contain no differences ($p < 0.001$).

participants with autism activated more in two left hemisphere areas: middle/superior frontal gyrus (26 voxels) and supramarginal gyrus (37 voxels). Control participants showed higher activation in the right middle temporal gyrus (37 voxels) (Figure 2b). We concluded that the differences in activation between the two groups were very small. The following areas were involved in the resting-state network for both groups: PCC, VACC, PREC, PCL, and bilaterally, MPFC, IPC, PHG, and ITC.

Discussion

Previously we have postulated the cortical underconnectivity theory of autism [1] on the basis of brain activity characteristics in a wide range of cognitive tasks. The present study extends the underconnectivity theory to the domain of a task free, resting state. The central finding of this study was that the participants with autism showed underconnectivity between anterior and posterior regions that activate in fixation. The medial structures anterior cingulate and posterior cingulate were functionally underconnected in autism relative to control participants. These findings reveal that functional underconnectivity in autism is not restricted to complex tasks, but also is prevalent during rest, so the lack of coordination among neural centers in autism is pervasive.

Lower functional connectivity between anterior and posterior medial cortex is interesting considering the possible role of these resting state (default-mode) centers. A number of studies indicate that both anterior medial cortex (medial frontal gyrus and/or anterior cingulate cortex) and posterior medial cortex (posterior cingulate cortex and/or precuneus) are involved in self-referential processing [16,17]. Studies have also reported impairments in self-consciousness in autism [18]. It is possible that people with autism might not be generating spontaneous and self-related thought processes during fixation. It is also possible that these regions are involved in emotional processing through the connections between ventral anterior cingulate and posterior cingulate cortex [17]. The link between PCC and VACC is believed by some to be important in connecting higher cortical (conscious) processing and more basic (possibly unconscious) processing related to autonomic state [8].

A second major result pertains to the correlation between fixation functional connectivity in autism and the corpus callosum size. fMRI studies during task performance have found such correlation before

[3,4]. It is interesting that this effect prevails even when participants are not doing a cognitive task. This indicates that whatever factor is depressing the functional connectivity in autism during task performance is also operative during a resting state. Finally, we found that the resting-state network activation was similar in both groups, unlike a previous study that found reduced activation in autism in the default mode network regions [19]. The source of this difference might be related to the task they used (the counting Stroop task) that produces activation related to inhibition which is seen in medial cingulate regions.

Conclusions. These results provide new converging evidence for the hypothesis that autism is a distributed neural systems disorder rather than a focal disorder and that the disorder is manifest even when people are not performing any particular task.

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