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Autistic Traits in Neurotypical Adults are Related to Cingulo-Insular Functional Connectivity

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Abstract

Objective—Based on the increased recognition of the dimensional nature of autistic traits, we examined their neural correlates in neurotypical individuals using the Social Responsiveness Scale-Adult version (SRS-A). The SRS-A measures autistic traits that are continuously distributed in the general population. Here, we establish a novel approach to examining the neural basis of autistic traits, attempting to directly relate SRS-A scores to patterns of functional connectivity observed for the pregenual anterior cingulate cortex (pgACC), a region commonly implicated in social cognition.

Methods—Resting state fMRI scans were collected in 25 neurotypical individuals (26.4 ± 5.6 y) who provided SRS-A completed by an informant who knew the participant in natural social settings. Whole brain corrected connectivity analyses were then conducted using the SRS-A as a covariate of interest.

Results—We found a significant negative relationship between SRS-A and pgACC functional connectivity with the anterior portion of mid-insula ($Z > 2.3$; $p < .05$, corrected). Specifically, low levels of autistic traits were observed when a substantial portion of the anterior mid-insula showed positive connectivity with pgACC. In contrast, elevated levels of autistic traits were associated with negative connectivity between the pgACC and the anterior mid-insula.

Conclusions—Resting state functional connectivity of the pgACC-insula social network was related to autistic traits in neurotypical adults. Application of this approach in samples with autism spectrum disorders is needed to confirm whether the pgACC- anterior mid insula circuit is dimensionally related to the severity of autistic traits in clinical populations.

Keywords

Autism - AJP0006; Brain Imaging Techniques - AJP0068

Appreciation of the likely dimensional nature of autistic symptoms (1–3) has led to the development of measures to evaluate autism-related social traits in the general population (2–4). The Social Responsiveness Scale (SRS) quantifies social reciprocity impairments that

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are characteristic of autism spectrum disorders (ASD; including autistic disorder, Asperger syndrome, and pervasive developmental disorders not otherwise specified) (3). The SRS, which has been extensively validated in both child and adult populations (3;5;6), provides a single measure of social impairment representing a “singular, continuously distributed underlying factor” in the population (7). As such, the SRS may allow investigators to study the neurobiology of autistic traits in both clinical and non-clinical samples.

In the current study we developed a protocol for examining the neural correlates of autistic traits, by measuring the relation between informant-provided SRS scores and resting state functional connectivity measures (FC) in neurotypical individuals. Resting-state fMRI has emerged as a powerful means of mapping and characterizing FC without the constraints of task-based approaches (8–10). Imaging and electrophysiological studies (11;12) examining intrinsic brain activity at rest in individuals with ASD support a model of compromised long-range cortico-cortical connectivity (13). Recently, two resting-state fMRI studies comparing high functioning adults with ASD to neurotypical controls (NC) (14;15) revealed reduced intrinsic connectivity of long-range circuits based in pregenual portions of anterior cingulate cortex (pgACC).

Involvement of pgACC in ASD is consistent with this region’s role in thinking about others’ thoughts and beliefs (16;17), which is qualitatively impaired in individuals with ASD. Furthermore, robust patterns of pgACC hypofunction in ASD were revealed by a recent voxel-wise meta-analysis of functional imaging studies of social processing (18). This accumulating evidence suggests that pgACC-based networks may underlie autistic traits. Other likely nodes include the posterior cingulate cortex (PCC), which is associated with self-referential processing and mentalizing (19), and the anterior insula which is linked to representing and/or monitoring the salience of one’s own and others’ emotions (20–22). Hypoactivation of both PCC and anterior insula was found in individuals with ASD compared to NC in a recent meta-analysis (18).

In the present study, we hypothesized that inter-individual differences in autistic traits would be related to differences in the functional connectivity of the pgACC even in a non-clinical population. Specifically, we predicted that higher SRS scores, indexing greater autistic traits, would be inversely related to the strength of FC in two specific circuits: pgACC – PCC and pgACC – anterior insula.

Methods

Study Design

We contacted 46 right-handed native English-speaking individuals (mean age $\pm$ SD 28.5 $\pm$ 6.9 y, 23 males) who participated in previously published resting-state studies (23–27). We asked that they select someone who knew them well in natural social settings to complete the adult-version of the SRS questionnaire (SRS-A) (5). All participants had received an unstructured clinical assessment by psychiatrists and were found to have no past or current psychiatric or neurological illness.

The 25 participants who provided questionnaires constituted this study sample (26.4 $\pm$ 5.6 y; 9 males); 20 had completed three resting-state scans as part of a test-retest study (24). The first of these three scans was collected 5–16 months prior to the other two which were performed 45 minutes apart in the same session. Four participants had two resting-state scans (45 minutes apart) and a single subject had only been scanned once. As detailed in the participant level analyses, all available scans were used to derive a best single estimate of FC for each participant. The study was approved by the institutional review boards of New

York University School of Medicine and New York University. Signed informed consent was obtained prior to participation, which was compensated.

Social Responsiveness Scale-Adult Version

The SRS-A is a 65 item questionnaire designed to be completed by an informant who knows the proband in naturalistic social settings (spouses/partners, relatives, or close friends for the SRS-A). Total SRS-A raw scores range from 0, corresponding to high social competence, to 195, corresponding to significant social impairment as observed in individuals with severe ASD. Scores between 60 and 80 are associated with mild forms of ASD (5).

fMRI Data Acquisition

Data were collected on a Siemens Allegra 3.0 Tesla scanner equipped for echo planar imaging (EPI). Resting-state fMRI scan entailed 197 continuous EPI functional volumes (TR = 2000ms; TE = 25 ms; flip angle = 90; 39 slices, matrix = 64×64, FOV = 192 mm; acquisition voxel size = 3×3×3 mm; duration: 6.5 minutes). During each scan, participants were instructed to rest with their eyes open while the word “Relax” was centrally projected in white, against a black background. For spatial normalization and localization, a high-resolution T1-weighted anatomical image was also acquired using a magnetization prepared gradient echo sequence (MPRAGE, TR = 2500 ms; TE = 4.35 ms; TI = 900 ms; flip angle = 8; 176 slices, FOV = 256 mm).

Image Preprocessing

As detailed in prior work (23;27) data were processed using both AFNI (version AFNI_2008_07_18_1710, <http://afni.nimh.nih.gov/afni>) and FSL (version 3.3, www.fmrib.ox.ac.uk). Image preprocessing using AFNI consisted of (1) slice time correction for interleaved acquisitions using Fourier interpolation, (2) 3D volume registration using least-squares alignment of three translational and three rotational parameters for motion correction, and (3) despiking of extreme time series outliers using a continuous transformation function. Preprocessing using FSL consisted of (4) mean-based intensity normalization of all volumes by the same factor, (5) spatial smoothing (Gaussian kernel of FWHM 6mm), (5) temporal high-pass filtering (Gaussian-weighted least-squares straight line fitting with sigma = 100.0s), (6) temporal low-pass filtering (Gaussian filter with HWHM = 2.8s), and (7) correction for time series autocorrelation (pre-whitening). Pre-whitening renders successive time points independent of one another, thus improving the validity of subsequent statistical analyses.

Seed Selection

We used a spherical region of interest (ROI; diameter = 10mm) centered at the same pgACC location revealed to be hypoactive in ASD by a meta-analysis of imaging studies (18). The Talairach coordinates of the pgACC generated from the meta-analysis were transformed into Montreal Neurological Institute (MNI) space using the algorithm implemented by Matt Brett (28). The resulting pgACC ROI was centered at x=0; y=47; z=9. To obtain the timeseries of the pgACC ROI of each subject and each available scan, we (1) transformed each subject's timeseries into MNI space using a 12 degree of freedom (DOF) linear affine transformation implemented in FLIRT (voxel size = 2×2×2 mm), and (2) calculated the mean timeseries for the pgACC ROI by averaging across all voxels within the seed. Further, as previously described (23;27), we extracted the timeseries of nine nuisance signals: global signal, white matter, cerebrospinal fluid, and six motion parameters.

Participant-Level Analyses

For each participant and each scan, we performed a multiple regression analysis (using the general linear model implemented in FSL's FEAT) including the timeseries of the pgACC ROI and the nine nuisance covariates as predictors. To ensure that the pgACC timeseries represented regionally specific neural activity, the mean timeseries of the pgACC ROI was orthogonalized with respect to the nine nuisance signals using FSL's FILM. This analysis produced individual subject-level maps of all positively- and negatively-predicted voxels for the pgACC predictor. The second level included a fixed-effects model for each participant with multiple resting-state scans (i.e., three scans for 20 participants and two scans for four participants). This step served to combine all FC maps available for a given participant into one, using an equal weighting.

Group-Level Analyses

Group-level analyses were carried out using the mixed-effects model implemented in FSL FLAME. In addition to the group mean vector, demeaned informant-based SRS-A score and three nuisance variables (sex, age and number of resting-state scans used for each participant) were included in the model. Corrections for multiple comparisons were carried out at the cluster level using Gaussian random field theory (min $Z > 2.3$; cluster significance: $p < 0.05$, corrected). This group-level analysis produced two types of thresholded Z-score maps: (1) maps of the significant positive and negative FC of the pgACC across the entire sample; and (2) positive and negative pgACC FC in relation to SRS-A informant results (i.e., regions in which pgACC connectivity is predicted by SRS-A scores). Finally, to examine the stability of our connectivity measures over time, we computed short- and long-term voxel-wise intraclass correlation of pgACC connectivity using the approach described in Shehzad et al. (24) on the data from the 20 participants with three scans available, over two sessions (5–16 months, intersession; and 45 minutes a part, intrasession).

Results

SRS-A

The informants who provided SRS-A scores consisted of nine partners/spouses (36%), 14 close friends (56%), and two relatives (8%). As expected in a sample of unaffected screened volunteers, total SRS-A scores ranged between 5 and 59 which is below the minimum score associated with clinically significant impairment; the mean (29.9 ± 16.0) was essentially identical to that of prior representative samples in adults (5).

Positive and Negative Connectivity of the pgACC Network

Positive Connectivity—Consistent with its role in social processes, pgACC was significantly correlated with brain regions implicated in social cognition. These include bilateral ventral and dorsal medial prefrontal cortex/ACC regions involved in mentalizing and emotional processes (16;17;29) and posterior cingulate and precuneus which are involved in awareness of the self (19;30). Superior frontal cortex, superior and middle temporal gyrus, portions of lateral occipital cortex and angular gyrus were also positively correlated to spontaneous pgACC timeseries. Finally, ventral aspects of anterior insula (vAI), implicated in evaluating one's own and other's emotions (20;31), were positively correlated with pgACC.

Negative Connectivity—In contrast, pgACC was negatively correlated with primary sensory and motor cortices, as well as regions involved in the dorsal attentional network (superior parietal cortices, dorsal precuneus, and lateral and medial occipital cortices) and in

motor control (bilateral inferior frontal gyrus, cingulate motor area, supplementary motor cortex). Negative correlations with pgACC were also observed in posterior aspects of the mid-insula (pMI), adjacent to the posterior insula. The pattern of negative connectivity observed in pMI did not extend into posterior insula, which is commonly implicated in the primary experience of somatic and visceral sensations (32). See Figure 1 for the group maps of positive and negative patterns of correlation with the pgACC. See Supplementary Table 1 for the peaks of significant positive and negative correlations.

pgACC Connectivity Predicted by the SRS-A

Positive Relationships—Analyses including SRS-A scores as a covariate of interest revealed that FC of the pgACC with a left hemisphere cluster in lateral occipital cortices, superior parietal cortex, and angular gyrus was positively related to SRS-A scores. Increased SRS-A scores predicted increased positive FC between pgACC and these left hemisphere cortical regions (see Figure 2).

Negative Relationships—We found a significant relationship between SRS-A scores and pgACC/anterior mid-insula (aMI) connectivity ($R^2=.44$ and $.56$ for right and left insula regions, respectively). Specifically, lower SRS-A scores (i.e., greater social competence) were associated with higher positive connectivity between these two regions. Examination of the FC between pgACC and aMI and SRS-A ratings indicated that individuals with high SRS-A scores (i.e., those who are socially less able) are characterized by negative pgACC/aMI FC, while those with lower SRS-A scores are characterized by positive connectivity between these two regions (see Figure 2).

Based on these findings, we examined individual participants' FC patterns between pgACC and aMI in greater detail by trisecting the group based on SRS-A scores. Individuals in the lowest SRS-A tercile exhibited the highest degree of positive connectivity between pgACC and aMI, as indexed by the number of significant positively correlated voxels within the aMI mask. In contrast, for individuals scoring in the highest tercile, the majority of significantly correlated voxels in the aMI region were negatively correlated with pgACC. We summarized these contrasts as the difference between the percentage of significant positively and negatively correlated voxels between pgACC and aMI for each hemisphere in Figure 3. To test for ordered differences in the three SRS-A subgroup medians we ran the Jonckheere-Terpstra test (33). There was a significant ordered difference among the three groups such that the more negative the difference between the percentage of positively and negatively correlated voxels, the higher were the SRS-A scores (J-T statistic = 35 and 50; Z score = -3.4 and -2.7 ; asymptotic significance $p=0.001$, and 0.007 , for left and right hemispheres, respectively).

To confirm the above results, we conducted secondary analyses. We repeated group-level statistics, treating individuals in the highest and lowest terciles as separate groups. As summarized in Figure 4, the results of between-group (highest tercile vs. lowest) analyses revealed significant voxel-wise differences. Specifically, the lowest SRS-A tercile group exhibited significantly greater positive FC between the pgACC seed region and a cluster centered in left aMI. In contrast, the highest SRS-A tercile showed significantly greater positive pgACC FC with a left hemisphere region comprising lateral occipital cortex, superior parietal gyrus, and angular gyrus.

Test-Retest Reliability of pgACC Connectivity

Using the analytical approach detailed in Shehzad et al. (24) we computed intraclass correlation values (ICC) of the pgACC FC both over the long- and short-term in the 20 subjects who participated in the test-retest study. As illustrated in Figure 5, key components

of the pgACC-based network showed moderate to high ICC's ($>.5$), including posterior cingulate cortex, insular cortex, anterior cingulate cortex and the inferior frontal gyrus. Most relevant to the present work, the aMI region, in which pgACC FC was found to be directly related to SRS-A scores, exhibited high reliability over both the long- and short-term (ICC=.68 and .77, respectively).

Discussion

The present work represents an initial application of a protocol developed to examine the neural correlates of autistic traits as indexed by the SRS-A in neurotypical individuals using resting state fMRI. As hypothesized, we found that individual differences in autistic traits in a non-clinical population were related to pgACC FC. Several studies have linked individual behavioral performance to patterns of FC observed during rest (e.g., 34). The present work extends this by relating informant-based measures of social skills directly to pgACC connectivity.

Consistent with one of our two predictions, we found that autistic traits were related to pgACC connectivity with insula, although this relationship was specifically limited to the anterior mid-insula (aMI) rather than anterior insula per se. Discussing the implications of this finding requires considering the pattern of pgACC connectivity with the various insula sub-regions. The insula can be subdivided broadly into anterior, mid-insula and posterior insula, with the anterior insula comprising ventral (vAI) and dorsal regions (35). The posterior insula, commonly involved in primary representations of visceral and somatic sensation (32), showed no connectivity with pgACC, in agreement with a recent report (22). In contrast, the anterior and mid-insula sub-regions, implicated in maintaining higher order representations of sensation and emotion (20;32;36), exhibited a significant pattern of pgACC FC along an anterior/posterior gradient, extending from positive to negative connectivity. At the anterior-most extent of the insula, the ventral anterior insula (vAI), along with a broader network of structures commonly implicated in social cognition, such as the retrosplenial complex, was positively connected with the pgACC. In contrast, at the posterior extent of this gradient, the posterior mid-insula (pMI) was negatively connected with the pgACC, along with a network of structures implicated in somatic and self-focused attention (primary somatosensory cortices, superior parietal cortices and primary motor cortices). Between these, an intervening aMI region exhibited variable patterns of pgACC FC across participants. Thus, the pattern of differential pgACC connectivity in anterior and middle insula sub-regions appears to reflect differential involvement of the vAI and pMI in social cognition and somatic and self-focused attention, respectively.

These findings of differentiable mid-insula sub-regions are noteworthy when considered in the context of current models of insula organization, which tend to treat the mid-insula as a single sub-region (37). Specifically, we found that the aMI appears to function as a transition zone between the vAI, which is related to social cognition, and the pMI, which is involved in somatic attention. As indicated by our secondary analyses, when a substantial portion of the aMI showed a positive connectivity pattern similar to that observed in vAI, low levels of autistic traits were observed. However, when the valence of FC in aMI resembled that of the pMI (i.e., was negatively correlated with pgACC), we observed elevated levels of autistic traits. These results have broad implications for future efforts to relate inter-individual differences in behavior to functional connectivity. Specifically, they highlight the need to interrogate connections exhibiting a high degree of variability across participants, as opposed to limiting analyses to a priori connections of interest, or to only those showing consistent patterns of connectivity across subjects.

We did not detect a significant relationship between SRS-A scores and pgACC FC with PCC. The PCC is strongly implicated in the development of theory of mind (38), and consistent patterns of PCC hypofunction have been noted in ASD (18). Further, initial studies of FC in ASD reported decreased connectivity between PCC and pgACC (14;15). Our study used a non-clinical screened sample which may not recapitulate brain-behavior relationships encountered in clinically diagnosed individuals. Future work with affected individuals will help to clarify this divergence from the literature.

Finally, we also found that increased SRS-A ratings were related to increased FC between pgACC and higher order sensory processing regions (superior parietal gyrus, lateral occipital cortex and angular gyrus). These regions are often abnormally hyperactive in individuals with ASD (18). As such, although not hypothesized, our findings suggest the need to consider abnormal patterns of connectivity beyond hypoconnectivity alone.

In considering the imaging approach employed in the present work, it is important to note that while SRS-A scores could also be related to task activation-based approaches, combining the SRS-A with resting state fMRI has several advantages. First, measuring resting-state FC avoids potential confounds associated with task-based approaches such as practice or floor/ceiling effects and sensitivities to experiment design parameters (9). Second, resting-state data can simultaneously delineate entire multiple networks which, are usually only partially observable in task-based studies depending on the selected contrasts (8). Despite concerns about the unconstrained nature of the resting state, patterns of intrinsic connectivity during rest have been found to be remarkably replicable across individuals and across research labs (39). Furthermore, Shehzad et al. (24) demonstrated the substantial quantitative stability of inter-individual differences in resting-state FC measures. Specifically, moderate ($ICC > 0.5$) to high ($ICC 0.7 - 0.95$) short- and long-term test-retest reliability was found for measures of resting state FC (24). The present work provides further support for the applicability of resting state fMRI approaches in studies of inter-individual differences in FC, by demonstrating moderate to high test-retest reliability for key components of the pgACC network.

Our findings should be interpreted in light of several limitations. First, seed-based approaches to mapping FC require a priori selection of a seed of interest. We selected the pgACC due to its prominence in models of ASD-related abnormalities and its emergence in our recent meta-analysis of ASD. Future work will entail investigating the intrinsic connectivity of other regions of interest potentially implicated in the social impairments associated with ASD (18). Second, we maximized the representativeness of individual parameter estimates by combining data from all available scans. Since 20% of the sample did not have three available scans, we considered the possibility that our results might have been influenced by differences in number of scans. However, we accounted for potential differences at the group level by including the number of scans as a covariate. We also repeated analyses limited to a single scan per participant and to the 20 participants who provided three scans; neither differed substantially from our main findings (see Supplementary Figure 1). Finally, significant controversy continues to surround the interpretation of negative relationships in functional connectivity (40). Thus, while patterns of positive and negative connectivity differentiate insula sub-regions, caution should be taken when interpreting the meaning of negative relationships.

In summary, the present work demonstrates the utility of resting state fMRI for mapping functional connectivity in relation to the SRS-A, a continuous measure of autistic traits in the general population. This approach led us to identify pgACC connectivity with the aMI as a candidate marker of social competence in a neurotypical sample. Application of this method in future studies examining patients with ASD should allow confirmation of whether

this circuit is a locus of dysfunction in autism that is dimensionally related to the severity of autistic traits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Reference List

1. Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry*. 1977; 18(4):297–321. [PubMed: 562353]
2. Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I. The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry*. 2001; 42(2):241–251. [PubMed: 11280420]
3. Constantino JN, Todd RD. Autistic traits in the general population: a twin study. *Archives of General Psychiatry*. 2003; 60(5):524–530. [PubMed: 12742874]
4. Hurley RS, Losh M, Parlier M, Reznick JS, Piven J. The broad autism phenotype questionnaire. *J Autism Dev Disord*. 2007; 37(9):1679–1690. [PubMed: 17146701]
5. Constantino JN, Todd RD. Intergenerational transmission of subthreshold autistic traits in the general population. *Biol Psychiatry*. 2005; 57(6):655–660. [PubMed: 15780853]
6. Constantino JN, Davis SA, Todd RD, Schindler MK, Gross MM, Brophy SL, Metzger LM, Shoushtari CS, Splinter R, Reich W. Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the autism diagnostic interview-revised. *J Autism Dev Disord*. 2003; 33(4):427–433. [PubMed: 12959421]
7. Constantino JN, Gruber CP, Davis S, Hayes S, Passanante N, Przybeck T. The factor structure of autistic traits. *J Child Psychol Psychiatry*. 2004; 45(4):719–726. [PubMed: 15056304]
8. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci*. 2007; 8(9):700–711. [PubMed: 17704812]
9. Buckner RL, Vincent JL. Unrest at rest: Default activity and spontaneous network correlations. *Neuroimage*. 2007; 37(4):1091–1096. [PubMed: 17368915]
10. Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med*. 1995; 34(4):537–541. [PubMed: 8524021]
11. Horwitz B, Rumsey JM, Grady CL, Rapoport SI. The cerebral metabolic landscape in autism. Intercorrelations of regional glucose utilization. *Arch Neurol*. 1988; 45(7):749–755. [PubMed: 3260481]
12. Murias M, Webb SJ, Greenson J, Dawson G. Resting state cortical connectivity reflected in EEG coherence in individuals with autism. *Biol Psychiatry*. 2007; 62(3):270–273. [PubMed: 17336944]
13. Minshew NJ, Williams DL. The new neurobiology of autism: cortex, connectivity, and neuronal organization. *Arch Neurol*. 2007; 64(7):945–950. [PubMed: 17620483]
14. Cherkassky VL, Kana RK, Keller TA, Just MA. Functional connectivity in a baseline resting-state network in autism. *Neuroreport*. 2006; 17(16):1687–1690. [PubMed: 17047454]
15. Kennedy DP, Courchesne E. The intrinsic functional organization of the brain is altered in autism. *Neuroimage*. 2008; 39:1877–1885. [PubMed: 18083565]
16. Amodio DM, Frith CD. Meeting of minds: the medial frontal cortex and social cognition. *Nat Rev Neurosci*. 2006; 7(4):268–277. [PubMed: 16552413]

17. Gilbert SJ, Spengler S, Simons JS, Steele JD, Lawrie SM, Frith CD, Burgess PW. Functional specialization within rostral prefrontal cortex (area 10): a meta-analysis. *J Cogn Neurosci*. 2006; 18(6):932–948. [PubMed: 16839301]
18. Di Martino A, Ross K, Uddin LQ, Sklar AB, Castellanos FX, Milham MP. Functional Brain Correlates of Social and Nonsocial Processes in Autism Spectrum Disorders: An Activation Likelihood Estimation Meta-Analysis. *Biol Psychiatry*. 2009; 65(1):63–74. [PubMed: 18996505]
19. Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain*. 2006; 129(Pt 3):564–583. [PubMed: 16399806]
20. Singer T. The neuronal basis and ontogeny of empathy and mind reading: review of literature and implications for future research. *Neurosci Biobehav Rev*. 2006; 30(6):855–863. [PubMed: 16904182]
21. Sridharan D, Levitin DJ, Menon V. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc Natl Acad Sci U S A*. 2008; 105(34):12569–12574. [PubMed: 18723676]
22. Taylor, KS.; Seminowicz, DA.; Davis, KD. Hum Brain Mapp. 2008. Two systems of resting state connectivity between the insula and cingulate cortex. In Press
23. Margulies DS, Kelly AMC, Uddin LQ, Biswal BB, Castellanos FX, Milham MP. Mapping the functional connectivity of anterior cingulate cortex. *Neuroimage*. 2007; 37(2):579–588. [PubMed: 17604651]
24. Shehzad, Z.; Kelly, AMC.; Reiss, PT.; Gee, DG.; Gotimer, K.; Uddin, LQ.; Lee, SG.; Margulies, DS.; Roy, Krain A.; Biswal, BB.; Petkova, E.; Castellanos, FX.; Milham, MP. Cerebral Cortex. 2008. The Resting Brain: Unconstrained yet Reliable. In Press
25. Roy, Krain A.; Shehzad, ZE.; Margulies, DS.; Kelly, AMC.; Uddin, LQ.; Gotimer, K.; Biswal, B.; Castellanos, FX.; Milham, MP. Functional Connectivity of the Human Amygdala using Resting State fMRI. *Neuroimage*. 2008 In Press.
26. Kelly AMC, Di Martino A, Uddin LQ, Shehzad Z, Gee DG, Reiss PT, Margulies DS, Castellanos FX, Milham MP. Development of anterior cingulate functional connectivity from late childhood to early adulthood. *Cereb Cortex*. 2008 In Press.
27. Di Martino A.; Scheres, A.; Margulies, DS.; Kelly, AMC.; Uddin, LQ.; Shehzad, Z.; Biswal, B.; Walters, JR.; Castellanos, FX.; Milham, MP. Functional connectivity of human striatum: a resting state FMRI study. *Cereb Cortex*. 2008; 18(12):2735–2747. [PubMed: 18400794]
28. Brett, M. The MNI brain and the Talarach Atlas:
<http://www.mrc.cbu.cam.ac.uk/Imaging/mnispace.html>; 1999. p. 12-19.
29. Gallagher HL, Frith CD. Functional imaging of 'theory of mind'. *Trends Cogn Sci*. 2003; 7(2):77–83. [PubMed: 12584026]
30. Northoff G, Bermpohl F. Cortical midline structures and the self. *Trends Cogn Sci*. 2004; 8(3):102–107. [PubMed: 15301749]
31. Sanfey AG, Rilling JK, Aronson JA, Nystrom LE, Cohen JD. The neural basis of economic decision-making in the Ultimatum Game. *Science*. 2003; 300(5626):1755–1758. [PubMed: 12805551]
32. Craig AD. How do you feel? Interoception the sense of the physiological condition of the body. *Nat Rev Neurosci*. 2002; 3(8):655–666. [PubMed: 12154366]
33. Bewick V, Cheek L, Ball J. Statistics review 10: further nonparametric methods. *Crit Care*. 2004; 8(3):196–199. [PubMed: 15153238]
34. Andrews-Hanna JR, Snyder AZ, Vincent JL, Lustig C, Head D, Raichle ME, Buckner RL. Disruption of large-scale brain systems in advanced aging. *Neuron*. 2007; 56(5):924–935. [PubMed: 18054866]
35. Augustine JR. The insular lobe in primates including humans. *Neurol Res*. 1985; 7(1):2–10. [PubMed: 2860583]
36. Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ. Neural systems supporting interoceptive awareness. *Nat Neurosci*. 2004; 7(2):189–195. [PubMed: 14730305]
37. Mesulam MM, Mufson EJ. Insula of the old world monkey. I. Architectonics in the insulo-orbito-temporal component of the paralimbic brain. *J Comp Neurol*. 1982; 212(1):1–22. [PubMed: 7174905]

38. Gobbini MI, Koralek AC, Bryan RE, Montgomery KJ, Haxby JV. Two takes on the social brain: a comparison of theory of mind tasks. *J Cogn Neurosci*. 2007; 19(11):1803–1814. [PubMed: 17958483]
39. Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF. Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A*. 2006; 103(37):13848–13853. [PubMed: 16945915]
40. Murphy K, Birn RM, Handwerker DA, Jones TB, Bandettini PA. The impact of global signal regression on resting state correlations: Are anti-correlated networks introduced? *Neuroimage*. 2008 In Press.

Figure 1. Surface maps for the pregenual anterior cingulate cortex (pgACC) functional connectivity across all participants (n=25)(a)

(a) Significant positive (orange) and negative (blue) connectivity for the pgACC ($Z > |2.3|$, cluster significance: $p < .05$, corrected). Surface maps were generated using the Analysis of Functional NeuroImages Surface Mapper (AFNI SUMA) package. The bottom axial and sagittal figures show the location of the pgACC region of interest (ROI) used to map functional connectivity ($x=0$, $y=47$, $z=9$ in Montreal Neurological Institute standard space).

Figure 2. Pregenual anterior cingulate (pgACC) functional connectivity (FC) related to the Social Responsiveness Scale –Adult version (SRS-A)(b)

(b) Surface maps depict regions for which pgACC FC is related to the SRS-A scores across participants ($n=25$) ($Z > |2.3|$, cluster significance: $p < .05$, corrected). Connectivity between pgACC and a cluster centered in anterior mid-insula (red) was negatively related to SRS-A bilaterally, while a cluster centered in the left hemisphere parietal/occipital cortices (green) was positively related to SRS-A. Scatter plots depict relationships between individual SRS-A scores and the parameter estimates of pgACC FC for (A) left anterior mid-insula, (B) left parietal/occipital cortices, and (C) right anterior mid-insula, and ($R^2=0.56; 0.51, 0.44$; for A, B, C, respectively; $p < 0.001$).

Figure 3. Inter-individual differences in pregenual anterior cingulate (pgACC) functional connectivity (FC) with anterior mid-insula (aMI)(c)

(c) Tricolor bar-plots depict the percentage of voxels within the aMI cluster mask showing significantly positive pgACC FC (red), negative pgACC FC (blue), or no significant connectivity (gray), for participants scoring in the lowest ($n=8$), intermediate ($n=9$) and highest ($n=8$) Social Responsiveness Scale –Adult (SRS-A) tercile. Scatter plots depict the difference between the percentage of voxels positively connected minus the percentage of voxels negatively connected to pgACC in the aMI masks for each of the three SRS-A terciles. Significantly ordered differences between the three SRS-A groups were found, such that the more positive the difference (i.e., more voxels in the mask positively connected to pgACC) the lower the SRS-A rating (Jonckheere-Terpstra based Z-score = -3.4 and -2.7 , for left and right hemispheres, respectively; $p < 0.01$).

Figure 4. Pregenual anterior cingulate cortex (pgACC) connectivity in the lowest and highest terciles per the Social Responsiveness Scale-Adult version (SRS-A)(d)

(d) Surface maps showing voxels with positive (orange) and negative (blue) pgACC FC for SRS-A subgroups (lowest tercile, highest tercile; $n=8$ for each group; mean age 26.7 ± 3.5 and 26.9 ± 3.3 ; 2 and 3 males; mean SRS-A Score 11.8 ± 5.2 and 48.9 ± 6.8 , respectively). Bottom panel represents statistical surface maps for direct comparisons between the two SRS-A-based subgroups. The group of participants in the lowest SRS-A tercile showed greater positive connectivity between anterior mid-insula (red) in the left hemisphere and pgACC. In contrast, the highest SRS-A tercile group showed greater positive connectivity in parietal/occipital cortices (green) in the left hemisphere ($Z > |2.3|$, cluster significance: $p < .05$, corrected). LH: left hemisphere; RH= right hemisphere.

Figure 5. Test-retest reliability for pregenual anterior cingulate cortex (pgACC) functional connectivity (FC)(e)

(e) Surface maps depicting voxel-wise intraclass correlation (ICC) values for the pgACC FC over the long-term/intersession (scans 1 and 2 separated by 5–16 months) and short-term/intra-session (scans 2 and 3 separated by 45 minutes) in the 20 participants who completed three scans. The upper map depicts the stability of parameter estimates for pgACC/left anterior mid-insula (aMI) FC across scans 1 and 2; the lower map shows the intrasession stability across scans 2 and 3 ($R^2 = .45$ and $.60$; $p < 0.01$ and $p < .001$, respectively). At the top left of the figure are the sagittal and axial images of the pgACC ROI ($x=0$, $y=47$, $z=9$ in Montreal Neurological Institute standard space); at the top right are sagittal and axial images of the left aMI mask ($x=34$, $y=0$, $z=-2$ in Montreal Neurological Institute standard space).