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Local resting state functional connectivity in autism: Site and cohort variability and the effect of eye status

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Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with prominent impairments in sociocommunicative abilities, which have been linked to anomalous brain network organization. Despite ample evidence of atypical long-distance connectivity, the literature on local connectivity remains small and divergent. We used resting-state functional MRI regional homogeneity (ReHo) as a local connectivity measure in comparative analyses across several well-matched low-motion subsamples from the Autism Brain Imaging Data Exchange and in-house data, with a grand total of 147 ASD and 184 typically developing (TD) participants, ages 7–18 years. We tested for group differences in each subsample, with additional focus on the difference between eyes-open and eyes-closed resting states. Despite selection of highest quality data and tight demographic and motion matching between groups and across samples, few effects in exactly identical loci (voxels) were found across samples. However, there was gross consistency across all eyes-open samples of local overconnectivity (ASD > TD) in posterior, visual regions. There was also gross consistency of local underconnectivity (TD > ASD) in cingulate gyrus, although exact loci varied between mid/posterior and anterior sections. While all eyes-open datasets showed the described gross similarities, the pattern of group differences for participants scanned with eyes closed was different, with local overconnectivity in ASD in posterior cingulate gyrus, but underconnectivity in some visual regions. Our findings suggest that fMRI local connectivity measures may be relatively susceptible to site and cohort variability and that some previous inconsistencies in the ASD ReHo literature may be reconciled by more careful consideration of eye status.

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Keywords

Autism spectrum disorder; functional MRI; local connectivity; cingulate gyrus; visual cortex; default mode network

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impaired social communication and interaction, as well as repetitive behaviors and restricted interests. After decades of neuroimaging research, biomarkers with high degrees of sensitivity and specificity (brain features occurring in all or most individuals with ASD, but not in those without ASD) remain elusive. Reasons include the neurofunctional complexity and heterogeneity of the disorder, with ASD being an umbrella term for an unknown number of neurodevelopmentally distinct subtypes ((Geschwind and State 2015). An additional problem is the lack of established best practices in imaging analysis (e.g., Koldewyn et al. 2014; Nair et al. 2014).

In the past decade, some broad consensus has emerged viewing ASD as a disorder of brain network connectivity (Müller 2007; Vissers et al. 2012; Wass 2011). However, this apparent consensus obscures a wide diversity of findings, stretching from predominant functional underconnectivity (Schipul et al. 2011) to mixed patterns reflecting network specificity (Abbott et al. 2015) or age (Nomi and Uddin 2015) to predominant overconnectivity (Shih et al. 2011; Supekar et al. 2013). The vast majority of studies have investigated long distance connectivity, whereas the evidence on local connectivity remains limited. The technique of choice to test local connectivity in functional MRI has been regional homogeneity (ReHo) – a method based on signal correlations in small clusters of neighboring voxels that was initially developed for cluster purification (Zang et al. 2004). Two early ReHo studies of ASD (Paakki et al. 2010; Shukla et al. 2010) reported divergent results, presumably due to differences in crucial methodological factors (e.g., resting state vs. task-activated data, treatment of head motion, size of local clusters tested). However, methodologically more rigorous recent studies have also produced partial inconsistencies. ReHo findings from the large-sample Autism Brain Imaging Data Exchange (ABIDE) showed extensive local overconnectivity in ASD in right frontal cortex (Di Martino et al. 2014), whereas studies in smaller samples indicated local overconnectivity in posterior regions (Keown et al. 2013; Maximo et al. 2013) that was not seen at all in the ABIDE study. A more recent study using a subset of ABIDE data reported underconnectivity in posterior regions with additional age-related changes (Dajani and Uddin 2015). However, this study applied relatively liberal head movement criteria and included resting data from both eyes-open and eyes-closed state, which have been shown to be associated with substantial differences in ReHo (Liu et al. 2013).

Given the inconsistencies in findings, we conducted a comparative ReHo study implementing several analysis pipelines in a number of samples, including both ABIDE and in-house data. Although there are potential sources of variability that may affect multi-site reproducibility and reliability (e.g., differences in pulse sequences, signal-to-noise ratios,

smoothing; Friedman et al., 2008; Friedman, Glover, and The FBIRN Consortium, 2006), several studies across a wide range of typical and patient populations and in both resting state and task-based fMRI have indicated high inter-site reliability (Brown et al., 2011; Sutton et al., 2008; Turner et al., 2013). The divergent findings from previous ReHo studies of ASD therefore call for further investigation. In the present study, we examined whether inconsistencies could be resolved (i) through use of consistent analysis pipelines and (ii) through strict separation of data acquired during eyes-open vs. eyes-closed conditions.

Methods

Participants

Data consisted of in-house anatomical and resting state functional MRI (rs-fMRI) scans (labeled “SDSU”), as well as data from the Autism Brain Imaging Data Exchange (ABIDE), a grassroots initiative dedicated to aggregating and sharing previously collected anatomical and rs-fMRI data sets from individuals with ASD and age-matched TD controls (Di Martino et al. 2014).

Six different low-motion datasets were analyzed: a Grand Total group including data from participants with eyes open and closed during resting state scan from both ABIDE, NYU, and SDSU (Grand Total-EO+EC; $N=331$; $ASD=147$; $TD=184$); a Grand Total group including only data from participants with eyes open (EO) during resting state scan (Grand Total-EO; $N=257$; $ASD=110$; $TD=147$); data from SDSU including only participants with eyes open during resting state scan (SDSU-EO; $N=53$; $ASD=26$; $TD=27$); data from New York University (NYU) including only participants with eyes open (NYU-EO; $N=63$; $ASD=25$; $TD=38$); data from ABIDE including only participants with eyes open and excluding participants from SDSU and NYU groups (ABIDE-EO; $N=141$; $ASD=59$; $TD=82$); and data from ABIDE including only participants with eyes closed (ABIDE-EC; $N=72$; $ASD=30$; $TD=42$). Within each subsample, groups were matched on age, nonverbal IQ, and head motion (root mean square of displacement – RMSD; see Table 1). Subsample (ABIDE-EO, ABIDE-EC, NYU, SDSU) by group analyses of variance (ANOVAs) showed marginal effects of subsample on age ($F(3,323)=2.341$, $p=0.073$) and nonverbal IQ ($F(3,287)=2.659$, $p=0.049$). While a strong effect of subsample on head motion ($F(3,323)=11.420$, $p<0.001$) was found, the mean difference in RMSD was minimal at 0.016 (and did not substantially affect the pattern of results; see “Limitations and perspectives” below and Supplementary Figure 3). Additionally, there were no main effects of group on age ($F(1,323)=0.291$, $p=0.590$), nonverbal IQ ($F(287)=0.045$, $p=0.832$), or head motion ($F(1,323)=0.624$, $p=0.430$), as well as no site by group interaction effects. Finally, there was no main effect of subsample on ADOS scores ($F(3,106)=1.907$, $p=0.133$) in the ASD group.

Diagnoses in the ASD group were based on the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) at all sites, and additionally the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994) at some sites. However, ADOS data were unavailable for a total of 37 participants (as stated in Table 1). Exclusionary criteria for both ASD and TD groups varied based on acquisition site (See Supplementary Table 1 for list of diagnostic and exclusionary criterion per acquisition site). All participants and caregivers gave informed consent in accordance with the responsible Institutional Review Boards.

MRI data acquisition

All data were acquired from 3 Tesla scanners, but type of scanner and image acquisition parameters varied across ABIDE acquisition sites (see Supplementary Table 2 for sites included in subsamples; acquisition protocols available at: fcon_1000.projects.nitrc.org/indi/abide/).

Resting state imaging data for SDSU participants were acquired on a GE 3T MR750 scanner with an eight-channel head coil at the Center for Functional MRI at the University of California, San Diego. High-resolution structural images were acquired with a standard FSPGR T1-weighted sequence (TR: 11.08ms; TE: 4.3ms; flip angle: 45°; FOV: 256mm; matrix: 256×256; 180 slices; 1mm³ resolution). Functional T2-weighted images were obtained in a 6:10-minute scan using a single-shot gradient-recalled, echo-planar pulse sequence consisting of 185 whole-brain volumes (TR: 2000ms; TE: 30ms; slice thickness: 3.4mm; flip angle: 90°; FOV: 220mm; matrix: 64×64). The first five time points were discarded to allow for T1 equilibration effects, leaving 180 time points (6 minutes) for analysis.

Resting state imaging data for NYU participants were acquired on a Siemens 3T MR2004A scanner with an eight-channel head coil. High-resolution structural images were acquired with a (TR: 2530ms; TE: 3.25 ms; flip angle: 7°; FOV: 256mm; matrix: 256×256; 128 slices; 1.3mm³ resolution). Functional T2-weighted images were obtained in a 6 minute scan (TR: 2000ms; TE: 15ms; slice thickness: 4mm; flip angle: 90°; FOV: 240mm; matrix: 240×240).

Data preprocessing

All resting state fMRI data were processed using Analysis of Functional NeuroImages Software (AFNI; Cox, 1996) and FMRI software library (FSL; Smith et al., 2004). Functional images were motion, slice-time, and field map corrected. Functional images were registered to the anatomical images using FSL's FLIRT (Jenkinson et al., 2002), and images were resampled to 3mm isotropic voxels and standardized to the MNI152 template atlas space via FSL's nonlinear registration tool (FNIRT). FMRI time series were bandpass filtered ($0.008 < f < 0.08$ Hz) to isolate spontaneous low-frequency BOLD fluctuations using a second-order Butterworth filter. As differences in smoothness between single-subject datasets would directly impact time series correlations between neighboring voxels, we consistently set the effective smoothness of all datasets to a Gaussian FWHM of 6mm, using AFNI's 3dBlurToFWHM. All nuisance regressors were band-pass filtered using the second-order Butterworth filter ($0.008 < f < 0.08$ Hz) (Power et al., 2012; Satterthwaite et al., 2013). A total of 16 nuisance regressors were applied, including six rigid-body motion parameters acquired from motion correction and their first derivatives, as well as signal from cerebral white matter and lateral ventricles, and their derivatives. Individual participant masks were created to remove signal from cerebral white matter and lateral ventricles using FSL's FAST automated segmentation (Zhang et al., 2001).

Motion was quantified as framewise displacement (FD) between two consecutive time points (calculated based on six dimensional rigid-body motion parameters). Any time point with

FD >0.2mm as well as the following two time points were censored, or “scrubbed” (Power et al. 2014). Participants with fewer than 80% of time points remaining after censoring were excluded from analyses. Average head motion, defined as the root mean square of displacement (RMSD), did not significantly differ between groups (ASD vs. TD).

Regional Homogeneity

Regional homogeneity implements Kendall’s coefficient of concordance (KCC) and relies on rank order correlations between time series to assess the homogeneity of a given center voxel and its neighboring voxels. ReHo can thus be considered a measure of local connectivity (Anderson et al. 2014; Lopez-Larson et al. 2011). KCC within a cluster of voxels is equal to the parameter W , which ranges from 0 to 1, with higher values indicating greater homogeneity (Zang et al. 2004):

$$W = \frac{\sum (R_i)^2 - n (\bar{R})^2}{\frac{1}{12} K^2 (n^3 - n)}$$

In a previous study (Maximo et al. 2013), we tested the effect of varying cluster sizes of 7, 19, and 27 voxels on ReHo in comparisons between TD and ASD samples, and observed most robust group differences for 27 voxels. For the present study, we therefore computed ReHo for a cluster size of 27 voxels. A gray-matter mask was used to avoid partial-volume effects. Voxel-wise ReHo maps were obtained for each participant using AFNI’s 3dReHo for a cluster size of 27 voxels. In each participant, KCC for each voxel was then standardized to a ReHo z -score by subtracting the mean KCC across all brain voxels and dividing it by the standard deviation. Between-group differences (ASD vs. TD) were examined with two-sample t -tests. Monte-Carlo simulations were applied to correct for multiple comparisons (Forman et al. 1995), using AFNI’s 3dClustSim (version post May 2015), to obtain a corrected significance level of $p < .05$ (using a voxelwise threshold of $p < .01$ uncorrected and a minimum cluster volume of 37 voxels for a corrected threshold at $p < .05$).

While KCC standardization, as described above, is a common default procedure in the ReHo literature because it reduces artifactual effects of individual variability (e.g., differences in motion across participants), it renders the technique insensitive to potential global group differences in local connectivity. We therefore also ran parallel analyses without standardization. Analyses were also performed both with and without global signal regression (GSR), given the ongoing debate highlighting both the virtues of GSR in denoising (Power et al. 2014) and its potential drawbacks, such as the creation of spurious anti-correlations (Murphy et al. 2009), which may confound between-group comparisons (Abbott et al. 2015; Gotts et al. 2013). Findings from these additional analyses are presented in Supplementary Figures 1 and 2 for completeness.

Results

Analyses with GSR and ReHo standardization

Between-group effects for the analyses with GSR and ReHo standardization for each subsample are presented in Figure 1A (for cluster listings see Supplementary Table 3). For the ReHo pipeline with GSR, group differences in the ABIDE-EO subsample included local overconnectivity (ASD > TD) in right middle temporal gyrus, and across left calcarine and bilateral lingual gyri, and underconnectivity in bilateral thalamus, right insula and inferior frontal gyrus, and left posterior cingulate cortex and precuneus. The ABIDE-EC analysis yielded local overconnectivity in the ASD group across bilateral precuneus, left middle temporal gyrus, left superior frontal gyrus, and right angular/middle temporal gyrus, contrasted by local underconnectivity across right paracentral and postcentral gyri, right cuneus and lingual gyrus, right superior and middle occipital gyri, left precentral and postcentral gyri. For the NYU subsample, group differences included local overconnectivity in left calcarine, lingual, and middle temporal gyri, and local underconnectivity across left superior medial and mid orbital gyri, left anterior cingulate cortex, right rolandic operculum, and right superior and middle frontal gyri. For the SDSU subsample, local overconnectivity in the ASD groups was detected in left calcarine cortex and cuneus, right superior and middle temporal gyri, right temporal pole, and right amygdala, whereas local underconnectivity occurred in bilateral paracentral as well as anterior and middle cingulate cortices, right SMA, and left cerebellum. The Grand Total-EO subsample yielded local overconnectivity in calcarine cortex, lingual gyrus and precuneus bilaterally, right superior temporal and supramarginal gyri, and bilateral middle temporal gyrus, contrasted by local underconnectivity across bilateral middle, anterior, and posterior cingulate cortices, bilateral thalamus, left cerebellum, and rolandic operculum bilaterally. The Grand Total-EO+EC ReHo analysis showed local overconnectivity in the ASD group across bilateral middle temporal gyrus, left calcarine cortex and lingual gyri, right cerebellum, left fusiform gyrus, left precuneus, and right supramarginal gyrus. Local underconnectivity was detected across bilateral middle and anterior cingulate cortices, bilateral thalamus, and right rolandic operculum.

Note that Grand Total is included here because it represented the largest available sample, although it should be understood that this sample was inclusive of other subsamples and the detected effects thus reflect a summary of those subsamples rather than an independent finding.

Analyses without GSR and standardization

Results for analyses without GSR and without ReHo standardization were mostly similar to those from standardized ReHo with global signal regression pipeline, with some exceptions. For the Grand Total- EO+EC subsample, local overconnectivity in left middle temporal gyri, left lingual gyrus, and left precuneus, and underconnectivity in bilateral thalamus was *not* seen in the non-GSR non-standardized analysis, and overconnectivity in right middle frontal gyrus and underconnectivity in left rolandic operculum were detected *solely* in this analysis (see Supplementary Figure 1A and Supplementary Table 4 for complete details). Results for analyses without GSR but with ReHo standardization were largely similar to those without

ReHo standardization and are presented in Supplementary Figure 1B (for cluster listings, see Supplementary Table 5).

Eye status

Two-sample *t*-tests directly comparing ABIDE eyes open and eyes closed subsamples yielded extensive regions of significantly greater local connectivity for EO compared to EC participants in occipital regions extending into temporal and parietal lobes, as well as in mostly ventral frontal and anterior cingulate regions. Reduced local connectivity for the same contrast was seen in mid-cingulate, paracentral, and (mostly medial) temporal regions. These patterns were mostly similar in ASD and TD groups (see Figure 1 and Supplementary Table 3; for findings from non-GSR and non-standardized analyses, see Supplementary Figure 2 and Supplementary Tables 4–5).

Analyses with added site and motion regressors

To examine effects driven by site-specific sample sizes and motion, two-sample *t*-tests were calculated with site and head motion (RMSD) as nuisance covariates. These analyses showed similar patterns of effects (Supplementary Figure 3), with the exception of ABIDE-EO, which had the lowest ratio of participants to number of sites. Specifically, local overconnectivity (ASD > TD) was found only in right middle temporal gyrus. For the ABIDE-EC, Grand Total-EO, and Grand Total-EO+EC subsamples, results were consistent with primary analyses with the exception of small clusters of local connectivity that did not survive correction (e.g., local overconnectivity in left middle temporal and superior frontal gyri for ABIDE-EC; overconnectivity in bilateral middle temporal gyrus and underconnectivity in thalamus for Grand Total-EO; overconnectivity in visual regions and underconnectivity in thalamus for Grand Total-EO+EC). Analyses comparing ABIDE EO to EC in both ASD and TD groups yielded similar patterns of local over- and underconnectivity, albeit to a lesser extent. Nevertheless, when sites contributing fewer than 10 participants were removed from multi-site subsamples, largely similar patterns of effects were retained (Supplementary Figure 4).

Discussion

Despite relatively large, high-quality subsamples, which were matched for head motion and available demographics, between-group effects differed across datasets, suggesting that ReHo may be highly sensitive to site and cohort variability. Differences in sample size across datasets could explain variations in overall extent of significant between-group effects, which were in fact modest, but not the many differences in regional patterns of such effects.

Two regional effects – both reflecting local overconnectivity in posterior, visual regions in ASD – were detected across all samples (except ABIDE-EC) for both the GSR and the non-GSR pipelines, as well as Grand Total-EO with additional site and motion covariates. However, the exact location of these between-group effects varied between samples (Figure 1C). Medial clusters peaked around the calcarine fissure (V1) in most samples. Additional posterior clusters detected in all samples were located in lateral temporo-parietal cortex

(except for NYU, and for ABIDE-EC with added site and motion covariates). A further effect with relatively high consistency (detected in all samples except NYU) was local underconnectivity in cingulate cortex. Again, exact locations of this effect varied greatly across samples.

Surprisingly, the robust local overconnectivity in right prefrontal cortex reported in the large-sample original ABIDE study (Di Martino et al. 2014) could not be replicated. A few methodological differences may have played a role: Di Martino and colleagues excluded sites with $N < 6$, but included datasets with $< 80\%$ remaining time points after censoring. Censoring was only performed on time points of motion peaks in their study, whereas we additionally censored two subsequent time points. More importantly, the much wider age range in Di Martino et al. (7–64 years vs. 7–18 years in ours) may account for differences in findings. Related to this, Dajani and Uddin (2015) reported local overconnectivity effects in right prefrontal cortex that were age-specific. However, their finding of a small right inferior frontal overconnectivity cluster only in 7–11 year-old children with ASD, but neither in adolescents nor in adults, could not account for the differences between our findings and those by Di Martino et al. (2014). Note that samples in Dajani and Uddin (2015) were exclusively drawn from the NYU data in ABIDE, resulting in small subsamples of 20 participants per group in each age bin, and that data from eyes open and eyes closed conditions were combined in these samples (see below). Itahashi and colleagues (2015) reported extensive local overconnectivity in right prefrontal cortex in adults with ASD (ages 19–50 years), consistent with the location of findings in Di Martino et al. Since the finding by Itahashi et al. comes from a large and independent sample (not included in ABIDE), it may provide a replication of the original ABIDE findings – however, with the potential caveat that these effects may be found only in adults. If so, this caveat could in turn explain why no corresponding effect was detected in our study, which was limited to children and adolescents under the age of 18 years.

‘General local overconnectivity’ in ASD is more myth than fact

Starting with Belmonte et al. (2004) and Just et al. (2004), the idea of a general principle of atypically reduced long-distance connectivity but increased local connectivity in ASD has survived through repeated cross-citations in many reviews (e.g., Maximo et al. 2014; Minshew and Williams 2007; Vissers et al. 2012; Wass 2011), based on surprisingly slim evidence. The hypothesis of general long-distance underconnectivity in ASD has been questioned elsewhere (Müller et al. 2011; Nair et al. 2014; Rudie and Dapretto 2013; Supekar et al. 2013). Functional MRI ReHo studies, while reporting many often inconsistent regional findings, have in fact given a clear answer to the second part of the claim: *There is no general local overconnectivity in ASD*. Instead every single autism ReHo study published to date (Dajani and Uddin 2015; Di Martino et al. 2014; Itahashi et al. 2015; Jiang et al. 2015; Maximo et al. 2013; Paakki et al. 2010; Shukla et al. 2010) has detected region-specific mixtures of atypically increased and reduced local connectivity in ASD.

However, there are a few caveats to be considered. Aside from the coarse spatial scale at which ReHo fMRI assesses ‘local connectivity’ (see below “Limitations and perspectives”), the ReHo pipeline implemented in almost all of the cited studies includes a standardization

step, which converts KCC into *z*-scores, normalizing effects to a distribution around zero in each participant. While this has advantages with respect to differing data quality and the detection of regionally specific effects, any global differences between a clinical and a control population will be lost. It is therefore theoretically possible that mixed between-group effects reported in all of the above studies were artifacts of ReHo standardization. Maximo et al. (2013), however, showed similar regional patterns of mixed over- and underconnectivity in ASD even in the absence of standardization. Nonetheless, given the relatively small sample size in this earlier study, we also analyzed all data without any steps that would have resulted in such normalization (i.e., without standardization and without GSR, which has also been shown to distort group differences in some instances (Abbott et al. 2015; Gotts et al. 2013). Although between-group effects were overall slightly less extensive, most regional effects were similar between standardized and non-standardized pipelines. In particular, even without standardization, there was a mix of local under- and overconnectivity effects, indeed with a slight predominance of the former. This mixture of connectivity effects was also shown in analyses including site and head motion as nuisance covariates and excluding sites that contributed fewer than 10 participants. This corroborates that *general* local overconnectivity in ASD at the coarse spatial scale of fMRI ReHo does not exist.

Cingulate cortex is locally underconnected

Findings of atypically reduced local ReHo in cingulate gyrus in ASD were relatively consistent across samples, although exact loci differed substantially. Underconnectivity in anterior cingulate cortex (ACC) was observed in Grand Total as well as SDSU and NYU eyes open samples (although not in the ABIDE-EO and EC cohorts). These findings are consistent with evidence of reduced gray matter in ACC from voxel-based morphometry, as reported in a voxelwise meta-analysis (Yang et al. 2016), as well as cytoarchitectonic anomalies in ACC of postmortem brains from children and adults with ASD (Simms et al. 2009; Uppal et al. 2014).

Reduced distal functional connectivity between medial prefrontal cortex/ACC and posterior cingulate cortex (PCC)/precuneus is one of the best-replicated findings in ASD (Abbott et al. 2015; Assaf et al. 2010; Burrows et al. 2016; Doyle-Thomas et al. 2015; Monk et al. 2009; von dem Hagen et al. 2013; Washington et al. 2013). In light of the findings by Burrows et al. (2016), this underconnectivity can be interpreted as reduced interplay between self-referential and other-referential processing (thinking about oneself vs. thinking about other people), which may be considered a fundamental component of social cognition. Moreover, the middle cingulate cortex (MCC), hypothesized to be crucial for cognitive processes involved during social exchanges and perspective-taking, has been shown to be diminished in ASD (Lu et al., 2015). Our additional finding of reduced *local* connectivity both in anterior and more posterior sections of the cingulate gyrus further suggests that reduced interplay between the two zones is accompanied by less coordinated activity *within* each of them. Aside from 'other-referential' thought, PCC is considered to be important for arousal and awareness, balance between internal and external attention, and environmental change detection (Leech and Sharp 2014).

The ACC also plays a crucial role as a hub of the salience network (SN), which provides a bridge between cerebral cortical networks and limbic and autonomic systems that are crucial for homeostatic, emotional, and visceral functions (Dosenbach et al. 2007; Seeley et al. 2007). Furthermore, the SN acts as a pivot in switching between task-positive and task-negative states (Sridharan et al. 2008), thus being a prime modulator of cognitive state (Dosenbach et al. 2007; Seeley et al. 2007). Disturbances of the SN have been found to be associated with behavioral impairment (Greicius 2008; Uddin et al. 2013). In ASD, decreased activation of SN regions has been observed during inhibition tasks (Agam et al. 2010; Kana et al. 2007) and in association with skin conductance response (Eilam-Stock et al. 2014), presumably indicating atypical autonomic functioning. Interconnectivity within the SN and with the amygdala has been found to be reduced in several studies of ASD (Abbott et al. 2015; Ebisch et al. 2011; Kana et al. 2007; von dem Hagen et al. 2013).

Visual cortex is locally overconnected

Local overconnectivity in visual cortex was one of the two broadly consistent findings across multiple datasets, including a Grand Total (EO+EC) with N=331. Although as mentioned, previous ReHo studies have differed, the finding is in agreement with some reports on local connectivity in ASD (Keown et al. 2013; Maximo et al. 2013; Washington et al. 2013). The divergent finding in Di Martino et al. (2013) may be partly attributed to the inclusion of eyes-closed data (see below). However, since we detected small local overconnectivity clusters in visual cortex even in the Grand Total sample that included EC data, other methodological differences mentioned earlier (e.g., the much wider age range up to 64 years in Di Martino et al.) were probable additional factors.

Overconnectivity in posterior cortices with visual functions may relate to extensive evidence suggesting a potential ‘special’ status of vision in the uneven neuropsychological profile commonly seen in ASD. This includes islands of atypically enhanced visual function (Simmons et al. 2009), as observed in visual search (Joseph et al. 2009; Kaldy et al. 2013) and in some studies on the embedded figures test (Eussen et al. 2016; Horlin et al. 2016; Keehn et al. 2009). Numerous studies of vision in ASD have suggested a preference for local processing, possibly at the expense of global gestalt processing (reviewed in Dakin and Frith 2005), in agreement with the hypothesis of ‘weak central coherence’ (Happé 1999). Another explanatory proposal has been generally enhanced visual perception in ASD (Mottron et al. 2006). The special cognitive-behavioral status of vision in ASD is supported on the neurobiological level by evidence of atypical participation of visual cortices across various tasks (Samson et al. 2012), including some non-visual ones (Jao Keehn et al. 2016; Kana et al. 2006; Shen et al. 2012).

Eye status has dramatic effect on ReHo patterns

Some previous resting state functional connectivity MRI (rs-fcMRI) studies of ASD have included EO and EC data in combination (e.g., Cerliani et al. 2015; Dajani and Uddin 2015; Di Martino et al. 2014; Itahashi et al. 2015). Our findings, however, suggest that eye status during rest may have dramatic effects on local synchronization of activity across almost the entire brain. First, between-group effects in ABIDE-EC diverged greatly from the three EO samples, with bilateral local *underconnectivity* in visual cortex close to V1 (similar to

findings from Itahashi et al. (2015)), and extensive overconnectivity in bilateral precuneus and PCC for EC. Direct comparison between EO and EC samples showed robust effects of eye status on ReHo patterns, with much greater local functional connectivity for EO in extensive posterior (visual) and some frontal regions, contrasted by extensive clusters of greater ReHo for EC in mostly limbic and subcortical regions (mid-portions of the cingulate gyrus, medial temporal lobe, thalamus). Similar patterns were observed in both TD and ASD groups (Figure 1B; Supplementary Figure 3B). Although EO and EC conditions included different cohorts (no participants scanned under both EO and EC conditions were included), samples were well matched and it is unlikely that the robust differences detected by us were cohort effects. Several previous rs-fMRI studies have directly compared EO and EC states. The regional patterns of our findings were largely consistent with those reported by Liu et al. (2013) and are probably related to similar patterns observed for the amplitude of low frequency fluctuations (Liu et al. 2013; Wang et al. 2015). Additionally relevant to effects in visual cortex observed by us, Zou et al. (2009) found that thalamic connectivity with visual regions was more strongly negative for EC than for EO.

In a first approach, the effects of EO status on activity in visual cortex may seem trivial, seemingly indicating that EC may be preferable, as it avoids visual stimulation that may confound intrinsic functional connectivity (iFC) effects. However, aside from low-pass filtering applied here (and commonly in the iFC literature), which will minimize any effects of visual stimulus processing in higher frequency domains, additional considerations speak against such a simple conclusion. Empirical evidence shows that EO states, especially when eyes are fixated (on a cross), have greater test-retest reliability for BOLD effect and cerebral blood flow measurements than EC states (Patriat et al. 2013; Zou et al. 2015), suggesting that EO states are better controlled whereas EC states are subject to undesirable variability. The most obvious example is the onset of drowsiness and sleep, which is difficult to monitor or prevent in EC conditions. Onset of sleep may be associated with substantial changes in BOLD correlations (Spoormaker et al. 2010; Tagliazucchi et al. 2012). Even beyond sleep, it is possible that mind wandering may be more intense in EC than in EO conditions because the visual link to the outside world that is typically maintained throughout the awake state is interrupted. Notably, we found that ReHo was higher in the two main nodes of the default mode network (PCC, medial prefrontal cortex) in EO than in EC datasets, possibly indicating that an expected mental ‘default state’ was maintained more reliably in the EO condition (for comparison of dynamic changes within the default mode network between ASD and TD cohorts, see Falahpour et al. 2016).

Limitations and perspectives

As mentioned, fMRI ReHo can assess ‘local connectivity’ only at a relatively coarse spatial scale (9mm³ for each 27-voxel cluster). It can therefore not be directly inferred what cytoarchitectonic patterns (and anomalies) described in postmortem studies of ASD (reviewed in Palmen et al. 2004) may be reflected in atypically increased or decreased ReHo (cf. Schumann and Nordahl 2011). Specifically, it may be tempting to relate local BOLD correlations to hypotheses of minicolumnar anomalies (Casanova et al. 2006) and excitation/inhibition imbalance (Nelson and Valakh 2015; Rubenstein and Merzenich 2003). Reduced intercolumnar inhibition (and thus greater neuronal cross-excitation) might in principle be

associated with greater synchronization of local neuronal activity. However, supportive evidence of such a link from combined MR spectroscopy of gamma-aminobutyric acid (GABA) and resting state fMRI has, to our knowledge, only come from two studies in motor cortex (Sampaio-Baptista et al. 2015; Staggs et al. 2014), and great caution is warranted in the absence of more direct evidence.

There were trade-offs between data quality (low motion) and loss in sample size. To maintain overall large sample sizes, sites contributing small numbers of datasets were included in some analyses. Differences in sample size and statistical power across different analyses, however, may have affected the findings, yet additional analyses covarying sample size and head motion showed that similar patterns of effects were retained.

Furthermore, since each site contributed different cohorts of participants, site-specific differences could not be distinguished from those related to the known heterogeneity of ASD. If idiopathic ASD, the population studied here, is understood as a clinical umbrella label for possibly hundreds of etiologically distinct rare disorders (Geschwind and State 2015), complete convergence across cohorts may in fact not be expected. Sites differed in their exact inclusionary and exclusionary criteria (Supplementary Table 1), and despite our matching procedures, some differences in findings may relate to cohort differences. Admittedly, the included datasets were not ideally suited for the purposes of the present study. Unfortunately, no dataset exists that would even approach what would be optimal (i.e., very large samples of ASD and TD participants, each scanned multiple times on different scanners and with different imaging protocols, both with eyes open and with eyes closed). However, we carefully matched samples group-wise and across sites, covarying for potentially confounding factors, and applied strict quality control criteria to minimize effects of cohort heterogeneity. In addition, acquisition of low-motion fMRI data in the awake state was almost exclusively limited to high-functioning individuals with ASD across sites. The findings presented here may thus not reflect local functional connectivity at the lower end of the spectrum.

Conclusions

Our study shows that local connectivity between-group effects from rs-fMRI are sensitive to site and cohort differences, even when data are selected for high quality and low motion and when groups are tightly matched for demographics and motion. However, local overconnectivity in posterior (mostly visual) regions and underconnectivity in cingulate cortex were detected both in some smaller ASD samples acquired under controlled conditions and large Grand Total samples with greater site variability, lending confidence to the finding. Some inconsistencies in overall patterns of findings and the surprising variability of exact loci of similar findings across samples may be in part explained by robust differences for data acquired in eyes open vs. eyes closed conditions. Our study generates two recommendations: First, careful attention needs to be paid to between-site factors of variability in the use of large consortium datasets in fMRI studies, especially those examining local connectivity. Sample size alone will not directly translate into confidence in findings. Second, mixing of data acquired under eyes open and eyes closed

conditions can confound findings because between-group differences in some regions may have opposite polarity for the two conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Surface renderings of regional homogeneity differences between ASD and TD groups for each subsample (A), of differences between ABIDE eyes open and eyes closed cohorts shown separately for ASD and TD groups (B), and of cluster overlap between subsamples (C), showing only independent subsamples (excluding Grand Total). Only results from pipeline with global signal regression and standardized ReHo are shown (see Supplementary Figure 1 for findings from other pipelines). The two columns on the left depict the left hemisphere and the two columns on the right depict the right hemisphere ($p < .05$, corrected).

Table 1

Participant Demographics

Sample	Group	N	% eyes open	Sex	Age		IQ		RMSD		ADOS Total		Avg % time pts post-censor:
					Mean (SD), Range		Mean (SD), Range		Mean (SD), Range		Mean (SD), Range		
					<i>t</i> -test, <i>p</i> -value*		<i>t</i> -test, <i>p</i> -value		<i>t</i> -test, <i>p</i> -value		<i>t</i> -test, <i>p</i> -value		
ABIDE-EO	TD	82	100	14 female	13.7 (2.67), 8.39–17.9		102.32 (12.58), 76–127		0.049 (0.014), 0.024–0.104		–		92.70
	ASD	59		6 female	13.67 (2.6), 8–17.94		102.43 (16.8), 64–137		0.049 (0.015), 0.022–0.087		11.88 (4.36), 3–21		92.60
ABIDE-EC					<i>t</i> (139)=0.048, <i>p</i> =0.962		<i>t</i> (110)=0.04, <i>p</i> =0.968		<i>t</i> (139)=0.032, <i>p</i> =0.974		[29 scores missing]		
	TD	42	0	6 female	13.34 (2.4), 7.26–17.5		107.45 (13.21), 72–137		0.059 (0.02), 0.026–0.107		–		95.10
	ASD	30		3 female	13.33 (2.55), 7.15–17.17		107.4 (14.87), 83–129		0.058 (0.016), 0.029–0.09		11.26 (3.31), 6–19		92.30
					<i>t</i> (70)=0.031, <i>p</i> =0.976		<i>t</i> (70)=0.016, <i>p</i> =0.988		<i>t</i> (70)=0.043, <i>p</i> =0.966		[7 scores missing]		
NYU-EO	TD	38	100	11 female	12.13 (2.76), 6.47–17.7		108 (16.86), 67–135		0.045 (0.012), 0.026–0.073		–		94.30
	ASD	25		3 female	12.08 (2.53), 8.51–17.88		107.76 (17.04), 72–149		0.045 (0.012), 0.024–0.067		12.44 (4.56), 6–22		94.20
					<i>t</i> (61)=0.067, <i>p</i> =0.947		<i>t</i> (61)=0.055, <i>p</i> =0.956		<i>t</i> (61)=0.052, <i>p</i> =0.959				
SDSU-EO	TD	27	100	5 female	13.83 (2.26), 8.7–17.6		106.89 (17.19), 53–136		0.051 (0.024), 0.017–0.095		–		95.50
	ASD	26		3 female	13.93 (2.43), 9.2–17.7		106.04 (18.47), 53–140		0.050 (0.021), 0.019–0.098		13.62 (4.53), 7–24		93.80
					<i>t</i> (51)=0.162, <i>p</i> =0.872		<i>t</i> (51)=0.017, <i>p</i> =0.863		<i>t</i> (51)=0.027, <i>p</i> =0.979				
Grand Total-EO	TD	147	100	30 female	13.3 (2.7), 6.5–17.9		104.92 (15.13), 53–136		0.048 (0.016), 0.017–0.119		–		93.80
	ASD	110		12 female	13.4 (2.6), 8–17.9		104.89 (17.27), 53–149		0.048 (0.016), 0.019–0.098		12.56 (4.40), 2–24		92.90
					<i>t</i> (255)=0.174, <i>p</i> =0.862		<i>t</i> (224)=0.015, <i>p</i> =0.988		<i>t</i> (255)=0.086, <i>p</i> =0.932		[29 scores missing]		
Grand Total-EO+EC	TD	184	79.9	36 female	13.47 (2.64), 8.01–17.9		104.70 (14.11), 67–137		0.052 (0.018), 0.016–0.119		–		93.80
	ASD	147	79.6	16 female	13.46 (2.63), 7.15–17.94		104.51 (16.65), 53–140		0.052 (0.017), 0.019–0.098		12.27 (4.00), 3–24		92.90
					<i>t</i> (329)=0.037, <i>p</i> =0.970		<i>t</i> (293)=0.105, <i>p</i> =0.916		<i>t</i> (329)=0.043, <i>p</i> =0.965		[37 scores missing]		

* Two-sample *t*-test comparing ASD and TD groups in each sample.