Supplementary Online Content


eAppendix. Supplementary Methodological Details

eFigure 1. Visualization of Main Steps of Independent Component Analysis and Calculation of Asymmetry Indices

eFigure 2. ICA Components From FDE Analysis

eFigure 3. Additional Matched True-Signal ICA Components From Auto-dimensionality Analysis That Did Not Significantly Differ in Asymmetry Indices

eFigure 4. Correlations Between Asymmetry Indices for ICA Networks and Diagnostic and IQ Scores in the ASD Group

This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix. Supplementary Methodological Details

Participants
Four participants with ASD were on medications: one on Risperdal, another on Risperdal and Concerta, a third on Vyvanse and Oxcarbazepine, and a fourth on Seroquel and Lithium.

Preprocessing
Field map correction was performed with in-house software (ppge4) developed by the Center for Functional MRI at the University of California, San Diego, to remove distortions resulting from magnetic field inhomogeneity (http://fmri.ucsd.edu/download/ppge4). This script calls the field map correction function provided by FSL Fugue. Using the “align_epi_anat” python script in AFNI, functional data were slice-time corrected, motion corrected by aligning to the first time point (using “3dvolreg” within “align_epi_anat”), skull-stripped (using “3dSkullStrip” within “align_epi_anat”), co-registered to the anatomical image, and standardized to the N27 ‘Colin’ brain in Talairach space. BOLD signal spikes (>2.5 SD) were replaced with an average of the time point before and after using “3dDespike” in AFNI. The resulting images were then smoothed with an isotropic Gaussian smoothing kernel at 10 mm full-width at half-maximum (FWHM). “3dAutomask” was used to remove any signal occurring outside the brain.

Head motion was estimated by first computing the sum of squared differences between neighboring time points, using the data output from AFNI’s “3dvolreg”. The magnitude of displacement between the brain at time point t and the next time point t+1, detected in spatial realignment of functional images, was roughly estimated as the Euclidian norm (i.e., root sum of squares) of the six temporal derivatives of movement in translational (mm) and rotational (°) directions:

\[ A = \sqrt{(\dot{z})^2 + (\dot{y})^2 + (\dot{x})^2 + (\dot{\alpha})^2 + (\dot{\beta})^2 + (\dot{\gamma})^2} \]

Then, the root mean square of this displacement was obtained to describe the total motion within a session, using the formula:

\[ RMED = \sqrt{\frac{1}{2}(1 - \frac{1}{2})} \]

Four ASD and two TD participants were excluded because this total head motion measure exceeded 1.5 mm. (Note that with inclusion of rotational motion measures, a total head motion measure in mm units is only an approximation).

Independent Component Analysis
For the Fixed Dimensionality Estimation (FDE) analyses, temporal concatenation group independent component analysis (TC-GICA) was performed with a set dimensionality of 20 components and voxel-wise variance normalization using MELODIC v3.02 for 20 iterations separately for each group. These 20 iterations of 20 components each were concatenated and TC-GICA was performed on this concatenated dataset with a set dimensionality of 20 components and without voxel-wise variance normalization. The purpose of this step was to extract the most consistent 20 components across the iterations. For analogous procedure, see online supplement in Smith et al.3

For the Automated Dimensionality Estimation (ADE) analyses, temporal concatenation group independent component analysis (TC-GICA) was performed with automatic dimensionality estimation using the Laplace approximation to the Bayesian evidence components and voxel-wise variance normalization implemented within MELODIC v3.02 for 20 iterations separately for each group. The ADE procedure reduced the data to 86 dimensions for the TD group and 55 dimensions for the ASD group. These 20 iterations were concatenated for each group and TC-GICA was performed on this concatenated dataset with a set dimensionality of 86 and 55 components, for the
TD and ASD group, respectively, and without voxel-wise variance normalization. Again, the purpose of this step was to extract the most consistent components across the iterations.

The spatial and temporal aspects of the resultant components from each run (FDE and ADE) in each group (TD and ASD) were visually inspected, and artifactual components were excluded from further analyses, following procedures detailed in Kelly et al. Components with less than 80% of the voxels located in gray matter were also considered noise and excluded, given that the BOLD signal predominantly originates from gray matter. To obtain an estimate of the percentage of voxels located in the gray matter, a gray matter mask was obtained by automated segmentation (FSL FAST) of the standard anatomical volume. Six segments were estimated, the segments comprising the gray matter were combined, and the resultant mask was dilated by one voxel (i.e., 1 mm) in all directions, to protect against exclusion of true gray matter voxels. Note that a gray matter mask based on a standard brain will underestimate the true gray matter compartment of a cluster derived from a group analysis because of morphological variability within the group, slight imprecisions of spatial normalization, and the lower spatial resolution and greater smoothness of functional images.

Asymmetry Index Calculation and Between-Group Comparison

The true-signal components from each analysis (FDE, ADE) were matched between-groups (TD, ASD) at a spatial correlation threshold of $r > 0.5$. Dual regression was used to obtain single-subject level spatial components from each group level spatial component. This procedure involves two sequential linear regression steps:

$$Y_i = X_i \beta + \epsilon_i^1 \quad (1)$$
$$Y_i = X_i \beta_i + \epsilon_i^2 \quad (2)$$

where $n$ is the total number of subjects in each group. $Y_i$ is a four dimensional matrix containing the pre-processed resting-state data for the $i$-th individual, $S$ is a set of all spatial components obtained from group-level ICA, and $\epsilon_i^1$, $\epsilon_i^2$ are the residual errors from each regression step, respectively. From Eq. (1), $X_i$ corresponds to a set of time series estimated from $Y_i$ for the spatial components in $S$. In the second step (Eq. 2), the component maps are finally recovered in the $i$-th individual by regressing the resting-state data $Y_i$ onto the predictor time series $X_i$ from Eq. 1, creating a set of estimated beta coefficient maps $B_i$ with the same dimensions as $S$. For calculating the asymmetry index, negative beta coefficients were replaced with zeros. This step was motivated by the uncertain significance of negative BOLD correlations, as debated in the functional connectivity MRI literature. More specifically, negative effects most likely indicated that voxels were not part of a given component, further warranting their exclusion from asymmetry index calculation. The within-group $t$-tests on the single-subject level component map obtained with dual regression are presented in Figure 1 and eFigures 2 and 3. Note that these are not the original component maps from temporal concatenation ICA.

Hemispheric masks based on the N27 brain were created using “3dAutomask” in AFNI. The cerebellum was manually excluded from the mask, and the mask was separated into left and right hemisphere masks. An asymmetry index $(R - L)/(R + L)$ was calculated for each participant and each component, using the average beta coefficients extracted from the dual regression step for all voxels with positive intensities per hemisphere. Permutation tests (10,000 iterations) were performed on each component to test for differences between TD and ASD groups, while controlling for age.

References


eFigure 1. Visualization of main steps of Independent Component Analysis and calculation of asymmetry indices

For details, see main text.
eFigure 2. ICA components from FDE analysis (fixed dimensionality of 20). Images are displayed in neurological convention (left side is left hemisphere). In each panel, a TD component is shown at the top and the matched ASD component at the bottom (with spatial correlation coefficient shown on the left). Asymmetry indices are stated next to the group labels. Components are labeled as L, left-lateralized (AI < -0.3); (L), weakly left-lateralized (AI: -0.3 to -0.1); B, bilateral (AI: -0.1 to 0.1); (R), weakly right-lateralized (AI: 0.1 to 0.3); R, right-lateralized (AI > 0.3). The p-values at the bottom right of each panel are derived from between-group permutation tests of asymmetry indices controlling for age. Matching of components to those from previous studies is abbreviated below as: mtL, matched to Laird et al., 10 and mtS, matched to Smith et al. 3 Components shown with no background are true-signal components and are matched between groups (r > 0.5). Components with a red background are noise components that were also matched between groups (r > 0.5). Components with a blue background are true-signal components that were not matched between groups. Components with a gray background are noise components that were not matched between groups. Panel A: mtS (r = 0.716) & mtL (r = 0.838) “medial visual area” component. B: mtS (r = 0.347) & mtL (r = 0.433) “right fronto-parietal” component. In the TD group, ADE components C (r = 0.558) and K (r = 0.443) from Figure 1 were matched to this FDE component. C: mtS (r = 0.634) & mtL (r = 0.410) “sensorimotor” component. In the TD group, ADE component F (r = 0.473) from Figure 1 was matched to this FDE component. D: mtS (component 2, r = 0.282; component 3, r = 0.391) and mtL (r = 0.587) “visual” component. In the TD group, ADE components B (r = 0.497) & I (r = 0.481) from Figure 1 and component B (r = 0.175) from Figure S1 were matched to this FDE component. E : mtL (r = 0.467) “auditory” component. In the TD group, ADE component J from Figure 1 was matched (r = 0.340) to this FDE component. F: mtS (r = 0.438) & mtL (r = 0.537) “left fronto-parietal” component. In the TD group, ADE components H (r = 0.442) from Figure 1 and E from Figure S1 (r = 0.275) were matched to this FDE component. G: mtL (r = 0.599) component “associated with action and somesthesia corresponding to speech, such as overt reading or recitation, chewing or swallowing, and flexion/extension of the tongue.” In the TD group, ADE component D from Figure S1 was matched (r = 0.420) to this FDE component. H: mtL (r = 0.581) component that “was related to cognitive control of visuomotor timing and preparation of executed movements.” In the TD group, ADE components A (r = 0.523) & C (r = 0.245) from Figure S1 were matched to this FDE component. I: mtS (r = 0.567) & mtL (r = 0.644) “default mode network” component. J: mtS (r = 0.634) “cerebellum” component. K: mtS (r = 0.391) “executive control” component. In the TD group, ADE component G (r = 0.372) from Figure 1 was matched to this FDE component. L: mtL (r = 0.599) component “related to audition (including tone and pitch discrimination), music, and speech [as well as] phonological discrimination and oddball discrimination.” M: mtL (r = 0.401) “sensorimotor” component.

Several additional components identified in the TD group were not matched to ASD components: Q: mtS (r = 0.371) “auditory” component. In the TD group, ADE component D (r = 0.421) from Figure 1 was matched to this FDE component. R: mtS (r = 0.375) “frontoparietal” component. S: mtS (r = 0.305) “default mode” component. Similarly, several additional components identified in the ASD group were not matched to TD components: U mtL (r = 0.616) component that was related to “olfaction, gustation, and emotion, with a strong preference for reward and thirst tasks.” V mtS (r = 0.331) & mtL (r = 0.542) “sensorimotor” component.
eFigure 3. Additional matched true-signal ICA components from auto-dimensionality analysis that did not significantly differ in asymmetry indices. This figure complements the set of components shown in Figure 1 of the main text. Images are displayed in neurological convention. In each panel, the TD component is shown at the top and the matched ASD component at the bottom (with spatial correlation coefficient shown on the left). Asymmetry indices are stated next to the group labels. Asymmetry labels (L, B, R etc.) are determined as described in eFigure 2. The p-values at the bottom right of each panel are derived from between-group permutation tests of asymmetry indices controlling for age. Matching of components to those from previous studies is abbreviated below as: mtL, matched to Laird et al.,\textsuperscript{10} and mtS, matched to Smith et al.\textsuperscript{3} Panel A: component “related to cognitive control of visuomotor timing and preparation of executed movements” (mtL); B: component implicated in “visuospatial processing and reasoning” (mtL); C: component “related to cognitive control of visuomotor timing and preparation of executed movements” (mtL); D: “sensorimotor” component (mtL and mtS); E: “left fronto-parietal” component (mtL and mtS); F: component “related to cognitive control of visuomotor timing and preparation of executed movements” (mtL).
eFigure 4. Correlations between asymmetry indices for ICA networks and diagnostic and IQ scores in the ASD group. Asymmetry indices are on the y-axis (positive numbers reflect rightward asymmetry), diagnostic and IQ scores are on the x-axis. Higher diagnostic scores reflect greater symptom severity.