Neuron, Volume *75*

Supplemental Information

Unreliable Evoked Responses in Autism

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Figure S1 (related to all main figures): This figure shows the design of our experiments.

Figure S2 (related to main Figure 1): This figure shows how we selected our regions of interest (ROIs) based on responses presented in main Figure1.

Figure S3 (related to main Figures 2, 3, & 4): This figure shows how we calculated response amplitude and trial-by-trial variability – the basis for most of the presented results.

Figure S4 (related to main Figure 2): This figure shows that the results presented in Figure 2 were also apparent when assessing other trial types (adapted, un-adapted, no-test, and when combining all three) using the same analysis.

Figure S5 (related to main Figure 2): This figure shows that the results presented in Figure 2 were also apparent when assessed using a different methodology – GLM analysis.

Figure S6 (related to main Figure 7): This figure shows an additional behavioral analysis (trial-by-trial reaction time variability), which is related to the task responses presented in Figure 7.

Figure S7 (related to all main figures): This figure contains control analyses showing that head motion was not significantly different across groups. This is an important control for interpreting all of our results.

Table S1 (related to all main figures): This table contains behavioral scores of the individuals with autism who participated in the experiments. This is important information for interpreting the entire study.

Table S2 (related to main Figure 1): This table lists the locations of our regions of interest, which were selected based on the response maps presented in main Figure 1.

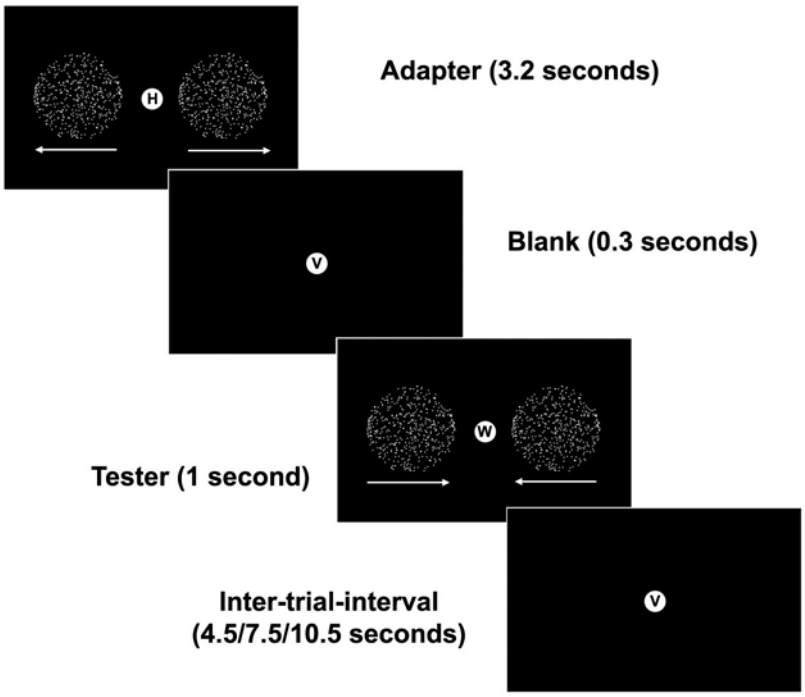

Figure S1 (related to all main figures): Experiment design. All three sensory experiments (visual, auditory, and somatosensory) followed the same rapid event-related temporal structure, which was designed to enable assessment of response amplitude, variability, and adaptation. Each trial was 4.5 seconds long and contained a 3.3 second adapter followed by 0.2 seconds of blank/rest and a 1 second test. Inter-trial intervals were 4.5, 7.5, or 10.5 seconds (in randomly shuffled order). Each run contained 12 adapted trials, 12 un-adapted trials, and 12 trials of the adapter without a test condition. Most subjects participated in two runs of each experiment.

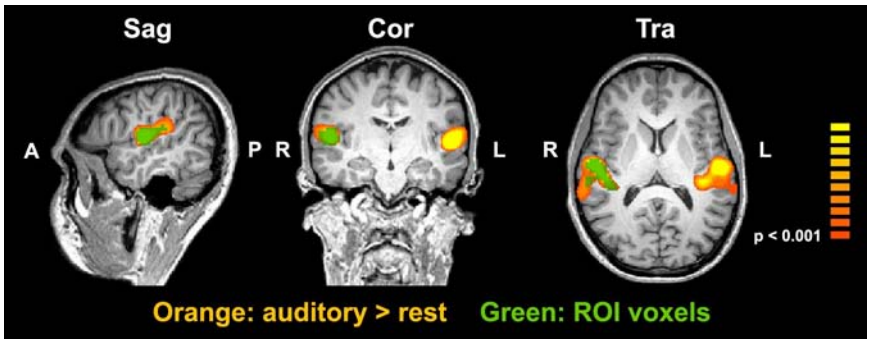

Figure S2 (related to main Figure 1): Selection of right auditory ROI in an example subject. Orange: responses to the auditory stimulus. Green: 200 adjacent functional voxels with the strongest activation were selected.

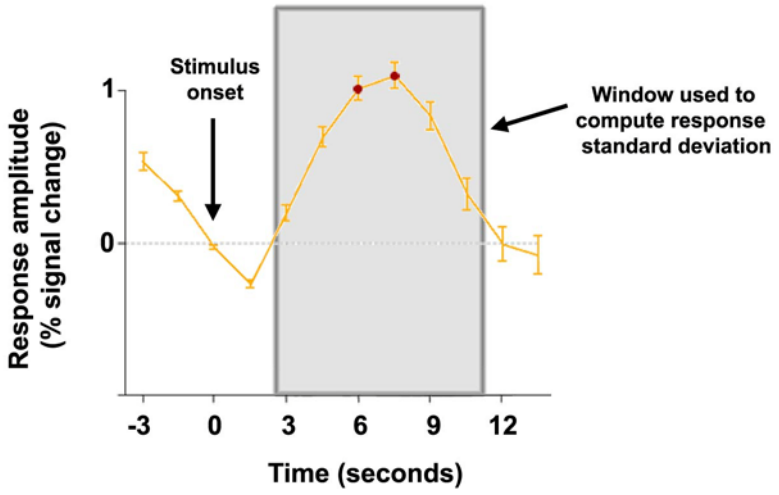

Figure S3 (related to main Figures 2, 3, & 4): Demonstration of the "trial-triggered average" analysis for an example subject in the visual stimulation experiment. For each voxel in each ROI, the fMRI time-course was divided by its mean to compensate for distance from the RF coil and convert to percent signal change. The resulting normalized time-courses were averaged across voxels to generate a single time-course for each ROI. We segmented these time-courses according to stimulus onset so as to generate single trial segments of 9 time-points containing the fMRI response following each stimulus presentation. The curve in orange shows the average fMRI response that evolves over several time-points following stimulus onset. Response amplitude was computed by averaging across time-points 4 and 5 post-stimulus, which corresponded to the peak of the hemodynamic response (marked in red). Response standard deviation was determined by computing the standard deviation across trials for time-points 2-7 post-stimulus (marked by gray window) and averaging across them to yield a single value. This analysis was performed separately for each of the three trial types: adapted, un-adapted, and no-test (Supplementary Figure 4). This analysis was carried out separately for each run and averaged across runs belonging to the same sensory experiment (in most subjects we had 2 runs of each). An equivalent analysis was performed on time-points where a button was pressed (indicating a letter repeat) to assess response amplitude and standard deviation in the motor ROIs (Figure 7).

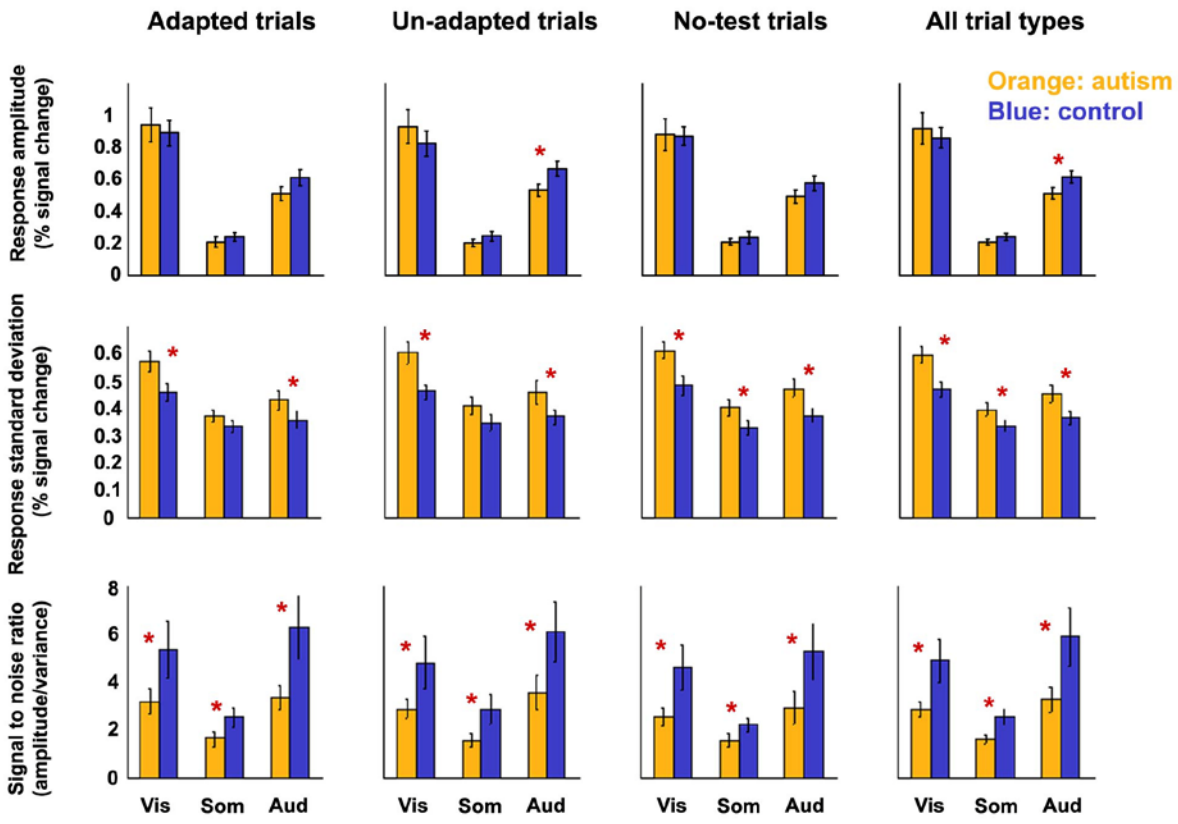

Figure S4 (related to main Figure 2): Comparison of responses across trial types (adapted, un-adapted, no-test, and when combining all three). Trial-by-trial response estimates were carried out with a "trial-triggered average" analysis (Supplementary Figure 3). Response amplitude (top), standard deviation across trials (middle), and signal-to-noise ratios (bottom) were very similar across all trial types. Signal-to-noise ratios were, however, always significantly smaller in the autism group compared to the control group, demonstrating that poor response reliability in autism was a robust finding across all trial types in all three experiments. Orange: autism group. Blue: control group. Red asterisk: significant difference between groups ($p<0.05$, one tailed t-test). Error bars: standard error across subjects.

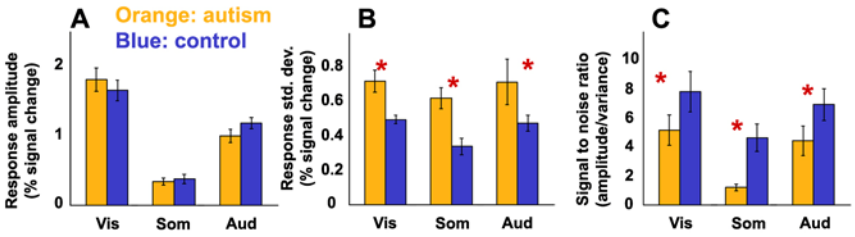

Figure S5 (related to main Figure 2): Complementary linear regression analysis, same format as Figure 2. A: Mean response amplitudes B: Standard deviations of response amplitude across trials. C: Signal-to-noise ratios. Orange: autism group. Blue: control group. Red asterisk: significant difference between groups ($p<0.05$, one tailed t-tests). Error bars: standard error across subjects.

The general linear model used in this analysis contained a single column for each trial, which was convolved with a canonical HIRF to create a model of the expected hemodynamic response (Boynton et al., 1996) for each trial separately. We then estimated the response amplitude of each trial using linear regression and calculated the mean and standard deviation across trials for each subject.

This analysis was performed using data from 10 subjects with autism and 10 matched controls who participated in two runs of each sensory experiment. We identified each ROI using one run and estimated the response amplitudes using the second run, thereby guaranteeing that the ROI-selection did not introduce any statistical bias in the response amplitudes. This analysis revealed equivalent results to those of the trial-triggered average analyses presented in Figure 2, confirming the finding of poor response reliability in autism when using an alternative method for estimating trial-by-trial responses and when selecting the ROIs using statistically-independent data.

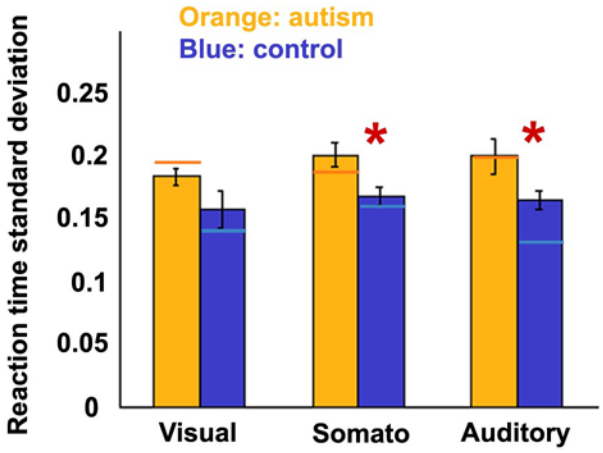

Figure S6 (related to main Figure 7): Behavioral variability. Trial-by-trial reaction time variability was larger in the autism group (orange) as compared with the control group (blue). Vertical bars show results after equating performance across groups. Darker orange and lighter blue lines show original results before equating performance for comparison. Asterisks: significant difference across groups after equating performance ($p<0.05$, one tailed t-test). Error bars: standard error across subjects.

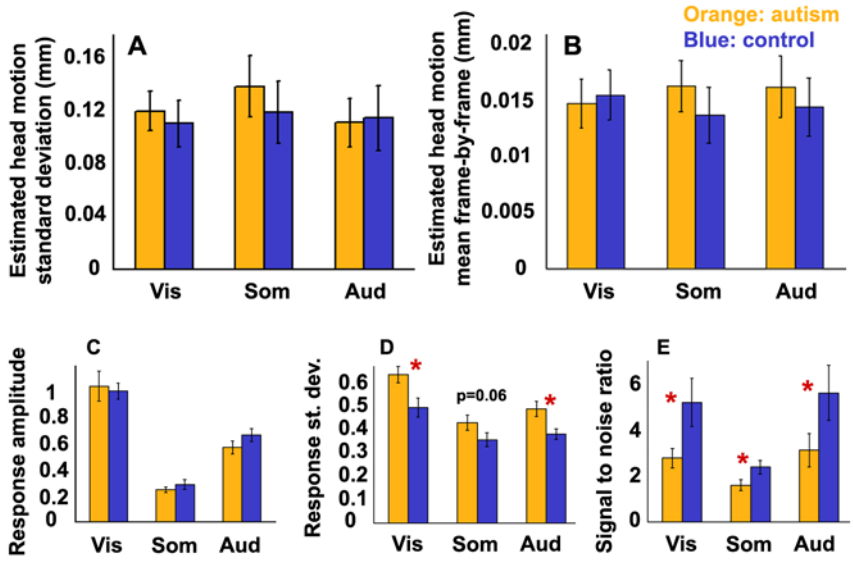

Figure S7 (related to all main figures): Comparison of head motion across groups and re-analysis of data after projecting out head motion estimates. A: Standard deviation of head motion estimates in each experiment (average of 3 rotation and 3 translation estimates). B: Mean absolute frame-by-frame movement estimates. There were no significant differences across groups in any of the experiments ($p>0.2$, one tailed t-test). C-E: ROI analysis (same format as Figure 2) after projecting out head motion parameters. C: Mean response amplitudes. D: Standard deviations of response amplitudes across trials. E: Signal-to-noise ratios. Response amplitudes were not significantly different across groups ($p > 0.1$ in all experiments, one tailed t-test). Standard deviations were significantly larger in the autism group in the visual and auditory experiments and almost significant in the somatosensory experiment ($p=0.06$). Signal-to-noise ratios were significantly smaller in the autism group in all experiments. Orange: autism group. Blue: Control group. Asterisks: significant difference across groups ($p<0.05$, one tailed t-test). Error bars: standard error across subjects.

Given the recent focus on possible head motion confounds when comparing fMRI results across patient and control populations (Power et al., 2012; Van Dijk et al., 2012), we made every effort to ensure that the interpretation of our results was not confounded by head motion. We, therefore, performed two different analyses to assess head motion throughout the fMRI experiments. First, we computed the standard deviation of each head motion parameter throughout each run and then averaged across the 6 parameters. Runs containing more head movements yield head motion parameters that have larger variability. Second, we computed the

absolute head movement from each volume to the one preceding it in time. We computed the mean frame-by-frame head motion for each head motion parameter and then averaged across the six parameters. Runs containing more head movements exhibit larger frame-by-frame changes. Neither of these measures were statistically significant across groups.

In another analysis, we removed the contribution of head motion to the fMRI measurements by orthogonal projection. Specifically, we created a regressor from the time-series of each motion parameter, and projected it out of each voxel's time series in the following manner. Let y be the time series measured during the experiment, and let x be a head movement regressor time series. We computed a residual time series r as: r = y-(y*x/norm(x)*x/norm(x)). Removal by projection ensured that the residual time series r was orthogonal to the removed component x such that both the correlation and the dot product between the time series of the voxel and any of the head motion estimators equaled zero. An equivalent procedure would be to perform a multivariate regression of the head motion estimators with each voxel time-course, and retain the residuals of the regression.

Response amplitudes, standard deviations, and signal-to-noise ratios using the projected-out data (where head motion contributions were removed) yielded equivalent results to those presented in the main text.

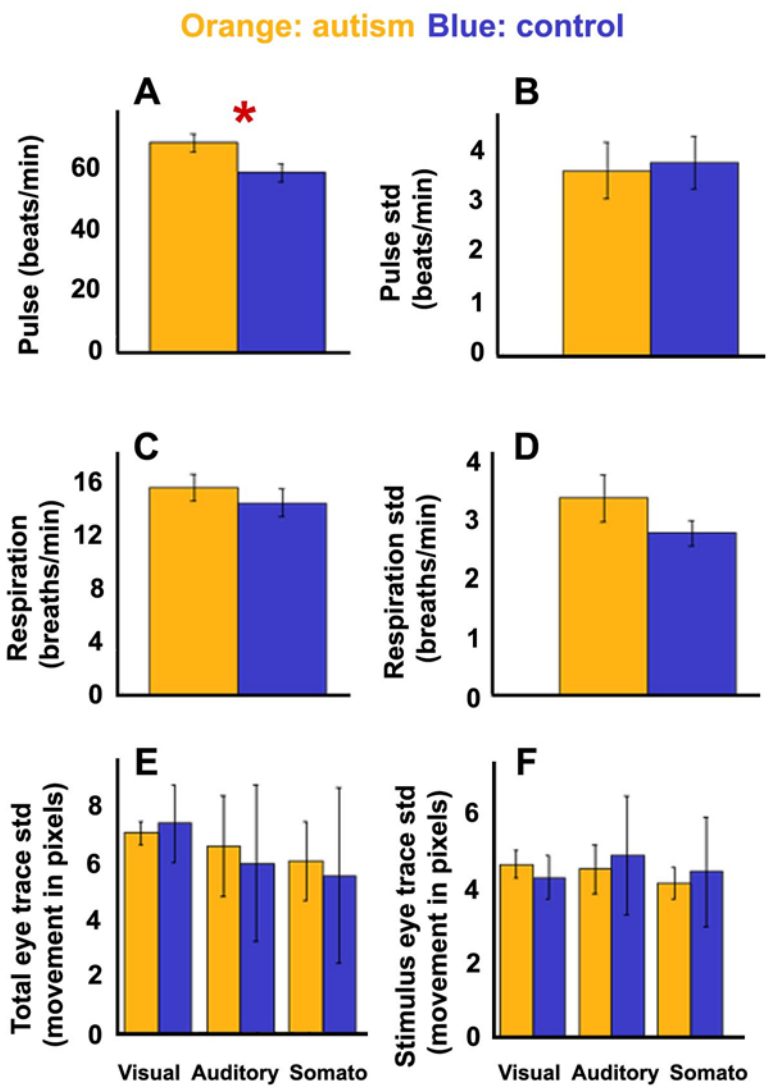

Figure S8 (related to all main figures): Comparison of heart rate, respiration, and eye traces across groups. Heart rate and respiration were measured in a separate resting state fMRI experiment performed during the same session as sensory experiments. A: Average heart rate. B: Heart rate standard deviation over time. C: Average respiration rate. D: Respiration standard deviation over time. E: Standard deviation of eye position, for each experiment. F: Standard deviation of eye position computed during 500 ms windows following stimulus onsets. Unstable fixation would have translated into larger standard deviation values. Orange: autism group. Blue: Control group. Red asterisks: significant differences across groups ($p<0.05$, one tailed t-test).

ADOS social	ADOS communication	ADOS stereotypical	ADI social	ADI communication	ADI stereotypical	Full IQ
7	5	1	19	16	8	103
8	4	2	21	17	6	123
7	5	3	27	20	6	107
8	5	1	27	22	5	124
9	5	1	22	15	5	108
6	2	3	20	16	7	104
10	6	3	15	9	6	121
6	5	6	19	11	4	127
7	3	4	10	8	6	123
7	4	1	21	16	8	116
13	6	3	10	16	3	118
13	5	3	20	13	3	134
10	5	2	20	12	4	95
9	4	3	20	17	7	100
Means						
8.6	4.5	2.6	19.4	14.9	5.6	114

Table S1 (related to all main figures): Behavioral scores of the individuals with autism who participated in the experiments. Participants were recruited and assessed by the Center for Excellence in Autism Research (CeFAR) at the University of Pittsburgh. ADOS and IQ measures were determined by CEFAR staff within 1 year of the fMRI experiments. All subjects, except one, met criteria for full autism such that the combined ADOS score in the social and communication domains equaled 10 or higher. The subject with a combined score of 8 had, in

previous years, met criteria for full autism and was, therefore, included in the study. All subjects met criteria for full autism using the ADI scale (communication > 8, social > 10, and stereotypical behaviors > 3).

Five individuals with autism were taking medications, three subjects were taking SSRIs, one was taking Risperdal and Depakote, and one was taking Ambien and SNRI. We performed a trial triggered-average analysis while excluding these 5 individuals and found similar results to those presented in the paper (increased trial-by-trial variability and decreased signal-to-noise in autism), but with weaker statistical significance as would be expected from the diminished statistical power (smaller group size).

Potential subjects with autism were excluded if they had an underlying etiology (e.g., tuberous sclerosis), active seizures, mood disorders, or a history of acquired brain injury. Exclusion was based on neurological history, chromosomal analysis, metabolic testing, and clinical evaluation. None of the subjects with autism had a history of epilepsy, four had a previous diagnosis of depression, and two had a previous diagnosis of anxiety.

ROI name	Autism				Control			
	X (std)	Y (std)	Z (std)	Size (std)	X (std)	Y (std)	Z (std)	Size (std)
Left Vis	-14 (2)	-87 (4)	-6 (5)	200	-14 (1)	-89 (4)	-8 (4)	200
Right Vis	11 (1)	-83 (5)	-6 (4)	200	10 (2)	-87 (4)	-7 (4)	200
Left Aud	-51 (4)	-25 (5)	7 (2)	200	-53 (3)	-24 (3)	7 (3)	200
Right Aud	52 (3)	21 (3)	6 (3)	200	52 (2)	-20 (6)	7 (3)	200
Left Som	-53 (5)	-31 (5)	16 (4)	200	-52 (6)	-32 (8)	15 (7)	200
Right Som	44 (4)	-24 (6)	16 (3)	200	41 (3)	-27 (4)	18 (5)	200
Mot	-40	-28	49	365	-40	-29	52	352
Left vPM	-43	-5	16	288	-43	-1	19	243
Right vPM	44	8	14	298	42	12	15	315
Left aIPS	-26	-56	43	330	-35	-55	40	313
Right aIPS	28	-56	39	257	34	-51	43	316

Table S2 (related to main Figure 1): Region of interest locations (Talairach coordinates) and sizes along with standard deviations (in parentheses) across subjects. Sensory ROIs were defined in each subject separately and were of a fixed size. Motor ROIs were identical across subjects of each group as they were defined manually using each group's multi-subject SPM map (see Figure S2).

Supplemental References:

Boynton, G.M., Engel, S.A., Glover, G.H., and Heeger, D.J. (1996). Linear systems analysis of functional magnetic resonance imaging in human V1. J Neurosci *16*, 4207-4221.

Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., and Petersen, S.E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage *59*, 2142-2154.

Van Dijk, K.R., Sabuncu, M.R., and Buckner, R.L. (2012). The influence of head motion on intrinsic functional connectivity MRI. Neuroimage *59*, 431-438.