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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample sizes and statistical information are included in the supplemental tables/information. For mouse studies, calculations were based on the following formula: n=1+2C(s/d)2 (with C calculated for p = 0.05, power 0.9). For human studies, no formal power analysis was performed, but the samples are larger than those in previously published studies, as stated in the Methods.

2. Data exclusions

   Describe any data exclusions.

   For mouse studies, ROUT methodology in Graphpad Prism was utilized to determine the presence of outliers. For human studies, participants were excluded primarily due to data quality control (in children with autism and neurotypical control children) and technical issues leading to unusable data (in neurotypical adults). Exclusion criteria are described in the Methods.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   In the mouse studies, all attempts at replication were successful. An additional cohort was performed to further demonstrate reproduction of results that RCrusI inhibition was sufficient to generate ASD-relevant behaviors, and results corroborated initial findings. In the human studies, no replication samples were conducted.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   For mouse studies, available litters with appropriate mice and controls were used for procedures or behavioral testing. A statement to this point is included in the Methods ("Mice", page 33-34). For human studies, neurotypical adults were randomly assigned to a tDCS condition. In children with and without ASD, clinical profile determined the groups.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   In the mouse studies, all behavioral assays were performed in blinded fashion (Methods, "Behavioral Analysis", page 39-42). In the neurotypical humans, participants were blind to the tDCS condition received.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study. Graphpad prism, SPM12, MATLAB

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company. None

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species). n/a

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used. n/a

b. Describe the method of cell line authentication used. n/a

c. Report whether the cell lines were tested for mycoplasma contamination. n/a

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use. n/a

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

L7/Pcp2-Cre (L7Cre) transgenic mice13 were attained from Jackson Laboratories. L7cre; Tsc1flox/flox (mutant) animals were generated by crossing L7/Pcp2-Cre (L7Cre) transgenic mice with floxed Tsc1 mice (Tsc1flox/flox) 14 to yield L7Cre;Tsc1 flox/+ progeny. These progeny were then crossed with one another to ultimately yield Tsc1flox/flox and L7Cre;Tsc1flox/flox (mutant) mice. These were then used as breeders with Tsc1flox/flox mice crossed to L7Cre;Tsc1flox/flox mice. Only male animals were used for behavioral experiments. Mice were of mixed genetic backgrounds (C57Bl/6J, 129 SvJae, BALB/cJ). Littermate controls were used for all behavioral experiments. Specific ages are detailed in Methods ("Behavioral Analysis", page 39-42) for behavior.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Full details in Methods (pages 26-28). Thirty-five participants (23 females, 12 males; mean age=23.79 ± 2.71 years) took part in this study. The study was approved by the Georgetown University Medical Center Institutional Review Board. All participants provided written informed consent and were compensated for their time. Participants were all right-handed, native English speakers with no history of neurological conditions, head trauma, psychiatric or developmental disorders, and no contraindications for tDCS or MRI. Participants were randomly assigned to either the anodal (n=20; 6 males) or sham (n=15; 6 males) tDCS group upon arrival at the scanning center. We prioritized assignment of participants to the active tDCS condition. We excluded one subject from all imaging analyses due to the presence of scanner artifact unrelated to the current experiment, leaving a final sample of 34 participants (23 females, 11 males; mean age=23.75 ± 2.74 years; n=19 anodal [5 males], n=15 sham [6 males]). We also excluded one additional participant from the during-tDCS analyses due to scanner malfunction during tDCS data collection (during tDCS, n=33; 22 females, 11 males; mean age=23.82 ± 2.76 years; n=18 in the anodal group [5 males] and n=15 in the sham group [6 males]).

Participants with autism spectrum disorder. To examine functional connectivity in a group of participants with autism spectrum disorder (ASD), we analyzed resting-state functional magnetic resonance imaging (rsfMRI) scans collected from children aged 8-13 years old. Eighty-one children had a diagnosis of ASD (mean age=10.36±1.44), and eighty-one were typically developing (TD) children (mean age=10.39±1.17) drawn from a larger sample of 195 TD children with usable rsfMRI data (Table S4). Data were acquired as part of an on-going study at the Center for Neurodevelopmental and Imaging Research (CNIR) at the Kennedy Krieger Institute (KKI). Participants were recruited via advertisements posted in the community, local pediatricians' and psychologists' offices, local schools, social service organizations, chapters of the Autism Society of American, the Interactive Autism Network (IAN) database, outpatient clinics at Kennedy Krieger Institute, and word of mouth. The study was approved by the Johns Hopkins Medical Institutional Review Board. Written consent was obtained from a parent/guardian and assent was obtained from the child.

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MRI Studies Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Experimental design

1. Describe the experimental design.	Human imaging: Resting-state functional MRI Mouse imaging: Structural MRI: Using deformation based morphometry the volume differences were assessed and compared between the TSC1 mutant and the WT mouse.
2. Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.	N/A
3. Describe how behavioral performance was measured.	N/A

Acquisition

4. Imaging	
a. Specify the type(s) of imaging.	Human imaging: Resting-state fMRI Mouse imaging: Structural Imaging using a T2 - Weighted Fast Spin Echo Sequence
b. Specify the field strength (in Tesla).	Human imaging: 3T Mouse imaging: 7T
c. Provide the essential sequence imaging parameters.	Human (tDCS): resting state fMRI; echo planar imaging; field of view 64x64, slice thickness 3.2mm, orientation axial, TE 30ms, TR 2500ms, flip angle 90 Human (ASD and controls): resting state fMRI; single-shot partially parallel (SENSE) gradient-recalled echo-planar sequence; field of view 84x84, slice thickness 3mm, orientation axial, TE 30ms, TR 2500ms, flip angle 70 Mouse imaging: T2- weighted, 3-D fast spin-echo sequence was used, with a cylindrical acquisition of k-space, and with a TR of 350 ms, and TEs of 12 ms per echo for 6 echoes, field-of-view of 20 x 20 x 25 mm^3 and matrix size = 504 x 504 x 630 giving an image with 0.040 mm isotropic voxels. Total imaging time for this sequence is currently ~14 h.
d. For diffusion MRI, provide full details of imaging parameters.	N/A
5. State area of acquisition.	Whole brain scans were acquired in all protocols

Preprocessing

6. Describe the software used for preprocessing.

Human (tDCS): CONN toolbox 15e; preprocessing: removal of first 5 volumes, slice-time correction, realignment and unwarping, ART scrubbing, normalization to MNI space, smoothing (8mm FWHM).

Human (ASD and controls): SPM12 and in-house MATLAB code; preprocessing: slice time correction, realignment, normalization, linear detrending, nuisance regression using aCompCor, smoothing (6mm FWHM), and temporal filtering (.01-.1 Hz pass band).

Mouse imaging: MR related distortions due to the multiple mouse acquisition were corrected prior to registration. This was done with a home built distortion correction pipeline. No other preprocessing was performed prior to registration and volume calculation.

7. Normalization

a. If data were normalized/standardized, describe the approach(es).

Human Imaging: Normalization to MNI space using individual T1 scan

Mouse Imaging: Registrations were preformed with a combination of mni_autoreg tools (Collins et al. 1994) and ANTS (advanced normalization tools) (Avants et al. 2008, 2011).

b. Describe the template used for normalization/transformation.

Human (tDCS): MNI 152 T1

Human (autism and controls): ICBM152

Mouse imaging: No template was used.

8. Describe your procedure for artifact and structured noise removal.

Human (tDCS) ART scrubbing for outlier detection; CompCor strategy for removal of signal from grey matter, white matter, and cerebrospinal fluid.

Human (ASD and controls): MATLAB script to flag spikes in motion; aCompCor was used to estimate spatially coherent noise components from tissues not expected to exhibit BOLD signals and remove them from the data: tissue probability maps of white matter and cerebral spinal fluid (CSF) were restricted using a 99% probability threshold and eroded using MATLAB's imerode function. The CSF mask was further constrained to areas within the ALVIN mask of the ventricles (Kempton et al., 2011). Principal components (PCs) explaining 50% of the variance in white matter and 50% of the variance in CSF were regressed from rsfMRI data along with linearly detrended versions of the six rigid body realignment parameters and the first derivative of each linearly detrended realignment parameter.

Mouse imaging: n/a

9. Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Human (tDCS) Default outlier removal as implemented in ART toolbox, including threshold value for global-signal (z-value; default 9) and threshold value for subject-motion (mm; default 2). Only n=6 participants had any volumes removed, and the max number of volumes removed for any one participant was 19 (out of ~480 volumes).

Human (ASD and controls) A matlab script was used to flag and exclude spikes greater than 3 mm or 3 degrees in the one-back differences of the six rigid body realignment estimates. Several time points were discarded from the beginning/end of eight scans (4 ASD) to uphold these criteria, but each participant retained at least 5 min 10 s of data. For spikes at the beginning of the scan, all frames prior to the spike plus two after were discarded. For spikes at the end of the scan, the spike plus all frames after the spike were discarded. Mean framewise displacement was calculated using the realignment estimates, compared across groups, and included as a covariate in brain-behavior models to minimize the impact of lingering motion-induced artifacts.

Mouse imaging: N/A

Statistical modeling & inference

10. Define your model type and settings.

Human (tDCS): At the first level, General Linear Model was used to correlate seed BOLD timecourse and all other voxels in the brain; At the second level, two-tailed independent t-test was used to compare correlation coefficients between groups (anodal, sham)

Human (ASD and controls): Two-tailed independent t-test was used to compare groups (ASD, TD)

Mouse imaging: Connectivity between the cerebellum and the cortex in the mouse was assessed using anatomical covariance (Evans, 2013). Correlations were calculated between the volumes of the cerebellar and cortical regions using Pearson's method.

11. Specify the precise effect tested.

Human Imaging: Comparisons were between groups

Mouse: This tests anatomical covariance between to regions which can be used as a measure of connectivity (Evans, 2013)

12. Analysis

a. Specify whether analysis is whole brain or ROI-based.

Human (tDCS): Whole brain

Human (ASD and controls): ROI-to-ROI

Mouse imaging: Analysis was done on the whole brain as well as ROI based

b. If ROI-based, describe how anatomical locations were determined.

Human (ASD and controls): ROIs for pairwise functional connectivity estimation were based on adult whole brain functional connectivity data.

Mouse: The ROIs included the entire brain volume, and we defined in the following three papers (Dorr et al. 2008; Ullmann et al. 2013; and Steadman et al. 2014)

13. State the statistic type for inference. (See Eklund et al. 2016.)

Human (tDCS): Voxel level p<0.001, cluster level FDR p<0.05

Human (ASD and controls): p<0.05, one ROI-to-ROI comparison of connectivity estimates

14. Describe the type of correction and how it is obtained for multiple comparisons.

Human (tDCS) Cluster level FDR p<0.05 estimated as per CONN toolbox

Human (ASD and controls) N/A, one comparison was made, no multiple comparisons correction needed

Mouse imaging: Multiple comparisons were controlled for using the False Discovery Rate

15. Connectivity

a. For functional and/or effective connectivity, report the measures of dependence used and the model details.

Human (tDCS): Bivariate correlations implemented in CONN for seed-to-voxel analyses. Covariates included the realignment parameters and outlier scrubbing as implemented in CONN and the ART toolbox in CONN, respectively.

Human (ASD and controls): Pearson correlation converted to z-scores using Fisher's transform. To maximize statistical power, we balanced the distribution of demographic and rsfMRI data quality covariates in the ASD and TD groups. Demographic covariates included age, gender, handedness, intellectual ability as measured by the general ability index derived from either WISC-IV or WISC-5, and socioeconomic status (Hollingshead, 1975). Mean framewise displacement, receiving coil type, and scan length were included as rsfMRI quality measures. Because of the large number of covariates, we used the MatchIt package in R (Ho et al., 2011) to calculate a propensity score for each subject and to then find the 81 ASD-TD pairs with the smallest distance between them to minimize selection bias (Stuart, 2010). All ASD subjects were used and the 81 TD subjects were drawn from a larger sample of 195 TD subjects with usable rsfMRI data.

Mouse imaging: Pearson correlation

b. For graph analysis, report the dependent variable and functional connectivity measure.

N/A

16. For multivariate modeling and predictive analysis, specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.

N/A