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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	MNI_autoreg tools.
Data analysis	RMINC package (https://github.com/Mouse-Imaging-Centre/RMINC). the CONN toolbox (version 18b; www.nitrc.org/projects/conn, RRID:SCR_009550).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

N/A

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Repeated measures ANOVA design (mixed model ANOVA with covariance structure of compound symmetry) with 2 between factors (group, treatment) and 1 within factor (social vs. object) estimates 10/group for social testing. PASS V12 was used to estimate sample size. Mixed models ANOVA design and sample size estimates were performed.
Data exclusions	N/A
Replication	For each behavioral experiment, multiple cohorts were used to increase replication. All tests were performed with examiner blinded to genotype/condition.
Randomization	In almost all cases (exception = rotarod and water maze testing due to learning component of these tests), animals were tested for both testing conditions (for chemogenetic experiments: CNO AND VEH; for optogenetics: light OFF AND light ON). animals were randomly chosen for injections with control construct vs. chemo/optogenetic construct.
Blinding	As noted above, examiners were blinded to testing condition/genotype.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	L7/Pcp2-Cre (L7Cre) transgenic mice1 were obtained from Jackson Laboratories. PCcre; Tsc1flox/flox (PC-Tsc1 mutant) animals were generated by crossing L7/Pcp2-Cre (PCCre) transgenic mice with floxed Tsc1 mice (Tsc1flox/flox)2 to yield PCCre;Tsc1 flox/+ progeny. These progeny were then crossed with one another to ultimately yield Tsc1flox/flox and PCCre;Tsc1flox/flox mice. These were then used as breeders with Tsc1flox/flox mice crossed to PCCre;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. Ai14 Td tomato reporter mice were utilized with AAV1- cre injections for tracing studies.
Wild animals	N/A
Field-collected samples	N/A
Ethics oversight	The University of Texas Southwestern and John Hopkins University Institutional Animal Care and Uses Committee approved all experimental protocols in this study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Structural MRI The human brain imaging data comes from the Province of Ontario Neurodevelopmental Disorders (POND) network as well as data collected in a series of experiments in the lab of MJT. All scans were acquired on the same 3T Siemens magnet, though there was an upgrade between the Tim Trio and the Prisma in between. A total of 1222 scans from 786 subjects were processed. T1 scans (Trio: TR/TE: 2300/2.96ms; FA: 9°; FOV: 192 x 240 x 256mm; 1.0mm isotropic voxels; scan time: 5 min; Prisma: TR/TE: 1870/3.14ms, FA: 9°, FOV: 192 x 240 x 256mm, 0.8mm isotropic voxels) were processed. Cerebellar volumes were segmented

using the MAGeT pipeline23,24. Cortical volumes were extracted from the CIVET v2.1 pipeline25-27. After quality control, 902 scans (425 ASD, 477 CTRL) from 648 subjects (285 ASD, 368 CTRL) were considered for analysis. Age range was 2-50 years, with a mean age of 13 years.

Functional MRI
Eight typically-developing adults (mean age 20.75 ± 1.91, all male) and eight adults with ASD (mean age 26.13 ± 9.79, all male) provided written, informed consent to participate in the study. The American University Institutional Review Board approved the study. Resting-state functional connectivity data were acquired at the Center for Functional and Molecular Imaging (CFMI), Georgetown University with a 3T Siemens Prisma scanner. Acquisition parameters were: TR = 2.5 s, TE = 30 ms, 47 interleaved slices, 3.2 mm slice thickness, 3.2 mm voxel size, 90 degree flip angle, 140 volumes (5min 50sec duration). Participants also completed a high-resolution T1 MP-RAGE structural scan (acquisition parameters: TR = 1.9 s, TE = 2.52 ms, 176 interleaved slices, 1.0 mm slice thickness, 0.98 mm voxel size, 9 degree flip angle). Data analyses were performed using the CONN toolbox (version 18b; www.nitrc.org/projects/conn, RRID:SCR_009550) implemented in Matlab (version R2018b). Group Multivariate Pattern Analysis (MVPA) was conducted using CONN with 3 factors and a dimensionality reduction of 64. Functional connectivity maps were thresholded at p<0.001 with FDR-corrected cluster p<0.05.

Recruitment

Structural:
Controls were recruited through advertising in public transit, in hospitals, and on social media. Inclusion criteria were age <18 years, and a clinical diagnosis of ADHD, ASD, or OCD. Controls had no developmental diagnosis, and no first-degree family history of such. Participants were recruited into the study based on their primary psychiatric diagnosis. Standardized research assessments confirmed the primary clinical diagnosis using established metrics (the Autism Diagnostic Observation Schedule—241, the Autism Diagnostic Interview-Revised (ADI-R)).

Functional MRI
Participants were recruited via advertisements on campus at American University and through research collaborators at local institutions, including the NIH. Inclusion criteria: male, aged 18-45, able to provide written, informed consent, have no contra-indications for MR scanning, and be able to complete the relevant study sessions. Typically developing participants must have no current or prior diagnosis of a neurological or neurodevelopmental disorder, no history of significant head injury, and not be taking any psychotropic medications. Participants with ASD must have a prior diagnosis of autism spectrum disorder.

Ethics oversight

The studies were approved by the Institutional Review Board of American University (human functional MRI) and for POND Network (Holland Bloorview Kids Rehabilitation Hospital, Hospital for Sick Children, McMaster Children's Hospital) Institutional Review Boards.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Magnetic resonance imaging

Experimental design

Design type

structural or functional connectivity (resting state)

Design specifications

no tasks performed during

Behavioral performance measures

no tasks performed during

Acquisition

Imaging type(s)

structural and functional

Field strength

mouse 7T, human 3T

Sequence & imaging parameters

Mouse
A 7T MRI scanner (Agilent, Palo Alto, CA) was used to acquire all images. Images were acquired between 2012 and 2017. In this time, the scan protocol was modified to increase throughput and resolution while maintaining consistent SNR and tissue contrast. All mice were scanned using a T2 weighted Fast Spin Echo (FSE) sequence, with 3 different sets of parameters. The majority of mice were scanned using Sequence 3.
sequence1: TR = 325ms, TE = 10ms/echo for 6 echoes (the centre of k-space was acquired on echo 4), 4 averages, FOV = 14 x 14 x 25 mm3, a matrix size of 432 x 432 x 780, and 32 µm isotropic voxels
sequence2: TR = 2000ms, TE = 42ms/echo for 6 echoes, FOV = 25 mm × 28 mm × 14 mm, a matrix size of 450 × 504 × 250, and 56 µm isotropic voxels
sequence3:TR = 350ms, TE = 12ms/echo for 6 echoes, FOV = 20 x 20 x 25 mm3, a matrix size of 504 x 504 x 630, and 40µm isotropic voxels
Human
Structural: T1 scans (Trio: TR/TE: 2300/2.96ms; FA: 9°; FOV: 192 x 240 x 256mm; 1.0mm isotropic voxels; scan time: 5 min; Prisma: TR/TE: 1870/3.14ms, FA: 9°, FOV: 192 x 240 x 256mm, 0.8mm isotropic voxels) were processed.
Functional: resting state TR = 2.5 s, TE = 30 ms, 47 interleaved slices, 3.2 mm slice thickness, 3.2 mm voxel size, 90 degree flip angle, 140 volumes; T1 MPRAGE scans TR = 1.9 s, TE = 2.52 ms, 176 interleaved slices, 1.0 mm slice thickness, 0.98 mm voxel size, 9 degree flip angle

Area of acquisition

Whole brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software

Mouse
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.
Human structural:
Cerebellar volumes were segmented using the MAGeT pipeline23,24. Cortical volumes were extracted from the CIVET v2.1 pipeline25-27.
Functional:
CONN toolbox 18b was used for preprocessing. Preprocessing involved: removal of first 5 volumes, realignment and unwarping, slice-time correction, ART scrubbing, segmentation and normalization to MNI space, smoothing (8mm FWHM).

Normalization

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

Human: Normalization to MNI space using individual T1 scans.

Normalization template

Human: MNI 152 T1

Mouse: No template was used.

Noise and artifact removal

Mouse: N/A

Human: ART scrubbing for outlier detection using CONN toolbox version 18b

Volume censoring

Mouse: N/A

Human: 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 0.9). Only n=7 participants had any volumes removed, and the max number of volumes removed for any one participant was 8 (out of 150 volumes).

Statistical modeling & inference

Model type and settings

Mouse: 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.

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

Functional:
Group Multivariate Pattern Analysis (MVPA) was conducted using CONN toolbox version 18b with 3 factors and a dimensionality reduction of 64 principal components. Analyses contrasted functional connectivity patterns in typically developing vs. ASD groups. Functional connectivity maps were thresholded at $p<0.001$ with FDR-corrected cluster $p<0.05$. Seed-to-voxel post-hoc analyses were conducted to examine the functional connectivity from the regions of interest emerging from the group comparisons. The seed-to-voxel group comparisons were thresholded at $p<0.001$ with $k>50$.

Effect(s) tested

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

Functional: Comparison in MVPA functional connectivity maps between groups; and group comparison in seed-to-voxel connectivity with regions of interest emerging from the MVPA analysis.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☒ Both

Anatomical location(s)

mouse: whole brain
human: whole brain and seed-to-voxel. Seeds for functional connectivity estimation were based on whole brain functional connectivity data emerging from the MVPA analysis.

Statistic type for inference
(See Eklund et al. 2016)

Human functional: Voxel level $p<0.001$, cluster level FDR $p<0.05$

Correction

Structural: Multiple comparisons were controlled for using the False Discovery Rate
Functional: Cluster level FDR $p<0.05$ estimated as per CONN toolbox

Models & analysis

n/a	Involved in the study
☐	☒ Functional and/or effective connectivity
☒	☐ Graph analysis
☐	☒ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Structural:
Pearson correlation

Functional: The MVPA analyses estimates the multivariate connectivity pattern at each voxel by calculating the pairwise connectivity between that voxel and the rest of the brain. Principal components analysis is used to characterize the connectivity patterns, first on a subject level (with 64 principal components) and then across participants, where the 3 strongest components were retained. Bivariate correlations were 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.

Multivariate modeling and predictive analysis

The MVPA analyses estimates the multivariate connectivity pattern at each voxel by calculating the pairwise connectivity between that voxel and the rest of the brain. Principal components analysis is used to characterize the connectivity patterns, first on a subject level (with 64 principal components) and then across participants, where the 3 strongest components were retained. Bivariate correlations were 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.