nature research

Corresponding author(s): Craig M. Powell

☐ Initial submission ☐ Revised version ☒ Final submission

# Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but 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 size was chosen based on prior experience of the investigators with similar experiments previously published. The authors have published numerous peer reviewed papers demonstrating clear positive findings with similar sample sizes for the types of experiments included. Furthermore, for many experiments, the sample sizes are far above the standard in the field (example, behavioral experiments with N=20 or more are quite rare).

2. Data exclusions

Describe any data exclusions.

No data points were excluded from analysis in any experiment depicted in this manuscript.

3. Replication

Describe whether the experimental findings were reliably reproduced.

While we did not attempt to independently replicate every experiment in this manuscript, several biological replicates are intrinsic to the manuscript. For example, the decrease in synaptic transmission in Kctd13 mutant mice was replicated under initial control conditions, then again once each in the vehicle controls for each of the two drugs tested, verifying that the initial finding was very reproducible (in both extracellular field recordings and in whole-cell patch clamp recordings). Loss of Kctd13 protein was reliably replicated. Beta-galactosidase staining was biologically replicated.

4. Randomization

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

Randomization was performed by blinding the investigator to genotype and allowing them to choose each subject blindly.

5. Blinding

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

No animals or slices were excluded from the analysis. Randomization was performed by blinding the investigator to genotype and allowing them to choose each subject blindly. For studies using pharmacological agents, the investigator was aware of the agent being used, but was not aware of the genotypes of the animals used. Investigators were not aware of the specific group to which an animal was assigned when doing the experiment or analysis until after completion.

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 in 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 clear description of 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. | Statistica, Excel, Graphpad Prism were used to analyze data in this study. |

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

## 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. | All unique materials will be made available by the corresponding author on request. Should such requests become unduly burdensome, the corresponding author may deposit such materials in a widely accessible repository such as Jackson Laboratories or other Murine Mouse resources. |

9. Antibodies

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

Western blot, antibodies, RhoA activity
Fresh hippocampal tissue was isolated via rapid decapitation/dissection of males and females and lysed in ice-cold RIPA buffer (#89901, ThermoScientific, Waltham, MA) supplemented with Halt Protease & Phosphatase Inhibitor cocktail (#78446, ThermoScientific, Waltham, MA). Individual samples were sonicated, centrifuged at 4°C for 1 min at 1000 RPM, diluted in 4x Laemmli sample buffer containing β-mercaptoethanol (Bio-Rad, Hercules, CA), and incubated at 100°C for 5 min. Protein concentration was determined by BCA Protein Assay (#23225, ThermoScientific, Waltham, MA). Samples were diluted with sample buffer such that 5-25 µg of protein were loaded per lane onto Criterion TGX gels either 10% or 12% (Bio-Rad). Gels were run and then transferred to nitrocellulose membranes, blocked in Odyssey Blocking Buffer ( #927-40000, LI-COR, Lincoln, NE), and incubated with primary antibody overnight at 4°C. Membranes were washed in 5% TBS-T followed by a one-h secondary antibody incubation at room temperature using infrared fluorescence IRDye® anti-Rabbit (#926-32212, LI-COR, Lincoln, NE) or anti-mouse (#926-68071, LI-COR, Lincoln, NE) antibodies at 1:10,000. Proteins were visualized with Odyssey fc® imager (LI-COR, Lincoln, NE). Signals were quantified using Image Studio Lite (LI-COR, Lincoln, NE), normalized to β-actin or gapdh and analyzed using Microsoft Excel and Graphpad Prism (La Jolla, CA). Antibodies used: KCTD13 (HPA043524, Atlas Antibodies, Stockholm, Sweden 1:400), RhoA (2117, Cell Signaling, Danvers, MA, 1:1000), actin (#MABT825, Sigma-Aldrich, St. Louis, MO, 1:50000). Both KCTD13 and RhoA antibody bands were verified using corresponding knockout brain tissue (RhoA KO brain tissue was a gift from Dr. Kim Tolias).

Non-fluorescent immunohistochemistry: Mixed male and female mice were transcardially-perfused with 0.1 M PBS followed by 4% PFA (paraformaldehyde, NC9245948, Fisher, Hampton, NH). Brains were removed, fixed with PFA for 24 h and dehydrated with 30% sucrose. Tissue was coronally sectioned in a series of ten (1:10) on a sliding microtome at 30 µm. Slide mounted sections were incubated with 3% of $H_2O_2$, 10% methanol in 0.1 M PBS and then blocked in 5% serum, 0.2% Triton X in 0.1 M PBS. Primary antibodies were diluted in the antibody solution (5% serum in 0.1 M PBS) and were applied overnight at room temperature. Slides were next incubated for 1 h with secondary antibodies (1:3000, Vector Labs, Burlingame, CA) followed by an avidin-biotin-HRP complex formed with VECTASTAIN® ABC kit (PK-4000, Vector labs, Burlingame, CA). A 10-min exposure to Metal-Enhanced DAB (#34065, ThermoScientific, Waltham, MA) was performed to visualize the stain. Dehydration was performed with a series of 5-min ethanol dilutions (75%, 95%, 100%) and CitriSolv (04-355-121, Fisher, Hampton, NH) incubation prior to coverslipping with DPX mounting medium. For experiments involving BrdU staining, tissue was treated with 2 M HCL for 30 min and washed with 0.1 M Borax solution before blocking the endogenous peroxidase. Antibodies used: BrdU (1:400, #6326, Abcam, Cambridge, United Kingdom), Doublecortin (1:3000, #8066, Santa Cruz, Dallas, TX), Ki67 (1:400, MA5-14520, ThermoScientific, Waltham, MA).

Fluorescent immunohistochemistry: 20-µm cryosectioned E15.5 brain sections or 30-µm slide-mounted adult brain sections were incubated in blocking solution (0.2 % Triton X, 10 % Serum, 1 % BSA in 0.1 M PBS) for 1 h. Primary antibodies were diluted in 5 % serum and 0.1 M PBS and were incubated overnight. Secondary antibodies (1:1000, Invitrogen, Carlsbad, CA) were applied for 1 h at room temperature. After extensive washing, sections were counterstained with DAPI (5 mg/mL, D3571, ThermoFisher, Waltham, MA) for 1 min. Prolong gold mounting media (ThermoFisher, #P36930) was used to secure coverslips. Antibodies used: GFP (1:1000, ab13970), Tbr1 (1:100, ab31940), Tbr2 (1:100, ab23345), Ctip2 (1:100, ab18465), Satb2 (1:10, ab92446, Abcam, Cambridge, United Kingdom), Cux1 (1:50, 13024, Santa Cruz, Dallas, TX), Tuj1 (1:1000, mms-435p, BioLegend San Diego, CA).

10. Eukaryotic cell lines

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

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

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

No eukaryotic cell lines were used.

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

No eukaryotic cell lines were used.

## 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.

Kctd13 mutants were created using targeted embryonic stem cells (ES line: VGB6, Strain: C57BL/6NTac) were obtained from the University of California, Davis Knockout Mouse Project (KOMP) Repository. These ES cells carry the targeting construct designed to replace the entire Mus musculus Kctd13 gene (from second reading frame to seventeenth base pair after stop codon of the gene, total 16,080 bp). This construct was created as a ZEN-UB1 cassette (assembled by combining a LacZ-p(A) gene and a hUBCpro-neo-p(A) cassette flanked by 2 loxp sequences). The neo cassette provided a positive selection marker for the targeted ES clones. The LacZ-p(A) gene provided a reporter for Kctd13 promoter activity. ES clones were injected into blastocysts (background: Albino c57BL/6) to generate chimeras at the Transgenic Facility of University of Texas Southwestern Medical Center at Dallas. Resulting chimeric mice were bred with C57BL/6J mice to confirm germline transmission identified by polymerase chain reaction (PCR) with three primers as follows: forward (1): SD-sense (CTCGGATCTTTGAGGAGACAC), reverse: SD (TGTGGCTGATAGCACTGTCC), forward (2): NeoFwd (TCATTCTCAGTATTGTTTTGCC). WT DNA produced a 546 bp band. KI (knock-in targeting construct to replace Kctd13 gene) DNA produced a 408 bp band. To confirm correct targeting, genomic DNA from WT and KI mice were analyzed by Southern blotting with a probe that distinguished between the WT and KI Kctd13 alleles. The WT DNA resulted in a 5,790 bp band while KI DNA resulted in a 9,454 bp band (not shown). KI mice were further crossed with germline-cre expressing, B6.C-Tg(CMV-Cre), Cgn/J mice (The Jackson Laboratory, Bar Harbor, ME, background: C57BL/6J) to remove the hUBCpro-neo-p(A) cassette. Successful removal of this selection cassette was identified by PCR using three primers as follows: forward: SU-sense-1 (GCGGTATTCTCCATCCACATGAACAAGG), reverse (1): SU-anti-1 (TGGGACTAGGGAGCCTGGAATGAACTG), reverse (2): LacZ-Rev (GCTGGCTTGGTCTGTCTGTCCTAGC). WT DNA produced a 659 bp band while KO (knockout Kctd13 and neo) DNA produced a 487 bp band (not shown). We also performed rt-PCR (reverse transcriptase-PCR) to confirm loss of Kctd13 mRNA in the KO mice (forward: GTGAAGCTTCTACACAACCGCAG, reverse: AAGATGTTCAGTGTCTCCTCAAAG, band=267 bp). The resulting constitutive Kctd13 deletion mice were bred as het X het for all experiments. All animal care and use were approved by the UT Southwestern Medical Center Institutional Animal Care and Use Committee and are compliant with US government principles regarding the care and use of animals, Public Health Service Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. Animal husbandry was performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

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.

The study did not involve human research participants.

nature research

Corresponding author(s): Craig M. Powell

☐ Initial submission ☐ Revised version ☒ Final submission

# MRI Studies Reporting Summary

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

## Experimental design

| | |
|---|---|
| 1. Describe the experimental design. | Mice were scanned Ex-Vivo<br><br>A multi-channel 7.0 Tesla MRI scanner (Agilent Inc., Palo Alto, CA) was used to image the brains within the skulls. 16 custom-built solenoid coils were used to image the brains in parallel71,72.<br><br>Two different age groups different sequences were required. In order to get the contrast required for image registration at p7, a 3-D diffusion weighted fast spin-echo sequence with an echo train length of 6 was used, with a TR of 270 ms, first TE of 30 ms, and a TE of 10 ms for the remaining 5 echoes, two averages, field-of-view 14 x 14 x 25 mm3 and a matrix size of 250 x 250 x 450 yielding an image with 0.056 mm isotropic voxels. 6 high b-value images (b=2147 s/mm2) in 6 orthogonal directions were acquired. This was done to acquire an improved gray/white matter contrast at the young age. Total imaging time was 14 h. The high b-value images were averaged to create a single diffusion weighted image that had the contrast required for image registration. For the adult brains, a T2-weighted 3D fast spin-echo sequence was also 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, two averages, field-of-view of 20 x 20 x 25 mm3 and matrix size = 504 x 504 x 630 giving an image with 0.040 mm isotropic voxels73. The current scan time required for this sequence is also ~14 hours.<br><br>To visualize and compare any differences in the mouse brains the images from each age group are linearly (6 parameter followed by a 12 parameter) and non-linearly registered together separated by age group. We then used Deformation based morphometry to determine the volume difference between groups both regionally and voxelwise. |
| 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. | Two scans were performed on Ex-Vivo Samples of two different age groups.<br><br>A 3D - Diffusion Weighted FSE sequence was used to assess differences in the younger age group and a regular 3D FSE sequence was used for the adult group.<br><br>As this was not an fMRI study no block or trials per session and/or subject is required for this testing. |
| 3. Describe how behavioral performance was measured. | N/A for MRI experiments in this paper |

## Acquisition

4. Imaging

| | |
|---|---|
| a. Specify the type(s) of imaging. | Structural MRI |
| b. Specify the field strength (in Tesla). | Methods, page 30 A multi-channel 7.0 Tesla MRI scanner (Agilent Inc., Palo Alto, CA) |
| c. Provide the essential sequence imaging parameters. | Methods, page 30 A multi-channel 7.0 Tesla MRI scanner (Agilent Inc., Palo Alto, CA) was used to image the brains within the skulls. 16 custom-built solenoid coils were used to image the brains in parallel71,72.<br><br>For the two different age groups different sequences were required. In order to get the contrast required for image registration at p7, a 3-D diffusion weighted fast spin-echo sequence with an echo train length of 6 was used, with a TR of 270 ms, first TE of 30 ms, and a TE of 10 ms for the remaining 5 echoes, two averages, field-of-view 14 x 14 x 25 mm3 and a matrix size of 250 x 250 x 450 yielding an image with 0.056 mm isotropic voxels. 6 high b-value images (b=2147 s/mm2) in 6 orthogonal directions were acquired. This was done to acquire an improved gray/white matter contrast at the young age. Total imaging time was 14 h. The high b-value images were averaged to create a single diffusion weighted image that had the contrast required for image registration. For the adult brains, a T2-weighted 3D fast spin-echo sequence was also 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, two averages, field-of-view of 20 x 20 x 25 mm3 and matrix size = 504 x 504 x 630 giving an image with 0.040 mm isotropic voxels73. The current scan time required for this sequence is also ~14 hours. |
| d. For diffusion MRI, provide full details of imaging parameters. | For the younger age group DTI was performed to increase the contrast between the gray and white matter at the young age when myelination is at its infancy. The parameters are as follows:<br><br>a 3-D diffusion weighted fast spin-echo sequence with an echo train length of 6 was used, with a TR of 270 ms, first TE of 30 ms, and a TE of 10 ms for the remaining 5 echoes, two averages, field-of-view 14 x 14 x 25 mm3 and a matrix size of 250 x 250 x 450 yielding an image with 0.056 mm isotropic voxels. 6 high b-value images (b=2147 s/mm2) in 6 orthogonal directions were acquired. The images used for registration and analysis were an average of the high b-value images (in the 6 orthogonal directions). |
| 5. State area of acquisition. | The whole brain was imaged at both the younger and older age groups. We used pre-existing atlases that included full brain coverage. For the older age group this included 159 different regions encompassing the entire brain volume. For the younger age group the atlas included 59 different regions again covering the entire brain volume. There are further delineations in the older age atlas for the cortex and cerebellum which accounts for the different number of regions between two groups. |

## Preprocessing

| | |
|---|---|
| 6. Describe the software used for preprocessing. | All of our software is home-built using a combination of R, Perl, Python and C. The only preprocessing that is used prior to the registration is a distortion correction, which is used to eliminate the distortions created by an inhomgeneous magnetic field. |

7. Normalization

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

N/A

b. Describe the template used for normalization/ transformation.

N/A

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

N/A

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

N/A

## Statistical modeling & inference

10. Define your model type and settings.

For regional measurements p-values are calculated using a two tailed t-test and voxelwise measurements we are using a linear model to determin differences in volume. These are both controlled for multiple comparisons using the false discovery rate.

See Methods Page 30

11. Specify the precise effect tested.

The Jacobian determinants are used after deformation based morphometry to determined significant differences in the voxelwise measurements. Standard two-tailed t-tests are used on regional volumes either measured as absolute volume in mm^3 or relative volume as a % total brain volume.

Methods, page 30 & Extended Data page 49

12. Analysis

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

The whole brain is examined voxelwise as well as a regional ROI assessment encompassing the whole brain volume.

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

Pre existing atlases were used to calculate the volume of 159 different regions throughout the whole brain volume.

Please see references:

Dorr et al. NeuroImage 2008
Ullman et al. NeuroImage 2013
Steadman et al. Autism Research 2012

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

T-Values

See Methods, page 30 & Extended Data page 49

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

We used the False Discovery Rate to control for Multiple comparisons. Please see reference Genovese et al. 2002.

See Methods, page 30 & Extended Data page 49

15. Connectivity

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

N/A

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.

The neuroanatomy was assessed and volume was measured as either absolute volume in mm3 or relative volume (% total brain volume). Methods, page 30 & Extended Data page 49. Supplemental Data Table S2 & Supplemental Data Table S3.