Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported.
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the page number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

<table>
<thead>
<tr>
<th>FIGURE NUMBER</th>
<th>WHICH TEST?</th>
<th>PAGE</th>
<th>n DESCRIPTION</th>
<th>PAGE</th>
<th>WHICH PAGE</th>
<th>ERROR BARS</th>
<th>PAGE</th>
<th>WHICH PAGE</th>
<th>STATISTIC VALUE</th>
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<tbody>
<tr>
<td>1a</td>
<td>one-way ANOVA</td>
<td>4</td>
<td>mice from at least 3 litters/group</td>
<td>4</td>
<td>4</td>
<td>mean +/- SEM</td>
<td>4</td>
<td>F(3, 36) = 2.97</td>
<td>4</td>
<td></td>
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<tr>
<td>2</td>
<td>unpaired t-test</td>
<td>6</td>
<td>slices from 10 mice</td>
<td>6</td>
<td>6</td>
<td>mean +/- SEM</td>
<td>6</td>
<td>t(28) = 2.808</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>linear regression</td>
<td>4</td>
<td>Discovery cohort (young adults of European ancestry)</td>
<td>3</td>
<td>N/A</td>
<td>raw datapoints plotted</td>
<td>N/A</td>
<td>p = 0.011</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1c</td>
<td>linear regression</td>
<td>6</td>
<td>Replication cohort (adolescents of European ancestry)</td>
<td>3</td>
<td>N/A</td>
<td>raw datapoints plotted</td>
<td>N/A</td>
<td>p = 0.001</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>linear regression</td>
<td>7</td>
<td>Postmortem cohort</td>
<td>7</td>
<td>N/A</td>
<td>raw datapoints plotted</td>
<td>N/A</td>
<td>p = 0.039</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

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## Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?  
   If so, what figure(s)?

   Statistical parametric map of voxel t-values from our fMRI results is overlaid onto a canonical structural brain image provided by MRIcron visualization software (http://www.mccauslandcenter.sc.edu/mricross/mricron/).

   Figures 1a and 1c.

2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?  
   If so, on what page(s) is this reported?

## Statistics and general methods

1. Is there a justification of the sample size?  
   If so, how was it justified?  
   On what page(s)?

   Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

   Genetic main effects on neural phenotypes have been reported in samples as small as 28 individuals (e.g., Hariri et al., Science 2002; Egan et al., Cell 2003). One prior study reported epigenetic effects on a neural phenotype in 84 individuals. Thus, we are confident that our current sample of 80 individuals in conjunction with our stringent statistical thresholds and controls sufficiently guard against Type I error. Moreover, we now report replication in an independent cohort of 96 individuals.
2. Are statistical tests justified as appropriate for every figure?

   On what page(s)?

   Statistical tests (e.g., linear regression) used are standard for the type of analysis conducted in this study. Thus, no explicit justification is provided. Statistical tests are described in detail on p. 12 of Supplementary Methods.

   a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

   Statistical methods are summarized in the "Statistical Analysis" section on p. 12 of Supplementary Methods.

   b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?

   Where is this described?

   The data (percent methylation and amygdala reactivity) did not violate the normality assumption. The normality of the data distributions is largely visible in the scatterplots provided. Thus, results from explicit tests of normality are not reported for those variables.

   SLC6A4 mRNA levels did deviate from normality. Thus, a square root transformation was applied to normalize their distribution. These procedures are described on p. 4 of Supplementary Methods.

   c. Is there any estimate of variance within each group of data?

   Is the variance similar between groups that are being statistically compared?

   Where is this described?

   N/A (No experimental groups compared)

   d. Are tests specified as one- or two-sided?

   Results from two-sided tests are reported throughout the manuscript (now stated on p. 13 of Supplementary Methods).

   e. Are there adjustments for multiple comparisons?

   Stringent familywise-error rate correction combined with an extent threshold of 10 voxels is applied to the neuroimaging data analysis (Supplementary Methods, p. 7). The remaining analyses are not adjusted for multiple comparisons.

3. Are criteria for excluding data points reported?

   Was this criterion established prior to data collection?

   On what page(s) is this described?

   Participants were excluded for excessive motion, task noncompliance or equipment failure during fMRI data acquisition, as well as for exhibiting wrong sequence patterns in the methylation data analysis (Supplementary Methods, p. 1-5).

4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.

   If no randomization was used, state so.

   On what page(s) does this appear?

   No randomization used, as there are no distinct experimental groups.

5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?

   If no blinding was done, state so.

   On what page(s)?

   No group assignment was used.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?  
On what page(s)?  
N/A

7. Is the species of the animals used reported?  
On what page(s)?  
N/A

8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?  
On what page(s)?  
N/A

9. Is the sex of the animals/subjects used reported?  
On what page(s)?  
N/A

10. Is the age of the animals/subjects reported?  
On what page(s)?  
N/A

11. For animals housed in a vivarium, is the light/dark cycle reported?  
On what page(s)?  
N/A

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?  
On what page(s)?  
N/A

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?  
On what page(s)?  
N/A

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?  
On what page(s)?  
N/A

  a. If multiple behavioral tests were conducted in the same group of animals, is this reported?  
On what page(s)?  
N/A

15. If any animals/subjects were excluded from analysis, is this reported?  
On what page(s)?  
See item #3 above.

  a. How were the criteria for exclusion defined?  
  Where is this described?
b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

Where is this described?

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?
   a. Is antibody catalog number given?
      On what page(s) does this appear?
   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
      On what page(s) does this appear?

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?
   On what page(s)?
   a. Were they recently authenticated?
      On what page(s) is this information reported?

Data deposition

Data deposition in a public repository is mandatory for:
   a. Protein, DNA and RNA sequences
   b. Macromolecular structures
   c. Crystallographic data for small molecules
   d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available here. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad.

1. Are accession codes for deposit dates provided?
   On what page(s)?

Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

No custom software or scripts were used.
2. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.  

N/A

### Human subjects

1. Which IRB approved the protocol?  
   Where is this stated?

   The relevant ethics committees at Duke University, University of Texas Health Center at San Antonio and the University of Pittsburgh approved all procedures involving the Discovery, Replication, and postmortem cohorts, respectively. This is now stated, along with study-specific consent information, on pages 1, 3 and 4 of Supplementary Methods.

2. Is demographic information on all subjects provided?  
   On what page(s)?

   Demographic information, including age and sex, is provided in Supplementary Methods for all subjects as follows:
   - Discovery cohort - page 1
   - Replication cohort - page 3
   - Postmortem cohort - page 4

3. Is the number of human subjects, their age and sex clearly defined?  
   On what page(s)?

   See item #2 in this section.

4. Are the inclusion and exclusion criteria (if any) clearly specified?  
   On what page(s)?

   Inclusion/exclusion criteria are specified on p. 1-5 of Supplementary Methods.

5. How well were the groups matched?  
   Where is this information described?

   No experimental groups were created.

6. Is a statement confirming that informed consent was obtained from all subjects included?  
   On what page(s)?

   See item #1 in this section.

7. For publication of patient photos, is a statement confirming that consent to publish was obtained included?  
   On what page(s)?

   N/A

### fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?  

   Yes. Participants were excluded for excessive motion, incidental neurological findings, poor fMRI data quality or task accuracy < 75%. In addition, we only focused on participants of Caucasian ancestry for the purposes of the current analysis.
1. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?

SPM was used for all analyses, as stated on p. 4 of Supplementary Methods. Additional parameters are stated on p. 6-7 of Supplementary Methods.
11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? On what page(s)?

MNI space was used (p. 6 of Supplementary Methods).

12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? On what page(s)?

A 12-parameter affine model was used (p. 6 of Supplementary Methods).

13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?

MNI space was used. Anatomical ROIs were based on the automatic anatomical labeling (AAL) system in the WFU Pickatlas.

14. Were any additional regressors (behavioral covariates, motion etc) used?

Motion regressors were included (p. 6 of Supplementary Methods).

15. Is the contrast construction clearly defined?

Yes (p. 7 of Supplementary Methods).

16. Is a mixed/random effects or fixed inference used?

A random effects model was used (p. 7 of Supplementary Methods).

a. If fixed effects inference used, is this justified?

N/A

17. Were repeated measures used (multiple measurements per subject)?

No repeated measures were used.

a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?

N/A

18. If the threshold used for inference and visualization in figures varies, is this clearly stated?

The same threshold was used for inference and visualization.

19. Are statistical inferences corrected for multiple comparisons?

Yes, a stringent familywise error rate correction coupled with an extent threshold was used to preclude Type I error in both in vivo cohorts.

a. If not, is this labeled as uncorrected?

N/A

20. Are the results based on an ROI (region of interest) analysis?

Yes.

a. If so, is the rationale clearly described?

Yes.

b. How were the ROI’s defined (functional vs anatomical localization)?

The ROI’s were anatomically defined, based on the WFU PickAtlas, as described on p. 7 of Supplementary Methods.

21. Is there correction for multiple comparisons within each voxel?

A stringent familywise error rate correction coupled with an extent threshold of 10 contiguous voxels was used to preclude Type I error. This correction was applied across voxels. No within-voxel correction was applied.
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined? 

All significance statistics are reported on the voxel-level. However, we also apply an extent threshold of 10 contiguous voxels to define significant activation.

Additional comments

Additional Comments