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 (section, e.g. Results, & paragraph number).
- 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 paragraph 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>SECTION &amp; PARAGRAPH #</th>
<th>Exact Value</th>
<th>Defined?</th>
<th>SECTION &amp; PARAGRAPH #</th>
<th>Reported?</th>
<th>Exact Value</th>
<th>SECTION &amp; PARAGRAPH #</th>
<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>one-way ANOVA</td>
<td>Methods para 8</td>
<td>9, 9, 10, 15 mice from at least 3 litters/group</td>
<td>error bars are mean +/- SEM</td>
<td>Fig. legend</td>
<td>p = 0.044</td>
<td>Fig. legend</td>
<td>F(3, 36) = 2.97</td>
<td>Fig. legend</td>
<td></td>
</tr>
<tr>
<td>results para 6</td>
<td>unpaired t-test</td>
<td>Results para 6</td>
<td>15 slices from 10 mice</td>
<td>error bars are mean +/- SEM</td>
<td>Results para 6</td>
<td>p = 0.0006</td>
<td>Results para 6</td>
<td>t(28) = 2.808</td>
<td>Results para 6</td>
<td></td>
</tr>
<tr>
<td>FIGURE NUMBER</td>
<td>WHICH TEST?</td>
<td>SECTION &amp; PARAGRAPH #</td>
<td>n</td>
<td>DESCRIPTIVE STATS (AVERAGE, VARIANCE)</td>
<td>P VALUE</td>
<td>DEGREES OF FREEDOM &amp; F/t/z/R/ETC VALUE</td>
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<tr>
<td>1a</td>
<td>Linear regression model</td>
<td>Fig. 1 legend</td>
<td>104</td>
<td>Full data plotted in fig. 1a</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>1b</td>
<td>Linear regression model</td>
<td>Fig. 1 legend</td>
<td>104</td>
<td>Mean corrected beta</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>1c</td>
<td>Linear regression model</td>
<td>Fig. 1 legend</td>
<td>113 (STG) and 110 (PFC)</td>
<td>Mean corrected beta</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>1d</td>
<td>Linear regression model</td>
<td>Fig. 1 legend</td>
<td>108</td>
<td>N/A</td>
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<td>N/A</td>
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<tr>
<td>1e</td>
<td>Cross-cortex meta analysis using Fisher's method</td>
<td>Fig. 1 legend</td>
<td>104 (EC) and 113 (STG) and 110 (PFC)</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>2a</td>
<td>Linear regression model</td>
<td>Fig. 2 legend</td>
<td>327 (cross-cortex London) and 142 (PFC Mount Sinai)</td>
<td>Percentage corrected methylation score for individual probes between mean of Braak 0 and mean of Braak VI</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>2b</td>
<td>Linear regression model</td>
<td>Fig. 2 legend</td>
<td>144 STG and 142 PFC</td>
<td>Mean corrected beta</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>*- 2c</td>
<td>Linear regression model</td>
<td>Fig. 2 legend</td>
<td>144 STG and 142 PFC</td>
<td>Mount Sinai Brain Bank samples (amyloid analysis)</td>
<td>Fig. 2c legend</td>
<td>full data plotted</td>
<td>Fig. 2c Y axis label</td>
<td>P=2.35E-4 (cg11823178 in PFC), P=9.93E-3 (cg05066959 in PFC), P=4.99E-4 (cg11823178 in STG) and P=5.65E-4 (cg05066959 in STG)</td>
<td>Fig. 2c legend</td>
<td>t(142) = 2.80 (cg11823178 in STG), t(142) = 3.47 (cg05066959 in STG), t(140) = 3.69 (cg11823178 in PFC) and t(140) = 2.55 (cg05066959 in PFC)</td>
</tr>
<tr>
<td>*- 2d</td>
<td>Linear regression model</td>
<td>Fig. 2 legend</td>
<td>51</td>
<td>Oxford Brain Bank EC samples</td>
<td>Fig. 2d legend</td>
<td>Mean corrected beta +/- SEM</td>
<td>Fig. 2d Source Data</td>
<td>P = 1.10E-03 (8:41519302), P = 9.91E-05 (8:41519304), P = 2.64E-03 (8:41519308), P = 1.80E-02 (8:41519348), P = 2.05E-02 (8:41519399), P= 0.196 (8:41519411), P = 8.09E-03 (8:41519417), P= 0.294 (8:41519420)</td>
<td>Fig. 2d Source Data</td>
<td>t(57) = 3.44 (8:41519302), t(57) = 4.19 (8:41519304), t(57) = 3.15 (8:41519308), t(57) = 2.44 (8:41519348), t(53) = 2.39 (8:41519399), t(53) = 1.31 (8:41519411), t(52) = 2.75 (8:41519417), t(51) = 1.06 (8:41519420)</td>
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<tr>
<td>*- 2e</td>
<td>Meta analysis</td>
<td>Fig. 2 legend</td>
<td>104 EC (London), 51 EC (Oxford), 113 STG (London), 144 STG (Mount Sinai), 56 STG (Oxford), 110 PFC (London), 142 PFC (Mount Sinai), 62 PFC (Oxford), 108 CER (London)</td>
<td>Meta-analysis across all three cohorts</td>
<td>Labeled in Fig. 2e</td>
<td>Percentage methylation difference (effect size) between Braak 0 and Braak VI</td>
<td>Fig. 2e X axis label</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>*- 2f</td>
<td>Meta analysis</td>
<td>Fig. 2 legend</td>
<td>104 EC (London), 51 EC (Oxford), 113 STG (London), 144 STG (Mount Sinai), 56 STG (Oxford), 110 PFC (London), 142 PFC (Mount Sinai), 62 PFC (Oxford), 108 CER (London)</td>
<td>Meta-analysis across all three cohorts</td>
<td>Labeled in Fig. 2f</td>
<td>Percentage methylation difference (effect size) between Braak 0 and Braak VI</td>
<td>Fig. 2f X axis label</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
### Representative figures

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

   - N/A

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, where is this reported (section, paragraph #)?

   - N/A

### Statistics and general methods

1. Is there a justification of the sample size?
   
   If so, how was it justified?

   Where (section, paragraph #)?

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

   - Sample size is justified in the Power section of the Online Methods. A conservative power calculation using methylome data from this and other ongoing studies in our lab suggests we are well-powered to identify DNA methylation differences of ~5% between groups for the majority of probes on the Illumina 450K array based conservatively on a case-control t-test with an array-wide Bonferroni threshold and the observed distribution of beta-value variances for the entorhinal cortex data set. Further details are provided in the Power section of the Online Methods.

2. Are statistical tests justified as appropriate for every figure?
   
   Where (section, paragraph #)?

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

   Statistical methods for each experiment are summarized in the Data analysis section of the Online Methods.

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

   This is not explicitly tested, but tests for normality are not particularly useful for small samples, and the tests are reasonably robust to minor deviation. The full data for key probes is scatter plotted.

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

   N/A (continuous predictor)

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

   Linear regression is a double-sided test
Multiple testing is discussed in the power calculation, but as is usual in studies of this kind, nominal p-values are presented and evaluated against genome-wide criteria.

Data points were filtered out probewise using a 4 * IQR criterion. These extreme outliers were by inspection clearly the result of single nucleotide polymorphisms. Details are provided in the Data analysis section of the Online Methods.

Samples were run on arrays by tissue and randomized with respect to age and gender, with details provided in the Methylomic profiling section of the Online Methods.

Samples were assigned a code number, as detailed in Supplementary Table 2, for the experiment, which was independent of age, gender or diagnosis. This code was used throughout the experiment, with details in the Methylomic profiling section of the Online Methods.

N/A

N/A

N/A

N/A

N/A
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
   Where (section, paragraph #)?
   N/A

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
   Where (section, paragraph #)?
   N/A

   a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
      Where (section, paragraph #)?
      N/A

15. If any animals/subjects were excluded from analysis, is this reported?
   Where (section, paragraph #)?
   N/A

   a. How were the criteria for exclusion defined?
      Where is this described (section, paragraph #)?
      N/A

   b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.
      Where is this described (section, paragraph #)?
      N/A

— Reagents —

1. Have antibodies been validated for use in the system under study (assay and species)?
   N/A

   a. Is antibody catalog number given?
      Where does this appear (section, paragraph #)?
      N/A

   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
      Where does this appear (section, paragraph #)?
      N/A

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?
   N/A

   a. Were they recently authenticated?
      Where is this information reported (section, paragraph #)?
      N/A
Data deposition

Data deposition in a public repository is mandatory for:

- Protein, DNA and RNA sequences
- Macromolecular structures
- Crystallographic data for small molecules
- 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?
   Where (section, paragraph #)?
   Yes, data is deposited in GEO under accession number GSE43414. This is provided in the Data analysis section of the Online Methods.

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.
   N/A

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 (section, paragraph #)?
   South East London REC 3 provided ethical approval for the study under reference 10/H0808/114, with details provided in the Subjects and samples section of the Online Methods.

2. Is demographic information on all subjects provided?
   Where (section, paragraph #)?
   Summary demographic data is provided in Supplementary Table 1. Detailed demographic data for the “discovery” cohort is provided in Supplementary Table 2.

3. Is the number of human subjects, their age and sex clearly defined?
   Where (section, paragraph #)?
   Summary age and gender information (mean +/- SD) and the number of human subjects in each cohort is provided in Supplementary Table 1. Age and gender for individual samples is provided for the “discovery” cohort in Supplementary Table 2.
4. Are the inclusion and exclusion criteria (if any) clearly specified?

Where (section, paragraph #)?

Samples were included in the quantitative analysis if a Braak score was provided by the source Brain Bank. For the case-control analysis used for blood, samples were included if they were clinically classified as either control or AD. Details of sample numbers used for quantitative and case-control analyses are provided in the Data analysis section of the Online Methods, and are summarized in Supplementary Table 1. On an individual probe level, samples were excluded from analysis with beta values lying more than four times the interquartile range from the mean as they were likely to be a result of rare polymorphisms, with details provided in the Data analysis section of the Online Methods.

5. How well were the groups matched?

Where is this information described (section, paragraph #)?

To account for gender and age differences between groups we regressed out the effects of age and sex were regressed out before subsequent analysis. Details are provided in the Data analysis section of the Online Methods.

6. Is a statement included confirming that informed consent was obtained from all subjects?

Where (section, paragraph #)?

Where blood samples were collected during life as part of the Alzheimer’s Research UK funded study “Biomarkers of AD Neurodegeneration”, informed consent was given according to the Declaration of Helsinki (1991) and is described in the Subjects and samples section of the Online Methods.

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?

Where (section, paragraph #)?

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?

N/A

   a. If yes, is the number rejected and reasons for rejection described?

   Where (section, paragraph #)?

   N/A

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

   N/A

   Where (section, paragraph #)?

3. Is the length of each trial and interval between trials specified?

   N/A

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.

   N/A

5. Is the task design clearly described?

   Where (section, paragraph #)?

   N/A
<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>How was behavioral performance measured?</td>
<td>N/A</td>
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<tr>
<td>7</td>
<td>Is an ANOVA or factorial design being used?</td>
<td>N/A</td>
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<tr>
<td>8</td>
<td>For data acquisition, is a whole brain scan used?</td>
<td>N/A</td>
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<tr>
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<td>If not, state area of acquisition.</td>
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<tr>
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<td>a. How was this region determined?</td>
<td>N/A</td>
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<tr>
<td>9</td>
<td>Is the field strength (in Tesla) of the MRI system stated?</td>
<td>N/A</td>
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<tr>
<td></td>
<td>a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?</td>
<td>N/A</td>
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<td>b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?</td>
<td>N/A</td>
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<tr>
<td>10</td>
<td>Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>11</td>
<td>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? Where (section, paragraph #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>12</td>
<td>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? Where (section, paragraph #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>13</td>
<td>How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?</td>
<td>N/A</td>
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<tr>
<td>14</td>
<td>Were any additional regressors (behavioral covariates, motion etc) used?</td>
<td>N/A</td>
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<tr>
<td>15</td>
<td>Is the contrast construction clearly defined?</td>
<td>N/A</td>
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<tr>
<td>16</td>
<td>Is a mixed/random effects or fixed inference used?</td>
<td>N/A</td>
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<tr>
<td></td>
<td>a. If fixed effects inference used, is this justified?</td>
<td>N/A</td>
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<tr>
<td>17</td>
<td>Were repeated measures used (multiple measurements per subject)?</td>
<td>N/A</td>
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<td>a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?</td>
<td>N/A</td>
</tr>
</tbody>
</table>
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?  
   N/A

19. Are statistical inferences corrected for multiple comparisons?  
   a. If not, is this labeled as uncorrected?  
   N/A

20. Are the results based on an ROI (region of interest) analysis?  
   a. If so, is the rationale clearly described?  
   N/A
   b. How were the ROI’s defined (functional vs anatomical localization)?  
   N/A

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

22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?  
   N/A

Additional comments

Additional Comments