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.

| FIGURE NUMBER | TEST USED | WHICH? | SECTION & PARAGRAPH # | n | DEFINED? | REPORTED? | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | P VALUE | DEGREES OF FREEDOM & F/T/Z/R/ETC VALUE |
|---------------|-----------|--------|-----------------------|---|----------|----------|--------------------------------------|---------|--------------------------------------|
| 1a | one-way ANOVA | Fig. legend | 9, 9, 10, 15 | mice from at least 3 litters/group | Methods para 8 | error bars are mean +/- SEM | Fig. legend | p = 0.044 | F(3, 36) = 2.97 |
| results para 6 | unpaired t-test | Results para 6 | 15 | slices from 10 mice | Results para 6 | error bars are mean +/- SEM | Results para 6 | p = 0.0006 | t(28) = 2.808 |
| FIGURE NUMBER | WHICH TEST? | SECTION & PARAGRAPH | n | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | P VALUE | DEGREES OF FREEDOM & F/T/Z/R/ETC VALUE |
|--------------|-------------|---------------------|---|------------------------------------|---------|-------------------------------------|
| 2b           | paired t-test | Fig. legend         | 1548, 1548, 1548 | up-regulated genes in WT control neuron | Fig. 2b | p=6.51E-27 (WT 0hr vs KO 0hr); p=5.24E-78 (WT 1hr vs KO 1hr) | t(3094)=10.9466 (WT 0hr vs KO 0hr); t(3094)=19.8081 (WT 1hr vs KO 1hr) |
| 2e           | Unpaired t-test | Fig. legend         | 3,3,3,3,3,3,3 | technical replicates from cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. 2e | p=0.004 (Arc); p=0.003 (Btg2); p=0.022 (Cyrl1); p=0.002 (Egr3); p=0.011 (Npas4); p=0.016 (Pcsk1) | t(4)=24.9433 (Arc); t(4)=31.5490 (Btg2); t(4)=9.4574 (Cyrl1); t(4)=54.8099 (Egr3); t(4)=16.0747 (Npas4); t(4)=13.5977 (Pcsk1) |
| 3a           | No statistical analysis applied (Histogram plots) | Fig. legend         | 1548, 1548 | up-regulated genes in WT control neuron | Results para 5 | p=1.284E-05 | D = 0.0879 (WT +KCl vs KO +KCl) |
| 3c           | two-tailed KS test | Fig. legend         | 1548, 1548 | up-regulated genes in WT control neuron | Results para 5 | p=5.38849E-0 | t=20.944 (WT KCl- vs WT KCl+); t=9.5021 (WT KCl+ vs KO KCl+) |
| 3d           | paired t-test | Fig. legend         | 1548, 1548 | up-regulated genes in WT control neuron | Result para 5 | p=0.00019816 2 (WT KCl- vs WT KCl+); p=0.00087533 9 (WT KCl+ vs KO KCl+) | t=19.9576 (WT KCl- vs WT KCl+); t=12.1616 (WT KCl+ vs KO KCl+) |
| 3e           | Unpaired t-test | Fig. legend         | 4,4,4,4 | technical replicates from cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. 3e | p=5.38849E-0 6 (WT KCl- vs WT KCl+); p=0.000449 014 (WT KCl+ vs KO KCl+) | t=15.4007 (WT KCl- vs WT KCl+); t=9.5004 (WT KCl+ vs KO KCl+) |
| 3f           | Unpaired t-test | Fig. legend         | 4,4,4,4 | technical replicates from cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. 3f | p=0.00021207 9 (WT KCl- vs WT KCl+); p=0.000194 012 (WT KCl+ vs KO KCl+) | t=15.2007 (WT KCl- vs WT KCl+); t=7.5804 (WT KCl+ vs KO KCl+) |
| 3g           | Unpaired t-test | Fig. legend         | 4,4,4,4 | technical replicates from cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. 3g | p=0.00022107 9 (WT KCl- vs WT KCl+); p=0.000194 012 (WT KCl+ vs KO KCl+) | t=15.4007 (WT KCl- vs WT KCl+); t=7.5804 (WT KCl+ vs KO KCl+) |
| Step | Number | Description | Fig. Legend | N | Treatment Groups | Error Bars | Results |
|------|--------|-------------|-------------|---|-----------------|-----------|---------|
| 3h   |        | No statistical analysis applied (Histogram plots) |              |    |                  |           |         |
| 4a   |        | one-way ANOVA |              | 10,10,10 | age-matched mice used / group | error bars are mean +/- S.E.M. | Fig. 4a |
| 4b   |        | one-way ANOVA |              | 10,10,10 | age-matched mice used / group | error bars are mean +/- S.E.M. | Fig. 4b |
| 4c   |        | one-way ANOVA |              | 10,10,10 | age-matched mice used / group | error bars are mean +/- S.E.M. | Fig. 4c |
| 4d   |        | Unpaired t-test |              | 4,4,4,4,4,4 | technical replicates of cortical regions of age- and sex-matched littermates | error bars are mean +/- S.D. | Fig. 4d |
| S5a  |        | Unpaired t-test |              | 473, 473, 473, 473 | up-regulated genes in WT neuron | error bars are mean +/- S.D. | Fig. 5a |
| S5b  |        | Unpaired t-test |              | 3,3,3,3 | technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | error bars are mean +/- S.D. | Fig. 5b |
| S5d  |        | Unpaired t-test |              | 454, 454, 454, 454 | up-regulated genes in WT neuron | error bars are mean +/- S.D. | Fig. 5d |
- **S5h**  
  Unpaired t-test  
  Fig. legend  
  4,4,4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos  
  Fig. legend error bars are mean +/- S.D.  
  Fig. S5h  
  p=0.3465 (Arc eRNA- KCl-); p=0.0011 (Arc eRNA+, KCl+); p=0.2598 (Arc eRNA+, KCl-); p=0.0190 (Arc eRNA+ KCl+); p=0.6038 (Fos eRNA-, KCl-); p=0.0128 (Fos eRNA+ KCl+); p=0.0120 (Npas4 eRNA+ KCl+); p=0.4110 (Nr4a1 eRNA-, KCl-); p=0.0084 (Nr4a1 eRNA+ KCl+)  
  Materials and Methods

- **S6e**  
  Unpaired t-test  
  Fig. legend  
  4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos  
  Fig. legend error bars are mean +/- S.D.  
  Fig. S6e  
  p=0.904 (Npas4); p=0.494 (Arc)  
  Materials and Methods

- **S6f**  
  Unpaired t-test  
  Fig. legend  
  4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos  
  Fig. legend error bars are mean +/- S.D.  
  Fig. S6f  
  p=0.363 (Npas4); p=0.014 (Arc)  
  Materials and Methods

- **S6g**  
  Unpaired t-test  
  Fig. legend  
  4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos  
  Fig. legend error bars are mean +/- S.D.  
  Fig. S6g  
  p=0.0074 (shC KCl- vs shPhf8 KCl-); p=0.0052 (shC KCl+ vs shPhf8 KCl+); p=0.0476 (Arc); p=0.3708 (Btg2); p=0.1894 (Cyr61); p=0.3875 (Egr3); p=0.2828 (Npas4); p=0.3663 (Psck1).  
  Materials and Methods

- **S6j**  
  Unpaired t-test  
  Fig. legend  
  3,3,3,3 3 biological replicates of cortical neurons/condition  
  Fig. legend error bars are mean +/- SEM  
  Fig. S6j  
  t(4)= 5.0265 (shC KCl- vs shPhf8 KCl-); t(4)= 5.5407 (shC KCl+ vs shPhf8 KCl+)  
  Materials and Methods

- **S6k**  
  Unpaired t-test  
  Fig. legend  
  3,3,3,3 3 biological replicates of cortical neurons/condition  
  Fig. legend error bars are mean +/- SEM  
  Fig. S6k  
  t(4)= 0.8241 (Arc); t(4)= 1.0072 (Btg2); t(4)= 1.5791 (Cyr61); t(4)= 0.9688 (Egr3); t(4)= 1.2397 (Npas4); t(4)= 1.0180 (Psck1).  
  Materials and Methods

- **S7c**  
  two-tailed KS test  
  Fig. legend  
  1548, 1548 up-regulated genes in WT control neuron  
  Fig. legend  
  p=0.00192  
  Materials and Methods

- **S7d**  
  Unpaired t-test  
  Fig. legend  
  4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos  
  Fig. legend error bars are mean +/- S.D.  
  Fig. S7d  
  p=0.000461591 (WT KCl- vs WT KCl+); p=0.13018453 (WT KCl+ vs KO KCl+); t(6)=4.9448 (WT KCl- vs WT KCl+); t(6)=2.3124 (WT KCl+ vs KO KCl+)  
  Materials and Methods
| Page | Section | Details |
|------|---------|---------|
| 7e   | Unpaired t-test | Fig. 4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=0.00016313 (WT KCl- vs WT KCl+); p=0.00023733 (WT KCl+ vs KO KCl+) | Fig. legend | t(6)=7.6956 (WT KCl- vs WT KCl+); t(6)=5.2179 (WT KCl+ vs KO KCl+) |
| 7f   | Unpaired t-test | Fig. 4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=0.1085 (WT KCl- vs WT KCl+); p=0.00021372 (WT KCl+ vs KO KCl+) | Fig. legend | t(6)=5.2133 (WT KCl- vs WT KCl+); t(6)=6.2577 (WT KCl+ vs KO KCl+) |
| 7g   | Unpaired t-test | Fig. 4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=1.373212E-05 (WT KCl- vs WT KCl+); p=0.00052120 (WT KCl+ vs KO KCl+) | Fig. legend | t(6)=7.4153 (WT KCl- vs WT KCl+); t(6)=4.3140 (WT KCl+ vs KO KCl+) |
| 7h   | Unpaired t-test | Fig. 4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=6.03266E-07 (WT KCl- vs WT KCl+); p=1.305662E-07 (WT KCl+ vs KO KCl+) | Fig. legend | t(6)=37.8704 (WT KCl- vs WT KCl+); t(6)=24.5796 (WT KCl+ vs KO KCl+) |
| 7i   | Unpaired t-test | Fig. 4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=0.1110 (WT KCl- vs WT KCl+); p=0.0099 (WT KCl+ vs KO KCl+) | Fig. legend | t(6)=7.7317 (WT KCl- vs WT KCl+); t(6)=4.6164 (WT KCl+ vs KO KCl+) |
| 7j   | two-tailed KS test | Fig. legend | 1548, 1548 | up-regulated genes in WT control neuron | Results para S | p=2.20E-16 | Fig. legend | D = 0.1545 (-KCl vs +KCl) |
| 7k   | Unpaired t-test | Fig. legend | 4,4,4,4 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=0.400 (Npas4); p=0.006 (Arc) | Fig. legend | t(6)=2.6156 (Npas4); t(6)=4.1147 (Arc) |
| 7l   | Unpaired t-test | Fig. legend | 3,3,3,3 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=0.002 (Npas4); p=0.007 (Arc) | Fig. legend | t(4)=7.2086 (Npas4); t(4)=5.1777 (Arc) |
| 7m   | Unpaired t-test | Fig. legend | 3,3,3,3 technical replicates of cortical neurons derived from a pool of 8-12 e15.5 embryos | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=0.002 (Npas4); p=0.00001 (Arc) | Fig. legend | t(4)=7.5372 (Npas4); t(4)=18.3843 (Arc) |
| 7n   | ANOVA test | Fig. legend | 16, 17 | number of age and sex-matched mice used/group | Materials and Methods | error bars are mean +/- S.E.M. | Fig. legend | p=0.111 | Fig. legend | F(31)=2.701 |
| 7o   | ANOVA test | Fig. legend | 16, 17 | number of age and sex-matched mice used/group | Materials and Methods | error bars are mean +/- S.E.M. | Fig. legend | p=0.7449 | Fig. legend | F(31)=0.108 |
| 7p   | ANOVA test | Fig. legend | 16, 17 | number of age and sex-matched mice used/group | Materials and Methods | error bars are mean +/- S.E.M. | Fig. legend | p=0.0550 | Fig. legend | F(31)=0.2577 |
| 7q   | Unpaired t-test | Fig. legend | 3,3,3,3,3,3 | technical replicates of cortical regions from young (4-month-old) or old (28-month-old) mice | Fig. legend | error bars are mean +/- S.D. | Fig. legend | p=0.189587999 (LSD1); p=0.000122016 (LSD1c); p=3.74585E-05 (LSD1n) | Fig. legend | t(4)=1.4330 (LSD1); t(4)=3.9409 (LSD1c); t(4)=4.2848 (LSD1n) |

Materials and Methods
### Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?  
   If so, what figure(s)?
   
   *Fig. 1b, Fig. S1b, Fig. S1d-g, Fig. S3a-e, Fig. 4b, Fig. 4e, Fig. 4i, Fig. 5b-c, Fig. 6h, Fig. S8a, Fig. 8f-g*

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 #)?
   
   *Materials and Method, paragraph #1, #3, #5*

### 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.
   
   *Yes.  
   10 mice for each group.  
   In Materials and Methods section, para 5.*

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?
   
   *Yes.
   In Materials and Method section, para 10.*

   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 (section, paragraph #)?
   
   *Yes.
   In Materials and Method section, para 10.*

   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 (section, paragraph #)?
   
   *No.*

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

   e. Are there adjustments for multiple comparisons?  
   
   *No.*

3. Are criteria for excluding data points reported?  
   Was this criterion established prior to data collection?  
   Where is this described (section, paragraph #)?
   
   *No.*
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.
   Where does this appear (section, paragraph #)?
   No randomization and blinding were employed.
   In Materials and Methods section, para 10.

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.
   Where (section, paragraph #)?
   No blinding were employed.
   In Materials and Methods section, para 5.

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
   Where (section, paragraph #)?
   Yes.
   In Materials and Methods section, para 1 and 5.

7. Is the species of the animals used reported?
   Where (section, paragraph #)?
   Yes.
   In Materials and Method section, para 1 and 5.

8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
   Where (section, paragraph #)?
   Yes.
   In Materials and Method section, para 1.

9. Is the sex of the animals/subjects used reported?
   Where (section, paragraph #)?
   Yes.
   In Materials and Methods section, para 5.

10. Is the age of the animals/subjects reported?
    Where (section, paragraph #)?
    Yes.
    In Materials and Method section, para 5.

11. For animals housed in a vivarium, is the light/dark cycle reported?
    Where (section, paragraph #)?
    Yes.
    In Materials and Method section, para 5.

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
    Where (section, paragraph #)?
    Yes.
    In Materials and Method section, para 1.

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
    Where (section, paragraph #)?
    Yes.
    In Materials and Method section, para 5.

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
    Where (section, paragraph #)?
    Yes.
    In Materials and Methods section, para 5.
1. If multiple behavioral tests were conducted in the same group of animals, is this reported?  
Where (section, paragraph #)?

| Yes. | In Materials and Methods section, para 5. |

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

| No. |

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

| Yes. |

2. Cell line identity

| No. |

2. Cell line identity

| Yes. | In Materials and Methods section, para 4. |

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

| Yes. |

2. Cell line identity

| No. |

2. Cell line identity

| Yes. | In Materials and Methods section, para 4. |

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

| Yes. |

2. Cell line identity

| No. |

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| Yes. | In Materials and Methods section, para 4. |

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

| Yes. |

2. Cell line identity

| No. |

2. Cell line identity

| Yes. | In Materials and Methods section, para 4. |
**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.

We encourage publication of Data Descriptors (see Scientific Data) to maximize data reuse.

| Question                                      | Answer |
|-----------------------------------------------|--------|
| 1. Are accession codes for deposit dates provided? | Yes. In Materials and Methods section, para 15. |

**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.

2. If computer code was used to generate results that are central to the paper’s conclusions, include a statement in the Methods section under "Code availability" to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability.

**Human subjects**

1. Which IRB approved the protocol?
   Where is this stated (section, paragraph #)?

2. Is demographic information on all subjects provided?
   Where (section, paragraph #)?

3. Is the number of human subjects, their age and sex clearly defined?
   Where (section, paragraph #)?

4. Are the inclusion and exclusion criteria (if any) clearly specified?
   Where (section, paragraph #)?
5. How well were the groups matched?
   Where is this information described (section, paragraph #)?

6. Is a statement included confirming that informed consent was obtained from all subjects?
   Where (section, paragraph #)?

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?
   Where (section, paragraph #)?

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?
   a. If yes, is the number rejected and reasons for rejection described?
      Where (section, paragraph #)?

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?
   Where (section, paragraph #)?

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

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.

5. Is the task design clearly described?
   Where (section, paragraph #)?

6. How was behavioral performance measured?

7. Is an ANOVA or factorial design being used?

8. For data acquisition, is a whole brain scan used?
   If not, state area of acquisition.
   a. How was this region determined?
9. Is the field strength (in Tesla) of the MRI system stated?
   a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?
   b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?

10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?

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? Where (section, paragraph #)?

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? Where (section, paragraph #)?

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

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

15. Is the contrast construction clearly defined?

16. Is a mixed/random effects or fixed inference used?
   a. If fixed effects inference used, is this justified?

17. Were repeated measures used (multiple measurements per subject)?
   a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?

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

19. Are statistical inferences corrected for multiple comparisons?
   a. If not, is this labeled as uncorrected?
20. Are the results based on an ROI (region of interest) analysis?
   a. If so, is the rationale clearly described?
   b. How were the ROI’s defined (functional vs anatomical localization)?

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

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

Additional comments

Additional Comments