nature neuroscience

Corresponding Author: Berislav Zlokovic

Manuscript Number: NN-A51966-T

Manuscript Type: Article

Main Figures: 8

Supplementary Figures: 14

Supplementary Tables: 1

Supplementary Videos: 0

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.

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
example	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	Fig. legend	F(3, 36) = 2.97	Fig. legend
example	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	Results para 6	t(28) = 2.808	Results para 6
+ -												

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ -	1c	ANOVA followed by Tukey's posthoc tests	Fig. Legend	6,6	n=6/group	Fig. Legend	Mean ± s.d	Fig. Legend	p = 0.00002, p = 1.4x10^-6	Fig. Legend	n/a	n/a
+ -	1e	Student's t-test	Fig. Legend	9,7	n=9 controls (Braak 0-I) and 7 AD cases	Fig. Legend	Mean ± s.d.	Fig. Legend	p = 7.8x10^-7, p = 8.6x10^-9	Fig. Legend	n/a	n/a
+ -	1f-i	Pearson and Spearman rank correlation analysis	Fig. Legend	50,50,37, 28	Each point in f-i is an individual value from 50 (f-g), 37 (h) and 28 (i)	Fig. Legend	n/a	n/a	calculated based on R value	Fig. Legend	r=-0.82 (f), r=-0.83 (g), r=0.71 (h), r=-0.82 (i)	1f-i
+ -	2b	Student's t-test	Fig. Legend	3,4	from 3-4 mice per group	Fig. Legend	Means + s.e.m	Fig. Legend	p = 0.0058, p = 0.0189	Fig. Legend	n/a	n/a
+ -	2c-f	Student's t-test	Fig. Legend	6,6	n=6 mice per group	Fig. Legend	Means + s.e.m.	Fig. Legend	p = 0.0043, p = 0.0128, p = 0.0002, p = 0.0011,	Fig. Legend	n/a	n/a
+ -	3c	Student's t-test	Fig. Legend	5,6	n=5-6 mice per group	Fig. Legend	means + s.e.m.	Fig. Legend	p = 5.6x10^-5, p = 2.8x10^-8	Fig. Legend	n/a	n/a
+ -	3f	Student's t-test	Fig. Legend	5,6	n=5-6 mice per group	Fig. Legend	means + s.e.m.	Fig. Legend	p = 7.5x10^-6, p = 1.3x10^-5	Fig. Legend	n/a	n/a
+ -	3g-h	Student's t-test	Fig. Legend	12,14	n=12-14 mice per group	Fig. Legend	means + s.e.m.	Fig. Legend	p = 4.2x10^-5, p = 0.012	Fig. Legend	n/a	n/a
+ -	4d	paired Wilcoxon Signed Rank Test	Fig. Legend	10,10	n=10 mice per group	Fig. Legend	means + s.e.m.	Fig. Legend	p < 0.01	Fig. Legend	n/a	n/a
+ -	4e	Student's t-test	Fig. Legend	10,10	n=10 mice per group	Fig. Legend	means + s.e.m.	Fig. Legend	p = 0.0063, p = 0.0111	Fig. Legend	n/a	n/a
+ -	4f-g	Student's t-test	Fig. Legend	10,10	n=10 mice per group	Fig. Legend	means + s.e.m.	Fig. Legend	p = 0.0013, p = 0.007	Fig. Legend	n/a	n/a
+ -	5d	Student's t-test	Fig. Legend	9,9	3 primary isolates in triplicate	Fig. Legend	Mean ± s.d.	Fig. Legend	p = 2.4x10^-7, p = 0.0002	Fig. Legend	n/a	n/a
+ -	5f	ANOVA followed by Tukey's posthoc tests	Fig. Legend	9,9	3 primary isolates in triplicate	Fig. Legend	Mean ± s.d.	Fig. Legend	p = 3.04, p = 3.9x10^-13, p = 1.9x10^-10	Fig. Legend	n/a	n/a
+ -	6g	ANOVA followed by Tukey's posthoc tests	Fig. Legend	9,9,9	3 primary isolates in triplicate	Fig. Legend	Mean + s.e.m.	Fig. Legend	p =5.7x10^-15, p = 2.4x10^-15	Fig. Legend	n/a	n/a

+ -	7d	Student's t-test	Fig. Legend	9,9	3 primary isolates in triplicate	Fig. Legend	Mean + s.e.m.	Fig. Legend	p = 4.2x10^-6, p = 0.89, p = 5.3x10^-10	Fig. Legend	n/a	n/a
+ -	7k	ANOVA followed by Tukey's posthoc tests	Fig. Legend	9,9,9,9	3 primary isolates in triplicate	Fig. Legend	Mean + s.e.m.	Fig. Legend	p = 1.8x10^-17, p = 0.4613, p = 6.5x10^-15	Fig. Legend	n/a	n/a
+ -	8a	Student's t-test	Fig. Legend	8,8	8 isolates in triplicate for control and AD monolayers	Fig. Legend	Mean + s.e.m.	Fig. Legend	p = 0.0066, p = 0.0001	Fig. Legend	n/a	n/a
+ -	8b	ANOVA followed by Tukey's posthoc tests	supplementary methods	8,8,8,8,8	8 isolates in triplicate for control and AD monolayers	Fig. Legend	Mean + s.e.m.	Fig. Legend	p = 0.0002, p = 2.8x10^-5, p = 9.9x10^-8,	Fig. Legend	n/a	n/a
+ -	8h	Student's t-test	Fig. Legend	6,6	6 cultures for each rs3851179 variant in triplicates	Fig. Legend	Mean + s.e.m.	Fig. Legend	p = 0.0006, p = 6.4x10^-5	Fig. Legend	n/a	n/a
+ -	8i	Student's t-test	Fig. Legend	6,6	6 cultures for each rs3851179 variant in triplicates	Fig. Legend	Mean + s.e.m.	Fig. Legend	p = 0.00079	Fig. Legend	n/a	n/a
+ -	3i	Student's t-test	Fig. Legend	12,14	n=12-14 mice per group	Fig. Legend	Mean + s.e.m.	Fig. Legend	p = 0.0478, p = 0.0014	Fig. Legend	n/a	n/a
+ -												

Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

   If so, what figure(s)?

Figure 1a,b & d; Figure 2a & b; Figure 3d & e; Figure 4 b & c; Figure 5a-c, e, g & i; Figure 6a-c; Figure 7 a-c,e-g, i,l-m; Figure 8a, d-h

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 #)?

Yes, the number of samples and the number of experiments/ replicates were stated in each figure legend.
The number of animals varies from 6-14, depending on the analysis;

For cells, it is stated in the supplementary method, section of "Primary human brain endothelial cell cultures", 4th paragraph: "at least 20 cells from 5 different randomly selected fields in each culture", and in the legends of figures 6&7: "3 primary isolates in triplicates"

There was no limitation in repeatability.

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, it is stated in the supplementary method, section of "Statistical analysis", 1st paragraph:
"Sample sizes were calculated using nQUERY assuming a two-sided alpha-level of 0.05, 80% power, and homogeneous variances for the 2 samples to be compared, with the means and common standard deviation for different parameters predicted from published data and our previous studies."

2. Are statistical tests justified as appropriate for every figure? Where (section, paragraph #)?	Yes. It is summarized in the supplementary method, section of "Statistical analysis", 1st paragraph:"Data were analyzed by Student's t-test for comparison between two groups; or by multifactorial analysis of variance (ANOVA) followed by Tukey's post hoc tests for multiple comparisons; or paired Wilcoxon's signed rank test for paired non-parametric comparison." For every figure, the statistical tests were listed in the corresponding legend.
a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?	Yes. It is stated in the supplementary method, section of "Statistical analysis", 1st paragraph:"Data were analyzed by Student's t-test for comparison between two groups; or by multifactorial analysis of variance (ANOVA) followed by Tukey's post hoc tests for multiple comparisons; or paired Wilcoxon's signed rank test for paired non-parametric comparison."
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. It is stated in the supplementary method, section of "Statistical analysis", 1st paragraph:"Shapiro-Wilk test was used to test normality of the data, F test was conducted to ensure that the data meets the assumptions of the tests and the variance was similar between the groups that are statistically compared. "
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 #)?	Yes. It is stated in the supplementary method, section of "Statistical analysis", 1st paragraph:"F test was conducted to ensure that the data meets the assumptions of the tests. The variance was similar between the groups that are statistically compared. "
d. Are tests specified as one- or two-sided?	Two-sided tests were used.
e. Are there adjustments for multiple comparisons?	Yes. For multiple comparisons, Tukey's post hoc tests were used for adjustments after ANOVA.
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	No data was excluded. It is stated in the supplementary method, section of "Animals", 1st paragraph:"All animals were included in the study."
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 #)?	It is stated in the supplementary method, section of "Animals", 1st paragraph:"All animals were randomized for their genotype information. "
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 #)?	It is stated in the supplementary method, section of "Animals", 1st paragraph:"All experiments were blinded; the operators responsible for the experimental procedures and data analysis were blinded and unaware of group allocation throughout the experiments. "

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	It is stated in the supplementary method, section of "Animals", 1st paragraph:"All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California with National Institutes of Health guidelines. "
7. Is the species of the animals used reported? Where (section, paragraph #)?	It is stated in the supplementary method, section of "Animals", 1st paragraph that mice were used in this study.
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	It is stated in the supplementary method, section of "Animals", 2nd paragraph that Picalm+/- mice used in this study were on C57BL/6-Thy1.1 background. It is stated in the supplementary method, section of "Animals", 3rd paragraph that, for APPsw/0; Picalm+/- mice, experiments were performed using age-matched littermates to minimize confounding effects of background heterogeneity all
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Both sexes were used in this study. For mice, It is stated in the supplementary method, section of "Animals", 1st paragraph:"Animals of both sexes 3, 6 and 9 month old were used in the experiments" For human post-mortem study, the gender information is listed in Supplementary table 1 & 2.
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Yes. For mice, It is stated in the supplementary method, section of "Animals", 1st paragraph:"Animals of both sexes 3, 6 and 9 month old were used in the experiments". For human post-mortem study, the age information is listed in Supplementary table 1 & 2.
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	Yes. It is stated in the supplementary method, section of "Animals", 1st paragraph:"Mice were housed in plastic cages on a 12 h light cycle with ad libitum access to water and a standard laboratory diet".
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	No. However, "All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California with National Institutes of Health guidelines.", which means the maximum mice per cage is 5.
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	No. However we followed the standard protocol for all the behavior test, including overnight testing for both nesting and borrowing.
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?	Yes. In figure 3a-b, baseline Abeta level prior to the compound E administration was reported.
a. If multiple behavioral tests were conducted in the same group of animals, is this reported? Where (section, paragraph #)?	No. The animals were analyzed with all 4 behavior tests, including nesting, borrowing, NOL and NOR. None of these tests were performed in consecutive days.

15. If any animals/subjects were excluded from analysis, is this reported?

    Where (section, paragraph #)?

    No animals/subjects were excluded. It is stated in the supplementary method, section of "Animals", 1st paragraph:"All animals were included in the study."

    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)?

   Yes, we only chose antibodies validated by previous publications, either from our own group (ref: Bell et al Neuron 2010; Bell et al Nature 2011; Sagare et al Nature communication 2013; Winkler et al Nature neuroscience 2015), or from other labs (Armulik et al Nature 2010, etc.)

   a. Is antibody catalog number given?

      Where does this appear (section, paragraph #)?

      Yes, all the informations were given in the supplementary method, section of "Reagents"

   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

      Where does this appear (section, paragraph #)?

      Yes, we only chose antibodies validated by previous publications, either from our own group (ref: Bell et al Neuron 2010; Bell et al Nature 2011; Sagare et al Nature communication 2013; Winkler et al Nature neuroscience 2015, or from other labs (Armulik et al Nature 2010, etc.)

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

   Where (section, paragraph #)?

   Yes. We used primary human brain endothelial cell cultures established from rapid brain autopsies from the frontal cortex (area 9/10) from neurologically intact age-matched controls and AD patients as we previously described (ref. Wu et al Nature medicine 2005; Bell et al Nature cell biology 2009; Zhu et al Blood 2010). The detailed information is listed in in the supplementary method, section of "Primary human brain endothelial cell cultures", paragraphs 1-4.

   a. Were they recently authenticated?

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

      Yes, we revalidated the cells in this study, the data is reported in figure 6a-b and supplementary figure 16a-c.

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.

1. Are accession codes for deposit dates provided? Where (section, paragraph #)?	N/A

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. 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.	N/A

Human subjects

1. Which IRB approved the protocol? Where is this stated (section, paragraph #)?	It is stated in the supplementary method, section of "Primary human brain endothelial cell cultures", 1st paragraph: "The autopsy study was approved by the Western Institutional Review Board (WRIB), Protocol #1534 WRIB; Study #1028052, “Human Autopsy Tissue Research Protocol.” "
2. Is demographic information on all subjects provided? Where (section, paragraph #)?	Yes, the demographic information was provided in supplementary table 1 (a&b).
3. Is the number of human subjects, their age and sex clearly defined? Where (section, paragraph #)?	Yes. A total of 20 controls and 30 AD individuals were used for histopathological analyses (supplementary method, section of "Human postmortem studies", 1st paragraph). Gender and age informations were provided in supplementary table 1 (a&b).

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   Where (section, paragraph #)?

   No subjects were excluded.

5. How well were the groups matched?

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

   The control and AD groups were age matched.\
   It is stated in the supplementary method, section of "Human postmortem studies", 1st paragraph that: "The incidence of vascular risk factors (e.g., hypertension, atherosclerosis, etc), the gender ratio, age, and the PMI were comparable between age-matched controls and AD patients. The cause of death in both groups was either cardiac or respiratory arrest."

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

   Where (section, paragraph #)?

   N/A

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?

   Where (section, paragraph #)?

   N/A

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

6. How was behavioral performance measured?

   N/A

7. Is an ANOVA or factorial design being used?

   N/A

8. For data acquisition, is a whole brain scan used? If not, state area of acquisition.	N/A
a. How was this region determined?	N/A
9. Is the field strength (in Tesla) of the MRI system stated?	N/A
a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?	N/A
b. Are the field-of-view, matrix size, slice thickness, and TE/TR/ flip angle clearly stated?	N/A
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?	N/A
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 #)?	N/A
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 #)?	N/A
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?	N/A
14. Were any additional regressors (behavioral covariates, motion etc) used?	N/A
15. Is the contrast construction clearly defined?	N/A
16. Is a mixed/random effects or fixed inference used?	N/A
a. If fixed effects inference used, is this justified?	N/A
17. Were repeated measures used (multiple measurements per subject)?	N/A
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?	N/A
19. Are statistical inferences corrected for multiple comparisons?	N/A

a. If not, is this labeled as uncorrected?	N/A
20. Are the results based on an ROI (region of interest) analysis?	N/A
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	N/A