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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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Experimental design

1. Sample size

Describe how sample size was determined.

Reported in the 'Statistical analysis' section of the Supplementary Methods.
In vivo antibody injection experiments are usually including between 7-10 animals. In this study, all experiments were performed at least twice and done so blind for the identity of the injected compound. This chosen sample size in most experiments satisfied the resource equation, where the error degrees of freedom in t-test or the denominator of F in the ANOVA was between 10-20.

2. Data exclusions

Describe any data exclusions.

Exclusion criteria are described in the text, specifically:
1) Supplement: Supplementary Methods: Statistical analysis: "Continuous dependent variables with a lower bound of 0 were routinely subjected to log10 transformation to satisfy assumptions of standard parametric statistical tests. Outliers were identified by Grubbs' test with an alpha level of 0.1, and instances where this occurred are described in the Figure statistics section."
2) Supplement: Figure statistics: Fig 3h: "Note that a data point in the GluA2 IgG treatment of our original data set had particularly low potentiation (26.6%). It was a significant outlier according to Grubbs' test, and so was removed from the graph and the analysis."
3) Supplement: Figure statistics: ED Fig 2d-e: "Analysis is of data from the same cells recorded in Fig 2a and b. However, to include blocking (and thus increase the power for comparison between treatments) two NA recordings could not be included since the data for the control was missing from one experiment block."
4) Supplement: Figure statistics: ED Fig 3a: "We had a notable upward sloping baseline in the ensemble mean of our original sample set (9 recordings). To exclude the possibility that run-up was responsible for the potentiation observed in this instance, we performed a correlation analysis on the baseline period of all the recordings in this figure panel and excluded any recordings with an absolute Pearson's correlation coefficient (r) > 0.5. Three recordings were removed based on this criterion."
5) Supplement: Figure statistics: ED Fig 4b: "An outlier (in NA pre-treatment: PPR = 3.214) was detected by Grubbs' test and removed from the graph and the analysis."

3. Replication

Describe whether the experimental findings were reliably reproduced.

The experimental findings were reliably reproduced

4. Randomization

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

End of the first paragraph of the 'Organotypic slice electrophysiology' section of the Supplementary Methods: "The order of recordings was randomized for (blinded) neutravidin vs. vehicle pre-treatment experiments."

Near the end of the 'Fear Conditioning' section of the Supplementary Methods in the main text: "Measurements of freezing behaviour were alternated between (blinded) experimental groups and the mice within each group were selected randomly."

5. Blinding

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

- End of the first paragraph of the 'Organotypic Slice Electrophysiology' section of the Supplementary Methods: "The order of recordings was randomized for (blinded) neutravidin vs. vehicle pre-treatment experiments."

- Near the end of the 'Fear Conditioning' section of the Supplementary Methods in the main text (page 26): "Measurements of freezing behaviour were alternated between (blinded) experimental groups and the mice within each group were selected randomly."

- Second paragraph of the 'In Vivo Electrophysiology' section of the Supplementary Methods: "The experimenter was blind to the antibody solution used in the experiments."

- Near the end of the 'Fear Conditioning' section of the Supplementary Methods: "The experimenter was blind to the antibody solution used in the experiments."

- Final sentence of the 'Biochemistry' section of the Supplemental Methods in the supplemental text: "The experimenter was blind to the antibody solution used in the experiments."

- First paragraph of the 'AMPAR endocytosis' of the Supplemental Methods: "The experimenter was blind to the antibody solution used in the experiments." and final sentence of the same section "The identity of samples' experimental treatment was revealed to experimenter after image analysis to avoid bias."

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.

Classical Excel, Graphpad and Metamorph softwares were mostly used.
In addition, the following code availability statement is now included at the end of the Methods section:
"Code availability. Custom python code used for protocols and analysis are available at GitHub (v0.1, https://github.com/acp29/penn). Custom matlab code for additional statistical functions (multicmp and epsilon, both v1.0) are available from Matlab Central File Exchange (#61659 and #61660 respectively)."

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.	There are no restriction on material availability. the anti-GluA2 antibody is available upon request from Eric Gouaux Lab.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Anti-GluA2 antibodies used in this study gave no staining in GluA2 knockout mice (see also Giannone et al., Biophysical journal 99, 1303-1310).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	n/a
b. Describe the method of cell line authentication used.	n/a
c. Report whether the cell lines were tested for mycoplasma contamination.	n/a
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.	n/a

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.	For in vivo and behaviour experiments, wild type males were used (inbred C57BL/6 background) aged between 3-5 months old. For acute slice electrophysiology experiments, C57Bl6 mice aged 8-9 weeks were used. Organotypic hippocampal slices were prepared from GluA2 knockout mice aged P6-8 (outbred Swiss NMRI background). Dissociated Hippocampal neurons were cultured from Sprague Dawley rat embryos (E18). All experiments were performed in accordance with the guidelines established by the European Communities Council (Directive 2010/63/EU of September 22, 2010) and were approved by the Animal Experimental Committee of Bordeaux Universities (CE50; A5012009).

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.	n/a