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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

The explanation for sample size is given in the manuscript, under "Sample size determination" (p. 43).

Because the experiments here were aimed at developing a technology, rather than testing a formal scientific hypothesis, the animal numbers in the paper were forecasted based upon past neuroengineering projects we have done, using the experience we have attained since starting the Synthetic Neurobiology group at MIT, similar to Klapoetke et al19. This follows guidelines recommended by the NIH63 regarding “experiments based on the success or failure of a desired goal.”

2. Data exclusions

Describe any data exclusions.

For recording in slices, data points (singular trials) were excluded if the recording was unstable, meaning the baseline potential changed by more than 5 mV during the recording. Cells were excluded if a gigaohm seal was lost or a high leak current appeared.

3. Replication

Describe whether the experimental findings were reliably reproduced.

For every experiment we conducted, we repeated it several time and then conducted a statistical test to check for an experimental effect. Reliability led to statistical significance.

4. Randomization

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

Animals and cultures were randomly assigned to the different experimental groups.

It appears in the methods section, paragraph " Randomization".

5. Blinding

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

There were no blind experiments.

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.	Software used: Matlab, origin8, IgorPro, pClamp10, Excel.

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.	We provided a Data availability statement (p.14-15): "All relevant data are available from the authors. Sequences are available in this manuscript under Supplementary information. Clones will be made available through the nonprofit DNA distributor Addgene, as well as the UNC and UPenn Viral cores".

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibodies were used in our study.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Chinese hamster ovary (CHO) ordered from Sigma-Aldrich, subclones of the parental CHO cell line originated by Puck in 1957 (The Journal of experimental medicine, 108, (1958-12-1)).
b. Describe the method of cell line authentication used.	Not known; presumably standard Sigma-Aldrich procedures were followed. Cells lot number 10D001
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were tested with Hoechst method.
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.	CHO cells are not in the list.

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.

Species: the species of animals were reported in in the Methods, under "Viral injections and whole-cell electrophysiology in brain slices" (p.33) and "Primary neuron culture, transfection and transduction " (p.29).

Strain: the strain of animals strains were well-characterized mice strains appearing in multiple publications from multiple labs.

Sex: for slice experiments we used male mice, and mentioned it under "Viral injections and whole-cell electrophysiology in brain slices".
For cultures, we do not know the sex of the mice used for the experiments.

Age: reported in the Methods, under "Viral injections and whole-cell electrophysiology in brain slices" and "Primary neuron culture, transfection and transduction ".

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