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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.

Sample sizes were chosen such that differences were confirmed at sufficiently high significance ($p \leq 0.05$). For the neuronal culture studies, a minimum of two culture preparations in the case of pairwise experiments (Figure 1F and G) or three culture preparations in the case of unpaired experiments (Figure 1A - 1E) were used to ensure the repeatability of the findings. No significant variations for similar conditions or experiments were observed between culture preps. The number of cell recordings per culture was not determined beforehand.

2. Data exclusions

Describe any data exclusions.

No culture preparations were excluded from this study. Electrophysiogical recordings were discarded prior to being analyzed if access to the cell was compromised or disrupted (see Methods Section; Electrophysiology paragraph).

3. Replication

Describe whether the experimental findings were reliably reproduced.

Independent trials of each experiment ($n \geq 3$) were performed to ensure reliability of our findings. All independent trials were successful and showed similar results.

4. Randomization

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

There was no method for randomization. For neuronal cultures, culture prep yielded enough neurons to be used in all conditions (see Methods section; Cell Culture paragraph). Thus, no randomization was necessary.

5. Blinding

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

Investigators were not blinded.

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.

Prism, Clamp Fit, Origin, FV10-ASW 3.1 acquisition software, Clampex 10

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.

No restriction.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

syb2 antibody (Bomba-Warczak et al., 2016)
syt1 antibody (Davis et al., 1999)
β-actin antibody (Cat. # 3700s, Cell Signaling Technology, Inc.)
Specificities of syt1 and syb2 antibodies have been validated by western blots using material from KO mice. β-actin antibody has been validated by Cell Signaling Technology, Inc.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No cell lines were used.

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.	Cultured rat cortical neurons were prepared from embryonic day 18-19 Sprague Dawley rats. The sex of the pups were not determined and neurons from all of the pups in a single litter were pooled during the culturing.

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.	this study did not involve human research participants.