nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

| For | all st | atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section. |
|-------------|-----------|---|
| n/a | Cor | nfirmed |
| | \square | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| \ge | | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| \ge | | The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| \boxtimes | | A description of all covariates tested |
| \ge | | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| | | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| \boxtimes | | For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable. |
| \boxtimes | | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| \times | | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| \boxtimes | | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |
| | | Our web collection on <u>statistics for biologists</u> contains articles on many of the points above. |
| | | |

Software and code

| Policy information about <u>availability of computer code</u> | | | | | | | | |
|---|--|--|--|--|--|--|--|--|
| Data collection | In vivo EPS: EP-Tracer_V1.05, Schwarzer Cardiotek Ex vivo EPS: Labchart v8.1.16, ADInstruments and EP-Tracer_V1.05, Schwarzer Cardiotek | | | | | | | |
| Data analysis | In vivo EPS: EP-Tracer_V1.05, Schwarzer Cardiotek Ex vivo EPS: Labchart v8.1.16, ADInstruments and EP-Tracer_V1.05, Schwarzer Cardiotek Excel 2013 (Microsoft Corporation USA) Origin® 2018 (OriginLab Corporation) | | | | | | | |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The source data underlying Figures 3, 7, 8, 10; Extended Data Figures 1-10 and Tables 3-6 are provided with this paper as Source Data files.

Field-specific reporting

K Life sciences

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to predetermine sample size. Sample sizes were chosen based on previous experience and considered to be sufficient based on publications in the field. Effort was taken to keep the number of animals at a minimum. |
|-----------------|---|
| Data exclusions | Possible technical problems during in-vivo EPS that lead to data exclusion were measurements in which the stimulus did not always lead to a capture/response, in which an animal's spontaneous heart rate was temporarily or permanently faster than the S1S1 stimulus interval, or premature termination of the experiment due to an uncontrollable stage of anaesthesia. All exclusion criteria were pre-established |
| Replication | All attempts at replication were successful. |
| Randomization | Randomization is not relevant to our study because no different experimental groups were compared. |
| Blinding | Blinding is not relevant to our study because no different experimental groups were compared. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

Methods

| n/a | Involved in the study | n/a | Involved in the study |
|-------------|-------------------------------|-------------|------------------------|
| \boxtimes | Antibodies | \boxtimes | ChIP-seq |
| \ge | Eukaryotic cell lines | \ge | Flow cytometry |
| \boxtimes | Palaeontology and archaeology | \ge | MRI-based neuroimaging |
| | Animals and other organisms | | |
| \boxtimes | Human research participants | | |
| \ge | Clinical data | | |
| \ge | Dual use research of concern | | |
| | | | |

Animals and other organisms

| Policy information about <u>st</u> | <u>udies involving animals; ARRIVE guidelines</u> recommended for reporting animal research | | | | |
|------------------------------------|---|--|--|--|--|
| Laboratory animals | WT mice were obtained by in-house breeding and maintained on a mixed C57BL/6N and 129/SvJ background. | | | | |
| | All in vivo measurements were carried out in male animals . Sexes were not mixed because there are significant sex differences in cardiac parameters. | | | | |
| | Age of animals used in the experiments: | | | | |
| | In vivo electrophysiology: 2-4 months | | | | |
| | Ex vivo electrophysiology: 3-4 months | | | | |
| Wild animals | No wild animals were used. | | | | |
| Field-collected samples | No field-collected samples were used. | | | | |
| Ethics oversight | All animal studies were approved by the Regierung von Oberbayern, were in accordance with German laws on animal experimentation, and were performed in compliance with widely accepted ethical standards. Effort was taken to keep the number of animals at a minimum | | | | |

Note that full information on the approval of the study protocol must also be provided in the manuscript.