

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 [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a | Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- 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.
- A description of all covariates tested
- 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)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

For data collection we employed custom tools written in the following softwares/programming languages:
- LabVIEW (v15.0.1.f10)
- Python3 (v3.10)
- C++

Data analysis

For data analysis we employed custom tools written in Python3 (v3.10), the open source image analysis software ImageJ (v1.54) and the commercial software Origin Pro 2022 (v9.95).

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 [data availability statement](#). 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](#)

Data underlying plots, custom-written LabVIEW/Python/C++ codes and all other relevant data are available from the corresponding authors upon reasonable request.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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

Field-specific reporting

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.

- Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size	No a priori calculation of sample size was performed.
Data exclusions	No data were excluded from the analyses.
Replication	Replications were considered successful if the optogenetic actuation elicited in the stimulation site a calcium transient with an amplitude exceeded 3 standard deviations above baseline level. All attempts at replication were successful.
Randomization	No randomization was performed. In the quantification of crosstalk, the two groups belonged to two different transgenic lines. In the quantification of activation probability and functional connectivity, only one experimental group was present.
Blinding	Blinding was not performed. In experiments involving two experimental groups (crosstalk evaluation), blinding was not necessary since results were analyzed on a whole-brain scale, thus excluding a selection of relevant results. In other experiments blinding was not relevant.

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

- n/a Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Clinical data
- Dual use research of concern

Methods

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

The study was performed on 5 days post fertilization (dpf) zebrafish (*Danio rerio*) larvae of the following strains:
 - Tg(*elavl3*:H2B-GCaMP6s; *elavl3*:ReaChR-TagRFP), on homozygous albino background (*slc45a2*^{b4/b4});
 - Tg(*elavl3*:H2B-GCaMP6s), on homozygous albino background (*slc45a2*^{b4/b4}).

Wild animals

This study does not involve wild animals.

Reporting on sex

Sex was not considered in this study since at the larval stage zebrafish do not show sex differentiation.

Field-collected samples

This study does not involve field-collected samples.

Ethics oversight

Experiments involving zebrafish larvae were carried out in compliance with European and Italian laws on animal experimentation (Directive 2010/63/EU and D.L. 4 March 2014, n.26, respectively), under authorization n.606/2020-PR from the Italian Ministry of Health.

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