

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	All data collection software is described in the methods section of the manuscript. Acquisition software used includes cellSens Dimension (Olympus, ver 4.2), Ethovision (Noldus, ver XT10), Clampfit (Axon Instruments, ver 10.7), and MicroManager (NIH).
Data analysis	All analysis is described in the methods section of the manuscript,. Analysis software used includes MATLAB (MathWorks, Inc., ver 2018a), ImageJ (NIH), Scanbox (Neurolabware), Chemdraw (PerkinElmer, ver 19.1.1.21), Instant JChem (ChemAxon), ACD Spectrus Processor (ACD Labs), and CalmAn (Github). Code that support the findings of this study are available at https://github.com/tadrosslab/DART.2

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](#)

Source data are provided for electrophysiology experiments at <https://github.com/tadrosslab/DART.2>. Imaging datasets, due to their large size, are available upon request and will be fulfilled by the corresponding author.

Human research participants

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

Reporting on sex and gender

Population characteristics

Recruitment

Ethics oversight

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

Life sciences study design

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

Sample size	Detailed in figure legend and methods. No statistical methods were used to predetermine sample size. Resulting sample sizes were decided according to the type of experiment performed (cultured neuronal assays, ex vivo electrophysiology, or animal behavior), informed by past work, to ensure that sample sizes were sufficient for statistical conclusions to be drawn.
Data exclusions	Detailed in methods. No data was excluded. As described in the methods, we obtained quality-control metrics for individual cells (in cultured neuronal assays) and for individual mice (in behavioral work) to quantify the extent of viral expression and/or tracer-DART capture. This allowed individual samples to be binned according to the efficacy of the manipulation.
Replication	Detailed in methods. All attempts at replication over multiple neuronal preparations and multiple mice were successful.
Randomization	Detailed in methods. In cultured neurons, data obtained to compare two or more reagents (e.g., YM90K.1DART.1 vs YM90K.1DART.2) was obtained with experimental groups tested side-by-side, such that all comparison groups were equally represented in each batch of cells. For slice, retina, and mouse behavior, positive vs negative groups (e.g., +HTP vs ddHTP mice) were interleaved and run side-by-side, using cage-mates when possible, and balancing groups to minimize confounding differences (e.g., animal sex, experimenter, time of year), with the goal of isolating the experimental variable of interest.
Blinding	Detailed in methods. Blinding was not included in this study, however all analyses were automated in a manner that prevented experimenter bias and influence on the resulting outcomes.

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	Involvement in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

n/a	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Cell lines were only used for AAV prep. The cell lines and their authentication was performed by the Duke Viral Vector core and Vector Builder.
Authentication	See above. Authentication was performed by the Duke Viral Vector core and Vector Builder.
Mycoplasma contamination	Duke Viral Vector Core and Vector Builder have verified that cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	N/A

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	Detailed in methods. Rats: Sprague-Dawley (mixed multiple males and females used for primary neuronal cultures, postnatal day 0 to 1). Mice: Drd1a-Cre (6 males, 8 females, 10-20 weeks old), DAT-Ires-cre (26 males, 21 females, 8-20 weeks old), SST-Cre (2 males, 1 female, P45-60), PV-Cre (22 males, 38 females, 12-24 weeks old), BACα6Cre-AxAl148D (6 males, 4 females, P50-60).
Wild animals	No wild animals were used in this study.
Reporting on sex	Detailed in methods. Relevant details related to sex and gender is provided above. Sex was considered in study design with regards to balanced representation in each of the experiments, and was determined based on vivarium assignment followed by experimenter verification. No sex-based differences were seen.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	Detailed in methods. All animal experiments were approved by the Duke Institutional Animal Care and Use Committee, an AAALAC accredited program registered with the USDA Public Health Service and the NIH Office of Animal Welfare Assurance, and conform to all relevant regulatory standards (Tadross protocols A160-17-06, A113-20-05, A091-23-04).

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