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Daniel Witvliet, Jeff Lichtman, Aravi Samuel, Corresponding author(s): Mei Zhen

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Reporting Summary

Nature Research 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 Research 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	firmed
		The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
\boxtimes		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.
	\boxtimes	A description of all covariates tested
	\boxtimes	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
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	\square	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	Neurons were segmented volumetrically in VAST 1.2.1 and skeletonized in CATMAID 2019.06.20						
Data analysis	Python 3.6.9 (Pandas 1.1.4, SciPy 1.4.1, Statsmodels 0.12.1), MATLAB R2020b. All code for data analysis is available at github.com/dwitvliet/ science-developmental-connectomics						

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data collection

Electron microscopy images were aligned in TrakEM2 1.3.2. Neurons were segmented volumetrically in VAST 1.2.1 and skeletonized in CATMAID 2019.06.20 Data analysis

All scripts and files used to generate all figures are available at https://github.com/dwitvliet/nature2021.

All data analysis was done in Python 3.6.9 (Pandas 1.1.4, SciPy 1.4.1, Statsmodels 0.12.1) and MATLAB R2020b.

Data availability

All electron microscopy images and volumetric reconstructions are available at *https://bossdb.org/project/witvliet2020*. Connectivity matrices for all datasets are available at *https://www.nemanode.org/* and as Supplementary Tables.

Field-specific reporting

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 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	The number of EM samples was selected to interrogate the developmental time course of maximum nervous system growth and plasticity. The number of adult samples was selected to validate animal-to-animal variability with respect to the one published
	connectome.
Data exclusions	
Replication	All annotations were replicated by three independent individuals to verify the consistency of each synaptic structure. All samples were drawn
	from an isogenic population to allow animal to animal comparisons and assess reproducibility.
Randomization	Randomization is not applicable in connectomics as the total number of mapped nervous systems is small.
Blinding	Annotations were done by three individuals working independently and blindly. All results were subsequently collected. Only consistent
0	annotations were reported in analysis.

Reporting for specific materials, systems and methods

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

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	We studied wild-type (Bristol N2) animals reared in standard conditions: 35x10mm NGM-plates, fed by OP50 bacteria, and raised at 22.5 degree C. The animals were within a few generations of the original stock acquired from Caenorhabditis elegans Genetics Center (CGC) in 2001.	
Wild animals	The study did not involve wild animals	
Field-collected samples	The study did not involve samples collected from the field	
Ethics oversight	No ethical considerations needed for working with C. elegans	

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

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Lichtman, Ara Corresponding author(s): Zhen

Jeff W. Lichtman, Aravinthan D.T. Samuel, Mei Zhen

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Competing interests

Policy information about competing interests

Competing interests declaration

In the interest of transparency and to help readers form their own judgements of potential bias, Nature Research journals require authors to declare any competing financial and/or non-financial interest in relation to the work described in the submitted manuscript.

🔀 We declare that none of the authors have competing financial or non-financial interests as defined by Nature Research.

We declare that one or more of the authors have a competing interest as defined by Nature Research.

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Prior to submission all listed authors must agree to all manuscript contents, the author list and its order and the author contribution statements. Any changes to the author list after submission must be approved by all authors.

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- A list of figures that have associated raw data

- A description of any restrictions on data availability

 \boxtimes We have provided a full data availability statement in the manuscript.

Mandated accession codes (where applicable)

Confirm that all relevant data are deposited into a public repository and that accession codes are provided.

🗌 All relevant accession codes are provided 🛛 Accession codes will be available before publication 🗌 No data with mandated deposition

Code availability

Policy information about availability of computer code

Code availability statement

For all studies using custom code or mathematical algorithm that is deemed central to the conclusions, the manuscript must include a statement under the heading "Code availability" describing how readers can access the code, including any access restrictions. Code availability statements should be provided as a separate section after the data availability statement but before the References.

 \bigotimes We have provided a full code availability statement in the manuscript

Data presentation

For all data presented in a plot, chart or other visual representation confirm that:

n/a Confirmed

- $\square \mid \boxtimes$ Individual data points are shown when possible, and always for $n \le 10$
- The format shows data distribution clearly (e.g. dot plots, box-and-whisker plots)
- 🛛 🔀 Box-plot elements are defined (e.g. center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers)
- | |
 ightarrow Clearly defined error bars are present and what they represent (SD, SE, CI) is noted

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Image integrity

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We have read Nature Research's image integrity policy and all images comply.

Unprocessed data must be provided upon request. Please double-check figure assembly to ensure that all panels are accurate (e.g. all labels are correct, no inadvertent duplications have occurred during preparation, etc.).

Additional policy considerations

Does not apply	Involved in the study
\boxtimes	Macromolecular structural data
\square	Unique biological materials
\square	Research animals and/or animal-derived materials that require ethical approval
\square	Human embryos, gametes and/or stem cells
\square	Human research participants
\square	Clinical data
	Does not apply

I certify that all the above information is complete and correct.

Typed signature Aravinthan D.T. Samuel

Date May 21, 2020

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