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Reporting Summary

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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
	\mathbf{x} 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.
x	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

Commercial imaging software was used for each camera. The Zyla camera (see Methods) uses Andor Solis. The Grasshopper and Basler cameras (see Methods) use commercial Point Grey and Basler interfaces, respectively.

Data analysis

Custom MATLAB 2018 scripts were used to analyze data except in the case of whole-brain imaging in behaving animals in which case we used the FIJI manual tracking plugin.

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/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.

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

A source data file have been provided with this submission and the manuscript includes a link to a BOX repository with all raw data. Upon request, we will also provide raw videos and analysis code

Field-specific reporting

Life sciences study design

Materials & experimental systems

All studies must dis	sclose on these points even when the disclosure is negative.
Sample size	Statistical analyses were not use to determine sample size before experiments. Rather, sample sizes were based on the scalability of experiments and are largely consistent with existing literature. This includes typically >20 animals for behavioral experiments and <10 animals for neural imaging experiments.
Data exclusions	Approximately 1% of animals were damaged by the microfluidic loading process, leading to severely impaired locomotion. We removed these animals from the analyses.
Replication	Rather than performing one experiment with a single population of animals, our experiments consist of multiple trials across multiple days with age-matched animals. Each individual trial typically includes imaging 1-15 animals simultaneously. These individual trials show the same trends as the full data set averaged across all trials.
Randomization	Controls were often interleaved with each experiment on the same imaging platform but were not randomized.
Blinding	No blinding was used in these experiments.

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.

n/a	Involved in the study	n/a	Involved in the study		
×	Antibodies	x	ChIP-seq		
×	Eukaryotic cell lines	x	Flow cytometry		
x	▼ Palaeontology		MRI-based neuroimaging		
	🗶 Animals and other organism	ms			
x	Human research participants				
x	▼ Clinical data				
,					
Animals and other organisms					
Policy information about <u>studies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research					
Laboratory animals C. elegans. Specific mutant		. elegans. Specific mutant a	nd transgenic strains are listed in the Methods.		
Wild animals No wild animals were u		lo wild animals were used			
Field-collected samples Study did not involve field		tudy did not involve field sa	mples.		
Eth	Ethics oversight IACUC or other ethical oversight is not required for research involving invertebrates.		ght is not required for research involving invertebrates.		

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