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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 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	No software was used.
Data analysis	The source code of DeepACSON is available at https://github.com/aAbdz/DeepACSON under the MIT License. We used Microscopy Image Browser (http://mib.helsinki.fi) to pre-process the collected 3D EM images. ACSON pipeline was used for the automated segmentation of 3D EM images of the white matter, available at https://github.com/aAbdz/ACSON under the MIT License.

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

The datasets used in the study will be available upon request to the corresponding author.

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	The brain of five rats was imaged ipsi- and contralaterally, resulting in ten EM datasets. Due to the investment required to obtain such a big dataset, we selected the sample size. But, each sample contains tens of thousands of myelinated axons. We applied nested (hierarchical) statistical analysis, which captures the variability of the measurements in all levels, optimizing the statistical power.
Data exclusions	No data were excluded.
Replication	We segmented white matter in ten EM datasets using the described pipeline and methods successfully. All experimental parameters used for the segmentation are described in detail in Materials and Methods section.
Randomization	To qualitatively evaluate the segmentation at the object-level, we randomly sampled every dataset for five non-overlapping windows of size 300 x 300 voxels (10 datasets, 50 samples, and total 6848 axonal cross-sections).
Blinding	The investigations were blinded. The expert had no access to the dataset ID nor the sampling location for the qualitative evaluations.

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
[×]	[ ] Human research participants
[×]	[ ] Clinical data
[×]	[ ] Dual use research of concern

Methods

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

Animals and other organisms

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

Laboratory animals	Adult male Sprague-Dawley rats, 10 weeks old.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal procedures were approved by the Animal Care and Use Committee of the Provincial Government of Southern Finland and performed according to the guidelines set by the European Community Council Directive 86/609/EEC.

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