Additional Review Protocol Methods

This file contains additional description of review methods. The pre-registered review protocol from which this is derived can also be accessed here: https://osf.io/5tfsd.

Search methods and study selection

We searched Ovid Embase, Ovid Medline, and Web of Science Core Collection in collaboration with a medical librarian. Searches were developed using a combination of relevant keywords and subject headings. Using Covidence, one reviewer screened the titles and abstracts identified and screened them in for further review based on the eligibility criteria. Subsequently, one reviewer reviewed the full text of these articles and decided which articles should be included. We also reviewed the reference list of the most relevant included papers to identify additional studies for inclusion. If any studies were identified outside of the formal search process and were deemed to meet the inclusion criteria, they were included as well. They were identified as being found outside of the formal search process in the table describing the included studies.

Data collection

For each publication included, at least one reviewer extracted data variables. If the information for a data variable was not described in the publication, then it was noted as "not recorded". For a randomly chosen subset of the studies (20%, chosen via https://www.random.org/), a separate reviewer independently evaluated the extracted data for accuracy, to ensure that the data extraction procedure was dependable. There were no substantial disagreements about the data variables recorded.

For each publication, at least one study type was recorded: (a) time series, (b) correlational, or (c) case report. Time series studies report data on changes of a structural feature measured at one or more postmortem time points in comparison with the baseline state, which is the condition with no PMI, either explicitly or implicitly. Correlational studies evaluate the relationship between a structural feature and the PMI in a cohort of brains, either quantitatively or qualitatively. Case report studies record data on cell morphometry visualized in a single brain after a naturalistic PMI, in the sense that the PMI was not manipulated for the purpose of the study.

Data variables were extracted for each study based on the type of study. For all studies, the following data variables were extracted: species, general microscopy method (*i.e.*, light microscopy or electron microscopy), visualization method specifics (*e.g.*, staining methods), brain region, the structural feature measured, and the decomposition outcome. The structural feature refers to the aspect of cell morphometry studied, such as axons, dendrites, synapses, or myelin. If no one major category of structural feature were clearly identified, the structural feature was recorded as "general cell membrane".

For time series and case report studies, the following additional variables were extracted: storage temperature, brain location during the PMI (*i.e.*, *in situ* or *ex situ*), and the cell type of focus (if any). For time series studies, the following additional data variables were extracted: the initial preservation method, at least one time point, and the decomposition outcome at that time point relative to baseline will be extracted. For defining the time point of a time series type of observation in a cohort with variable PMIs, the median PMI of the cohort was used. For correlational studies, the following additional data variables were extracted: the sample size, the PMI range considered, and the disease studied, if any. For case reports, the following additional data variables were extracted: storage location notes.

The decomposition outcome extracted included any descriptions from the text of the decomposition of the morphology of cell membranes during the PMI relative to the baseline state. Where possible, direct quotes were extracted. If relevant to the grade, qualitative reviewer impressions from any representative figures were recorded. Statements about general tissue morphology were assumed to include cell membrane morphology, because cell membrane morphology is generally a key focus in the analysis of histologic images. For example, if the authors state that there is no change in "cellular morphology", this is assumed to include cell membrane morphology.

For time series studies, in the case that relative statements are made about the condition between successive PMI data points, a comparison to the baseline condition may be possible by "chaining" together statements made by authors. For example, if authors note that there is no or minimal decomposition for a first time point compared to baseline, and then describe that there is no change at the second time point relative to the first one, then the outcome at the second time point would be that there is no or minimal decomposition relative to baseline. When there are multiple time points measured in a study, in the case that no change is described for a given structural feature at a given time point, it was assumed that there was no change observed for that structural feature at that time point. For example, if a study notes that damage to a structural feature began to be observed at a particular time point, then the most recent time point measured prior to that time point would be recorded as having no damage observed.

Grading the severity of decomposition outcomes

For each decomposition outcome extracted in the time series studies, at least two raters independently graded the outcomes for each structural feature using the following decomposition severity scale:

0. **Absent/minimal decomposition**: No or minimal differences of the feature compared to baseline state. Example adjectives might include "no differences" or "almost no change".

1. **Partial decomposition**: Observable differences of the feature due to changes in the postmortem interval, but no significant difficulty in interpretation, making inference of the original state seem possible. For example, loss of intensity of a label without a significant change in localization of the label. As another example, structural artifacts may be observed, but they are able to be distinguished from the true underlying morphology. If cell diameter measurements are reported, it is assumed that the decomposition of cell membrane morphology is not so severe as to lead to an inability to measure cell diameters. Example adjectives might include "adequate" or "no significant change".

2. **Severe decomposition**: Significant difficulty in interpretation of cell membrane morphology due to decomposition, making inference of the original state uncertain. For example, loss of intensity of a label such that the cell membranes are not easily recognizable, or a significant change in the distribution of the label used for visualization. As another example, structural artifacts are present that severely affect cell membrane morphology, such as due to compression. Example adjectives might include "significantly altered" or "poorly defined".

3. **Near-total/total decomposition**: Near-complete or complete inability to appreciate the original state of the feature, making inference of the original state likely impossible with the available data. Example adjectives might include "unable to detect".

This grading process is meant to produce subjective scores of the decomposition severity so that readers of the review can easily visualize and conceptualize broad trends of what has been reported in the literature at different time points. To clarify any ambiguity, readers can refer to the text-based description of the decomposition outcome and/or the publication where the data was reported.

The grading was performed by multiple raters in an independent fashion. For each observation, one rater reviewed the full text of the study, while other raters reviewed the extracted decomposition outcome, including any figures if relevant, referring to the full text where needed for context. A pilot round of grading was performed with all the raters using multiple representative studies to ensure that the raters were using the same general framework.

The interrater reliability of the grades was calculated with the intraclass correlation (ICC) statistic, specifically using a single-rating, absolute-agreement, two-way random-effects model. If a rater specified a range of two grades instead of a single grade for an observation, then one of the two grades was chosen randomly (by using https://www.random.org/) for calculating the interrater reliability score. Grades discussed in the pilot round of grading were not included in the interrater reliability calculation. The ICC value and its 95% confidence interval was interpreted based on the guidelines of Koo and Li (Koo and Li, 2016).

For determining the final grade, if there was a discrepancy between the first two grades, then the final grade was decided by coming to a consensus between them. If there was a discrepancy of two or more grade scales between the two grades, then the grade from a third independent rater was used to arbitrate between them.

Differences between the protocol and the review

Compared to the original protocol, the realist synthesis style of review was adopted. Case reports were added as an additional study type and the “other” study type category was removed. The data extraction and grading procedures were updated as well, in part to accommodate the larger than expected number of studies included in the review.

Search strategy

Embase

Embase Classic+Embase

1	exp central nervous system/
2	brain.mp.
3	nervous system.mp.
4	cortex.mp.
5	1 or 2 or 3 or 4
6	exp postmortem interval/
7	((post mortem or postmortem) adj2 (interval or time or delay or change* or period)).mp.
8	interval after death.mp.
9	time elapsed since death.mp.
10	time since death.mp.
11	6 or 7 or 8 or 9 or 10
12	exp autolysis/
13	(autolysis or autolytic).mp.
14	12 or 13
15	11 or 14
16	exp histology/
17	exp ultrastructure/
18	exp microscopy/
19	exp microscope/
20	Neurohistology.mp.
21	Histology.mp.

22 Ultrastructure.mp.
23 Microscop*.mp.

24 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25 exp nerve cell/
26 Synapse.mp.
27 Dendrite.mp.
28 Axon.mp.
29 Myelin.mp.
30 25 or 26 or 27 or 28 or 29
31 morphology.mp.
32 24 or 30 or 31
33 5 and 15 and 32

Representative microscopy images

The brain tissue used for microscopy was obtained and de-identified at the Icahn School of Medicine at Mount Sinai in accordance with its policies, regulations, and institutional review board recommendations. For light microscopy, formalin fixed tissue from the frontal cortex was paraffin embedded, and consecutive sections with a thickness of 5-7 µm were prepared using a microtome as previously described (McKenzie et al., 2022). These sections were then placed on glass slides, deparaffinized, and stained with hematoxylin, eosin, and Luxol fast blue, followed by imaging at 40X resolution with a Phillips Ultra Fast Scanner.

For electron microscopy (EM), regions of the frontal cortex were dissected by hand and were fixed in a solution of 2% paraformaldehyde and 2% glutaraldehyde in 0.1M sodium cacodylate buffer. An adapted version of the NCMIR protocol was used to provide additional and enhanced contrast for the material (Deerinck et al., 2010). Briefly, this protocol uses multiple methods of chemical fixation that are standard for EM. After the fixation, the sample is then dehydrated through a graded ethanol series, infiltrated with Embed 812 epoxy resin (EMS), and polymerized at 60C for 72 hours. Semithin sections (0.5 µm) were obtained using a Leica UC7 ultramicrotome (Leica, Buffalo Grove, IL) and counterstained with 1% toluidine blue to determine the regions of interest within layers 2/3 of the cortex. Ultra-thin sections (80 nm) were collected onto nickel slot grids (EMS, FCF2010-Ni) and the grids were imaged on an HT7700 transmission electron microscope (Hitachi High-Technologies, Tokyo, Japan) using an advantage CCD camera (Advanced Microscopy Techniques, Danvers, MA). Images were adjusted for brightness and contrast using Adobe Photoshop CS4 11.0.1.

References

Deerinck, T.J., Bushong, E.A., Thor, A., Ellisman, M.H., 2010. NCMIR methods for 3D EM: a new protocol for preparation of biological specimens for serial block face scanning electron microscopy. Microscopy 1, 6–8.

Koo, T.K., Li, M.Y., 2016. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J. Chiropr. Med. 15, 155–163. https://doi.org/10.1016/j.jcm.2016.02.012

McKenzie, A.T., Woodoff-Leith, E., Dangoor, D., Cervera, A., Ressler, H., Whitney, K., Dams-O'Connor, K., Wu, Z., Hillman, E.M.C., Seifert, A.C., Crary, J.F., 2022. Ex situ perfusion fixation for brain banking: a technical report. Free Neuropathol. 3, 22–22. https://doi.org/10.17879/freeneuropathology-2022-4368