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Electron Microscopy of Brain Tissue Samples

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Livia Hecke Morais[1], Mark Ladinski[1]

[1]California Institute of Technology

ASAP Collaborative Rese…

Livia Hecke Morais
California Institute of Technology

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We use this protocol and it's working

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Abstract

Protocol has been approved by the California Institute of Technology's Institutional Animal Care and Use Committee (IACUC).

Brain perfusion

1 Mice were anesthetized with 150 uL pentobarbital (Euthasol), and their hearts were punctured.

2 Mice were perfused with 50 mL of 37°C PBS followed by 50 mL of 37°C 4% paraformaldehyde (PFA) at a flow rate of 6 mL/min.

3 Brains were dissected and immediately placed in a cold (4°C) fixative solution of 3% glutaraldehyde, 1% paraformaldehyde (Electron Microscopy Sciences, EMS), 5% sucrose in 0.1M sodium cacodylate
trihydrate.

Samples preparation

4 Tissue blocks were transferred to brass high-pressure freezing planchettes (Ted Pella, Inc.) prefilled with buffer containing 10% 70kD Ficoll (extracellular cryoprotectant).

5 Planchettes were placed into a high-pressure freezing machine (Bal-Tec HPM010) and ultra-rapidly frozen.

6 Planchettes with vitrified tissue samples were transferred under liquid nitrogen to cryotubes (Nunc) containing a frozen solution of 2% Osmium tetroxide (EMS), 0.05% uranyl acetate (EMS) in acetone.

7 Tubes were placed in a freeze-substitution machine (Leica Microsystems AFS2) and freeze-substituted as follows:

1. -90°C for 72 hrs.
2. Warm to -20°C over 24 hours.
3. Hold at -20°C for 12 hours
4. Warm to 4°C over 1 hour.

8 Samples, still within planchettes, were rinsed 3x with cold (4°C) acetone and brought to room temperature.

9

1. Samples were infiltrated into Epon-Araldite resin (EMS) as follows:
2. 2:1 (Acetone:Resin) for 1 hour
3. 1:1 for 1 hour

4. 1:2 for 1 hour

10 Samples transferred into 100% resin and removed from the planchettes.

11 Samples were allowed to infiltrate into resin for 24 hours, with gentle agitation.

12 Brain tissue samples were placed into resin containing accelerator (DMP30) and flat-embedded between two Teflon-coated glass microscope slides. Resin was polymerized for 24-48 hrs at 60°C.

13 Embedded brain tissue blocks were examined with a dissecting microscope to select optimum regions. These were excised with scalpel and reglued onto plastic sectioning stubs.

14 Semi-thin (170 nm) sections were cut with a ultramicrotome (Leica Microsytems UC6) using a diamond knife (Diatome, Ltd.). Sections were collected onto formvar-coated copper-rhodium 1mm slot grids (EMS) and stained with 3% uranyl acetate and lead citrate.

Imaging

15 Grids were imaged with a transmission electron microscope (Thermo-Fisher Tecnai T12-G2, 120k eV) equipped with a Gatan US1000 2k x 2k CMOS camera. Large-area montaged images of brain regions were collected automatically using the SerialEM software package.

16 Images were subsequently aligned and analyzed using the IMOD software package and AIVIA image analysis software (version 10.5.0; Leica Microsystems) with pixel classifier machine learning software to identify mitochondria.