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Electron Microscopy of Brain Tissue Samples

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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

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

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

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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.

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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.

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Semi-thin (170 nm) sections were cut with a ultramicrotome (Leica Microsystems 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

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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.

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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.