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Supplemental information

Preserving extracellular space for high-quality optical and ultrastructural studies of whole mammalian brains

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Supplemental Information

Table S1. List of immunoprobes and dilution ratios, related to Figure 3 and the STAR Methods.

Name of immunoprobes	Manufacturer and catalog number	Dilution
NeuN	Abcam, ab190195	1:100
GFP, A647	Invitrogen, A31852	1:100
PV	Abcam, ab11427	1:250
SST	Millipore Sigma, MAB 354	1:50
CR	Sigma-Aldrich, C7479	1:50
PSD-95	Antibodies Incorporated, 75-028	1:200
Anti-rat Fab2 A488	Jackson ImmunoResearch, 712-546-150	1:100
Anti-rabbit Fab2 A594	Jackson ImmunoResearch, 711-586-152	1:100
Anti-mouse IgG2a A488	ThermoFisher, A-21131	1:100
biotinylated WFA	Adipogen, VC-B-1355-M002	1:5000
Streptavidin A488	ThermoFisher Scientific, S11223	1:1000

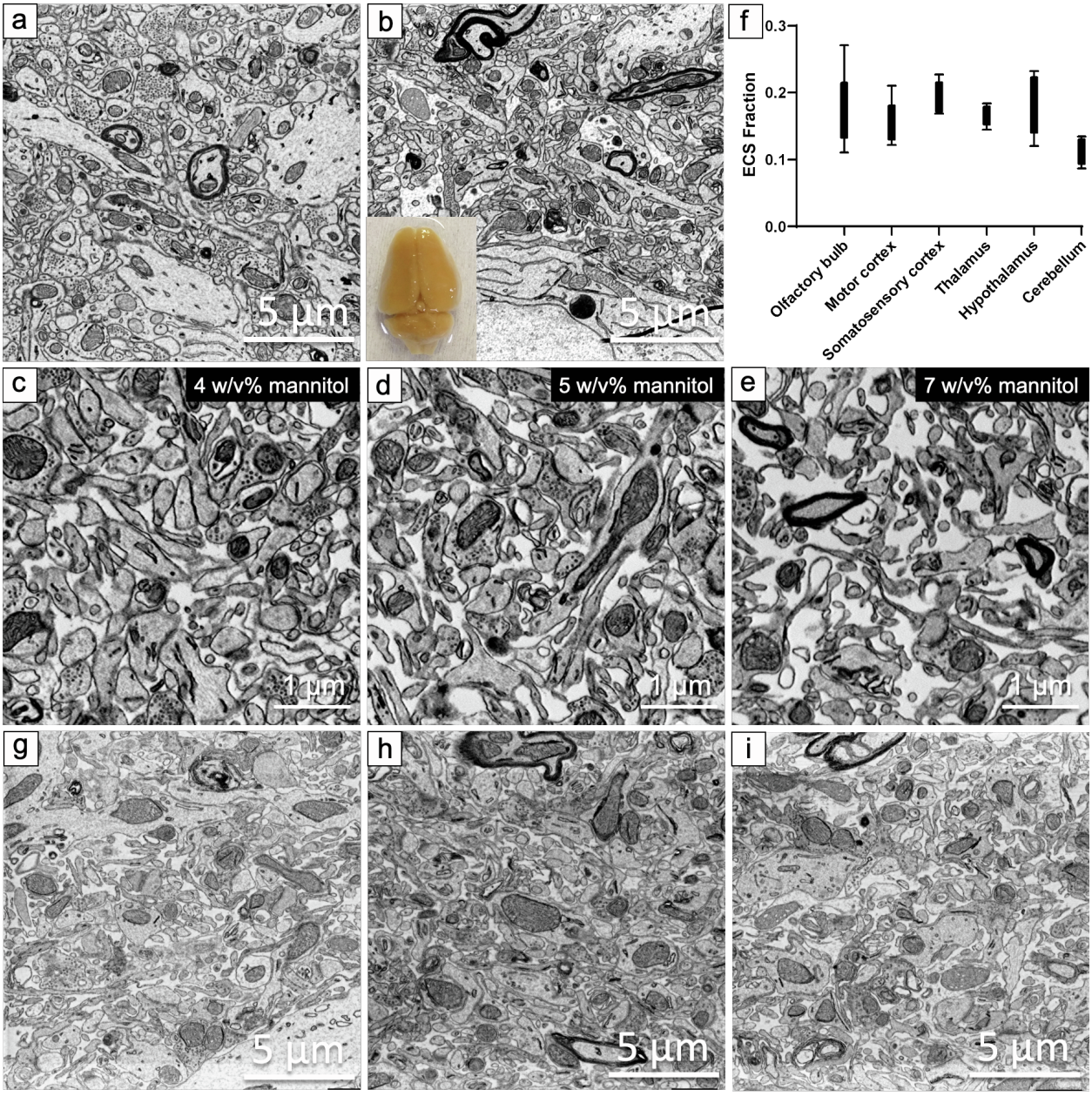

Figure S1: Parameter optimization for the ECS-preserved perfusion method, related to Figure 1. (a) When mannitol-added fixative was perfused after blood clearing (i.e., two-step perfusion), very little ECS was observed in the cortex. (b) When mannitol-added fixative was infused immediately after the opening of the BBB with a 15 w/v% mannitol in aCSF (i.e., three-step perfusion), no ECS was preserved in the cortical samples. (inset) The brain also showed an obvious volume shrinkage. (c-e) EM images were obtained in the center of 500 µm-thick brain slices that were incubated in a mannitol-containing aCSF and then transferred to a mannitol-containing fixative to determine the optimal mannitol concentration for ECS preservation: (c) 4 w/v% mannitol; (d) 5 w/v% mannitol; (e) 7 w/v% mannitol. (f) The ECS fraction in the perfusion-fixed mouse brain was measured in several brain regions of three different mouse brains. The olfactory bulb had an ECS fraction of 16.3 ± 6.9%, the motor cortex had 16.9 ± 3.1%, the somatosensory cortex had 17.3 ± 2.7%, the thalamus had 16.8 ± 1.6%, the hypothalamus had 18.7 ± 4.7%, and the cerebellum had 10.7 ± 2.0%. (g-i) EM images of the mouse brain cortices from three preparations showed that ECS was preserved in different samples using the mannitol-assisted four-step perfusion method.

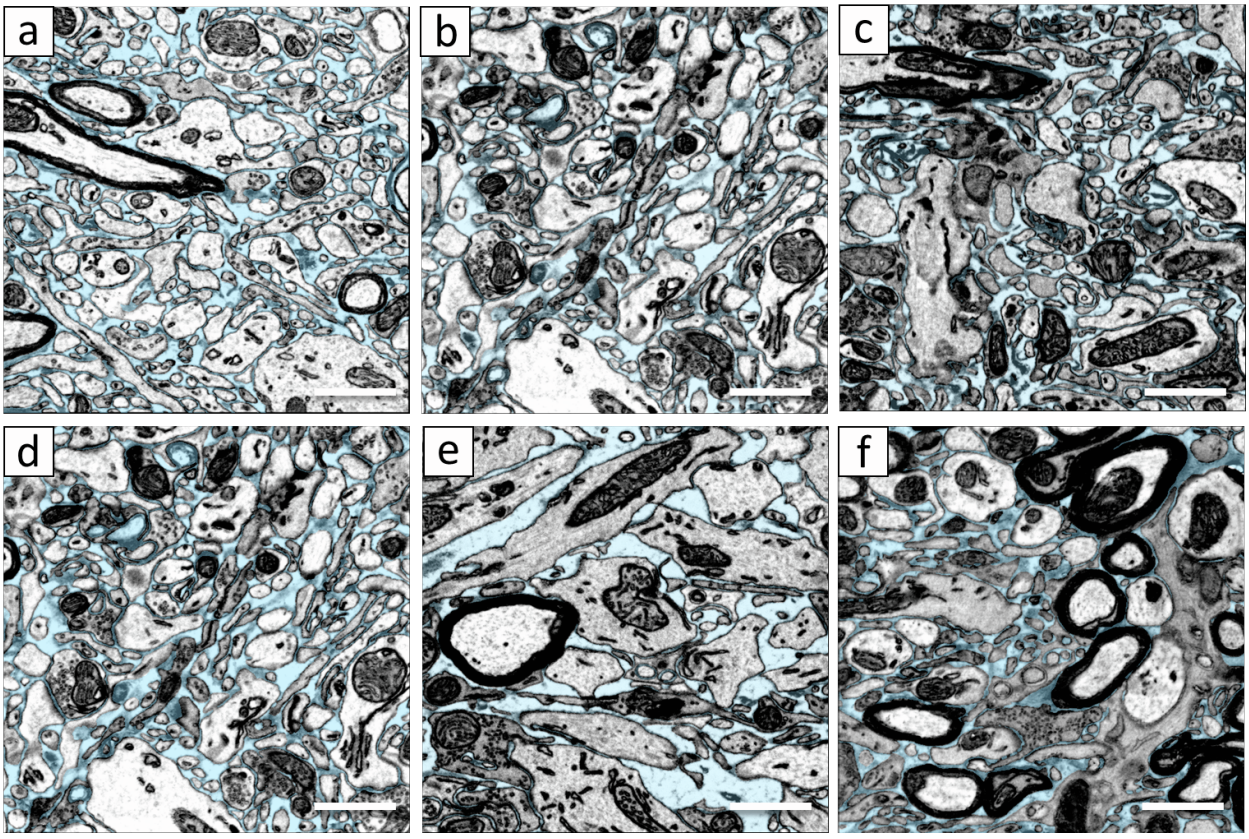

Figure S2: ECS was also preserved in rat brains using the four-step perfusion, related to Figure 1. EM images were taken from (a) motor cortex, (b) somatosensory cortex, (c) visual cortex, (d) olfactory bulb, (e) hippocampus, and (f) thalamus. Scale bars: 1 μm.

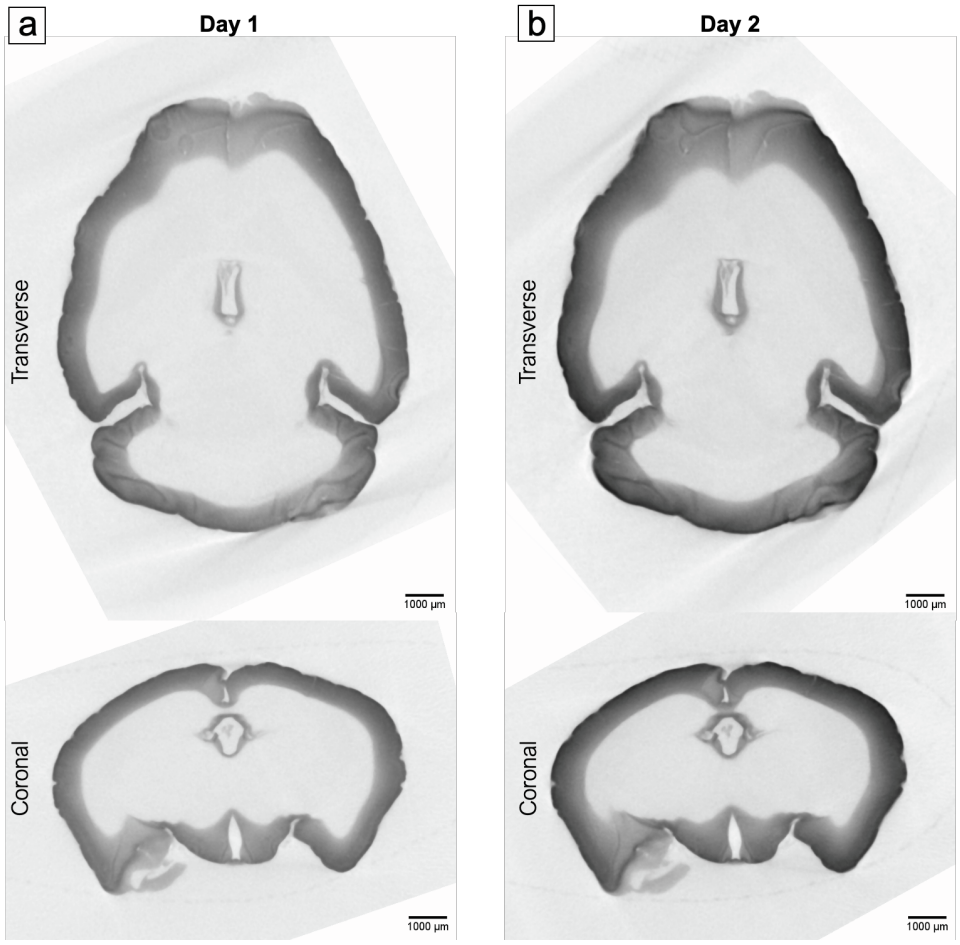

Figure S3: ROTO cannot be applied to whole brain staining, related to Figure 2. Ferrocyanide reduced osmium was applied on whole mouse brains that were fixed with ECS preservation, after one day (a) and two days (b) at room temperature. The results showed that the reduced osmium could penetrate up to 800 μm from the pia but did not progress further after 2 days. This finding suggests that the ROTO protocol is not suitable for whole mouse brain preparation due to its limited penetration depth.

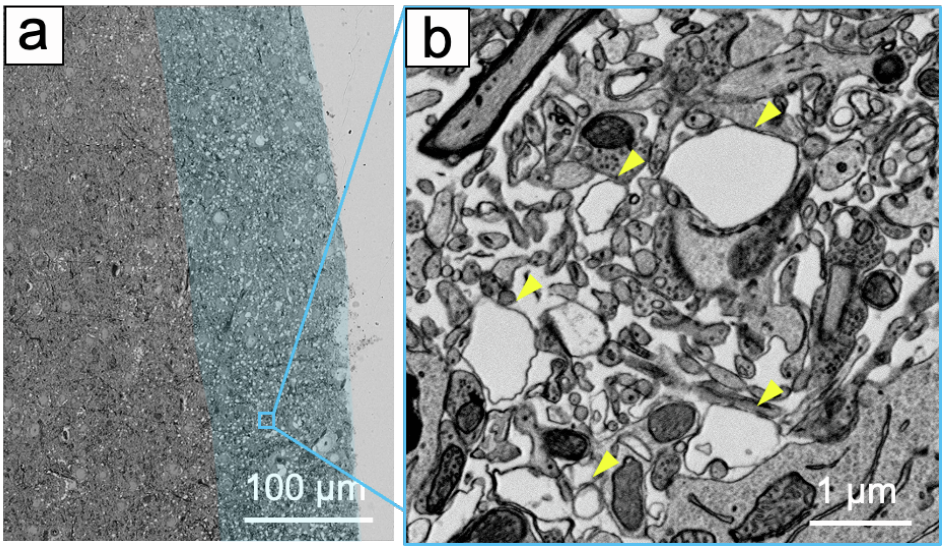

Figure S4: Vibratoming artifacts on unfixed brain tissues, related to Figure 3. (a) Vibratoming of acute brain slices can generate artifacts in the superficial part of the vibratome section (e.g., vacuolar structures in the blue-shaded region). (b) A higher magnification electron micrograph of empty vacuoles from the blue-shaded region in panel b (yellow arrows). Brain slices from transcardially fixed samples did not show this artifact.