Horseradish peroxidase (HRP) uptake/trafficking

1. Mice are anesthetized and placed in a stereotaxic apparatus with a mouse adapter (BenchMark Stereotaxic model from myNeuroLab, 477001).
2. The scalp is shaved and a midline incision made starting slightly behind the eyes, exposing the skull area. A hole is drilled in the skull and the coordinates for HRP perfusion into the lateral ventricle are AP -0.22 mm to Bregma, ML 1.0 mm to Bregma, and DV 2.5 mm to cranium, according to The Mouse Brain, in Stereotaxic Coordinates, by Keith B. J. Franklin and George Paxinos, Academic Press, ISBN 0-12-266070-6.
3. 20 µl of a 5% solution of HRP (Type VI-A, Sigma, P6782) in PBS are delivered into the lateral ventricle at a rate of 1 µl/min.
4. 24 hours after the perfusion, mice are fixed by cardiac perfusion using 2% PFA, 2% glutaraldehyde containing 0.025 % $CaCl_2$ in 0.1 M sodium cacodylate buffer, pH 7.2.
5. Brain sections (50-µm thick) are cut on a Vibratome and rinsed in cacodylate buffer 5 times to wash out the fixative.
6. Sections are then incubated in a DAB solution, made according to manufacturer's instructions (DAB Substrate Kit, SK-4100, Vector Laboratories) and filtered with a 0.22-µm syringe-filter, for 5-10 min at room temperature.
7. Sections are washed several times in cacodylate buffer, then either mounted onto gelatin-coated slides for light microscopic analysis, or post-fixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide for 30 min, dehydrated and embedded for EM. The tissue is not stained en bloc, nor ultrathin sections post-stained with heavy metals.