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Immunogold ultrastructural localization of neural antigens in Lowicryl HM20 embedded, freeze substituted rat brain tissue.

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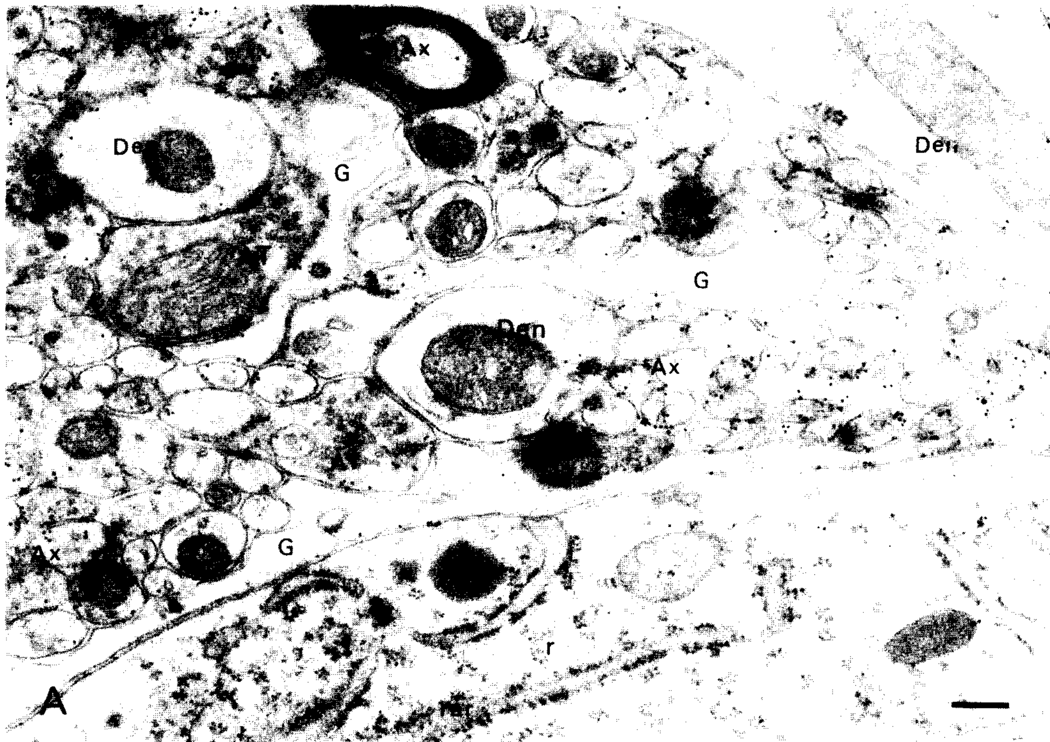
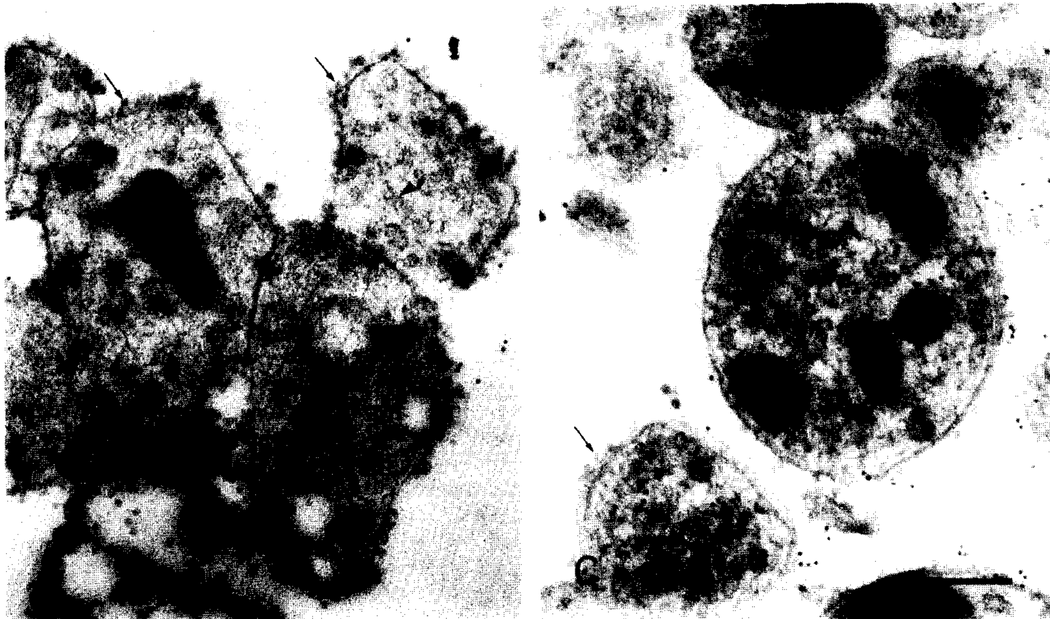
Ultrastructural localization of several neuronal antigens has been successfully performed by immunogold labelling of ultrathin melted cryosections. Labelling density is high, but integrity of the sections is not sufficient to obtain overviews and to trace specific structures in the brain tissue. To meet these requirements, we examined the suitability of freeze substitution and Lowicryl HM20 embedding to localize several neural antigens in large and serial sections at the ultrastructural level. The mesencephalon of rat brain was perfusion-fixed with 2% glutaraldehyde and 4% paraformaldehyde, cryoprotected and frozen in liquid nitrogen. Freeze substitution was performed with anhydrous methanol and 0.5% uranyl acetate at -90°C. We studied the ultrastructural localization of the following antigens: B-50, a presynaptic membrane-anchored protein, glial fibrillary acidic protein, a subunit of glial intermediate filaments, myelin basic protein, a component of central nervous system myelin, ACTH, a neuropeptide present in dense core granules and synaptophysin (p38), an integral protein of synaptic vesicle membranes. Dehydration by freeze substitution preserves the ultrastructure of the mesencephalon very well, allowing precise immunogold single and double localization of the various antigens on large and serial sections. An ultrathin Lowicryl section of the mesencephalon (Fig. A) shows part of a cell body with rough endoplasmic reticulum (rer) and free ribosomes (r). In the surrounding neuropil, myelinated axons (MAx), small unmyelinated axons (Ax), axon terminals (AT) filled with small synaptic vesicles, glial cell processes (G) and dendrites (Den) are clearly recognized. B-50 immunoreactivity, as shown by 10 nm gold particles, is found at the plasma membrane of unmyelinated axons and axon terminals, but absent from dendrites and glial cell processes. To examine the effect of aldehyde fixation prior to freezing, synaptosomes were isolated and either chemically fixed with 3% glutaraldehyde or cryo-fixed in liquid propane with a Reichert KF80. The ultrastrucuture of the cryofixed synaptosomes (Fig. B, C) is well preserved after freeze substitution and Lowicryl embedding, allowing detection of small synaptic vesicles of different sizes (arrowheads). B-50 immunoreactivity in cryofixed synaptosomes (Fig. B) is increased compared to chemically fixed synaptosomes, and shows localization at the plasma membrane and, in addition, at vesicles. Immunoreactivity for synaptophysin in cryo-fixed freeze substituted synaptosomes (Fig. C) is comparable to aldehyde prefixed synaptosomes, indicating that aldehydes do not neccessarily affect immunoreactivity of all neuronal antigens. In conclusion, fixed brain tissue can be successfully dehydrated by freeze substitution and embedded in Lowicryl. Large and serial sections can be processed, allowing localization of scarcely distributed antigens and double labelling on serial adjacent sections. The excellent morphological preservation and conservation of antigenicity of a variety of proteins suggests that this method can have wide applications for post embedding immunocytochemistry on brain tissues.

Legend:
A. An ultrathin section of rat mesencephalon, fixed by perfusion and embedded in Lowicryl HM20 by the freeze substitution method. B-50 is detected with primary antibodies and 10 nm gold particles coupled to the secondary antibodies. B-50 immunoreactivity is present at plasma membranes of small axons (Ax) and axon terminals (At), but absent from dendrites (Den), glial processes (G) and a cell body. r = ribosomes, rer = rough endoplasmic reticulum. B, C. Ultrathin sections of rat brain





synaptosomes, cryofixed and embedded in Lowicryl HM20 as described above and incubated with polyclonal antibodies to B-50 (B) and monoclonal antibodies to synaptophysin (C). B-50 immunoreactivity (B) is found at the plasma membrane as well as at vesicles (arrowheads). Synaptophysin immunoreactivity (C) is only found at vesicles. Small arrows indicate Percoll particles, used to prepare density gradients for the isolation of the synaptosomes. Scale bars = 0.2 μm.