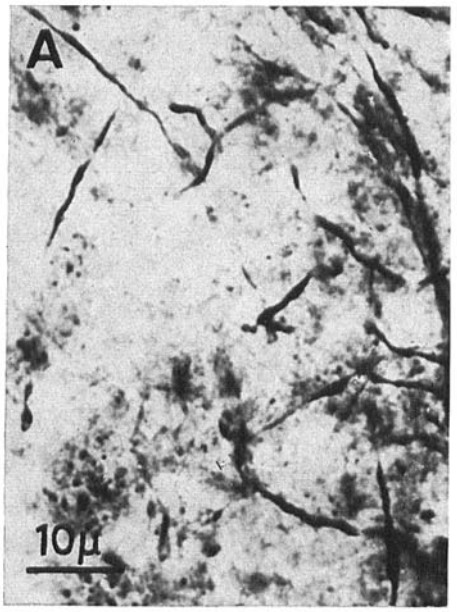
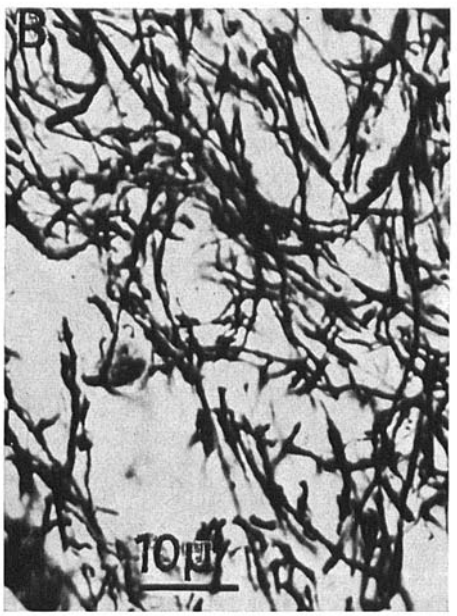
Neuronal Argyrophilia in Early Degenerative States: A Light and Electron-Microscopic Study of the GLEES and NAUTA Techniques

Studies on the mechanism of silver impregnation suggest that the much-used GLEES and NAUTA-GYGAX methods have highly specific, although fundamentally different, reaction sites[1-5]. Attractive as this notion of high tinctorial selectivity appears to be at first glance, it is not entirely clear how to reconcile it with the great diversity of histological structures, inside as well as outside the central nervous system, which respond positively to silver impregnation of both the GLEES and NAUTA types. It therefore appeared desirable to re-investigate the matter by a combined light- and electron-microscopic study.

In this study the fornix was cut electrolytically in 3 rats approximately 4 months of age. After 2, 3 and 4 days respectively the animals were killed by an overdose of Nembutal, and perfused transcardially with physiological saline followed by 10% buffered formalin solution (pH 7.7). After 2 days in formalin, the brains were sectioned on a freezing microtome at 50–100 μ, and sections were impregnated according to the techniques of GLEES[6] and NAUTA-GYGAX[7]. From some of the impregnated sections, pieces from the lateral or posterior part of the medial mammillary nucleus were excised and post-fixed in an osmic acid solution, dehydrated in ethanol, and imbedded in Epon for subsequent electron-microscopic examination. An unoperated animal of the same age was used as a control.

The light-microscopic appearance of the normal medial mammillary nucleus prepared according to the NAUTA-GYGAX and GLEES methods is seen in Figures 1A and B, respectively. The appearance of the medial mammillary nucleus in the NAUTA-GYGAX sections 2 days after fornix section is seen in Figure 2. Many irregular argyrophilic bodies, probably representing individual degenerated boutons, are seen. The GLEES stained sections on the second day of the degeneration process did not show any terminals and had an appearance closely comparable to the normal state. 3 days after fornix lesion both the NAUTA-GYGAX sections (Figure 3A) and the GLEES sections (Figure 3B) show a massive terminal degeneration. The GLEES technique failed to demonstrate degenerated





Figs. 1A and B. Photomicrographs of the medial mammillary nucleus in a non-operated animal. Intact nerve fibres are impregnated both in the NAUTA-GYGAX section (A) and in the GLEES section (B). However, no synaptic end-formations are visible.

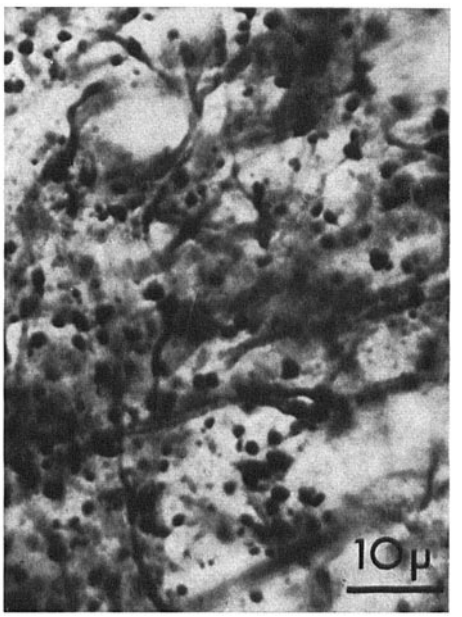


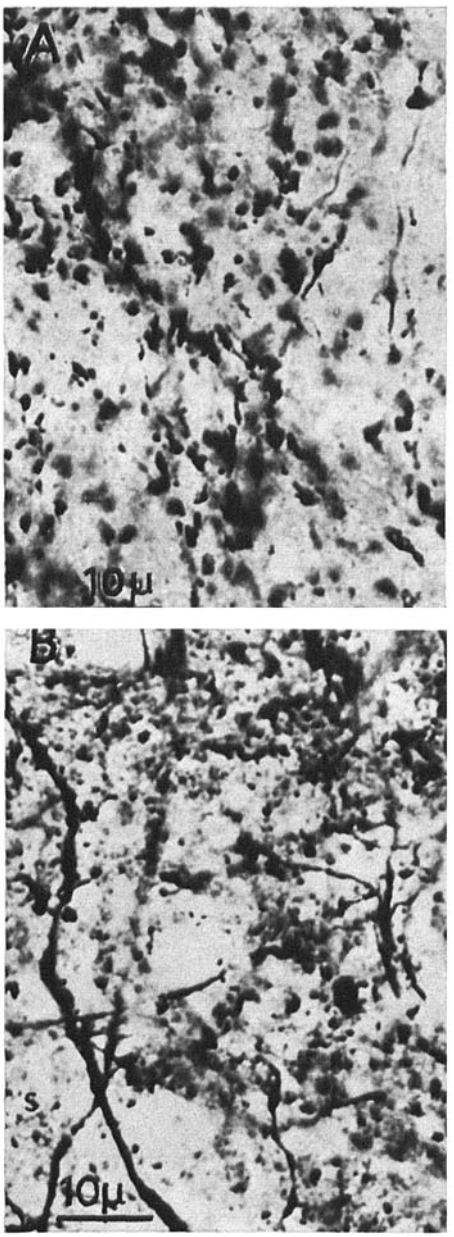
Fig. 2. The medial mammillary nucleus in an animal sacrificed 2 days after a fornix section. Many irregular and drop-shaped argyrophilic bodies, probably representing individual degenerated boutons, are visible (NAUTA-GYGAX method).

[1] D. H. L. EVANS and L. H. HAMLYN, J. Anat. *90*, 193 (1956).
[2] E. G. GRAY and L. H. HAMLYN, J. Anat. *96*, 309 (1962).
[3] R. W. GUILLERY and H. J. RALSTON, Science *143*, 1331 (1964).
[4] R. D. LUND and L. E. WESTRUM, Science *151*, 1397 (1966).
[5] R. P. EAGER and R. J. BARRNETT, J. comp. Neurol. *126*, 487 (1966).
[6] P. GLEES, J. Neuropath. exp. Neurol. *5*, 54 (1946).
[7] W. J. H. NAUTA, in *New Research Techniques of Neuroanatomy* (Ed. W. F. WINDLE; Charles C. Thomas, Springfield, Illinois 1957).

boutons after a 4-day post-surgical survival period. In such material the NAUTA-GYGAX method, by contrast, produced pictures closely resembling those found in animals with 3 days survival time.

The electron-microscopic appearance of NAUTA-GYGAX impregnated sections 2 days after fornix section is shown in Figures 4 and 5. The erratic deposition of silver particles in the tissue is obvious, and almost everywhere silver can be found. However, in the axons (Figure 4) and the boutons (Figure 5), a particularly heavy accumulation of silver has taken place. Silver was often seen in relation to neurofilaments of the axons. The myelin and the mitochondria, however, were relatively free of silver. When such 'silver-powdered' terminals (see Figure 5) are seen under the much lower magnification of the light microscope they will very likely have the appearance of solid black bodies. No GLEES-impregnated section suitable for electron-microscopic study was obtained from the mammillary body. Fortunately, a GLEES-impregnated section reasonably suitable for subsequent electron-microscopic study was obtained from the apparently normal cerebral cortex of another rat. An axon appearing in the corresponding electron-micrograph is shown in Figure 6. Despite the obvious imperfections of the material, it is noteworthy that the silver particles accumulated in the axon are confined almost exclusively to axoplasmal regions occupied by neurofilaments.

PETERS[8], using a reduced silver method, showed electron-microscopically that the silver had a tendency to be deposited along the filaments of the normal nerve fibre, and in more recent studies, GRAY and HAMLYN[2] and GUILLERY[9] suggested that the presence of neurofilaments is a prerequisite for positive findings with the GLEES method. However, this issue appears to be controversial. In the present study no ring-like structures suggesting the impregnation of a ring of neurofilaments were seen in GLEES sections from the mammillary body (Figure 3B), nor does WALBERG[10] consider the presence of neurofilaments necessary for positive GLEES impregnation. Regarding the NAUTA-GYGAX method, GUILLERY and RALSTON[3] found neurofilaments and synaptic vesicles



Figs. 3A and B. Massive terminal degeneration in the medial mammillary nucleus in an animal sacrificed 3 days after fornix lesion. NAUTA-GYGAX (A) and GLEES (B) methods, respectively.

[8] A. PETERS, Q. Jl microsc. Sci. *96*, 317 (1955).
[9] R. W. GUILLERY, in *Progress in Brain Research* (Ed. M. SINGER and J. P. SCHADE; Elsevier, Amsterdam 1965), vol. 14, p. 57.
[10] F. WALBERG, J. comp. Neurol. *122*, 113 (1964).

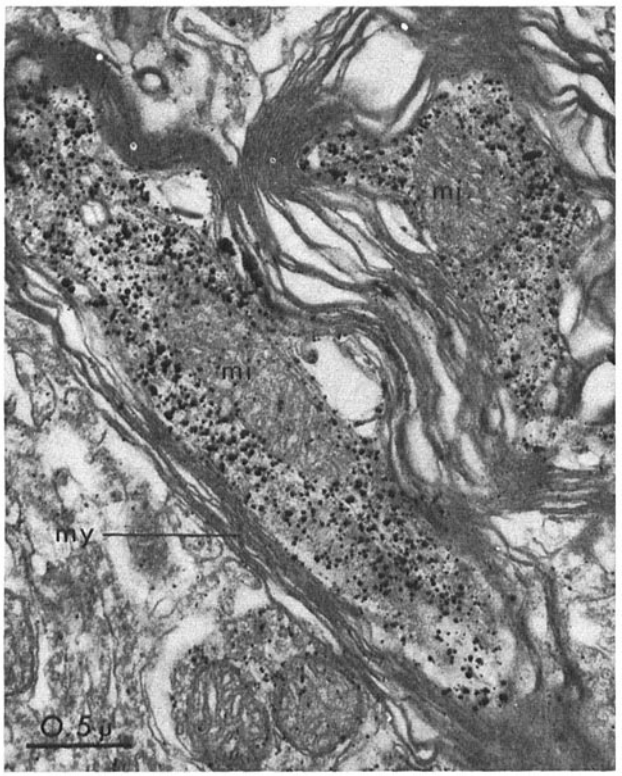
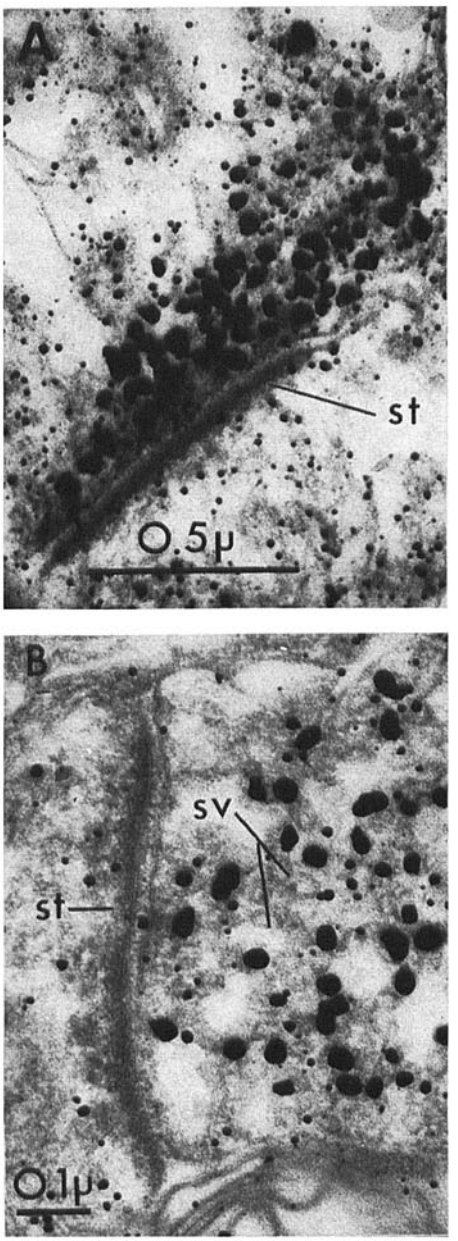


Fig. 4. Electron-micrograph of a NAUTA-GYGAX impregnated section showing 2 myelinated axons in the mammillary body. The animal had undergone unilateral fornix section 2 days before sacrifice, but it is uncertain whether the axons shown are normal or degenerating. There are very few silver particles found in the myelin (my) or in the mitochondria (mi), whereas the rest of the axon is powdered with silver.

free from silver and assumed that the argyrophilia is dependent upon another hitherto unidentified component of the axoplasm, whereas LUND and WESTRUM[4] found silver granula especially in relation to synaptic vesicles and mitochondria. However, there seems to be general agreement in previous studies that the neurofilaments are not argyrophilic in the suppressive NAUTA-GYGAX method[3-5].

It must be emphasized that the results of the present study are in less than complete agreement with the results obtained by previous investigators. Argyrophilia, however, is influenced by many physical as well as chemical factors, and it may be suggested that the form and sites of metallic silver deposition may depend upon numerous and often subtle variables in the technical procedures followed. It is a well-known fact, for instance, that the results with the NAUTA-GYGAX technique are highly dependent upon such variables as the permanganate treatment, one of the less controllable steps in the process. The different results obtained by various investigators may be a consequence of apparently minor differences in the silver procedure used. This may, in fact, result in different patterns of silver deposition in the tissue. Consequently, it may not be possible to categorically relate results obtained with these silver techniques to any specific morphological component of the axon[11].



Figs. 5A and B. Electron-micrographs of NAUTA-GYGAX impregnated sections, showing synaptic endings in the medial mammillary nucleus of an animal in which the fornix was sectioned 2 days before sacrifice. (Compare the light microscopic appearance in Figure 2.) The silver particles are concentrated within the terminals which can be identified by the synaptic thickenings (st) and the synaptic vesicles (sv).

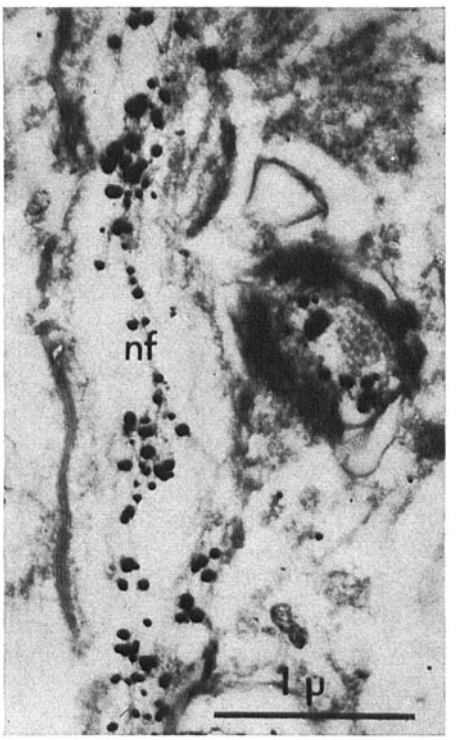


Fig. 6. Electron-micrograph of a GLEES impregnated section showing myelinated axons in the cortex of a normal rat. Practically all the silver particles show a relation to neurofilaments (nf).

Zusammenfassung. Die Grundlagen für die Silbermethoden nach NAUTA und GLEES werden elektronenmikroskopisch untersucht und die Verteilung der Silbergranula im Gewebe festgestellt.

L. HEIMER[12] and R. EKHOLM

Department of Anatomy, University of Göteborg (Sweden), 7th October 1966.

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[12] Present address: Department of Psychology, Massachusetts Institute of Technology, Cambridge (Massachusetts 02139, USA).