The staining of degeneration in the nervous system of the octopus by modified silver methods

By R. D. LUND

(Department of Anatomy, University College, London, W.C. 1)

With 1 plate

Summary

The Nauta-Gygax and Glees methods used on mammalian material for demonstrating degenerating nerve fibres have been modified for similar use on Octopus vulgaris. The results compare well with the Cajal method which has been employed up to the present. Degenerating fibres stain more intensely than with the Cajal method and many of the normal fibres are differentially suppressed.

Introduction

Degenerating axons of cephalopod material can be stained with a Cajal method (Sereni and Young, 1932). Whilst this has proved extremely valuable for demonstrating principal fibre connexions, it becomes more difficult to interpret the results when identifying degenerating fibres which are diffusely distributed within neuropil, or in tracts composed mainly of normal fibres. This is largely because there is no differential staining of degenerating and normal fibres, such as can be found in vertebrate material stained by the Nauta and Gygax method (Nauta and Gygax, 1954). A further difficulty is that degenerating fibres stained by the Cajal method often do not appear as grossly distorted as vertebrate degenerating fibres stained by the Glees method (Glees, 1946) or either Nauta and Gygax method (Nauta and Gygax, 1951, 1954). However, none of these techniques for vertebrates is satisfactory when used on the octopus brain. It has therefore been considered valuable to adapt them for use on such material.

A large number of variants have been tried. The two outlined below are found to be the most successful in that they consistently produce reliable results, and stain a maximum amount of degeneration when compared with Cajal-stained material.

The preliminary study has been made on Octopus vulgaris in which one optic tract has been cut intracranially. Post-operative survival times of one to six days have been used with a tank temperature of 20 to 25°C.

Experimental procedure

Modification of the Nauta-Gygax method

1. Fix material in 10% formol saline for one to six weeks.
2. Cut frozen sections at 30 μ.
3. Wash in distilled water.

4. Place in 50% ethyl alcohol (100 parts) and 0.88 ammonia solution (1 part) for 6 h.
5. Wash in three changes of distilled water.
6. Place in 10% silver nitrate for 12 to 18 h.
7. Wash briefly in distilled water.
8. Transfer to the following ammoniacal silver bath for one min:
   - Silver nitrate (4.5%) 20 ml
   - Ethyl alcohol 10 ml
   - Ammonia (0.88) 2 ml
   - Sodium hydroxide 2.5% 1.4 ml
9. Transfer to the standard Nauta reducer for one min:
   - Distilled water 400 ml
   - Ethyl alcohol 45 ml
   - Formalin 10% 13.5 ml
   - Citric acid 1% 13.5 ml
10. Wash briefly in distilled water and fix in 5% sodium thiosulphate for one min.
11. Wash, mount, dehydrate, clear, and cover.

Modification of the Glees method

Stages 1–6 as above
7. Wash for 30 min in distilled water.
8. Transfer to the standard ammoniacal silver bath for one min:
   - Silver nitrate 20% 15 ml
   - Ethyl alcohol 10 ml

Add concentrated ammonia until the solution clears, and then add a further six drops.
9. Transfer through three changes of 3% formalin in tap water.
10. Wash briefly in distilled water and fix in 5% sodium thiosulphate.
11. Wash, mount, dehydrate, clear, and cover.

This method stains frozen sections mounted on gelatinized slides particularly successfully. Sections are mounted under water, left to dry over night and stained exactly as above. This is useful for maintaining the serial order of sections.

Figs. 1 to 4. The modified techniques applied to the octopus brain, after section of one optic tract.
1. Degeneration in the lateral superior frontal lobe ipsilateral to the lesion. Glees modification. 3 days’ survival.
2. Absence of degeneration in the contralateral lobe on the same section as 1, stained in the same manner.
3. A small degenerating tract (arrow) distributing in the contralateral optic lobe. Nauta-Gygax modification. 2 days’ survival.
4. A clump of degeneration and typical degeneration granules in the optic commissure. Glees modification on mounted sections. 3 days’ survival.
When used on mammalian material, the method proved unsatisfactory, failing to stain any fibres at all.

**Discussion**

Both techniques resemble the Bielschowsky method more closely than do either unmodified Nauta and Gygax or Glees methods. As would be expected, degeneration appearing as a neurofibrillar proliferation is stained well. However, a considerable number of normal fibres known to contain neurofibrils can be suppressed without suppression of degenerating fibres. This indicates that other factors than the presence of neurofibrils are responsible for the specific staining properties.

Each method discriminates degenerating fibres more clearly from the background than does the Cajal. They are evident even at low power as is seen in figs. 1, 2, and 3. Normal fibres tend to be more heavily stained in the Glees than the Nauta and Gygax modification for unmounted sections, but the best differential staining of degenerate fibres occurs using the Glees modification on mounted sections. However, in each method degenerating fibres can be differentiated from stained normals, since they appear as small granules rather than continuous fibres.

In addition clumps of degeneration are found in larger tracts, generally not orientated in line with the fibres. They become more numerous and more bizarre in shape after longer survival times. One such clump is shown in fig. 4. The positively stained material does not appear to be related to a macrophage cell. The exact time for which degeneration continues to stain positively has not yet been adequately investigated. For the optic tract an optimum is reached at about three days with a tank temperature of 22–24° C. However, some of the degeneration can still be stained after a survival time of six days at a temperature of 20° C. The relation of fibre diameter and of tank temperature to optimum survival time for the staining of degeneration are at present under investigation. On the whole, it would appear that longer survival times can be used, particularly with the Glees modification, than with the Cajal method.

Further investigations of the application of the method after damage to other systems than the optic tract have so far shown little difference in staining properties from those described here.

I should like to thank Professor J. Z. Young, and Drs. J. Messenger, R. Martin, and A. Packard for providing operated material, as well as Dr. J. Parris for considerable help in this project, and Mr. A. Aldrich for assistance with the photography.

**References**