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Number of fibres per sheath cell and internodal length in cat cranial nerves

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INTRODUCTION

Structures similar to the nodes of Ranvier of peripheral nerve fibres are well known to occur in the central nervous system (Feindel, Allison & Weddell, 1948; Allison & Feindel, 1949; Bodian, 1951; Pease, 1955; Uzman & Villegas, 1960; Metuzals, 1965; Laatsch & Cowan, 1966; Peters, 1966). The length of the internodes of central nerve fibres has been estimated as being nearly the same as that of peripheral nerve fibres, and both show similar relations between internodal length and axon calibre (Hess & Young, 1949; Thomas & Young, 1949; Bodian, 1951; Haug, 1967). Each internode of a myelinated mammalian peripheral nerve fibre is formed by a single Schwann cell whereas a single oligodendroglial cell may myelinate more than one fibre (Cajal, 1928; Hortega, 1928; Bunge, 1968). The only specific investigation, however, on the number of axons supplied by an 'average' oligodendroglial cell appears to be that of Peters & Proskauer (1969) who concluded that one oligodendrocyte of rat optic nerve myelinates 30–50 fibres.

The goal of the present investigation was to obtain data on the average number of fibres sheathed by one oligodendroglial cell, including data on internodal length. Cranial nerves are particularly suitable for such studies, as one and the same fibre population passes through the central and peripheral portion of the nerve, while its sheath cell population changes abruptly at the glia–Schwann cell border (Tarlov, 1937).

MATERIAL AND METHODS

Six adult female cats, 2–3 kg in weight, were sacrificed under deep Pentothal anaesthesia (Diabutal, 30 mg/kg); the central nervous system was fixed by perfusion from the heart at a pressure approximating normal blood pressure and/or by flooding the basilar subarachnoid space with fixative (2·5 % glutaraldehyde in 0·1 M phosphate buffer, pH 7·2–7·4, chilled at 4 °C).

After removing the head the cranial nerve roots were exposed ventrally by removing the clivus, carefully avoiding stretching or dislocating the roots; cold fixative was continually flushed around the roots during preparation, and the exposed roots were postfixed by immersion of the specimens in chilled fixative for 24 h. The cranial nerves III, V and VIII were then removed bilaterally in such a way as to include the glia–Schwann cell border with generous proximal and distal segments of the roots as well as some of their intracerebral course. Specimens were transferred to cold buffered sucrose for storage.

Tissues were embedded in Maraglas, cut approximately 1 μm thick on a Porter–Blum ultramicrotome and stained with *p*-phenylene diamine in 7% isopropyl alcohol. Other specimens were embedded in paraffin and stained with haematoxylin and eosin or with the periodic acid–Schiff method. Additional frozen sections were obtained from specimens fixed with formalin ammonium bromide and stained with Cajal's gold sublimate method for astrocytes. Nuclear densities were determined at various distances from the glia–Schwann cell border in the proximal and distal portions of the roots. To this end, paraffin-embedded longitudinal sections of the roots, cut parallel to fibre axes, were photographed with a Zeiss Ultraphot photomicroscope and printed on 16×20 in paper at a final enlargement of $320\times$. The glia–Schwann cell border was identified, and 30–40 parallel lines were drawn 5 mm apart proximal and distal to the border. All nuclei, exclusive of endothelial nuclei, were counted for each interval. No attempt was made to correct the data for shrinkage due to fixation and processing. All counts were made in longitudinal sections because counting nuclei in sections cut tranverse to fibre axes would introduce unacceptable error due to the great variation in the long axis of Schwann cell nuclei. The difference between the short axis of the Schwann cell nuclei and the diameter of glial nuclei in our specimens was too small to warrant correction of the crude counts by Abercrombie's (1946) formula; such correction would also be irrelevant as our counts compare cell densities in two portions of the same section. Differential counts of oligodendroglia and astrocytes in the central portion of the roots were based on silver impregnations of astrocytes; the latter were selected because the results were, in our hands, more consistent than those obtained by methods for the impregnation of oligodendroglia. The total number of glial nuclei was counted along with the number of nuclei encompassed by argyrophilic cytoplasm having long processes characteristic of astrocytes.

Fibre spectra proximal and distal to the glia–Schwann cell border were determined in transverse sections of plastic-embedded material, cut precisely perpendicular to fibre axis, and approximately 1–2 mm on either side of the glia–Schwann cell border. Photographs were taken with oil-immersion lenses at a primary magnification of $1000\times$ and enlarged to $5100\times$. One hundred axons were selected at random for each proximal and distal segment of the nerves by drawing a grid of equidistant lines across the prints then measuring each fibre touched by one of the lines. This method of random sampling reduced the need for measuring larger numbers of fibres. The calibres of fibres were determined in terms of axon circumference, using a Dietzgen plan meter (Friede & Samorajski, 1967, 1968); previous electron microscopic studies have shown that this method is more accurate than measuring diameters, in that it is affected neither by the irregularities of normal fibre profiles nor by shrinkage or redistribution of axoplasm along the fibre during fixation and processing of the tissue. Histograms were plotted and mean axon circumferences as well as *t* values for the differences were calculated.

RESULTS

The glia–Schwann cell border, separating the central and peripheral portions of the cranial nerve roots, transected the tissue in a straight or undulating plane. The loose texture of the tissue ('Aufhellungzone') found proximal to the glia–Schwann

cell border in non-perfused material (Tarlov, 1937) was absent in our specimens. This zone probably represents an artefact due to delayed fixation and, perhaps, slight stretching of the nerve during removal. The proximal surface of the glia–Schwann cell border is lined with astrocytic footplates (Tarlov, 1937; Maxwell, Kruger & Pineda, 1969), which are known to swell and disintegrate rapidly with delayed fixation. Accumulation of glycogen granules (PAS reaction) just proximal to the glia–Schwann cell border of our specimens was consistent with the high glycogen content of glial footplates observed in electron micrographs (Maxwell *et al.* 1969).

Significant differences in nuclear densities were observed between the central and the peripheral portions of the roots (Table 1). Counts in silver-impregnated sections indicated that approximately 20% of the glial nuclei in the proximal portion of the roots were astrocytes. This value was consistent with the frequency of nuclei having the features generally considered characteristic of astrocytes in conventionally stained preparations. If one assumes, then, that approximately 80% of the glia cells in the proximal portion of the root were sheath cells, sheath cell ratios ranged

Table 1. *Nuclear densities proximal and distal to the glia–Schwann cell border*

Distance from glia–Schwann border*	Cat 1			Cat 2			Cat 3		
	Proximal	Distal	Prox./dist.	Proximal	Distal	Prox./dist.	Proximal	Distal	Prox./dist.
				Oculomotor nerve					
0–156 μm	26	57	1/2·2	35	45	1/1·3	22	38	1/1·9
156–312 μm	29	36	1/1·2	30	30	1/1·0	17	34	1/2·0
312–468 μm	18	46	1/2·5	12	33	1/2·8	23	28	1/1·2
486–624 μm	12	—	—	—	41	—	—	36	—
Totals	83	139	1/1·7	77	108	1/1·4	62	100	1/1·6
Totals corrected for astrocytes	68	139	1/2·1	62	108	1/1·7	50	100	1/2·0
				Trigeminal nerve					
0–156 μm	31	78	1/2·5	18	45	1/2·5	42	69	1/1·6
156–312 μm	27	54	1/2·0	21	40	1/2·0	46	54	1/1·2
312–468 μm	21	40	1/2·0	15	37	1/2·5	—	—	—
468–624 μm	—	—	—	15	44	1/2·9	—	—	—
Totals	79	172	1/2·2	69	166	1/2·4	88	123	1/1·4
Totals corrected for astrocytes	63	172	1/2·7	55	166	1/3·0	70	123	1/1·8
				Acoustic nerve					
0–156 μm	61	8	1/1·4	83	164	1/2·0	44	157	1/3·6
156–312 μm	52	94	1/1·4	57	74	1/1·3	65	84	1/1·3
312–468 μm	41	98	1/2·4	31	110	1/3·6	59	133	1/2·6
468–624 μm	15	—	—	47	85	1/1·8	36	113	1/3·1
Totals	145	280	11/1·8	218	433	1/2·0	204	447	1/2·2
Totals corrected for astrocytes	123	280	1/2·3	174	433	1/2·5	163	447	1/2·7

* The distances correspond to 10 lines in Fig. 1.

between 1–2 and 1–3 for the two portions of the roots; the average ratio was higher for the acoustic than for the oculomotor nerve. Comparison of the total and corrected counts in Table 1 indicates that any reasonable extent of error in determining astrocytic density would change the ratios by less than the extent of variation between nerve samples.

As the myelin sheaths are not continuous across the glia–Schwann cell border, there was an alignment of all the nodes of Ranvier and consequently, also, of the entire

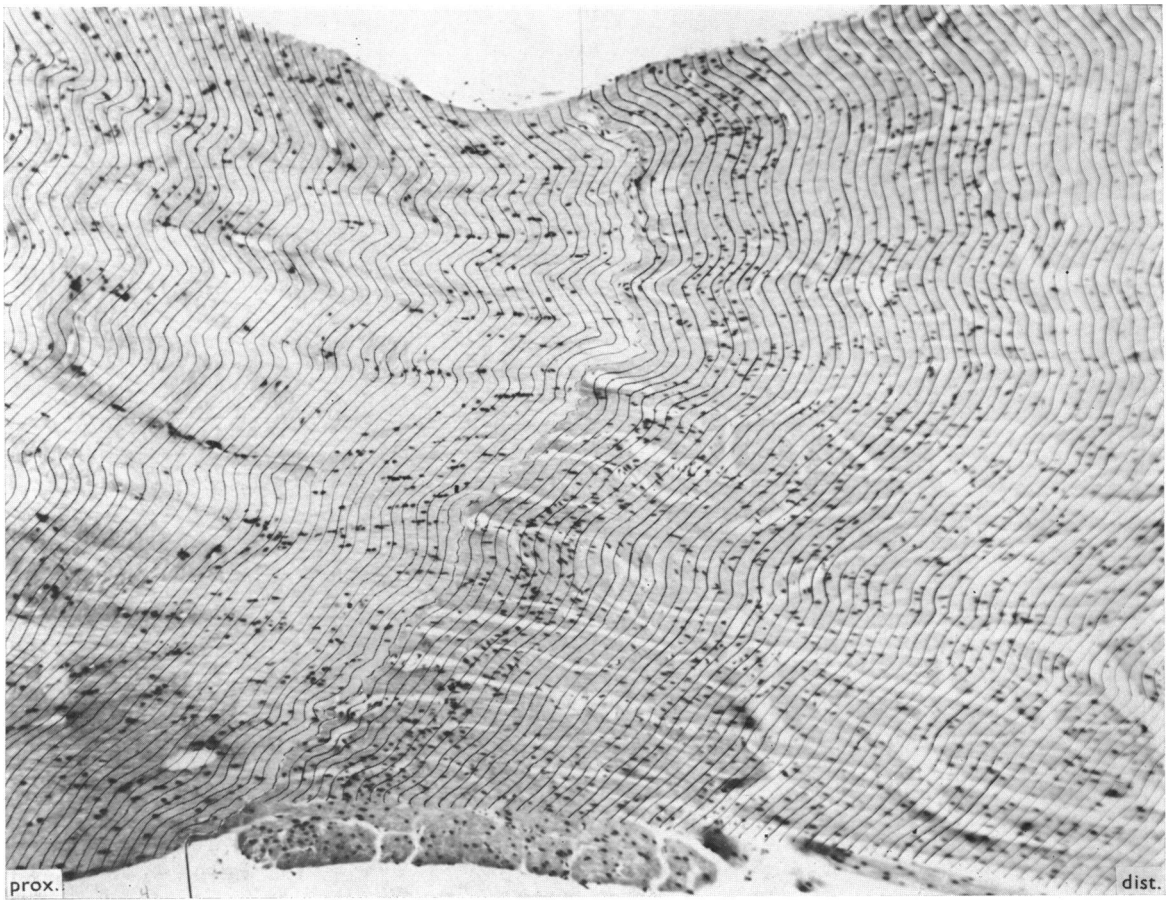

Fig. 1. Sample of preparations used for determining the density of nuclei in relation to distance from the glia–Schwann cell border. The number of nuclei was determined for a continuous strip of tissue having equal area for each interval between two parallel lines. The alignment of the first and second Schwann cell generations is evident. This illustration is a photograph of a 16×20 in print and some sharpness is lost in the reproduction.

first generations of internodes. Nuclear density was low, therefore, on either side of the Schwann cell border, corresponding to the aligned proximal and distal halves of the first generations of internodes. Increase in nuclear density occurred approximately 125 μm from the borderline, probably marking the middle of the first genera-

tion of internodes (Figs. 1, 2). Beyond this point, nuclear densities were uniform throughout the roots, except in the distal portion of the eighth nerve, where two clearly defined peaks marked two aligned generations of Schwann cells (Fig. 2). The accurate alignment of Schwann cells in this nerve was explained by its very

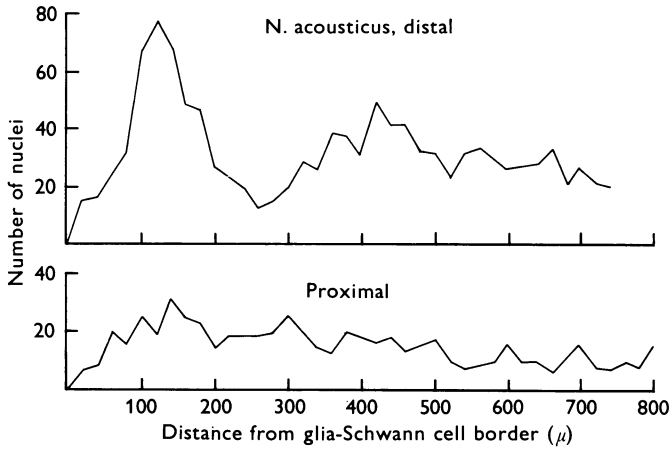

Fig. 2. Nuclear density at various distances from the glia–Schwann cell border in the acoustic nerve. The alignment of two generations of Schwann cells in the distal portion is evident by the corresponding peaks in nuclear density.

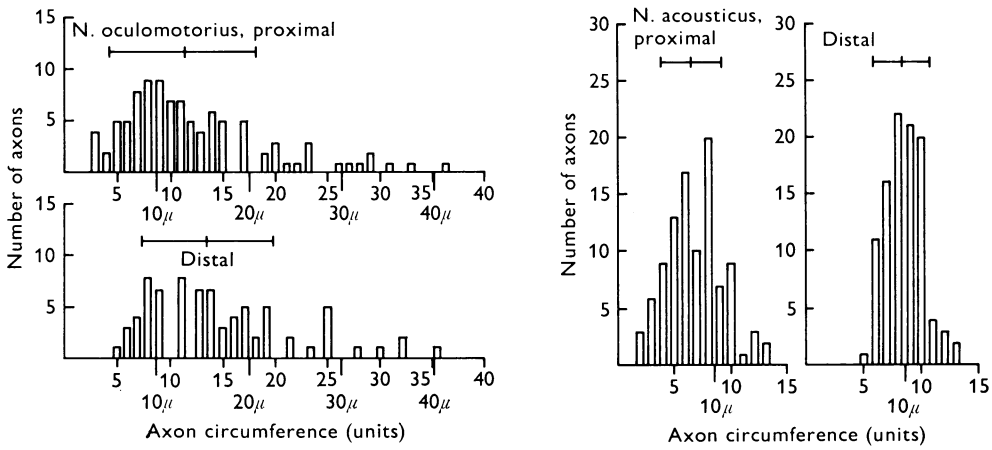

Fig. 3. Fibre spectra based on axon circumferences show a uniform fibre population for acoustic nerves, and more scatter for oculomotor nerves. The bar on top signifies the mean value and standard deviation. Differences between proximal and distal portions are statistically not significant.

uniform fibre spectrum. Nuclear densities increased at nearly the same distance proximal and distal to the border, corresponding to an average internodal length of approximately 250 μm (see Discussion).

Spectra of axon calibres proximal and distal to the glia–Schwann cell border were alike for each set of nerve roots. The acoustic nerve showed a unimodal fibre

spectrum with little variation in axon calibres, whereas there was much greater variation in the third and fifth nerves (Fig. 3). All nerve roots showed more fibres of slightly smaller calibre in the central portion than in the distal portion of the root; however, none of the differences in calibre spectra were statistically significant.

DISCUSSION

The portions of cranial nerve roots proximal and distal to the glia–Schwann cell border contain one and the same fibre population. Yet there are many fewer oligodendroglial cells in the central portion than there are Schwann cells in the distal portion, where one Schwann cell is known to myelinate only a single axon. A lower sheath cell density in the proximal portion of the root may indicate: (1) that peripheral and central internodes differ greatly in length, or (2) that the number of axons myelinated by a given sheath cell differs on either side of the border. These two factors are interdependent to the degree that determination of one determines both. The relationship would be modified if there were a significant population of non-myelinated fibres which would possess no oligodendroglial envelopes in the central portions of the roots. A search of the 1 μm sections indicated that non-myelinated fibres were either absent in our material or at least not present in sufficient numbers to alter our figures.

Previous determinations have shown that the internodes of central and peripheral fibres of the same calibres are nearly equal in length (Hess & Young, 1949; Thomas & Young, 1949; Bodian, 1951; Haug, 1967). Estimates of internodal length in the present study were based on a different principle – that is, the assumption that the position of the nucleus marks the middle of the internodes. This has been established for Schwann cells, where the nucleus is normally positioned exactly in the middle of each internode (Lubinska, 1959). Accordingly, we observed alignment of the first generation of Schwann cell nuclei distal to the glia–Schwann cell border, or even alignment of two generations of nuclei in the acoustic nerve, where the fibre spectrum was very uniform. The alignment of oligodendroglial nuclei proximal to the border was less pronounced, which could be expected if one cell myelinated two or more fibres of different sizes. The assumption that the oligodendroglial nucleus is positioned in the centre of a central internode, therefore, is not as well supported as for Schwann cells. Nevertheless, the length of the zone of low nuclear density on either side of the Schwann cell border was nearly the same in all of our samples, which is consistent with the widely held assumption that central and peripheral internodes are of the same lengths for fibres of equal calibre. Average internodal lengths calculated from the alignment of the first nuclear generations proximal and distal to the glia–Schwann cell border was 250 μm for fibres having 3·1 μm axon diameter (4·5 μm total diameter). These dimensions are similar to those reported by Hess & Young (1949), Bodian (1951) and Haug (1967).

None of the axon calibre spectra showed statistically significant changes between the proximal and distal portions of the roots; hence axon calibre may be ignored as a factor affecting sheath cell densities. However, the consistent prevalence of thin fibres in the proximal samples may indicate that axon calibre increased slightly in the distal portion (Wulfhekel, 1969); such an increase might be concealed by the

degree of variation in the fibre spectrum. It was considered unlikely that greater numbers of measurements in the present material would give an explicit answer to this problem; the study of isolated fibres or of a nerve with extremely uniform calibre spectrum may be a more appropriate model. Our data, also, do not answer the question as to whether the known difference in interperiod width between central and peripheral myelin affects sheath thickness, or the number of turns of myelin lamellae, respectively.

If one accepts the conclusion that there is no significant difference in the length of central and peripheral internodes, it follows, from our data, that the average oligodendroglial cell of cat cranial nerve roots supplies two to three axons. Illustrations of metallic impregnations of oligodendroglial cells (Hortega, 1928; Stensaas & Stensaas, 1968) indicate that some of them may myelinate four or more fibres. In cat cranial nerve roots, cells myelinating four or more fibres are either exceptions or, if present, compensated by a proportionate number of cells supplying only one axon.

Our observations are difficult to reconcile with the calculations by Peters & Proskauer (1969) who concluded that one oligodendrocyte of adult rat optic nerve may be connected to 30–50 fibres. This figure is far greater than our estimate, even if reasonable values for the various sources of error in our method are compounded, including our estimates of internodal length, the counts of astrocyte density, the number of non-myelinated fibres, the determinations of axon calibres and subjective error in counting. These authors used an indirect method of calculation based on the frequency of nodes in electron micrographs of cross-sections of the nerve, a value for the length of a node, the number of fibres per nerve and the number of oligodendroglial cells per mm^3 of tissue calculated from counts in 1 μm thick sections. The magnitude of the sources of error in this system is difficult to assess as it is difficult to quantify some of the parameters, such as the ease of identification of nodes in cross-section, the definition of the length of a node and the determination of the thickness of sections of plastic-embedded material.

Another factor entering the interpretation of sheath cell–fibre ratios is that fibre systems may differ in terms of the number of axons supplied by a sheath cell, a possibility suggested by the observation that certain morphological types of oligodendroglia tend to be characteristic for some fibre tracts (Hortega, 1928). The differences between axon sheath cell ratios in acoustic and oculomotor nerves in our samples (Table 1) suggest that an oligodendroglial cell of the acoustic nerve supplies more axons than one of the oculomotor nerve.

SUMMARY

Comparison of oligodendroglial and Schwann cell densities in the proximal and distal portions of the third, fifth and eighth cranial nerves of cats indicates that an 'average' oligodendroglial cell myelinates two to three axons. An estimate of internodal length shows similar values proximal and distal to the glia–Schwann cell border. No statistically significant alteration of axon calibres is found proximal and distal to the border, although a slight change may be concealed by variations in the fibre spectrum.

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