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CONTENTS OF THE OPTIC NERVE OF A SMALL CETACEAN

William W. Dawson, G. Marion Hope, Robert J. Ulshafer, Minnie N. Hawthorne and Robert L. Jenkins
Departments of Opthalmology and Physiology, College of Medicine, University of Florida, Gainesville, Florida, United States (W.W.D., G.M.H., R.J.U., M.N.H.) and Marineland of Florida, St. Augustine, Florida, United States (R.L.J.)

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Abstract

Nerve fibre and axon circumferences were digitized from electron micrographs of twelve mid-peripheral and central regions of an adult dolphin's *(Tursiops truncatus)* optic nerve. The data base of 822 fibres allowed the analysis of fibre size spectra, related axon dimensions, estimation of fibre total, quantity of non-neural space and fibre-axon relations. Giant fibres were found to be larger than in any known mammal but not at the expense of the small fibre contribution to the spectrum. The modal value for g (axon diameter/fibre diameter) was unusually small while myelin investment was more extensive than in any known mammal. Non-neural tissue and extracellular space occupy the major portion of this unusually large cranial nerve.

Introduction

In his editorial introduction of a major compilation of comparative sensory physiology of the last decade, CRESCITELLI (1977) registers his amazement at the breadth of the adaptations of the visual apparatus. These adaptations appear not only as variations in anatomy and physiology imposed by the environment but as various strategies employed by different forms to solve the same environmental problems. Because of their environment, whales occupy a place of particular interest in the study of mammalian anatomy and physiology. Marine precursors of the modern whale, 6m long, have been reconstructed from fossil remains found in southern

Georgia, Alabama and north Florida. Although their bone age is 45-50 million years by carbon dating, their phylogenetic linkage to both modern terrestrial and marine mammals is firmly established (MATTHEWS, 1978). There is little doubt that whales were initially terrestrial quadrupeds, later became amphibious and finally became fully marine. Thus this great order, as the terrestrial mammals' only full-time marine relative, developed its visual system under the influence of the common terrestrial environment. But for the last 40 or so million years it has had superimposed upon this background many conflicting needs imposed by a totally marine subsistence.

Superficially, the visual apparatus of marine dolphins appears roughly equivalent to that of its terrestrial relatives. However, the recent availability of quality tissue shows that there are aspects of the retinal (DRAL, 1977; DAWSON, 1980) and other eye tissues which are major adaptations of its obviously mammalian organization. For example, some inner-retinal cell types have elaborations and dimensions greater than any other recognized mammalian neural cell, while the opic nerve fibre spectrum, as seen by light microscopy (LM), extends to axon diameters greater than those found in any other mammalian optic nerve (DAWSON *et al.*, 1982). However, electron microscopic (EM) analyses of the optic nerve (HUGHES and WÄSSLE, 1976; STONE and CAMPION, 1978) disclose a tendency for LM to underestimate the total number of neurons, overestimate the contribution of larger fibre diameters and underestimate the contribution of fibres less than 1 μm in diameter. With the addition of new data processing capability we have further quantified the fibre spectrum of the dolphin optic nerve. Samples from the optic nerves used in the LM study (DAWSON *et al.* 1982) were prepared for EM. EM photographs were digitally analyzed for axon and myelin sheath circumferences. The results confirm the unique giant axon finding (DAWSON *et al.*, 1982), show a significant quantitative contribution by fibres under 1 μm in diameter, and establish unique sheath dimensions and unusual set of relationships between axons and their myelin sheaths.

Methods

Details of the methods for tissue preparation of optic nerves from *Tursiops truncatus* (Atlantic bottle-nosed dolphin) were described previously (DAWSON *et al.*, 1982). Briefly, less than 3 hr postmortem optic nerves were cut cross sectionally, and a segment taken approximately 1 cm from the globe was immersion fixed in buffered neutral formalin and subsequently postfixed in osmium. For optic nerves, this method has been critically evaluated by BISHOP *et al.* (1971). The tissue was subdivided into one peripheral and two central samples, each approximately 1 mm^2. These were embedded in Epon and 500 angstrom sections were cut perpendicular to the long axis of the nerve by a Sorval ultramicrotome. The thin sections were mounted on copper grids, magnified for examination and photographed in a Zeiss 9S transmission electron microscope. Photographs were digitized by manual tracings on a graphics pad. A small, general purpose computer stored the results on magnetic disc as a data base for further analysis. The data base consisted of digitized tracings of the external sheath circumferences and axon circumferences for 822 neurons (204 core, 618 mid periphery). From these, the various dimensional characteristics of the fibre[1], sheath and axon were calculated and analyses were made.

[1]Fibre refers to the axon plus the myelin wrappings or sheath.

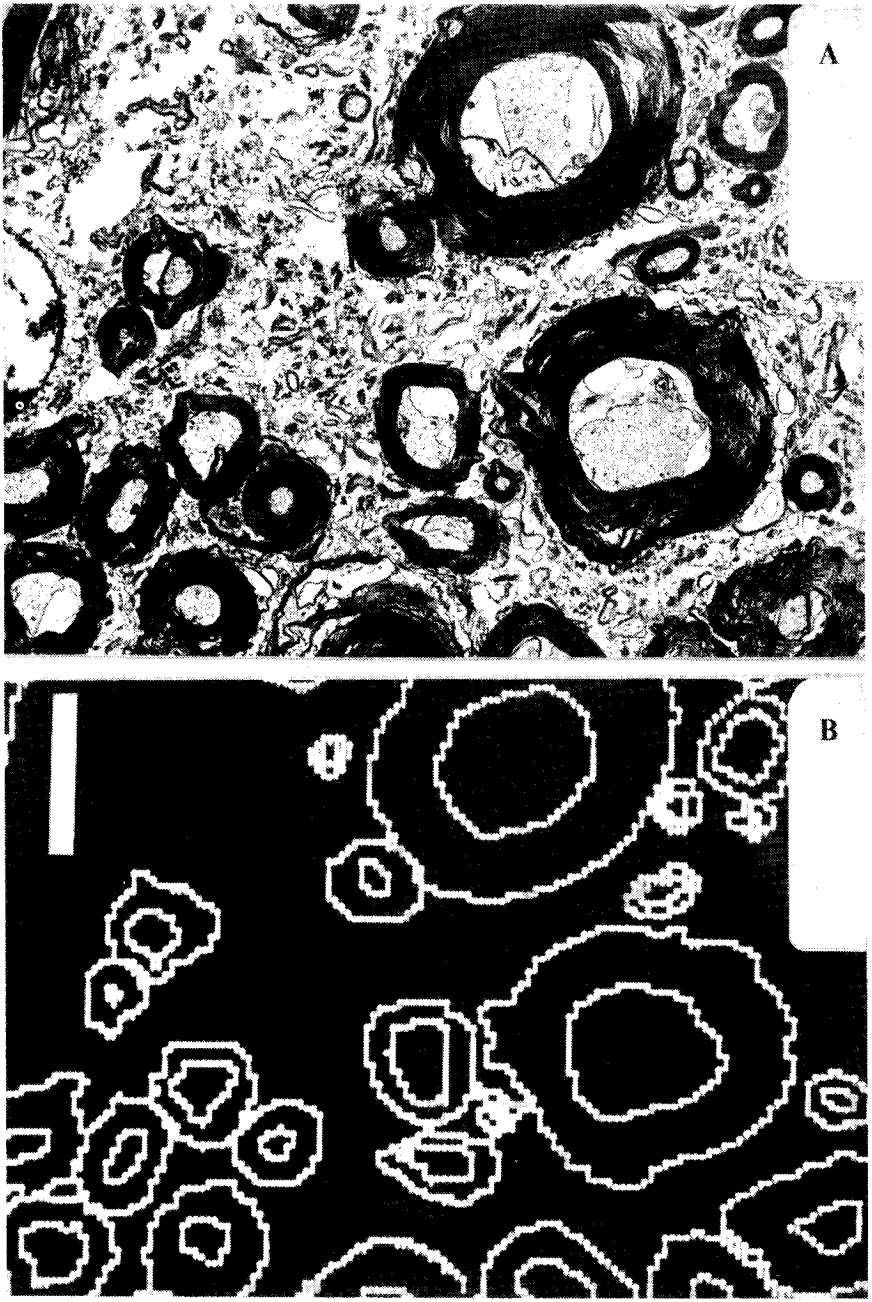

Fig. 1. A portion of one optic nerve photomicrograph digitized for storage in the data base. The photomicrograph A was digitized and then displayed (B). The scale (B) equals 5 microns.

Results

In agreement with the LM findings (DAWSON *et al.*, 1982) insignificant differences were found between the central and peripheral regional samples. 36.1% of the area of the central samples is occupied by fibres (7.3% axons, 28.8% sheath) while 49.5% of the area of the peripheral samples is occupied by fibres (9.6% axon, 39.9% sheath). Data from both regions were pooled for the remaining analyses.

Fig. 1 shows a portion of an EM micrograph (A) of one tissue sample and (B) its video image after the axon and sheath circumferences were digitally encoded. The digital resolution and scaling resulted in 0.08 micron data base resolution. The figure shows that thick sheaths and large extra-neuronal spaces are common in this tissue.

In the previous LM analysis of the dolphin optic nerve (DAWSON *et al.*, 1982) the size regions 1-3 micron and >3 micron were emphasized. Fibres less than 1 micron in diameter were poorly resolved and were not measured or were measured with minimal accuracy. To facilitate comparison, analyses from the EM data base (Fig. 2) are divided into ranges; (A) ≤ 1 micron, (B) >1 to < 3 micron, and (C) ≥ 3 micron. Thirty-eight per cent of the fibres are 3 micron or larger in diameter with the largest fibre diameter in the data base being 15.7 micron. Thirty-six percent of the fibres were in the intermediate class, while the smallest category (26%) included fibres of 1 micron or less. In the latter category, the smallest fibre which we found was 0.27

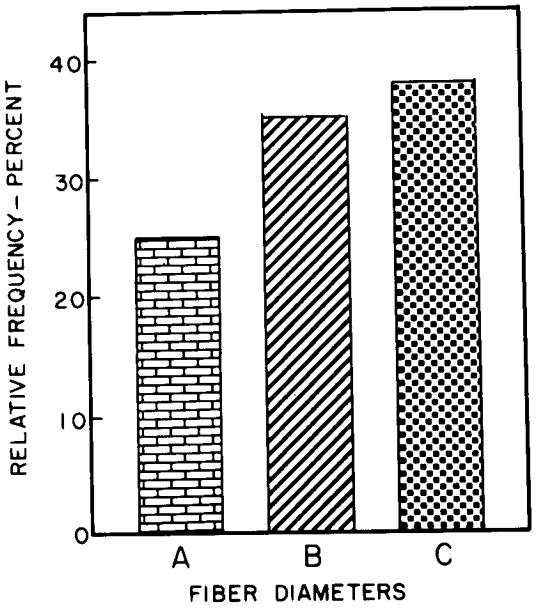

Fig. 2. Frequency of fibre diameters found in three categories. A. includes those resolved best by EM (≤ 1 micron). B. includes those most representative of LM studies of mammalian optic nerves (>1 to < 3 micron). C. includes the size range unique to this species (>3 micron. Analysis of 822 fibres.

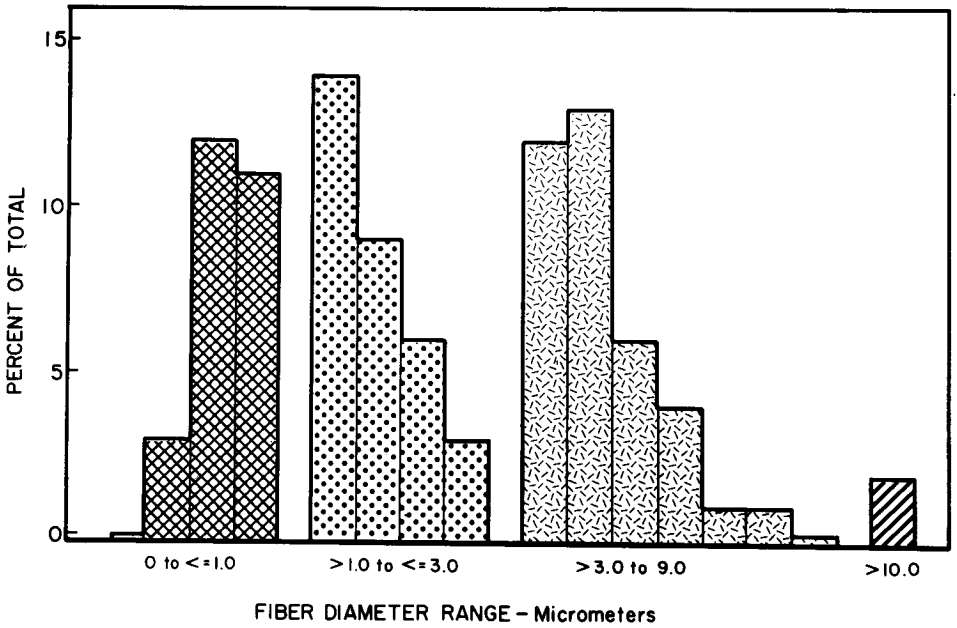

Fig. 3. More detailed analysis of the fibre -diameter, frequencey spectrum. Range 0 1.0 micron is divided into 0.25 micron bins, range 1.0 to 3.0 micron is divided into 0.5 micron bins and range 3.0 to 9.0 is divided into 1 micron bins. Bins including 0.1 and 8.5 micron are empty.

micron in diameter. In a more detailed analysi of fibre diameter (Fig. 3) the 0-1 micron range has been divided into 0.25 micron bins, the intermediate range into 0.5 micron bins and the 3-9 micron range into 1 micron bins. The most frequent fibre diameters are in the 0.5-1 micron range and account for 23% of all fibres in our sample. The second most common size occurred in the 1-1.5 micron range which accounted for 14% of the sample. Remaining elements of the fibre spectrum cover a broad range of sizes but drop off gradually from the peak around 1 micron without any significant secondary peak. As in the previous LM paper (DAWSON *et al.*, 1982) the band above 10 micron is a significant portion of the distribution.

Each of the 822 fibres is plotted as a point in Fig. 4 where the y-axis is sheath thickness and the x-axis is axis cylinder (axon) diameter. The Pearson r correlation value for this distribution is 0.67 ($P < 0.005$). Inspection shows a much greater dispersion of points around the regression line above 1 micron than below it. Sheath thickness ranges from a minimum at 0.1 to a maximum at 7.5 micron and frequently equals 1 or more axon diameters.

The physiological importance of axon/sheath relationships justifies the special emphasis on its analysis. One expression is g where $g = d/D$, d is the axon diameter and D is the fibre diameter (RUSHTON, 1951). Consistent with the division of fibre diameters described in Fig. 2 (A,B ranges) the frequency of units displaying g values from 0.1-0.9 is described in Fig. 5. The symmetry of each distribution persists regardless of fibre size with the mode falling at $g = 0.4$. In a one-way analysis of variance for the effect of fibre size upon g, $F = 2.1$ which is significant at the 0.05 level. Thus there is a weak but significant tendency for g to vary directly with fibre

diameter. For fibres <1.0 micron, mean g = 0.39 (SD = 0.19) for fibres >1 micron, mean g = 0.41 (SD = 0.12) and for fibres 4 micron in diameter, mean g = 0.42 (SD = 0.14).

An overall picture of the 822 fibres from 12 digitized EM photographs indicates that 8.8% of the area of the nerve face was devoted to axons, 36.0% to myelin and 44.8% to fibres. Fibre density ranged from 26-77 X 10^3 fibres per mm^2. The mean density was 48.4 X 10^3 fibres per mm^2. If the nerve face area is corrected for 10% gross non-neural tissue regions (DAWSON *et al.*, 1982), 9% thin section compression (HUGHES and WÄSSLE, 1976) and 8.98 mm^2 is used as mean face area, we estimate a total fibre count of 390,000.

Discussion

Fibre count. The LM study by DAWSON *et al.* (1982) showed an unexpected departure from the usual mammalian optic nerve fibre spectrum in the significant contribution of fibres with diameters greater than 4 and even greater than 12 micron. Consequently, a major motivation for this research was to determine if the apparent bias toward large fibres was accompanied by a reduction or an absence, in this species, of very small fibres. HUGHES and WÄSSLE (1976) and STONE and CAMPION (1978) show that estimates of optic nerve fibre content generally increase when the method of analysis is shifted from LM to EM. Further, POTTS *et al,* (1972a) have provided the only automated whole-count of primate optic axons and argue that the small number of fibres with diameters greater than 2.5 micron which they report are artifacts of pathology. It is not surprising then that the number of optic nerve fibres we estimate using EM technology is greater than the 147,000 (LM) reported by MORGANE and JACOBS (1972),

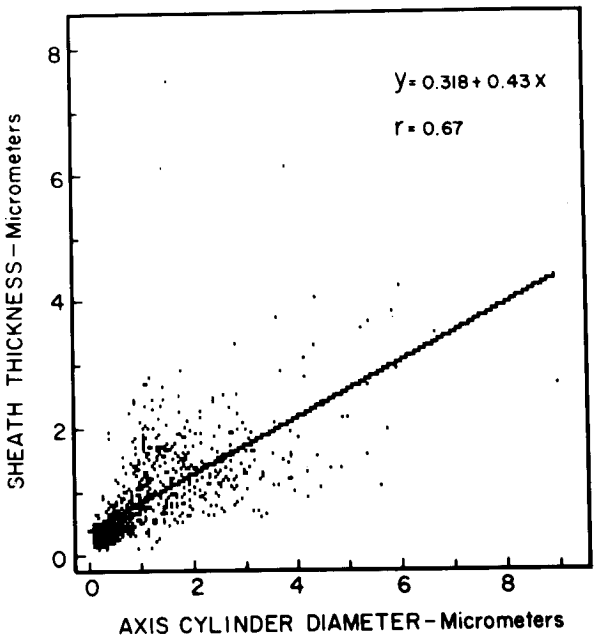

Fig. 4. Relation between sheath thickness and axon diameter for 822 fibres. The insect provides the equation for the regression line and the Pearson correlation coefficient.

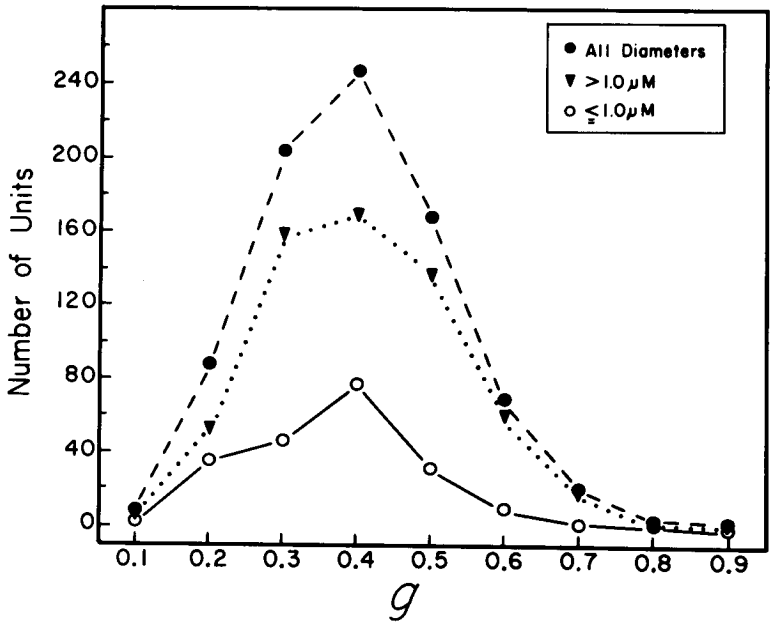

Fig. 5. The distribution of g for 822 fibres divided into three size categories (inset).

185,000 (LM) by DAWSON (1980) and most recently by DAWSON *et al.* (1982) where an estimated total of 157,000 (LM) was based on a sample of 6,335. From the EM data base it was found that on the average there are 48.4 X 10^3 fibres per mm^2 in the *Tursiops* optic nerve. However, variation between individual nerves described by DAWSON *et al.* (1982) and a wide range of fibre densities (26-77 X 10^3 per mm^2) were among our 12 EM samples. The data base of 822 fibres is probably insufficient to insure high accuracy. Probably this can be achieved only when each fibre is machine-counted with EM resolution. This has not been done, but would deserve unusual confidence. Our 390,000 fibre estimate by EM is roughly 2 X the previous LM based estimates, consistent with the increment found by HUGHES and WÄSSLE (1976). The fibre spectral distribution and characteristics of myelination which are the major topics of this paper are relatively less sensitive to the problems which contribute variation to estimated counts.

Fibre size spectrum. The fibre diameter ranges used in Fig. 2 were chosen to allow the easiest comparison with the previous LM study of dolphin optic nerve (DAWSON *et al.*, 1982). That paper showed that, from a survey of several terrestrial mammals, there are no optic nerve fibres larger than 8 micron (except cat, where about 2.5% are larger than 8 micron). Even when including the very small fibres (Fig. 2, category A) the major overall contribution to the spectrum is found in fibres >3 microns in diameter. Only 25% of the fibres in the dolphin nerve were included in the $0 \leq 1$ micron range. Fig. 3 provides a more detailed examination of these three distribution ranges. Except for the unique group of fibres larger than 10 micron in diameter, this spectrum is typically mammalian. That is, in several species there is a peak at 1-2 micron with two minima, one at about 0.3-0.5 micron with a second minimum (which is the upper end of the distribution) between 3 and 10 micron (cat, DONOVAN, 1967; cat, FRIEDE

et al., 1971; rat, HUGHES, 1978). The shapes of the distributions for rat and cat are quite similar except that there is variation toward the large fibre end of the spectrum with only a few fibres in the cat optic nerve reaching 10 micron in diameter. The shape of these distributions is faithfully replicated in the human and monkey data (POTTS *et al.*, 1972). POTTS *et al.* (1972) present spectra of "fibre area" data. If diameters are calculated to make the comparison one finds the peak at 0.5 micron with the range extending from 0.25-2.5 micron. The primate distributions are narrow in contrast to those of rat, cat and very narrow when compared to dolphin. However, careful examination of the POTTS *et al.*, 1972a text indicates that the terms "fibre" and "axon" are used interchangeably. Their page 983 states, "it can be seen by inspection of Fig. 1 that each optic nerve fibre is surrounded by a myelin sheath". Therefore, their "fibre-area distributions" are actually axon-area distributions. A correction can be estimated by assuming $g = 0.7$. The result would move their peak fibre diameter to approximately 1 micron with the upper end of their distribution extended to about 3 or 4 micron. This fibre diameter distribution is more consistent with other terrestrial mammalian species. HUGHES (1978) ably defends the desirability of analyses based on fibre dimensions and their relation to physiological function.

Myelinization. The importance of the myelin wrappings which constitute the nerve sheath has been reviewed and discussed by RUSHTON (1951). At one time it was thought that axons were unmyelinated below a certain "critical diameter" between 1 and 2 micron (DUNCAN, 1934). From the preceeding discussion of the optic nerve fiber spectrum it is clear that, at least in this cranial nerve, the critical diameter concept is no longer valid. None of the authors cited above reported the existence of non-myelinated fibres in the optic nerve. FRIEDE and SAMORAJSKI (1967) reported only myelinated fibres in the vagus or sciatic nerves of mice while FRIEDE and HU (1967) found no fewer than 4 lameller wrappings in the human optic nerve. In our dolphin tissue we saw no fewer than 5 wrappings. Fig. 4 shows how axis cylinder (axon) diameter varies with sheath thickness ($r = 0.67$, $p < 0.05$). We found the best agreement between these two variables for fiber diameters < 2 micron. This agrees with a report by BISHOP *et al.* (1971) who used fixation methods like ours and includes the mouse, hedgehog, green frog optic nerve and the cat optic tract. In none of these analyses on land animals was the sheath thickness range nearly so great as that reported in Fig. 4 for dolphin. Among these species BISHOP *et al.* (1971) compare the ratios of sheath thickness to axon diameters (not to be confused with g) which lie between 0.1 and 0.2 except for the green frog optic nerve where there was no variation in sheath thickness. The comparable ratio for dolphins (Fig. 4) is 0.5. This indicates much heavier myelination. The maximum sheath thickness in the data base was 7.5 micron. BISHOP *et al.* (1971) reported a single sheath about 3.2 micron thick in the cat cervical dorsal column. But in several other species and central tracts maxima ranged from 1.5 to 0.16 micron.

It has been accepted for many years that the conduction characteristics of myelinated fibres are more dependent upon the characteristics of the myelin sheath than upon the axon. A review of empirical data on conduction in myelinated fibres by RUSHTON (1951) examined the physical characteristics of sheath thickness, space constant, conduction velocity, internodal distance and nodal clefts. RUSHTON concluded that all medullated nerves are made of the same specific materials; and if nodal cleft size is assumed to be equal, voltage time and space relations should depend entirely upon fibre size. He described a relatively constant factor g which is the ratio of axon diameter to fibre diameter and was found to lie close to 0.6 in all cases and which is optimal for the spread of current from node to node. This theory and related arguments were

reviewed and elaborated in 1967 by FRIEDE and SAMORAJSKI who conclude that the range of g in the mouse vagus and sciatic nerves lies between 0.5-0.9 depending upon fibre size. A recent review by BERTHOLD (1978) cites results from ultrastructural studies of rat, rabbit, mouse and dog tissue which conform to RUSHTON's predictions. But BERTHOLD (1978) noted some departure for axons > 10 micron in diameter where the sheath thickness becomes more asymptotic than anticipated by the theoretical relationship. For the mammalian sheath sizes cited from WILLIAMS and WENDELL-SMITH (1971) some ratios as small as 0.47 were measured in peripheral, ventral root axons. But for dolphin optic nerve, Fig. 5 demonstrates very little variation in the modal value for g, regardless of fibre diameter, as predicted by RUSHTON (1951). Extensive comparative statistics are not available. From the data base the mean values for g (0.4) are notably less than predicted by RUSHTON, even admitting the variability discussed by BERTHOLD (1978). Many optic fibres of the dolphin deviate significantly from the g values usually found in terrestrial animals and from the range of g values proposed as most efficient for all myelinated fibres. This appears due to unusually thick myelin deposits rather than small axons.

Specializations. The LM analysis by DAWSON *et al.* (1982) has been partially confirmed by our EM results which show an unexpectedly large number of fibres larger than 10 micron in the dolphin optic nerve. Since it is well established that conduction velocity and sheath dimensions are linearly related (RUSHTON, 1951), it is probable that peak conduction velocity in the dolphin optic nerve is greater than in any other known mammal. This probability requires the assumption that the nodal characteristics of these fibres are typical of those found in terrestrial mammals. Evidence to the contrary is not available. But increased myelin investment as a compensation for an inefficient nodal structure would be a remarkable adaptation. A more conservative, ethologically based argument for increased conduction velocity has been made (DAWSON, 1980). This argument is based on the need for rapid visual target acquisition in the turbid marine visual environment.

While the increased myelinization of this nerve would naturally contribute to larger nerve trunk diameters, the 3.4 mm dolphin optic nerve diameter cited by DAWSON *et al.* (1982) is still much greater than can be easily explained by the fibre count alone. The previous analysis (DAWSON *et al.*, 1982) showed that although the optic nerve was largest, the axon density in the *Tursiops* optic nerve is least among several representative diurnal mammals. The data presented in this paper requires that the number of axons in the optic nerve be revised upward. Accepting the upper limit in the range of reasonable total axon estimates (390,000) and the morphometric measures from DAWSON *et al.* (1982) axon densities were recalculated. Nevertheless, Fig. 6 shows that dolphin remains at the low end of the axon-density continuum with approximately 48,000 axons/mm^2 with human at the high end with approximately 222,000 axons/mm^2. Monkey and cat fall between these two limits.

The sparseness of neuronal tissue in other nervous system structures in dolphins has been noticed by other authors. In his review KRUGER (1964) remarked on the scarcity of CNS neurons mentioned in 12 papers since 1876. Relatively more quantitative data is available on the contents of the retina than on the *Tursiops* brain. Measures from DAWSON *et al.* (1973), DRAL (1977), DAWSON (1980) and DAWSON *et al.* (1982) describe the retinal thicknesses between about 370 and 425 micron. Comparable measures in non-foveate diurnal land mammals are: dog, 112-240 micron; cat, 115-220 micron; horse, 110-220 micron; cow, 220

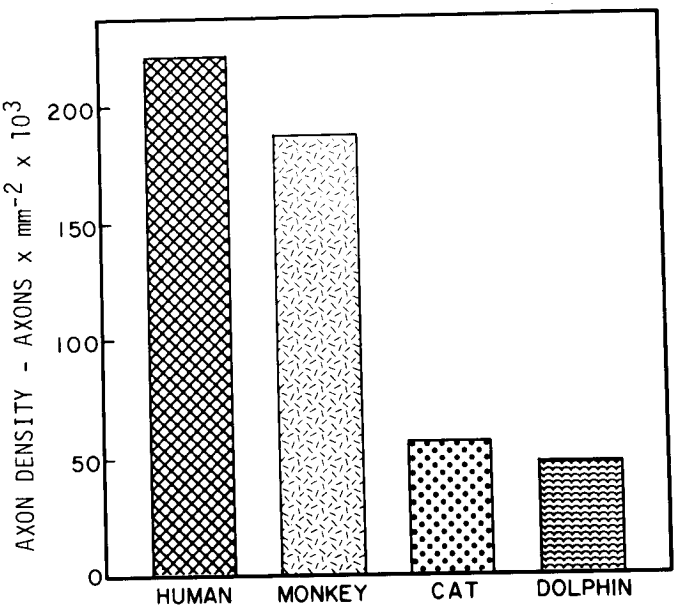

Fig. 6. Optic nerve axon density for three common terrestrial mammals (from DAWSON *et al.*, 1982) and for the Atlantic bottle-nosed dolphin. All are based on EM measures.

micron (PRINCE *et al.*, 1960). These measurements agree well with the human retinal thicknesses (80-230 micron, excluding fovea) pictured but not measured by VAN BUREN (1963). Large eyes (cow, horse) do not necessarily have thick retinas.

Conclusions

We conclude that the dolphin retina is about two times as thick as those of these representative land mammals. Further, the thickness increment cannot be explained by elevated neuron content. In the retina the layering of neuron cell bodies provides for easy evaluation of density by the staining of nuclei with the Nissl techniques. None of the published work from this laboratory or from DRAL (1977) demonstrates any unusual increase in retinal nuclear layer dimensions. If the neurons are not responsible for this exceptional thickness, only two other explanations are apparent; (1) glial elaboration and (2) increased extraneuronal space. Although retinal data are not available, our EM data base has demonstrated great glial-myelin elaboration in the dolphin optic nerve and more than 50% extraneuronal space in that tissue. VAN HARREVELD (1972) estimates 12-20% as the normal extracellular space content in terrestrial mammal central nervous systems. The dolphin's exceptional retinal thickness is consistent with these comparative numbers.

The importance of the size peculiarity of dolphin CNS structures may justify the tentative hypothesis that brain and retina responded to the same environmental influences, The typical neuron is at the center of, or linked to, each of three metabolically relevant compartments; the glia and their extensions, the extracellular space and the blood of the capillary network and/or

its analog, the cerebro-spinal fluid. MORGANE and JACOBS (1972) call the dolphin lateral ventricles "extremely large and prominent". Among these compartments the least understood are the physiology of the brain's glia and the contents of the extracellular space. There has been some needed attention given to retinal glia, their ion distribution role, enzymatic functions and glycogen distributions. KUWABARA *et al.* (1959), KUWABARA and COGAN (1961) and KUWABARA (1965) argue that retinal glia provide mandatory support for the neurons. On other grounds, this relationship has been formalized by HYDEN (1967) who views the neuron-glia system as a functional metabolic unit. The transport roles of fluids in the large extracellular compartment seem clear. They are complemented by the prodigious size of the blood compartment in dolphins (about 40 liters) suggested by the perfusions described by MORGANE and JACOBS (1972).

In aggregate these data seem sufficient to document the importance of the extra-neuronal compartments and the probability of their existence in unusual magnitude in dolphins. In the optic nerve they participate in a major maintenance task. If we assume a 150 mm optic nerve length, calculation from the dolphin data base shows that it contains a 2.32×10^5 mm^2 area of axon membrane which must be supported. But the center of the matter is why, in contrast to its terrestrial relatives, should this mammalian species have a requirement for so vast a storage facility for high energy metabolites, gases and salts? The acceptable answer must also intimately involve the needs which make it different from its terrestrial relatives. We believe that the crucial factor is its marine environment and the imposed respiratory patterns.

LILLY (1967, 1978) has emphasized the gross size and weight of the *Tursiops* brain which is reported to be similar to that of the human brain and which he speculatively associates with "intelligence". But experiments show that the unusually large optic nerve and retina of the dolphin eye does not contribute special visual acuity or sensitivity (for review see DAWSON, 1980). We have demonstrated, in this case, that size is an artifact of an exceptional development of the non-neural elements. Perhaps the large size of the dolphin brain is really not neural but a consequence of similar special development of the extra-neural, metabolically relevant compartments. Such highly developed vegetative support and reserves would be of considerable value to a smaller nervous system subjected to long periods of apnea as a consequence of environmental pressures.

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