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Retinofugal fibres change conduction velocity and diameter between the optic nerve and tract in ferrets

Gary E. Baker & Michael P. Stryker*

Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, UK

In earlier studies of central nervous fibre tracts, it was tacitly assumed that individual axons are relatively uniform along their length. In the retinofugal pathway in particular, axon diameter, myelin thickness and correlated conduction properties[1,2] have been treated as constant throughout the optic nerve, chiasm and tract. We report here that the conduction velocities of fibres contributing to the early components of the compound action potential are significantly greater in the optic tract than in the optic nerve of ferrets, and also that the diameters of the largest retinofugal fibres increase from nerve to tract[3]. This observation raises significant questions about the developmental mechanisms in the central nervous system that relate the axons, their diameters, and the glia with which they are myelinated. In addition, it indicates that studies that have relied on the constancy of conduction velocity along the retinofugal course[4] may require reappraisal.

Five normally pigmented adult ferrets were studied using the arrangement of electrodes shown in Fig. 1*a*. Records of the compound action potential (Fig. 1*b–g*) were obtained with electrodes positioned: intraretinally near the optic disk; around the optic nerve about 1 mm behind the eye; in the optic nerve about 3 mm distal to its point of fusion with the contralateral nerve; in the optic chiasm contralateral to the midline; and at the contralateral optic tract close to the lateral geniculate nucleus (Fig. 1*a*). Each record shows two major conduction latency modes, one (t_1) with a short latency negative peak, and a second (t_2) with a slower peak[5]. This form of compound action potential is similar to that previously found in studies of the cat retinofugal pathways[6,7]. Each conduction latency mode in the compound action potential is believed, on the basis of studies of the peripheral nervous system[8] and various investigations of the retinofugal pathways[9–11], to reflect the presence of a subpopulation of fibres with similar conduction velocity.

The t_1 and t_2 conduction velocities were determined for the optic nerve and optic tract from measurements of conduction times and distances. Conduction times for the nerve and tract were calculated by two methods. First, from recordings made with the intraretinal electrode (RET) near the optic disk, we took the tract conduction time to be the difference between the latencies of the peak t_1 and t_2 negativities elicited from optic tract (OT) and optic chiasm (OX) stimulation. Similarly, we took the nerve conduction time to be the difference between the latencies of the potentials elicited by intracranial optic nerve (ONc) and intraorbital optic nerve (ONo) stimulation. Figure 1*h* shows that these measurements depend only negligibly on stimulus intensity over the range used. The measured distances were divided by these conduction times to yield a single estimate of conduction velocities. These measures of latency incorporate a period of delay, the utilization time, between stimulus onset and spike initiation in the stimulated fibres. This utilization time has been described as being negligibly small for optic tract stimulation in the cat[12] and ~100 μs in the rat optic nerve[13]. If the utilization time is equal at all of the stimulating electrodes, it would have no effect on the conduction velocities measured by this method, as the same delay would be subtracted from each latency. This measure of conduction velocity is largely independent of which points on the t_1 and t_2 waveforms are chosen for the latency measurement, as similar waveforms are measured in the various stimulus conditions. Figure 2*a*, *b* shows that the t_1 velocities in the optic tract were consistently much greater than those in the nerve. The difference between nerve and tract velocities was smaller and more variable for the t_2 responses, but the measured tract velocities were greater than or equal to those in the nerve in every case.

If the utilization times do differ greatly for the different stimulating electrode placements, the previous method may be invalid[14]. We therefore measured conduction times by a second method, recording the peak t_1 and t_2 negativities of antidromic responses at ONo and orthodromic responses at OT after identical OX stimulation (Fig. 1*f*, *g*). Using this strategy, a single utilization time is common to latencies measured in both nerve and tract. The optic tract velocities measured by this method were consistently greater than those in the nerve, even when the utilization time is assumed to be zero. The difference between the mean values of tract and nerve velocities was 36% for the t_1 mode and 20% for the t_2 mode. These differences would, of course, increase if a realistic value was assumed for the utilization time. Therefore, although the increase from nerve to tract was smaller when measured by this second method, indicating that the utilization time may not have been constant along the retinofugal pathway as assumed for the previous method, the differences between nerve and tract illustrated above are not artefacts of unequal utilization times.

Figure 3*a–e* shows that in each of the animals studied physiologically, there were many more axons with a diameter >5 μm in the optic tract than in the optic nerve. In the ferret optic tract large-calibre axons are known to be of retinal origin[3]. If we assume that the largest axons in the nerve increase their diameters to become the largest axons in the tract, we can estimate the magnitude of this increase graphically, as in Fig. 3*f*, by shifting the nerve axon population to the right until it is superimposed on the equivalent number of largest axons in the tract. For an axon of 5 μm diameter in the nerve, the average increase in diameter estimated by this method was 16.9% (range, 5–40%, $n = 5$). The data of Figs 2 and 3 show, therefore, that the largest and fastest optic tract axons are consistently larger and faster than those in the nerve, with no overlap between the two samples. These data do not determine the velocity–diameter relationship, however, because the anatomical data were obtained from the extreme upper tail of the axon diameter distribution, whereas the physiological measurements probably reflect the majority of the many thousands of axons in each axon diameter class.

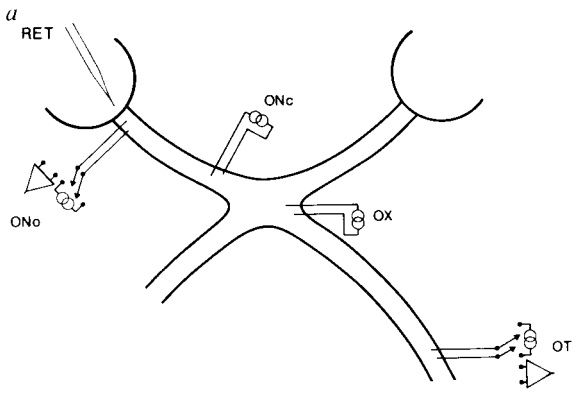
In the retinofugal pathway of the cat, each of the peaks of the compound action potential has been associated with a physiologically and anatomically distinct cell type, the fastest (t_1), with large diameter, called Y- or α-cell fibres and the slower (t_2), with predominantly intermediate diameter, called X- or β-cell fibres[15–17]. We have not studied the slowest (t_3) latency group, associated with W-cell fibres[18,19]. It is therefore reasonable to infer from our data that the conduction velocities of both Y- and X-cells increase along their central course. Consequently, calculations of conduction velocity from measurement of conduction time along only a segment of the retinofugal pathway are likely to be inaccurate. For example, to infer the total extraretinal conduction time from measures of conduction velocity in the optic tract may underestimate the total conduction time[4].

In the developing ferret optic nerve, glia have an interfascicular arrangement distally and a radial organization more centrally[20], and different distributions of glial precursor cells have been identified between the eye and chiasm in the developing optic nerve of rats[21]. The fascicular arrangement of fibres in the adult optic nerve changes abruptly to a non-fascicular organization just rostral to the chiasm, with a partial segregation

* Present address: Department of Physiology and Neuroscience Graduate Program, University of California, San Francisco, California 94143-0444, USA.

of fibre diameter types similar to that in the optic tract[22]. Each retinal-ganglion-cell axon thus passes through three different glial environments. It has long been recognized that the conduction velocity in the unmyelinated, intraretinal segment is much lower than that in the more central segment[19]. The results presented here show that conduction velocity and axon diameter also differ between the nerve and tract environments. We suggest that the change in axon diameter may be a result of the change in glial type as the axons pass from one region of the central pathway to the other, but the precise region in which the change in diameter occurs remains to be defined. It will, therefore, be interesting to determine whether the change described here is gradual, extending over a considerable distance, or local, occurring at the site where the glial environment of the axons changes.

The stable relationship between axon diameter and myelin thickness over the course of regeneration and development has



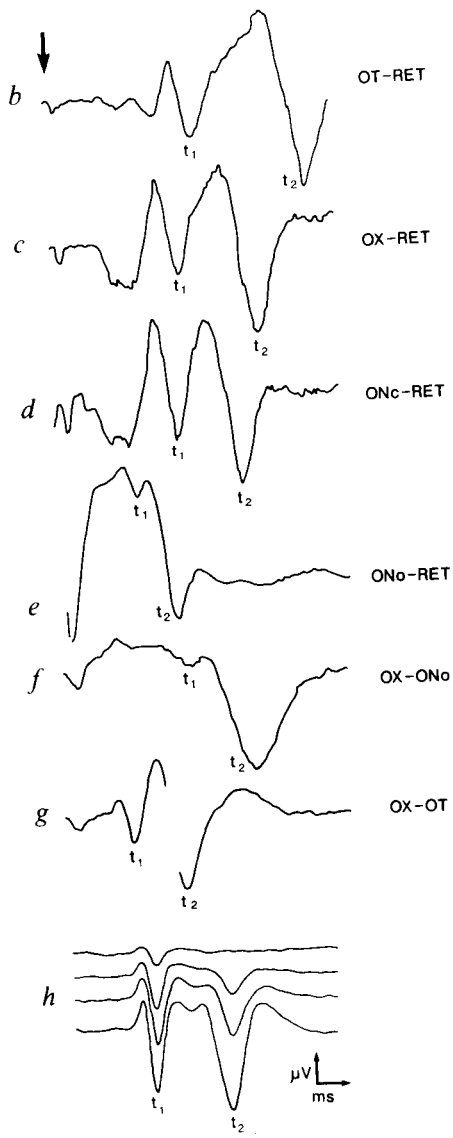
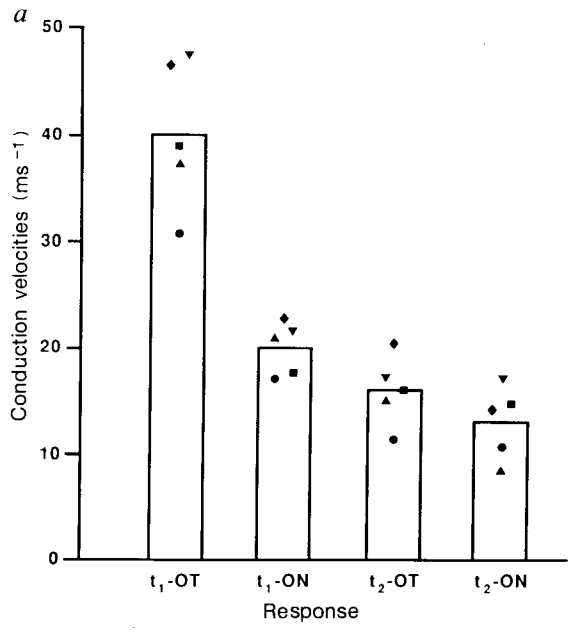


FIG. 1 *a*, Schematic drawing to illustrate the arrangement of electrodes. *b*–*e*, Antidromic potentials recorded at the optic disk (RET) following stimulation of: *b*, optic tract (OT); *c* optic chiasm (OX); *d* intracranial optic nerve (ONc); *e*, intraorbital optic nerve (ONo). *f*, Antidromic potential recorded at ONo following stimulation at OX. *g*, Orthodromic potential recorded at OT following stimulation at OX. Conduction distances (in this animal): OT – ONo, 29.5 mm; OX – ONo, 17.9 mm; ONc – ONo, 13.4 mm. *h*, Series of antidromic potentials recorded at RET following stimulation of increasing intensity at OT. Note that the conduction latencies for t_1 and t_2 remain nearly constant throughout the series. Calibration: *b*, *c*, *d*, 20 μV, 0.5 ms; *e*, 50 μV, 0.5 ms; *f*, 20 μV, 0.2 ms; *g*, 50 μV, 0.2 ms; *h*, 200 μV, 0.5 ms. Stimulus onset is indicated by arrow.

METHODS. Anaesthesia was induced with xylazine (Rompun; 1 mg kg^{-1}) and ketamine (Vetalar: 15 mg kg^{-1}) intramuscularly and maintained by intravenous sodium pentobarbitone (Sagatal: 10 mg kg^{-1}). During recording, animals were placed in a stereotaxic frame and rectal temperature was regulated at 38.0 ± 0.5 °C. After the tissues behind the globe were dissected, a bipolar electrode manufactured from two pieces of Teflon-coated stainless steel wire (7 × 125 μm outer diameter) stripped of the insulation at the tips, was hooked onto the intraorbital optic nerve. A concentric bipolar irridium electrode (Rhodes) was advanced through the vitreous body onto the region of the optic disk through a scleral puncture. Craniotomies were made and, after reflection of the dura, bipolar electrodes were positioned at the optic chiasm and tract using coordinates previously computed for the ferret[30]. The reference electrode for recording was placed in the exposed tissue of the scalp. The positions of intracranial stimulating electrodes were finally adjusted to evoke the lowest threshold responses at the optic disk. Compound action potentials were recorded (a.c. coupled, 0.1 Hz–10 kHz) using a high impedance head stage (Neurolog NL100) and displayed on a storage oscilloscope (Tek 5113) from which they were photographed (Polaroid C5). Conduction latencies from stimulus onset to the negative peak of each latency mode were measured directly from the photographic records using a digital caliper. Responses were recorded at each electrode following constant current stimuli (50 or 100 μsec duration; variable intensity) delivered to the other electrode sites. Electrode configuration: concentric bipolar electrodes were used for RET, ONc, OX and in some experiments for OT. ONo was a colinear bipolar electrode inserted normally to the course of the optic nerve, with <250 μm tip separation parallel to the course of the optic nerve. When the OT electrode was not concentric bipolar, it was a parallel bipolar electrode (with the tips separated by 2 mm in the coronal plane) lowered on a vertical axis to stimulate optic tract axons rising nearly vertically. All other electrodes (OX, ONc, ONo) were advanced along a course normal to the axons stimulated. Electrode position and effects of stimulus polarity: in every case, electrodes were sited at the low threshold point with the cathode actually in or on the optic nerve or tract (confirmed histologically). Cathodal stimulation was always effective at lower (usually very much lower) intensities than anodal stimulation. In cases in which the OT electrode was parallel bipolar instead of concentric bipolar, unipolar stimulation (using a distant extracranial reference) was found to yield identical latencies over the range of stimulus intensities used for measurement of responses. Effects of stimulus intensity: at every site, a series of stimulus intensities was used, ranging from subthreshold through gradually increasing steps, to suprathreshold for both the t_1 and t_2 responses. The records measured were those of the lowest intensity at which a clear response was observed for the component measured. Thus, in many cases, the t_1 response rose to maximum (and in some cases shortened in latency) before the t_2 response had attained moderate size; in such cases we measured the t_1 and t_2 latencies from different records, each at an intensity at which it had attained less than its half-maximal size. We usually observed very little latency shift during manifold increases in stimulus intensity. Stimulus spread: none of the range of stimulus intensities used in this study was capable of activating optic axons at shorter latencies from OX than from ONc, indicating that stimulus spread equal to or greater than the distance separating them did not take place from those electrodes.



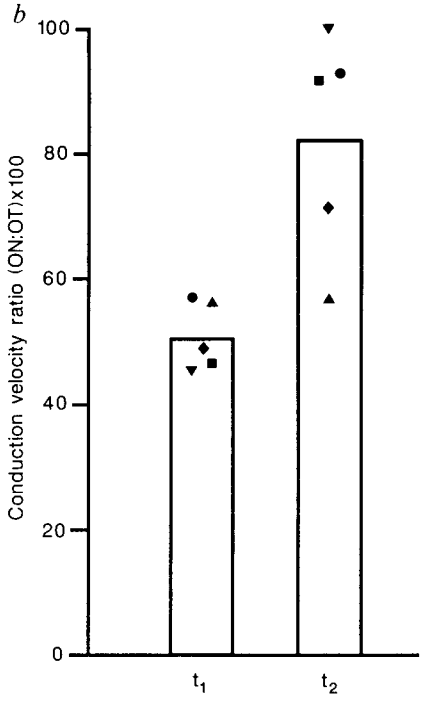


FIG. 2 *a*, Conduction velocities for t_1 and t_2 responses in optic nerve (ON) and tract (OT). *b*, Conduction velocities in optic nerve as a percentage of velocity in the optic tract for each animal. Bars show the means; individual animals are coded respectively by a triangle, inverted triangle, square, diamond and circle.

METHODS. At the end of recording, electrolytic lesions were made at the tips of the centrally sited electrodes, and animals were perfused with a solution of 3% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate-buffered saline. Segments of optic nerve and tract contralateral to those from which recordings had been made were removed and retained for subsequent fibre analysis. Conduction distances were measured along the course of the optic nerve and tract from the back of the globe to visible electrode sites and landmarks (fusion of the optic nerves, stereotactically directed knife cuts) using a length of fine silk thread. For measurements of distances between deep sites, blocks of tissue were embedded in gelatin-albumin, sectioned on a Vibratome at 100 μm and stained with cresyl violet. The course of the optic tract was reconstructed using an X–Y microscope stage encoder (Boekeler) and the distance separating lesion sites calculated.

been interpreted as indicating that growing axons must inform the associated glial cells of just how many turns of myelin to produce[23,24]. The observations reported here indicate that, as for dorsal root ganglia *in vitro*[25] and *in vivo*[26], this interaction seems to be a two-way affair, with the glial environment influencing the final axon diameter as well.

Changes of conduction velocity and diameter have been reported between peripheral nerve roots and the spinal cord[27,28] but the identity and origin of the myelinating cells, Schwann cells peripherally and oligodendrocytes centrally, changes completely at the central–peripheral boundary[29]. The change observed here takes place entirely within the central nervous system along a fibre pathway that has generally been regarded

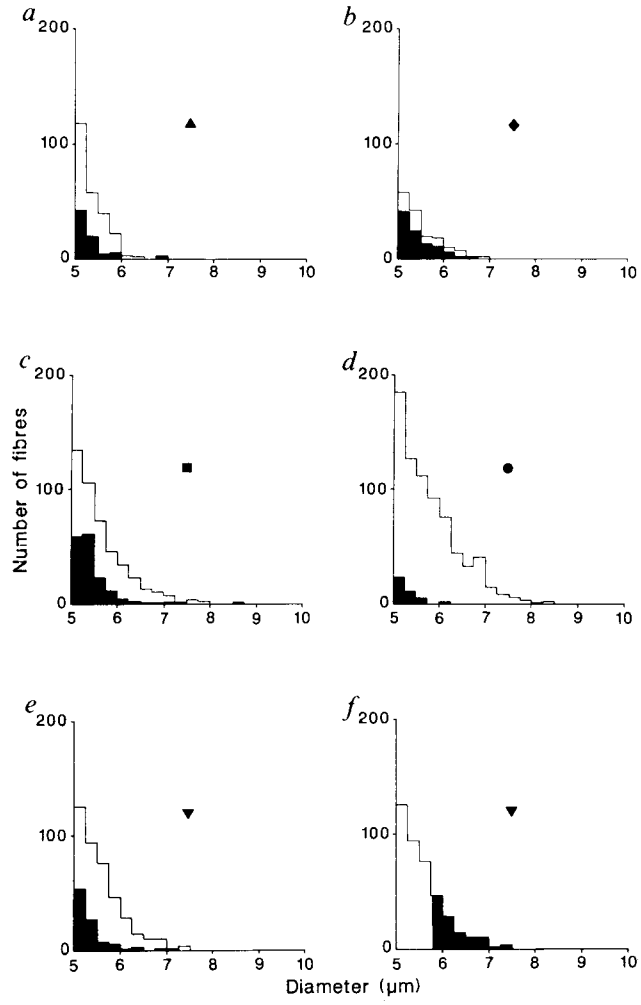


FIG. 3 *a–e*, Diameter distribution of all axons with an internal diameter of >5 μm in the optic nerve (shaded) and tract of each of the animals that were studied physiologically. *f*, Optic nerve axon diameter distribution in the animal shown in *e*, shifted rightward by 0.81 μm to match the distribution of the largest axons in the optic tract.

METHODS. Optic nerve and tract segments were sectioned transverse to their course at 200 μm on a Vibratome, post-fixed in 1% osmium tetroxide, dehydrated through graded ethanols and embedded in Epon. Semi-thin sections (1 μm) were stained with 1% *p*-phenylenediamine. The internal circumferences of all axons of internal diameter ⩾4 μm (as judged by comparison with standard circles) in each nerve and tract were drawn from semi-thin sections at magnification ×1,500 using a drawing tube attached to a microscope. The circumference of each profile drawn was then measured using an image analysis system (Kontron IPS), which also calculated the diameters and constructed the histograms. The data presented here are restricted to axons with an internal diameter >5 μm, to eliminate the possibility of incomplete sampling.