J. Anat. (1994) 184, pp. 511–518, with 10 figures Printed in Great Britain

Trigeminal innervation of the scleral spur in cynomolgus monkeys

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(*Accepted 26 September 1993*)

ABSTRACT

Degeneration induced by intracranial section of the ophthalmic branch of the trigeminal nerve was used as a marker to trace the ocular passage and termination of fibres in ocular filtration angle structures of 3 cynomolgus monkeys. Fine supraciliary nerves branched from precorneal nerves as they entered the sclera from the uvea, passed forwards radially in the lamina fusca, entered the scleral spur, turned circumferentially and terminated. Many of the fibres were degenerated. Other less numerous branches advanced in the ciliary muscle close to the lamina fusca and were distributed either to the spur or to the trabecular meshwork. Spur fibres were approximately 4 times as numerous as trabecular fibres. The position, arrangement and ophthalmic identity of most of the spur terminals suggest a capacity to record tension produced by ciliary muscle contraction.

Key words: *Macaca fascicularis*; eye; ciliary muscle; trabecular meshwork; ophthalmic nerve; tension receptors.

INTRODUCTION

Trigeminal nerve fibres are present in the chamber angle tissues of primate eyes. Little information is available regarding their incidence apart from comments that they occur infrequently in monkey trabeculae, as determined by induced degeneration studies (Ruskell, 1976) and that moderate numbers of substance P-like and rather fewer calcitonin gene-related polypeptide immunoreactive fibres are present in the trabeculae of man and monkeys (Laties et al. 1981; Stone & Kuwayama, 1985; Stone & McGlinn, 1988). Both neuropeptides probably indicate a trigeminal source, but other sources cannot be excluded (Kuwayama & Stone, 1987; Uusitalo et al. 1989). Most of these studies refer to the trabecular meshwork and comparatively little attention has been given to the adjacent scleral spur. Unlike the trabecular terminals, which are strategically positioned to relate to events associated with the movement of aqueous humour, a potential role for spur terminals is not obvious, and this no doubt accounts for the disparity in interest between these 2 regions of the filtration angle. Furthermore, it is arguable that the spur merely provides passage for fibres destined for the trabeculae. The purpose of this report is to show that this is not the case, and that the spur is a specific target for nerve terminals, more generously innervated than the trabeculae.

MATERIALS AND METHODS

Three young cynomolgus (*Macaca fascicularis*) monkeys, 2 male and 1 female, were sedated parenterally with 2–3 mg/kg ketamine and anaesthetised with 15–25 mg/kg Sagatal (pentobarbital sodium) given via the saphenous vein or intraperitoneally. The skin of the head was incised sagittally and frontally, the temporalis muscle detached from the skull on both sides and reflected downwards. Five holes were drilled through the skull, positioned to allow removal of the calvarium in one piece. Removal was completed by sawing between the drill holes at the perimeter of the calvarium. A miniature circular saw blade attached to the drill drive was used for this purpose and a narrow metal spatula was inserted between dura mater and

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skull from one hole to the next to protect the brain during sawing. The exposed area of the brain was extended on the left side by removing temporal bone with nibblers to a position almost opposite the cranial floor. Dura mater was cut and reflected to expose the brain surface and the temporal lobe was elevated with a broad curved spatula to reveal the trigeminal ganglion. The ophthalmic nerve sheath was split parallel to the nerve axis close to the trigeminal ganglion. The nerve was freed from sheath attachments, separated from adjacent structures with a hook, divided and reflected and a short piece removed. This method was used to minimise the risk of injury to adjacent nerves and to the cavernous sinus. Most importantly, damage to the filaments of the cavernous sinus plexus had to be avoided. The dura mater was sutured, the calvarium replaced, held in position by strapping the temporalis muscles together across the crown with strong sutures, and the wound closed.

One animal was killed 3 d after operation, the other 2 at 7 and 8 d respectively. The longer times are optimal for displaying wallerian degeneration and, risking incomplete degeneration, the shorter time was chosen to minimise disorganisation of terminals. Animals were sedated, anaesthetised and given an injection of the anticoagulant heparin sodium (1500 units). The external jugular veins and the inferior vena cava were cut and 3 l of a 2% glutaraldehyde, 3% paraformaldehyde, cacodylate-buffered solution were perfused through the heart for approximately 10 min using a Sigmamotor pump. The left and right eyes were removed and stored in fixative at approximately 4 °C and dissected while immersed in buffered sucrose using a dissection microscope.

Eyes were cut equatorially and segments of the anterior half, measuring about 2 mm in width at the filtration angle, were prepared. The segments were postfixed in 1% unbuffered osmium tetroxide, dehydrated and embedded in Araldite. Interrupted serial semithin transverse sections (parallel to the scleral spur) of the segments were cut, commencing at the ora serrata, and stained with 1% toluidine blue in an equal volume of 2.5% sodium carbonate. Intervals between retained sections varied from 200 μm to full serials depending on position and content. Sample ultrathin sections were cut for electron microscopy and mounted on unfilmed copper grids, and stained with a saturated solution of uranyl acetate in 70% ethanol followed by 0.4% lead citrate in 0.1 M sodium hydroxide. Counts of degenerated and normal nerve fibres were made using the absence of normal axoplasm as the criterion for degeneration; for unmyelinated nerve fibres, Schwann cells showing evidence of hypertrophy and without enclosed axons were also accepted as evidence of degeneration. Left and right eyes were prepared similarly and the latter were used as controls. No evidence of degeneration was found in the controls. A few of the segments were trimmed, discarding all but the spur region, and meridional sections cut.

The intracranial segment of the ophthalmic nerve was inspected using a dissection microscope to assess the outcome of the lesions. In 1 animal the lesion appeared incomplete and in another a fine strand of the ophthalmic nerve bridged the gap created by the lesion. The nerves were removed together with the adjacent tissues, including the internal carotid artery, cavernous sinus and retro-orbital nerves and prepared for histology. Nerve fibres in the ophthalmic nerve, cut about 5 mm anterior to the wound and examined with a light microscope, revealed apparently complete degeneration in 1 preparation (ML85 – 7 d survival), approximately 90% degeneration in another (ML84 – 3 d) and only 60% in the 3rd (ML86 – 8 d). There was no evidence of damage to the cavernous sinus plexus.

RESULTS

Ciliary nerves, seen at the level of the ora serrata and pars plana, were composed largely of medium-size myelinated fibres characteristic of postganglionic parasympathetic fibres of the ciliary ganglion. Most of the ciliary nerves also contained compact groups of unmyelinated and small myelinated fibres. The latter branched selectively from the ciliary nerves, mainly at pars plicata level, and the largest of them entered the

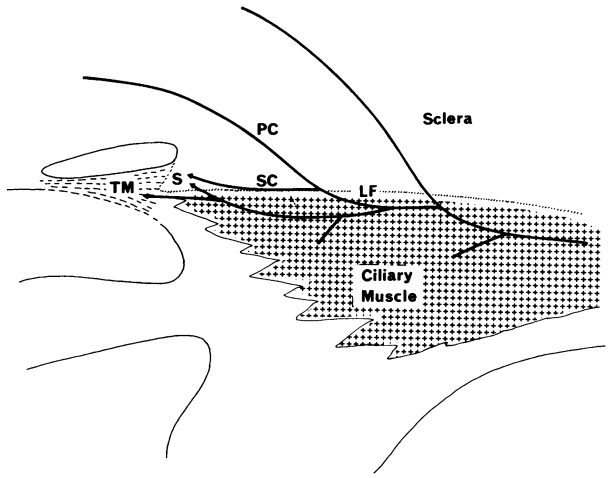
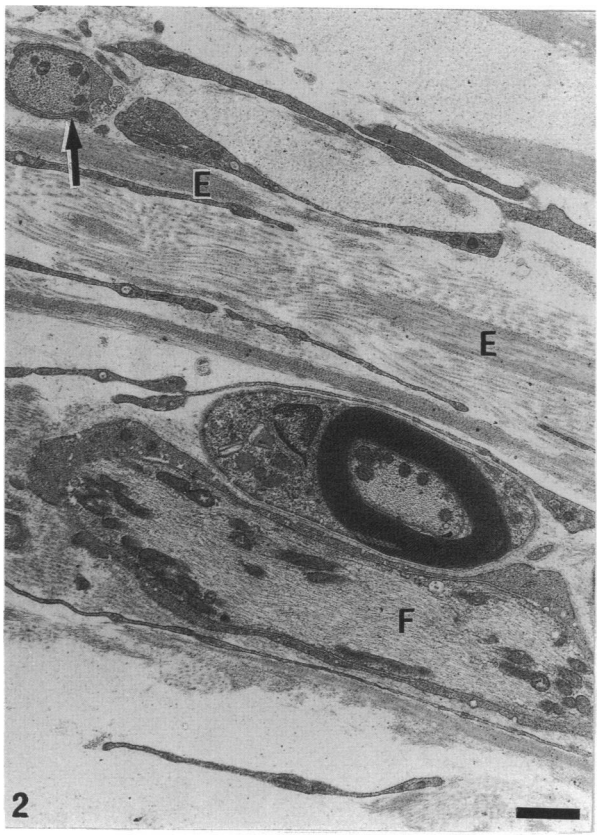


Fig. 1. Course of ciliary nerve branches entering the scleral spur (S) and trabecular meshwork (TM). LF, lamina fusca; PC, precorneal nerve; SC, supraciliary nerve.



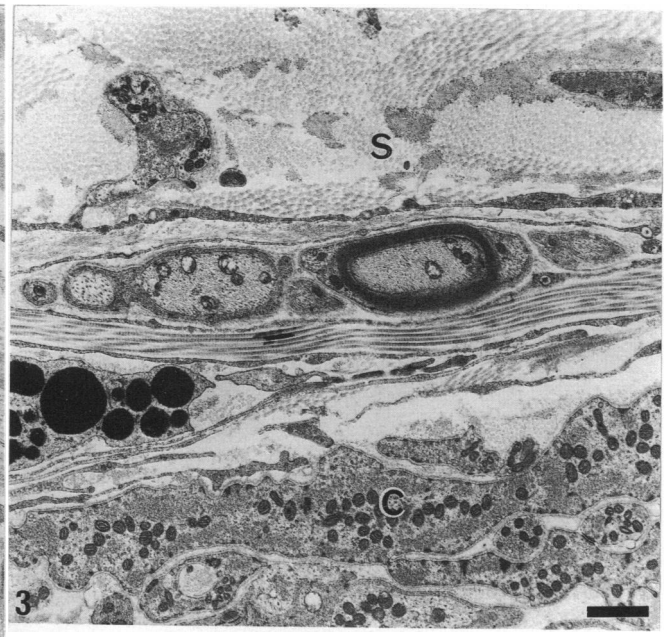


Fig. 2. Part of a supraciliary nerve buried in scleral spur collagen close to the cilioscleral border. The larger nerve fibre (F) contains aggregations of mitochondria and has an appearance typical of an axon having just emerged from its myelin sheath. It is partly enclosed, together with a myelinated fibre, by thin fibroblast processes. An axon without a fibroblastic investment is also present (arrow). E, elastic fibres. Control. Bar, 1 μm.

Fig. 3. A supraciliary nerve interposed between the ciliary muscle (C) and scleral spur (S) within the lamina fusca. Control. Bar, 1 μm.

sclera and continued to the cornea; they will be referred to as the precorneal nerves. As they crossed the uveoscleral interface, small superciliary branches, typically containing 1 or 2 small myelinated fibres and 3 or 4 unmyelinated bundles, passed forwards towards the chamber angle (Fig. 1). Supraciliary nerves advanced in the lamina fusca tissue at the interface, or nearby in the deepest layers of the sclera, or outermost layers of the ciliary muscle (Figs 1–3). Other tiny branches, of similar composition, advanced within the ciliary muscle. Supraciliary nerves were numerous, with a frequency of 3.6 ± 2.17 nerves per mm of scleral circumference measured close to the spur in the 3 animals. At the filtration angle the supraciliary nerves entered the spur and turned circumferentially within it to run parallel to the spur collagen. Branches lying a little deeper within the ciliary muscle either turned outwards to enter the spur and become disposed in the same manner as the others, or they passed forwards into the trabecular meshwork. Some nerves lost their perineurium before and others after changing direction, and an incomplete fibroblast-like investment was often substituted (Fig. 2). Having entered the scleral spur, no nerve could be traced subsequently to the trabecular meshwork or cornea. The meshwork lying immediately anterior to the scleral spur, the scleral trabeculae, was free of nerve fibres.

Many of the larger, unmyelinated nerve fibres within the spur displayed local thickenings or beads where the predominant axonal content of neurofilaments was supplemented by aggregates of mitochondria mainly disposed peripherally, and a few small clear vesicles (Figs 2, 8). Less frequently, smaller axons also displayed beads almost filled with small clear (35–55 nm) and large granular (65–85 nm) vesicles and mitochondria with relatively few neurofilaments (Fig. 9). Both forms are characteristic of terminals.

In material prepared from the operated sides evidence of degeneration within ciliary nerves was

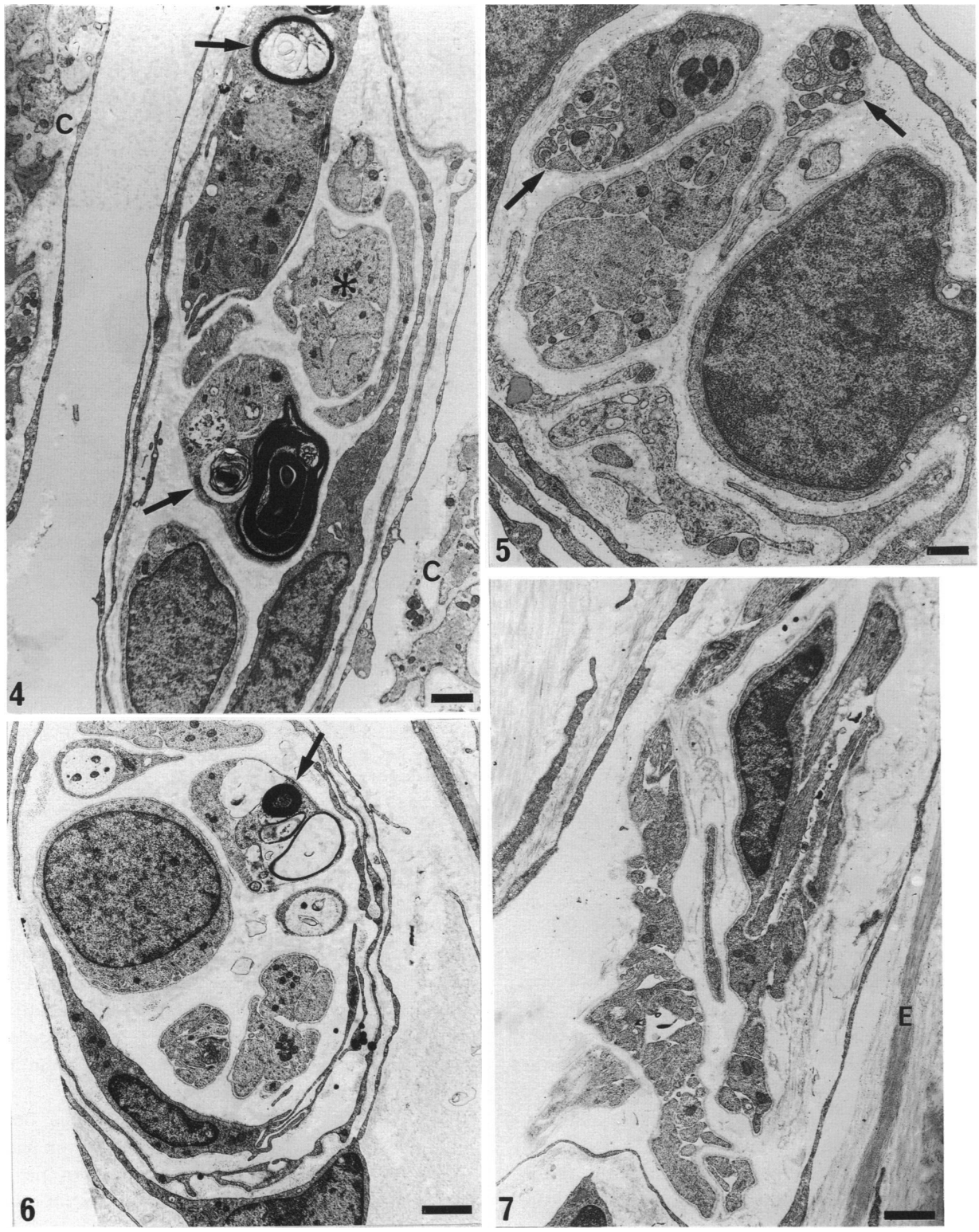


Fig. 4. A supraciliary nerve lying between fascicles of ciliary muscle (C) close to the ciliioscleral border; 8 d after neurotomy. All nerve fibres are degenerated. The 2 myelinated fibres (arrows) lack axoplasm, the myelin is disrupted and the Schwann cell cytoplasm is hypertrophic. Axoplasm is absent from an unmyelinated bundle which also shows hypertrophy and multiple processes (asterisk). Bar, 1 μm.

Fig. 5. A small unmyelinated supraciliary nerve with all nerve fibre bundles exhibiting degeneration but with 2 retaining axons of normal appearance (arrows); 8 d after neurotomy. Bar, 0.5 μm.

Fig. 6. A scleral spur nerve containing 4 bundles without enclosed axons, 1 with a Schwann cell nucleus, 1 degenerated myelinated nerve fibre (arrow), and 2 single unmyelinated axons with possible axoplasmic changes; 8 d after neurotomy. Bar, 1 μm.

Fig. 7. A group of degenerated nerve fibre bundles oriented circumferentially in the scleral spur; 3 d after neurotomy. The 4 or 5 bundles shown are mostly composed of membrane-bound Schwann cell fragments without enclosed axons. E, elastic fibre. Bar, 1 μm.

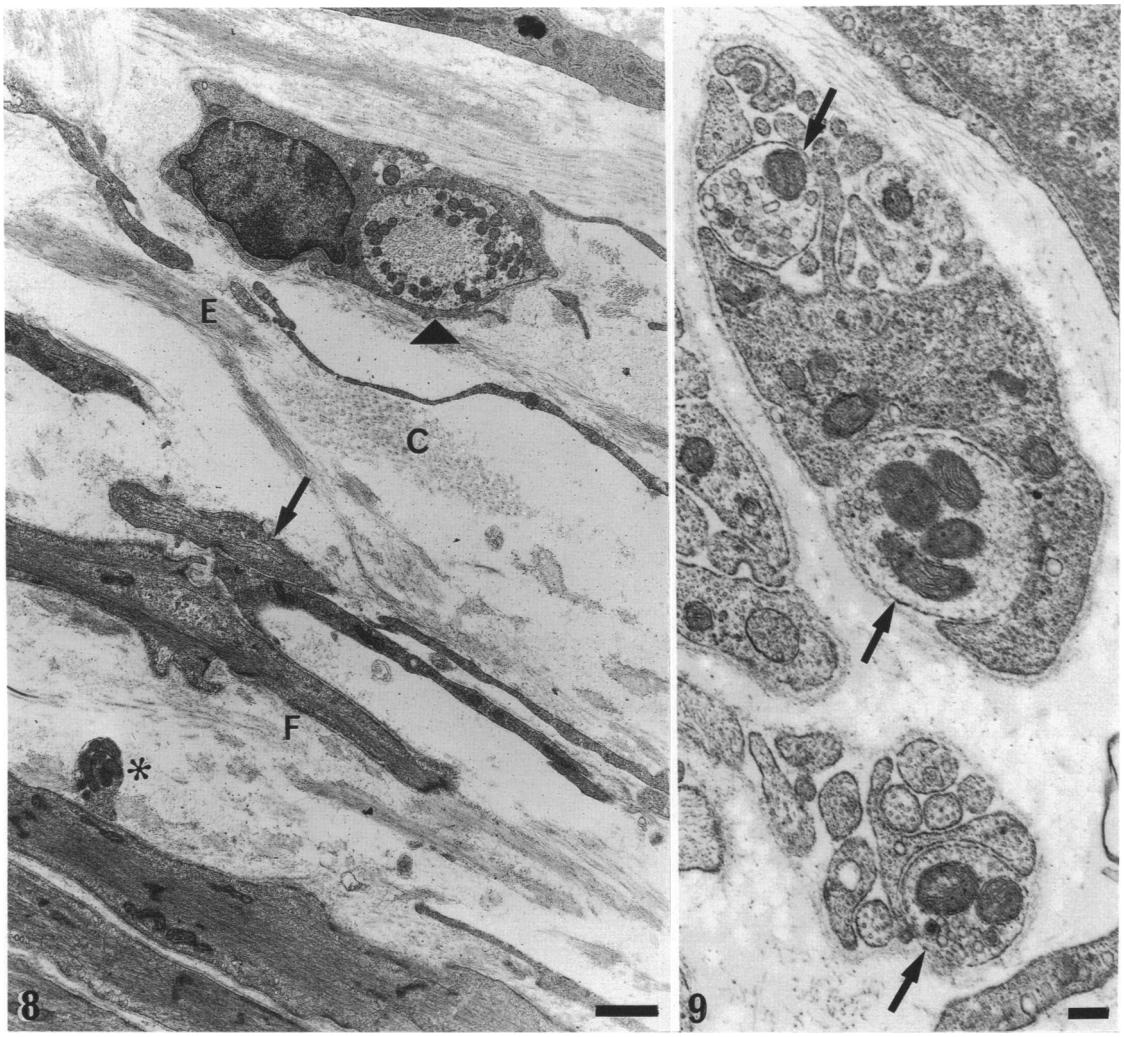


Fig. 8. Meridional section through the scleral spur showing an example of a larger axon with neurofilaments and peripherally disposed aggregates of mitochondria characteristically found in local expansions and suggestive of terminal form (arrowhead). A nerve fibre (arrow) and terminal (asterisk) contact ciliary muscle fibres. One of the muscle fibres (F) has made a shallow penetration of the spur connective tissue. C, collagen; E, elastic fibre. Control. Bar, 1 μm.

Fig. 9. Detail from Figure 5 with examples of a 2nd type of terminal containing mitochondria and small granular vesicles (arrows). Two of them also contain a large granular vesicle. The terminals have retained a normal appearance 8 d subsequent to ophthalmic neurotomy. Bar, 2 μm.

restricted to the small fibre groups that subsequently formed the precorneal nerves and, further forwards, degeneration was identified in the supraciliary branches and in spur nerve fibres. The various features indicating induced degeneration are illustrated in Figures 4–7 and described in the legends. Many of the beaded fibres, principally the vesicle-rich variety, and some myelinated fibres retained a normal appearance on the operated sides. None of the changes expressed on the operated sides were found in control material.

Spur and ciliary muscle terminals in control material were compared to seek evidence of distinguishing features. But both forms of spur terminal were also present in the ciliary muscle, although the ratios were different in the 2 structures. As expected, of the 2 types, vesicle-rich terminals were by far the more abundant in the muscle, whereas mitochondrion-rich terminals were more common in the spur.

Most of the nerves in the scleral spur in 2 of the animals contained degenerated fibres, whereas in the 3rd, following an incomplete lesion, they formed a minority. As counts could only be made reliably from transverse sections of nerves they were made exclusively from the radially oriented supraciliary nerves before they turned circumferentially into the spur (see Table and Fig. 10). The same anterior eye segments were used to count trabecular nerve fibres distal to the level of the spur. The proportion of supraciliary nerve fibres degenerated, grouping unmyelinated and mye-

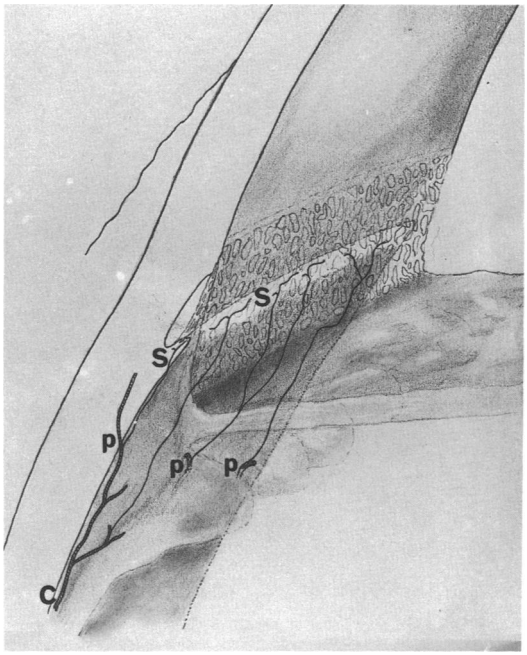


Fig. 10. Summary drawing to show the distribution of supraciliary nerves to the scleral spur. Supraciliary nerves branch directly from ciliary nerves (c) or from their scleral/corneal branches as they leave the ciliary body at p. A majority of supraciliary nerves change direction to run parallel to the scleral spur ring (s).

linated fibres together, were 48%, 85% and 18% respectively. The total proportions of myelinated (66%) and unmyelinated (58%) fibres degenerated were similar. Unmyelinated fibre bundles with induced changes but containing axons of normal appearance (Fig. 5) were included in the degenerated group in the Table.

Most of the nerve fibres entering the trabecular meshwork were single or in groups of 2 or 3 and without a perineurium, and more than a third of them were myelinated. Fibre orientation was mainly radial. Occasionally a larger nerve with a perineurial investment looped into the trabeculae transiently before returning to the ciliary muscle. With adequate illumination and magnification such nerves could often be identified in tissue blocks before sectioning. Other fibres, of terminal form, were seen adjacent or close to slender ciliary muscle fibre extensions into the trabecular meshwork. Excluding these 2 groups, in counts made from the operated sides, only 14% of the total unmyelinated fibre bundles (n = 459) and 34% of myelinated fibres (n = 138) seen in the angle tissues of the 3 animals were located in the trabecular meshwork. The remainder entered the scleral spur (Table); 26% (n = 65) of unmyelinated and 26% (n = 47) of myelinated trabecular fibres were degenerated.

DISCUSSION

On entering the ciliary body the ciliary nerves are composed of trigeminal, sympathetic and oculomotor postganglionic parasympathetic fibres (which contribute by far the most to nerve bulk). As they advance to the pars plicata the fibres are sorted. Most trigeminal fibres leave the ciliary nerves to enter the sclera and subsequently the cornea and, as shown here, some enter the scleral spur. Before considering these further it is worth noting that, apart from a few trigeminal fibres within nerves whose location indicated the iris as their destination, other trigeminal fibres in the ciliary body were remarkably scarce. Those present were nearly all located close to or within the lamina fusca and directed to the scleral spur, so trigeminal innervation of the ciliary muscle was sparse. Substance P-like nerve fibres (presumably of trigeminal origin) in the human ciliary muscle were reported to be most numerous in the longitudinal portion close to the scleral spur, and some were present in the lamina fusca, consistent with the present results (Stone & Kuwayama, 1985). On the other hand, moderate numbers were noted in all regions of the human ciliary muscle.

For several reasons, discussed elsewhere (Ruskell & Simons, 1987), the method of intracranial ophthalmic neurotomy close to the trigeminal ganglion fails to induce degeneration of all fibres peripheral to the lesion. Of these, the disposition of many somata beyond the trigeminal ganglion in the ophthalmic

Table. *Supraciliary nerve and trabecular fibre analysis following ophthalmic neurotomy*

Animal*	Length of sclera** (mm)	No. of nerves	No. with degenerated fibres	Unmyelinated degenerated/ total fibres	Myelinated degenerated/ total fibres
ML84[3]	14.4	35	23	37/100 (2/15)	30/40 (5/11)
ML85[7]	10.3	55	49	174/200 (15/24)	26/35 (5/12)
ML86[8]	13.8	48	10	16/94 (0/26)	4/16 (2/24)

Trabecular fibres are bracketed. The lesion in ML86 was incomplete. * The number of postoperative days is indicated as a superscript. ** Circumferential length cut at or close to the spur.

nerve presents the greatest problem; removal of the full intracranial length of the nerve is required to produce degeneration of all the distal fibres. To achieve this without damaging other nerves is impracticable, and removal of short lengths is not without hazard. Failure to obtain a full transection of the nerve in one of the preparations is attributable to an excess of caution, and the complete or nearly complete lesion in the other 2 does not ensure that all axons distal to the lesion would be degenerated. Moreover, any maxillary nerve fibres contributing to the sensory supply of the eye (Ruskell, 1974), would not have been recognised. Consequently, the degenerated fibres identified in the scleral spur nerves are unlikely to represent the full sensory complement.

In animal ML86, with a partial lesion, only 18% of the spur fibres displayed degeneration (Table). In the other 2 the proportions were 85% and 48%; the lower figure was found in the animal killed 3 d after surgery, indicating that this interval is probably inadequate if identification of the full complement of lesioned fibres is to be attempted. A majority of the nerve fibres in the spur of 1 animal was therefore of ophthalmic origin and, taking the technical limitations into account, a substantial proportion and possibly a majority of nerve fibres were arguably ophthalmic in the other 2. The remainder were presumably autonomic.

The presence of autonomic terminals in the scleral spur is to be expected, bearing in mind its association with the ciliary muscle. A proportion of the autonomic terminals were adjacent to those ciliary muscle fibres making shallow penetrations into the spur. Where this relationship was identified the terminals were not included in the counts. Whether or not the remainder were destined to be similarly related could not be determined. Innervated contractile fibroblasts, noted recently in the human and monkey scleral spur, may be alternative targets (Tamm et al. 1992). They were described as circularly oriented, spindle-shaped cells with characteristics only partly shared by ciliary muscle cells and surprisingly, in view of the present findings, penetration of the scleral spur by ciliary muscle fibres was not observed. The penetrating muscle fibres noted here often turned obliquely on entering the spur, but isolated circularly oriented cells had the appearance of conventional fibroblasts with no particular association with nerve fibres. Spur cells with the morphological correlates of contractility are therefore regarded here as smooth muscle cells rather than fibroblasts.

An alternative to the conclusion that nerves identified in the spur terminate there, is the possibility that they are in transit and subsequently pass to the trabecular meshwork or the cornea. The spur, as its name indicates, is connected to the sclera only at its base and spur nerves would first have to take a recurrent path to the sclera in order to gain access to the cornea. Serial sections offered no evidence that this unlikely course was taken. The dense innervation of the cornea stems from nerves that pass from the sclera to the cornea and these were followed in the present study. They crossed from the sclera in the outer two-thirds of its thickness and rarely took a deeper course; those that did, invariably entered the cornea directly, unrelated to the spur.

As the trabecular meshwork is partly a forward extension of the spur, there was an obvious possibility that spur nerves might continue into the meshwork. However, careful examination of the spur/trabecular interface, using serial sections, failed to reveal any bridging fibres, and nerve access to the trabecular meshwork was exclusively at uveal level. Even without this evidence the low numbers of fibres found in the trabecular meshwork compared with those of the spur, indicate that had transfer occurred it could only apply to a small fraction of the spur fibres. The values given in the Table, totalled for the 3 eyes, show a fourfold greater incidence of fibres in the spur.

The absence of accessing of spur fibres to adjacent tissues and the demonstration of the ophthalmic origin of a substantial proportion of their number, permits the proposal that this structure is a principal target for sensory nerve endings. The marked reorientation of fibres on entering the spur and the beaded form of many of the axons in control material are consistent with this view. The purpose of a sensory role for the scleral spur is not obvious and the comments that follow are necessarily speculative.

The ciliary muscle is firmly attached to the scleral spur by fibrous tissue and by shallow penetration of the spur by some meridional fibres. Attachment to the outer coat being much weaker elsewhere, the spur is, in effect, the muscle origin and on contraction the ciliary muscle imposes tension on the spur, displacing it backwards and inwards (Grierson et al. 1978). The spur terminals appear disposed to function as mechanoreceptors capable of recording tension. The predominantly circumferential fibre orientation constitutes a ring, coincident with that formed by scleral spur collagen, and ciliary muscle contraction would exert a force directed to expand the ring.

Displacement of the spur with accommodation causes a widening of the base of the trabecular prism (as seen in section), increasing the volume occupied by trabecular tissue and spacing of the meshes (Grierson

et al. 1978). Increased aqueous outflow and intraocular pressure reduction in drug-induced ciliary muscle contraction is attributed to the pull on the meshwork (Kaufman & Bárány, 1976). Accepting a mechanoreceptor function for the spur terminals, it follows that increments in outflow and spur tension may be correlated, providing an indirect measure of flow. That they may be employed as the sensory arm of a reflex mechanism contributing to regulation of aqueous production and intraocular pressure is unlikely when the multiplicity of factors influencing flow other than ciliary muscle contraction are considered, such as blood flow changes, various factors affecting rate of aqueous production, eye and eyelid movements and posture. Terminals within the meshwork itself are better placed to register flow changes.

On the reasonable assumption that they respond to the sum of ciliary muscle pull, spur receptors have the capacity to transmit information on the state of contraction of the ciliary muscle, and this is suggested as a more tenable role, i.e. they act as muscle proprioceptors. The concept of receptors lying external to muscle responding to muscle-induced tension is by no means novel and in the present instance it has the virtue of being in the one position likely to permit monitoring of contraction of all parts of the muscle. Golgi tendon organs of voluntary muscle have similar characteristics and although an exact parallel in other smooth muscles does not spring to mind, tension receptors associated with smooth muscle have been described. Morphologically unspecialised sensory receptors, for example, are located in adjacent connective tissue rather than in the smooth muscle itself in the gut (Cevero & Foreman, 1990) and in the walls of arteries (Simons & Ruskell, 1988). A requirement for recording ciliary muscle contraction is unclear, but perhaps the rapid microfluctuations of accommodation under steady viewing conditions (Campbell et al. 1959) incorporate corrective adjustments in response to spur terminal feedback. The signalling of fatigue is another possible role, contributing subjectively to asthenopia.

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