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Innervation of the lacrimal gland in the cynomolgous monkey: a retrograde tracing study

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ABSTRACT

Retrograde transport of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was used to study the localisation of neurons that innervate the lacrimal gland of the cynomolgous monkey. WGA-HRP-labelled neurons were localised in the ipsilateral trigeminal, superior cervical and ciliary ganglia and in the ipsilateral and contralateral pterygopalatine ganglia. In the trigeminal ganglion WGA-HRP-labelled somata were found in the ophthalmic part (18%) and the maxillary part (5%). Identification of labelled neurons in the ciliary and pterygopalatine ganglia indicates a dual parasympathetic innervation of the lacrimal gland. There is no known pathway to account for the contralateral location or pterygopalatine neurons. These novel findings are incorporated in a concept of a neural control mechanism for the lacrimal gland.

Key words: *Macaca fascicularis*; WGA-HRP; trigeminal ganglion; parasympathetic ganglia; superior cervical ganglion.

INTRODUCTION

In primates the lacrimal gland, the meibomian glands and the glands of Krause and Wölfring all contribute to tear production (Duke-Elder & Wybar, 1961; Jones, 1973; Iwamoto & Jakobiec, 1982). Most of the tear fluid is produced by the lacrimal gland. Secretion by the lacrimal gland is regulated by the autonomic and sensory nervous systems via a reflex arc (Drummond & Lance, 1992). According to the classical concept, the lacrimal gland in the primate is innervated parasympathetically via the pterygopalatine ganglion (Mitchell, 1953; Duke-Elder & Wybar, 1961). Morphologically the pterygopalatine ganglion in the monkey is ovoid with conical extensions (Ruskell, 1971; Baljet et al. 1989). The orbital rami issue from 6 or fewer dorsal extensions (Wilson, 1984). From the brainstem, preganglionic nerve fibres run from the superior salivary nucleus along the greater superficial petrosal nerve and the vidian nerve through the pterygoid canal to the pterygopalatine ganglion (Spencer et al. 1990; Ten Tusscher et al. 1990).

Histochemically, acetylcholinesterase-positive nerve fibres have been detected in and between the acini in the lacrimal gland of mammals (Ehinger, 1966*a*). In the rat and guinea pig, adrenergic nerve fibres have been found in the vascular plexus of the gland but not in the acini and secretory ducts (Ehinger, 1964). Other histochemical and electron microscopy studies of the lacrimal gland of the rat (Nikkinen et al. 1984, 1985; Lee et al. 1985), guinea pig (Nikkinen et al. 1984, 1985), cynomolgous monkey (Ruskell, 1971) and man (Ruskell, 1975) have also revealed sympathetic nerve endings on arterioles and in the interstices of the gland originating in the superior cervical ganglion.

Electrostimulation of the cervical sympathetic trunk of the cat caused an immediate increase in lacrimation (Whitwell, 1961). This could not be confirmed by Botelho et al. (1966), although stimulation of the lacrimal nerve, including nerve fibres from the pterygopalatine ganglion, caused an increase of outflow. Pharmacological studies on in vitro slices of rabbit lacrimal gland have indicated the presence of both alpha and beta-adrenergic receptors and cholinergic receptors, indicating a sympathetic and

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parasympathetic mechanism for control of lacrimal protein secretion (Bromberg, 1981).

Studies on the distribution of autonomic postganglionic nerve fibres to the lacrimal gland in the cynomolgous monkey showed fine nerves, the lacrimal rami, extending from the retro-orbital plexus (both parasympathetic and sympathetic) or directly from the pterygopalatine ganglion (parasympathetic) towards the gland (Ruskell, 1965, 1971; Baljet et al. 1989). After superior cervical or pterygopalatine ganglionectomy, light and electron microscopy studies (Ruskell, 1967, 1969) revealed that all sympathetic and some parasympathetic nerve fibres in the lacrimal gland had degenerated. The precise localisation of the parasympathetic cell bodies that innervate the lacrimal gland was not established. A selective retrograde tracing study can provide information on specific neurons involved in lacrimal gland secretion. In order to examine this, the wheat germ agglutinin-horseradish peroxidase (WGA-HRP) retrograde tracing technique has been performed in the cynomolgous monkey.

MATERIALS AND METHODS

Six adult cynomolgous monkeys (*Macaca fascicularis*) of both sexes, weighing between 3.2 and 8.0 kg, served as experimental subjects. Each animal was anaesthetised with a mixture of ketamine, xylazine (Rompun) and atropine in the proportions 10, 1 and 0.1 mg/kg, respectively. A solidified piece of WGA-HRP (Sigma) in Willospon (Willpharma), prepared by soaking a small piece of Willospon in a 10% WGA-HRP phosphate-buffered solution, was implanted surgically in the lacrimal gland of the right orbit.

The animals were anaesthetised 48 or 72 h after surgery with ketamine (0.4 ml/kg) followed by 5000 units thromboliquine/kg. Next the animals were killed by an overdose of pentobarbital and perfused directly through the internal carotid artery with 2 l phosphate-buffered saline, pH 7.4, at 37 °C, perfused with 2–3 l of fixative, containing 0.1 M phosphate-buffered 2% paraformaldehyde and 2.5% glutaraldehyde, pH 7.4, and finally with 0.1 M phosphate buffer containing 10% sucrose at 4 °C.

Immediately after perfusion of the animals, the orbital fat, parts of the upper eyelid and the cornea were dissected and processed in the same way as the brainstem and ganglia in order to determine whether WGA-HRP had leaked into these tissues. The lacrimal gland was also removed and processed to assess the extent of the labelled area. The brainstem, the trigeminal, superior cervical, pterygopalatine, ciliary, geniculate (NVII), and nodose (NX) and the 1st 3 spinal ganglia on both sides were dissected. Transverse sections (50 μm) of the brainstem were cut with a cryostat microtome from the level of the anterior part of the superior colliculus up to the spinal cord. In addition 25 μm serial cryostat sections of the ganglia from both sides were mounted directly on chrome-alum-gelatin-coated slides; sections on the slides were then tested for the presence of WGA-HRP activity using either the tetramethylbenzidine (TMB) procedure (Mesulam, 1978; van der Werf & Baljet, 1989; Zaborszky & Reimer, 1989) or the gold-substituted silver peroxidase (GSSP) procedure (Van den Pol & Görcs, 1986; Prins et al. 1993). All samples were examined by bright and dark-field microscopy for the presence of WGA-HRP-labelled neurons. Serial cryostat sections have been made of all ganglia. Unfortunately, producing cryostat sections seldom results in a complete series. Therefore cell counting was performed on the morphologically optimal series of cryostat sections of ganglia of 3 monkeys (nos 53, 98, 2847). Because of some missing sections in the remaining series, cell counts were not performed in these ganglia.

A series of camera lucida drawings was made of every 4th 25 μm cryostat section of the ipsilateral trigeminal (monkey 53), superior cervical (monkey 98) and ciliary (monkey 53) and the ipsilateral pterygopalatine (monkey 2847) ganglia in order to count WGA-HRP-labelled neurons and to estimate the number of labelled somata in the ganglia innervating the lacrimal gland. Only those neurons with a nucleus were counted and their diameter measured. Using this method, double counting of neurons can be avoided, although some neurons will be missed. The monkeys used in this study previously participated in behavioural studies at the University of Utrecht. The animal experiments were carried out under the responsibility of the ethical committee of The Royal Netherlands Academy of Arts and Sciences acting in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

RESULTS

At 48 and 76 h after retrograde tracing, labelled somata were seen in the various ganglia except in the ipsilateral geniculate (NVII), nodose (NX) and 1st 3 spinal ganglia. Except in the pterygopalatine ganglion, labelled somata were never observed in the contra-

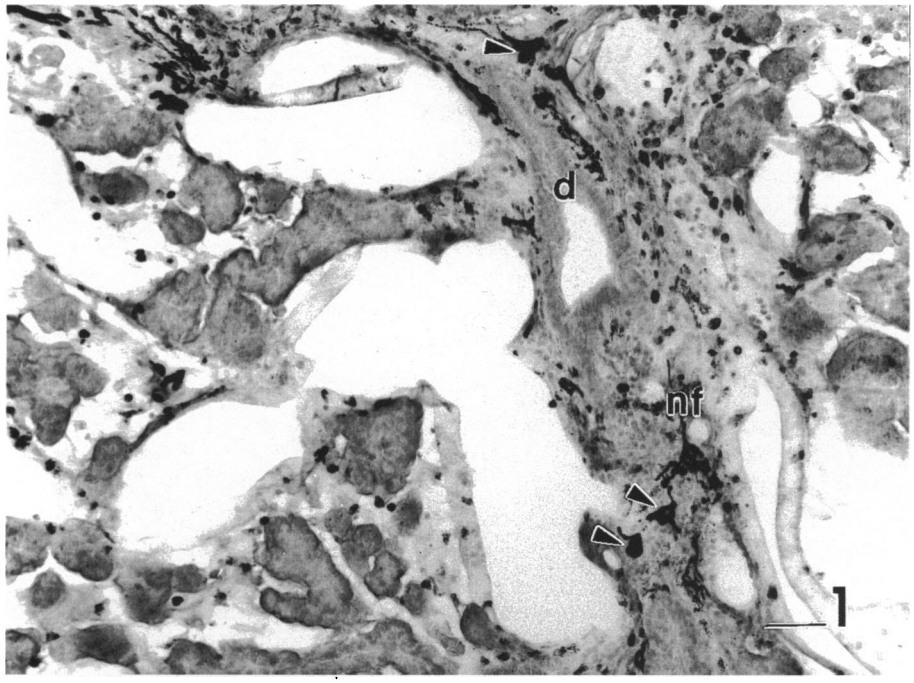

Fig. 1. Brightfield light micrograph of WGA-HRP-labelled somata of neurons (arrows) in the wall of a duct of the lacrimal gland (GSSP procedure); d, duct; nf, nerve fibre. Bar, 50 μm.

lateral ganglia of any of the animals. Leakage of the tracer was not detected in cryostat sections of the orbital fat, parts of the upper eyelid and whole cornea.

Lacrimal gland

After inserting WGA-HRP implants, the tracer was restricted to the orbital lacrimal gland. Cryostat sections revealed the presence of WGA-HRP-labelled somata of neurons in the walls of the duct system (Fig. 1) and many WGA-HRP-labelled nerve fibres in the stroma, duct system and acini.

Trigeminal ganglion

In all 6 monkeys, WGA-HRP-labelled somata of varying size were distributed in the ipsilateral trigeminal ganglion opposite the origin of the ophthalmic and maxillary nerves, but not opposite the mandibular nerve (Fig. 2). Counts of labelled somata in the trigeminal ganglion of monkey 53 revealed 18% of neurons in the ophthalmic part ($\leqslant$ 1% of 18% in the transitional zone between the ophthalmic and maxillary parts) and 5% in the maxillary part (Fig. 3, Table). In the trigeminal ganglion, WGA-HRP-labelled neurons varied in size; small as well as large cell bodies (larger than 60 μm) were positive for the WGA-HRP tracer.

Superior cervical ganglion

Many large (30–40 μm) and small (20–30 μm) WGA-HRP-labelled somata (Fig. 4) were distributed randomly ipsilaterally throughout the spindle-shaped superior cervical ganglion of all 6 monkeys. Counts of labelled somata in serial sections of the superior cervical ganglion of monkey 98 revealed the presence of 31% of neurons in the cranial part, 41% in the

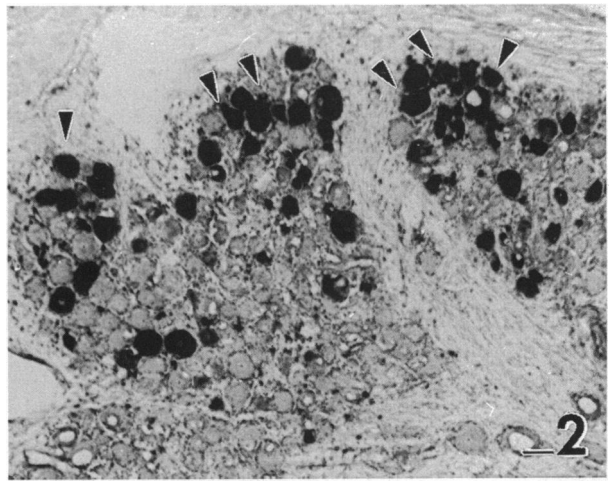

Fig. 2. Brightfield light micrograph of WGA-HRP-labelled somata (arrows) in the ophthalmic and maxillary parts of the trigeminal ganglion (TMB procedure). Bar, 50 μm.

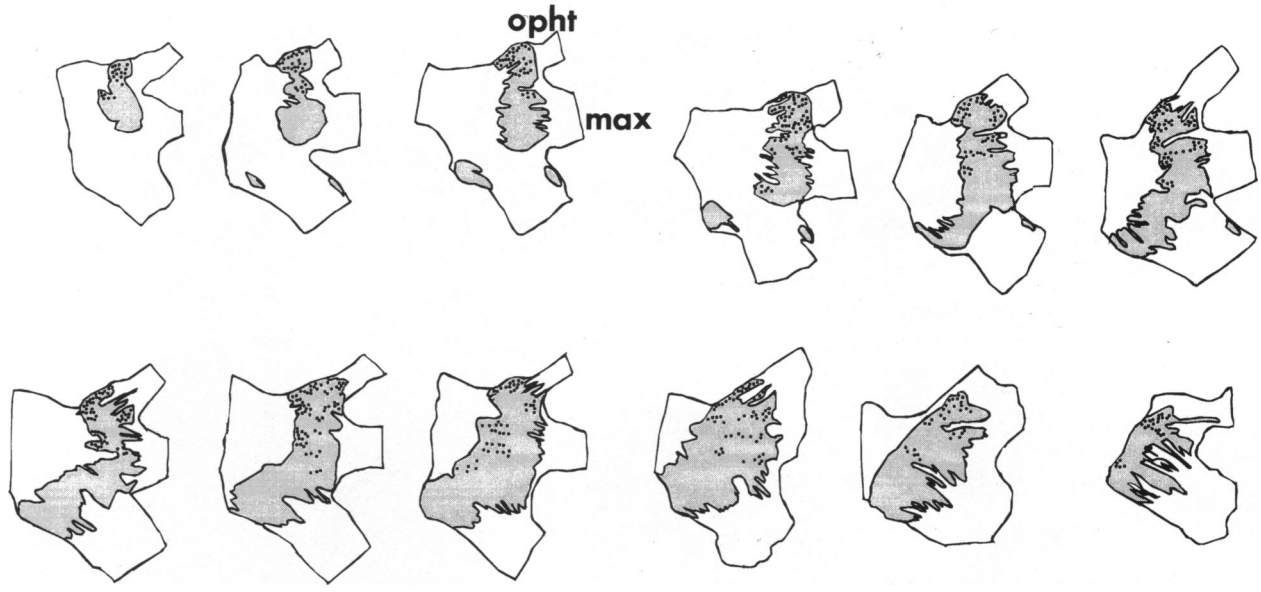

Fig. 3. Schematic drawing of serial cryostat sections at 100 μm intervals illustrating the distribution of WGA-HRP-labelled somata (solid circles) in the trigeminal ganglion of monkey 53: 75% of the labelled somata are located in the ophthalmic part (opht), 20% in the maxillary part (max), 5% in the transitional zone (tz).

Table. *Number of labelled neurons (lab.) in each ganglion after WGA-HRP injection into the lacrimal gland**

Ganglia	Trigeminal				Superior cervical									
	Ophthalmic		Maxillary		Cranial		Central		Caudal		Pterygopalatine		Ciliary	
Section	Lab.	Total no.	Lab.	Total no.	Lab.	Total no.	Lab.	Total no.	Lab.	Total no.	Lab.	Total no.	Lab.	Total no.
1	67	340	3	471	0	0	72	156	32	104	27	76	19	242
2	140	457	10	487	26	112	56	140	48	168	94	227	24	471
3	107	295	12	830	41	141	56	172	42	172	87	280	14	905
4	95	215	61	1009	62	186	173	287	56	198	115	436	3	885
5	144	581	73	1248	80	241	179	409	54	207	69	387	4	673
6	194	714	71	1414	82	244	75	166	27	216	20	295	3	712
7	111	603	79	1258	14	73	28	214	0	0	23	431	—	—
8	114	33	114	1500	—	—	—	—	—	—	5	329	—	—
9	131	744	117	1474	—	—	—	—	—	—	0	28	—	—
10	157	1257	29	620	—	—	—	—	—	—	—	—	—	—
11	148	1024	26	825	—	—	—	—	—	—	—	—	—	—
12	66	543	27	737	—	—	—	—	—	—	—	—	—	—
Total	1347	7506	622	11973	305	997	639	1544	259	1065	440	2489	67	3878
(%)	18	—	5	—	31	—	41	—	24	—	18	—	2	—

* Counts of WGA-HRP-labelled somata within the trigeminal (monkey 53), superior cervical (monkey 98), pterygopalatine (monkey 2847) and ciliary (monkey 53) ganglia. Values were obtained from every 4th 25 μm cryostat section.

central part and 24% of neurons in the caudal part of the ganglion (Fig. 5, Table).

Pterygopalatine ganglion

WGA-HRP-positive somata were localised in the ipsilateral and *contralateral* pterygopalatine ganglion of all 6 monkeys. In the ipsilateral pterygopalatine ganglion WGA-HRP-labelled somata were detected in the upper ovoid part of the ganglion and its extensions (Fig. 6). Counts of WGA-HRP-labelled somata in serial sections of the ipsilateral pterygopalatine ganglion of monkey 2847 revealed a total of 440 (18%) labelled neurons (Fig. 7, Table). In the contralateral ganglion granular WGA-HRP-labelled somata were present (Fig. 8).

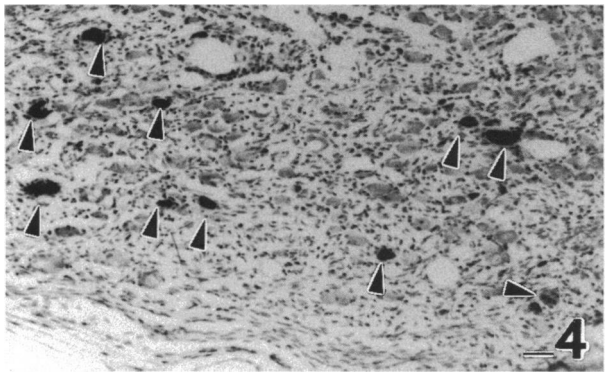

Fig. 4. Brightfield light micrograph of large and small WGA-HRP-labelled somata (arrowheads) in the superior cervical ganglion distributed randomly throughout the ganglion (GSSP procedure). Bar, 50 μm.

Ciliary ganglion

WGA-HRP-labelled somata were found in the ipsilateral ciliary ganglion of all 6 monkeys (Fig. 9). Counts of labelled somata in serial sections of the ciliary ganglion in monkey 53 revealed the presence of 67 cell bodies (Fig. 10, Table). WGA-HRP-labelled nerve fibres were detected between the somata throughout the ciliary ganglion.

DISCUSSION

The results of the present study provide information on the localisation of neurons that innervate the orbital lacrimal gland of the cynomolgous monkey. Sensory (afferent), sympathetic (efferent) and parasympathetic (efferent) innervation of the gland was determined. Afferent innervation was restricted to the ophthalmic (18%) and maxillary (5%) parts of the trigeminal ganglion; efferent sympathetic innervation was found for the superior cervical ganglion and parasympathetic innervation for the pterygopalatine and ciliary ganglia.

Controls

The method used for selective application of the tracer to the lacrimal gland produced no detectable leakage into adjacent tissues. There was no difference in neuron labelling in the ganglia examined between specimens killed 48 and 72 h after treatment. Varying survival times can alter the number of labelled neurons in the involved ganglia (Ten Tusscher et al. 1988); this was not the case in this study.

Lacrimal gland

Acetylcholinesterase histochemical analysis of cryostat sections of the lacrimal gland from the cynomolgous monkey showed a meshwork of bundles of thick and thin nerve fibres in the stroma and cells of the acini, around the ductal system and as a perivascular network (Baljet et al. 1989; van der Werf & Baljet, 1989). Nerve fibres in the lacrimal gland of the Japanese macaque (Matsumoto et al. 1992) and the salivary glands of cebid monkeys (Rossoni et al. 1992) exhibited the same pattern of acetylcholinesterase-positive fibres as seen in the cynomolgous monkey (Baljet et al. 1989), but the relationships with nerve plexuses were not mentioned by the authors. Investigations on the rabbit, guinea pig and monkey, using the fluorescence method for catecholamines developed by Falck (Falck et al. 1962), revealed adrenergic nerves only on the blood vessels which supply the lacrimal gland (Ehinger, 1964, 1966*b*). In this study cell bodies and nerve fibres of neurons were localised in the ductal system. Immunohistochemical studies of rat (Nikkinen et al. 1984; Leblanc & Landis, 1988), rabbit (Uddman et al. 1980; Butler et al. 1984), guinea pig (Nikkinen et al. 1984), and the monkey (Matsumoto et al. 1992), neuropeptide Y, vasoactive intestinal polypeptide, substance P and calcitonin gene-related peptide were observed at

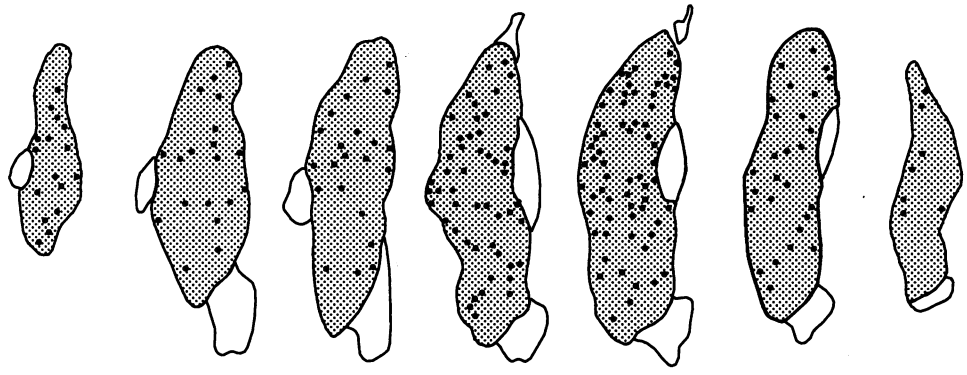

Fig. 5. Schematic drawing of serial cryostat sections at 100 μm intervals illustrating the distribution of WGA-HRP-labelled somata in the superior cervical ganglion of monkey 98.

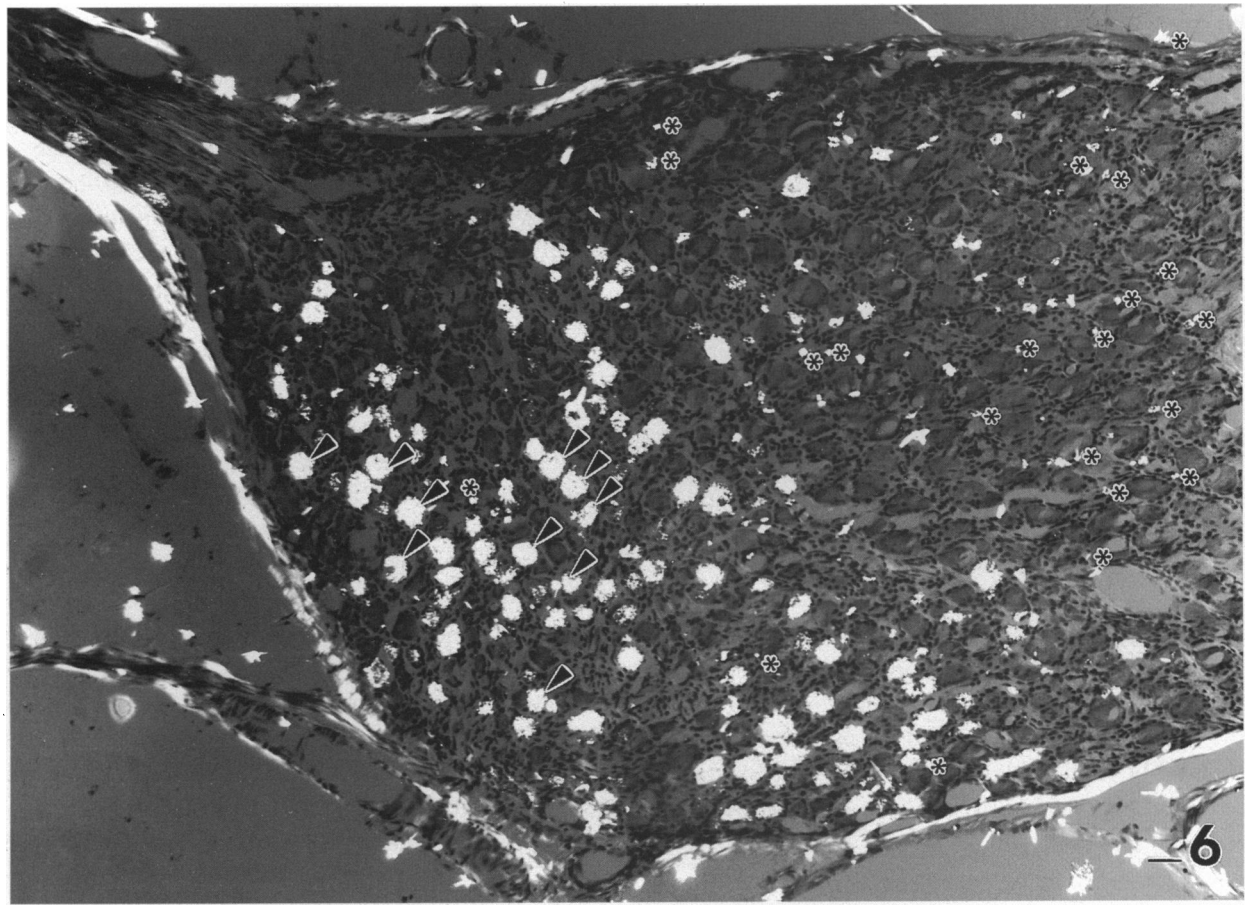

Fig. 6. Darkfield light micrograph of WGA-HRP-labelled somata (arrowheads) in the ipsilateral pterygopalatine ganglion (TMB procedure). Artefacts of small TMB cristal fragments are marked by asterisks. Bar, 50 μm.

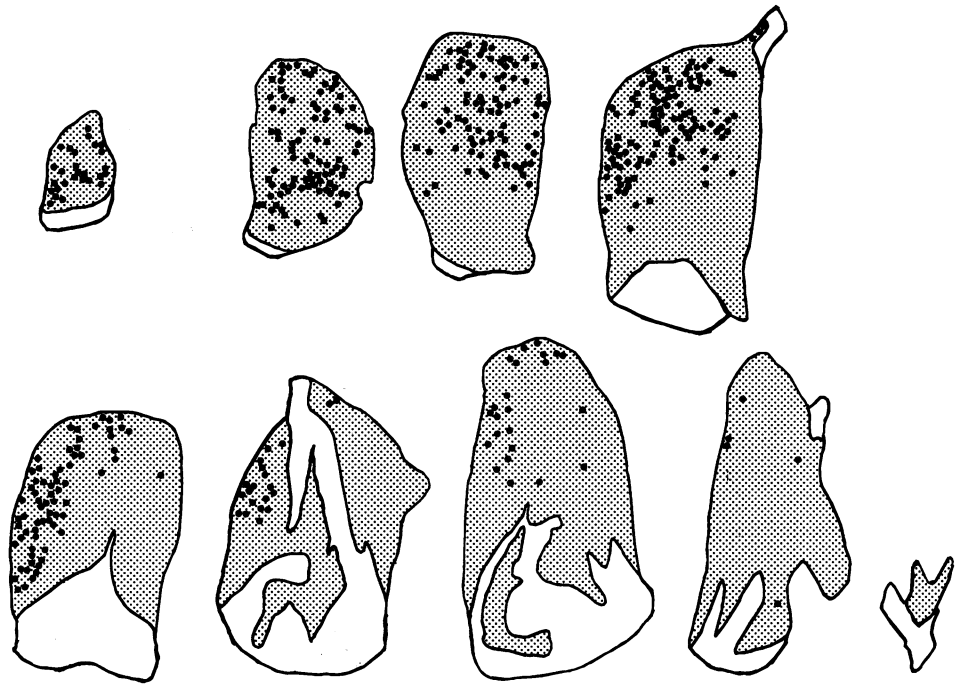

Fig. 7. Schematic drawing of serial cryostat sections at 100 μm intervals illustrating the distribution of WGA-HRP-labelled somata in the ipsilateral pterygopalatine ganglion of monkey 2847. A concentration of labelled somata is localised in the upper part of the ganglion.

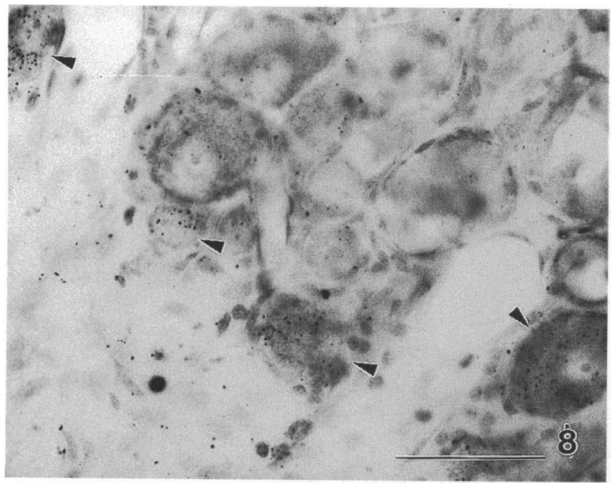

Fig. 8. Brightfield light micrograph of WGA-HRP-labelled somata (arrows) in the contralateral pterygopalatine ganglion (GSSP procedure). Granular WGA-HRP-labelled somata are present in the orbital rami of the ganglion. Bar, 50 μm.

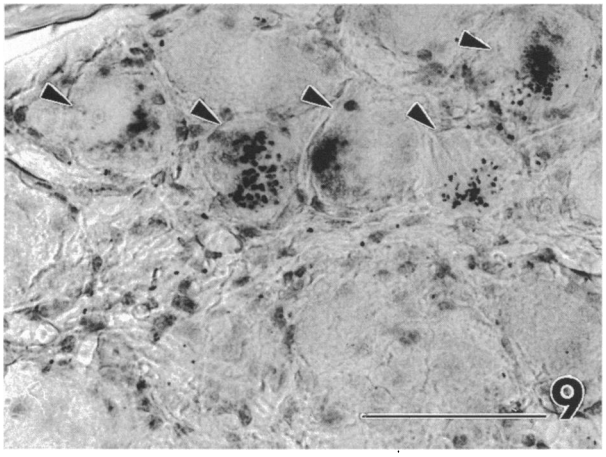

Fig. 9. Brightfield light micrograph of WGA-HRP-labelled somata (arrows) in the ciliary ganglion (TMB procedure). Most of the labelled somata are located in the periphery of the ganglion. Bar, 50 μm.

different sites along the nerve fibres in the lacrimal gland. After VIIth nerve (pterygopalatine) degeneration in the rabbit (Butler et al. 1984), vasoactive intestinal polypeptide-positive content in the lacrimal gland was almost completely absent. Biologically active peptides, such as vasoactive intestinal polypeptide, induced fluid and protein secretion by the lacrimal gland in a dose-dependent manner (Dartt et al. 1988), while cholecystokinin octapeptide stimulated only fluid production and not protein secretion. These nerves were presumed to be parasympathetic; our finding of acetylcholinesterase in autonomic (pterygopalatine) nerve fibres in the lacrimal gland supports this theory.

Trigeminal ganglion

The present study of the cynomolgous monkey has demonstrated that afferent neurons are concentrated in the ophthalmic and maxillary parts of the ipsilateral trigeminal ganglion. A somatotopic distribution was present in the medial lateral region of the ophthalmic part. In contrast to classical concepts (Duke-Elder & Wybar, 1961; Jones, 1973; Iwamoto & Jakobiec, 1982), i.e. that neurons which contribute to innervation of the lacrimal gland are localised only in the ophthalmic part of the trigeminal ganglion, WGA-HRP-labelled somata of various sizes were also detected in the maxillary part of this ganglion. As branches of the lacrimal nerve subserving the skin traverse the gland, tracer uptake 'en passage' may be possible. The surgical implantation method used gives little diffusion of the tracer in the gland. Occasionally some tracer uptake into skin nerve fibres was found, so some of the labelled cells in the trigeminal ganglion can contribute to skin innervation. There are 2 pathways in lacrimal gland innervation of the cynomolgous monkey: via the lacrimal nerve and via the zygomaticofacial nerve. These pathways are supported by observations in whole mount preparations of human fetus orbits, in which networks of autonomic and sensory nerve fibres in the anterior orbital part were found (Baljet et al. 1989).

Superior cervical ganglion

The results reveal that the lacrimal gland of the cynomolgous monkey is sympathetically innervated by numerous neurons located in the ipsilateral superior cervical ganglion. Other studies confirm a sympathetic innervation of the lacrimal gland (Ehinger, 1964, 1966*b*; Ruskell, 1967, 1969; Pick, 1970; Nikkinen et al. 1984; Baljet et al. 1989; van der Werf & Baljet, 1989; Ten Tusscher et al. 1990; Matsumoto et al. 1992). Unilateral superior cervical ganglionectomy caused degeneration of sympathetic nerve fibres in the acini and duct system of the lacrimal gland of the cynomolgous monkey (Ruskell, 1969), which led to dysregulation in the composition of tear fluid. In rats (Ten Tusscher et al. 1990) retrograde-tracing experiments showed both ipsilateral and contralateral contributions from superior cervical ganglion neurons, whereas a contralateral distribution in monkeys was absent. Presumably the disparity can be attributed to a species difference. The number of labelled superior cervical neurons may be relatively high in this study, although retrograde fluorescent studies in the submaxillary salivary gland

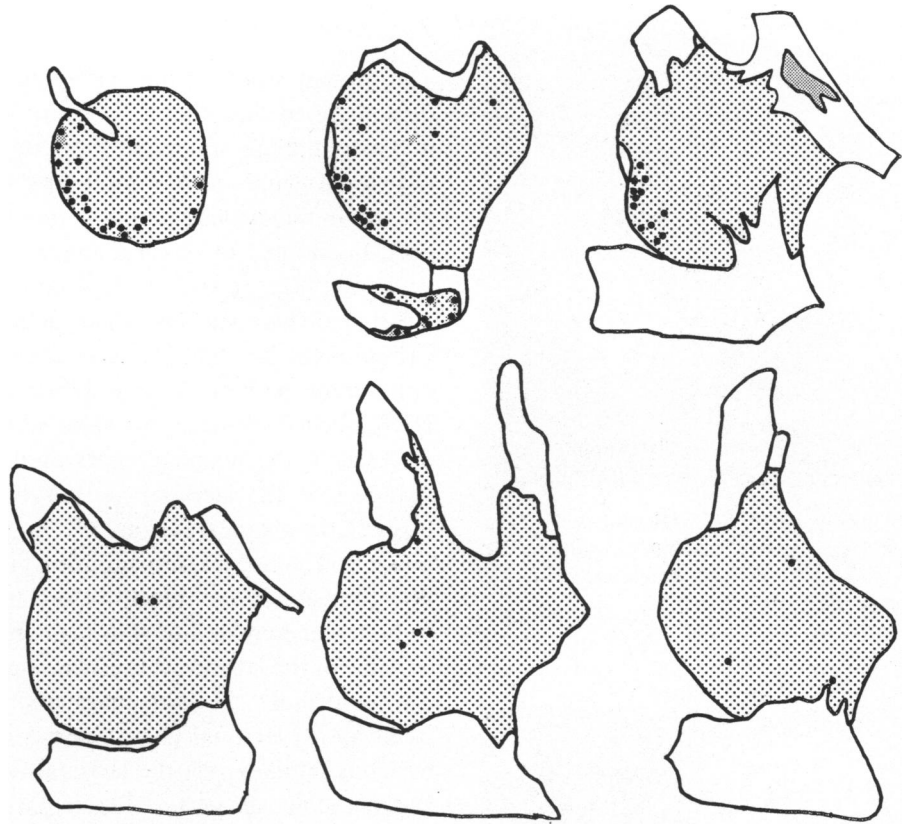

Fig. 10. Schematic drawing of serial cryostat sections at 100 μm intervals illustrating the distribution of WGA-HRP-labelled somata in the ipsilateral ciliary ganglion of monkey 53. A concentration of labelled somata is localised in the peripheral part of the ganglion.

in the rat revealed more than 5000 labelled neurons in the superior cervical ganglion (Flett & Bell, 1991)!

Pterygopalatine ganglion

According to the classical concept, the lacrimal gland in the primate is innervated exclusively via the ipsilateral parasympathetic pterygopalatine ganglion (Mitchell, 1953). In this study implantation of the WGA-HRP tracer into the lacrimal gland resulted in detection of labelled somata in the lacrimal gland itself, and in the ipsilateral and contralateral pterygopalatine ganglia. The presence of autonomic-like neurons in the lacrimal gland is not exceptional: in human and monkey choroids hundreds of neurons were found in the region around the fovea (Flügel et al. 1994*a*, *b*). These neurons have autonomic properties and may be described as 'displaced neurons originating from the pterygopalatine ganglion'. WGA-HRP-labelled somata detected in the ipsilateral pterygopalatine ganglion were localised in the upper part of the ovoid ganglion. In the contralateral ganglion, labelled cell bodies were detected near the orbital rami. Never more than 20 neurons were counted in a ganglion, this is less than 5% in comparison with labelled neurons on the ipsilateral side. No obvious nerve pathway for the passage of nerves from the pterygopalatine ganglion to the opposite orbit is known; a perivascular nerve pathway from the lacrimal gland along the ophthalmic artery out of the orbit, via internal carotid arteries at the circle of Willis towards the contralateral ophthalmic artery and thus to the contralateral pterygopalatine ganglion is perhaps feasible. The functional aspects of this finding need to be investigated further. However, it is well known that intraocular pressure of both eyes decreases after treatment of one eye with betablockers.

Preliminary results of our anterograde tracing studies in the ophthalmic part of the trigeminal ganglion of cynomolgous monkeys (van der Werf, 1993) revealed labelled terminals on cell bodies of the pterygopalatine ganglion. In rats after anterograde labelling of the trigeminal ganglion, labelled terminals were also observed in the pterygopalatine ganglion both by light and electron microscopy (Beckers et al. 1991). These nerve fibres contain both calcitonin gene-related peptide and substance P and can form pericellular baskets around vasoactive intestinal polypeptide or choline acetyltransferase-positive somata (Suzuki et al. 1989). In the rat pterygopalatine ganglion cells, innervating the lacrimal gland, are vasoactive intestinal polypeptide positive (Leblanc &

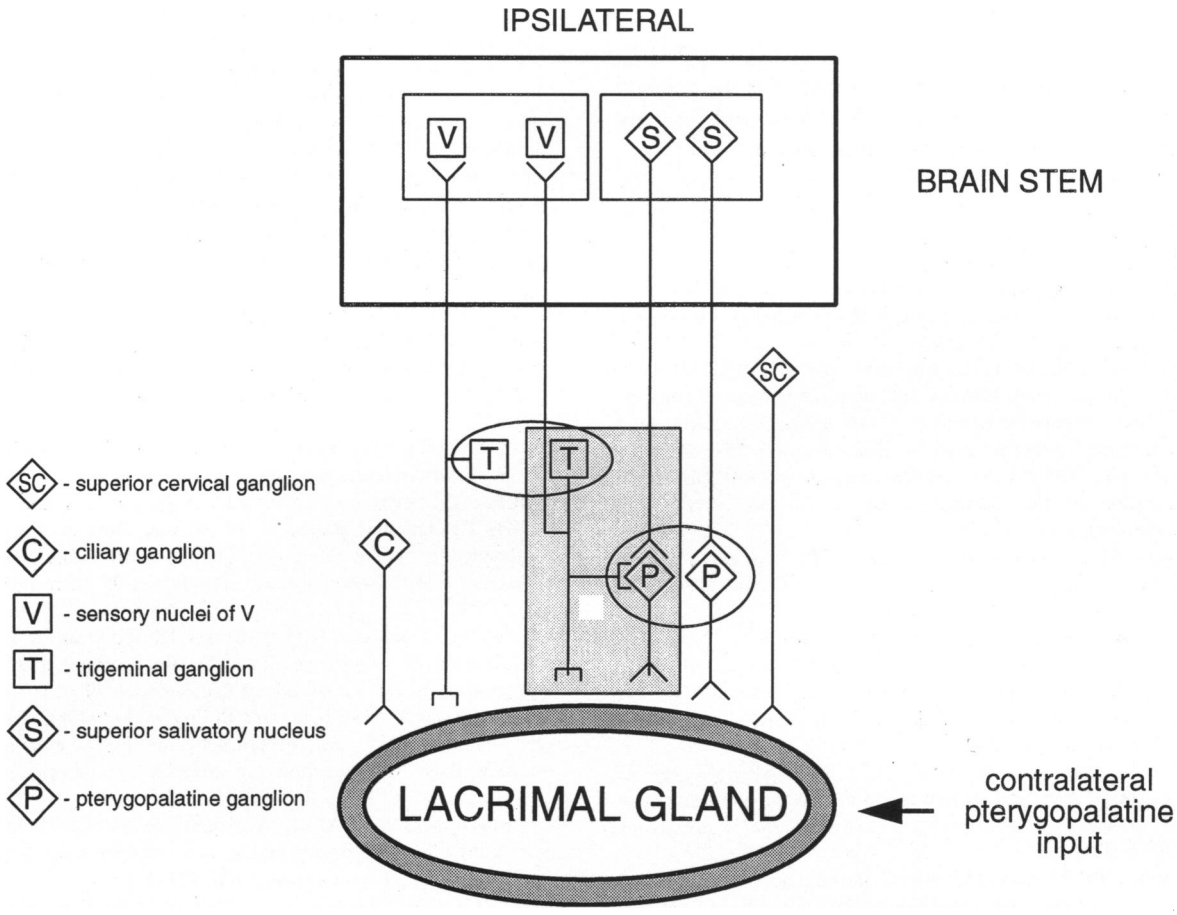

Fig. 11. Diagram of the cynomolgous monkey lacrimal innervation based on tracing experiments in the lacrimal gland.

Landis, 1988). It can be speculated that in the monkey, pterygopalatine ganglion cells innervating the lacrimal gland contain vasoactive intestinal polypeptide and may be surrounded by calcitonin gene-related peptide and substance P-positive nerve fibres from the trigeminal ganglion. Consistent with the results in rats (Beckers et al. 1991) it is likely that pterygopalatine cell bodies with their sensory trigeminal terminals are involved in an axon reflex modulating parasympathetic activity for lacrimal gland secretion (Drummond & Lance, 1992).

Ciliary ganglion

The results of this study reveal that parasympathetic innervation of the lacrimal gland is not exclusively a function of the pterygopalatine ganglion, the ciliary ganglion also making a minor contribution. WGA-HRP-labelled somata located in the ciliary ganglion or in the accessory part of the ciliary ganglion have the morphological characteristics of ciliary neurons. The possibility that these neurons are ectopic pterygopalatine or superior cervical neurons can therefore be excluded. Other retrograde tracing studies (van der Werf, 1993, van der Werf et al. 1993) in eyelids of the cynomolgous monkey revealed no labelled cell bodies in the ciliary ganglion, indicating that leakage of the tracer is unlikely to account for the present results. Moreover, different survival times, i.e. 48 and 72 h, always revealed WGA-HRP-labelled somata in the ciliary ganglion. WGA-HRP-labelled nerve fibres in the ciliary ganglion can originate from the superior cervical and/or pterygopalatine ganglia running through the ciliary ganglion (Tan et al. 1995).

Acetylcholinesterase studies on the ciliary ganglion of cynomolgous monkeys (Baljet et al. 1989) revealed that all neurons were positive for acetylcholinesterase, indicated that these neurons may have the capacity to fulfil a lacrimal secretomotor function.

CONCLUSIONS

In the cynomolgous monkey the lacrimal gland is innervated by the trigeminal, superior cervical, ciliary and pterygopalatine ganglia, including the contralateral pterygopalatine ganglion (Fig. 11). A particular finding is the contribution of ciliary ganglion neurons in lacrimal gland innervation, this possibly having a modulating effect on the efferent innervation of the lacrimal gland.

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REFERENCES

BALJET B, VAN DER WERF F, OTTO AJ (1989) Autonomic pathways in the orbit of the human fetus and rhesus monkey. *Documenta Ophthalmologica* **72**, 247–264.

BECKERS HJM, KLOOSTER J, VRENSEN GFJM, LAMERS WPMA (1991) Ultrastructural identification of trigeminal nerve terminals in the pterygopalatine ganglion of rats: an anterograde tracing and immunohistochemical study. *Brain Research* **557**, 22–30.

BOTELHO SY, HISADA M, FUENMAYOR N (1966) Functional innervation of the lacrimal gland in the cat. *Archives of Ophthalmology* **76**, 581–588.

BROMBERG BB (1981) Autonomic control of lacrimal gland protein secretion. *Investigative Ophthalmology and Visual Science* **20**, 110–116.

BUTLER JM, RUSKELL GL, COLE DF, UNGER WG, ZANG SQ, BLANK MA et al. (1984) Effects of VIIth (facial) nerve degeneration on vasoactive intestinal polypeptide and substance P levels in ocular and orbital tissues of the rabbit. *Experimental Eye Research* **39**, 523–532.

DARTT DA, SHULMAN M, GRAY KL, ROSSI SR, MATKIN C, GILBARD JP (1988) Stimulation of rabbit lacrimal gland secretion with biologically active peptides. *American Journal of Physiology* **17**, G300–G306.

DRUMMOND PD, LANCE JW (1992) Pathological sweating and flushing accompanying the trigeminal lacrimal reflex in patients with cluster headache and in patients with a confirmed site of cervical sympathetic deficit. *Brain* **115**, 1429–1445.

DUKE-ELDER S, WYBAR KC (1961) The anatomy of the visual system. In *System of Ophthalmology*, vol. 2 (ed. Duke-Elder S), pp. 559–581. London: Kimpton.

EHINGER B (1964) Adrenergic nerves to the eye and its adnexa in rabbit and guinea-pig. *Acta Universitatis Lundensis, Sectio II* **20**, 3–23.

EHINGER B (1966*a*) Cholinesterases in ocular and orbital tissues of some mammals. *Acta Universitatis, Sectio II* **2**, 3–15.

EHINGER B (1966*b*) Adrenergic nerves to the eye and to related structures in man and the cynomolgous monkey (*Macaca irus*). *Investigative Ophthalmology* **5**, 42–52.

FALCK B, HILLARP NÅ, THIEME G, TORP A (1962) Fluorescence of catecholamines and related compounds condensed with formaldehyde. *Journal of Histochemistry and Cytochemistry* **10**, 348–354.

FLETT DL, BELL C (1991) Topography of functional subpopulations of neurons in the superior cervical ganglion of the rat. *Journal of Anatomy* **177**, 55–66.

FLÜGEL C, KAUFMAN P, LÜTJEN-DRECOLL E (1994*a*) Association of a choroidal ganglion cell plexus with the fovea centralis. *Investigative Ophthalmology and Visual Science* **35**, 4268–4272.

FLÜGEL C, TAMM ER, MAYER B, LÜTJEN-DRECOLL E (1994*b*) Species differences in choroidal vasodilative innervation: evidence for specific intrinsic nitrergic and VIP-positive neurons in the human eye. *Investigative Ophthalmology and Visual Science* **35**, 592–599.

IWAMOTO T, JAKOBIEC FA (1982) Lacrimal glands. In *Ocular Anatomy, Embryology and Teratology* (ed. Jakobiec FA), pp. 761–782. Philadelphia: Harper and Row.

JONES LT (1973) Anatomy of the tear system. *International Ophthalmology Clinics* **13**, 3–22.

LEBLANC GG, LANDIS SC (1988) Target specificity of neuropeptide Y-immunoreactive cranial parasympathetic neurons. *Journal of Neuroscience* **8**, 146–155.

LEE Y, TAKAMI K, KAWAI Y, GIRGIS S, HILLYARD CJ, MACINTYRE I et al. (1985) Distribution of calcitonin gene-related peptide in the rat peripheral nervous system with reference to its coexistence with substance P. *Neuroscience* **15**, 1227–1237.

MATSUMOTO Y, TANABE T, UEDA S, KAWATA M (1992) Immunohistochemical and enzyme histochemical studies of peptidergic, aminergic and cholinergic innervation of the lacrimal gland of the monkey (*Macaca fuscata*). *Journal of the Autonomic Nervous System* **37**, 207–214.

MESULAM MM (1978) Tetramethylbenzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *Journal of Histochemistry and Cytochemistry* **26**, 106–117.

MITCHELL GAG (1953) *Anatomy of the Autonomic Nervous System*, p. 356. Edinburgh: Livingstone.

NIKKINEN A, LEHTOSALO JI, UUSITALO H, PALKEMA A, PANULA P (1984) The lacrimal glands of the rat and the guinea pig are innervated by nerve fibres containing immunoreactivities for substance P and vasoactive intestinal polypeptide. *Histochemistry* **81**, 23–27.

NIKKINEN A, UUSITALO H, LEHTOSALO JI, PALKEMA A (1985) Distribution of adrenergic nerves in the lacrimal glands of guinea-pig and rat. *Experimental Eye Research* **40**, 751–756.

PRINS M, VAN DER WERF F, BALJET B, OTTO AJ (1993) Calcitonin gene-related peptide and substance P immunoreactivity in the monkey trigeminal ganglion, an electron microscopic study. *Brain Research* **629**, 315–318.

ROSSONI RB, MACHADO ABM, MACHADO CRS (1992) Autonomic innervation of the salivary glands in cebid monkeys: a histochemical study. *Acta Anatomica* **143**, 211–218.

RUSKELL GL (1965) The orbital distribution of the sphenopalatine ganglion in the rabbit. In *The Structure of the Eye* (ed. Rohen JW), pp. 355–368. Stuttgart: Schattauer.

RUSKELL GL (1967) Vasomotor axons of the lacrimal gland of monkeys and the ultrastructural identification of sympathetic terminals. *Zeitschrift für Zellforschung* **83**, 321–333.

RUSKELL GL (1969) Changes in nerve terminals and acini of the lacrimal gland and changes in secretion induced by autonomic denervation. *Zeitschrift für Zellforschung* **94**, 261–281.

RUSKELL GL (1971) The distribution of autonomic post-ganglionic nerve fibres to the lacrimal gland in monkeys. *Journal of Anatomy* **109**, 229–242.

RUSKELL GL (1975) Nerve terminals and epithelial cell variety in the human lacrimal gland. *Cell and Tissue Research* **158**, 121–136.

SPENCER SE, SAWYER WB, WADA H, PLATT KB, LOEWY AD (1990) CNS projections to the pterygopalatine parasympathetic preganglionic neurons in the rat: a retrograde transneuronal viral cell body labelling study. *Brain Research* **534**, 149–169.

SUZUKI N, HARDEBO JE, OWMAN C (1989) Trigeminal fibre collaterals storing substance P and calcitonin gene-related peptide associated with ganglion cells containing choline acetyltransferase and vasoactive intestinal polypeptide in the sphenopalatine ganglion of the rat. An axon reflex modulating parasympathetic ganglionic activity? *Neuroscience* **30**, 595–604.

TAN CK, ZHANG YL, WONG WC (1995) A light- and electron microscopic study of tyrosine hydroxylase-like immunoreactivity in the ciliary ganglia of monkey (*Macaca fascicularis*) and cat. *Histology and Histopathology* **10**, 27–34.

TEN TUSSCHER MPM, KLOOSTER J, VRENSEN GFLM (1988) The innervation of the rabbit's anterior eye segment: a retrograde tracing study. *Experimental Eye Research* **46**, 717–730.

TEN TUSSCHER MPM, KLOOSTER J, BALJET B, VAN DER WERF F, VRENSEN GFJM (1990) Pre- and post-ganglionic nerve fibres of the pterygopalatine ganglion and their allocation to the eyeball in rats. *Brain Research* **517**, 315–323.

UDDMAN R, ALUMETS J, EHINGER B, HÅKANSON R, LORÉN I, SUNDLER F (1980) Vasoactive intestinal peptide nerves in ocular and orbital structures of the cat. *Investigative Ophthalmology and Visual Science* **19**, 878–885.

VAN DEN POL AN, GÖRCS T (1986) Synaptic relationships between neurons containing vasopressin, gastrin-releasing peptides, vasoactive intestinal polypeptides and glutamate decarboxylase immunoreactivity in the suprachiasmatic nucleus: dual ultrastructural immunohistochemistry with gold-substituted silver peroxidase. *Journal of Comparative Neurology* **252**, 507–521.

VAN DER WERF F (1993) *Autonomic and sensory innervation of some orbital structures in the primate*. PhD Thesis, University of Amsterdam.

VAN DER WERF F, BALJET B (1989) An acetylcholinesterase thick section method for the topographical studies of autonomic nerve fibres in deep-frozen specimens. *Journal of Histotechnology* **12**, 47–50.

VAN DER WERF F, BALJET B, PRINS M, OTTO AJ (1993) Innervation of the superior tarsal (Müller's) muscle in the cynomolgous monkey: a retrograde tracing study. *Investigative Ophthalmology and Visual Science* **34**, 2333–2340.

WHITWELL J (1961) Role of the sympathetic in lacrimal secretion. *British Journal of Ophthalmology* **45**, 439–445.

WILSON JE (1984) Pterygopalatine ganglion cytology in monkeys. *Journal of Anatomy* **139**, 307–317.

ZABORSZKY L, HEIMER L 91989) Combinations of tracer techniques, especially HRP and PHA-L, with transmitter identification for correlated light and electronmicroscopic studies. In *Neuroanatomical Tract-tracing Methods 2, Recent Progress* (ed. Heimer L, Zaborszky L), pp. 49–97. New York: Plenum.