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Morphology of the accessory ciliary ganglion of the cat

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Summary. A microdissection of the orbital nerves of the cat was made paying particular attention to the accessory ciliary ganglion. The structures were studied with the light microscope by histological and histochemical techniques. The accessory ciliary ganglion consisted of cells similar to those in the main ganglion. The neurons of the accessory ciliary and those of the main ciliary ganglion emitted no specific fluorescence for catecholamines. After injection of horseradish peroxidase-wheat germ agglutinin conjugates into the chambers of the eye, most ganglion cells of the accessory and main ciliary ganglia were labeled. A few labeled ectopic cells were also found in proximity of the accessory ganglion in the lateral short ciliary nerve or in the communicating branch from the trigeminal nerve. The present results indicate that the accessory ciliary and ectopic ganglion cells are parasympathetic in nature and may innervate the intrinsic eye musculatures.

Key words: Ciliary ganglion – Parasympathetic ganglia – Oculomotor nerve – Autonomic ganglia – Eye innervation

Introduction

Since Lecco (1906) first described the presence of an accessory ciliary ganglion along the short ciliary nerve, this ganglion has been observed by several researchers, and it is now considered to be common to all or most mammalian species (Imai 1935a, b; 1936; Christensen 1936; Grimes and von Sallmann 1960; Kuchiiwa et al. 1989). However, since none of the previous studies have dealt with the histology of the ganglion, its nature was still unknown. This paper reports on the morphology of the accessory ciliary ganglion of the cat studied by light and fluorescence microscope techniques, and on the distribution of neurons that innervate structures surrounding the chambers of the eye studied with a neuroanatomic tracing method.

Materials and methods

A total of ten adult cats were used in the histological study. The cats were anesthetized deeply with ketamine hydrochloride (70 mg/kg) and perfused through the ascending aorta with physiological saline followed by 10% buffered formalin (pH 7.4). Both orbits were dissected under a binocular microscope. The accessory ciliary and main ciliary ganglia were removed, washed, dehydrated and embedded in paraffin. Serial sections were cut at 8 or 25 μm, mounted and prepared using gallocyanine or Holmes' silver method (Ráliš et al. 1973). A few tissues were treated with 1% osmium tetroxide and embedded in Epon 812 (Oken), and 1 μm semithin sections were made and stained with toluidine blue. Some preparations were stained with thionin (Kuchiiwa et al. 1989) and examined as whole-mounts in a light microscope. In addition, one whole orbit was embedded in celloidin, sectioned at 25 μm parallel to the equatorial plane of the eyeball and stained with hematoxylin-eosin. In three animals, fresh accessory and main ciliary ganglia were removed under deep anesthesia. The tissues were frozen in isopentane cooled with dry ice and acetone, sectioned in a Cold Tome (Sakura Seiki), dried and treated with formaldehyde gas for 1 h at 80° C (modification of the Falck and Hillarp method, 1962). These preparations were then examined under a fluorescence microscope.

Experiments using horseradish peroxidase-wheat germ agglutinin conjugates (WGA-HRP: prepared in the author's laboratory; Kuchiiwa et al. 1988) were performed in three adult cats, which were anesthetized by intramuscular injection of ketamine hydrochloride (35–50 mg/kg) and placed in a stereotaxic head holder. Local anesthesia (Xylocaine) was applied at all pressure points of the unilateral eyeball. The needle of a 100 μl microsyringe was inserted through the sclera approximately 2 mm posterior to the limbus, and 50 μl 5% WGA-HRP dissolved in saline was injected into the posterior and anterior chambers of the eye. The surface of the eyeball was flushed with saline immediately after puncture and withdrawal to minimize extraocular leakage of WGA-HRP. In addition, the puncture site was plugged with Aron alpha A (Sankyo) to minimize possible backflow of WGA-HRP from the eyeball. After a post-injection period of 43–51 h, the cats were re-anesthetized deeply and perfused through the ascending aorta with 1 L saline followed by 2 L 2.5% formalin and 1.25% glutaral-

dehyde in 0.1 M phosphate buffer at pH 7.4. The ganglia and brains were removed and immersed in cold buffer containing 30% sucrose, and 40 μm thick sections were cut on a freezing microtome. All sections of the ganglia and every second section of the brain were reacted for peroxidase histochemistry by the tetramethylbenzidine method (Mesulam 1978). Light- and polarized-illuminations were employed to identify WGA-HRP labeled cells.

Results

Accessory ciliary ganglion

The ciliary ganglion gave rise to a thick lateral and a finer medial short ciliary nerve. One or two fine communicating branches from the trigeminal nerve always fused with the lateral branch about 3–4 mm from the main ganglion; at this point of fusion there was a single prominent accessory ciliary ganglion (Fig. 1A, B). A number of autonomic nerves derived from the retro-orbital plexus in the rete mirabile or from the internal carotid nerve fused with the short and long ciliary nerves close to the eyeball rather than joining the accessory or main ciliary ganglion. The accessory ganglion bulged out slightly from the lateral branch so that it could be identified readily even in non-stained materials. In a few cases, however, the ganglion was poorly developed and required further microscopic observation of the stained specimens. It was ovoid in shape, about 1 mm long and 0.6 mm wide, situated inside the retractor bulbi and located lateral to the optic nerve.

The number of accessory ciliary ganglion cells varied from individual to individual, ranging from several tens to over three hundred. Most accessory ganglion neurons were ellipsoid and irregular in shape with their longest diameter paralleling the axis of the short ciliary nerve. A few neurons were ovoid, and these were generally located in the peripheral areas of the ganglion. The accessory neurons were multipolar, and their perikarya contained coarse Nissl granules and stained densely with toluidine blue. A pale spherical nucleus contained a single densely stained nucleolus in the proximity of its center. The accessory ciliary ganglion consisted of cells morphologically similar to those of the main ganglion, rather than pseudounipolar trigeminal or much smaller superior cervical ganglion cells (see Truex 1940; Gabella 1976), although the cells of the accessory ganglion were slightly smaller (measuring about 50×30 μm) than those of the main ganglion (measuring about 60×50 μm) (Fig. 1C, D).

Osmic acid preparations of sections of the lateral short ciliary nerve taken from between the main and accessory ciliary ganglion were composed entirely of small myelinated fibers of nearly uniform diameter (Fig. 2A). The communicating branch from the trigeminal nerve was composed of large myelinated, small myelinated and unmyelinated fibers (Fig. 2B). Whenever a union of short ciliary nerve and communicating branch occurred, the large myelinated fibers and the unmyelinated fibers from the trigeminal nerve could always be detected in the resulting mixed nerve, since they appeared in sharp contrast to the smaller, myelinated parasympathetic fibers of the short ciliary nerves (Fig. 2C). The small myelinated fibers of the two nerves, however, could not be distinguished from each other in the mixed nerve.

Ectopic ganglion cells

A few ectopic ganglion cells were often found in proximity to the accessory ciliary ganglion in the lateral short ciliary nerve or sometimes in the communicating branch from the trigeminal nerve (Figs. 1A, 2D). They were elongated multipolar cells, histologically similar to those of the accessory ganglion in shape, size and in their cytoplasmic and nuclear characteristics. These cells were labeled after injection of WGA-HRP into the chambers of the eye, as described below. No ganglion cells, i.e. the so-called episcleral ganglia of Axenfeld (1907), were observed on the sclera nor in the scleral canal.

Fluorescence microscopy

The perikarya of the accessory ciliary ganglion cells emitted no specific catecholamine fluorescence, nor did the main ciliary ganglion cells. A few fibers exhibiting specific fluorescence for catecholamines were found in the ganglion and probably represented perivascular adrenergic fibers or passing fibers coursing along the communicating branch to the eyeball, since no endings of the preganglionic fibers could be detected around the perikarya. Administration of nialamide (250 mg/kg) caused no recognizable changes in the appearance of the specific fluorescence of the tissue.

Intraorbital WGA-HRP injection

When WGA-HRP was injected into the chambers of the eye, WGA-HRP positive granules were observed in most ganglion cells in the accessory and main ciliary ganglion in every case (Fig. 2D). The ectopic cells in the short ciliary nerve and the communicating branch were also labeled. No other labeled cells were found in any of the peripheral branches of the oculomotor nerve.

In the brain stem, neither anterogradely labeled terminals nor retrogradely labeled perikarya were found in the nuclei of the oculomotor, trochlear, trigeminal, abducens and facial nerves. In the primary visual centers, anterogradely labeled terminals were not observed except in two cases in which labeled terminals were confined to a small portion of the superior colliculus, the pretectal area and the lateral geniculate nucleus.

Discussion

The results of the present study indicate that the accessory ciliary ganglion is parasympathetic in nature. As its

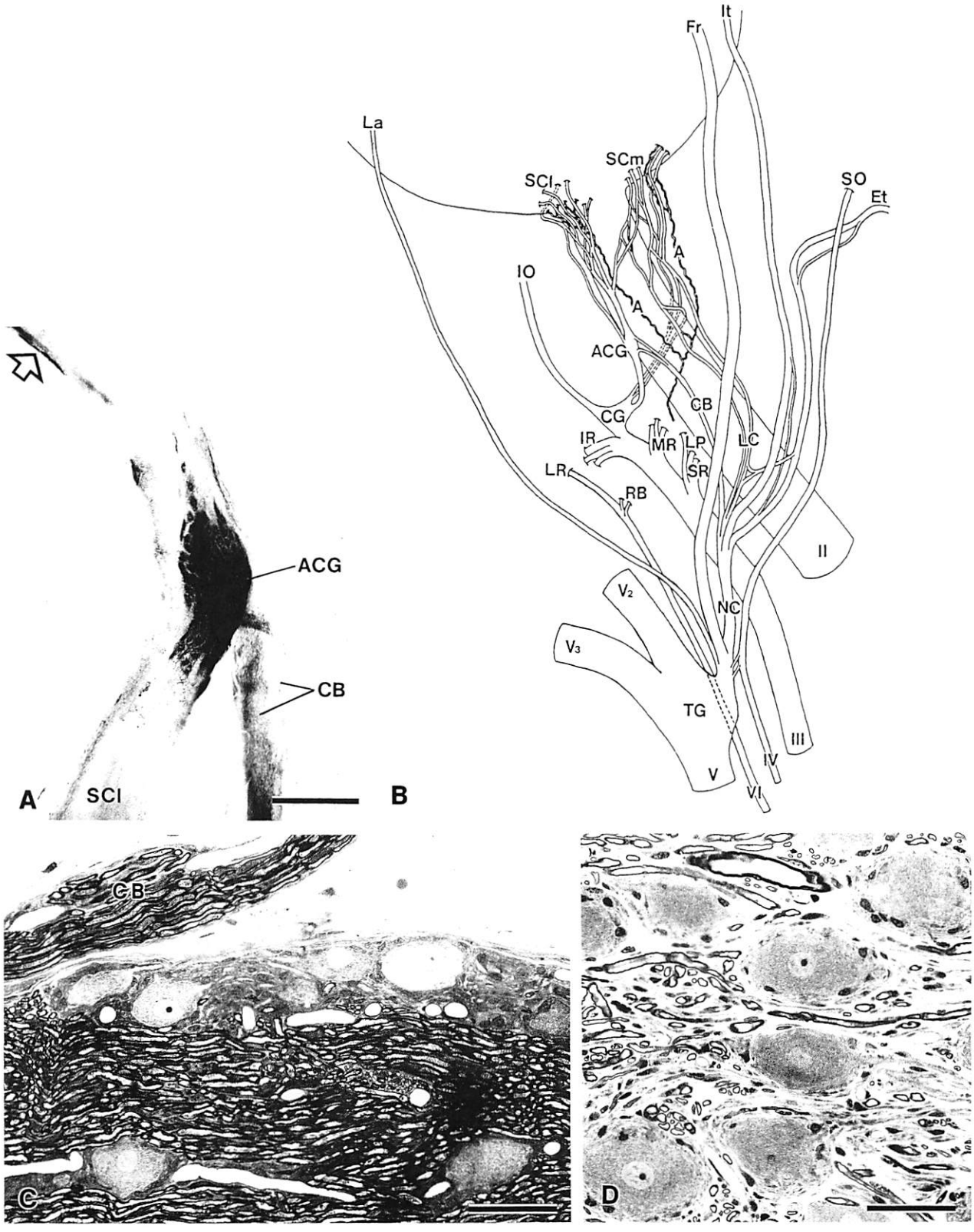

Fig. 1. **A** Whole-mount preparation of the accessory ciliary ganglion (*ACG*) stained with thionin. Bifurcated communicating branches from the trigeminal nerve join the lateral short ciliary nerve in proximity of the ACG. The *arrow* points to several slender ectopic cells. Scale 0.1 mm. **B** Diagram of nerves in the left orbit showing the location of the accessory ciliary ganglion viewed from above with the globe to the top. **C, D** Photomicrographs of semithin sections of the accessory (**C**) and main ciliary ganglion (**D**). Stained with toluidine blue, 1 μm thick, embedded in Epon 812. Scale 50 μm

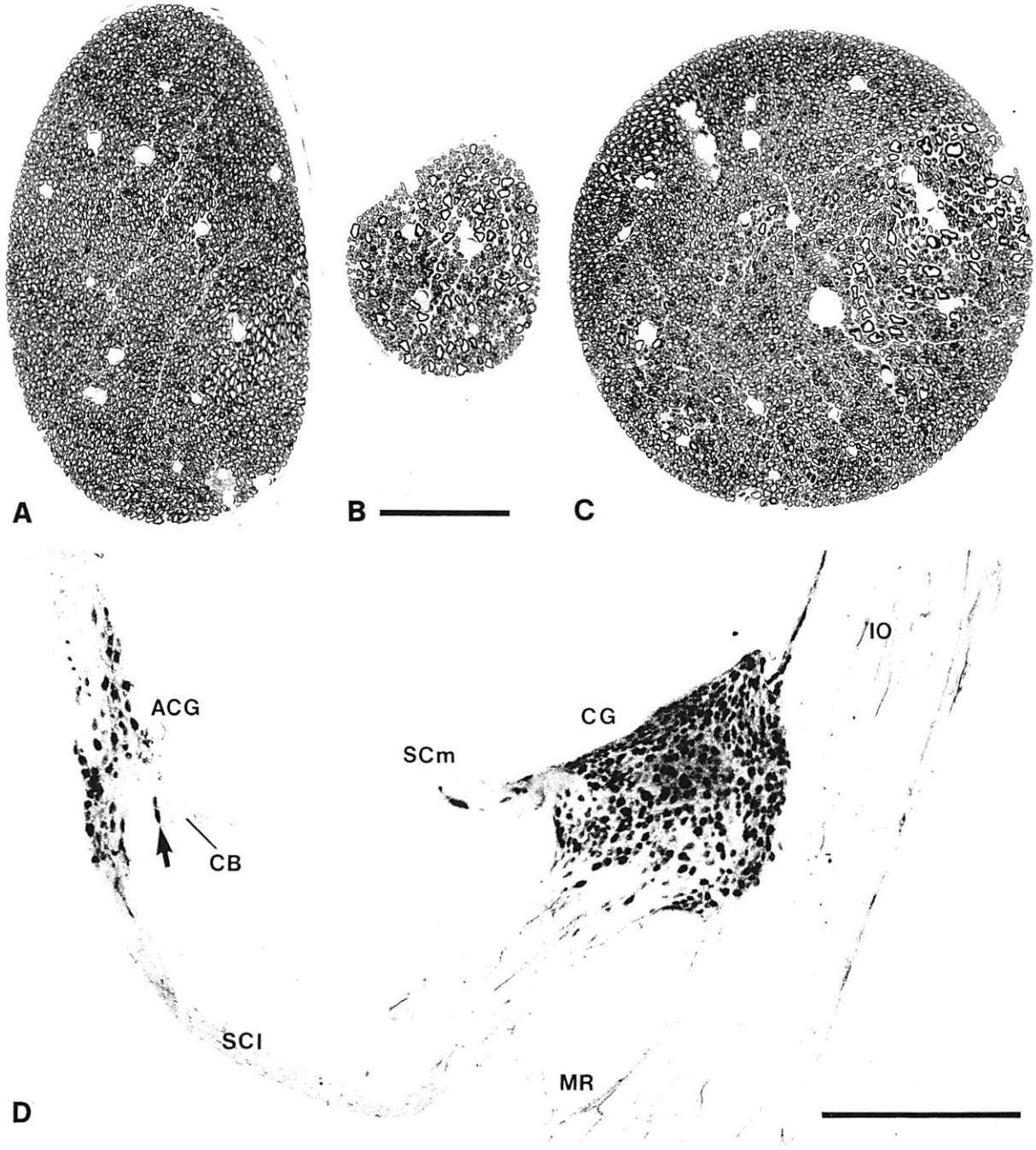

Fig. 2. A–C Photomicrographs of semithin sections of an osmic acid preparation stained with toluidine blue. 1 μm thick, embedded in Epon 812, scale 50 μm. **A** Section of the lateral short ciliary nerve taken from between the main and accessory ciliary ganglion. **B** Communicating branch from the trigeminal nerve. **C** The lateral short ciliary nerve after union of **A** and **B**. **D** Photomicrograph of a longitudinal section of the accessory and main ciliary ganglia treated with tetramethylbenzidine after injection of WGA-HRP into the chambers of the eye. The *arrow* points to labeled neurons in the communicating branch. 50 μm thick. Scale 1 mm

Abbreviations (Figs. 1, 2): *II*, optic nerve; *III*, oculomotor nerve; *IV*, trochlear nerve; *V*, trigeminal nerve; *V2*, maxillary nerve; *V3*, mandibular nerve; *VI*, abducens nerve; *A*, autonomic nerve; *ACG*, accessory ciliary ganglion; *CB*, communicating branch from the trigeminal nerve; *CG*, ciliary ganglion; *Et*, ethmoidal nerve; *Fr*, frontal nerve; *IO*, branch to the inferior oblique muscle; *IR*, branch to the inferior rectus muscle; *It*, infratrochlear nerve; *La*, lacrimal nerve; *LC*, long ciliary nerve; *LP*, branch to the levator muscle of the palpebra; *LR*, branch to the lateral rectus muscle; *MR*, branch to the medial rectus muscle; *NC*, nasociliary nerve; *RB*, branch to the retractor bulbi muscle; *SCl* lateral branch of the short ciliary nerve; *SCm*, medial branch of the short ciliary nerve; *SO*, branch to the superior oblique muscle; *SR*, branch to the superior rectus muscle; *TG*, trigeminal ganglion

name implies, it is assumed to be accessory to the main ciliary ganglion.

After extirpating the ciliary ganglion in monkeys, Foerster et al. (1936) found that although pupil reactions to light in the operated eyes were lost, the pupil reactions to convergence and accommodation were retained. This experiment showed that there must be two efferent pathways for pupillary contraction, one subserving the light reflex that passes through the ciliary ganglion, the other concerned with the contraction of the pupil that accompanies convergence and accommodation, mediated in some other ganglion and running to the sphincter muscle by some other route.

The accessory ciliary ganglion is believed to be common to all or most mammalian species, and it generally receives communicating branches from the trigeminal nerve (Kuchiiwa et al. 1989). The communicating branch contains many smaller myelinated fibers that are impossible to distinguish from fibers in the short ciliary nerve, and this branch also contains many acetylcholinesterase-positive fibers postulated to be parasympathetic (T. Kuchiiwa, personal communication). It is possible that the accessory ciliary ganglion is a station for the convergence-accomodation synkinetic contraction.

The ectopic ganglion cells located in proximity of the accessory ganglion are assumed to be identical to the accessory neurons. The existence of ectopic cells has been reported in man, monkey and rabbit (Peschel 1893; Ernyei 1936; Grimes and von Sallmann 1960). In man there are two types of ganglion cells, parasympathetic and sensory (Ernyei 1936). In the present study, however, a single population of cells could be detected. It is believed that in the cat there is a single type of ganglion cell, parasympathetic in nature.

Axenfeld (1907) described the presence of ganglion cells in human ciliary nerves close to the eye and the episclera and named them episcleral ciliary ganglia. These cells were found both singly and in clusters of up to 30. In the present study in the cat, however, ganglion cells could not be detected in these structures with either histological or hodological techniques.

In the WGA-HRP experiment no retrogradely labeled cells were found in the brain stem, indicating that no detectable leakage of the enzyme from the eyeball had occurred. Furthermore, orthogradely labeled terminals were not identified in the primary visual centers, or they were restricted to a small portion of the primary visual centers, indicating that the injected enzyme either was completely prevented from filtering into the retina by the vitreous body or was taken up by a small fraction of ganglion cells as a result of a moderate leakage toward the retina. This suggests that the enzyme was taken up mainly by structures surrounding the chambers of the eye, and that the postganglionic fibers of the accessory ciliary ganglion and the ectopic cells project to the intrinsic musculatures of the eye.

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