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Direct Parasympathetic Pathway from Midbrain to Ciliary Muscles in Cats and Monkeys

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Abstract: In cats and monkeys, we examined the parasympathetic component of the oculomotor complex, which directly innervates the ciliary muscle, using horseradish peroxidase (HRP). Labeled neurons of varying form and size were found in the Edinger-Westphal (EW) and the Perlia nuclei of the cat and in the anteromedian, EW, and Perlia nuclei of the monkey. Our study confirmed that a direct parasympathetic pathway exists from the midbrain to the ciliary muscles, and that accommodation is controlled in part by this direct link from the midsagittal region via a parasympathetic neuron of the oculomotor nuclear complex. **Jpn J Ophthalmol 1997;41:203–208** © 1997 Japanese Ophthalmological Society

Key Words: Cat, ciliary ganglion, direct pathway, horseradish peroxidase, midbrain, monkey.

Introduction

In addition to the parasympathetic oculomotor nerve that synapses in the ciliary ganglion and innervates the intraocular muscles, a direct parasympathetic pathway has been suggested by Westheimer and Blair[1] in their electrophysiological study of monkeys. Jaeger and Benebento[2] supported this hypothesis with observations of labeled neurons in the anteromedian (AM), Edinger-Westphal (EW), and Perlia nuclei following intraocular injection of horseradish peroxidase (HRP) in rabbits and monkeys. Another neuroanatomical study[3] found labeled neurons in the AM, EW, and Perlia nuclei using HRP or wheat germ aggatinin-horseradish peroxidase (WGA-HRP) in monkeys. Recent studies in our laboratory[4] suggested that these nonsynapsing pathways originate in the AM, EW, and Perlia nuclei in cats and, therefore, the parasympathetic nerve innervating the intraocular muscles has a direct nonsynapsing pathway in the iris and the ciliary body. It is not clear, however, whether this direct pathway innervates the iris sphincter, the ciliary muscles, or both, because HRP injected into the eyeball is taken up by the iris sphincter as well as the ciliary muscles. In the present experiment, HRP was injected into the eyeball after a unilateral total iridectomy, therefore affecting only the ciliary muscles. We studied the subnuclear division of the oculomotor complex, which directly innervates the ciliary muscles in cats and monkeys.

Materials and Methods

A total unilateral iridectomy was done in three cats (2.0–3.0 kg) and three monkeys (*Macaca fascicularis*; 2.5–3.5 kg) anesthetized with intramuscular ketamine hydrochloride (30 mg/kg) (Ketalar®, Sankyo, Tokyo) and intraperitoneal sodium pentobarbital (25 mg/kg). All animals were handled in accordance with the ARVO resolution on the care of animals in vision research. In the cats, the sclerocorneal limbus was incised and the iris removed using sclerocorneal scissors with wetfield bipolar coagulation. In the monkeys, the iris was removed with a cryo tip (Figure 1). After a month to allow post-surgical inflammation to subside, 5 μL 50% HRP was injected into the ipsilateral ciliary muscles under the scleral flap within a 1 mm area 2 mm posterior to the limbus, with a 10 μL Hamilton syringe. We confirmed the absence of HRP leakage, sutured the scleral flap, and sealed it with superglue (Alon Al-

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Figure 1. Photograph of monkey eyes after total iridectomy.

pha®, Konishi, Tokyo). After 48 hours, the animals were perfused transcardially with normal saline followed by a fixative (0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L pH 7.4 phosphate buffer) at room temperature. A final perfusion of 20% sucrose in 0.1 mol/L of the pH 7.4 phosphate buffer at 4°C was used to further harden the tissues. The brainstem and ciliary ganglion were removed and held in 30% sucrose with 0.1 mol/L pH 7.4 phosphate buffer at 4°C for 3 days. They were then sliced transversely into 30 μm sections with a freezing microtome; the sections were mounted on gel-coated slides and processed by Mesulam's trimethylbenzidine method.[5] After counterstaining with 0.5% neutral red, the sections were examined with both dark field and bright field light microscopy. Camera lucida drawings were made of every third section; photomicrographs of the neurons were taken with Kodachrome 64® film (Kodak, Rochester, NY, USA).

Results

Results were similar in all animals. Densely packed HRP-labeled neurons were seen in the ciliary ganglions of both cats and monkeys (Figures 2,3).

Localization of Labeled Cells

In the cats, the Perlia nucleus lying between the two oculomotor complexes ventral to the EW nucleus had the greatest density of labeled neurons; a large number were also found in the rostral half of the EW nucleus, dorsal to the oculomotor complex

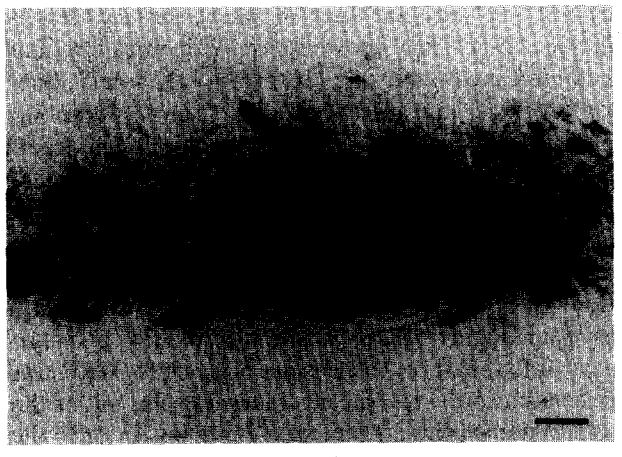

Figure 2. Light field photomicrograph of labeled cells in ciliary ganglion after HRP injection into cat ciliary muscle. Bar = 50 μm. × 120.

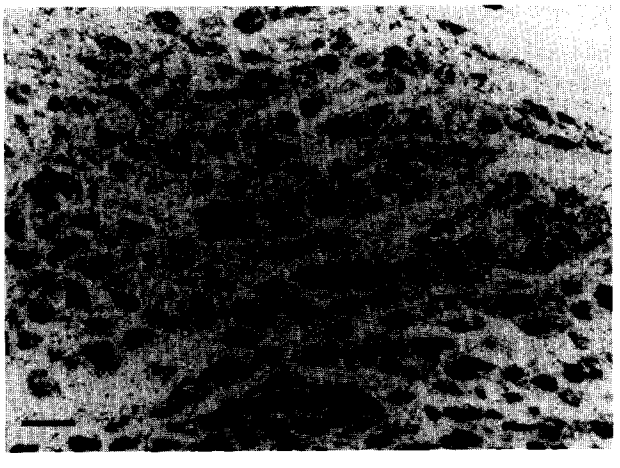

Figure 3. Light field photomicrograph of labeled cells in ciliary ganglion after HRP injection into monkey ciliary muscle. Bar = 50 μm. × 120.

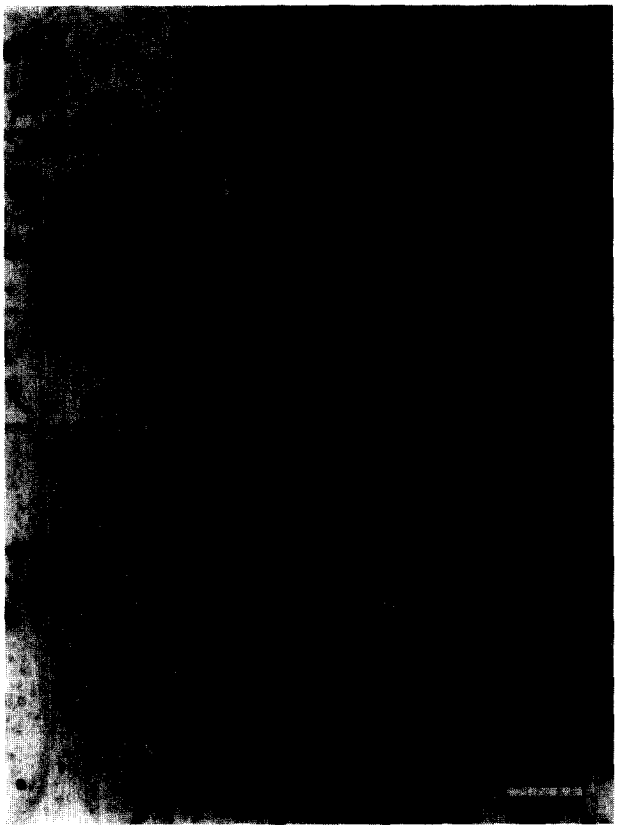

Figure 4. Dark field photomicrograph of labeled cells of Edinger-Westphal nucleus and Perlia nucleus in cat. Bar = 50 μm. × 180

(Figures 4,5). There were no labeled neurons in the AM nucleus or the ventral tegmental area (VTA).

In the monkeys, labeled neurons were found in the AM nucleus and the medial visceral columns (MVC) of the EW and Perlia nuclei. The AM nucleus is rostral and ventral to the oculomotor complex. Distribution density was similar in all nuclei (Figures 6,7).

Morphology of Labeled Neurons

In the cat Perlia nucleus, the labeled neuron body was spindle-shaped (mean size: 18.1 ± 3.2 μm); in the EW nucleus, the body was oval or round (mean size: 17.1 ± 2.0 μm). In the monkey, labeled neuron bodies were spindle-shaped in the AM nucleus (mean size: 21.4 ± 4.4 μm) and the Perlia nucleus (mean size: 20.5 ± 2.6 μm), but oval or round in the EW nucleus (mean size: 17.0 ± 3.4 μm). The labeled neuron body of the oculomotor complex was round and larger (mean: cats, 26.4 ± 4.4 μm; monkeys, 27.0 ± 4.3 μm) than in the other nuclei (Table 1).

Discussion

This investigation confirms the existence in cats and monkeys of a nonsynapsing pathway that bypasses the ciliary ganglion, and agrees with the findings of Jaeger and Benebento[2] on monkeys, Parelman et al on monkeys,[3] and Tanemoto et al on cats.[6]

Procedure

Total iridectomy caused retrograde degeneration of the parasympathetic nerve that innervates the iris,

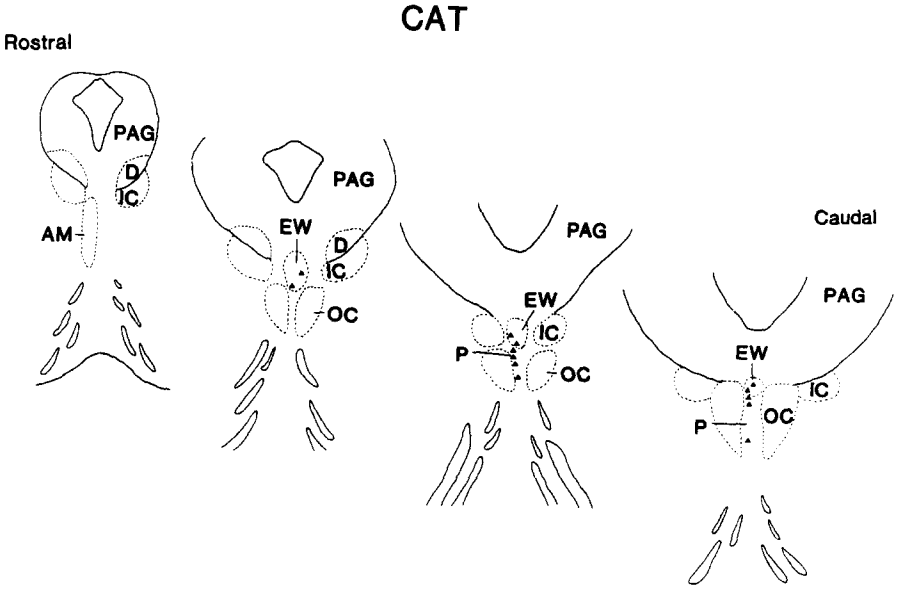

Figure 5. Distribution of labeled cells in midbrain after HRP injection into cat ciliary muscle. Triangles: labeled cells. AM: anteromedian nucleus. D: nucleus of Darkschwitsch. EW: Edinger-Westphal nucleus. IC: interstitial nucleus of Cajal. OC: oculomotor complex. P: nucleus of Perlia. PAG: periaqueductal gray.

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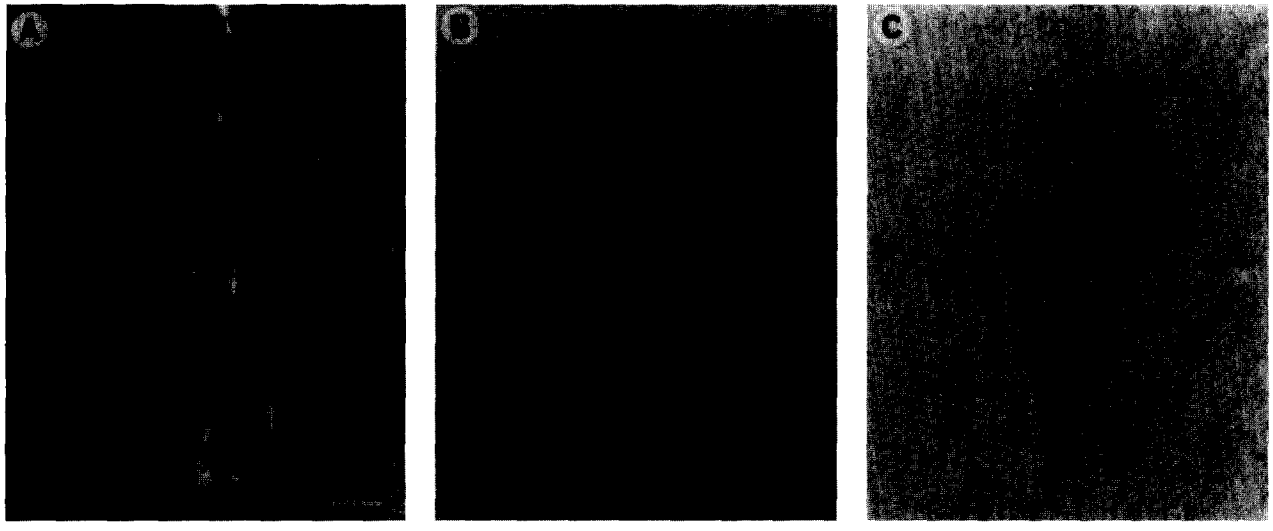

Figure 6. Photomicrograph of labeled cells in monkey midbrain. (**A**) Anteromedian nucleus in dark field. (**B**) Edinger-Westphal nucleus in light field. (**C**) Perlia nucleus in light field. Bar = 50 μm. × 100.

leaving only the ciliary body to take up the HRP. Tobari[7] has also described total degeneration of the ciliary ganglion following enucleation of the eyeball, with chromatolytic changes occurring in the ciliary ganglion 14–28 days after enucleation in a cat. Warwick[8] noted chromatolytic neurons in the ciliary ganglion 8–16 days after total iridectomy in monkeys. These studies indicate that iris-innervating neurons will have sufficiently degenerated 1 month after total iridectomy to abolish the uptake of HRP by the iris. Neurons then are labeled only by uptake from the ciliary body, and clearly demonstrate a nonsynapsing parasympathetic nerve that innervates only the ciliary body in monkeys and cats. The large concentrations of labeled neurons found in the ciliary ganglion indicate that the parasympathetic neurons that innervate the ciliary muscles synapse primarily in the ciliary ganglion.

Comparison With Previous Anatomical Studies

In the present study, labeled neurons were found in the AM, EW, and Perlia nuclei in monkeys, as in the work of Jaeger and Benebento[2] and Parelman et al.[3] Ruskell and Griffiths,[9] however, concluded from a morphological denervation study that the peripheral accommodation nerve pathway shares a relay in

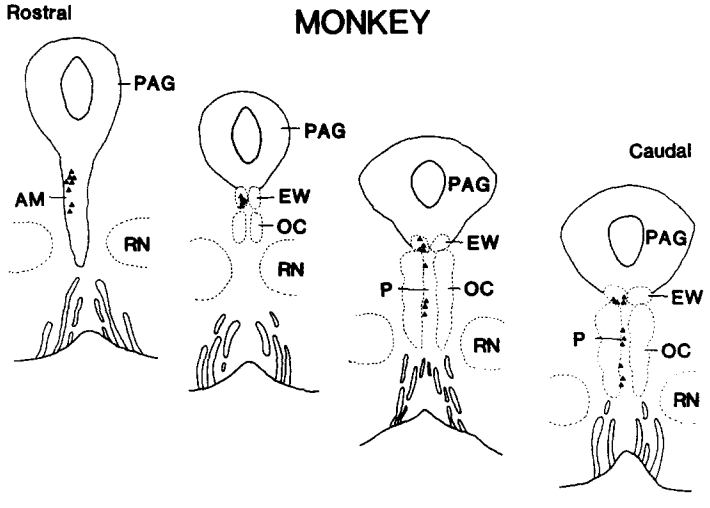

Figure 7. Distribution of labeled cells in the midbrain after HRP injection into monkey ciliary muscle. Triangle: labeled cells. AM: anteromedian nucleus. EW: Edinger-Westphal nucleus. OC: oculomotor complex. P: nucleus of Perlia. PAG: periaqueductal gray.

Table 1. Cell Sizes and Shape of Labeled Cells in Each Nucleus by HRP in μm (Means and Standard Deviations)

	Cat		Monkey	
	Size (μm)	Shape	Size (μm)	Shape
AM	—	—	21.4 ± 4.4	spindle
EW	17.1 ± 2.0	oval, round	17.0 ± 3.4	oval, round
Perlia	18.1 ± 3.2	spindle	20.5 ± 2.6	spindle
OC	26.4 ± 4.4	round	27.0 ± 4.3	round

Oculomotor complex was not labeled.

AM: Anteromedian nucleus. EW: Edinger-Westphal nucleus. Perlia: Perlia nucleus. OC: Oculomotor complex.

the ciliary ganglion with the pupillary constrictor nerve pathway. Our results indicate that the parasympathetic neurons innervating the ciliary body have both synapsing and nonsynapsing pathways in the ciliary ganglion, suggesting different accommodative functions for these pathways. Tanemoto et al[4] injected HRP into the ciliary body and iris of cats and found labeled neurons in the AM, EW, and Perlia nuclei. In our current study, the labeled neurons were noted in the EW and Perlia nuclei, but not in the AM nucleus, indicating the existence of a nonsynapsing pathway from the AM nucleus to the iris.

Comparison With Physiological Studies

Westheimer and Blair[1] concluded from electrophysiology studies in monkeys that the motor pathway for accommodation does not synapse in the ciliary ganglion. Hiraoka and Shimamura[10] agreed, finding a conduction time of 4.5 milliseconds from the AM nucleus to the iris sphincter, but 6.7 milliseconds from the postganglionic short ciliary nerve to the muscles, suggesting the existence of a direct pathway and a concentration of neurons primarily in the cat AM nucleus. Hultborn et al[6] concluded that the parasympathetic nerve of accommodation innervates the ciliary muscles directly because the latency of mass potential of the short ciliary nerve was about 2 milliseconds less than that of the iris sphincter in cats. Our present observations of cats supported the findings of these researchers.

Comparison With Clinical Studies

Our conclusions are reinforced by the studies of Ponsford et al,[11] Slamovits et al,[12] and Oono and Mukuno,[13] who found that application of 2.5% methacholine causes pupil constriction as the iris sphincter becomes hypersensitive to it, after an initial preganglionic oculomotor palsy. This indicates that preganglionic damage to the parasympathetic nerve causes degeneration of the postganglionic nerve. Oku et al,[14] observing early recovery of the pupillary reflex rather than accommodation in an orbital fracture with internal ophthalmoplegia, suggested that a direct parasympathetic pathway from the EW nucleus to the ciliary muscles existed. Earlier involvement of accommodation in the contralateral eye, with a unilateral tonic pupil, was also noted.

In the human eye, there appear to be two accommodation systems, dynamic and tonic.[15] The dynamic affects quick focusing from far to near, as in the direct nonsynapsing neurons of the present study, while the tonic is involved in slow continuous focusing. A dissociation of dynamic and static accommodation has also been reported in Fisher's syndrome.[16] These clinical reports strongly suggest the existence of a direct sympathetic pathway to the ciliary body in humans.

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