THE JOURNAL OF COMPARATIVE NEUROLOGY 340:577–591 (1994)

Localization of Preganglionic Neurons of the Accessory Ciliary Ganglion in the Midbrain: HRP and WGA-HRP Studies in the Cat

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ABSTRACT

Localization of preganglionic neurons of the accessory ciliary ganglion (ACG), including ectopic intraocular ganglion cells, was investigated in the cat with the aid of horseradish peroxidase (HRP) and HRP-conjugated wheat germ agglutinin (WGA-HRP) methods. When HRP or WGA-HRP was injected into the anterior and posterior chambers of the eye, no retrogradely labeled cells were found in the visceral oculomotor nuclei, although most neurons of the ACG and the main ciliary ganglion (CG) were intensely labeled. When a microsyringe needle was inserted into the ciliary body, the tracer diffused into the suprachoroid lamina and the intraocular ganglion cells, and a small number of labeled neurons appeared in the midplane between each side of the somatic oculomotor nuclei. After injection into the ACG, many labeled neurons were observed in the anteromedian nucleus, Edinger-Westphal nucleus, and midplane between the somatic oculomotor nuclei, their ventral continuations of the ventral tegmental area, and the periaqueductal gray. HRP/WGA-HRP injection into the CG labeled cells in all these areas and in the lateral border zones of the anteromedian, Edinger-Westphal and somatic oculomotor nuclei, and their ventral continuations of the ventral tegmental area. These findings indicate that the visceral oculomotor neurons which project to the ACG tend to be located more medially than those to the CG. © 1994 Wiley-Liss, Inc.

Key words: **ciliary ganglion, intraocular ganglion, oculomotor parasympathetic outflow, afferents, pupillary near reflex**

The accessory ciliary ganglion (ACG) is located on the short ciliary nerve and is believed to be common to all or most mammalian species. It consists of cells similar to those in the main ciliary ganglion (CG) and is assumed to be an accessory to the CG as its name implies (Kuchiiwa et al., '89; Kuchiiwa, '90a). However, the origins of the afferents in the brainstem and their function in control of intrinsic eye musculature remain obscure.

The connecting pattern of the ACG with the trigeminal branch is common to most mammals, although the location, number and degree of development vary greatly from species to species (Kuchiiwa et al., '89). Figure 1 schematically shows the location and the manner of the ACG connection in the cat. This animal is very useful for hodological study in the following respects: (1) it has a single prominent ACG, (2) its connection is very simple, (3) few ectopic ganglion cells are present in the ciliary nerves in the orbit, (4) and there is little individual variation (Kuchiiwa et al., '89; Kuchiiwa, '90a).

In the present study, preganglionic neurons of the cat ACG (including the ectopic intraocular ganglion neurons) within the brainstem were located by means of the retrograde axonal transport of horseradish peroxidase (HRP) and HRP-conjugated wheat germ agglutinin (WGA-HRP). The results were compared with those of the CG to clarify whether the projections to both ganglia are topographically organized. The findings are discussed in relation to the involvement of the ACG in control of the pupillary constriction in association with convergence and accommodation (pupillary near reflex), and the recent concept of a direct neural path from the midbrain to the ciliary muscle.

MATERIALS AND METHODS

Dissection of the intraocular ganglion

The presence, location, number, and histological characteristics of the intraocular ganglion cells were examined in

Accepted August 11, 1993.

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ten eyes enucleated from 10 adult cats, which were sacrificed for other studies (Kuchiiwa, '90a; Kuchiiwa et al., '92).

The cats (body weight 1.5–4.0 kg) were anesthetized with ketamine hydrochloride (30–70 mg/kg) and perfused with physiological saline followed by 10% buffered formalin or a mixture of 1.0–2.5% formalin and 1.25% glutaraldehyde (pH 7.4). One eye in each animal was enucleated and dissected under a binocular dissecting microscope. The ciliary nerves in the suprachoroid lamina, including those that were extremely fine, were removed as long and many as possible, then the adhering pigmented cells were eliminated completely with fine watchmaker forceps and soft brushes. The nerves were mounted on gelatinized slides, stained with thionine as described elsewhere (Kuchiiwa et al., '89) or by Holmes's silver impregnating method (Ráliš et al., '73). The preparations were examined as wholemounts under a light microscope.

Injections into the anterior and posterior chambers of the eye

Four adult cats (body weight 1.4–3.8 kg) were anesthetized with intramuscular ketamine hydrochloride (20–40 mg/kg) and intraperitoneal Nembutal (10–40 mg/kg), then local anesthesia (xylocaine) was applied at all pressure points of the eyeball. The needle of a microsyringe was inserted into the posterior chamber through the sclera approximately 3 mm posterior to the limbus so as to minimize extraocular leakage of the tracer and infiltration into the vitreous body, and 50 μl of 30% horseradish peroxidase (HRP; Toyobo, Grade IC) dissolved in sterile saline or 50 μl of 5% wheat germ agglutinin conjugated to HRP (WGA-HRP, prepared in the authors' laboratory; Kuchiiwa et al., '88) was injected into the anterior and the posterior chambers of the eye. The surface of the eyeball was flushed with saline immediately after puncture and withdrawal, and the puncture site was sutured and plugged with a surgical binding agent (Aron Alpha A: Sankyo) to minimize backflow of HRP/WGA-HRP from the eyeball. After a postinjection period of 45–51 hours, the cats were reanesthetized deeply and perfused through the ascending aorta with 1,000 ml of saline followed by 2,000 ml of 1.0–2.5% formalin and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer at pH 7.4. The brainstem, ACG and CG were removed and immersed in a cold buffer containing 30% sucrose, and 40 or 50 μm thick sections were cut on a freezing microtome. The eyeball was dissected and the intraocular ciliary nerves were removed from the suprachoroid lamina. All sections of the brain, ganglia and whole ciliary nerves were prepared for peroxidase histochemistry by the tetramethylbenzidine reaction method (Mesulam, '78), and treated with a 5% ammonium molybdate solution adjusted to pH 3.5 with 1N hydrochloric acid in

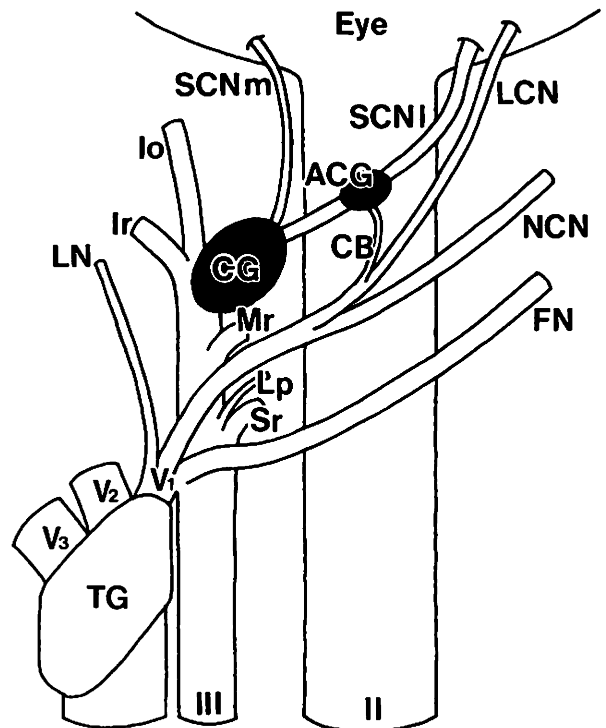

Fig. 1. Schematic diagram showing the location and the connecting pattern of the cat accessory ciliary ganglion (ACG) in the left orbit, viewed from above with the globe to the top. The main ciliary ganglion (CG) is fastened to the inferior trunk of the oculomotor nerve and gives rise to a heavy lateral and a finer medial short ciliary nerve. The lateral short ciliary nerve joined the communicating branch from the long ciliary nerve about 3–4 mm peripheral to the CG; at this point of fusion there is a single prominent ACG. See list of abbreviations for this and subsequent figures.

Abbreviations

3N	oculomotor nucleus
II	optic nerve
III	oculomotor nerve
ACG	accessory ciliary ganglion
AM	anteromedian nucleus
CB	communicating branch from the long ciliary nerve
CG	ciliary ganglion
CL	central linear nucleus
CP	cerebral peduncle
D	nucleus of Darkschewitsch
DBC	decussation of the brachium conjunctivum
DR	dorsal nucleus of raphe
EW	Edinger-Westphal nucleus
FN	frontal nerve
IC	interstitial nucleus of Cajal
IF	interfascicular nucleus
Io	branch to the inferior oblique muscle
IP	interpeduncular nucleus
Ir	branch to the inferior rectus muscle
LCN	long ciliary nerve
LN	lacrimal nerve
Lp	branch to the levator muscle of the palpebra
MLF	medial longitudinal fascicle
Mr	branch to the medial rectus muscle
NCN	nasociliary nerve
PAG	periaqueductal gray
PC	posterior commissure
PG	pontine gray
R	red nucleus
RF	retroflex bundle
RL	rostral linear nucleus
SC	superior central nucleus
SCNl	lateral branch of the short ciliary nerve
SCNm	medial branch of the short ciliary nerve
Sr	branch to the superior rectus muscle
TD	tegmental decussation
TG	trigeminal ganglion
V1	ophthalmic nerve
V2	maxillary nerve
V3	mandibular nerve
VTA	ventral tegmental area

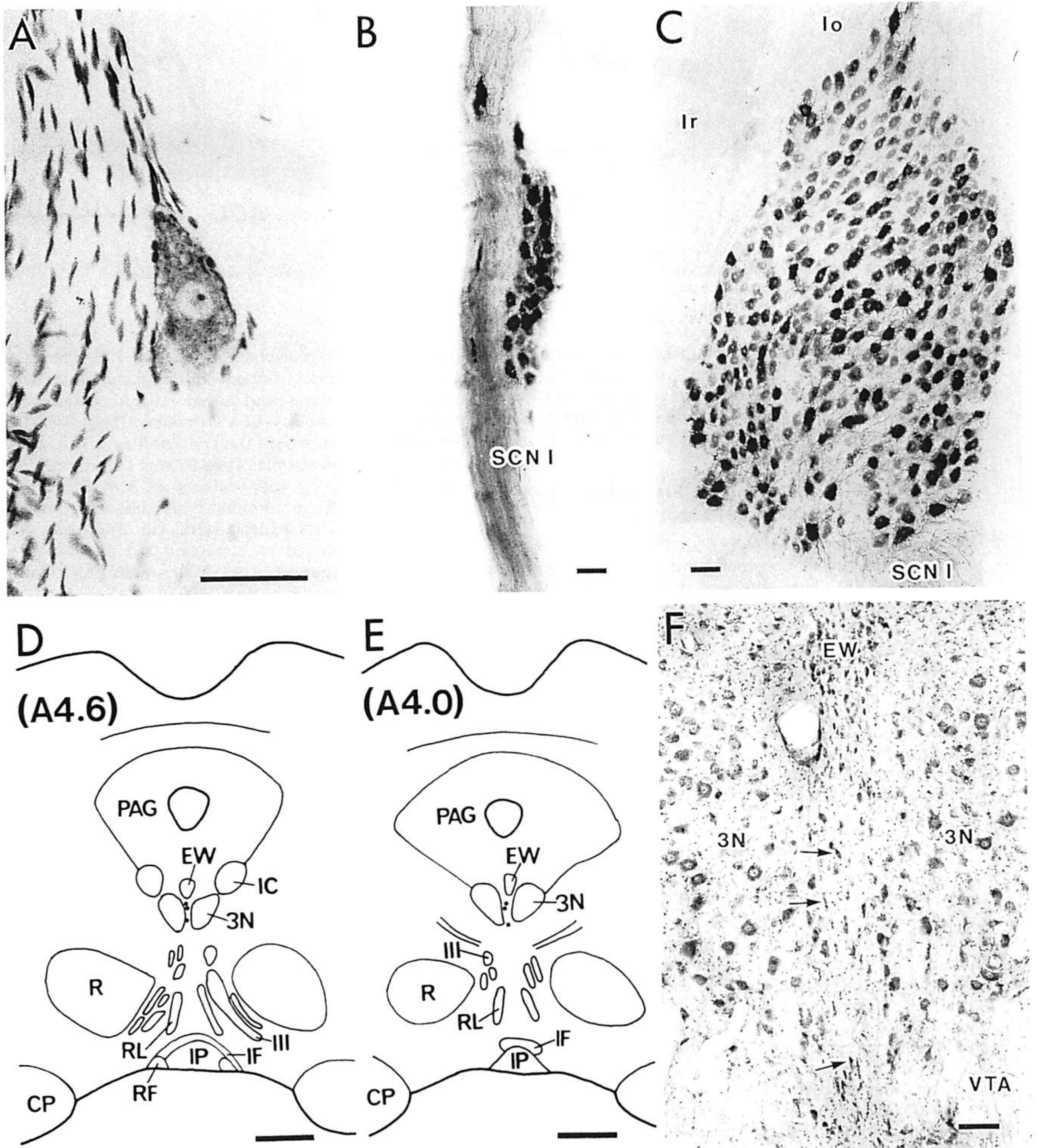

Fig. 2. **A:** Wholemount preparation of the intraocular ganglion neuron on the ciliary nerve in the eye, stained with thionine. **B,C:** Photomicrographs of longitudinal sections of the ACG (B) and the CG (C) treated with tetramethylbenzidine after injection of horseradish peroxidase (HRP) into the anterior and posterior chambers of the eye. Most cells were labeled with the tracer. Fifty μm thick sections. **D,E:** Diagram of frontal sections through the midbrain, showing locations of labeled cells (dots) following application of HRP into the ciliary bodies and suprachoroid laminae in both eyes. **F:** Photomicrograph of the frontal section through the rostral one-fifth of the somatic oculomotor nucleus at level of A.4.0 showing the location of retrogradely labeled neurons (arrows), the same preparation as E, counterstained with neutral red, 50 μm thick section. Bars = 50 μm for A; 100 μm for B, C and F; 1 mm for D and E.

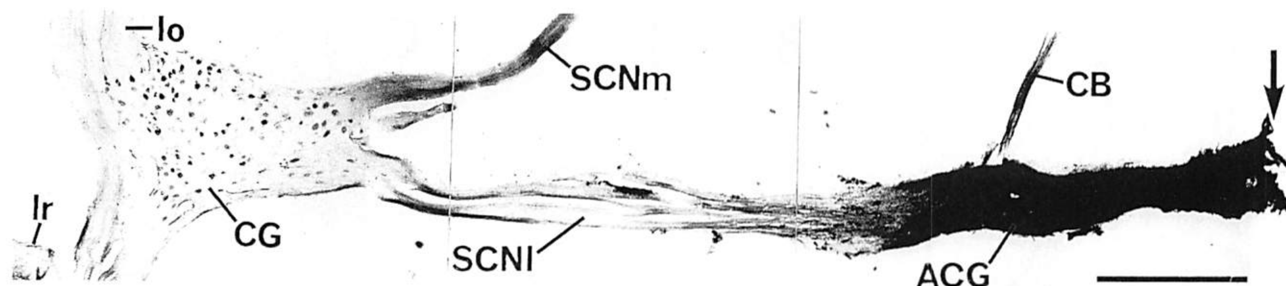

Fig. 3. Low power photomicrograph showing the site of WGA-HRP injection into the ACG. The arrow points to the peripheral cut end of the lateral short ciliary nerve, from which the glass micropipette was inserted. Fifty μm thick section. Bar = 1 mm.

order to stabilize the reaction products (modification of the method of Fujii and Kusama, '84). Part of each section was counterstained with neutral red. Light and polarized illuminations were employed to visualize HRP/WGA-HRP-labeled cells. Darkfield illumination was not useful for the ammonium molybdate preparations.

Injections into the ciliary body and suprachoroid lamina

Five adult cats (body weight 2.2–3.5 kg) were anesthetized as in the preceding experiment. Two of five animals had injections into both eyes and the remainder only into the left eye. The needle of a microsyringe was inserted through the center of the cornea and directed into the ciliary body. Eight separate injections of 5 μl of 30% HRP or 5% WGA-HRP solution were performed at intervals of about 45°. The surface of the eyeball was flushed with saline and the puncture site was sutured and plugged with the surgical binding agent. After 45–51 hours survival, the animals were reanesthetized and perfused with the fixative. The brainstem, ACG, CG, and the intraocular ciliary nerves were removed and subsequently processed histochemically in the same manner described above.

Injections into the ACG

Twelve adult cats (body weight 1.6–4.3 kg) were anesthetized as described above and placed in a stereotaxic head holder permitting easy access to the ACG. The skull was opened, the dura was cut over the left sylvian fissure, and the opening was blotted with cotton applicators to remove the cerebrospinal fluid in order to reduce the hemisphere. Then the anterior portion of the left brain was retracted caudally with a Langenbeck's flat hook to expose the upper wall of the orbit, and then the wall was removed. The ACG and the lateral short ciliary nerve were approached through the interspace between the superior and the lateral rectus muscles, and then the lateral short ciliary nerve was cut at a point approximately 2 mm peripheral to the ACG. A strip of plastic film was carefully pulled underneath the nerve to prevent leakage of the tracer to subjacent structures. A glass micropipette attached to a 1 μl Hamilton microsyringe was inserted through the peripheral cut end of the nerve and 0.1 μl of 50% HRP or 5% WGA-HRP dissolved in sterile saline was injected slowly into the ganglion manually under an operation microscope. Thereafter, the cut end of the nerve was plugged with Alon Alpha A (Sankyo) and the orbit was flushed with saline. After 45–51 hours, the cats were again anesthetized deeply and perfused with saline followed by a 1.0–2.5% formalin and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The ACG and the CG ipsilateral to the injection and the brainstem were removed immediately and stored in 0.1 M phosphate buffer containing 30% sucrose at 4°C for 2 or 3 days. Serial frontal or sagittal sections were then cut at 40 or 50 μm thickness on a freezing microtome. The sections were then processed histochemically by the protocol of Mesulam ('78) and treated with a 5% ammonium molybdate solution adjusted to pH 3.5 with 1N hydrochloric acid. The brain sections were counterstained with neutral red or treated by an acetylthiocholinesterase (ATChE) staining method as described elsewhere (Kuchiiwa, '90b).

Injections into the CG

Six adults cats (body weight 1.5–3.9 kg) were anesthetized and fixed in the stereotaxic head holder, then injected with a HRP or WGA-HRP solution into the CG. The ACG and the CG were exposed in the orbit microsurgically through the dorsal approach as described above. The lateral short ciliary nerve was cut between the ACG and the CG, a strip of plastic film was pulled underneath the nerve to prevent leakage of the tracer to subjacent structures, and the glass micropipette connected to the microsyringe was inserted into the CG through the cut end of the nerve to avoid damaging fibers to the inferior oblique and inferior rectus muscles. Thereafter, 0.2 μl of 50% HRP or 5% WGA-HRP solution was injected slowly into the ganglion. After 45–51 hours, the animals were perfused with the fixative. The brainstem and the CG ipsilateral to the injection were removed and processed as described above for histochemical localization of retrogradely labeled neurons.

RESULTS

Observations of the intraocular ganglion

In 9 of 10 cats, a small number of ectopically located ganglion cells were found in the intraocular ciliary nerves in the suprachoroid lamina in both silver impregnation and Nissl preparations (Fig. 2A). In one case, however, no neurons were found, although we did not have all the intraocular ciliary nerves. The maximum number observed in one eye was 8, with an average of 3.9. Most ganglion neurons were large, and elliptic or elongated multipolar cells, with their longest diameter paralleling the axis of the ciliary nerve. The cells were about 40–75 μm long and 25–50 μm wide. The perikarya had coarse Nissl granules

Fig. 4. Photomicrograph of frontal section showing the distribution of retrogradely labeled neurons (arrows) after applying WGA-HRP into the left ACG, through the anteromedian nucleus at A.5.9. Counterstained with neutral red; 50 μm thickness. Bar = 100 μm.

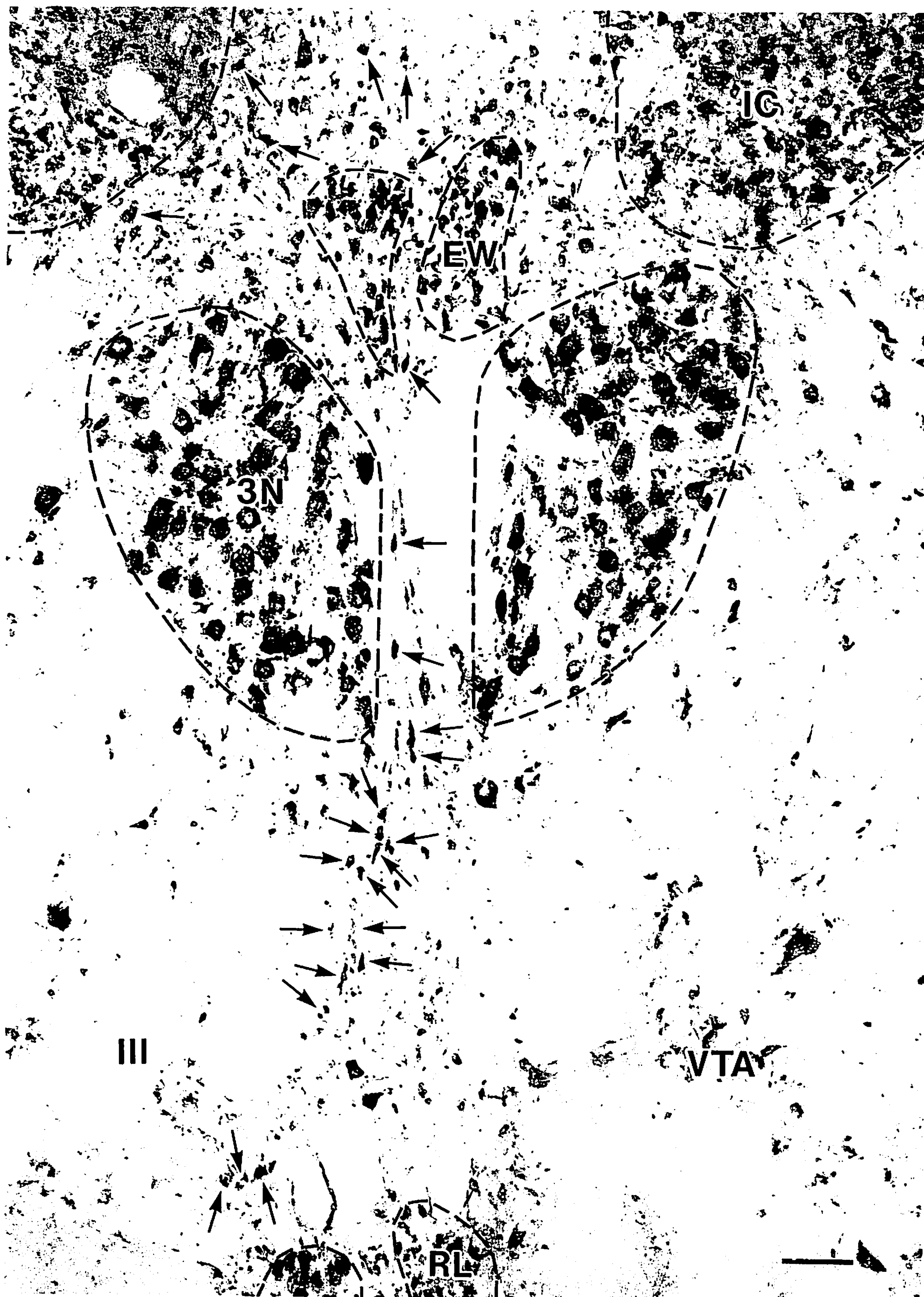

Fig. 5. Photomicrograph of frontal section showing the distribution of neurons labeled with WGA-HRP injected into the left ACG, at the level of A.4.7. The labeled neurons are indicated with arrows. The section was counterstained with neutral red; 50 μm thickness. Bar = 100 μm.

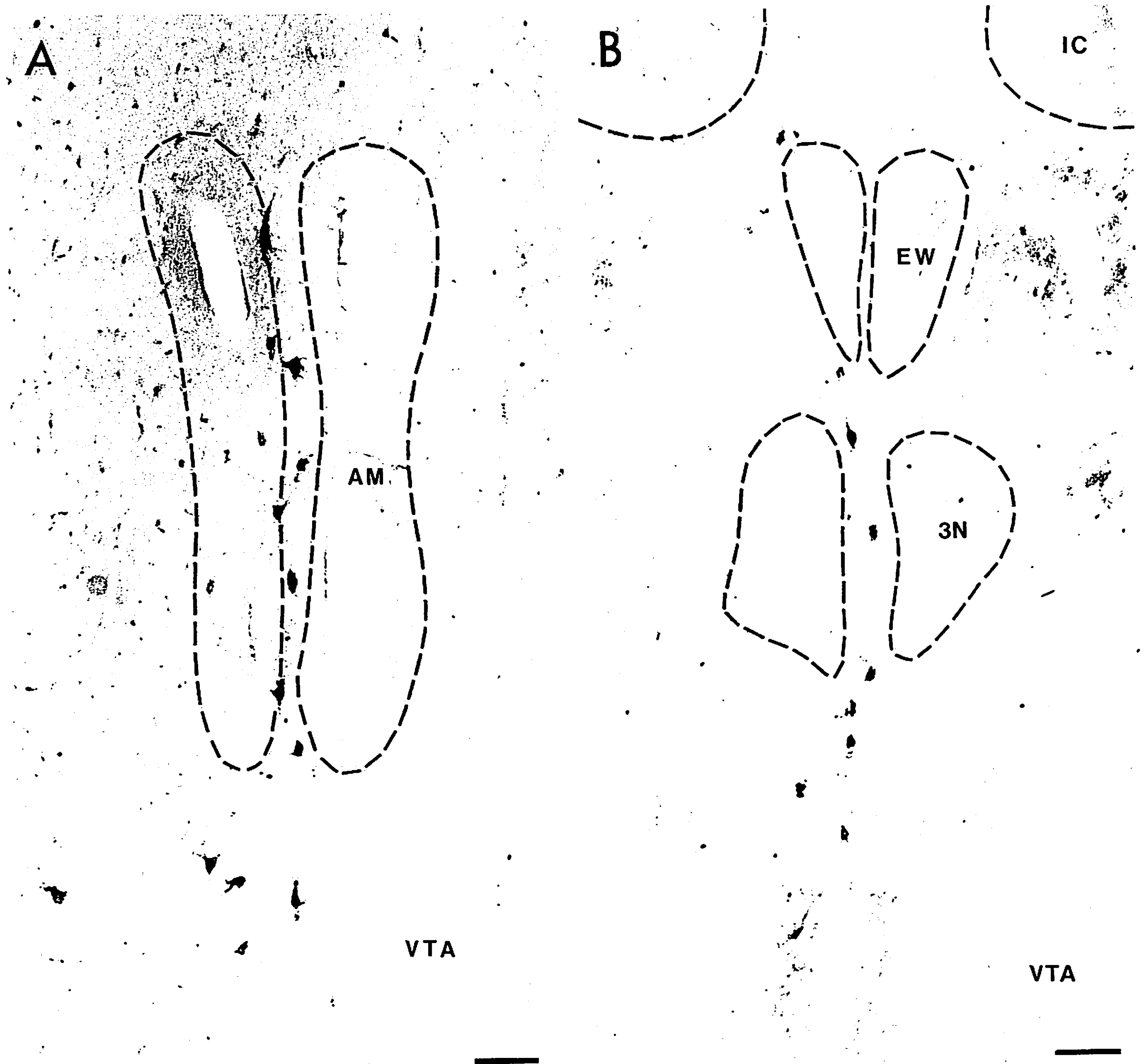

Fig. 6. Photomicrograph of frontal section showing the distribution of neurons labeled with WGA-HRP injected into the left ACG, at the caudalmost level of the anteromedian nucleus (**A**, A.5.2) and rostral level of the somatic oculomotor nucleus (**B**, A.4.6). These sections were treated with ammonium molybdate but not counterstained with neutral red; 50 μm thickness. Bars = 100 μm.

and the pale spherical or elliptical nucleus contained a single densely stained nucleolus. Most ganglion cells were histologically similar to those of the ACG in shape, size, and cytoplasmic and nuclear characteristics rather than pseudounipolar trigeminal or smaller superior cervical ganglion cells (see Gabella, '76; Lieberman, '76; Kuchiiwa, '90a).

Injections into the anterior and posterior chambers of the eye

After injection of HRP or WGA-HRP into the anterior and posterior chambers of the eye, most ganglion cells in the ACG and the CG were intensely labeled (Fig. 2B,C). Examination of the intraocular ciliary nerves did not reveal diffusion of the tracer into the suprachoroid lamina. In the brainstem, no retrogradely labeled somata were found in the rostral mesencephalic regions in which the visceral oculomotor neurons are located, namely, in the anteromedian nucleus, the Edinger-Westphal nucleus, the median region between the somatic oculomotor nucleus, the ventral tegmental area, and the ventral periaqueductal gray (Sugimoto et al., '77; Loewy et al., '78; Toyoshima et al., '80; Burde et al., '82; Maciewicz et al., '83). Neither retrogradely labeled neurons nor anterogradely labeled terminals were found in the nuclei of the somatic oculomotor, trochlear, trigeminal, abducens and facial nerves. In the primary visual centers, anterogradely labeled terminals were con-

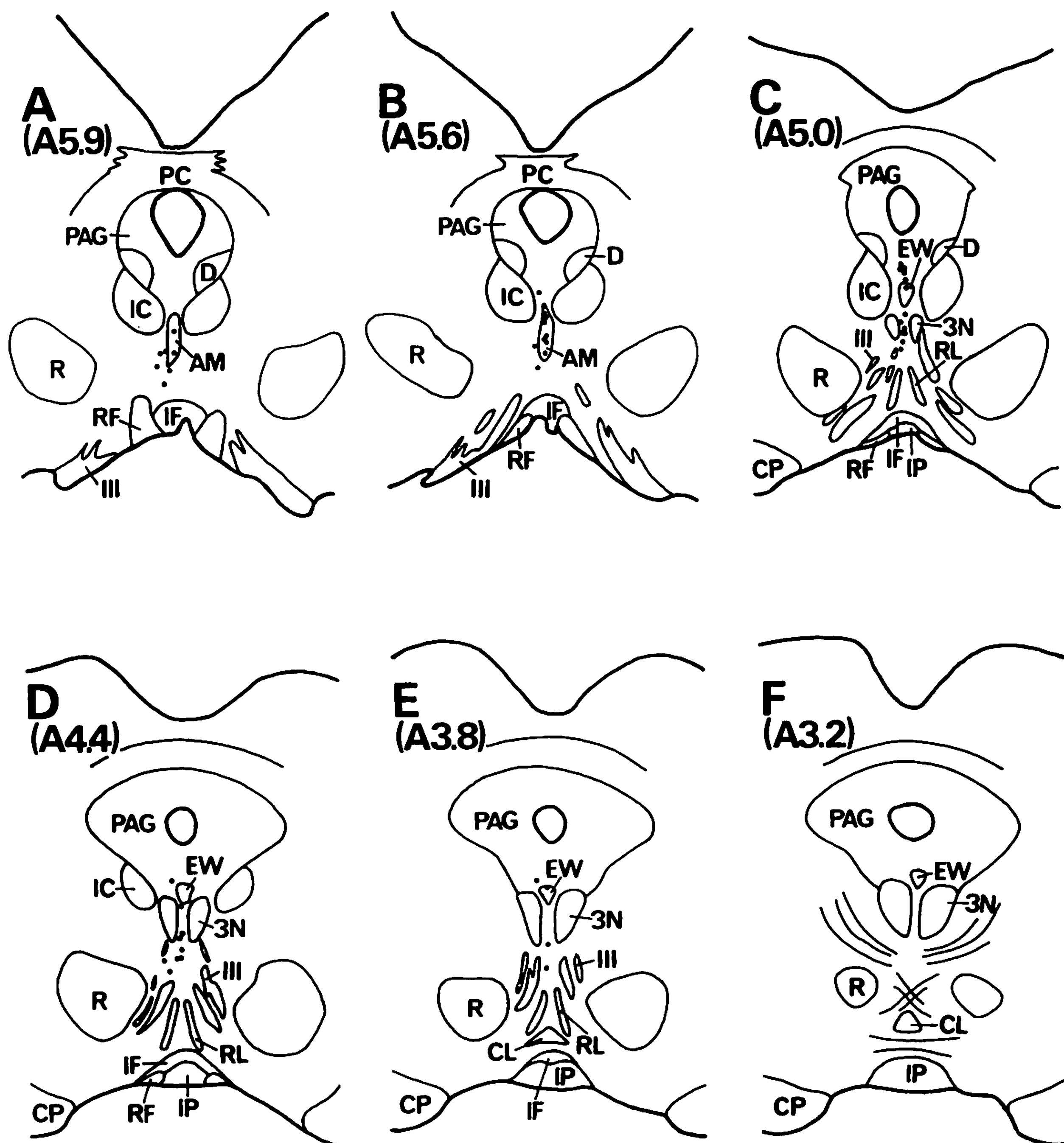

Fig. 7. A–F: Drawings of series of frontal sections through the midbrain rostral (A) to caudal (F), showing distribution of labeled cells after injecting WGA-HRP into the left ACG. WGA-HRP-filled cells are indicated by heavy black dots. Bar = 1 mm.

fined to a small portion of the lateral geniculate nucleus, superior colliculus, and the pretectal nuclei.

Injections into the ciliary body and suprachoroid lamina

Abundant labeled cells were observed in the ACG and the CG, although some areas of the ganglia remained unlabeled. The intraocular ciliary nerves contained dense HRP/WGA-HRP positive granules up to 4–5 mm from the ciliary body, indicating that HRP/WGA-HRP was indeed introduced into the suprachoroid lamina by inserting the microsyringe needle into the ciliary body. Subsequently, most intraocular ganglion cells became immersed in the tracers. In all brainstems studied, a few retrogradely labeled neurons were found in the midplane between each side of the somatic oculomotor nuclei at the level of A.5.0–3.5 (Fig.

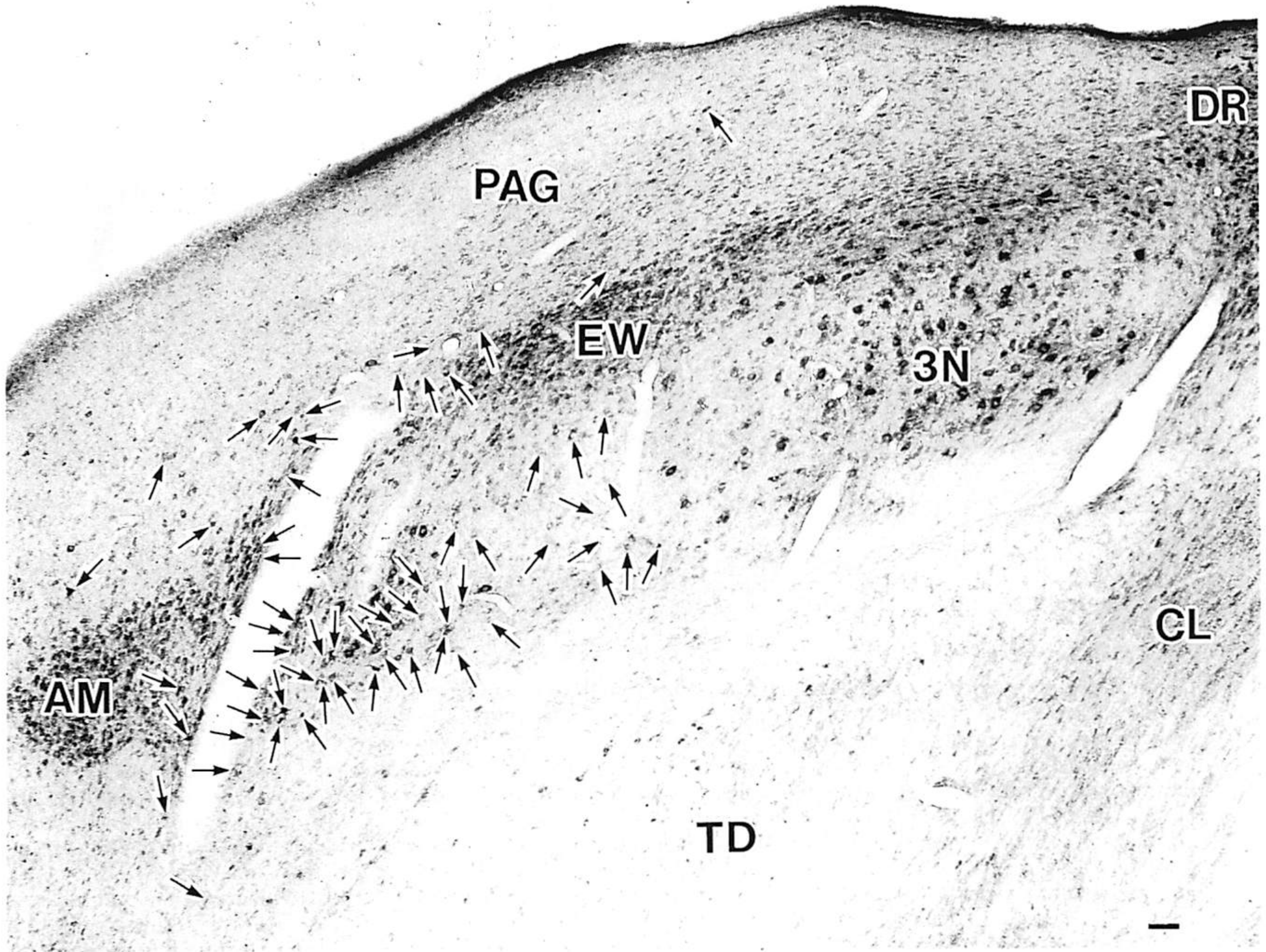

Fig. 8. Photomicrograph of a sagittal section through the midbrain 0.05 mm left to the midplane (L.0.05), showing the distribution of neurons labeled with WGA-HRP injected into the left ACG. The labeled neurons are indicated with arrows. Counterstained with neutral red; 50 μm thick section. Bar = 100 μm.

2D–F). No labeled cells were found in the other visceral oculomotor nuclei.

Injection into the ACG

The injected HRP or WGA-HRP was immersed throughout the ACG but did not diffuse into the CG, and most CG neurons were intensely labeled (Fig. 3). Neither labeled neurons nor terminals were observed in the nuclei of the somatic oculomotor, trochlear, trigeminal, abducens, or facial nerves.

Most labeled cells were restricted to the vicinity of the midplane and the paramedian regions of the rostral mesencephalon up to 0.6 mm left to the midplane (L.0.0–0.6), at the levels from A.6.0 to A.3.5 (Figs. 4–10).

In the frontal sections, at the level of the anteromedian nucleus (A.6.0–5.2), labeled neurons were distributed in an arc-shaped arrangement dorsoventrally from the ventral periaqueductal gray to the median region of the ventral tegmental area through the anteromedian nucleus (Figs. 4; 6A; 7A,B). At the level from the junction of the anteromedian and the Edinger-Westphal nuclei to the rostral two-fifths of the somatic oculomotor nucleus (A.5.2–3.5), a gentle arc-like arrangement of labeled neurons also appeared in close contact with the median plane (Figs. 5; 6B; 7C–E). Most labeled cells were located in the midplane between each side of the somatic oculomotor nucleus and the ventral continuation of the median region of the ventral tegmental area, dorsal to the rostral linear nucleus and between each side of the oculomotor root fibers. Moreover, a small number of labeled neurons appeared in the ventral periaqueductal gray and the median region of the Edinger-Westphal nucleus. Few labeled neurons were found more caudally than A.3.5 (Fig. 7F).

In sagittal sections, most labeled cells were distributed in the region between each side of the somatic oculomotor nuclei at the level of the median plane (Figs. 8, 10A), and the median region of the ventral tegmental area at the level of 0.2–0.4 mm left to the midplane (L.0.2–0.4; Figs. 9, 10B,C). At a more lateral level, only a few labeled cells were found in the median region of the ventral tegmental area (Fig. 10D,E).

The labeled cells in the midplane between the somatic oculomotor nuclei were small and medium in size. Spindle-shaped neurons whose major orientation was in the dorsoventral axis predominated in the frontal sections (Fig. 11A). However, 3–4 main dendrites were observed in these cells

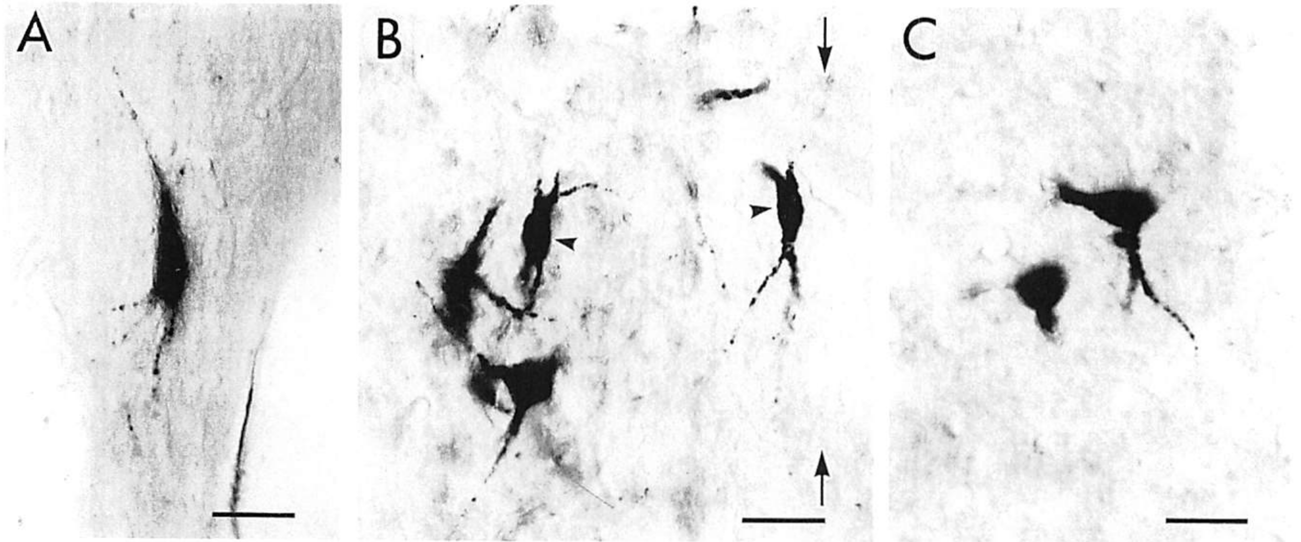

Fig. 11. Photomicrographs of labeled cells within the median region between the somatic oculomotor nuclei (**A**), the median region of the ventral tegmental area (**B**), and the ventral periaqueductal gray (**C**). Note the spindle appearance of neurons, especially in A and B (arrowheads). Arrows in B indicate the median plane; 50 μm thick section. Bars = 50 μm.

ciliary nerves in the orbit but are also located in the eyeball (Castro-Correia, '67). During the course of our study of oculomotor parasympathetic projections, we also found that, especially in man and monkey, clusters of cells are located in the intraocular ciliary nerves in the suprachoroid lamina, from the vicinity of the iris and ciliary body to the scleral canal, and sometimes behind the sclera. (The microganglia observed just behind the sclera of man have been long recognized as the episcleral ganglia of Axenfeld [Axenfeld, '07; Givner, '39].) In this study, we found several intraocular ganglion cells also in the cat. Their histological features and the location of the cells of the origin of the afferents suggest that they, at least in part, are ectopic cells from the ACG.

Direct neural path from the midbrain to the eye

When the anterior and posterior chambers of the eye were injected with HRP or WGA-HRP, no neurons in the visceral oculomotor nuclei were retrogradely labeled, although most neurons of the ACG and the CG were intensely labeled, indicating that the intraocular muscles had taken up the tracers in full, thus suggesting that the visceral oculomotor nuclei do not project directly to the intrinsic eye musculature.

When the tracer was injected by inserting the syringe needle into the ciliary body, a small number of neurons in the median region between the somatic oculomotor nuclei were retrogradely labeled. The intraocular ciliary nerves from these experiments provided evidence the tracer had diffused into the suprachoroid lamina and subsequently immersed the intraocular ganglion cells, suggesting that the oculomotor preganglionic parasympathetic fibers terminating the intraocular ganglion cells originate in the median region between the somatic oculomotor nuclei.

Direct intraocular projections from the midbrain have already been reported in the rabbit and monkey by Jaeger and Benevento ('80) and Parelman ('84). They observed HRP- or WGA-HRP-labeled neurons in the median plane of the somatic oculomotor nuclei following application of the enzyme into the eye, and subsequently argued for the existence of a direct parasympathetic neural path to the ciliary muscle. However, the investigators were unaware of the existence of intraocular ganglion neurons. In their studies, a microsyringe needle was inserted into the ciliary body, assuming that the tracers would diffuse into the suprachoroid lamina, and subsequently immerse the ganglion neurons. The intraocular ganglion cells of the monkey are several tens or over one hundred in number in a single eye, while scarcely any cells are found in the rabbit (unpublished data). They described that HRP or WGA-HRP labeled many cells in the midbrain in the monkey, but much fewer (Jaeger and Benevento, '80) or none (Parelman, '84) in the rabbit. It is speculated that some of the labeled neurons that they observed are the cells of origin of the afferents to the intraocular ganglion neurons but not those to the ciliary muscle.

The present investigation provides new anatomical evidence for the excellent reviews of Loewenfeld ('73) and Ruskell ('90) that reject the concept of the direct neural path to the intrinsic eye musculature, which challenges the conventional view of the oculomotor parasympathetic outflow.

The origin of the preganglionic fibers of the ACG

The HRP/WGA-HRP injections into the ACG revealed that the neurons in the median and paramedian regions of the rostral mesencephalon (that is, anteromedian and Edinger-Westphal nuclei, the median region between the somatic oculomotor nuclei, the ventral continuations of the median region of the ventral tegmental area, and the ventral portion of the periaqueductal gray) give rise to the oculomotor parasympathetic outflow to the ACG. Moreover, it becomes apparent that the intraocular ganglion neurons receive preganglionic fibers exclusively from the neurons located in the midplane between the somatic oculomotor nuclei.

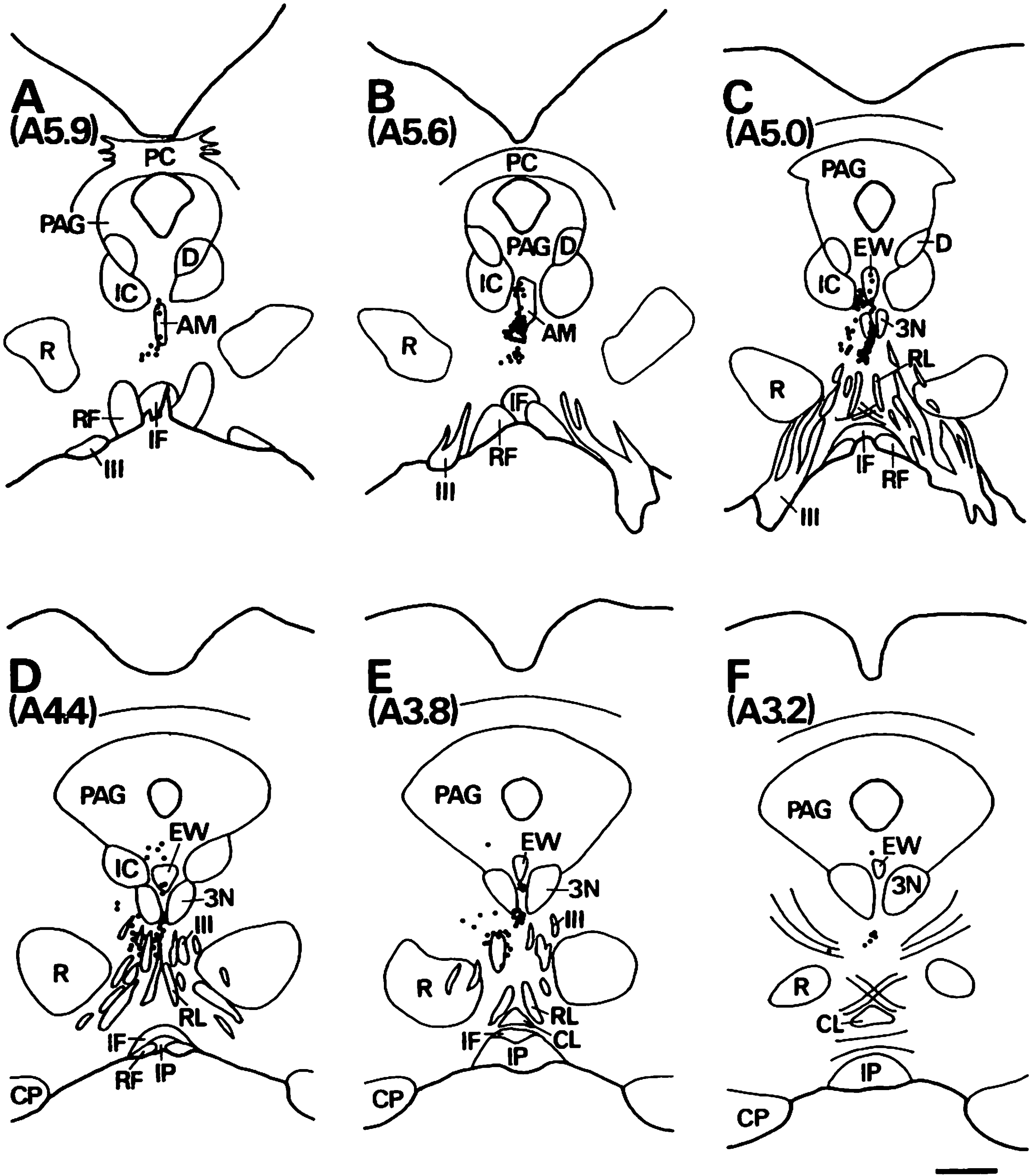

Fig. 12. **A–F:** A series of drawings of frontal sections showing distribution of retrogradely labeled neurons in the mesencephalon after injecting WGA-HRP into the left CG. WGA-HRP-labeled neurons are indicated by heavy black dots. Note that the labeled cells are located in the border zones of the anteromedian, Edinger-Westphal, and the somatic oculomotor nuclei as well as in the median regions in which labeled neurons were observed after injection into the ACG. **A** is most rostral, **F** is most caudal. Bar = 1 mm.

Furthermore, the present study confirmed that the afferents of the CG originate in the lateral border zones of the anteromedian, the Edinger-Westphal and the somatic oculomotor nuclei, and their ventral continuations of the ventral tegmental area, in addition to all the aforementioned median and paramedian regions giving rise to the preganglionic fibers to the ACG. These lateral border zones were labeled only after injection into the CG, indicating that they send preganglionic fibers exclusively to the CG. In the medial regions, since the number of labeled cells in the CG

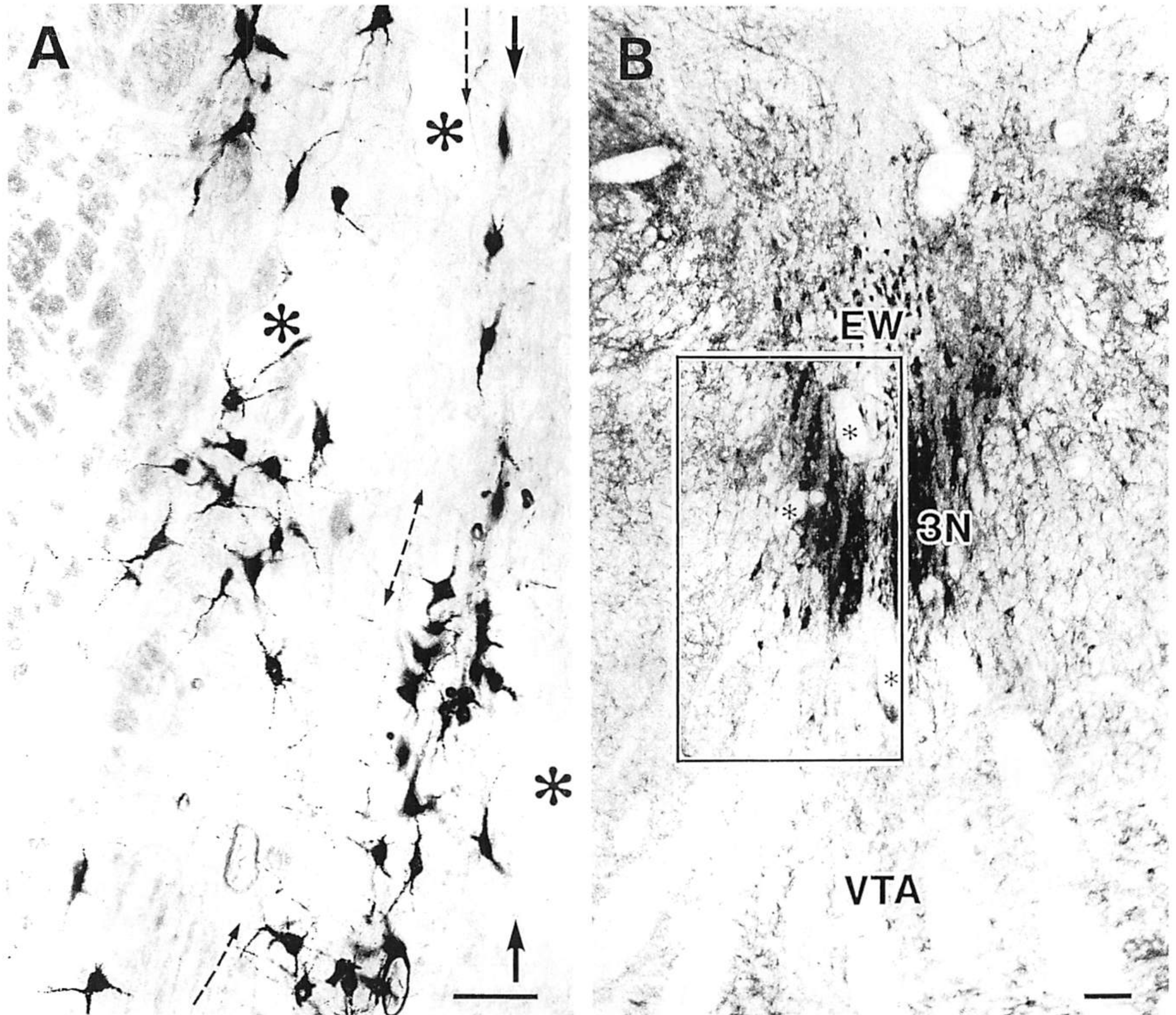

Fig. 13. Photomicrographs of transverse sections through the rostral tip of the somatic oculomotor nucleus, showing distribution of WGA-HRP-labeled neurons following injection into the left CG. **A** and **B** are the same section. The section was initially prepared for WGA-HRP histochemistry and photographed (A), then treated by an acetylthiocholinesterase (AThChE) staining method (B). Note that the labeled neurons are located in the lateral border zones of the anteromedian, Edinger-Westphal, and somatic oculomotor nuclei as well as in the median/paramedian regions. The neurons in the lateral border zones were labeled only after injection into the CG. A imaginary arc-like border line between the lateral border zones and the median/paramedian regions is indicated by broken arrows. Solid black arrows indicate the median plane. The rectangular frame indicates position of the left photomicrograph. Asterisks denote to the same vessels; 50 μm thick sections. Bars = 100 μm.

study was much greater than that in the ACG study except in the median region between the somatic oculomotor nuclei and at the middle level of the anteromedian nucleus, these regions are considered to send visceral oculomotor fibers to the CG as well as to the ACG.

In the median region between the somatic oculomotor nuclei and at the middle level of the anteromedian nucleus, the differences between the numbers of labeled cells in the ACG- and CG studies were not significant. If some preganglionic fibers originating in these regions pass through the CG to reach the ACG, since the both HRP and WGA-HRP are taken up by damaged fibers of passage and label the cells of origin retrogradely (Herkenham and Nauta, '77; Brodal et al., '83; Kuchiiwa et al., '88), HRP/WGA-HRP injections into the CG might label the origins of the afferents of the ACG as well as the CG. However, since it is known that some preganglionic fibers of the ACG bypass the CG by way of the communicating branch from the long ciliary nerve in the cat (Kuchiiwa et al., '93), it is possible that these regions may also give rise to oculomotor parasympathetic outflow to the CG as well as to the ACG.

It is concluded that the preganglionic nerve fibers of the ACG (including the intraocular ganglion) originate in the extremely confined areas of the median and paramedian regions of the rostral midbrain, whereas those of the CG arise more laterally, also. Although we can not judge

whether or not the projections to the ACG and the CG are topographically organized because of the above mentioned fibers of passage problem, it is certain that the preganglionic neurons of the ACG tend to be predominantly located more medially than those of the CG. If part of the pupillary near reflex originates in the median and paramedian regions of the rostral midbrain and is mediated in the ACG, this function should be relayed in more peripherally located ganglion neurons.

A possible functional role of the ACG

It has long been assumed that the neurons in the visceral oculomotor nuclei send their axons exclusively via the oculomotor nerve to the CG where they synapse and thus, function as the parasympathetic preganglionic outflow which mediates pupillary constriction and lens accommodation. However, after extirpating the CG in monkeys, Foerster et al. ('36) found that although pupil reactions to light in the operated eyes were lost, pupillary near reflex was retained. This experiment showed that there are two peripheral pathways for pupillary constriction, one subserving the light reflex that passes through the CG, the other concerned with the pupillary near reflex, mediated in other ganglion and running to the sphincter muscle by other routes.

While studying pupillo-constrictor pathways, we found that the ACG generally receives communicating branches from the trigeminal branch in most mammalian species (Kuchiiwa et al., '89), and the branch contains many fine myelinated fibers that are indistinguishable from parasympathetic fibers in the short ciliary nerve (Kuchiiwa, '90a). Moreover, we observed that some of the fibers in the communicating branch of the cat could be labeled by injecting an enzyme marker into the rostral midbrain including the visceral oculomotor nuclei (Kuchiiwa et al., '93). These results suggest that at least a part of preganglionic fibers of the ACG travel by way of the trigeminal nerve bypassing the CG. It is possible that the ACG is involved in pupillary near reflex.

ACKNOWLEDGMENTS

We thank Miss M. Ushikai and Mr. T. Shinyama for their expert technical assistance. This research was supported by the Kodama Foundation for Research of Medical Science and a Hirosaki University Special Research Grant, 1990, No. 20.

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