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Cytoarchitecture and Fibre Connections of the Edinger-Westphal Nucleus in the Filefish

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Cytoarchitecture and fibre connections of the Edinger–Westphal nucleus in the filefish

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SUMMARY

Many teleosts have an intraocular lens muscle which causes lens movement for visual accommodation. Central pathways for visual accommodation were traced in the filefish (*Novodon modestus*) using horseradish peroxidase (HRP) tracing method. After HRP injection into the lens muscle, large cells were labelled in the ciliary ganglion. After HRP injections into the ciliary ganglion, a compact cell group was retrogradely labelled in an area rostrodorsal to the somatic oculomotor nuclei in the midbrain. The nucleus consisted of about 90 cells which were slightly smaller than the somatic oculomotor neurons. This nucleus was considered to be homologous with the mammalian Edinger–Westphal nucleus. After injections of HRP into the Edinger–Westphal nucleus, cells in the nucleus of the posterior commissure were retrogradely labelled. Because the nucleus of the posterior commissure receives retinal projections (Ito *et al.* 1984), it is considered that the cells in the nucleus of the posterior commissure are the first central neurons in the pathway for visual accommodation in teleosts.

1. INTRODUCTION

Visual accommodation is an important function of the fish eye in various behaviour. Many teleosts can accommodate their eyes by moving the lens (Somiya & Tamura 1973). Efferent arrangements of the accommodatory system, i.e. the intraocular lens muscle and its associated nerve including the ciliary ganglion, have already been described in mackeral and bass (Somiya 1987; Wathey 1988*a*). Recently Wathey (1988*b*) found the teleost Edinger–Westphal (EW) nucleus, which sends information to the ciliary ganglion. However, there appears to be no literature on the afferent link of the accommodatory reflex in fishes. As far as we know, the accommodatory reflex, especially its afferent link, has not been fully examined and understood in any group of vertebrates (Reiner *et al.* 1983; Pilar 1984; Bando & Toda 1991; Gamlin & Reiner 1991).

The present paper describes the EW nucleus in the filefish, and then its afferent connection for visual accommodation is examined.

2. MATERIALS AND METHODS

The filefish (*Navodon modestus* Günther) was selected for the present study because of its well-developed visual system (Kishida 1979). Twenty specimens, 15–25 cm in length (1–2 years old, mature), were obtained from commercial sources. For cytoarchitecture and study of the normal fibre system of the oculomotor nuclei, two brains were fixed by immersion in Bodian II solution, embedded in paraffin, sectioned frontally and horizontally at 15 μm, and stained according to the Bodian–Otsuka method (Otsuka *et al.* 1960). Four ciliary ganglia were also fixed in glutaraldehyde, dehydrated, embedded in Epon, sectioned in 1–2 μm and stained with toluidine blue (Somiya 1987).

For HRP injections, the animals were first anesthetized by immersion in a 0.01% solution of tricaine methanesulfonate (MS-222) and positioned in a fish holder. The gills were perfused with aerated artificial seawater containing 0.005% MS-222 during all surgical procedures.

(*a*) *HRP injection into the lens muscle (musculus retractor lentis) and the ciliary ganglion*

To expose the lens muscle, the ventrolateral part of the eyeball was opened by cutting the cornea and iris. HRP was injected in the central part of the lens muscle using an insect pin coated with HRP paste (Sigma type VI). The ciliary ganglion was exposed by putting one eye aside rostrally, and HRP was injected in the ciliary ganglion using an insect pin. After the operation, the opening in the eyeball or the orbit was covered with clingfilm.

(*b*) *HRP injection into the extraocular muscles and optic nerve*

To define the extact position of the EW nucleus in the oculomotor complex, HRP was injected into the central part of three rectus (inferior, medial and superior) and two oblique (inferior and superior) muscles separately. HRP was injected in the optic

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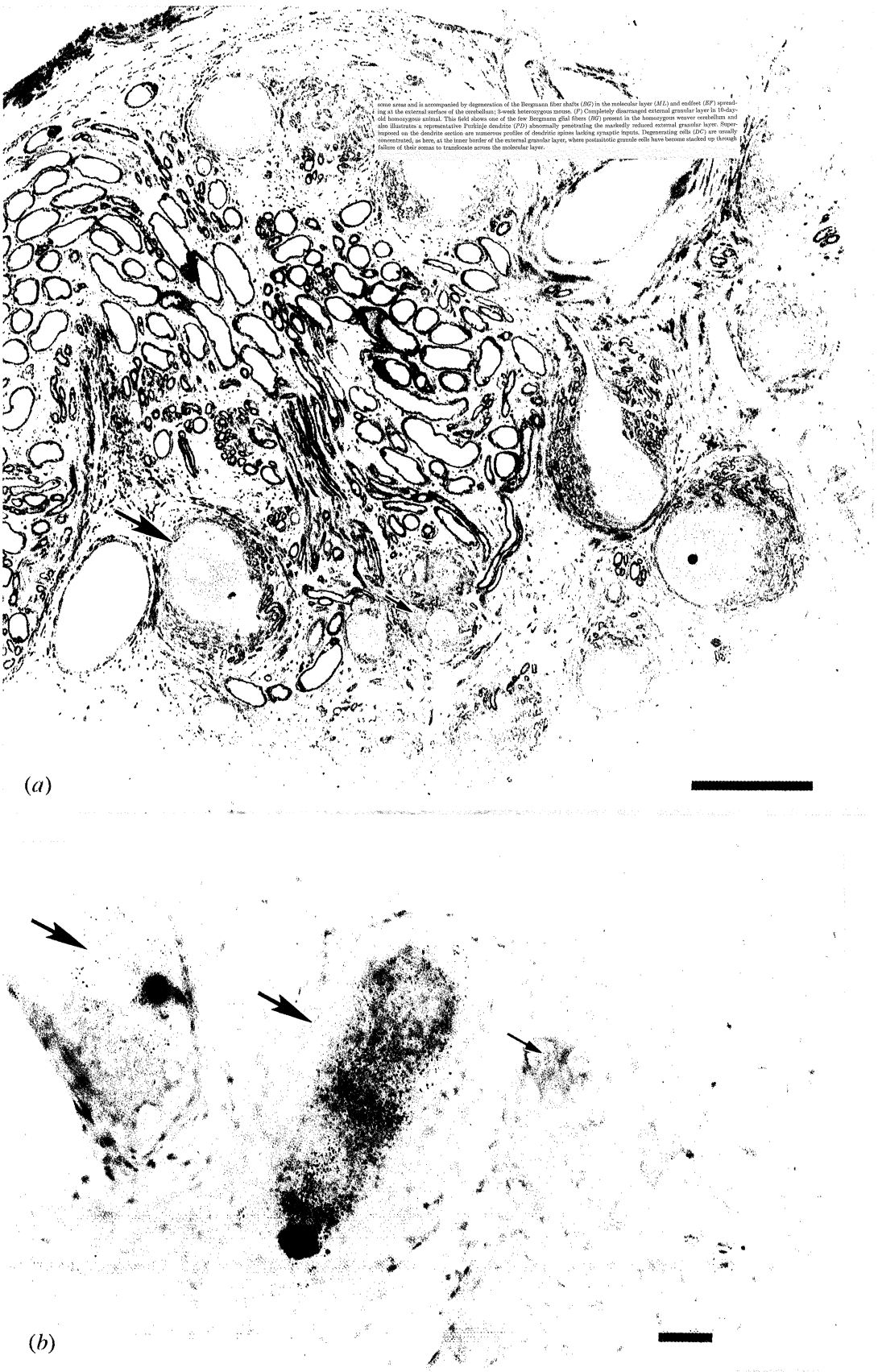

Figure 1. (*a*) Thick (1 micron) epoxy section of the ciliary ganglion stained with toluidine blue. Scale bar, 100 μm. (*b*) Enlarged photograph of the large ganglion cells which are HRP-positive. Scale bar, 10 μm. Large arrow indicates the large ganglion cell. Small arrow indicates the small ganglion cell.

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nerve to confirm the exact position of the nucleus of the posterior commissure.

(*c*) *HRP injections into the EW nucleus*

A part of the cranium was removed with a dental drill. The EW nucleus was exposed caudally from the posterior opening of the mesencephalic ventricle. A part of the valvula cerebelli was removed, and HRP was injected iontophoretically (0.2 μA, 0.5 Hz, and 15 min) into the EW nucleus using a glass micropipette with 30% solution of the HRP in saline. After the operation, the opening of the cranium was sealed with acrylic resin.

(*d*) *Procedures for HRP reaction*

Fish recovered from the anesthesia were kept in a tank maintained at 21–25°C. Three or four days after the operation, the fish was deeply anesthetized with MS-222 and perfused transcardially with saline followed by a solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The ciliary ganglia and brains were removed from the head and skull, embedded in egg yolk, postfixed in a fresh solution of the same fixative at 4°C for 24 h, and then stored in 0.1 M phosphate buffer (pH 7.4) containing 10% sucrose at 4°C for 24 h. Serial frontal sections were cut at 40 μm with a cryostat, then reacted with diaminobenzidine (DAB) following the procedure of Adams (1981). After the reaction, sections were mounted on slides with 1% gelatine in water, dried for 24 h at room temperature, and counterstained with 0.1% cresylviolet.

3. RESULTS

(*a*) *General arrangement of the accommodatory system*

The accommodatory system of the filefish eye consists of three elements; a single trapezoidal lens muscle with pigmented covering, the lens and its suspensory ligaments, and the short ciliary nerve which innervates the lens muscle. The efferent arrangements of the accommodatory system of the filefish are basically the same as those of bass (Somiya 1987; Wathey 1988*a*). The ciliary ganglion of the filefish lay on the eyeball ventrolaterally to the oculomotor nerve in the central part of the orbit. The size of the ciliary ganglion was about 1 × 2 mm.

The approximate size of the lens muscle was measured from photographs using a digitizer (Nikon, Cosmozone). The area of the lens (L) and the lens muscle (M) measured from the macroscopic photographs was 17.3 (lens diameter, 4.7 mm) and 4.6 mm^2 respectively, and their value of area percentage of muscle/lens (M/L%) was about 27.

(*b*) *HRP labelled cells in the ciliary ganglion*

The ciliary ganglion of the filefish contains about 250 cells (figure 1*a*). According to the soma size, cells were divided into two groups, a small cell group (smaller than 29 μm in long axis, $n = 166$) and the large cell group (greater than 30 μm in long axis, $n = 88$). The cell size (long axis) spectrum is shown in figure 2.

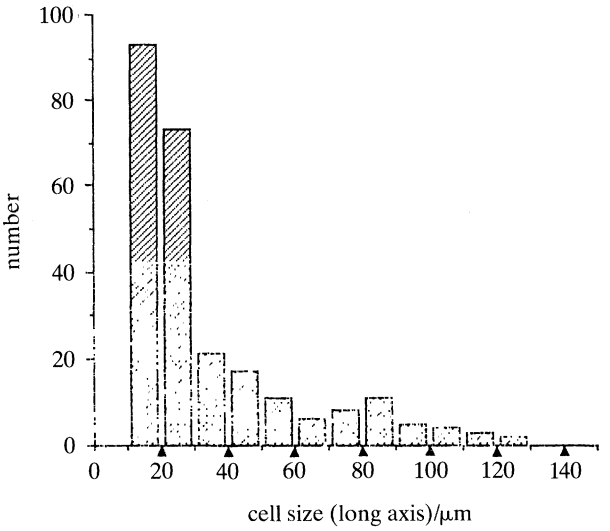

Figure 2. Histogram of the cell size (long axis) distribution in the ciliary ganglion.

Following HRP injection into the lens muscle, the large cells (greater than 50 μm) were labelled retrogradely (figure 1*b*). The average number of the labelled cells is about 50. They were spherical in shape.

(*c*) *The EW nucleus of the filefish*

After HRP injection into the ciliary ganglion, a discrete cell group was retrogradely labelled in an area rostrodorsal to the somatic oculomotor nuclei in the ipsilateral midbrain (figure 3). Labelled cells were not observed in the contralateral side. Schematically, the EW nucleus is shaped to a disk flattened dorsoventrally. The long axis of the EW nucleus was about 250 μm and the short axis was about 100 μm in the horizontal section (figure 4).

The EW nucleus comprises about 90 HRP labelled cells, which are oval, spindle-shaped or multipolar (figure 5). Mean area of the EW cells ($n = 88$) measured from the preparation, was about 400 (396 ± 11.8) μm^2. The somatic oculomotor cells were oval, rather than spindle-shaped. Mean size of the HRP labelled cells ($n = 66$) of the somatic nucleus for the inferior rectus, was about 500 (513 ± 19.2) μm^2. Thus the visceral EW cells were slightly smaller than the somatic oculomotor neurons.

The relative position of the EW nucleus in the oculomotor complex (including the trochlear nucleus) is schematically shown in figure 6. The EW nucleus lies in the most dorsal, rostral, and lateral part of the oculomotor complex. It was found that the oculomotor complex of the filefish was composed of one visceral (EW) nucleus and five somatic nuclei, i.e. the nuclei for the inferior rectus, medial rectus, superior rectus, inferior oblique and the trochlear nucleus for the superior oblique muscle. The nucleus for the

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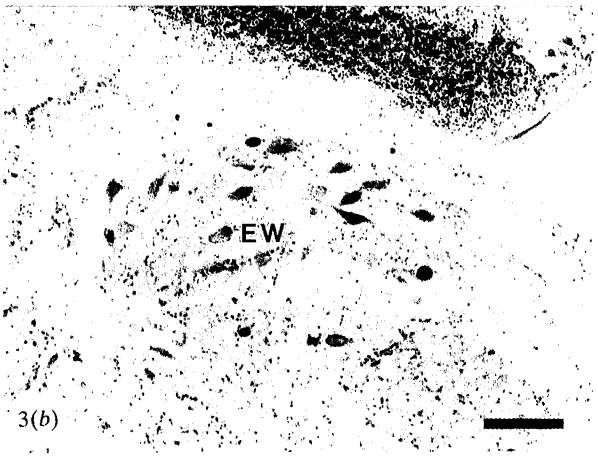

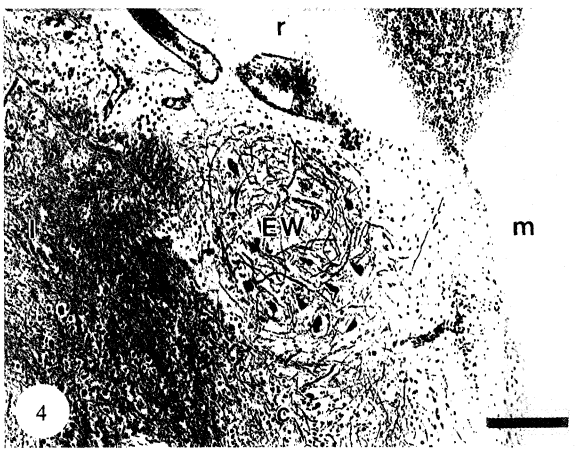

Figure 3. (*a*) Transverse section of the brain at the level of the EW nucleus. Small rectangle encloses a region of the EW nucleus shown in (*b*). Scale bar, 200 μm. (*b*) Transverse section of the EW nucleus showing HRP-positive cells retrogradely labeled from the ciliary ganglion. Scale bar, 50 μm. EW, Edinger–Westphal nucleus.

Figure 4. Horizontal section of the EW nucleus, stained with the Bodian–Otsuka method. Scale bar, 50 μm. EW, Edinger–Westphal nucleus; c, caudal direction; l, lateral direction; m, medial direction; r, rostral direction.

Figure 5. HRP-labeled neurons in the EW nucleus. Scale bar, 10 μm.

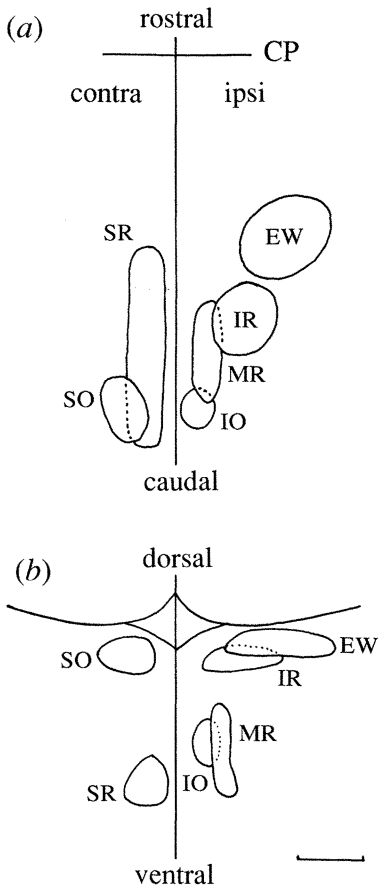

Figure 6. Schematic representation of motor neuron distribution in the oculomotor complex (including the trochlear nucleus) of the filefish representing horizontal (*a*) and coronal (*b*) sections. Lateralities are drawn for projections to the right eye. The oculomotor complex was composed of one visceral (EW) nucleus and five somatic nuclei, i.e. the nucleus for the inferior rectus (IR), the medial rectus (MR), the superior rectus (SR), the inferior oblique (IO) and the trochlear nucleus for the superior oblique muscle (SO). Scale bar, 300 μm. CP, Posterior commissure.

inferior rectus was the closest somatic nucleus to the EW nucleus.

(*d*) *Afferent connections to the EW nucleus*

After HRP injections into the EW nucleus, about 50 cells in the contralateral nucleus of the posterior commissure were retrogradely labelled (figure 7*a*,*b*). The labelled cells were spindle-shaped, and the soma size was about 10 μm × 20 μm (figure 7*c*). An HRP injected area is shown in figure 8.

4. DISCUSSION

(*a*) *Relative size of the lens muscle*

Measurement of the area percentage of muscle/lens (M/L%) is very useful in estimating and comparing the power of accommodation in different kinds of fish species of different size (Somiya 1987). The M/L% of several teleosts examined are as follows: bass (*Dicentrarchus*), about 20%; ayu (*Plecoglossus*), 10%; mackerel (*Scomber*), 6%; goldfish (*Carassius*), 2%; mullet (*Mugil*), 2% (Somiya 1987). The present study showed that the M/L% of the filefish is about 27%. This indicates that the filefish belongs to a fish species with the most powerful accommodation ability. Indeed, the filefish can move the lens for the accommodation about 0.5 mm in response to the electrical stimulation (lens diameter, 5.3 mm) (Somiya & Tamura 1973).

(*b*) *Accommodation reflex in teleost*

Using HRP and electrophysiological methods, Wathey (1988*a*,*b*) found the teleost EW nucleus and established the efferent link of the teleost accomodation reflex. That is, the teleost EW nucleus projects to the ipsilateral ciliary ganglion cells, and in turn, the post-ganglionic neurons innervate the lens muscle. Our results confirmed this efferent link of the accommodation reflex in the filefish.

It is found by the present experiments that cells in the nucleus of the posterior commissure project to the contralateral EW nucleus. It is well established that the nucleus of the posterior commissure receives direct retinal projections in teleosts (Vanegas & Ito 1983; Ito *et al.* 1984). Therefore, the cells in the nucleus of the posterior commissure are the first central neurons in the afferent link for the visual accommodation reflex in teleosts.

The hypothetical accommodation reflex in teleosts is shown in figure 9. Some 'signals' for visual accommodation may drive the specific retinal ganglion cells which project to the contralateral nucleus of the posterior commissure. The nucleus of the posterior commissure of the filefish receives two kinds of retinofugal fibres, fine (about 0.8 μm in diameter) and medium (about 1.3 μm) (Ito *et al.* 1984). Because the rate of accommodation is almost 10 times faster than human accommodation (Campbell & Westheimer 1960; Sivak & Howland 1973), the larger medium fibres may mediate the information for visual accommodation from the retina to the nucleus of the posterior commissure.

In terms of the cell number contributing to the teleost accommodation reflex, the present study indicates the following. About 50 cells in the nucleus of the posterior commissure project to the contralateral EW nucleus mainly through the posterior commissure. Then, about 100 cells of the EW nucleus relay the signal to the ipsilateral ciliary ganglion cells. Finally, about 50–100 large type ganglion cells control the lens muscle contraction.

(*c*) *Comparative aspects of the EW nucleus and its afferent connection for visual accommodation*

The existence of the visceral nuclei of the oculomotor complex was first reported in man by Edinger (1885), and Westphal (1887) first suggested its pupillary function from his clinical studies. Now the EW nucleus has generally been considered to be the nucleus of origin of the parasympathetic preganglionic fibres to the ciliary ganglion which in turn innervate the ciliary and pupillary muscles (Miller 1985).

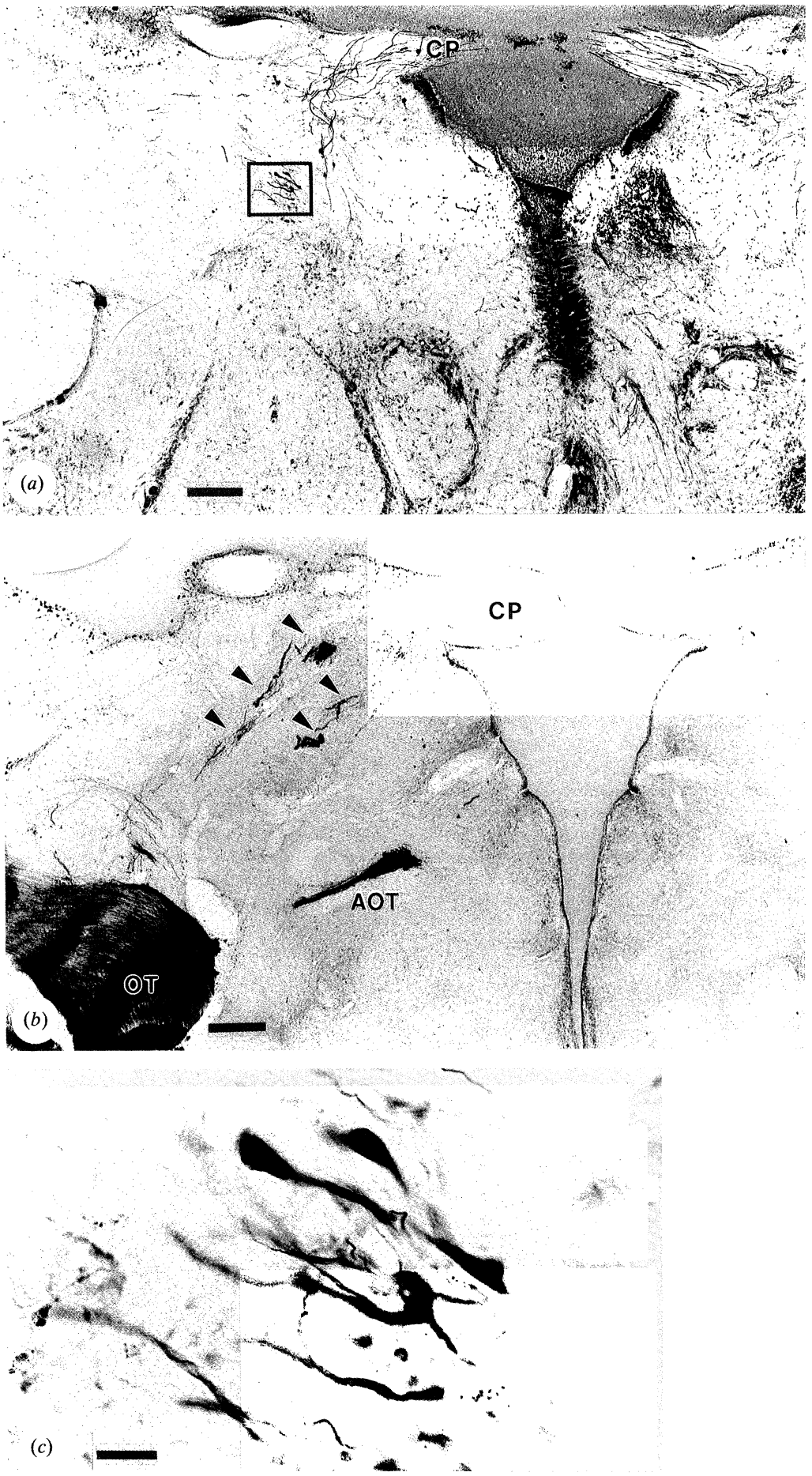

Figure 7. For description see opposite.

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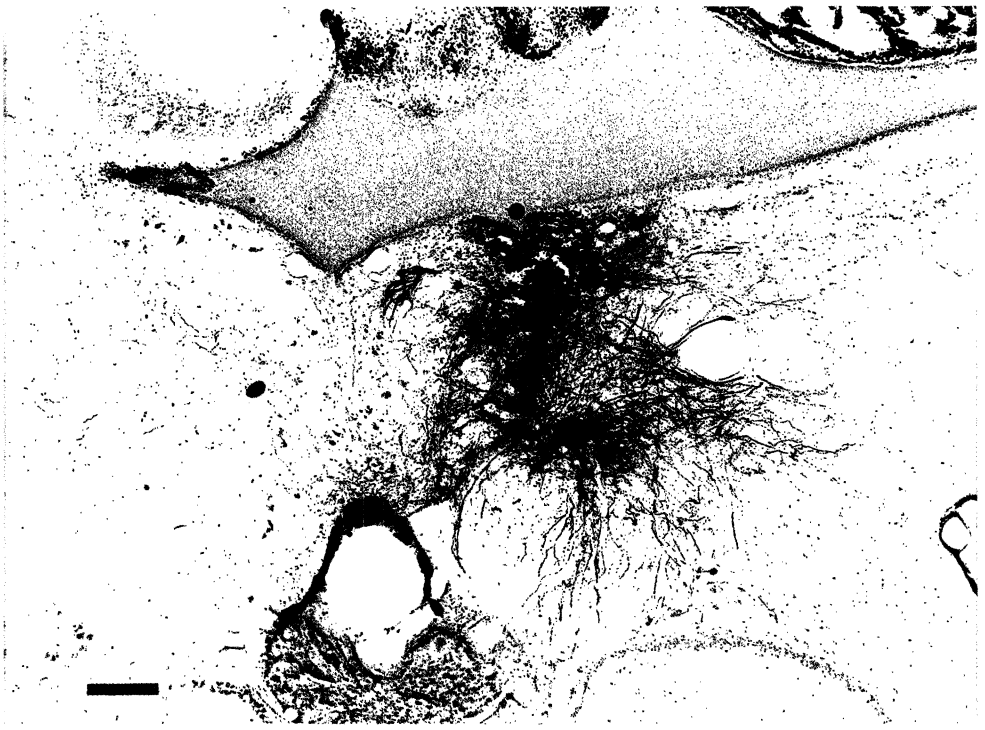

Figure 8. Transverse section of the brain showing an HRP injected area. The injection included the EW nucleus. Scale bar, 200 μm.

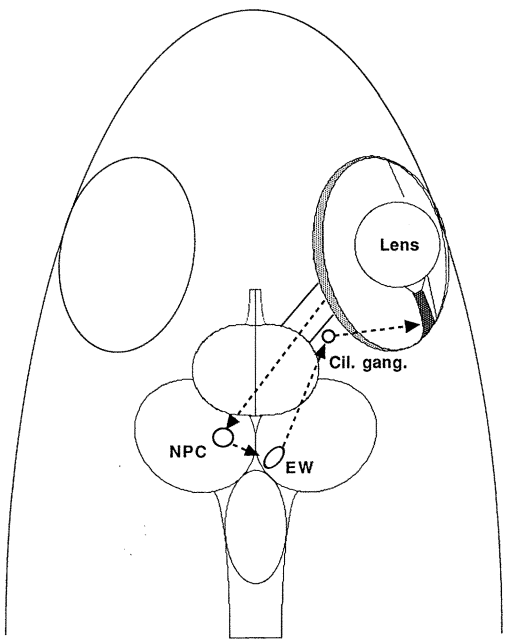

Figure 9. Hypothetical scheme for the central pathway of the teleost accommodation reflex. Cil.gang., ciliary ganglion; EW, Edinger–Westphal nucleus; NPC, nucleus of the posterior commissure.

Although the EW nucleus or its homologue has been described and assumed in many vertebrates classes, from elasmobranchs to mammals (Wathey 1988*b*), a precise concept of the EW nucleus has not been elucidated throughout vertebrates. To understand the specific properties of the teleost EW nucleus we compare here the structural organization of the teleost EW nucleus to that of mammals.

The oculomotor complex contains both somatic and visceral components. There are four somatic oculomotor nuclei in the complex which control four eye muscles respectively. The relative position of the four somatic nuclei is basically similar throughout osteognathostome (jawed) vertebrates (from bony fish to mammals) (Fritzsch *et al.* 1990). By contrast, the structural organization of the mammalian visceral nucleus of the oculomotor complex is quite different from that of teleosts. First, the teleost EW nucleus is a compact structure without any subdivision, whereas the EW complex in monkey is composed of at least three elements: the anteromedian nucleus, nucleus of Perlia, and EW nucleus (Warwick 1954; Carpenter & Peter 1970/71; Akert *et al.* 1980; Burde & Loewy 1980; Burde 1983). Secondly, cells in the teleost EW nucleus project mainly to the ciliary ganglion but the neurons

Figure 7. (*a*) Transverse section of the brain at the level of the nucleus of the posterior commissure. The small rectangle encloses a region of the nucleus of the posterior commissure shown in (*c*). Scale bar, 200 μm. (*b*) Transverse section of the brain showing the retinal projection to the nucleus of the posterior commissure. Arrows indicate the retinofugal fibres and terminal portions in the nucleus of the posterior commissure. Scale bar, 200 μm. (*c*) Enlarged photograph of a part of the nucleus of the posterior commissure showing HRP-positive cells retrogradely labeled from the EW nucleus. Scale bar, 10 μm. AOT, retinofugal terminal portion in nucleus of accessory optic tract; CP, posterior commissure; OT, optic tract.

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in the cat EW complex project mainly to the spinal cord, caudal trigeminal nucleus, cerebellum and several other brain stem nuclei (Loewy *et al.* 1978; Sugimoto *et al.* 1978). It is known in cat that afferents to the ciliary ganglion are primarily outside the EW and the anteromedian nucleus (Toyoshima *et al.* 1980). Thus recent studies show that the organization of the mammalian EW and the anteromedian nucleus is more complex than was originally thought.

The mammalian EW nucleus and its associated visceral nucleus may thus have a much more complex function than the teleost EW nucleus. Indeed, teleosts have neither a consensual pupillary reflex nor the 'near reflex' which involves a triad of processes consisting of accommodation, convergence and pupillary constriction. Furthermore, the pupillary mechanism in teleosts is quite different from that of mammals. In teleosts, the parasympathetic element of the oculomotor innervates the dilator muscle, while the sympathetic nerve innervates the constricting sphincter muscle (Young 1931, 1933; Nilsson 1983; Somiya 1987). It is also reported that the dilator muscle has no parasympathetic innervation in cod (Nilsson 1980). Possibly, the oculomotor (parasympathetic) constricting system which might be 'discovered' in ancestral land vertebrates, may have changed the simple EW nucleus into a more complex structure in the course of evolution.

In this paper, we showed that the retinorecipient cells in the nucleus of the posterior commissure send fibres for accommodation to the contralateral EW nucleus. Recently, Gamlin & Reiner (1991) suggested that cells in the medial and lateral mesencephalic reticular formation may be the source of afferents to the EW nucleus for accommodation in pigeon. They also consider that the avian lateral mesencephalic reticular formation may be comparable to the pretectal region which includes the nucleus of the posterior commissure in mammals. However, it should be noted here that the teleost nucleus of the posterior commissure is a direct retinorecipient nucleus (Ito *et al.* 1984), while the mammalian nucleus of the same name does not receive retinal projections (Berman 1977). In monkey, the pretectal olivary nucleus receives retinal inputs and sends fibres to the nucleus of the posterior commissure which in turn projects to the EW nucleus (Carpenter & Pierson 1973; Pierson & Carpenter 1974). In cats, the pretectal olivary nucleus is retinorecipient and sends signals for pupil constriction to the contralateral EW nucleus (Distler & Hoffmann 1989). Therefore, it is possible that the teleost nucleus of the posterior commissure contains two mammalian components, i.e. the pretectal olivary nucleus and the nucleus of the posterior commissure. HRP injection into the nucleus of the posterior commissure in teleosts will be the subject of future research to understand the basic plan of the vertebrate accommodation reflex.

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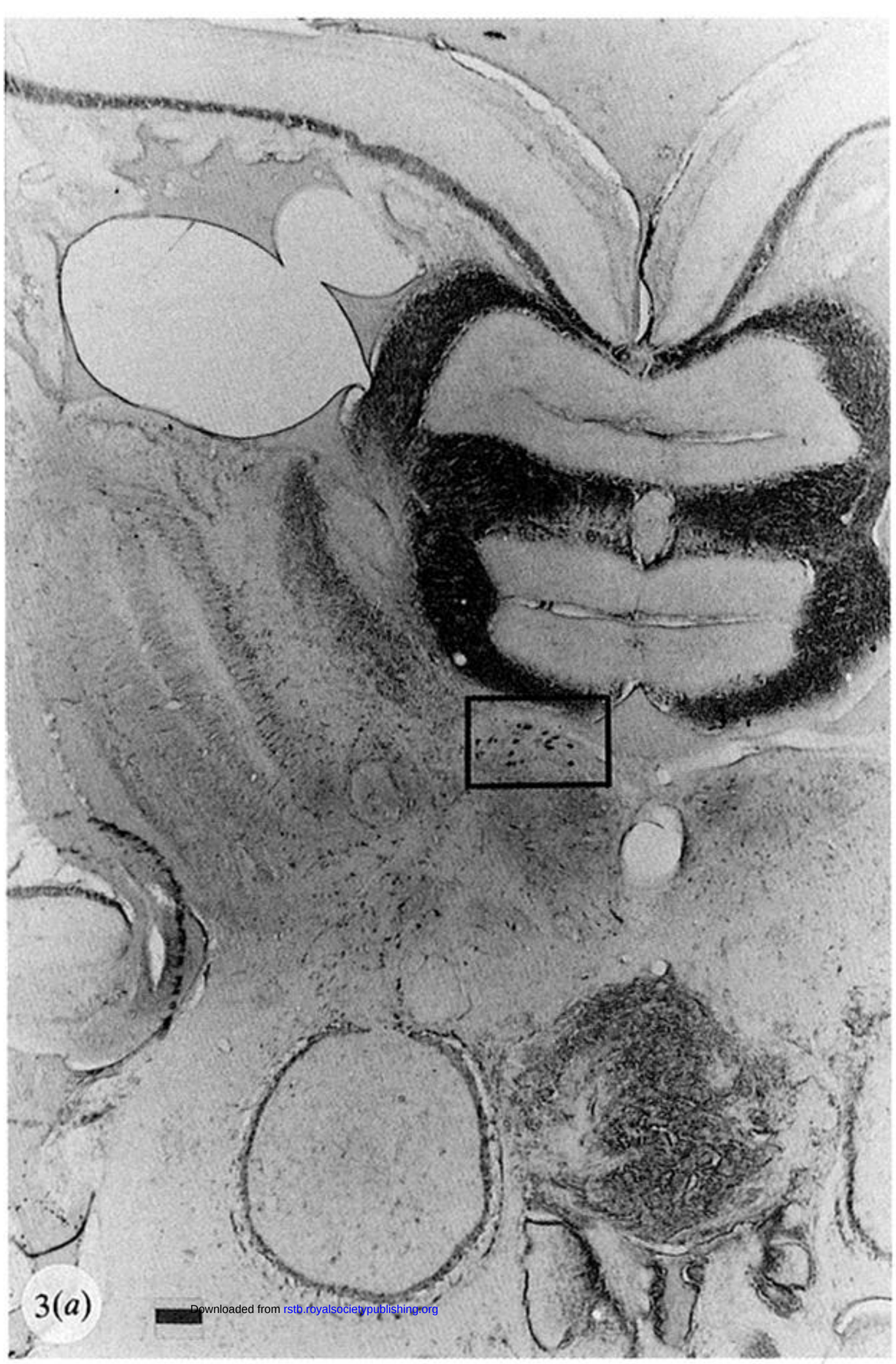
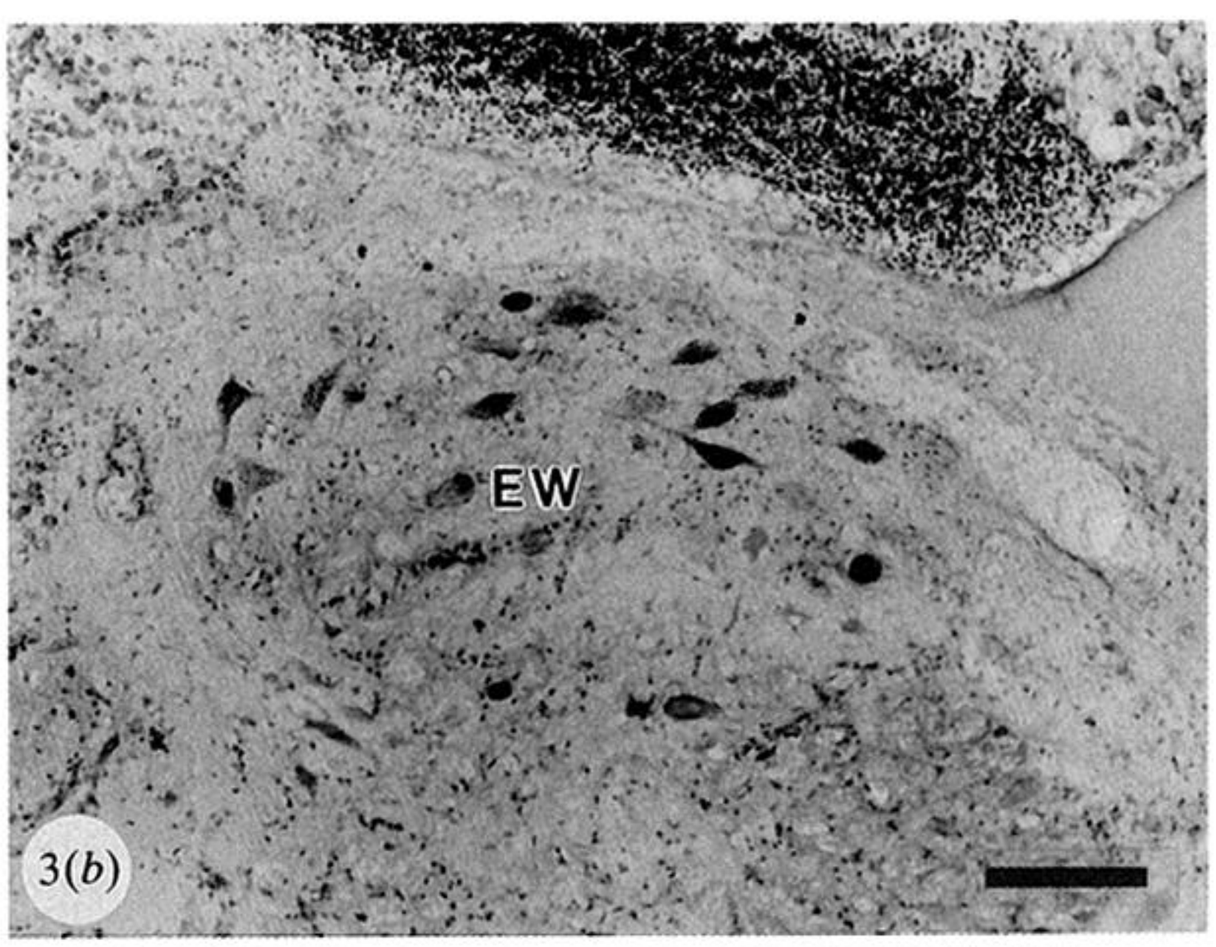
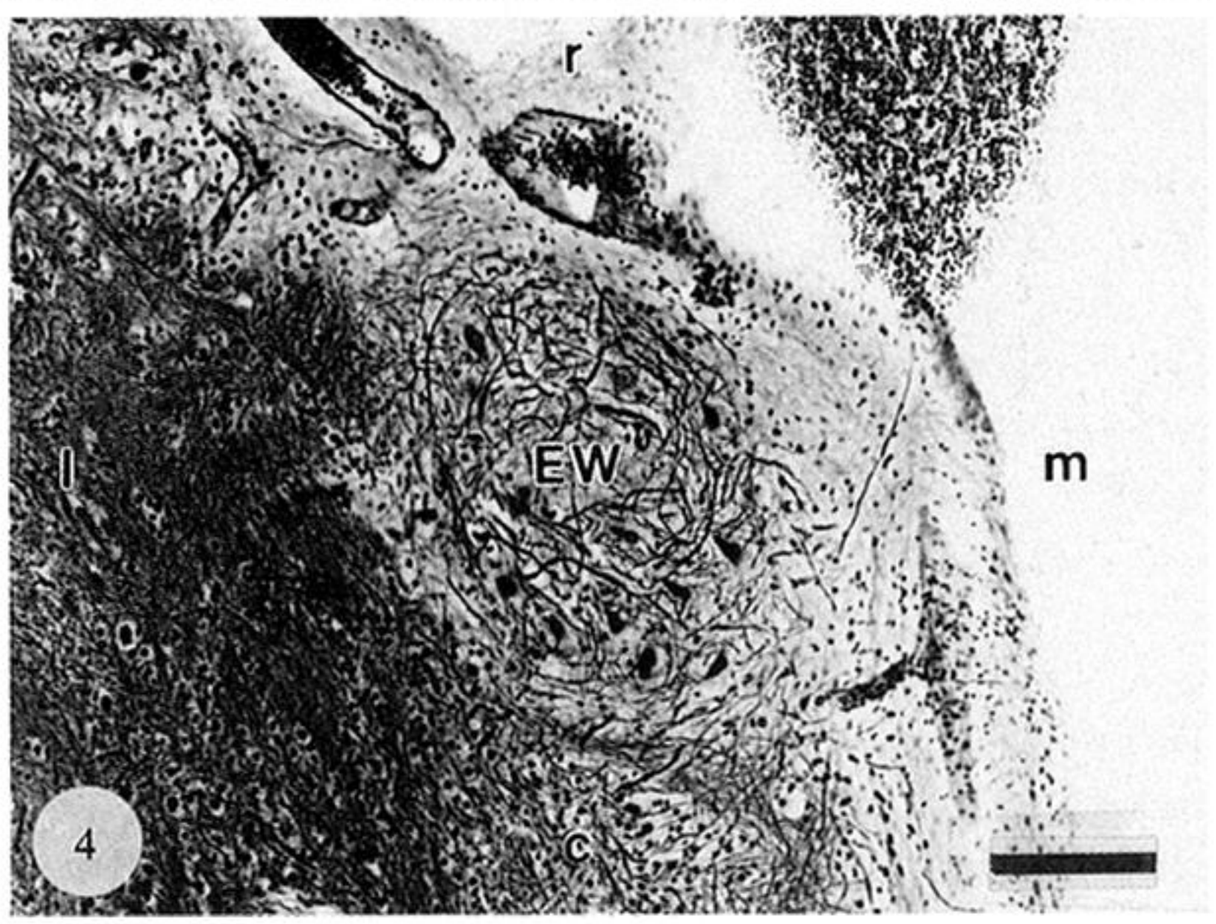
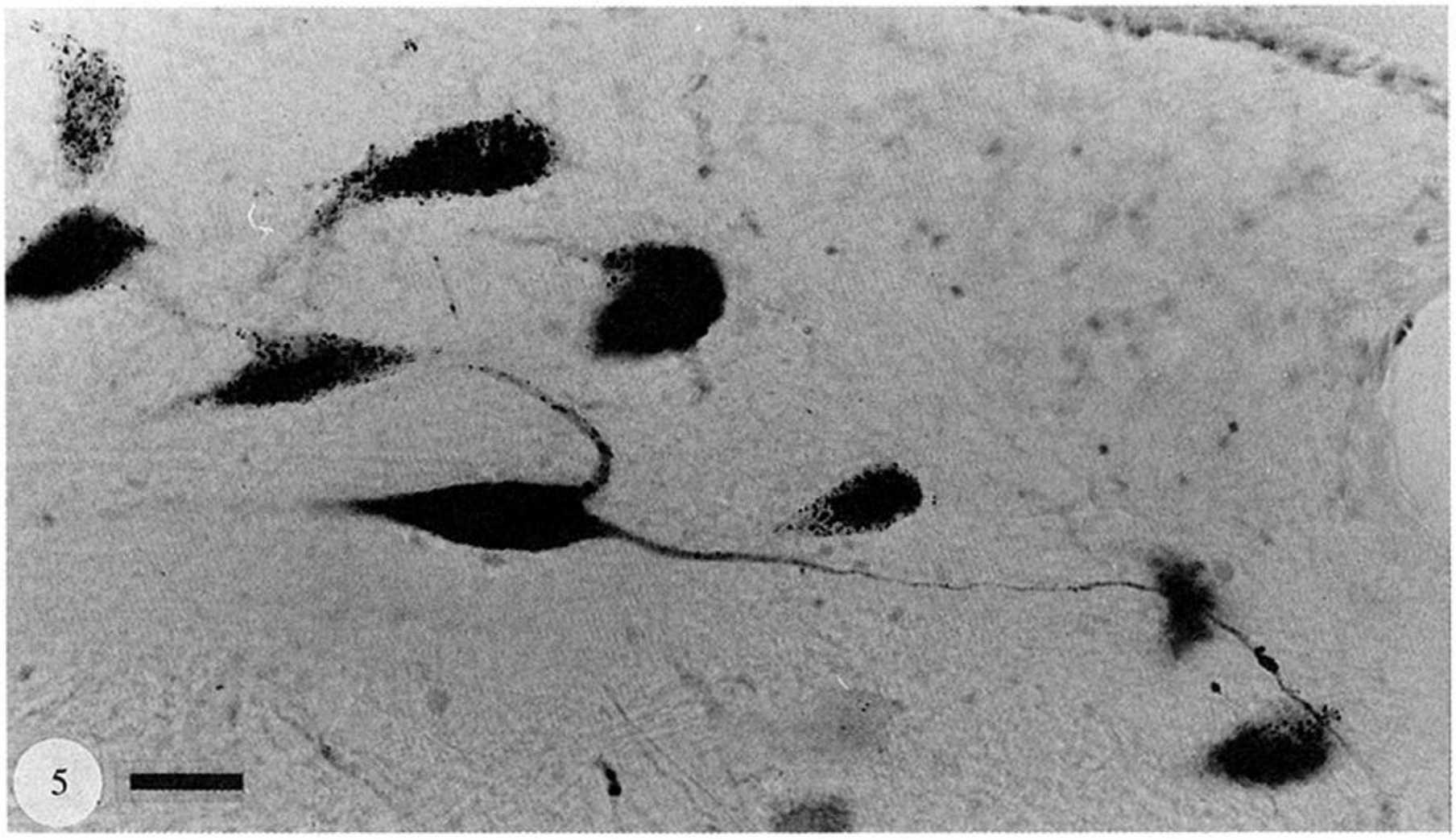
igure 1. (*a*) Thick (1 micron) epoxy section of the ciliary ganglion stained with toluidine blue. Scale bar, 100 μm. *b*) Enlarged photograph of the large ganglion cells which are HRP-positive. Scale bar, 10 μm. Large arrow ndicates the large ganglion cell. Small arrow indicates the small ganglion cell.





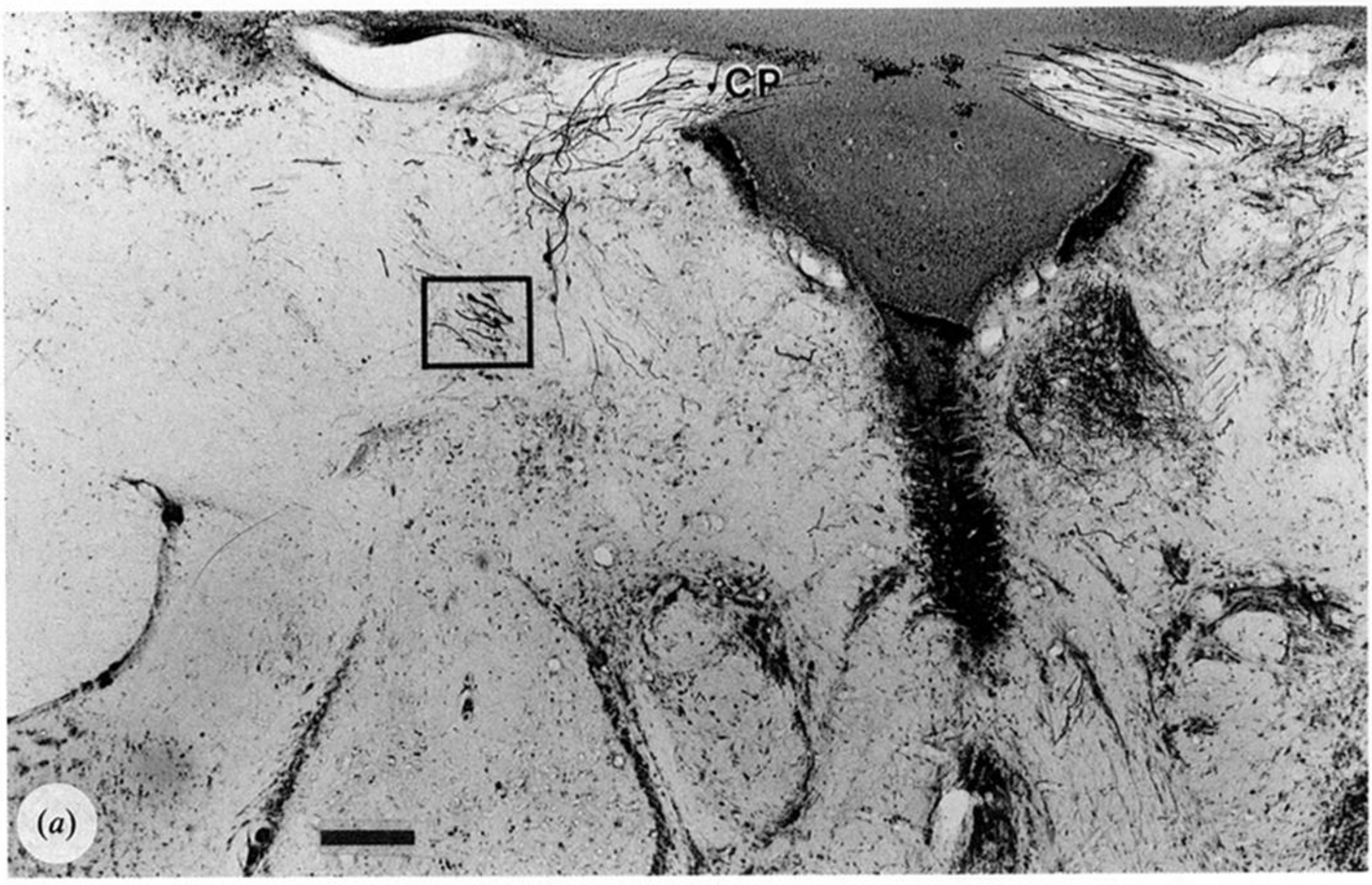


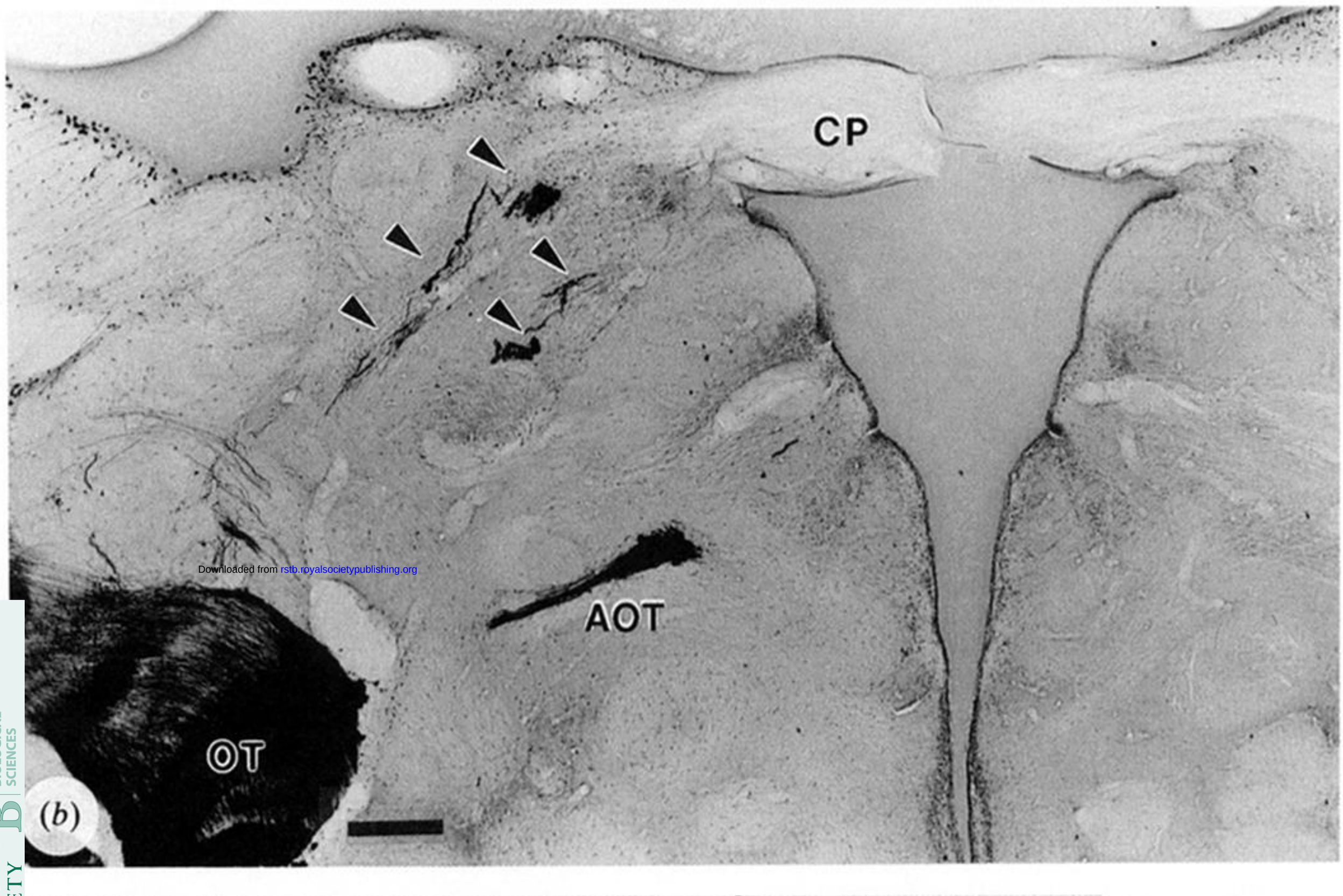


igure 3. (*a*) Transverse section of the brain at the level of the EW nucleus. Small rectangle encloses a region of the W nucleus shown in (*b*). Scale bar, 200 µm. (*b*) Transverse section of the EW nucleus showing HRP-positive cells trogradely labeled from the ciliary ganglion. Scale bar, 50 µm. EW, Edinger–Westphal nucleus.

igure 4. Horizontal section of the EW nucleus, stained with the Bodian–Otsuka method. Scale bar, 50 µm. EW, dinger–Westphal nucleus; c, caudal direction; l, lateral direction; m, medial direction; r, rostral direction.

igure 5. HRP-labeled neurons in the EW nucleus. Scale bar, 10 µm.





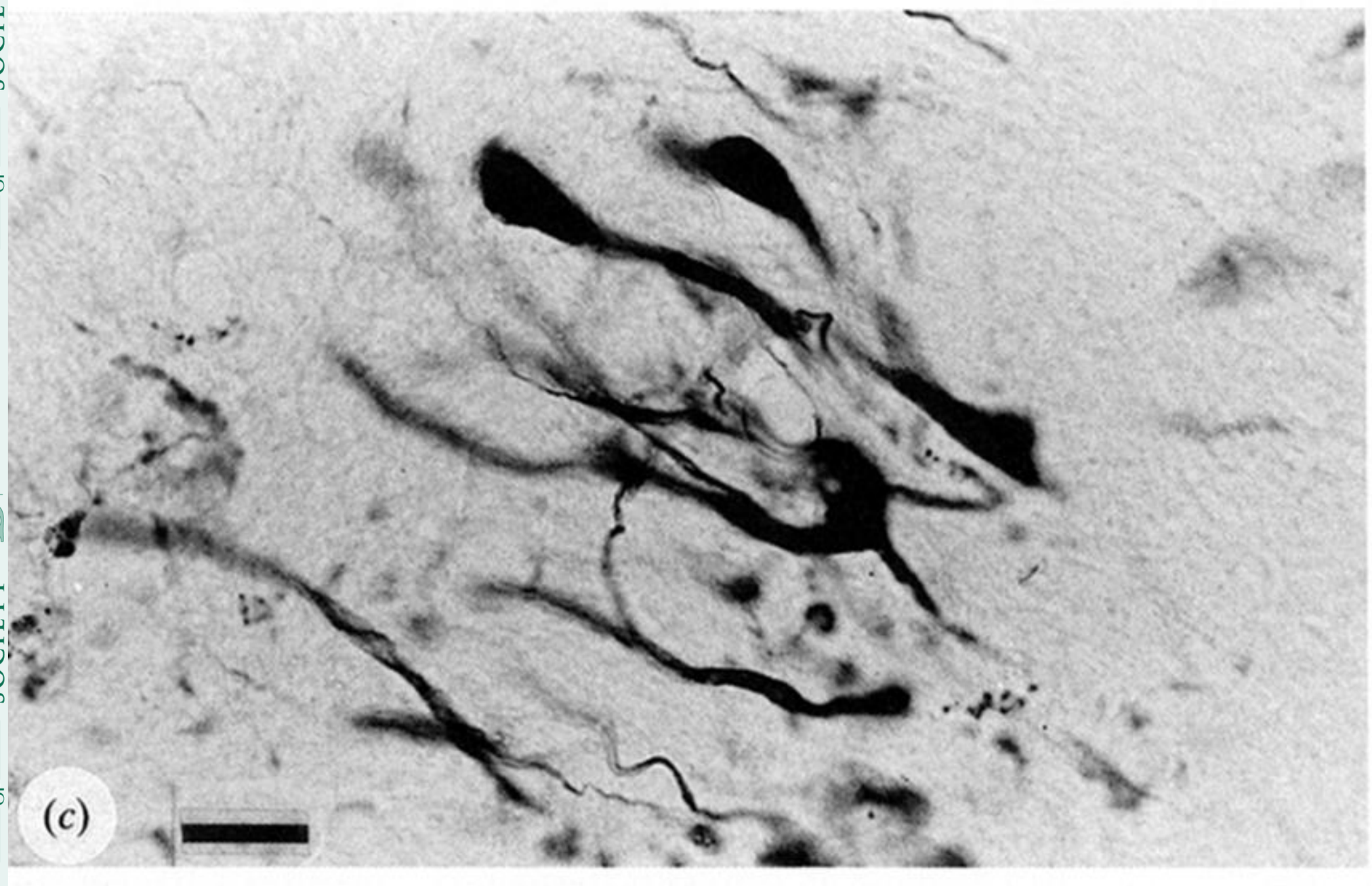


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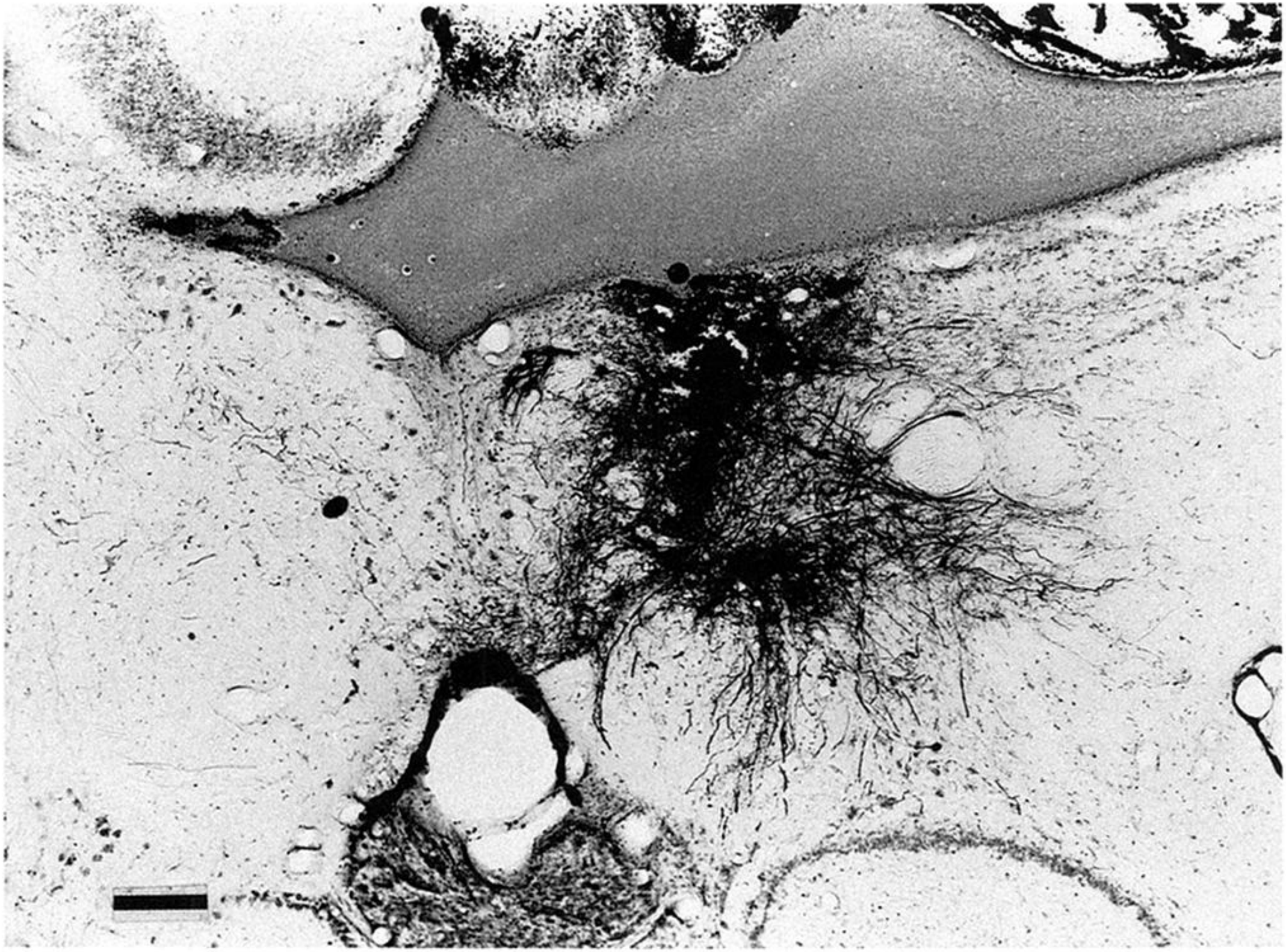
Figure 7. For description see opposite.



gure 8. Transverse section of the brain showing an HRP injected area. The injection included the EW nucleus. Scale bar, 200 μm.

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