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The Localization of the Ocular Parasympathetic Preganglionic Neurons and the Efferents from the Edinger-Westphal Nucleus to the Spinal Cord in the Rat: an HRP Study

Tetsuji MIURA* and Hajime SATOMI**

*Undergraduate Student** *and Department of Anatomy (Section 1)***
Sapporo Medical College, Sapporo, Japan
(***Chief*: *Prof. K. Takahashi*)

The localization of the ocular parasympathetic preganglionic neurons and the efferents from the Edinger-Westphal (EW) nucleus to the spinal cord in the rat were investigated by means of the horseradish peroxidase (HRP) method. The ocular preganglionic neurons, which were identified after HRP bathing of the oculomotor nerve, were located in the ventral part of the EW nucleus, the mesencephalic tegmental area immediately ventral to central gray and the medial-most part of the chief oculomotor nuclei. The finding that substantial numbers of EW neurons were labeled after HRP injection into the spinal cord confirmed the existence of EW-spinal projections in the rat. Moreover, the ocular parasympathetic preganglionic neurons in the EW nucleus and the EW-neurons projecting to the spinal cord seemed to be independent of each other due to their complementary distribution. (Received September 4, 1979 and accepted September 28, 1979)

1 Introduction

It is established that pupillary constriction and lens accommodation are mediated by parasympathetic nerves from the ciliary ganglion, to which preganglionic fibers are carried in the oculomotor nerve. However, the localization of the ocular parasympathetic preganglionic neurons still remains controversial. Although pronounced chromatolysis in the Edinger-Westphal (EW) nucleus after ciliary ganglionectomy was reported in the rhesus monkey[1], leading to the general assumption that the EW nucleus was the nucleus of origin of the preganglionic fibers to the ciliary ganglion[2,3], the same experimental procedure in the cat and the rabbit resulted in only equivocal evidence[4~6].

Recently, using the horseradish peroxidase (HRP) method, the ocular preganglionic neurons in the cat were reported to localize not only in the EW nucleus but in the rostromedial tegmental area and the central gray surrounding the oculomotor nuclear complex[7,8]. In addition, efferent projection from the EW nucleus to the spinal cord[7,9~11] and the cerebellum[11] were demonstrated by means of the HRP method. In the rat, however, it was reported that the preganglionic neurons located in the rostro-medio-ventral portion of the chief oculomotor nucleus but not in the EW nucleus[12].

In the present study, we attempted to identify the localization of the ocular parasympathetic preganglionic neurons in the rat by the HRP bathing method[13] and to confirm the efferents from the EW nucleus to the spinal cord by HRP injection into the spinal cord. From these experiments the difference in the distribution of the preganglionic neurons between the rat

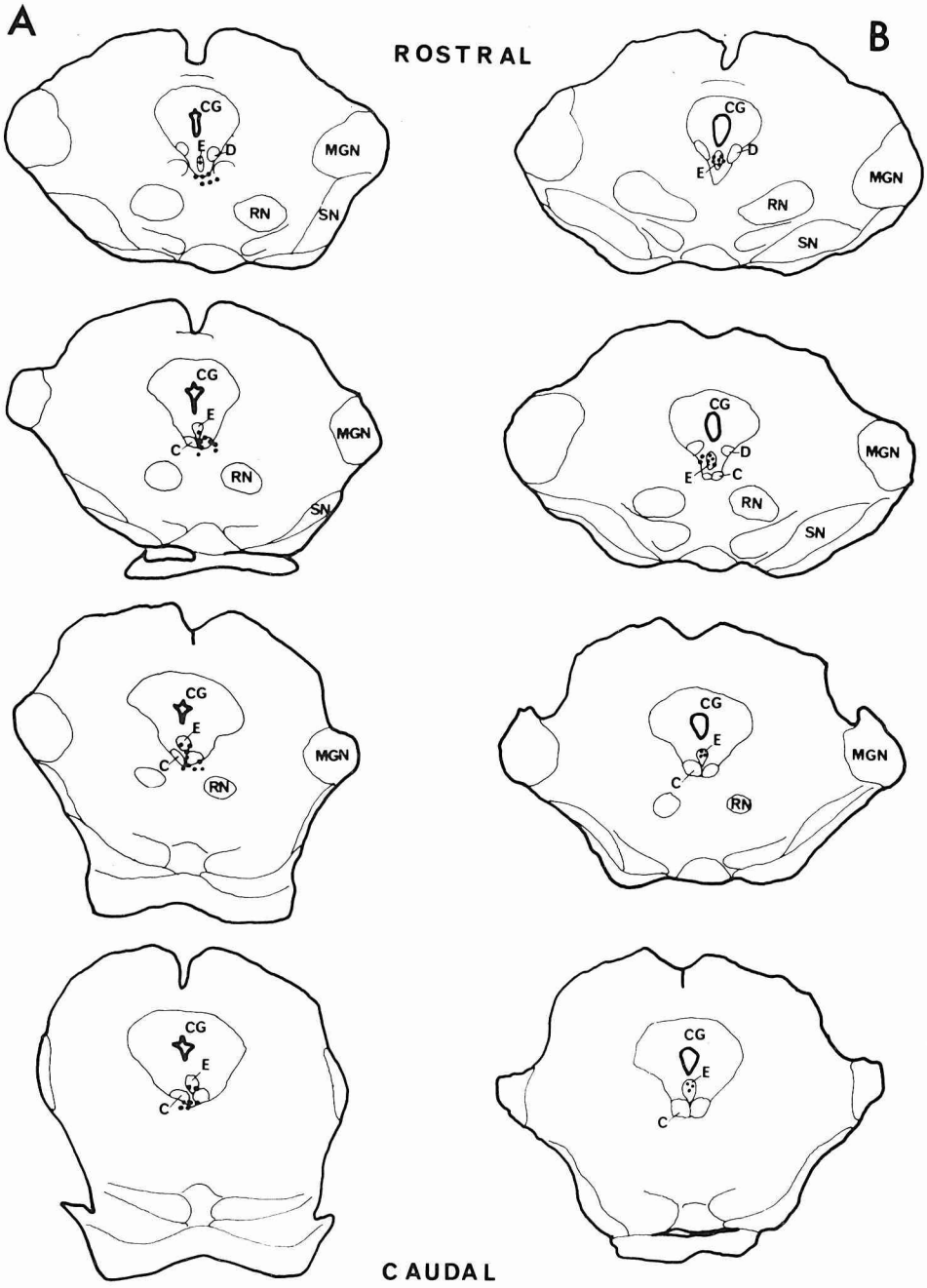

Fig. 1 A: Distribution of labeled neurons (dots) which were fusiform and small-sized in the oculomotor nuclear complex and its neighboring tegmental area after HRP bathing of the left oculomotor nerve. B: Distribution of labeled neurons (dots) in the oculomotor nuclear complex after HRP injection into L_5–L_6 spinal segments. Abbreviations: D, nucleus of Darkschewitsch; E, Edinger-Westphal nucleus; C, chief oculomotor nucleus; MGN, medial geniculate nucleus; CG, central gray; RN, red nucleus; SN, substantia nigra.

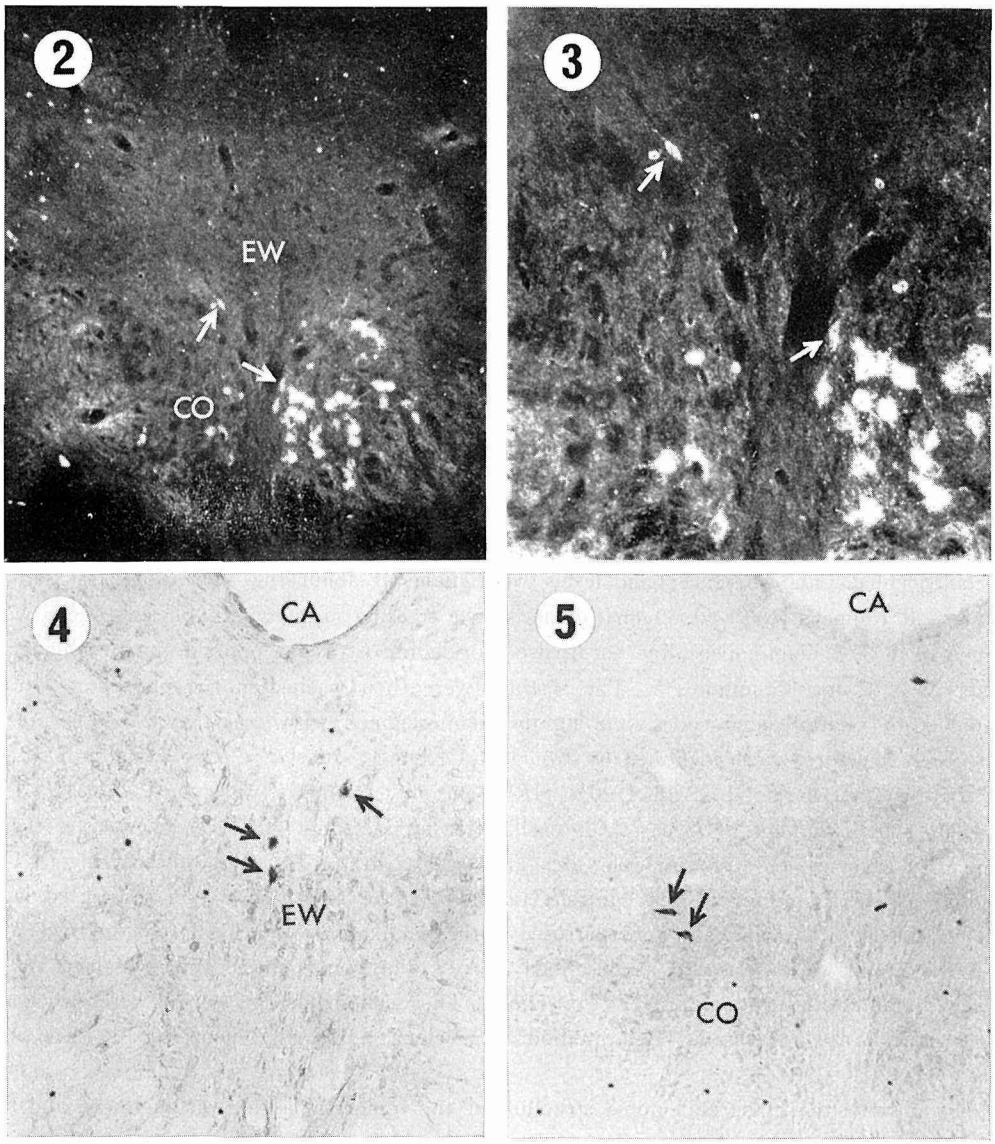

Fig. 2 A dark-field photomicrograph of a frontal section through the oculomotor nuclear complex. Arrows indicate the position of retrogradely labeled neurons in the EW nucleus after HRP bathing of the left oculomotor nerve. ×60. CO, chief oculomotor nucleus; EW, Edinger-Westphal nucleus.

Fig. 3 A higher magnification of Fig. 2. HRP-labeled neurons (arrowed) are small, fusiform and quite distinct from the large neurons labeled in the chief oculomotor nuclei. × 146.

Fig. 4 A photomicrograph of a frontal section through the oculomotor nuclear complex. Arrows indicate HRP-labeled neurons in the EW nucleus after HRP injection into left L_5–L_6 spinal segments. Neutral red staining. ×97. EW, Edinger-Westphal nucleus; CA, cerebral aqueduct.

Fig. 5 A photomicrograph of a frontal section through the oculomotor nuclear complex. Arrows indicate neurons labeled in the chief oculomotor nucleus after HRP injection into left C_2–C_3 spinal segments. Neutral red staining. ×97. CO, chief oculomotor nucleus; CA, cerebral aqueduct.

and the cat and the relationship between the preganglionic neurons and EW-neurons projecting to the spinal cord are discussed.

2 Material and Methods

Two groups of experiments were carried out on 11 Wistar rats of both sexes (200～300 g). Animals were anesthetized with ketamine (200 mg/kg, i. m.).

In the first series of 5 rats, the left temporal part of the skull was removed and the frontal protion of the cerebrum just medial to the left orbit was sucked away. Extraocular muscles and an optic nerve were cut and reflected. The oculomotor nerve was then identified and followed proximally with removal of the cranial base over the nerve root. The oculomotor nerve was then transected and its proximal stump was bathed in 20% HRP (Toyobo; Grade I-C) saline solution filled in a small rubber bag, which was sealed with an adhesive, Aron-alpha (Sankyo). After a period of 24 hours survival, the animals were perfused transcardially with 0.9% saline, followed by 1% glutaraldehyde and 2% paraformaldehyde in Millonig's phosphate buffer (pH 7.35, 4°C). The brains were removed and stored overnight in the phosphate buffer containing 20% sucrose. The following day, serial frontal sections through the mesodiencephalic and mesencephalic levels were made at about the same angle as that in König and Klippel's atlas[14] at 60 μm thickness on a freezing microtome. These sections were incubated in 3, 3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide in the phosphate buffer at 37°C for 35 minutes[15]. The sections were then washed in the phosphate buffer, mounted on gelatin-coated slides, and lightly counterstained with cresyl violet. The nuclei and other structures were identified by König and Klippel's atlas[14].

In the other 6 rats, 1～2 μl of 30% HRP solution was injected into the C_2–C_3, Th_2–Th_3, or L_5–L_6 spinal segments, two cases for each, after laminectomy of correspondent vertebrae. The HRP was injected into multiple sites unilaterally with a Hamilton microsyringe. The diffusion of HRP injected to the contralateral half of the spinal cord was recognized later histochemically. The animals were perfused with the fixative after 2～3 day survival and then the brains were sectioned as described above. The spinal cords were sectioned transversely to ascertain the site of HRP injection. The sections in this series were processed histochemically by Mesulam's HRP method[16], in which tetramethyl benzidine is used as a chromogen.

To observe the cytoarchitectonic structure of the midbrain, a rat was sacrificed and the serial sections were stained by the Klüver-Barrera method.

3 Results

HRP bathing of the oculomotor nerve. The neurons labeled with HRP were observed in the bilateral chief oculomotor nuclei, the EW nucleus and the mesencephalic tegmental area adjacent to the ventrolateral part of central gray in all cases. In 2 cases labeled neurons also were found in the contraleteral trochlear nucleus. HRP-labeled neurons were rarely found in the nucleus of Darkschewitsch and the dorsal raphe nucleus.

In the rostral one-third of the chief oculomotor nuclei, the majority of the neurons in the ipsilateral nucleus were labeled with HRP, and in the caudal two-thirds HRP-labeled neurons were also found in the contralateral nucleus, mainly occupying the dorsomedial portion of

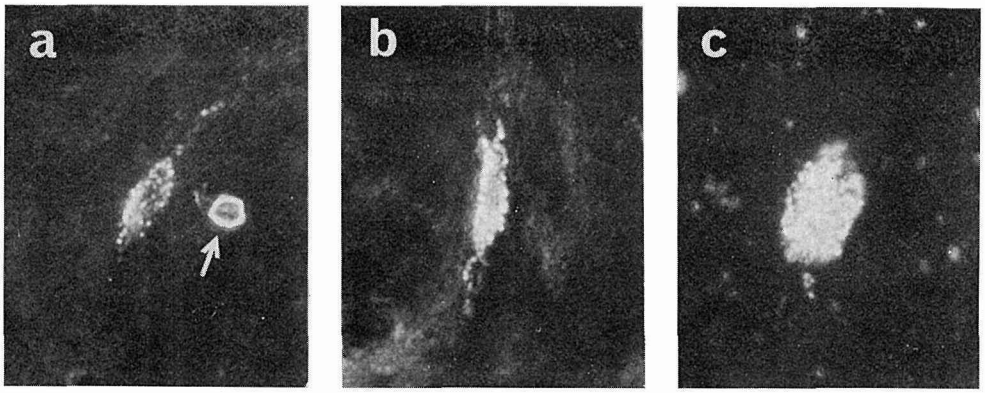

Fig. 6 Dark-field photomicrographs of HRP-labeled neurons. ×600. a: an EW-neuron labeled after HRP bathing of the oculomotor nerve. An arrow indicates a red blood cell. b: a neuron labeled in the tegmental area after HRP bathing of the oculomotor nerve. c: an EW-neuron labeled after HRP injection into L_5–L_6 spinal segments. The labeled neurons in the EW nucleus (a) and tegmental area (b) after the HRP bathing are small and fusiform. The EW-neuron labeled after the HRP injection (c) appears somewhat larger and rounder than the other two neurons.

the nucleus and increasing in number more caudally. The majority of the labeled neurons in the chief oculomotor nuclei were large (20～25 μm in diameter) and of motoneuron type. In the medial-most portion of the nucleus a few fusiform cells of small size (10～15 μm in diameter) were observed. Similar fusiform neurons of small size labeled with HRP were distributed in the EW nucleus (Figs. 2 and 3) and in the mesencephalic tegmental area immediately ventral to the central gray from the level of the rostral end of the EW nucleus to the caudal end of the red nucleus (Fig. 1A). The size and shape of the HRP-labeled EW-neurons were similar to those in the tegmental area (Figs. 6a and 6b).

In 2 cases, a part of the contralateral trochlear nucleus was labeled with HRP and this indicated that small amounts of HRP solution leaked out of the rubber bag and were taken up by the axons of the trochlear nerve. Nonetheless, the distribution of the HRP-labeled neurons in these cases was the same as that in the other cases except for the trochlear nucleus.

HRP injection into the spinal cord. In all cases, substantial numbers of neurons in the EW nucleus were labeled with HRP (Fig. 4). In the case of HRP injection into L_5–L_6 segments, up to 34 neurons were labeled. These neurons were observed throughout the rostrocaudal extent of the EW nucleus and appeared to locate the central part of the nucleus (Fig. 1B). The labeled neurons appeared somewhat larger and rounder than the EW-neurons labeled after HRP bathing of the oculomotor nerve (Fig. 6c). In addition, neurons about 20 μm in diameter in the chief oculomotor nucleus were, although a few, labeled with HRP in 3 cases (Fig. 5). In the tegmental area few neurons which corresponded to those labeled after HRP bathing of the oculomotor nerve were labeled after HRP injection into the spinal cord.

4 Discussion

The oculomotor nerves are believed to carry special somatic efferent (SSE), general visceral efferent (GVE), and general somatic afferent (GSA, proprioceptive) components. The GSA

component was recently suggested to be afferent fibers from the trigeminal ganglion to muscle spindles in the extraocular muscles[17]. Of the labeled neurons after HRP bathing of the oculomotor nerve, the majority of the neurons in the chief oculomotor nuclei were large and of motoneuron type and their distribution was very similar to those observed in the cat and the rabbit after HRP injection into the extraocular muscles[18,19]. Accordingly, these cells of motoneuron type were considered as the origin of the SSE component. As a result, the remaining HRP-labeled neurons which were fusiform and small-sized were probably those sending the GVE component. In other words, the ocular parasympathetic preganglionic neurons in the rat were assumed to locate in the ventral portion of the EW nucleus, the rostromedial tegmental area immediately adjacent to the mesencephalic central gray and the medialmost portion of the chief oculomotor nuclei. Sakai *et al.*[12] were not able to find the connection between the EW nucleus and the ciliary ganglia in the rat, based on the finding that after HRP injection in the location of the ciliary ganglion medium-sized spindle-shaped neurons in the rostro-medio-ventral portion of the chief oculomotor nucleus were labeled with HRP, but not those in the EW nucleus. In the present study the EW-neurons and the neurons in the tegmental area were labeled with HRP as well as the neurons in the chief oculomotor nuclei which seemed to correspond to those described by Sakai *et al.*[12] The discrepancy between our result and Sakai *et al.* 's may result from the different methods employed.

In recent studies in the cat, neurons in the EW nucleus, ventromedial tegmental area and rostroventral central gray were labeled after HRP incubation of the isolated preganglionic nerve to the ciliary ganglion[7] and after HRP injection into the proximal portion of the oculomotor nerve[8]. The labeled EW-neurons in these studies were located mainly in rostral and peripheral portion of the nucleus. Some electrophysiological studies[20,21] supported these areas as the source of the parasympathetic outflow. Our results in the rat were almost the same as the data in the cat but different in two points; firstly, we could not observe the HRP-labeled neurons in the central gray and secondly, the labeled neurons did not locate predominantly in the rostral portion of the nucleus, which appeared fewer than those in the cat. In this respect, it is interesting to note that in the rhesus monkey Warwick[1] found that most of the cells of the ipsilateral EW nucleus (pars caudalis) and of the ipsilateral half of the anteromedian nucleus (pars rostralis of the EW nucleus) were chromatolyzed after ciliary ganglionectomy or division of the oculomotor nerve. The proportion of the cells retrogradely degenerated in his experiment was less in the pars caudalis than in pars rostralis of the EW nucleus. These findings suggest that the preganglionic neurons may locate much more in the pars rostralis than in the pars caudalis in the monkey as in the cat and that the contribution of the EW nucleus to the GVE component may be larger in the monkey than in the cat in which EW-neurons degenerated equivocally after ganglionectomy[5,6] and only a small percentage of the neurons in the nucleus were labeled with HRP[7,8]. Generally the EW nucleus is described as well developed in higher animals[22], and ontogenetically there is a rostrocaudal gradient of differentiation in the oculomotor muclear complex[23]. From an electrophysiological study in the cat[20], it has been suggested that efferents from the pars rostralis of the EW nucleus controlled lens accommodation but not pupillary constriction. The differences in the contribution between the pars rostralis and the pars caudalis among these three species — the monkey, the cat and the rat, might result from the different process of development of the

oculomotor nuclear complex and relate how well accommodation function works in the species.

The findings that substantial numbers of EW-neurons were labeled after HRP injection into the spinal cord in the present study confirmed the existence of efferents from the EW nucleus to the spinal cord as far caudally as L_5-L_6 segments in the rat. The labeled neurons in the EW nucleus appeared larger and rounder than those after HRP bathing of the oculomotor nerve and seemed to locate in the central portion of the nucleus. The distribution of the labeled neurons after the HRP bathing and after the HRP injection appeared to complement and not to overlap. Therefore, it is unlikely that EW-spinal projection and the GVE component in the oculomotor nerve originate in the same neurons although it is conceivable that most of the preganglionic neurons may remain unlabeled under the condition of HRP bathing of the oculomotor nerve.

A few neurons in the chief oculomotor nuclei were also labeled after HRP injection into the spinal cord. The significance of these neurons is not clear at present. However, it might be possible that these neurons were dislocated EW-neurons projecting to the spinal cord, or some interneurons as those in the dorsolateral portion of the chief oculomotor nucleus projecting to the abducens nucleus in the cat[24].

In summary, the present study suggested that the ocular parasympathetic preganglionic neurons are located in the ventral part of the EW nucleus, the mesencephalic tegmental area ventral to central gray and in the medial-most portion of the chief oculomotor nuclei and that the ocular preganglionic neurons in the EW nucleus and EW-neurons projecting to the spinal cord may be independent of each other due to their complementary distribution.

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ラットにおける眼球への副交感性節前線維の起始細胞の局在と Edinger-Westphal 核より脊髄への投射について

—— HRP 法による検索 ——

三浦哲嗣*　里見肇**

札幌医科大学医学部学生*

札幌医科大学解剖学第1講座**(主任　高橋杏三教授)

要　約

ラットの眼球への副交感神経節前線維の起始細胞の局在と Edinger-Westphal (EW) 核から脊髄への投射系，およびこれらの関連を西洋ワサビペルオキシダーゼ (HRP) 法により検索した。

動眼神経根の近位断端をゴム袋中の HRP 液に浸漬することにより，副交感性と思われる HRP 陽性細胞が，EW 核の腹側部，中脳中心灰白質に近接する被蓋の部分，および動眼神経主核の最内側部に見出された。また脊髄の頸，胸，腰部への HRP 注射によって，EW 核のかなりの細胞が HRP でラベルされ，EW 核から脊髄への投射がラットにおいても確認された。EW 核内に存在する副交感性神経細胞と脊髄へ投射している細胞とは，その分布が相補的であり，動眼神経内の副交感性節前線維と，EW 核より脊髄への線維が同一の細胞から起始しているとは考え難い。