Distribution of Efferent Neurons Projecting to the Tectum and Cerebellum in the Rat Prepositus Hypoglossi Nucleus

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Distributions of efferent neurons projecting to the superior colliculus (SC), the pretectum, and the cerebellar cortex were studied in the rat prepositus hypoglossi nucleus (PRH) by retrograde labeling with wheat germ agglutinin-horseradish peroxidase (WGA-HRP). After injection of the tracer into the unilateral SC, small- and medium-sized neurons were labeled throughout the entire rostrocaudal extent of the PRH, with the highest proportion in the middle part of the nucleus. After application of WGA-HRP to the unilateral pretectal region, including the nucleus of the optic tract, numerous labeled neurons appeared in the bilateral PRH. The highest distribution was found in the most rostral part of this nucleus. Furthermore, small neurons of the supragenual nucleus of the facial nerve also were labeled. Injection of WGA-HRP into lobules VI–VII of the cerebellar vermis resulted in the labeling of mainly medium-sized neurons in the bilateral PRH, primarily in the caudal third. WGA-HRP-labeled neurons were less frequently observed in the PRH after unilateral injection into the flocculus and paraflocculus. Retrograde double labeling by fluorescent dyes showed that a few neurons in the middle part of the PRH sent divergent axons to vermal lobules VI–VII and the SC. Invest Ophthalmol Vis Sci 33:2567–2574, 1992

Since Graybiel and Hartwig1 first reported labeled neurons in the prepositus hypoglossi nucleus (PRH) after the injection of horseradish peroxidase (HRP) into the oculomotor nucleus, the PRH has been found to act as an important part of the brainstem circuitry that controls eye movements in relation to the cerebellar and vestibular systems.2-3 Neurophysiologic experiments have revealed that conversion of the velocity of eye movements to position information is performed mathematically by the process of integration, and that the neural integrator is located in a region that includes the medial vestibular nucleus and the PRH.4-5 Recent data have indicated that lesions in the brainstem region cause aberrant vestibular, optokinetic, saccadic, and pursuit eye movements.6

The efferent PRH projections to the cerebellum have been examined extensively.7-12 In monkeys, PRH neurons project axons as mossy fibers to the posterior vermis (lobules VI, VII, IX, and X) and to the flocculus and the paraflocculus. The heaviest projection found was to vermal lobule VII,9 and the presence of topographically organized projections from certain regions of the PRH to different cerebellar regions also has been noted.7,9

Stechison et al13 observed retrogradely-labeled neurons in contralateral PRH and in the nucleus intercalatus after wheat germ agglutinin (WGA)-HRP injection into the cat superior colliculus (SC). They found some topographic differences in the rostrocaudal distribution of labeled PRH cells after restricted collicular injection. The SC is considered to be a visuomotor integrator in the orienting reflex and in facilitating shifts in gaze.14-15 Furthermore, recent physiologic studies have disclosed that the nucleus of the optic tract, a component of the pretectal nuclear complex, may act as a visuomotor relay nucleus in the pathway mediating the optokinetic nystagmus.16-18 To understand the mechanisms of visuomotor control mediated by the PRH, it is necessary to study the intranuclear organization of the PRH neurons innervating the SC, the pretectal area, and the cerebellar cortex.

The purpose of the present study was to investigate the distribution and morphologic characteristics of ef-
ferent PRH neurons to the SC, the pretectal region, and the cerebellum. Furthermore, we also studied whether or not PRH neurons send collateral axons to the SC and the posterior vermal cortex.

**Materials and Methods**

The investigations in this report that used animals conformed to the ARVO Resolution on the Use of Animals in Research.

**Injection of WGA-HRP**

A total of 48 (Wistar) albino rats weighing 210–360 g were used in this study. Animals were anesthetized with sodium pentobarbital (30 mg/kg) and then fixed in a stereotaxic headholder. The SC, the pretectal area, the cerebellar vermis (lobules VI–VII), or the parafloccular and floccular cortices were surgically exposed. An aliquot (0.02–0.03 μl) of 10% WGA-HRP (Toyobo, Osaka, Japan) in physiologic saline was injected into one of the above-mentioned brain areas through a small glass micropipette connected to a 1 μl Hamilton microsyringe. At 2–3 d after the injection, the animals were deeply anesthetized and perfused transcardially with 200 ml of Ringer’s solution, followed by 500 ml of 1.0% paraformaldehyde and 1.25% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4). They were finally perfused with 500 ml of 10% sucrose in the same buffer. Both perfusates were cooled to 4°C before they were used. After perfusion, the brains were removed and placed in a 10% sucrose solution for 12 hr, after which they were cut into serial transverse sections with a thickness of 50 μm on a freezing microtome. Alternate sections were treated with tetrabenzyl benzidine (TMB), according to the method of Mesulam,19 and were counterstained with neutral red.

The proportion of labeled neurons in a given region of the PRH was determined as follows:

\[
\text{Number of labeled neurons} \times 100 \%
\]

\[
\text{Total number of labeled neurons in the PRH}
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The size of the labeled PRH neurons was determined from the long and short axes of randomly selected neurons filled with TMB reaction products.

**Injection of Fluorescent Tracers**

Two fluorescent dyes were used in this experiment—2% fast blue (FB; Sigma, St. Louis, MO) in distilled water and a 2% aqueous suspension of diamidino yellow dihydrochloride (DY; Sigma). Four adult Wistar rats weighing 250–300 g were anesthetized with sodium pentobarbital (30 mg/kg). After exposure of the SC by aspiration of the overlying cerebral cortex, unilateral injection of 2% FB (0.08–0.12 μl) was performed. Four days later, 0.08–0.12 μl of 2% DY was applied to the cerebellar vermis (lobules VI–VII). After 48 hr, the animals were deeply anesthetized and perfused transcardially with 200 ml of Ringer’s solution, followed by 500 ml of 4% paraformaldehyde in 0.1 mol/l phosphate buffer (pH 7.4, 4°C). The brains were stored for 24 hr in the same fixative with 10% sucrose at 4°C, then cut into serial transverse sections 30 μm thick. Every second section was mounted on gelatine-coated slides and air dried. The materials were examined with a Leitz (Heerburg, Switzerland) fluorescence microscope, equipped with a U-filter system that provided an excitation light of 369 nm. The extent of the injections and the distribution of labeled PRH neurons were examined with photomontages, with the locations of the labeled neurons plotted on the photomontages. For delineation of the brainstem structures, sections were stained with cresyl violet after the fluorescence examination.

**Results**

Unilateral injection of WGA-HRP into the SC revealed that labeled neurons were contralaterally distributed throughout the whole rostrocaudal extent of the PRH (Fig. 1), with the highest proportion in the middle part of the nucleus (Fig. 2). The tracer was widely deposited within the superficial and deeper layers of the SC. About 55% of the labeled PRH neurons had small oval cell bodies with long and short axes of 10–15 and 5–10 μm, respectively (Fig. 3B), and 45% of them were medium-sized neurons with elongated to polygonal cell bodies (long and short axes of 18–30 and 13–18 μm, respectively; Fig. 4A).

No large neurons with a long diameter of more than 35 μm were labeled in the PRH. In addition, a few labeled neurons were observed in the contralateral nucleus intercalatus (Fig. 3C), whereas no neurons were labeled in Roller’s nucleus. Nissl-stained preparations of the perihypoglossal nuclear complex revealed that the intercalatus was a small poorly developed parvicellular region between the hypoglossal nucleus and the dorsal motor nucleus of the vagus nerve. When the WGA-HRP-injected area extended to the deep collicular layers and invaded the dorsal to dorsolateral part of the central gray matter (Fig. 1), in addition to the labeling of PRH neurons, a considerable number of neurons were labeled in the supragenualis nucleus of the facial nerve (SG; Fig. 1). The SG was a collection of small cells that formed a rostral continuation of the PRH just dorsal to the genu of the facial nerve root. It consisted of relatively densely packed
small neurons with oval to fusiform cell bodies. Some reticular neurons just ventral to the PRH also contained TMB reaction products after injection of the SC (Fig. 1).

Unilateral WGA-HRP injection into the pretectal region, including the nucleus of the optic tract and the pretectal olivary nucleus, produced the bilateral labeling of PRH neurons (Fig. 5). The number of labeled PRH cells increased toward the more rostral part of the nucleus (Fig. 2). Furthermore, a large number of small neurons with oval to fusiform cell bodies. Some reticular neurons just ventral to the PRH also contained TMB reaction products after injection of the SC (Fig. 1).

Unilateral WGA-HRP injection into the pretectal region, including the nucleus of the optic tract and the pretectal olivary nucleus, produced the bilateral labeling of PRH neurons (Fig. 5). The number of labeled PRH cells increased toward the more rostral part of the nucleus (Fig. 2). Furthermore, a large number of SG neurons just dorsal to the genu of the facial nerve root were labeled on both sides (Fig. 3D).

Paramidline injection of the tracer into lobules VI–VII of the cerebellar vermis resulted in the labeling of a considerable number of neurons in the bilateral PRH (Fig. 6), with the highest proportion distributed in the caudal third of the nucleus (Fig. 2). Eighty two percent of the labeled PRH neurons were medium-sized cells with elongated to polygonal somata with long and short axes of 18–30 and 13–20 μm, respectively (Fig. 7A), while only 18% of them were small oval to ellipsoid neurons with long and short axes of 10–15 and 5–10 μm, respectively (Fig. 4B). A few large neurons were also labeled.

In contrast to vermal injections, a small number of PRH neurons were labeled after the unilateral intrafloccular and parafloccular injections. These cells scattered bilaterally in the caudal half of the nucleus (Figs. 2 and 6) and were more prominent in the contralateral PRH than in the ipsilateral one (Fig. 2). After cerebellar injection, a small number of the labeled cells were constantly observed in the bilateral Roller's nucleus (Fig. 7B), whereas no intercalatus neurons showed TMB reaction products.

The distribution of double-labeled PRH neurons was studied by the combined injections of two fluorescent dyes into the SC and vermal lobules VI–VII (Fig. 8). After SC injection, the boundary between the SC and the pretectum always was shown to be free of any invasion by fluorescent dye. Therefore, double label-
Fig. 3. (A) Unilateral injection of WGA-HRP into the superior colliculus. Bar = 1 mm. (B) Labeled small neurons in the contralateral PRH. (C) Labeled neuron (arrow) in the contralateral nucleus intercalatus. (D) Labeled neurons on the contralateral SG after unilateral WGA-HRP injection into the pretectal region, including the nucleus of the optic tract. Bars in (B and D) = 100 μm. Abbreviations, see Figure 1.

Fig. 4. Size distribution of efferent PRH neurons. Histograms (A and B) show the neurons projecting to the superior colliculus and to vermian lobules VI–VII, respectively.
After HRP injection into the cat SC, Edwards et al. observed labeled neurons in the bilateral PRH, with a preponderance to the contralateral side. However, the present finding of a contralateral PRH-SC projection in the rat is consistent with the results of McCrea and Baker and Stechison et al. Belknap and McCrea reported that neither retrogradely labeled neurons nor anterogradely labeled fibers were found in the SC after injection of WGA-HRP into the squirrel monkey PRH. However, recently, Hartwich-Young et al. have clearly demonstrated the existence of the contralateral PRH-SC projection in the rhesus monkey, as shown in cats and rats (the present data). Thus, it can be considered that eye-position information could reach the SC through this ascending pathway in many vertebrates, including the primates.

PRH projections to the cerebellar flocculus and paraflocculus have been reported. In the squirrel monkey, unilateral injection of WGA-HRP into the PRH results in heavy bilateral anterograde labeling of these cerebellar regions. The present study, however, showed that the number of PRH neurons pro-

**Discussion**

The present WGA-HRP study proved the presence of PRH-SC and PRH-cerebellar projections in the rat.
In the rat, the SG is a collection of small neurons that forms a rostral continuation of the PRH, and the density of the SG neurons seems to be higher than in the chimpanzee or the cat. In the cat, the SG is represented by a loose aggregation of small cells and it sends efferent fibers to the oculomotor nuclei. Brodal has pointed out some species difference in the nucleus. For example, the monkey SG is formed by medium-sized cells and begins at the lateral aspect of the rostral end of the PRH, from where these neurons project to the cerebellum. The present study showed that small SG neurons were constantly labeled when WGA-HRP was injected into the pretectal region and when the tracer invaded the dorsolateral part of the central gray matter just below the SC.

Ipsilateral descending projections from the pretectum to the PRH, including the nucleus of the optic tract, have been reported in the rat, cat, and rabbit. However, ascending projection from the PRH to the pretectum was not consistently observed. Although the present study did not define the terminal area in the pretectum, it is thought that neurons in the PRH and the SG gave off bilateral ascending projections to the flocculus and paraflocculus was smaller than that projecting to lobules VI–VII. It is possible that lobule VII receives the most abundant projection of fibers from the PRH.

Although labeled neurons were widely distributed throughout the PRH regardless of their projecting targets, we found that the efferent neurons appeared to have topographically preferential sites of origin. PRH neurons projecting to lobules VI–VII of the cerebellar vermis were distributed predominantly in the caudal third of the PRH. On the other hand, labeled neurons projecting to the SC were located mainly in the middle part of the nucleus. Moreover, most efferent neurons to the pretectal region were located in the rostral PRH on both sides. These findings are consistent with those of previous reports, in that the caudal PRH was more related to the cerebellum than the rostral part of this nucleus.

Fig. 7. (A) Neurons labeled in the PRH after WGA-HRP injection into lobules VI–VII of the cerebellar vermis. (B) Labeled neurons in the nucleus of Roller after intravermal injection. Bars = 100 μm. Abbreviations, see Figure 1.

Fig. 8. Distribution of retrogradely labeled neurons in the middle part of the PRH, after the combined injection of fast blue (FB) and diamidino yellow dihydrochloride (DY) into the superior colliculus and vermal lobules VI–VII, respectively. Triangle, FB positive neurons. Square, DY positive neurons. Asterisks, double-labeled neurons. Other abbreviations, see Figure 1.
Fig. 9. Double-labeled PRH neurons (large arrow) after the combined injection of FB and DY into the superior colliculus and the vermal lobules VI–VII, respectively. Small arrows, PRH neurons with the nucleus labeled by DY. Arrowheads, FB-labeled neurons. Bar = 100 μm.

The present results suggest that the PRH-pretectal projection may serve as an anatomical substrate by which information on the eye position could directly reach the pretectal region, especially the nucleus of the optic tract.16–18

Previous experiments have shown that PRH neurons are physiologically and morphologically heterogeneous, and that different types of cells tend to be regionally segregated.7–9,11,26 The intracellular injection of HRP showed that PRH neurons in the cat can generally be classified into three morphologically distinct types: (1) large multidendritic neurons; (2) medium-sized principal neurons; and (3) small neurons.26 McCrea and Baker11 also mentioned that the large multidendritic cells in the ventral PRH sent fibers to the flocculus and paraflocculus, while the small cells projected to the inferior olive and the principal neurons had connections with many brainstem structures. According to Gacek,27 however, the small neurons in the rostral PRH project to the oculomotor nuclei. The present study indicated that not only small but medium-sized PRH neurons extended axons to the SC. In contrast, the PRH neurons projecting to vermal lobules VI–VII were mainly medium-sized cells and only a few PRH cells more than 35 μm in long diameter had cerebellar projections.

Although the present study gave little information on the dendritic morphology of the projection neurons, it is likely that each efferent system of the PRH consists of fibers that project from several types of neurons and is possibly heterogeneous with respect to the signals transmitted and their synaptic effect on target neurons. Do efferent neurons of the PRH give rise to collaterals? In the cat, retrograde cellular changes occurred in small and large cells after cerebellar lesions, as previously reported by Brodal.22 These data indicate that many PRH neurons have axons that collateralize in two or more target regions. Yingcharoen and Rinvik28 have demonstrated branched projection from rostral PRH neurons to the oculomotor nucleus and the cerebellar flocculus and paraflocculus. The present double-labeling study clearly demonstrated that some neurons in the middle part of the PRH had branched axons innervating cerebellar lobules VI–VII and the SC.

The SC is an important supranuclear structure for central eye and head movements.14,15 Furthermore,
systematic mapping has disclosed that microstimulation of lobule VI of the cerebellar vermis produces saccades.\textsuperscript{29,30} Thus, these earlier findings and the present findings strongly suggest that PRH neurons play an important role in controlling visuomotor mechanisms.

Key words: prepositus hypoglossi nucleus, WGA-HRP labeled neurons, retrogradely double-labeled neurons, rat

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