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# Descending brainstem projections of the pedunculopontine tegmental nucleus in the rat*

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**Summary.** Descending brainstem projections from the pedunculopontine tegmental nucleus (PPN) were studied in the rat by use of the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L) and the retrograde tracer lectin-conjugated horseradish peroxidase (HRP-WGA). Results of these expeiments demonstrated prominent bilateral projections to the pontomedullary reticular nuclei, but direct connections to the motor and sensory nuclei of the cranial nerves could not be ascertained. The PPN fibers terminated mainly in the pontine reticular nuclei oralis and caudalis and in ventromedial portions (pars alpha and pars ventralis) of the gigantocellular reticular nucleus. A smaller number of labeled fibers distributed to more dorsal regions of the gigantocellular nucleus, lateral paragigantocellular, ventral reticular nucleus of the medulla and lateral reticular nucleus. Although a significant number of PHA-L labeled fibers was seen in two cases in the contralateral medial portion of the facial nucleus, and all cases exhibited a sparse predominantly ipsilateral projection to the lateral facial motor neurons, the retrograde tracing experiments have revealed that these facial afferents originated in the nuclei surrounding the PPN. The results are discussed in the context of PPN involvement in motor functions. It is suggested that the PPN may participate in a complex network involved in the orienting reflex.

**Key words:** Pedunculopontine nucleus – Descending projections – Reticular nuclei – Cranial nerve nuclei – Anterograde transport of PHA-L – Retrograde transport of HRP-WGA

## Introduction

The nucleus tegmenti pedunculopontinus (PPN) was first described in the human caudal mesencephalic tegmentum as being "bounded medially by the superior cerebellar peduncle, laterally by fibers of the medial lemniscus, and dorsally by the nuclei cuneiformis and subcuneiformis" (Olszewski and Baxter 1954). These authors further identified two subdivisions of the nucleus on the basis of cellular density. The smaller pars compacta "occupies the dorsolateral portion of the caudal half of the nucleus" and the "remainder of the nucleus constitutes the pars dissipata". In subprimate species, the nucleus is less clearly defined. Delineation of the PPN and its subdivisions has been variously made on the basis of cytoarchitectural features (Spann and Grofova 1989), basal ganglia input (Nauta 1979; Moon-Edley and Graybiel 1983), and cytochemistry (Woolf and Butcher 1986, 1989; Rye et al. 1987). In particular, this region of the pontomesencephalic tegmentum has been shown to contain numerous cholinergic neurons which form a major component of the Ch5 group as defined by Mesulam and co-workers (Mesulam et al. 1983). Both cholinergic and non-cholinergic cells of the PPN contribute in various proportions to the wealth of connections and functions attributed to this nucleus. The PPN has widespread connections with the basal ganglia, thalamus, and limbic structures and has been implicated in a wide variety of motor and non-motor functions.

The role of the PPN in the control of movement is well supported by both anatomical and physiological data. Convincing evidence exists that the PPN receives substantial input from several nuclei of the basal ganglia (Nauta and Mehler 1966; Kim et al. 1976; Nauta and Cole 1978; Beckstead et al. 1979; Nauta 1979; Larsen and McBride 1979; McBride and Larsen 1980; Jackson and Crossman 1981; Beckstead and Frankfurter 1982; Gerfen et al. 1982; Parent and DeBellefeuille 1982; Garcia-Rill et al. 1983a, b; Noda and Oka 1986; Granata and Kitai 1989; Nakamura et al. 1989; Spann and Grofova 1989; Grofova et al. 1991) and ascending efferents of the PPN reciprocate these basal ganglia projections (DeVito and Anderson 1982; Gerfen et al. 1982; Saper and Loewy 1982; Garcia-Rill et al. 1983a, b; Gonya-Magee and Anderson 1983; Jackson and Crossman 1983; Moon-Edley and Graybiel 1983; Woolf and

* This paper is dedicated to Professor Fred Walberg on the occasion of his 70th birthday.

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Butcher 1986; Rye et al. 1987). In addition, physiological experiments have identified a "midbrain locomotor region" in the decerebrate cat and rat (Mori et al. 1980; Garcia-Rill 1987b). This is located in the pedunculopontine region and includes the PPN. Furthermore, lesions of the PPN in the rat have been associated with impaired motor function (Kilpatrick and Starr 1981), and clinical studies have shown an associated between PPN cell loss in humans and movement disorders related to progressive supranuclear palsy (Zweig et al. 1985, 1987) and Parkinson's disease (Jellinger 1988; Zweig et al. 1989).

The pathways by which the PPN affects motor behavior have not yet been fully established. On the basis of the anatomical evidence, they may include both ascending projections to the basal ganglia nuclei as proposed by Moon-Edley and Graybiel (1983), and the descending projections to the brainstem and spinal cord as originally suggested by Jackson and Crossman (1983). Studies utilizing anterograde as well as retrograde tracers have repeatedly demonstrated that the PPN gives rise to a substantial descending projection to the pontomedullary reticular formation (Jackson and Crossman 1983; Moon-Edley and Graybiel 1983; Garcia-Rill 1986; Garcia-Rill and Skinner 1987a; Mitani et al. 1988; Rye et al. 1988; Nakamura et al. 1989; Woolf and Butcher 1989; Jones 1990; Robbins et al. 1990; Grofova et al. 1991) and a modest projection to the spinal cord (Jackson and Crossman 1983; Goldsmith and van der Kooy 1988; Rye et al. 1988; Spann and Grofova 1989). In addition, direct cholinergic projections from the PPN to the motor nuclei of cranial nerves 5, 7, and 12 have also been recently described (Woolf and Butcher 1989).

The present study was undertaken in order to detail the termination of descending PPN efferents in the pontomedullary reticular nuclei, and to confirm that some of these fibers also terminate in the sensory and motor nuclei of cranial nerves. To achieve this goal, we have employed a powerful anterograde tracing technique utilizing the transport of plant lectin *Phaseolus vulgaris*-leucoagglutinin (PHA-L). This technique is distinctly superior to previously used autoradiographic and HRP methods in both its sensitivity and clarity of labeling terminal arborizations and varicosities. Additionally, retrograde transport of HRP conjugated to wheat germ agglutinin (HRP-WGA) was used in control experiments. Preliminary accounts of this study have been published in abstract form (Grofova and Spann 1988; Keane and Grofova 1989).

## Material and methods

A total of 15 male Sprague-Dawley albino rats weighing 300–350 g were utilized for this study. Eight rats received unilateral injections of PHA-L in the PPN, while seven received unilateral injections of HRP-WGA in the facial nucleus. Both surgeries and perfusion were performed under deep anesthesia (sodium pentobarbital, 50–100 mg/kg, i.p.), and atropine sulfate solution (0.7 mg/kg) was administered i.m. prior to the surgery in order to prevent brain edema. Injections of both tracers were made iontophoretically using glass micropipettes with an inside tip diameter of 15–35 μm, and a positive 7 s pulsed 5 μA current. The stereotaxic coordinates were derived from the atlas of Paxinos and Watson (1986).

*PHA-L experiments.* Micropipettes filled with a 2.5% solution of PHA-L (Vector Labs) in 10 mM Tris buffer (pH 8.0) were inserted vertically through the ipsilateral hemisphere and tectum of the midbrain. Single iontophoretic depositions of PHA-L were made for 30–40 min. Following a survival period of 10 to 14 days, deeply anesthetized rats were perfused through the heart with a sodium-phosphate-buffered saline solution followed by a fixative consisting of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.15 M sodium phosphate buffer. The brains were immediately removed and stored overnight at 4° C in fixative. The following day, the brains were divided into a caudal block containing the caudal pons and medulla, and a left and right rostral block including the forebrain, midbrain, and rostral pons. Serial sections were cut at 30 μm on a vibratome in the coronal (caudal block) or sagittal (rostral block) plane. Sections were collected in Tris-buffered saline and processed for PHA-L immunohistochemistry using a modified biotin-avidin (Vector Labs) protocol by Gerfen and Sawchenko (1984). Immunoreacted sections were mounted onto gelatin-coated slides, air-dried, dehydrated, and lightly stained with cresyl violet. The sections were examined in a Leitz Orthoplan microscope, using bright-field illumination for the localization of PHA-L injections and the presence of labeled nerve fibers and terminal fields. The localization of the PHA-L deposit in the PPN was charted on a standard map of sagittal sections through the pontomesencephalic region containing the lateral and medial halves of the PPN. The distribution of the labeled fibers and plexuses in the brainstem was documented on projection drawings and photomicrographs of selected sections.

*HRP-WGA experiments.* Single unilateral injections were made in the medial or lateral portions of the facial nucleus using a 2% HRP-WGA solution in Tris buffer, delivered iontophoretically for 15 to 25 min according to Graybiel and Devor (1974). In order to minimize leakage of HRP-WGA along the needle track, the micropipette was not withdrawn until 10 min after the injection, and reversed polarity was applied during its withdrawal.

After a 24–48 h survival period, deeply anesthetized rats were perfused intracardially with a fixative consisting of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.15 M sodium phosphate buffer. The brains were blocked into right and left halves, and serial sections were cut sagittally at 30–50 μm on a freezing microtome or vibratome. HRP-WGA histochemistry using the chromogen tetramethyl benzidine (TMB) was performed according to Mesulam (1982). Some of the sections were additionally stabilized with ammonium molybdate (Olucha et al. 1985). Reacted sections were mounted on gelatin-coated slides and lightly counterstained with neutral red.

Sections were analyzed in bright-field illumination for the presence of retrogradely labeled cells in the PPN and surrounding regions. The labeled cells were plotted on projection drawings in representative cases using major blood vessels and fiber tracts as landmarks. Cell counts for all retrogradely labeled mesopontine nuclei were taken from alternating sections, and the numbers of labeled cells contained in a specific nucleus were pooled from all inspected sections.

The borders of the PPN and its subnuclei were identified according to previously established criteria (Spann and Grofova 1989). For clarity, the delineation of all other relevant structures was taken from the atlas of Paxinos and Watson (1986).

## Results

Before describing the experimental results, the normal boundaries of the pedunculopontine nucleus adopted in the present study will be considered briefly. The rat PPN is located medial to the nuclei of the lateral lemniscus and lateral to the decussation of the scp. It is caudally contiguous with the lateral and medial parabrachial nu-

clei (LPB and MPB) and is rostrally adjacent to the retrorubral field (RRF) and, more laterally, the retrorubral nucleus (RR) as defined by Paxinos and Watson (1986). The PPN borders ventrally on the nucleus reticularis pontis oralis (RPo), and dorsally on the cuneiform (Cnf) and mesencephalic reticular nuclei (Rmes). While both divisions of PPN can be distinguished in the rat, the PPNd comprises the bulk of the nucleus. The PPNd is particularly prominent medially, and the medial portion appears to receive a substantial input from the substantia nigra pars reticulata (Spann and Grofova 1988).

We have recently studied the distribution of cholinergic neurons in the PPN and surrounding regions in sagittal sections using ChAT immunohistochemistry (Spann and Grofova 1990). The cholinergic neurons are present in both subdivisions of the PPN but they do not respect the cytoarchitectural boundaries of the nucleus. Smaller numbers of cholinergic cells are also present in the adjacent nuclei such as the RRF, RR, rostral poles of the LPB and MPB as well as in the subpeduncular tegmental nucleus.

*PHA-L experiments*

PHA-L injections were directed toward the PPN region receiving afferents from the substantia nigra (i.e., the medial two thirds of the PPNd). They were intentionally large in order to label the entire outflow of this PPN region. Out of eight cases, five showed excellent anterograde labeling as well as precise localization of the injection in the PPNd. These five cases (PHA-L # 24, #25, #27, #28, #31) represent the core material for the present report (Fig. 1). The remaining three cases yielded less intense labeling and provided complementary data.

While centered in the medial portions of PPNd, all PHA-L injections involved the entire mediolateral extent of the PPN. Two zones of different labeling intensity were observed at the injection sites; a central zone in which the DAB reaction product obscured the cytoarchitecture, and a peripheral zone in which single neurons exhibiting Golgi-like labeling could be discerned (Figs. 2–6). In all animals, a few labeled neurons were observed in the surrounding nuclei. However, fiber tracts passing through the injection site were always free of label. The absence of labeling was particularly striking in the scp which was included in the central zone of all PHA-L injections (Figs. 2 and 3).

In case PHA-L #25 (Figs. 2, 4, 5), there was a dense deposit of PHA-L throughout the extent of the PPN. Occasional cells in the Cnf were also labeled along the course of the needle track. In addition, a few PHA-L labeled cells were observed rostrally in the RR (Fig. 4), ventrally in the RPo, caudally in the MPB (Fig. 5), and laterally in the nuclei of the lateral lemniscus. The inclusion of rostrally adjacent structures in the peripheral zone of PHA-L uptake was also observed in case PHA-L #31, where the rostroventral half of PPN was labeled in addition to individual neurons in the surrounding RR, RRF, and RPo. In cases PHA-L #28, PHA-L #27, and PHA-L #24, injections were also centered in PPN, but extended somewhat caudally to include neighboring regions of the MPB and LPB. The PHA-L injection in case PHA-L #28 (Fig. 3) was the most laterally placed of these, involving the caudoventral PPN and labeling several cells caudal to PPN in the MPB (Fig. 6) and ventrally in the RPo.

*Projections to the reticular nuclei*

The bulk of PPN descending projections were distributed within the reticular nuclei of the brainstem (Fig. 7).

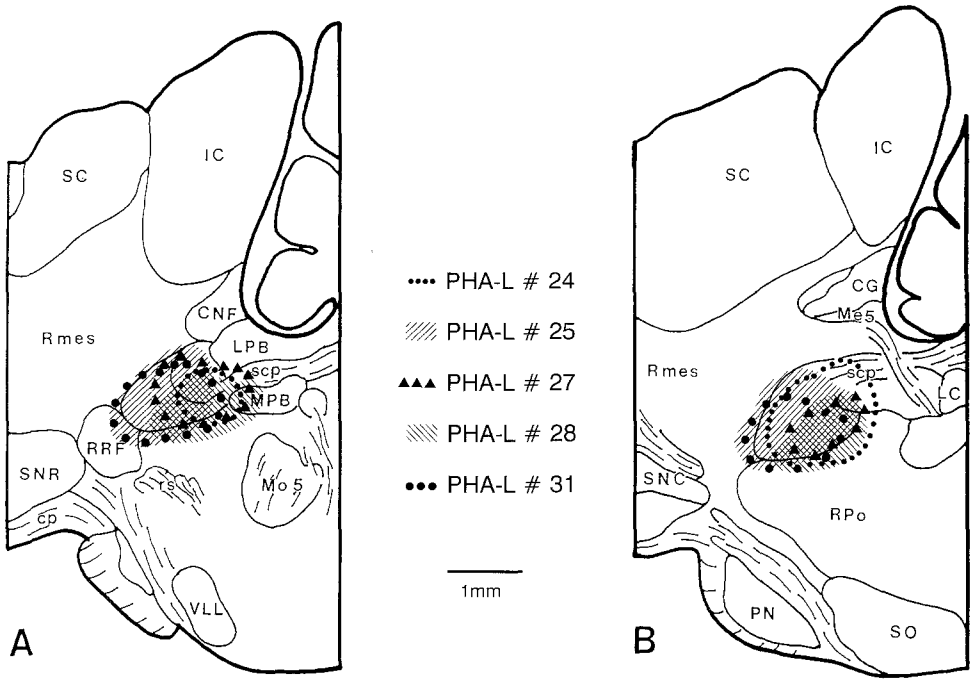
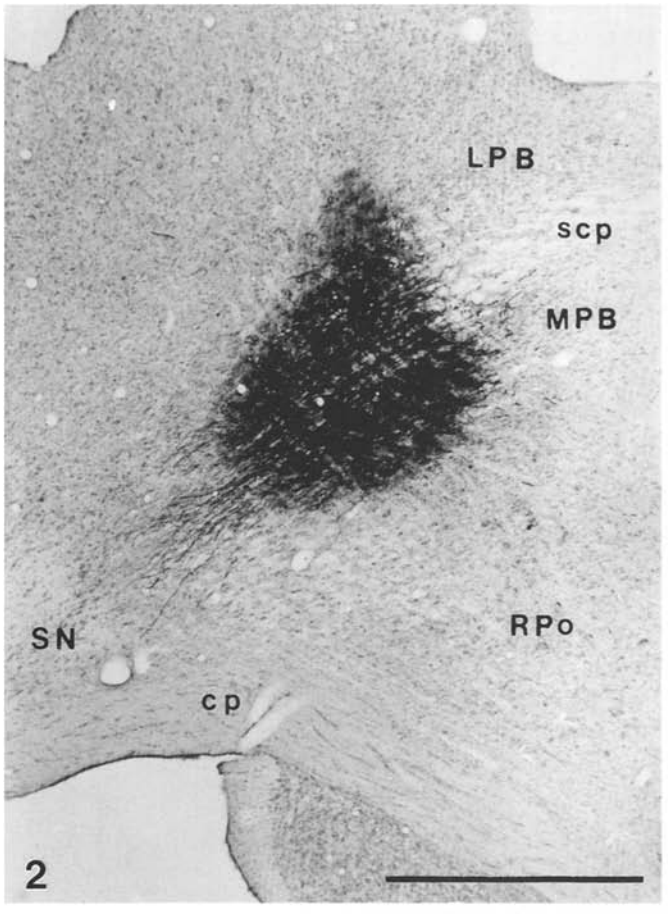
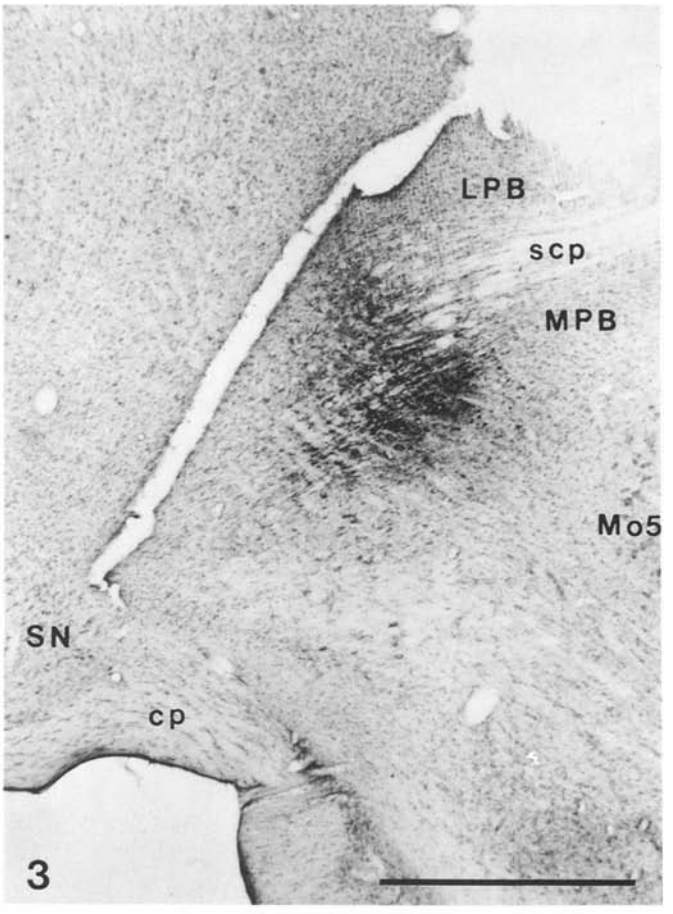
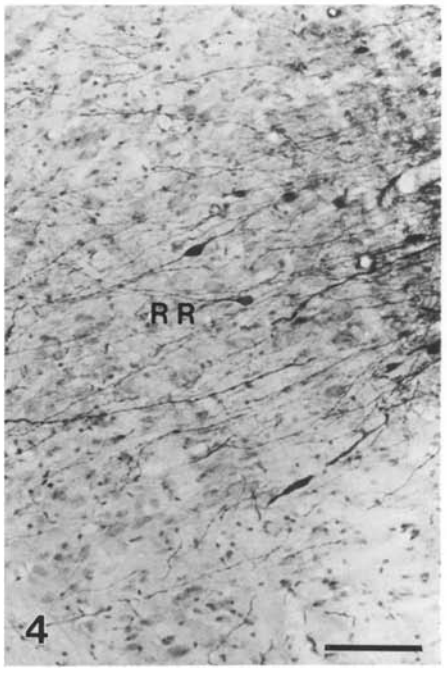
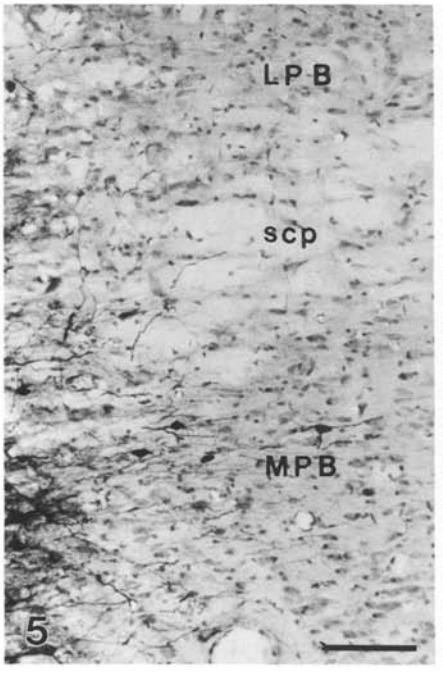


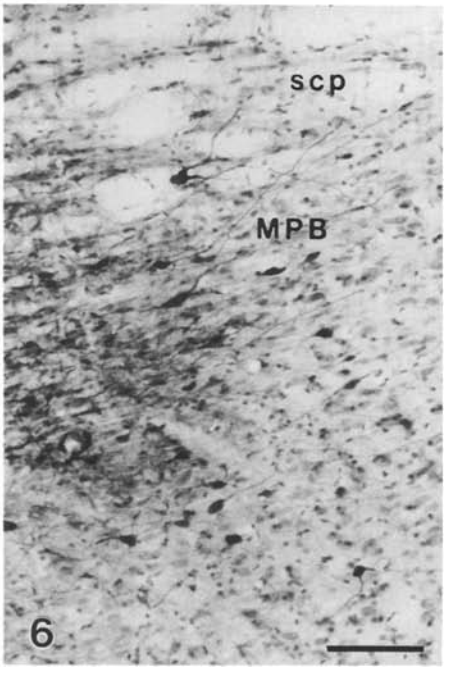

**Fig. 1A, B.** Drawings of sagittal sections through the lateral (**A**) and medial (**B**) halves of the PPN, showing the approximate position and extent of PHA-L injections in five representative cases. Two cases selected from this material are illustrated photographically in Figs. 2 and 3



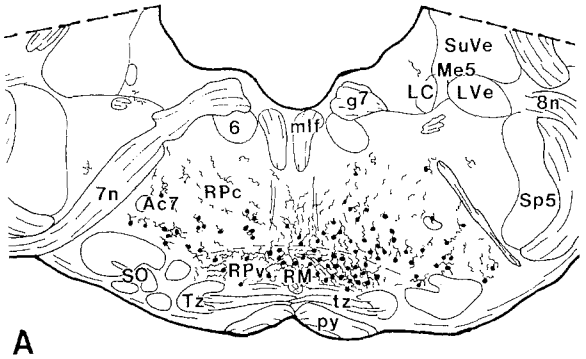



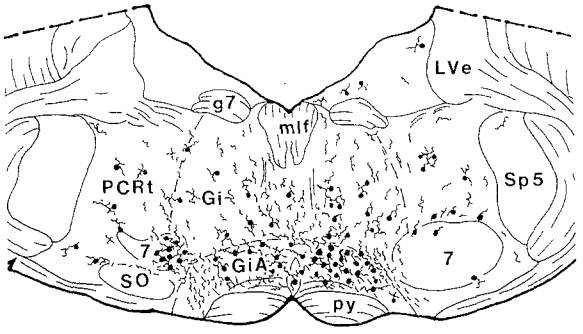



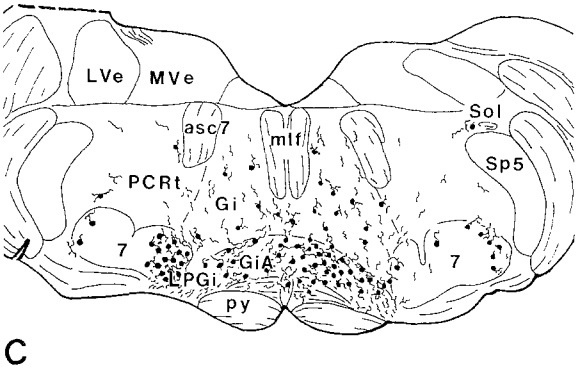



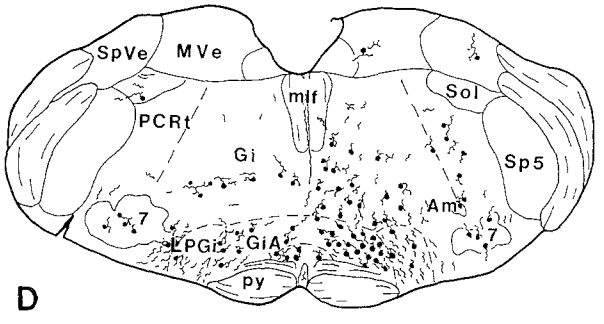

**Figs. 2 and 3.** Brightfield photomicrographs showing the center of PHA-L injection sites in cases #25 and #28 respectively. Notice the absence of label in the fibers of the superior cerebellar peduncle. Bars 1 mm

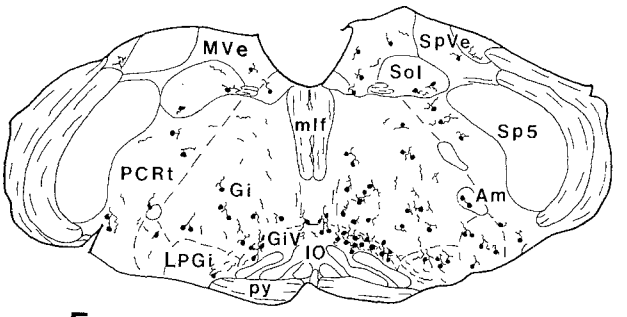
**Figs. 4–6.** Isolated Golgi-like labeled cells at the periphery of PHA-L injections illustrated in Figs. 2 and 3. In case #25, such cells were present in the retrorubral as well as the parabrachial nuclei (**Figs. 4 and 5**), while case # 28 exhibited scattered labeled neurons only in the parabrachial nuclei (**Fig. 6**). Bars 100 μm

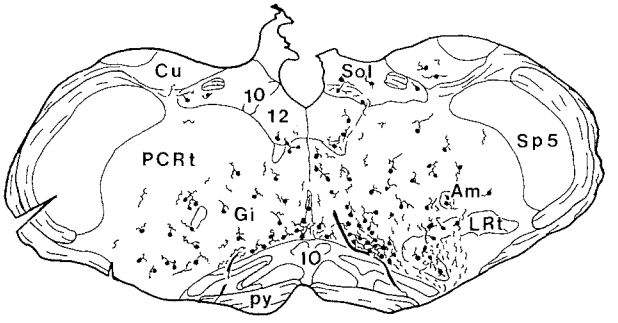


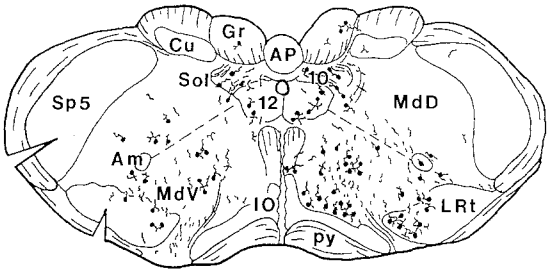



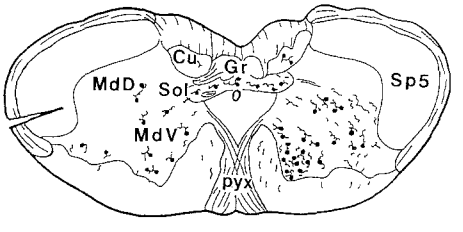
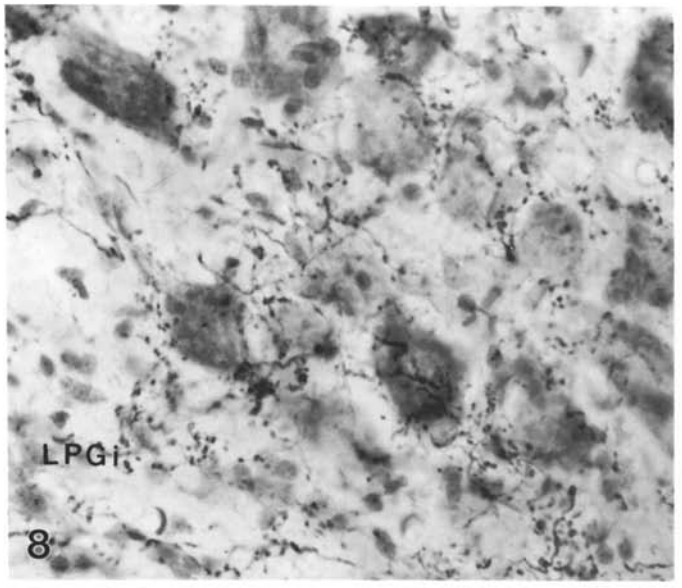




**Fig. 7A–H.** Projection drawings of coronal sections in case #25 illustrate the course and distribution of PHA-L labeled fibers from the caudal pons (**A**) to the pyramidal decussation (**H**). *Wavy lines* indicate labeled fibers en passant, while *dots* symbolize plexuses of fine varicose fibers, The incision on the lateral surface of the brainstem indicated the side contralateral to the PHA-L injection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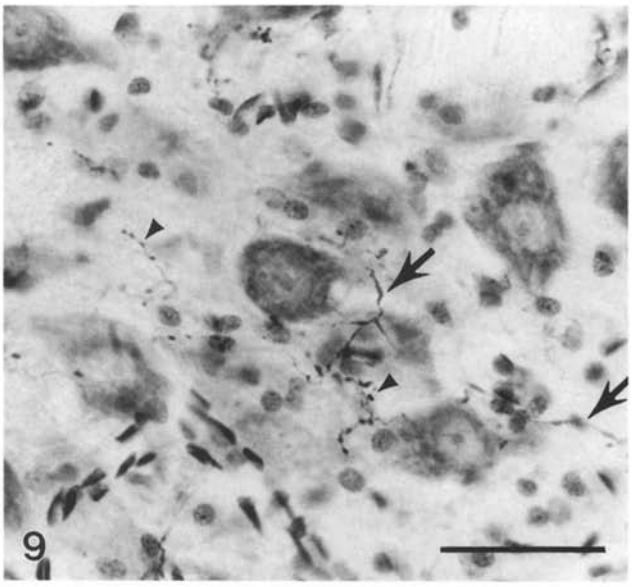
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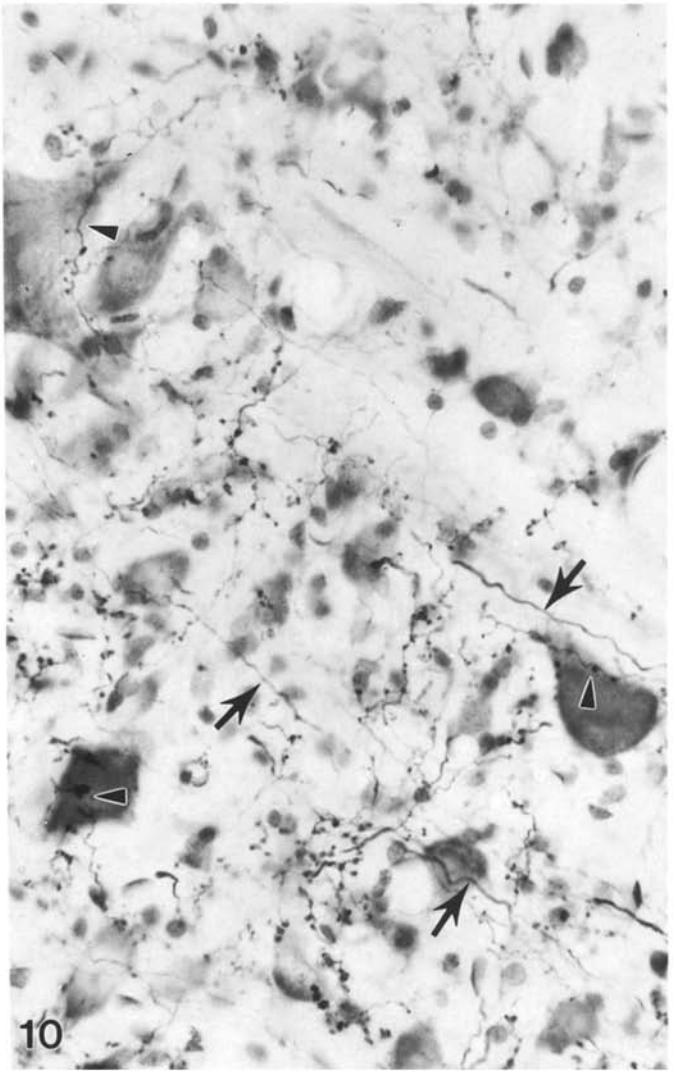
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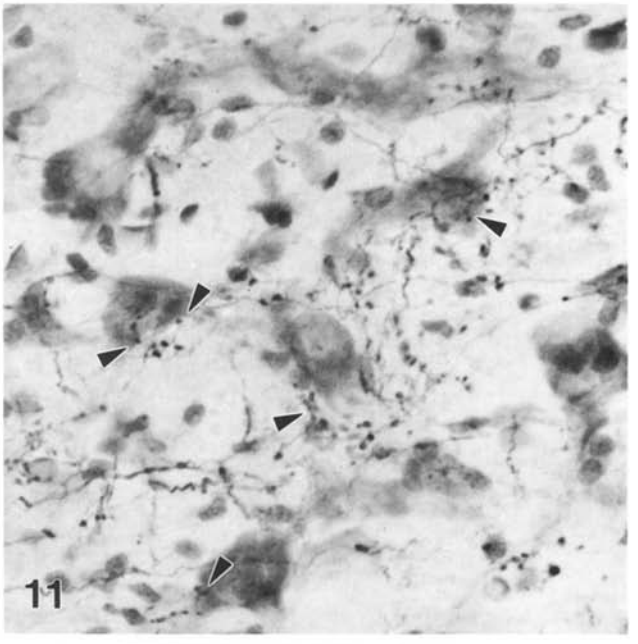
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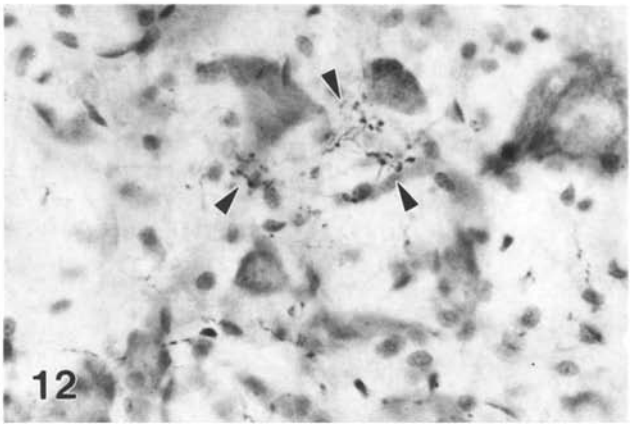
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Although the quantity of PHA-L labeled fibers varied in different experiments, the general pattern of labeling illustrated in Fig. 7 was consistent in all cases. From the injection site, labeled fibers coursed ventrally and medially through the RPo and RPc toward the ventral portion of the pontine reticular nucleus. During this course, numerous thin varicose branches of the labeled fibers were seen to terminate around large reticular neurons, especially in the RPc (Fig. 10). In the medulla, the labeled fibers were concentrated in the ventromedial portion of the reticular formation, particularly in the pars alpha and pars ventralis of the gigantocellular nucleus (GiA and GiV), and in the lateral paragigantocellular nucleus. Although terminal arborizations were seen along the course of labeled fibers in the gigantocellular nucleus, and even in the parvocellular reticular nucleus, their number was quite modest compared to the dense terminal plexuses of fine varicose fibers in the GiA and GiV (Figs. 11 and 12). In the caudal medulla, the number of labeled fibers decreased, but small patches of terminal arborizations were still observed around the cells in the ventral medullary reticular nucleus and in the lateral reticular nucleus.

Many labeled fibers crossed the midline in the caudal pons and rostral medulla to terminate in the corresponding reticular nuclei on the contralateral side. However, the terminal arborizations were always denser ipsilaterally.

*Projections to the cranial nerve nuclei*

Fine PHA-L labeled varicose fibers were seen in several motor and sensory cranial nerve nuclei. No labeled fibers were found in the motor trigeminal nucleus. However, two distinct distribution patterns were observed in the facial nucleus. One pattern, present in all cases, consisted of diffuse labeling in the ipsilateral facial nucleus (Fig. 9), while two cases (PHA-L #25 and PHA-L #31) additionally demonstrated a dense contralateral projection to the rostromedial two thirds of the facial nucleus (Fig. 8). All PHA-L experiments exhibited a sparse bilateral projection to the hypoglossal, ambiguus, and dorsal motor vagus nuclei. From the sensory nuclei, the ipsilateral as well as contralateral solitary nucleus consistently contained a few varicose fibers which occasionally formed fine plexuses in the lateral portion of the nucleus on the ipsilateral side. On the other hand, no labeled fibers were seen in the spinal trigeminal nucleus, and in the vestibular nuclei the labeling was inconsistent and very sparse.

*HRP experiments*

Results of PHA-L experiments suggested a topographical organization of PPN projections to the facial nucleus, with rostral portions of PPN projecting to the contralateral groups of medial facial neurons, and caudal portions of PPN projecting ipsilaterally primarily to the lateral half of the facial nucleus. To verify this organization, experiments utilizing the retrograde transport of HRP-WGA from lateral (N=3) or medial (N=4) portions of the facial nucleus were carried out.

*Lateral facial injections*

The HRP-WGA injection sites involving the lateral portions of the facial nucleus are shown in Fig. 13. Dense deposits of HRP-WGA were observed in the lateral third to half of the facial nucleus in all three lateral injections (CN7 #14, #16, #17). Despite all precautionary measures, HRP-WGA reaction product was also consistently present along the needle track and in parts of surrounding structures.

In case CN7 #17, the dense deposit of HRP-WGA was nearly completely confined to the lateral two thirds of the facial nucleus in its middle antero-posterior extent. A "halo" of diffused reaction product extended ventrally into the trapezoid body and rubrospinal tract, and into the reticular formation caudolateral to the facial nucleus. No retrogradely labeled cells were observed in the PPN. Labeled neurons were identified primarily in the ipsilateral principal trigeminal (Pr5), MPB, and Kölliker-Fuse (KF) nuclei (Fig. 14). Retrogradely labeled cells of the MPB were concentrated rostrally, near the caudal border of the PPN. A substantial number of labeled cells in the ipsilateral Pr5 and KF were also present at levels lateral to the PPN, while fewer neurons were labeled in the ipsilateral LPB and RPo. Several labeled neurons were observed in the contralateral red nucleus (RN). In addition, a few scattered retrogradely labeled cells were found in the ipsilateral RPc and contralaterally in the deep layers of the superior colliculus (SC).

Other injections of HRP-WGA in the lateral half of the facial nucleus exhibited larger diffusion of the reaction product into the surrounding tissue. In case CN7 #16, the injection was centered at the caudolateral edge of the facial nucleus and exhibited diffuse HRP-WGA reaction product with "Golgi-like" labeling of neurons in the caudolateral two thirds of the facial nucleus as well as the parvicellular reticular nucleus dorsolateral

**Figs. 8–12.** Brightfield photomicrographs of PHA-L labeled fibers in the facial nucleus and pontomedullary reticular nuclei. Bars 50 μm

**Fig. 8.** A dense plexus of varicose fibers surrounding ventromedial facial motoneurons contralateral to the PHA-L injection in case #25

**Fig. 9.** Dorsolateral group of facial motoneurons ipsilateral to the injection site in case #28. Thick fibers of an even diameter (*arrows*) divide into thin branches exhibiting multiple varicosities (*arrowheads*)

**Fig. 10.** A sagittal section through the nucleus reticularis pontis caudalis ipsilateral to the injection in case #25 displays thick fibers of even diameter descending to the medulla (*arrows*), and perpendicularly coursing thin varicose fibers which are often aligned along the cell bodies of large reticular neurons (*arrowheads*)

**Figs. 11 and 12.** Fine varicose fibers form plexuses (*arrowheads*) in the proximity of ipsilateral reticular neurons in the pars alpha (**Fig. 11**) and pars ventralis (**Fig. 12**) of the gigantocellular reticular nucleus

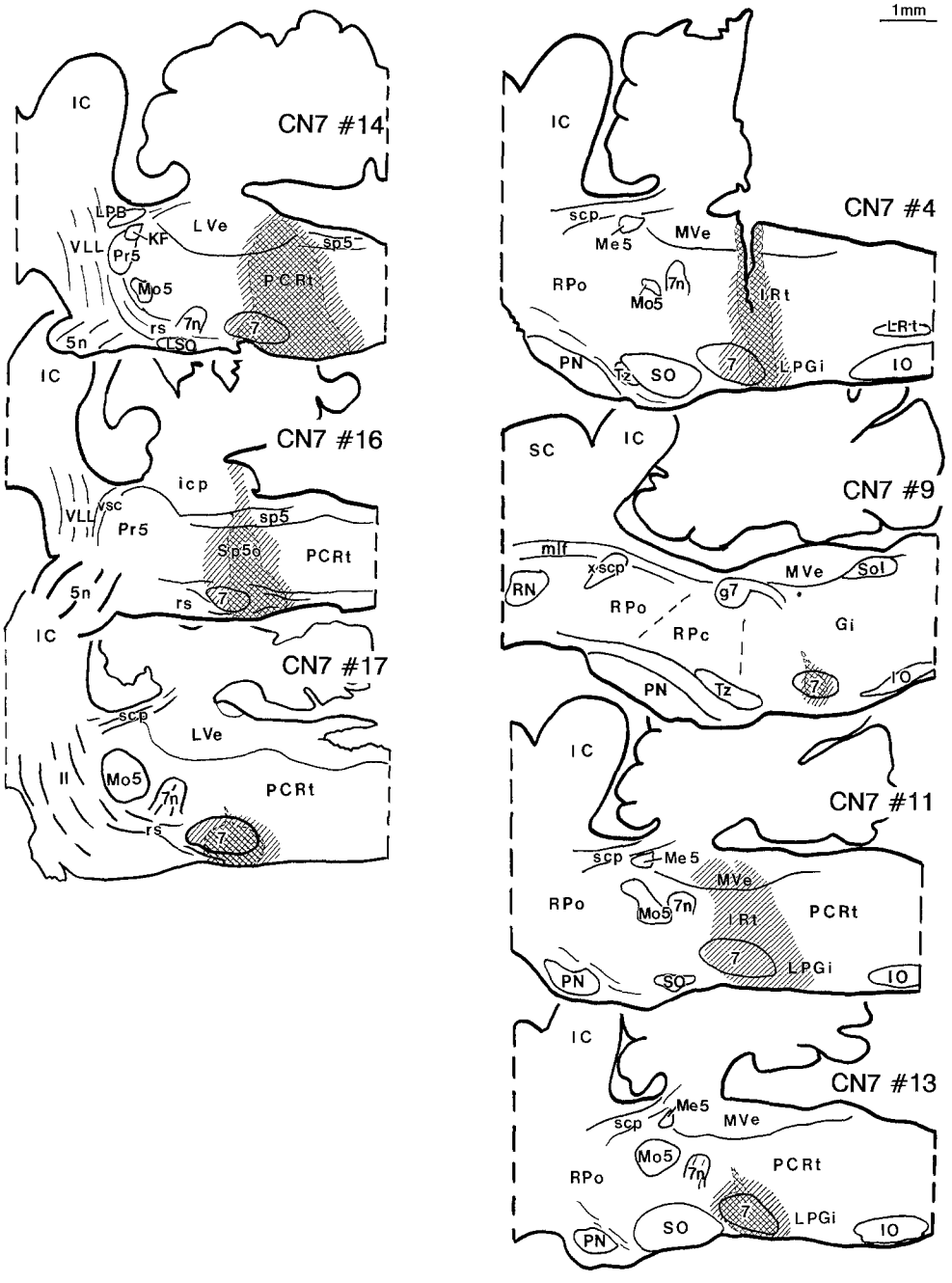


**Fig. 13.** Projection drawings of sagittal sections through the center of HRP-WGA injection sites involving the lateral (left column) and the medial (right column) portions of the facial nucleus. The position of dense reaction product around the pipette is indicated by *cross-hatching*. *Hatching* indicates a surrounding area of lighter diffusion which may be somewhat overestimated because of use of the sensitive chromogen, TMB

to the facial nucleus. In case CN7 #14, the injection was centered caudal to the facial nucleus and included only the caudal half of the lateral portions of the facial nucleus. HRP-WGA reaction product in case CN7 #14 was seen as far caudally as the rostral Am and extended dorsally into the reticular formation as well. In these cases, the PPN exhibited a few retrogradely labeled neurons in addition to heavy HRP labeling ipsilaterally in several nuclei caudally bordering the PPN (MPB, KF, and Pr5). Results are summarized in Table 1.

*Medial facial injections*

In four rats (CN7 #4, #9, #11, #13), HRP-WGA injections were centered in the medial half of the facial nucleus (Fig. 13). In case CN7 #13, dense HRP-WGA reaction product was almost totally confined to the medial half of the facial nucleus. However, slight encroachment of the underlying fibers of the trapezoid body and faint HRP-WGA reaction product was also evident in a small region of the lateral paragigantocellular nucleus adjacent to the facial nucleus. As observed after HRP-WGA injections in the lateral portions of the facial nucleus, there were no labeled cells well within the confines of the PPN (Fig. 15). Immediately rostral to the PPN, the contralateral RR and RRF were heavily labeled (Fig. 16). This projection was exclusively contralateral. Labeled RR cells were located caudally, abutting the rostral pole of PPN and often occupying the border region between these two nuclei. In addition, a more mod-

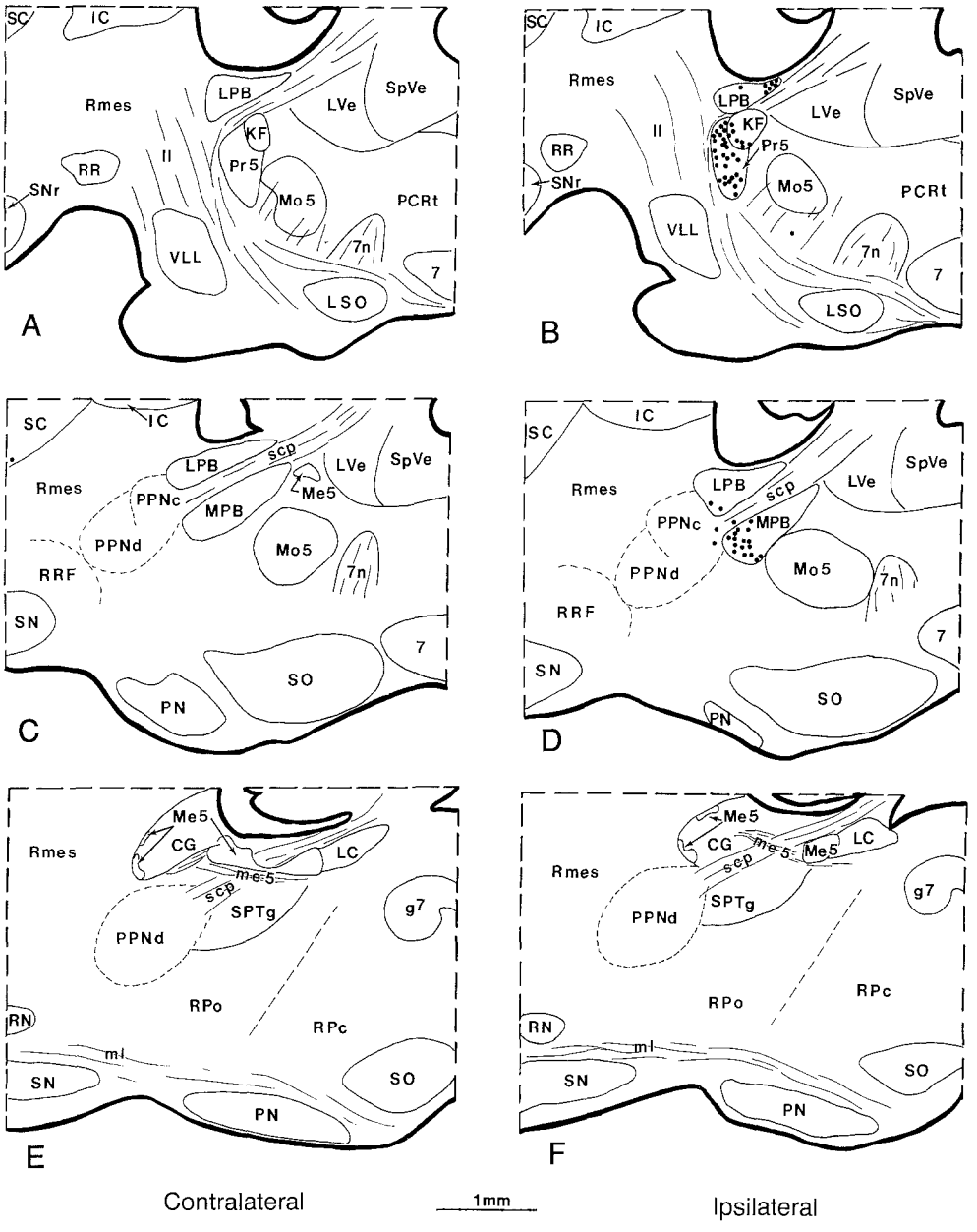


**Fig. 14A–F.** Projection drawings of lateromedially arranged sagittal sections through the pontomesencephalic region showing the distribution of HRP-labeled cells in the controlateral (**A–C**) and ipsilateral (**D–F**) nuclei surrounding the PPN, following HRP-WGA injection involving the lateral portion of the facial nucleus in case CN7 # 17. Sections illustrated in **A** and **D** are lateral to the PPN. Each *dot* represents one labeled cell

**Table 1.** Retrogradely labeled cells following lateral facial HRP-WGA injections

| Case CN7# | PPN | Pr5 | KF | MPB | LPB | RPo | RN | Other |
|---|---|---|---|---|---|---|---|---|
| #17 | 0 (0) | 139 (2) | 55 (0) | 118 (3) | 29 (0) | 18 (0) | 0 (11) | I: 5 RPc<br>C: 4SC, 1RR, 1CG, 1Rmes |
| #16 | 1 (0) | 35 (0) | 4 (0) | 8 (1) | 0 (0) | 4 (0) | 0 (11) | I: 0<br>C: 1 SuVe |
| #14 | 10 (5) | 307 (9) | 16 (14) | 182 (14) | 92 (0) | 75 (26) | 2 (212) | I: 8SubLC, 1Cnf 4SuVe, 2Rmes, 1 Mo5, 1 SNR<br>C: 23SubLC, 10SuVe, 16SC 1 Rmes, 1 CG |

Retrogradely labeled cells in the pontomesencephalic nuclei following HRP-WGA injections in the lateral portions of the facial nucleus were counted from alternating 50-μm-thick sections. Cell counts from the contralateral nuclei are in parentheses

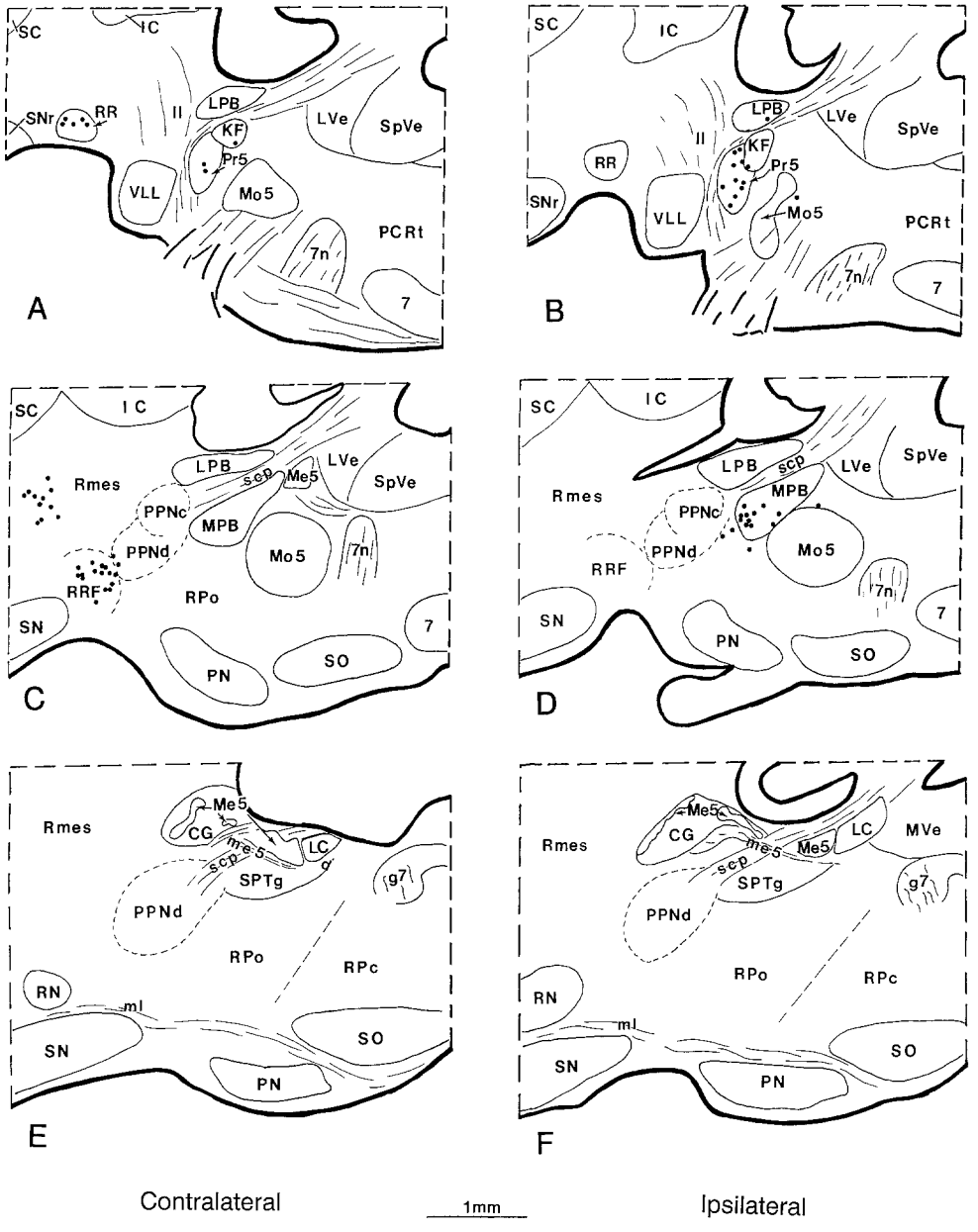


**Fig. 15A–F.** Projection drawings of sagittal sections illustrating the distribution of HRP-labeled cells in the contralateral (**A–C**) and ipsilateral (**D–F**) pedunculopontine region following HRP-WGA injection involving the medial part of the facial nucleus in case CN7 # 13. Each *dot* represents one labeled cell

erate number of labeled neurons was observed in the ipsilateral MPB. As in the pattern observed following lateral HRP-WGA injections, retrogradely labeled MPB cells were found primarily along the rostral and dorsal borders of MPB in a zone bordering the caudal PPN. The other mesopontine nuclei exhibiting HRP-labeled neurons included, in decreasing order: the Rmes, RPo, RRF, Pr5, central gray (CG), KF, RN and LPB.

In the other three rats with injections in medial portions of the facial nucleus, moderately dense HRP-WGA reaction product was observed in neighboring regions of the adjacent reticular nuclei. GiA was labeled medial to the facial nucleus in cases CN7 #11, CN7 #4, and CN7 #9, while the reticular formation caudodorsal to the facial nucleus was labeled only in cases CN7 #11, and CN7 #4. Results from these experiments confirmed those of case CN7 #13. There were essentially no labeled cells in the PPN following HRP-WGA injections into medial portions of the facial nucleus. Diffusely labeled neurons were observed in nuclei surrounding (but not including) the ipsilateral PPN, particularly the MPB and Pr5. Distinct labeling in the contralateral RR was present in all medial cases, even in one case (CN7 #9) with an extremely small injection site. Results are presented in Table 2. In one additional case (CN7 #18) in which the core area of the HRP-WGA injection included the entire extent of the facial nucleus and substantial portions of the surrounding reticular nuclei, significant numbers of HRP-labeled cells were observed in the PPN (136 cells ipsilaterally, 54 contralaterally).

## Discussion

The present results confirm the existence of previously reported descending PPN projections to the pontome-

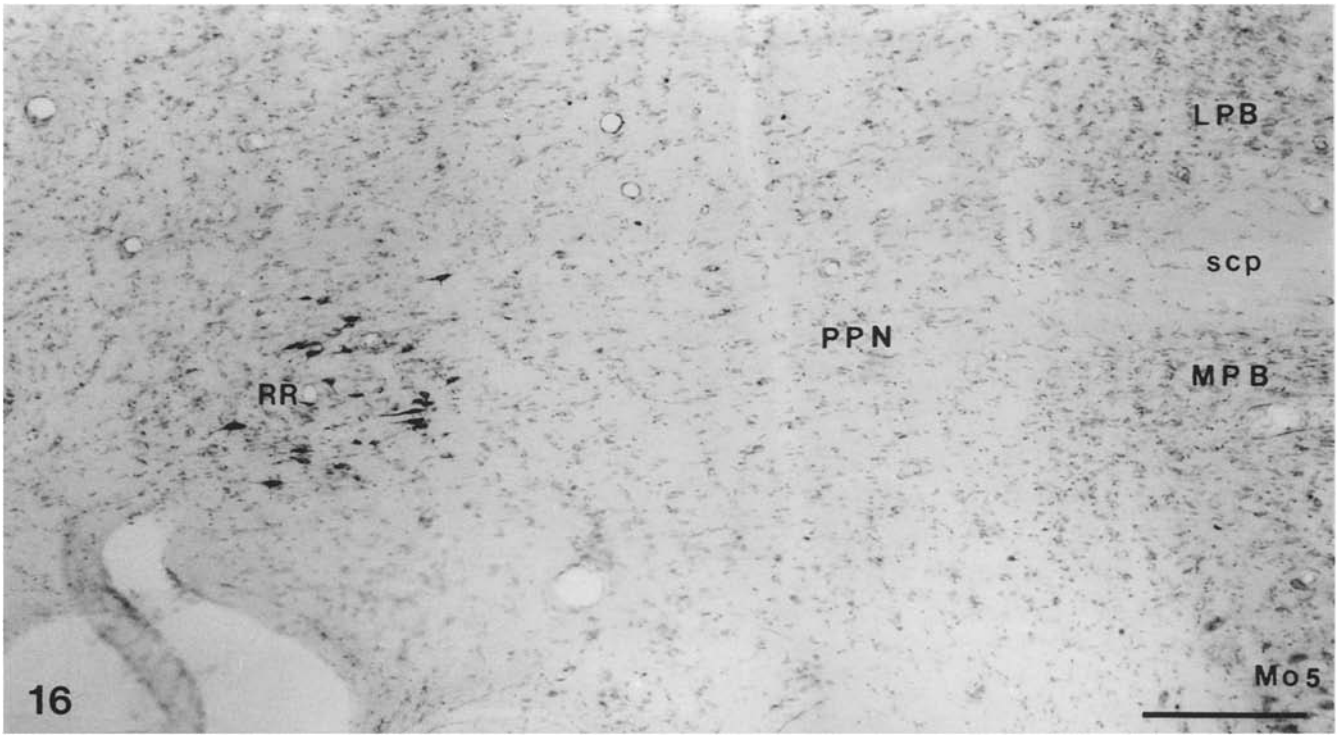


**Fig. 16.** Brightfield photomicrograph of labeled cells in the contralateral retrorubral nucleus following HRP-WGA injection involving the medial part of the facial nucleus in case CN7 #13. Bar 250 μm

**Table 2.** Retrogradely labeled cells following medial facial HRP-WGA injections

| Case CN7 # | RR | PPN | Pr5 | MPB | KF | LPB | Other |
|---|---|---|---|---|---|---|---|
| #13 | 0 (191) | 0 (1) | 11 (2) | 38 (0) | 9 (4) | 3 (0) | I: 17 RPo, 10 CG, 7 Rmes, 2 RN<br>C: 47 Rmes, 12 RRF, 3 RN, 2 RPo |
| #11 | 0 (21) | 1 (0) | 10 (0) | 32 (0) | 1 (0) | 4 (0) | I: 8 Me5, 7 SN, 5 RPo, 5 SuVe<br>C: 1 CG |
| # 9 | 0 (1) | 0 (0) | 0 (0) | 0 (0) | 0(0) | 0 (0) | None |
| # 4 | 0 (11) | 0 (0) | 0 (0) | 2 (0) | 0 (0) | 0 (0) | I: 1 CG<br>C: 1 RN, 1 CG |

Retrogradely labeled cells in the pontomesencephalic nuclei following HRP-WGA injections in the medial portions of the facial nucleus were counted from alternating 50-μm-thick sections. Cell counts from the contralateral nuclei are in parentheses

dullary reticular formation (Jackson and Crossman 1983; Moon-Edley and Graybiel 1983; Garcia-Rill 1986; Garcia-Rill and Skinner 1987a, b; Mitani et al. 1988; Rye et al. 1988; Nakamura et al. 1989; Woolf and Butcher 1989; Jones 1990; Robbins et al. 1990). These projections are partially crossed with a stronger ipsilateral component, and appear to terminate mainly in the pontine nuclei RPo, RPc, and RPv and in the ventromedial portion of the medullary reticular formation including the GiA and GiV. Our observations strongly suggest that a major proportion of these descending projections originates from the PPNd, including the region which receives the nigral input. On the other hand, our results do not support the existence of direct projections from the same region of the PPN to the sensory and motor nuclei of the cranial nerves. The cholinergic PPN projections to the motor nuclei of cranial nerves 5, 7, and 12 described by Woolf and Butcher (1989) probably originated from cholinergic neurons located just outside the cytoarchitectural borders of the PPN.

### *Technical considerations*

While PHA-L injections in the PPN clearly demonstrated two distribution patterns of labeled fibers in the facial nucleus, the HRP-WGA experiments failed to retrogradely label cells located in the core of the PPN. Although there exist occasional reports (Cliffer and Giesler 1988; Schofield 1989) that the PHA-L can be taken up by fibers passing through the injection site, most authors agree with the original observations of

Gerfen and Sawchenko (1984) that PHA-L does not appear to be taken up and transported effectively by fibers of passage. Our own observations fully support the latter notion. The PPN is traversed by several prominent fiber systems, including the superior cerebellar peduncle and the central tegmental tract which were unavoidably involved in all PHA-L injections. However, there was no evidence of PHA-L uptake and transport by these fiber systems. In fact, the fiber bundles of the scp traversing the center of PHA-L deposits were clearly discernible by the absence of reaction product (Figs. 2 and 3), and no labeled fibers could be traced to the red nucleus. Thus labeling of fibers "en passage" does not provide a reasonable explanation of the results of the anterograde tracing experiments.

It may be argued that the negative results obtained from the HRP experiments were due to technical failure. However, this seems unlikely since retrogradely labeled cells were consistently present in the areas surrounding the PPN. Furthermore, the PPN cells did exhibit distinct labeling in one of the experimental animals in which the HRP-WGA injection involved mostly the ventral part of the reticular formation adjacent to the medial aspect of the facial nucleus. Taken together, these observations indicate that the PPN does not give rise to the facial projections demonstrated in the experiments utilizing anterograde transport of PHA-L.

Most probably, the labeled fibers in the facial nucleus originated from scattered PHA-L labeled cells located within the nuclei surrounding the lateral aspect of the PPN. This conclusion is substantiated by careful comparisons between the distributions of HRP labeled cells following medial and/or lateral facial injections, and the distribution of single PHA-L labeled cells surrounding the dense center of PHA-L deposit. The HRP-WGA injections into the medial groups of facial motor neurons resulted in labeling of a discrete group of cells in the contralateral RR. Correspondingly, single labeled cells were seen in this region in the two PHA-L experiments (#25 and #31) which demonstrated a contralateral projection to the medial portion of the facial nucleus. On the other hand, retrogradely labeled neurons were most numerous in the ipsilateral MPB, KF, LPB and Pr5 following HRP-WGA injections centered laterally in the facial nucleus, and uptake of PHA-L by isolated cells within these regions was more prominent in cases PHA-L #28, #27 and #24 which demonstrated a diffuse projection terminating predominantly ipsilaterally in the lateral division of the facial nucleus.

*Afferents to the facial nucleus*

The retrograde labeling experiments confirmed ipsilateral afferent projections from the KF, MPB, and LPB to intermediate and lateral portions of the facial nucleus described previously in the rat (Saper and Loewy 1980; Hinrichsen and Watson 1983; Travers and Norgren 1983; Isokawa-Akesson and Komisaruk 1987), cat (Takeuchi et al. 1979; Holstege et al. 1986; Fort et al. 1989), and opossum (Panneton and Martin 1983). Projections from parabrachial nuclei to the lateral facial subnuclei may contribute to the control of oral musculature in respiratory and feeding behaviors.

In addition to parabrachial facial afferents, our data confirm a contralateral projection from RR to medial subdivisions of the facial nucleus (Hinrichsen and Watson 1983; Travers and Norgren 1983; Isokawa-Akesson and Komisaruk 1987). Similar observations have also been reported in the cat "paralemniscal zone" (Henkel and Edwards 1978; Takeuchi et al. 1979; Fort et al. 1989). Cytoarchitectural features of the rat RR resemble those described for the cat paralemniscal zone. This zone appears to receive projections from the superior colliculus and other structures known to be involved in visual and auditory orienting responses (Henkel 1981), and it has been suggested that the connection from this zone to the medial facial motoneurons may play a role in the pinnae-orienting response. Although no such function has been previously proposed for the rodent RR, it is interesting that the projection from the rat RR to the contralateral facial nucleus is restricted only to medial facial motoneurons which innervate the pinnae (Watson et al. 1982).

Our experiments also confirm a crossed rubro-facial pathway terminating in the lateral and intermediate facial subnuclei as described in the literature (Edwards 1972; Dom et al. 1973; Hinrichsen and Watson 1983; Panneton and Martin 1983; Travers and Norgren 1983; Isokawa-Akesson and Komisaruk 1987). Our data show this to be a sparse projection. The large number of RN cells labeled in case CN7 #14 is very likely due to uptake of HRP-WGA by damaged fibers of the rubrospinal tract passing caudolateral to the facial nucleus.

Finally, we have demonstrated a rather substantial projection from Pr5 to the lateral and intermediate facial subnuclei. Many neurons located just caudal to the ventral spinocerebellar tract (rostrolateral to Mo5) were labeled following HRP-WGA injections in both the medial and lateral portions of the facial nucleus. These were more numerous after lateral facial injections. Physiological studies demonstrating disynaptic responses of facial motoneurons to stimulation of the trigeminal nerve seem to support these findings (Tanaka et al. 1971). In contrast to our results, previous investigators have described Pr5 innervation of the facial nucleus to be rather sparse in the rat (Erzurumlu and Killackey 1979; Travers and Norgren 1983) and opossum (Panneton and Martin 1983). A projection from Pr5 to the lateral subdivisions of the facial nucleus may provide a pathway mediating the tactual guidance of oromotor behavior.

*Afferents to hypoglossal, dorsal motor vagus, ambiguus, and solitary nuclei*

While retrograde experiments were not carried out to clarify our findings of labeled fibers in the caudal cranial nerve nuclei (12, 10, Sol, and Am) following PHA-L injections centered in PPN, the literature supports the impression that these projections may also originate in nuclei surrounding the PPN. Several authors have shown

retrogradely labeled KF, and to a lesser extent MPB and LPB neurons, following HRP-WGA injections in several regions of the Sol in the rat (Fulwiler and Saper 1984; Rye et al. 1988; Herbert et al. 1990). The largest proportion of these cells has been reported in the KF, with the remaining retrogradely labeled cells surrounding the ventrolateral scp in the MPB and LPB nuclei. Rye and colleagues (1988) have additionally shown that while many cells were retrogradely labeled in the parabrachial nuclei following injections of HRP-WGA in the ventrolateral Sol region, only a few cells in the PPN were so labeled. In the present experiment, PHA-L injections sites did not include cells in the KF, but did include several cells of the rostral and middle regions of the LPB and MPB. This may explain the sparse distribution of PHA-L labeled fibers to Sol in our results.

Parabrachial projections to the ventrolateral medulla (including Am) and the hypoglossal nucleus have also been reported in the rat (Saper and Loewy 1980; Fulwiler and Saper 1984; Rye et al. 1988; Herbert et al. 1990) and in the pigeon (Wild et al. 1990). The ventrolateral medulla is widely thought to be involved in respiratory control (Feldman 1987; Ellenberger et al. 1990), and it has been suggested that the ventrolateral portion of the Sol also contributes to the control of respiration (Herbert et al. 1990). Moreover, the muscles controlling the patency of the upper airway are innervated by the hypoglossal (Lewis et al. 1971; Odutola 1976; Krammer et al. 1979) and dorsal motor vagus (Lewis et al. 1970) nuclei, and receive parabrachial input (Saper and Loewy 1980). On the basis of their connections with these respiratory and oral motor nuclei, it has been proposed that the parabrachial nuclei (particularly the KF) may contribute to the control of respiration (Herbert et al. 1990) and vocalization (Wild et al. 1990). While it seems unlikely that the PPN contributes directly to these functions, the present study can not rule out this possibility.

*Functional considerations*

The PPN has been implicated in motor control by virtue of its abundant connections with the basal ganglia and spinal cord projecting reticular nuclei, and by its colocalization within the physiologically identified mesencephalic locomotor region. Our data confirm that the descending PPN efferents distribute to the pontomedullary reticular nuclei and terminate mainly in the RPc, GiA, and GiV. These nuclei have been shown to project to somatic and autonomic motor columns in the medulla and spinal cord (Peterson 1980; Martin et al. 1981; Holstege and Kuypers 1982; Travers and Norgren 1983; Zemlan et al. 1984; Jones and Yang 1985; Kelland and Asdourian 1989), thus establishing a potential pathway by which PPN may affect motor behavior. The existence of a monosynaptic PPN-reticulo-spinal pathway has been proposed by Garcia-Rill and Skinner (1987a) on the basis of electrophysiological experiments. Since the reticular nuclei receiving input from the PPN project not only to the spinal cord but also to several motor and autonomic nuclei of the cranial nerves (Peterson 1980; Martin et al. 1981; Holstege and Kuypers 1982; Travers and Norgren 1983; Zemlan et al. 1984; Jones and Yang 1985), it is possible that the PPN may influence both the spinal and cranial motor systems indirectly, through a relay in the brainstem reticular formation. Clinical syndromes associated with neuronal loss in the PPN in humans seem to support this suggestion, since they invariably include disorders related to dysfunctions of the cranial nerves. In progressive supranuclear palsy (PSP), symptoms include unsteady gait, dysarthric or dysphonic speech, and impaired ocular movements, particularly in the vertical plane (Maher and Lees 1986; Jellinger 1988). Another syndrome associated with PPN cell loss is Meige syndrome, a rare disorder involving involuntary head turning, blepharospasm, and grimacing movements of the orofacial musculature (Tolosa and Marti 1988; Zweig et al. 1988).

In addition to the motor functions described above, the PPN has also been implicated in a wide variety of rhythmic behaviors including sleep-wake cycles, respiration, locomotion, and chewing (Garcia-Rill and Skinner 1988) as well as in sensory modulation (Basbaum and Fields 1980; Carstens et al. 1980; Katayama et al. 1984; Hylden et al. 1985). Furthermore, it has been suggested that the descending PPN projections to the reticular formation may play a role in the induction of motor atonia during REM sleep (Woolf and Butcher 1989; Jones 1990) and in the regulation of blood pressure and heart rate (Yasui et al. 1990).

In the light of various lines of evidence, it is tempting to speculate that the PPN may represent a part of a complex substrate underlying orienting reflexes. While the precise pathways involved in the orienting reflex are far from clear, several contributing nuclei have been identified (Fig. 17). In particular, the deep layers of the SC are necessary to elicit orienting behaviors (Peterson 1980). It is possible that the orienting reflex, characterized by turning of the head, pinnae and eyes toward a novel stimulus, may be executed via deep tectal efferents projecting directly to appropriate motor structures. Intermediate and deep layers of the SC project to the upper cervical segments, innervating axial musculature required for head turning (Huerta and Harting 1982),

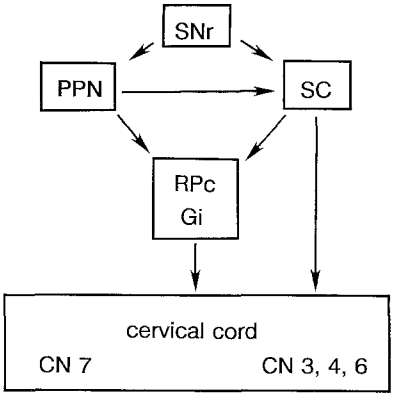


**Fig. 17.** Circuit diagram illustrates connections of some of the structures thought to be involved in the orienting reflex

to the cranial motor nuclei involved in extraocular and pinnae movements (Graham 1977; Keller 1979; Vidal et al. 1988) and to the pontomedullary reticular formation projecting to these cranial motor nuclei (Kawamura and Hashikawa 1978; Takeuchi et al. 1979; Edwards 1980; Panneton and Martin 1983; Isokawa-Akesson and Komisaruk 1987; Fort et al. 1989). Since the PPN sends descending projections to these same reticular nuclei (i.e., the medioventral RPc, GiA, and GiV), it is well situated to modulate neural activity occurring during the orienting response.

On the other hand, the SNr is in a position to influence both the SC and the PPN, which project either directly or indirectly to the motor nuclei required for execution of the orienting response. The SNr projects substantially to the ipsilateral deep layers of the SC (see Williams and Faull 1988 for references) as well as to the ipsilateral PPN. There seem to be considerable interconnections within this circuitry. In this regard, it is of interest to note that the PPN also sends input to the SC (Beninato and Spencer 1986; Woolf and Butcher 1986; Hall et al. 1989). Thus, nigral efferents may assist in coordinating the influence of the SC and PPN on the medial RPc, GiA, and GiV reticular nuclei.

Physiological observations of directionally specific behavioral abnormalities following unilateral lesions of the rat PPN (Kilpatrick and Starr 1981) also support a possible role of the PPN in the orienting reflex. Because of the multiplicity of nuclei involved in control of the orienting response, and the complexity of interconnections among them, further detailed studies will be required to elucidate the role of the PPN in the orienting reflex as well as other functions.

## Abbreviations

| | |
|---|---|
| *Am* | ambiguus nucleus |
| *AP* | area postrema |
| *Ac 7* | accessory facial nucleus |
| *asc 7* | ascending fibers, facial nerve |
| *CG* | central gray |
| *Cnf* | cuneiform nucleus |
| *Cu* | cuneate nucleus |
| *cp* | cerebral peduncle |
| *g7* | genu facial nerve |
| *Gi* | gigantocellular reticular nucleus |
| *GiA* | gigantocellular reticular nucleus, pars alpha |
| *GiV* | gigantocellular reticular nucleus, pars ventralis |
| *Gr* | gracile nucleus |
| *IC* | inferior colliculus |
| *icp* | inferior cerebellar peduncle |
| *IO* | inferior olive |
| *IRt* | intermediate reticular nucleus |
| *KF* | Kölliker-Fuse nucleus |
| *LC* | locus coeruleus |
| *ll* | lateral lemniscus |
| *vsc* | ventral spino-cerebellar tract |
| *xscp* | decussation of superior cerebellar peduncle |
| *3* | oculomotor nucleus |
| *4* | trochlear nucleus |
| *6* | abducens nucleus |
| *5n* | trigeminal nerve |
| *7* | facial nucleus |
| *7n* | facial nerve |
| *10* | dorsal motor nucleus of vagus |
| *12* | hypoglossal nucleus |
| *MPB* | medial parabrachial nucleus |
| *MVe* | medial vestibular nucleus |
| *PCRt* | parvicellular reticular nucleus |
| *PN* | pontine nucleus |
| *PPNc* | pedunculopontine tegmental nucleus, pars compacta |
| *PPNd* | pedunculopontine tegmental nucleus, pars dissipata |
| *Pr5* | principal sensory trigeminal nucleus |
| *py* | pyramidal tract |
| *pyx* | pyramidal decussation |
| *Rmes* | mesencephalic reticular nucleus |
| *RN* | red nucleus |
| *RPc* | reticularis pontis caudalis nucleus |
| *Rpo* | reticularis pontis oralis nucleus |
| *RR* | retrorubral nucleus |
| *RRF* | retrorubral field |
| *rs* | rubrospinal tract |
| *SC* | superior colliculus |
| *scp* | superior cerebellar peduncle |
| *LPB* | lateral parabrachial nucleus |
| *LPGi* | lateral paragigantocellular reticular nucleus |
| *LRt* | lateral reticular nucleus |
| *LSO* | lateral superior olive |
| *LVe* | lateral vestibular nucleus |
| *MdD* | medullary reticular nucleus, dorsal |
| *MdV* | medullary reticular nucleus, ventral |
| *Me5* | mesencephalic trigeminal nucleus |
| *me5* | mesencephalic trigeminal tract |
| *ml* | medial lemniscus |
| *mlf* | medial longitudinal fasciculus |
| *Mo5* | motor trigeminal nucleus |
| *SNc* | substantia nigra, pars compacta |
| *SNr* | substantia nigra, pars reticulata |
| *SO* | superior olive |
| *Sol* | nucleus of the solitary tract |
| *sol* | solitary tract |
| *sp5* | spinal trigeminal tract |
| *Sp5o* | spinal trigeminal nucleus, pars oralis |
| *SPTg* | subpeduncular tegmental nucleus |
| *SpVe* | spinal vestibular nucleus |
| *Tz* | nucleus of the trapezoid body |
| *tz* | trapezoid body |
| *VLL* | ventral nucleus of the lateral lemniscus |

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