Subcortical Afferents to the Nucleus Reticularis Tegmenti Pontis in the Rabbit: A Retrograde Horseradish Peroxidase Study

By

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Summary: The subcortical afferent projections to the nucleus reticularis tegmenti pontis (RT) were identified from the retrograde transport of horseradish peroxidase (HRP). HRP was injected into the medial and lateral parts of the RT, respectively. The major afferent inputs to the RT were found to arise ipsilaterally from the nucleus of the fields of Forel, the medial division of the medial mammillary nucleus, the region lateral to the red nucleus, and the posterior commissural nucleus; contralaterally from the anterior pretectal nucleus, the deep layer of the superior colliculus, and the supragenual nucleus; and bilaterally from the lateral habenular nucleus, the retrorubral nucleus, the central and posterior nuclei of the interpeduncular complex, the gray matter along the floor of the fourth ventricle, and the prepositus hypoglossi nucleus. Regions containing fewer labeled cells included the premammillary nucleus, the zona incerta, the nucleus pontis centralis oralis and the medial vestibular nucleus. In addition, the medial division of the medial mammillary nucleus, the lateral habenular nucleus, the central and posterior nuclei of the interpeduncular complex, and the supragenual nucleus were found to project mainly to the medial part of the RT. The superior colliculus, the retrorubral nucleus and the gray matter along the floor of the fourth ventricle projected mainly to the lateral part of the RT.

These results indicate that the medial part of the RT receives fibers mainly from the diencephalic limbic areas, whereas the lateral part of the RT receives fibers mainly from non-limbic (somatomotor, visuomotor and vestibular) areas.

The nucleus reticularis tegmenti pontis (RT) of the rabbit is situated lateral to the central superior nucleus, dorsal to the pontine gray proper, and ventromedial to the nucleus pontis centralis oralis. It is well known that the RT sends fibers to the cerebellar cortex and deep cerebellar nuclei (Chan-Palay, 1977; Brodal, 1980b, 1982; Blanks et al., 1983), and also receives fibers from the sensori-

motor cerebral cortical areas (Brodal and Brodal, 1971). The topographical organization of the cortico-ponto-cerebellar projections has thus been recognized (Brodal, 1980a).

On the other hand, recent anatomical studies employing anterograde autoradiographic methods, have suggested that subcortical afferent projections to the RT

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arise from the mammillary nuclei (Cruce, 1977, Veazey et al., 1982), the habenular nuclei (Herkenham and Nauta, 1977), the superior colliculus (Holstege and Collewyn, 1982), the pretectal nuclei (Berman, 1977), the zona incerta (Ricardo, 1981), and the vestibular nuclei (Gerrits et al., 1985). However, there are few comprehensive studies on the afferent projections to the RT, and some discrepancies exist among different authors concerning the details of these connections. Such discrepancies appear to have arisen partly because the habenular, tectal and vestibular inputs project not only to the RT but also to the central superior nucleus (Aghajanian and Wang, 1977; Zyo et al., 1978; Hayakawa and Zyo, 1982; Holstege and Collewyn, 1982) and the nucleus pontis centralis oralis (Kawamura et al., 1974; Graham, 1977; Gerrits et al., 1985), and partly because the degeneration method cannot avoid involvement of fibers-of-passage.

The present study was carried out in order to investigate the original subcortical nuclei projecting to the RT using a retrograde horseradish peroxidase (HRP) method, and to determine whether the projections to the RT are topographically organized.

Materials and Methods

In total, 28 adult female rabbits weighing 2.0-2.5 kg were used in this study. All surgical procedures were carried out under pentobarbital (30 mg/kg, i.v.) anesthesia.

The animals were placed in a Kopf stereotaxic apparatus and HRP conjugated with wheat-germ-agglutinin (WGA-HRP) or non-conjugated HRP (Sigma, Type VI) was injected into the pontine tegmental region by microinjection or microiontophoresis (Gonatas et al., 1979). For the microinjection, 20-40 nl of 30% HRP or 2% WGA-HRP (in 0.1 M Tris buffer, pH 8.6) was introduced through a 1 μ l Hamilton

syringe over a period of 10 min. For the iontophoretic injection, a fine tip glass pipet (O.D. 10-50 μ m) filled with 10% HRP was inserted, and the HRP was injected with a current of 2-4 μ A applied in an alternating mode (5s on/5s off) for 4-6 min according to the procedure of Graybiel and Devor (1974). The pipet was kept in situ for an additional 20 min after the injection in order to restrict the injection site.

Following 20-48 hours survival, the animals were perfused with 0.9% NaCl followed by 1.25% glutaraldehyde-1% paraformaldehyde (in 0.1 M P-buffer, pH 7.4), and finally with cold 10% buffered sucrose solution. The brains were removed and stored in phosphate buffer with 10% sucrose at 4°C until sectioning. Serial 40 μ m-thick frozen frontal sections were cut and every third section was reacted with tetramethyl benzidine (TMB) according to the method of Mesulam (1978). All reacted sections were counterstained with neutral red, and HRP-labeled cells were observed under bright-field or darkfield illumination. For cytoarchitectonic studies, serial 15 μ m-thick frontal or parasagittal sections of the formalin-fixed brain were stained by cresyl violet or Klüver-Barrera's method. The cytoarchitectural subdivisions and nomenclature of the rabbit brain were based primarily on the descriptions of Winkler and Potter (1911), Berman (1977) and Gerhard and Olszewski (1969).

Results

Control injections

HRP or WGA-HRP was injected into the central superior nucleus (cases G209 and G235), and the nucleus pontis centralis oralis (cases G225 and G230) as control experiments.

In the two cases of HRP injection into the central superior nucleus, the HRP de-

Fig. 1. Schematic diagrams of frontal sections showing the central parts of the injection sites in different regions of the pontine tegmental area. Shaded areas indicate injection sites, and thick lines indicate pipet tracks.

positis were found to be restricted within the ventral part (G209) or the dorsal part (G235) of the central superior nucleus (Figs. 1A and B). In these cases, many HRP-labeled cells were present bilaterally in the lateral habenular nucleus and the medial habenular nucleus, as well as several cells in the paraventricular nucleus of the thalamus (Fig. 2C). The labeled cells in the medial habenular nucleus were lay along the borders of the lateral habenular nucleus (Fig. 8). There were many labeled cells in the posterior nucleus of the interpeduncular complex (IP), and several labeled cells in the central and paramedian nuclei of the IP (Figs. 2D and E). Several labeled cells were also found in the nucleus of the diagonal band of Broca, the lateral hypothalamic nucleus, the dorsal raphe nucleus, the latero-dorsal tegmental nucleus, the nucleus pontis centralis oralis, the gray matter along the floor of the fourth ventricle, and the medial vestibular nucleus (Figs. 2A-I). A few labeled cells were present in the preoptic area and the retrorubral nucleus, but no labeled cells were observed in the mammillary, the subthalamic, the pretectal, or the perihypoglossal region (Table 1).

Slightly large WGA-HRP injections were made into the nucleus pontis centralis oralis

in cases G225 and G230. The center of the WGA-HRP deposits was more rostromedial in case G230 (Fig. 1A) than in case G225 (Fig. 1D). The distribution of WGA-HRP deposits in both cases was extended within the nucleus pontis centralis oralis. In case G225, many WGA-HRP-labeled cells were found ipsilaterally in the nucleus of the fields of Forel, contralaterally in the anterior pretectal nucleus, the lateral one-third of the deep layer of the superior colliculus, and the nucleus pontis centralis oralis; and bilaterally in the prepositus hypoglossi nucleus (Table 1). A few labeled cells were present in the ipsilateral nucleus of the posterior commissure, while sporadic but consistent labeled cells were observed ipsilaterally in the interstitial nucleus of Cajal, the nucleus of Darkschewitsch, and the retrorubral nucleus, and bilaterally in the medial vestibular nucleus. In case G230, there were also labeled cells in the bilateral midbrain periaqueductal gray matter, and just a few in the lateral hypothalamic nucleus, and the lateral habenular nucleus ipsilaterally (Table 1).

Injections into the RT

In the experiments involving large WGA-HRP injections, the WGA-HRP deposits were centered in the ventral part of the

Fig. 2. Schematic drawings of the distribution of HRP-labeled cells after injection into the central superior nucleus in case G209. The shaded area indicates the HRP injection site, and each dot indicates a few labeled cells.

Table 1. Distribution of HRP-labeled Cells.

The densities of labeled cells were as follows: +=0-1 cell per section; ++=2-5 cells per section; +++ = 6-10 cells per section; ++++ = more than 10 cells per section.

Area	Injection Sites									
	Nucleus reticularis tegmenti pontis					Control injection				
	G134	G246	G234	G242	G232	G223	G229	G209	G235	G225 G230
N. diagonal band of Broca	++	+						++	+	
Preoptic area	++	++						+		
Lateral hypothalamic n.	++++	+++			+	+	+	++		+
Premammillary n.	++	+			+	+				
Medial mammillary n.	++++	++++	+++	++						
Lateral mammillary n.	++	+								
Medial habenular n.	+++	+						+++	++	
Lateral habenular n.	++++	+++	+++	+++				++++	++++	+
Paraventricular n. of thalamus	+++	++						++	++	
N. of fields of Forel	++++	++++	++	++	++++	+++	++++			+++ ++
Zona incerta	++	+++	+		+					
Region lateral to red nucleus	++	+++	++		+++		++			+
Midbrain periaqueductal gray matter	+++	++					+			++
Posterior commissural n.	+	++++	+	+	++	++	+++			++
Anterior pretectal n.	++	++++	++	++	+++	++	++		+++	+++
Superior colliculus	++	+++	+		++	+	+		+++	++
Interstitial n. of Cajal	++	+								+
N. Darkschewitsch	++	+					+			+
Central n. of IP	++++	++	++	+				++	+	
Paramedian n. of IP	++++	++						++	++	
Posterior n. of IP	++	++	++	+				+++	++	
Retrorubral n.	+++	+++			+++	+	+++	+		+++
N. pontis centralis oralis	+++	+++			+	+		++	+	++++ ++
N. raphe dorsalis	++++							++		
Laterodorsal tegmental n.	++++	++						+++	++	
Gray matter along floor of fourth ventricle	++++	+++	+	+	++	++	++	++	+	
Medial vestibular n.	+	++	+	+	+	+	+	++	++	+
Supragenual n.	+++	++++	++	++	+++					
Prepositus hypoglossi n.	++++	++++	++	++	+++	++	+++			+++ ++

central superior nucleus (G134) (Figs. 1C and 5A) or the ventromedial part of the RT (G246) (Fig. 1B). In both cases, the WGA-HRP deposits extended bilaterally into the ventral part of the central superior nucleus, the RT, the lemniscus medialis, the dorsal part of the medial pontine nucleus, and the ventromedial part of the nucleus pontis centralis oralis. Numerous WGA-HRP-labeled neurons were found bilaterally in the medial mammillary nucleus (Figs. 6 and 7), and a few labeled cells were present in the lateral mammillary nucleus and the pre-mammillary nucleus (Table 1). According to Bleier's (1969) nomenclature, the medial mammillary nucleus of the rabbit is divided into medial, intermediate, basal, and posterior divisions. In this case, many labeled cells were found only in the bilateral medial divisions, and they formed a V-shape (Fig. 6). There were many labeled cells in the lateral habenular nucleus, the lateral part of the medial habenular nucleus, the nucleus of the fields of Forel (Fig. 9), and the zona incerta. In the medial habenular nucleus, many labeled cells were detected in case G134, but only a few were found in case G246 (Table 1). There were also labeled cells in the nucleus of the diagonal band of Broca, the preoptic area, the lateral hypothalamic nucleus, and the nucleus paraventricularis thalami.

In the midbrain, many labeled cells occurred in the nucleus of the posterior commissure, the anterior pretectal nucleus, the superior colliculus, mainly the lateral one-third of the deep layer, the region lateral to the red nucleus, the rostral part of the midbrain periaqueductal gray matter, and the central, paramedian and posterior nuclei of the IP, but no labeled cells were found in the nucleus of the optic tract. A few labeled cells were found in the interstitial nucleus of Cajal and the nucleus of Darkschewitsch. There were also many labeled cells in the prepositus hypoglossi nucleus, the supragenual

nucleus, the gray matter along the floor of the fourth ventricle, and the laterodorsal tegmental nucleus, several cells in the retrorubral nucleus, and the nucleus pontis centralis oralis; and just a few cells in the medial vestibular nucleus. In the nucleus raphe dorsalis, many labeled cells were present in case G134 but no labeled cells were observed in case G246 (Table 1).

Restricted HRP injections were made into the medial part of the RT in cases G234 and G242. The HRP deposits were found to be restricted within the unilateral mediodorsal part of the RT, and did not extend to the central superior nucleus (Figs. 1B, C and 5B). In case G234 (Fig. 3), many HRP-labeled cells were observed ipsilaterally in the medial division of the medial mammillary nucleus, and the nucleus of the fields of Forel; and bilaterally in the lateral habenular nucleus (Figs. 3A and B). Several labeled cells were detected in the central and posterior nuclei of the IP (Figs. 3D and 10), the ipsilateral region lateral to the red nucleus, the contralateral anterior pretectal nucleus (Fig. 3C), the contralateral supragenual nucleus, and the bilateral prepositus hypoglossi nucleus (Figs. 3F and G). There were also a few labeled cells ipsilaterally in the zona incerta, and the nucleus of the posterior commissure, contralaterally in the deep layer of the superior colliculus, and bilaterally in the gray matter along the floor of the fourth ventricle, and the medial vestibular nucleus. In case G242, no labeled cells were found in the zona incerta and the region lateral to the red nucleus (Table 1).

Experiments in which the HRP deposits involved the lateral part of the RT and the ventral edge of the nucleus pontis centralis oralis included case G232, G223 and G229 (Figs. 1C, D and 5C). In case G232 (Fig. 4), many HRP-labeled cells were found ipsilaterally in the nucleus of the fields of Forel, and the region lateral to the red nucleus; contralaterally in the anterior pretectal nucleus

G234

Fig. 3. Schematic drawings of the distribution of HRP-labeled cells after injection into the medial part of the nucleus reticularis tegmenti pontis (RT) in case G234. The shaded area indicates the HRP injection site, and each dot indicates a few labeled cells.

(Figs. 4B-D), and the superagenual nucleus (Fig. 13); and bilaterally in the retrorubral nucleus (Fig. 11) and the prepositus hypoglossi nucleus (Fig. 12). Several labeled cells were present in the ipsilateral nucleus of the posterior commissure, the contralateral deep layer of the superior colliculus (Figs. 4C and D), and the bilateral gray matter along the floor of the fourth ventricle (Fig.

4H). Only a few but consistently labeled cells were detected in the lateral hypothalamic nucleus, the premammillary nucleus and the medial vestibular nucleus ipsilaterally. No labeled cells were found in the mammillary nuclei, the habenula and the IP. In cases G223 and G229, the HRP deposits in the RT were centered more laterally than those of G232. The labeling patterns were

Fig. 4. Schematic drawings of the distribution of HRP-labeled cells after injection into the lateral part of the RT in case G232. The shaded area indicates the HRP injection site, and each dot indicates a few labeled cells.

almost the same in these cases, but no labeled cells were observed in the zona incerta and the supragenual nucleus (Table 1).

Discussion

The present results demonstrate that the RT receives its main inputs ipsilaterally from the medial division of the medial mammillary nucleus, the nucleus of the fields of Forel, the region lateral to the red nucleus, and the posterior commissural nucleus; contralaterally from the anterior pretectal nucleus, the deep layer of the superior colliculus, and the supragenual nucleus; and bilaterally from the lateral habenular nucleus, the central and posterior nuclei of the IP, the gray matter along the floor of the fourth ventricle, and the prepositus hypoglossi nucleus.

Although uninjured axons of fibers-of-passage may not appear to take up and transport free HRP (FitzGibbon et al., 1983), some problems do arise in the interpretation of the present findings for HRP labeled cells (Herkenham and Nauta, 1977). Brodal et al. (1983) have reported that the number of retrogradely WGA-HRP labeled cells taken up from the fibers-of-passage was much smaller than that after similar free HRP injections. When large WGA-HRP injections were made into the RT in the present study, the injection sites were extended to the fibers of the lemniscus medialis. In these cases, there were no labeled cells in the gracile or cuneate nuclei. In addition, when WGA-HRP cases were compared with HRP ones, the overall distribution of retrograde cell labeling was found to be similar (Table 1). Therefore, uptake of HRP from fibers-of-passage may not contribute significantly to the overall afferent pattern of the RT.

When large WGA-HRP injections were made into the RT, labeled cells were found in the nucleus of the diagonal band of Broca,

the preoptic area, the lateral hypothalamic nucleus, the medial habenular nucleus, the paraventricular nucleus of the thalamus, the paramedian nucleus of the IP, the dorsal raphe nucleus and the laterodorsal tegmental nucleus. However, all these nuclei were labeled in the case of either a large injection including the central superior nucleus or restricted injections into the central superior nucleus, but no labeled cells were observed in the case of restricted injections into the RT. These nuclei are therefore considered to project not to the RT but to the central superior nucleus.

Many authors have reported that the lateral habenular nucleus projects to the pontine tegmentum in the rat (Herkenham and Nauta, 1979), cat (Akagi and Powell, 1968; Zyo et al., 1978), rabbit (Cragg, 1961), opossum (Way and Kaelber, 1969; Smaha and Kaelber, 1973), and wallaby (Way, 1975). The present findings also indicate that the medial part of the RT and the central superior nucleus receive fibers from the lateral habenular nucleus, but neither the lateral part of the RT nor the nucleus pontis centralis oralis receive lateral habenular inputs. The lateral habenular nucleus thus projects to the paramedian pontine tegmental region including the medial pontine nucleus, the medial part of the RT, the central superior nucleus, the ventral and the dorsal tegmental nuclei of Gudden, the dorsal raphe nucleus, the laterodorsal tegmental nucleus, and the nucleus of the locus caeruleus.

The descending fibers from the medial habenular nucleus terminate at the IP in a topographical manner in the rat (Marchand et al., 1980; Contestabile and Flumerfelt, 1981) and the cat (Hayakawa and Zyo, 1982). In the present study, neurons in the lateral border of the medial habenular nucleus were also labeled after HRP injection into the central superior nucleus. Herkenham and Nauta (1979), in their an-

terograde autoradiographic tracing study in the rat, reported sparse projections from the medial habenular nucleus not to the RT but to the central superior nucleus. Descending projections from each of the subnuclei of the IP to the central superior nucleus and Gudden's tegmental nuclei have been reported in the rat (Liu et al., 1984; Shibata and Suzuki, 1984), rabbit (Hayakawa and Zyo, 1985) and cat (Smaha and Kaelber, 1973; Hayakawa et al., 1981). Our experiments also revealed that the medial part of the RT receives fibers from the central and posterior nuclei of the IP in the rabbit.

Our interesting finding in the present study was that many labeled cells were found only in the medial division of the ipsilateral medial mammillary nucleus after HRP or WGA-HRP injection into the medial part of the RT. Degenerating and autoradiographic tracing studies have demonstrated that the mammillary nuclei project to the pontine gray proper and the RT in the rat (Cruce, 1977), cat (Niimi et al., 1972), rabbit (Ban and Zyo, 1963), and cynomolgus monkey (Veazey et al., 1982). These antero-grade tracing studies did not show subnuclei of the medial mammillary nucleus projecting to the pontine tegmentum. When large WGA-HRP injections were made by us into the RT and the medial pontine nucleus, WGA-HRP-labeled cells were present in the lateral mammillary nucleus in cases G134 and G246. This suggests that the lateral mammillary nucleus projects not to the RT but to the medial pontine nucleus. Our previous experiments (Hayakawa and Zyo, 1984, 1985) have demonstrated that the medial division of the medial mammillary nucleus sends fibers to and receives fibers from the pars principalis of the ventral tegmental nucleus of Gudden, while the lateral mammillary nucleus sends fibers to and receives fibers from the dorsal tegmental nucleus of Gudden, ipsilaterally. Van der Kooy et al. (1978) reported in

the rat that the neurons of the medial part of the medial mammillary nucleus send fibers both to the ipsilateral anteroventral nucleus of the thalamus and into the mam-millo-tegmental tract. The neurons projecting into the mamillo-tegmental tract may thus send off collateral fibers, which terminate not only at the ventral tegmental nucleus of Gudden, but also at the medial part of the RT.

The RT is cytoarchitectonically clearly distinct from the nucleus pontis centralis oralis, but both the RT and the nucleus pontis centralis oralis receive many fibers from the nucleus of the fields of Forel, the zona incerta, the retrorubral nucleus, and the region lateral to the red nucleus. Many reports have indicated that the mid-brain and pontine reticular formation including the nucleus pontis centralis oralis and the RT, receive fibers from the nucleus of the fields of Forel and the zona incerta in the rat (Ricardo, 1981; Watanabe and Kawana, 1982; Shammah-Lagnado et al., 1983), cat (Kaelber and Smith, 1979) and rhesus monkey (Schnyder et al., 1985), but the RT received less fibers from the zona incerta in the present study. The major subcortical afferents to the medullary paramedian reticular nuclei are from the interstitial nucleus of Cajal, the nucleus of Darkschewitsch and the midbrain and pontine reticular formation (Elisevich et al., 1985). The RT receives few fibers from these nuclei, so that the RT may be included not in the medullary but in the pontine reticular core.

Although many authors have demonstrated the pretectal and superior collicular projections to the RT and the pontine reticular formation (Kawamura et al., 1974; Berman, 1977; Graham, 1977; Itoh, 1977; Kawamura and Hshikawa, 1978; Holstege and Collewyn, 1982), some discrepancies do exist concerning the original nuclei of the tecto-reticular projections. This is

partly because there are many subnuclei in the pretectum and layers in the superior colliculus (Kanaseki and Sprague, 1974), or partly because injection sites of tritium-labeled amino acids are liable to involve several subnuclei when using the anterograde autoradiographic tracing method. The present findings demonstrated that the anterior pretectal nucleus and the deep layer of the superior colliculus project contralaterally to the RT, while the nucleus of the posterior commissure projects ipsilaterally to the RT. On the other hand, recent electrophysiological studies have indicated that the RT neurons were antidromically activated from the flocculus and orthodromically activated from the optic tracts, so that the RT neurons could transfer visual signals to the flocculus (Maekawa et al., 1981; Miyashita and Nagao, 1984). However, the RT was not found to receive direct projections from the nucleus of the optic tract in the present experiments or in other autoradiographic anatomical studies (Berman, 1977; Itoh, 1977; Holstege and Collewyn, 1982). The RT also has connections with the prepositus hypoglossi nucleus and the supragenual nucleus as well as the flocculus (Brodal, A., 1952; Brodal, P., 1980b, 1982; Blanks et al., 1983), and less connections with the medial vestibular nucleus (Balaban, 1983; Gerritis, 1983). These results suggest that the RT, especially the medial part of the RT, may contribute to optokinetic movements through the cerebellar and vestibular nuclei as well as the diencephalic limbic nuclei.

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Abbreviations

a	: Anterior commissure	HM	: Medial habenular nucleus
AP	: Anterior pretectal nucleus	IM	: Intermediate division of medial mammillary nucleus
BM	: Basal division of medial mammillary nucleus	IP	: Interpeduncular complex
C	: Interstitial nucleus of Cajal	IPC	: Central nucleus of IP
CS	: Central superior nucleus of raphe	IPN	: Posterior nucleus of IP
D	: Nucleus of Darkschewitsch	IPP	: Paramedian nucleus of IP
DB	: Nucleus of diagonal band of Broca	LC	: Nucleus of locus caeruleus
F	: Nucleus of fields of Forel	LD	: Laterodorsal tegmental nucleus
flm	: Fasciculus longitudinalis medialis	LG	: Lateral geniculate body
HL	: Lateral habenular nucleus	LH	: Lateral hypothalamic nucleus

LL	: Ventral nucleus of lateral lemniscus	S	: Subthalamic nucleus
LM	: Lateral mammillary nucleus	SC	: Superior colliculus
lm	: Lemniscus medialis	SE	: Septal area
LP	: Lateral pontine nucleus	SG	: Supragenual nucleus
LR	: Region lateral to red nucleus	SN	: Substantia nigra
LV	: Lateral vestibular nucleus	tc	: Tractus corticospinalis
MG	: Medial geniculate body	TD	: Dorsal tegmental nucleus of Gudden
MM	: Medial division of medial mammillary nucleus	TDD	: Pars dorsalis of TD
MP	: Medial pontine nucleus	TDV	: Pars ventralis of TD
mt	: Fasciculus mamillothalamicus	TV	: Ventral tegmental nucleus of Gudden
MV	: Medial vestibular nucleus	TVP	: Pars principalis of TV
PC	: Nucleus of posterior commissure	TZ	: Nucleus of trapezoid body
PCO	: Nucleus pontis centralis oralis	Z	: Zona incerta
PH	: Prepositus hypoglossi nucleus	3	: Oculomotor nucleus
PM	: Premammillary nucleus	4	: Trochlear nucleus
PO	: Preoptic area	4VG	: Gray matter along floor of fourth ventricle
PV	: Nucleus paraventricularis thalami	5	: Nucleus motorius nervi trigemini
R	: Red nucleus	5M	: Nucleus mesencephalicus nervi trigemini
RD	: Dorsal raphe nucleus	5S	: Nucleus spinalis nervi trigemini
RP	: Rostroventral pontine nucleus	6N	: Abducens nerve
RR	: Retrorubral nucleus	7G	: Genu of facial nerve
RT	: Nucleus reticularis tegmenti pontis		

PLATES

Explanation of Figures

Plate I

- Fig. 5. Brightfield photomicrographs of injection sites. A, Large WGA-HRP injection site into the RT in case G134. B, HRP injection site into the medial part of the RT in case G234. C, HRP injection site into the lateral part of the RT in case G232.
- Fig. 6. Brightfield photomicrograph of numerous WGA-HRP-labeled cells in the medial division of the medial mammillary nucleus (MM) after WGA-HRP injection into the RT in case G134.
- Fig. 7. Darkfield photomicrograph of WGA-HRP-labeled cells in the medial division of the medial mammillary nucleus at the same section as in the Fig. 6. Scale bar: 100 μm .
- Fig. 8. Darkfield photomicrograph of HRP-labeled cells in the habenular complex in case G209. Arrows indicate HRP-labeled cells in the lateral edge of the medial habenular nucleus (MH), and the lateral habenular nucleus (LH). Scale bar: 100 μm .

Plate II

Fig. 9. Brightfield photomicrograph of many HRP-labeled cells in the ipsilateral nucleus of the fields of Forel after injection into the RT in case G134. Scale bar: 100 μ m.

Fig. 10. Darkfield photomicrograph of HRP-labeled cells in the posterior nucleus of the interpeduncular complex after injection into the medial part of the RT in case G234. Arrows indicate HRP-labeled cells. Scale bar: 100 μ m.

Fig. 11. Darkfield photomicrograph of HRP-labeled cells in the contralateral retrorubral nucleus after injection into the lateral part of the RT in case G232. Arrows indicate HRP-labeled cells. Scale bar: 100 μ m.

Fig. 12. Brightfield photomicrograph of HRP-labeled cells in the ipsilateral nucleus prepositus hypoglossi after injection into the lateral part of the RT in case G232. Arrows indicate HRP-labeled cells. Scale bar: 100 μ m.

Fig. 13. Brightfield photomicrograph of HRP-labeled cells in the contralateral supragenual nucleus after injection into the lateral part of the RT in case G232. Scale bar: 50 μ m.