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Collateral projections of trigeminal sensory neurons to both cerebellar paramedian lobules in the rabbit: demonstration by fluorescent double labeling study

Dorota Bukowska[1], Leszek Zguczyński[2], Barbara Mierzejewska-Krzyżowska[2] and Eleonora Sikora[2]

[1]Department of Neurobiology, University School of Physical Education, 10 Droga Dębińska St., 61-555 Poznań; [2]Department of Morphological Sciences, University School of Physical Education, 13 Estkowski St., 66-400 Gorzów, Poland

Abstract. Neurons in the trigeminal sensory nuclei (TSN) were indicated to project simultaneously to the cerebellar paramedian lobule (PML) of both sides by way of axon collaterals in the rabbit. Injections of Fast Blue and Diamidino Yellow made in twelve rabbits into the regions of the left and right PML cortex, respectively, resulted in retrograde double labeling of neurons in the principal trigeminal (Vp) and spinal trigeminal nuclei including subnucleus oralis (Vo), and rostral (Vir) and caudal (Vic) subnucleus interpolaris. Sparse labeling was seen among the root fibers of the facial nerve immediately adjacent to Vp. The data indicated that out of the total population of ipsilateral and contralateral single labeled neurons in TSN subdivisions, double labeled neurons constituted about 11% in Vo, 2% in Vp and Vir, and 1% in Vic. Thus, it seems that out of TSN neurons sending collateral projections to PML of both hemispheres, those in Vo project more frequently than those in Vp, Vir and Vic. The homotopic (corresponding) middle (c and d) PML sublobules receive collateral projections from neurons of all these TSN subdivisions, whereas the caudal (a and b) and rostral (e and f) sublobules seem to be targets of the projection from Vp and Vir. The findings suggest that some TSN neurons may exert simultaneous bilateral modulatory influences upon the regions in various sublobules of both PML.

Key words: trigeminal sensory neurons, cerebellar paramedian lobule, collateral projection, rabbit, retrograde fluorescent tracing

INTRODUCTION

The trigeminal sensory nuclei (TSN), the main target of primary afferent fibers mediating pain and tactile signals from orofacial structures, contain neurons which project to various regions of CNS such as the thalamus (Silverman and Kruger 1985, Phelan and Falls 1991, Li and Mizuno 1997a,b), superior and inferior colliculi (Silverman and Kruger 1985, Li and Mizuno 1997b,c), parabrachial region (Hayashi and Tabata 1991), oculomotor and accessory abducent nuclei (Van Ham and Yeo 1996b), cochlear nucleus (Li and Mizuno 1997a,c), inferior olive (Van Ham and Yeo 1992, Yatim et al. 1996), hypoglossal nucleus (Aldes and Boone 1985) as well as the spinal cord (Hayashi et al. 1984, Phelan and Falls 1991) and cerebellum. According to cytoarchitectonic criteria (Olszewski 1950, Taber 1961) TSN is divided into the mesencephalic trigeminal nucleus (Vmes), principal trigeminal nucleus (Vp) and spinal trigeminal nucleus (Vs). From rostral to caudal, Vs is further subdivided into the subnucleus oralis (Vo), rostral (Vir) and caudal (Vic) subnucleus interpolaris (Vi) and subnucleus caudalis (Vc).

A trigeminocerebellar projection is well documented to originate from Vp, Vo and Vi and, to a lesser extent, from Vc and to terminate mainly in the vermal lobule IX, the simple lobule, crus I and crus II of the ansiform lobule and the paramedian lobule (PML) in the cat, rat and mouse (Somana et al. 1980, Matsushita et al. 1982, Steindler 1985, Phelan and Falls 1991, Yatim et al. 1996). Few data are available on the trigeminocerebellar projection to PML in the rabbit. With the anterograde and retrograde WGA-HRP technique the TSN-PML projection is shown to arise bilaterally from Vp, the caudal half of Vo and the rostral two-thirds of Vi (Van Ham and Yeo 1992). Recently in our laboratory we have supported these findings and, in addition, revealed details of projections from TSN neurons to the various regions of PML with emphasis on the climbing fiber zones in the rabbit (Bukowska 1996). In the present paper we give evidence of collateral projections from single TSN neurons to the various regions of PML cortex of both sides by means of retrograde double labeling technique. Such connections have not been demonstrated in any species.

METHODS

Experiments were performed under aseptic conditions on 12 adult rabbits weighing 2.0-3.5 kg. Surgical procedures were carried out under general anesthesia with ketamine hydrochloride (Calypsol, 1 mg/kg) and promazin (0.38 mg/kg) injected intramuscularly. After fixation of the head of the animal in a stereotaxic frame (Narishige), small craniectomies were made to expose selected PML sublobules of both sides. In each rabbit pressure injections of 4-5% Fast Blue (FB) and 2% Diamidino Yellow (DY) were made into the cortex of left and right PML sublobules, respectively, using a glass micropipette (tip diameter 30-60 μm) connected to a 5 μm Hamilton microsyringe for 10-15 min. Following an 8-15 day survival period, animals were deeply anesthetized and sacrificed by transcardiac perfusion with 0.9% NaCl mixed with heparin, followed by 4% formaldehyde in 0.4 M phosphate buffer (pH 7.4) and 10% sucrose in the same buffer. Brains were removed, placed in a 20% sucrose solution overnight and then cut in the sagittal

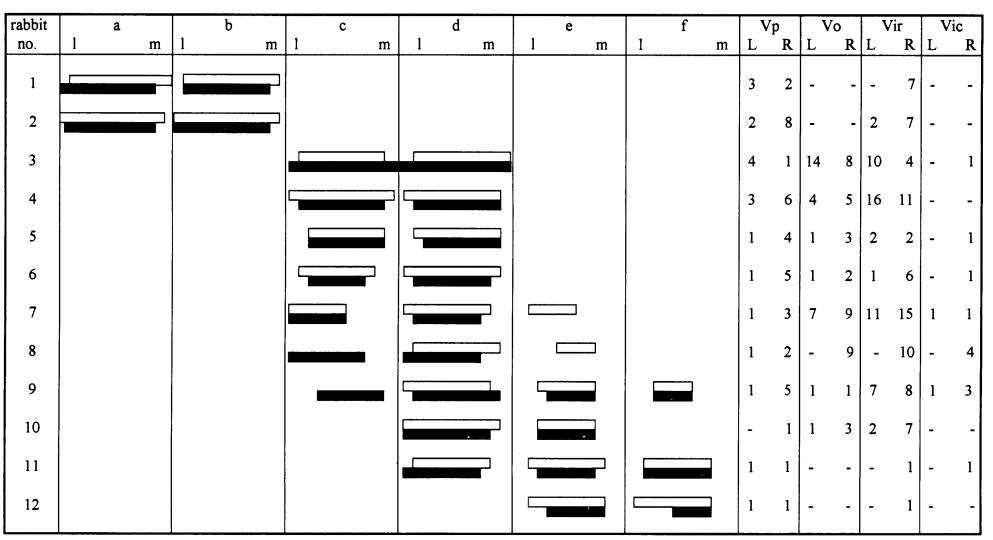

Fig. 1. Schematic representation of the mediolateral extent (length of bars) of FB (blank bars) and DY (black bars) injection sites of each case within the left (FB) and right (DY) PML sublobules a - f, and resulting number of double labeled neurons within TSN subdivisions. L, left side; R, right side; l, lateral side; m, medial side.

(cerebellum) and transverse (pons and medulla) planes on a freezing microtome (Reichert) at 40 μm thick. Serial sections were collected in phosphate buffer, mounted on chrom-alum gelatinized slides, coverslipped with Fluoromount (Serva) and examined under an epifluorescence microscope (Jenalumar, Carl Zeiss Jena and Optiphot-2, Nikon) at 410 nm light excitation wavelength. In each case, injection sites in PML were determined and reconstructed on diagrams of dorsal view and sagittal sections of PML, as well as locations of labeled neurons in TSN, which were transferred to diagrams of transverse sections of the pons and medulla. Criteria of defining borders between the TSN subdivisions were taken from the cytoarchitectonic atlas of the rabbit brainstem (Meessen and Olszewski 1949). Labeling with FB and/or DY was recognized by blue fluorescence in the neuroplasm and/or yellow fluorescence in the nucleus (Kuypers et al. 1980, Keizer et al. 1983). In each case, the numbers of both single and double labeled neurons were counted from all mounted serial sections. FB or DY single labeled neurons observed in all cases in the contralateral inferior olive served as a control for the effectiveness of uptake and retrograde transport of tracers (Zimny et al. 1989). The research reported herein was performed under guidelines established by the Declaration of Helsinki concerning the appropriate Care and Use of Animals in Research, and Polish Law on Animal Protection was respected.

RESULTS

Injection sites

Figure 1 illustrates the mediolateral extent of FB and DY injection sites within the sublobules of both PML and resulting retrograde double labeling (FB+DY-labeling) of trigeminal neurons within TSN subdivisions. All injections were restricted rostrocaudally to between two and four adjacent homotopic (corresponding) or, rarely, heterotopic (non-corresponding) PML sublobules of both sides (Fig. 2). In most cases, the injection site, including the diffusion area, covered superficial regions of the sublobule and did not extend deeper than the granular layer of the cortex. In three cases (nos. 3, 9 and 12) a little diffusion of tracers was seen in the white matter of the apical part of the sublobule. However, this incident had probably no influence on labeling pattern in TSN. Other cases where the tracers spread to deeper parts of sublobules were excluded from the present study.

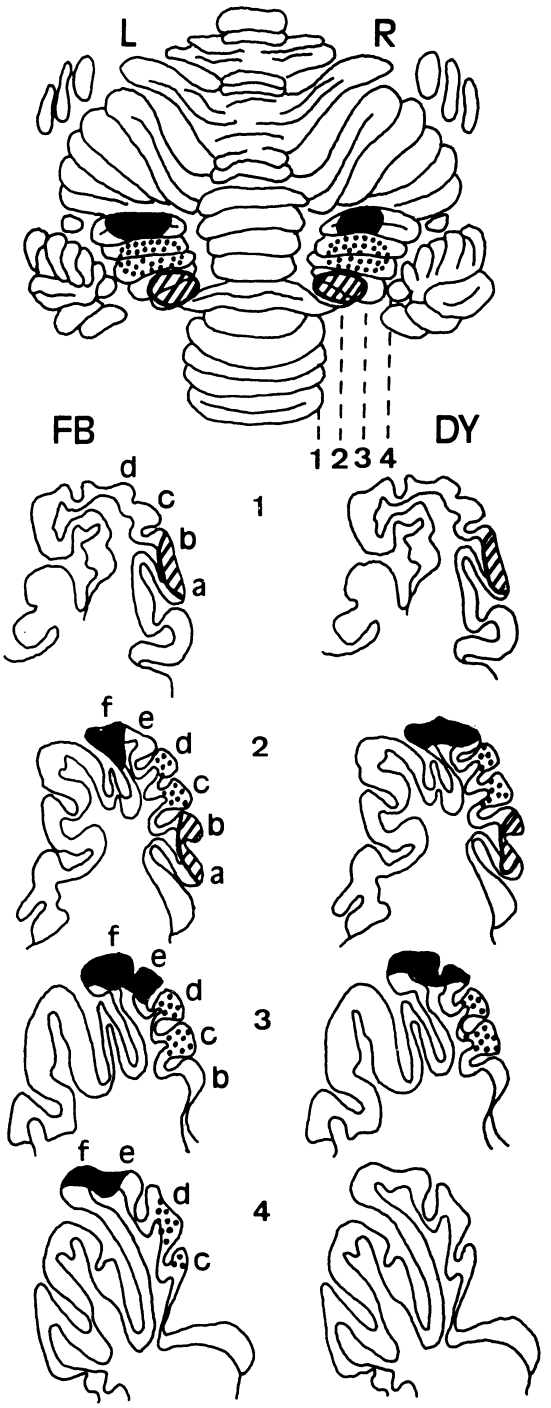

Fig. 2. Diagrams of dorsal view of the cerebellum (upper) and sagittal sections through the left (L) and right (R) PML showing the injection sites in the caudal (a, b), middle (c, d) and rostral (e, f) sublobules in cases no.1 (hatched area), no.4 (dotted area) and no.12 (black area), respectively.

TABLE I

Number of TSN single and double labeled neurons as a result of FB and DY injection into PML sublobules of both sides

Injections sites	Vp					Vo					Vir					Vic				
	single			double		single			double		single			double		single			double	
rabbit no.	i	c	total	total	%	i	c	total	total	%	i	c	total	total	%	i	c	total	total	%
a+b																				
1	140	98	238	5	2.1	4		4		0.0	236	120	356	7	2.0	101	38	139		0.0
2	185	113	298	10	3.4						301	102	403	9	2.2	111	30	141		0.0
c+d																				
3	160	14	174	5	2.9	99	28	127	22	17.3	619	64	683	14	2.0	81	12	93	1	1.1
4	232	53	285	9	3.2	52	12	64	9	14.0	701	85	786	27	3.4	66	8	74		0.0
5	111	20	131	5	3.8	38	6	44	4	9.1	312	48	360	4	1.1	49	8	57	1	1.8
6	125	29	154	6	3.9	27	5	32	3	0.0	397	67	464	7	1.5	47	9	56	1	1.8
c-f combination																				
7	99	35	134	4	3.0	62	21	83	16	19.3	608	92	700	26	3.7	57	13	70	2	2.9
8	114	38	152	3	2.0	69	26	95	9	9.5	462	64	526	10	1.9	78	15	93	4	4.3
9	398	95	493	6	1.2	22	8	30	2	6.7	623	156	779	15	1.9	133	26	159	4	2.5
10	166	24	190	1	0.5	30	14	44	4	9.1	312	59	371	9	2.4	64	10	74		0.0
11	261	103	364	2	0.5	48	12	60		0.0	337	61	398	1	0.3	70	9	79	1	1.3
e+f																				
12	110	51	161	2	1.2	35	6	41		0.0	226	30	256	1	0.4	54	9	63		0.0
total after all injections			2774	58	2.1			624	69	11.1			6082	130	2.1			1098	14	1.3

In each rabbit the number of neurons was counted from all serial sections of TSN; i, ipsilateral; c, contralateral; blank, no labeling.

Pattern of labeling

Among numerous single labeled neurons in TSN, much smaller numbers of neurons were double labeled in bilateral Vp, Vo, Vir and Vic (Table I and Fig. 1). They were distributed in these TSN regions where single labeled neurons were present. No single and double labeling was observed in Vc or Vmes.

Single labeled neurons were seen bilaterally with a clear ipsilateral preponderance. Considering all cases of injections, they appeared the most numerously in Vir (6082 cells). Their total number in Vp, Vic and Vo was lower (2774, 1098 and 624 cells, respectively). The number of single labeled neurons in Vir ranged from 256 (injection into e + f sublobules) to 786 (injection into c + d sublobules), in Vp from 131 (injection into c + d sublobules) to 493 (injection into c - f sublobules), in Vic from 56 (injection into c + d sublobules) to 159 (injection into c - f sublobules) and in Vo from 0 (injection into a + b sublobules) to 127 (injection into c + d sublobules). It seems that more or less variations in the number of both single and double labeled neurons observed in the individual cases arise from differences in the mediolateral and rostrocaudal extents of injections within individual PML sublobules. Since distribution of single labeled neurons after unilateral injection of FB, DY or HRP into PML was described previously (Bukowska 1996), it was omitted here to avoid unnecessary repetitions. Thus, only FB+DY-labeled neurons were taken into account in the present study.

With regard to all cases of injections the total number of double labeled neurons was the highest in Vir (130 cells) and lower in Vo, Vp and Vic (69, 58 and 14 cells, respectively). In Vp labeled neurons were found

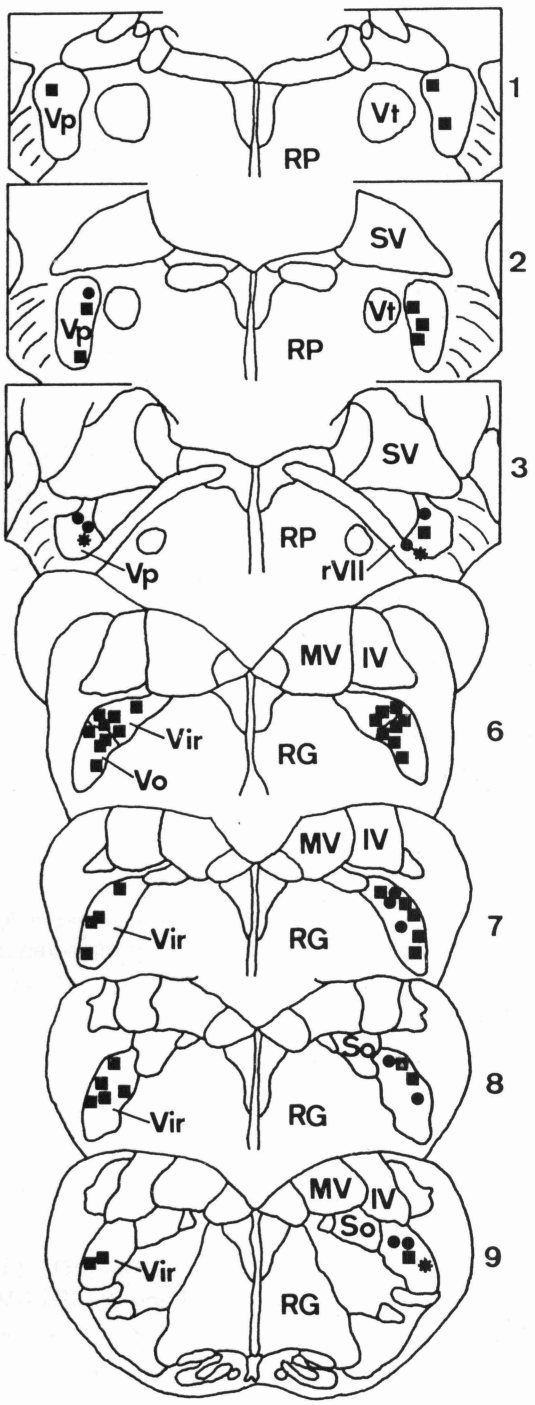

Fig. 3. Distribution of double labeled neurons in TSN as a result of injections in the left (FB) and right (DY) PML (Fig. 2) showed on diagrams of transverse sections through the pons and medulla from rostral to caudal, in cases no.12 (asterisk), no.1 (circles) and no.4 (squares). The levels of sections correspond to those in Fig. 5. Each symbol represents one double labeled cell body.

throughout its entire length apart from the caudal- (level 4) and rostral-most poles (Figs. 3 and 5). Most frequently they appeared in the central part (levels 2-3) occupying mainly medial locations (Fig. 4A). In some cases (nos. 1, 2, 7 and 12) labeled neurons occurred among the root fibers of the facial nerve immediately adjacent to Vp. Labeling in Vo was confined to its caudal half with the

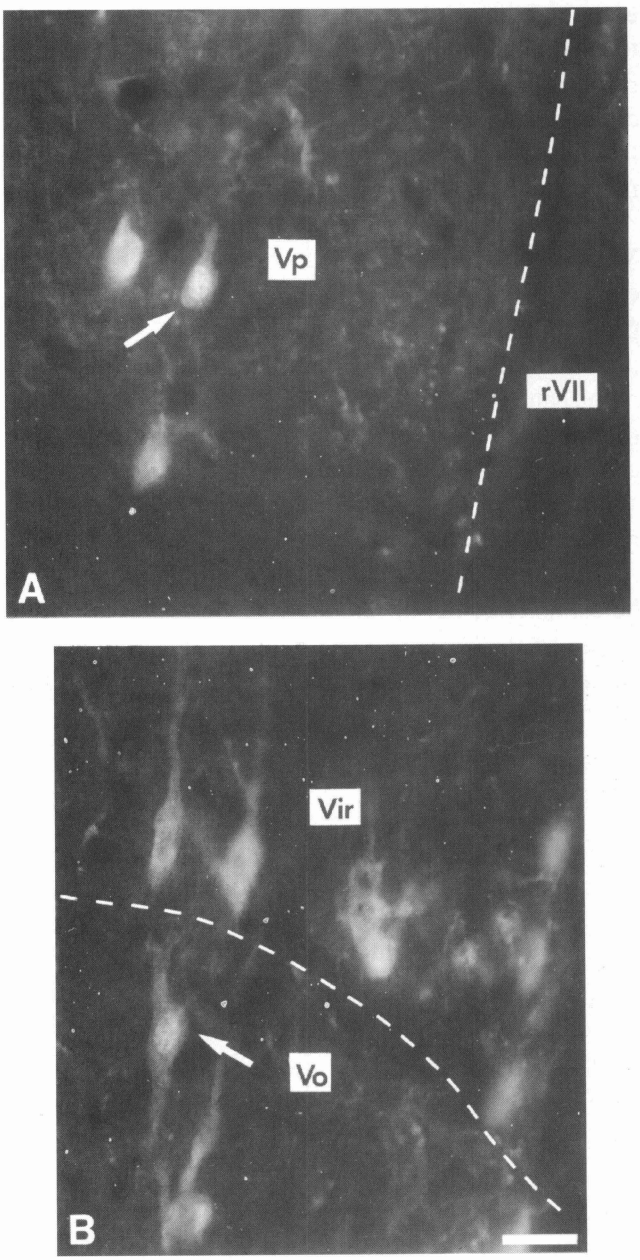

Fig. 4. Photomicrographs of FB+DY- labeled neurons (indicated by arrows) in the left (A) Vp between the levels 2 and 3 in case no.1, and (B) Vo near the level 6 in case no.7. Note filled fluorescing cytoplasm (blue) and brightly fluorescing nucleus (yellow). Bar for A and B represents 40 μm.

exception that single FB+DY-labeled neurons were found in addition more rostrally (level 5) in cases nos. 3 and 8. Within the caudal half of Vo, they were seen scattered throughout the nucleus at more rostral levels (between levels 5 and 6) while they tended to be distributed in the dorsal and dorsomedial regions bordering to Vir at more caudal levels (from level 6) (Fig. 4B). In most cases labeled neurons in Vir were present throughout the entire rostrocaudal extent of the nucleus with preference to the dorsal and lateral locations. However, some cases resulted in no labeling in the rostral (nos. 1 and 12) and caudal (nos. 3 and 6) poles of Vir. As regards Vic, a weak labeling was found in its rostral aspect (level 10), mainly in the medial and lateral regions (Fig. 5).

Of the total population of single labeled neurons in Vp, Vo, Vir and Vic, double labeled neurons in these nuclei constituted 0.5 - 3.9% (average 2.1%; 1 - 10 Vp cells per rabbit), 0.0 -19.3% (average 11.1%; 0 - 22 Vo cells per rabbit), 0.3 - 3.7% (average 2.1%; 1 - 27 Vir cells per rabbit) and 0.0 - 4.3% (average 1.3%; 0 - 4 Vic cells per rabbit), respectively. It is interesting that the highest percentage of double labeled neurons out of TSN subdivisions was noted in Vo, where the total number of ipsilateral and contralateral labeling was the lowest.

In spite of injections covering various PML sublobules, no clear-cut differentiation was found in localization of FB+DY-labeled neurons within TSN although certain correspondence in reference to the caudal (a and b), middle (c and d) and rostral (e and f) sublobules could be detected (Table I and Figs.1 and 3). Thus, labeling in Vp and Vir was recognized as a result of injection into all three homotopic PML sublobules (cases nos. 1-6 and 12) whereas labeling in Vo and Vic was identified only following injections into the homotopic middle sublobules (cases nos. 3-6).

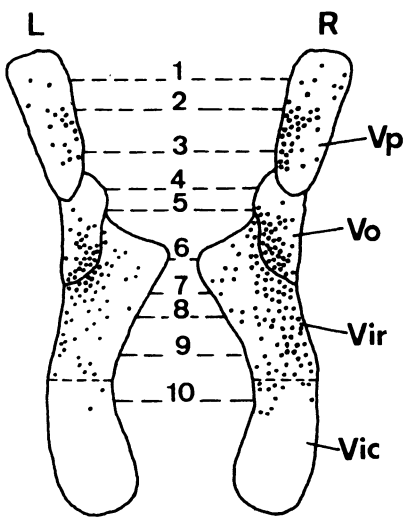

Fig. 5. Diagram of horizontal reconstruction of TSN illustrating areas of origin (dots) within the trigeminal subdivision of collateral projection to PML of both sides as recognized in the present study. L, left side; R, right side.

DISCUSSION

The present report provides evidence of bilateral projections from neurons of some TSN subdivisions to PML sublobules of both sides by way of axon collaterals. Until now, such connections have not been studied in any species. It has been found that the cells of origin of the TSN-PML branching projection and those of TSN-PML ipsilateral and contralateral projections occupy a common area within TSN; however, the former ones are considerably fewer in number. The findings concerning distribution of PML projecting neurons in the ipsilateral and contralateral TSN subdivisions confirm those obtained previously (Bukowska 1996). The collateral projections indicated herein take their origin from Vp, Vo, Vir and Vic (Fig. 5). No evidence has been found for projections from Vc and Vmes. Divergent axons arise mainly from neurons in the medial region of Vp, the caudal half of Vo and the dorsal and lateral regions of Vir. Those from Vic are scarce and originate predominantly from neurons in the medial and lateral regions of its rostral part. Sporadic projections are derived, in addition, from neurons within the root fibers of the facial nerve adjacent to Vp. The data show that the total number of FB+DY-labeled neurons is 130 in Vir, 69 in Vo, 58 in Vp and 14 in Vic. However, with regard to the total population of ipsilateral and contralateral PML projecting neurons in TSN subdivisions, neurons sending their branched axons to PML of both sides form about 11% in Vo, about 2% in Vp and Vir, and about 1% in Vic (Table I). The results indicate that out of TSN neurons projecting to both PML by way of axon collaterals, those in Vo project more frequently than those in Vp, Vir and Vic.

It appears from the present data that all sublobules of both PML are recipients for the collateral projection, although contribution of the middle sublobules is most significant. The homotopic middle (c and d) sublobules seem to receive a projection which is derived from Vp, Vo, Vir and Vic. The homotopic caudal (a and b) and rostral (e and f) sublobules are preferred for similar projections from Vp and Vir. The cells of origin of projection

to various sublobules of the left and right PML do not constitute a distinct population in a specific region within TSN but their distribution overlaps in a wide extent.

Although the present findings are the first to demonstrate the existence of interhemispheric axonal branching in trigeminocerebellar projection, in a more general context, they are not so surprising. That is, there is literature available on a similar type of collateralization in the other main cerebellar mossy fiber system, i.e., the pontocerebellar system in the rat (Mihailoff 1983) and cat (Rosina and Provini 1984). In the rat (Mihailoff 1983) combined Nuclear Yellow (NY) and FB bilateral injections in homotopic and heterotopic lobules of the cerebellar hemispheres, including PML, revealed the presence of double labeled neurons in the nucleus reticularis tegmenti pontis (NRTP) and pontine nuclei (PN). As regards PML, injections involving homotopic medial and lateral portions of the left and right PML resulted in a modest number of double labeled NRTP and PN neurons and no difference in their distribution pattern. In the cat (Rosina and Provini 1984) axonal collateral branching of pontocerebellar fibers to both hemispheres (crus I and crus II of the ansiform lobule) were also reported, however, PML was not taken under study.

In addition to receiving projections from TSN neurons, PML is influenced directly by other projections, e.g., secondary vestibular afferents (Grottel et al. 1991) and afferents from neurons within and outside the motor trigeminal nucleus, which are likely to be premotor neurons for orofacial motor nuclei (Bukowska and Grottel 1997). Furthermore, PML is the target of optokinetic projections mediated through the nucleus reticularis tegmenti pontis (see Grottel et al. 1988, for Ref.) as well as direct projections from the nucleus prepositus hypoglossi (Zimny and Grottel 1995) and nucleus "k", which are known to play a role in the control of ocular movements (Grottel et al. 1986). It is also evidenced that neurons in Vc, Vi, Vo and Vp receive periocular and corneal afferent inputs and thus they may be involved in the eyeblink reflex (Pellegrini et al. 1995, Van Ham and Yeo 1996a). In light of the above reports it is assumed that TSN neurons indicated in the present study that project to PML of both hemispheres by way of axon collaterals may be engaged in bilateral coordination of orofacial movements with simultaneous adjustment of eye and head position in response to tactile, thermoceptive or nociceptive stimulation of orofacial structures.

TSN neurons have been in addition demonstrated to receive projections from the cerebral cortex (Tashiro 1982) and brainstem nuclei such as parabrachial nucleus (Yoshida et al. 1997), red nucleus (Davis and Dostrovsky 1986), locus coeruleus (Simpson et al. 1997) tegmental reticular area (Herbert et al.1997), gigantocellular reticular nucleus, raphe magnus nucleus, periaqueductal gray (Beitz et al. 1983) and others. Since double labeled neurons in the present study occupied similar locations within TSN in comparison with neurons demonstrated to receive inputs from above functionally heterogenous regions, all these neurons could be considered as potential sources of bilateral modulating influences on various sublobules of both PML. It has been also shown that TSN contains neurons which issue collaterals to both the cochlear nucleus and inferior colliculi (Li and Mizuno 1997c), cochlear nucleus and ventrobasal thalamus (Li and Mizuno 1997a) as well as ventrobasal thalamus and inferior colliculus (Li and Mizuno 1997b). It is not unlikely that some of the neurons indicated in the present study may project in addition to the above-described recipient brainstem centers by sending their branching axons. This suggestion could be tested in further electrophysiological and multiple labeling investigations.

ACKNOWLEDGEMENT

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ABBREVIATIONS

IV	-	inferior vestibular nucleus
MV	-	medial vestibular nucleus
RG	-	gigantocellular reticular nucleus
RP	-	pontine reticular nucleus
rVII	-	root of the facial nerve
So	-	nucleus of the solitary tract
SV	-	superior vestibular nucleus
Vic		caudal subnucleus interpolaris
Vir	-	rostral subnucleus interpolaris
Vo	-	subnucleus oralis
Vp	-	principal trigeminal nucleus
Vt	-	motor trigeminal nucleus

REFERENCES

Aldes L. D., Boone T. B. (1985) Organization of projections from the principal sensory trigeminal nucleus to the hypoglossal nucleus in the rat: an experimental light and elec-

tron microscopic study with axonal tracer techniques. Exp. Brain. Res. 59: 16-29.

Beitz A. J., Wells W., Shepard R. D. (1983) The location of brainstem neurons which project bilaterally to the spinal trigeminal nuclei as demonstrated by the double fluorescent retrograde tracer technique. Brain Res. 258: 305-312.

Bukowska D. (1996) Trigeminocerebellar projection to the paramedian lobule with emphasis on the climbing fibre zones: a retrograde tracing study in the rabbit. J. Brain Res. 37: 159-172.

Bukowska D., Grottel K. (1997) Cells in and outside the motor trigeminal nucleus projecting to the cerebellar paramedian lobule. Neuroreport 8: 2953-2956.

Davis K. D., Dostrovsky J. O. (1986) Modulatory influences of red nucleus stimulation on the somatosensory responses of cat trigeminal subnucleus oralis neurons. Exp. Neurol. 91: 80-101.

Grottel K., Zimny R., Jakielska D. (1986) The nucleus "k" of Meessen and Olszewski efferents to the cerebellar paramedian lobule: a retrograde tracing histochemical (HRP) study in the rabbit and the cat. J. Hirnforsch. 27: 305-322.

Grottel K., Zimny R., Jakielska D., Ostrowska A. (1991) Non-reciprocal connections between the vestibular nuclei and the cerebellar paramedian lobule, with special reference to the climbing fiber zones: an analysis in the rabbit using the retrograde horseradish peroxidase method. Neurosci. Res. 12: 185-200.

Grottel K., Zimny R., Kowalski K. (1988) The pontocerebellar efferents from the pontine reticular tegmental nucleus in the rabbit: differential projections to the paramedian sublobules as revealed with tracing of retrograde axonal transport of horseradish peroxidase. J. Hirnforsch. 29: 203-216.

Hayashi H., Sumino R., Sessle B. J. (1984) Functional organization of trigeminal subnucleus interpolaris: nociceptive and innocuous afferent inputs, projecting to thalamus, cerebellum, and spinal cord, and descending modulation from periaqueductal gray. J. Neurophysiol. 51: 890-905.

Hayashi H., Tabata T. (1991) Distribution of trigeminal sensory nucleus neurons projection to the mesencephalic parabrachial area of the cat. Neurosci. Lett. 122: 75-78.

Herbert H., Klepper A., Ostwald J. (1997) Afferent and efferent connections of the ventrolateral tegmental area in the rat. Anat. Embryol. 196: 235-259.

Keizer K., Kuypers H. G. J. M., Huisman A. M., Dann O. (1983) Diamidino-yellow dihydrochloride (DY.2HCl): a new fluorescent retrograde neuronal tracer, which migrates only very slowly out of the cell. Exp. Brain Res. 51: 179-191.

Kuypers H. G. J. M., Bentivoglio M., Catsman-Berrevoets C. E., Bharos A. T. (1980) Double retrograde neuronal labeling through divergent axon collaterals using two fluorescent tracers with the same excitation wavelength which label different features of the cell. Exp. Brain Res. 40: 383-392.

Li H., Mizuno N. (1997a) Collateral projections from single neurons in the dorsal column nucleus to both the cochlear nucleus and the ventrobasal thalamus: a retrograde double-labeling study in the rat. Neurosci. Lett. 222: 87-90.

Li H., Mizuno N. (1997b) Collateral projections from single neurons in the dorsal column nuclei to the inferior colliculus and the ventrobasal thalamus: a retrograde double-labeling study in the rat. Neurosci. Lett. 225: 21-24.

Li H., Mizuno N. (1997c) Single neurons in the spinal trigeminal and dorsal column nuclei project to both the cochlear nucleus and the inferior colliculus by way of axon collaterals: a fluorescent retrograde double-labeling study in the rat. Neurosci. Res. 29: 135-142.

Matsushita M., Ikeda M., Okado N. (1982) The cells of origin of the trigeminothalamic, trigeminospinal and trigeminocerebellar projections in the cat. Neuroscience 7: 1439-1454.

Meessen H., Olszewski J. (1949) A cytoarchitectonic atlas of the rhombencephalon of the rabbit. Springer-Verlag, Basel.

Mihailoff G. A. (1983) Intra- and interhemispheric collateral branching in the rat pontocerebellar system. A fluorescence double-label study. Neuroscience 10: 141-160.

Olszewski J. (1950) On the anatomical and functional organization of the spinal trigeminal nucleus. J. Comp. Neurol. 92: 401-413.

Pellegrini J. J., Horn A. K. E., Evinger C. (1995) Origin of ascending intratrigeminal evoked blink reflex. I. Neuronal circuits. Exp. Brain Res. 107: 166-180.

Phelan K. D., Falls W. M. (1991) A comparison of the distribution and morphology of thalamic, cerebellar and spinal projection neurons in rat trigeminal nucleus interpolaris. Neuroscience 40: 497-511.

Rosina A., Provini L. (1984) Pontocerebellar system linking the two hemispheres by intracerebellar branching. Brain Res. 296: 365-369.

Silverman J. D., Kruger L. (1985) Projections of the rat trigeminal sensory nuclear complex demonstrated by multiple fluorescent dye retrograde transport. Brain Res. 361: 383-388.

Simpson K. L., Altman D. W., Wang L., Kirifides M. L., Lin R. C.-S., Waterhouse B. D. (1997) Lateralization and functional organization of the locus coeruleus projection to the trigeminal somatosensory pathway in rat. J. Comp. Neurol. 385: 135-147.

Somana R., Kotchabhakdi N., Walberg F. (1980) Cerebellar afferents from the trigeminal sensory nuclei in the cat. Exp. Brain Res. 38: 57-64.

Steindler D. A. (1985) Trigeminocerebellar, trigeminotectal, and trigeminothalamic projections: a double retrograde axonal tracing study in the mouse. J. Comp. Neurol. 237: 155-175.

Taber E. (1961) The cytoarchitecture of the brain stem of the cat. I. Brain stem nuclei of cat. J. Comp. Neurol. 116: 27-69.

Tashiro T. (1982) Distribution of cortical cells projecting to the main sensory trigeminal nucleus in the cat: a study with the horseradish peroxidase technique. Exp. Neurol. 78: 561-573.

Van Ham J. J., Yeo C. H. (1992) Somatosensory trigeminal projections to the inferior olive, cerebellum and other precerebellar nuclei in rabbits. Eur. J. Neurosci. 4: 302-317.

Van Ham J. J., Yeo C. H. (1996a) The central distribution of primary afferents from the external eyelids, conjunctiva, and cornea in the rabbit, studied using WGA-HRP and B-HRP as transganglionic tracers. Exp. Neurol. 142: 217-225.

Van Ham J. J., Yeo C. H. (1996b) Trigeminal inputs to eyeblink motoneurons in the rabbit. Exp. Neurol. 142: 244-257.

Yatim N., Billig I., Compoint C. (1996) Trigeminocerebellar and trigemino-olivary projections in the rats. Neurosci. Res. 25: 267-283.

Yoshida A., Chen K., Moritani M., Yabuta N. H., Nagase Y., Takemura M., Shigenaga Y. (1997) Organization of the descending projections from the parabrachial nucleus to the trigeminal sensory nuclear complex and spinal dorsal horn in the rat. J. Comp. Neurol. 383: 94-111.

Zimny R., Grottel K. (1995) Non-reciprocal connections between the perihypoglossal nuclei and the cerebellar paramedian lobule in rabbits and their relationship to the climbing fiber zones. Brain Behav. Evol. 46: 339-353.

Zimny R., Grottel K., Jakielska D., Ostrowska A. (1989) Topographic and zonal pattern of olivocerebellar projection to the paramedian lobule in the rabbit: an experimental study with an HRP retrograde tracing method. Neurosci. Res. 7: 173-198.

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