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Topography and axonal collaterals of trigeminocerebellar projection to the paramedian lobule and uvula in the rabbit cerebellum

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Short communication

Abstract. To study projections of the trigeminal sensory nuclei (TSN) to the rostral parts of the paramedian lobule (PML) and of the uvula of the rabbit cerebellar cortex, the retrograde double fluorescent labeling method was used. Injections of Fast Blue (FB) into PML and Diamidino Yellow (DY) into the uvula, resulted in prominent labeling neurons with FB bilaterally and with DY ipsilaterally, in the principal trigeminal nucleus, subnucleus oralis, and rostral and caudal subnucleus interpolaris. We observed topographical arrangement of neurons in such a fashion that FB labeled cells were localized in the medial and DY labeled cells in the lateral regions of TSN. Apart from this small number of double FB+DY labeled neurons (n=138) were found in the narrow common region of single labeling. This implies that PML and the uvula receive independent trigeminal sensory information from neurons in separate regions of TSN. However, some trigeminal neurons may also exert simultaneous influences upon these hemispheral and vermal components by way of axonal branchings.

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The trigeminal sensory nuclei (TSN) mediate in transmission of sensory information from orofacial regions to the thalamus (Mantle-St John and Tracey 1987, Patrick and Robinson 1987, Phelan and Falls 1991), various centers of the brainstem (Aldes and Boone 1985, Dauvergne et al. 2002, Godefroy et al. 1998, Ndiaye et al. 2002, Silverman and Kruger 1985, Van Ham and Yeo 1992, Zerari-Mailly et al. 2001), spinal cord (Phelan and Falls 1991), and the cerebellum.

TSN includes the mesencephalic (Vme), principal (Vp) and spinal nuclei. The latter is further divided rostrocaudally into the subnucleus oralis (Vo), rostral (Vir), and caudal (Vic) subnucleus interpolaris (Vi), and subnucleus caudalis (Vc) (Olszewski 1950, Taber 1961).

Anatomical data collected in the rat (Mantle-St John and Tracey 1987, Phelan and Falls 1991, Silverman and Kruger 1985, Yatim et al. 1996), cat (Ikeda and Matsushita 1992, Matsushita et al. 1982, Somana et al. 1980), mouse (Steindler 1977), sheep (Saigal et al. 1980), tree shrew (Patrick and Haines 1982), pigeon (Arends and Zeigler 1989), and monkey (Faull 1977) is agrees that the trigeminocerebellar projection arises from Vp, Vo and Vi, and achieves some regions of the cerebellar cortex, mainly lobules VI–VIII, IX (uvula) and X of the vermis as well as the simple lobule and paramedian lobule (PML) of the hemisphere. In the rabbit, the trigeminocerebellar projection was studied with WGA-HRP technique (Van Ham and Yeo 1992). It arises from the rostral two-thirds of Vi, from caudal Vo and strongly from Vp, and terminates in the simple lobule, ansiform lobule, PML and the uvula, exhibiting a similar projection pattern. In our previous material in the rabbit we studied trigeminal projection to PML and, in general, we confirmed the sources of projection from TSN described by Van Ham and Yeo (1992), but in addition we revealed that some trigeminocerebellar fibers may diverge to supply PML of both sides (Bukowska et al. 1998) as well as may diverge inside of unilateral PML (Bukowska et al. 2003).

It was shown electrophysiologically that trigeminal mossy fiber signals evoked by tactile stimulation of the perioral and other facial structures reached the rostral parts both PML and the uvula in the rat (Shambes et al. 1978). Moreover, the rostral three and rostral two sublobules of PML and of the uvula, respectively, were proved to receive trigeminal sensory projection also in the cat, with use of HRP labeling (Somana et al. 1980).

In the above context, the interest of the present paper is focused on the study whether there are some differences in the trigeminocerebellar projection from TSN subdivisions to the hemispheral component – rostral PML and vermal component – rostral uvula, and whether collateral interlobular projection to these two cerebellar targets also exists. In this aim the double fluorescent retrograde technique was employed in the rabbit.

The experiments were performed on seven adult New Zealand white rabbits weighing 2.1–2.4 kg anes-

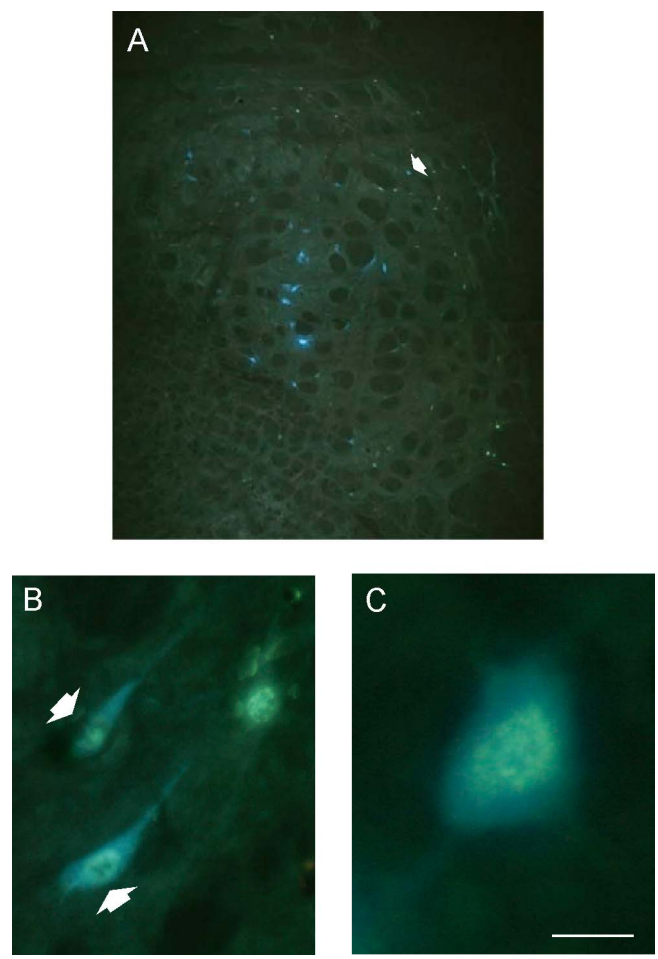

Fig. 1. Photomicrographs of retrogradely labeled neurons in TSN resulting from FB and DY application into PML and the uvula, respectively. (A) A low magnification of the right Vir (at level between IX and X of Fig. 3) showing the clearly segregated populations of FB (medially; blue fluorescence in the neuroplasm) and DY (laterally; yellow fluorescence in the nucleus) labeled neurons with one FB+DY double labeled cell (arrow). (B) Two FB+DY double (arrows) and one DY single labeled neurons in Vir. (C) A magnification of FB+DY double labeled cell in Vic. Scale bar is 385 μm for A, 47 μm for B, and 16 μm for C.

thetized with a mixture of ketamine (50 mg/kg) and promazine (19 mg/kg). The animal head was immobilizated in a stereotaxic frame and two craniectomies were made to expose PML and the posterior vermis on the right side. After opening the dura, pressure injections of 4–5% Fast Blue (FB) and 2% Diamidino Yellow (DY) were made (total 2.5–3.5 µl) in several (3–6) points to the rostral parts of PML and of the uvula, respectively, using a glass micropipette (tip diameter 40–65 µm) secured on a 5-µl Hamilton microsyringe. Following a survival period of 8–11 days, the rabbit were deeply re-anesthetized and transcardially perfused with saline followed by 20% formalin and then by 10% sucrose, all phosphate-buffered. The cerebellum and brainstem were removed, and infiltrated by immersion with 30% sucrose overnight at 4°C. Then they were cut into sagittal (cerebellum) and transverse (pons and medulla) frozen 40-µm-thick serial sections. Two out of three sections were mounted and examined using the Optiphot-2, Nikon (Japan) and Jenalumar (Germany) fluorescent microscopes at 380-nm and 410-nm light excitation wavelength. Neurons labeled with FB and/or DY were recognized by blue fluorescence in the neuroplasm and/or yellow fluorescence in the nucleus as shown in Fig. 1 (Keizer et al. 1983, Kuypers et al. 1980). This report was performed according to the Polish Law on Animal Protection and the guidelines established by the Declaration of Helsinki concerning Care and Use of Animal in Research.

In all cases analyzed in this study injections involved various rostrocaudal and mediolateral extents of the cerebellar cortex in sublobules d, e and f of the rostral PML (FB), and in sublobules a and b of the rostral uvula (DY). An example of the pair of injections is demonstrated for representative case 20 in Fig. 2 (upper).

In each case examined, FB and DY labeled neurons were detected in all TSN divisions apart from Vme and Vc, with a clear, over two times, predominance of FB labeling (Table I). Labeled neurons with FB were seen bilaterally with ipsilateral preponderance over two times in Vp and four times in Vc, and almost three times in Vir and five times in Vic. The most numerous FB filled cells were found in Vir (*n*=12315) and in less extent in Vp (*n*=2692), Vic (*n*=2663), and Vo (*n*=1326). DY neurons were labeled exclusively on the ipsilateral side, the most frequently in Vic (*n*=4552) and Vir (*n*=3615), rarely in Vo (*n*=185) and occasion-

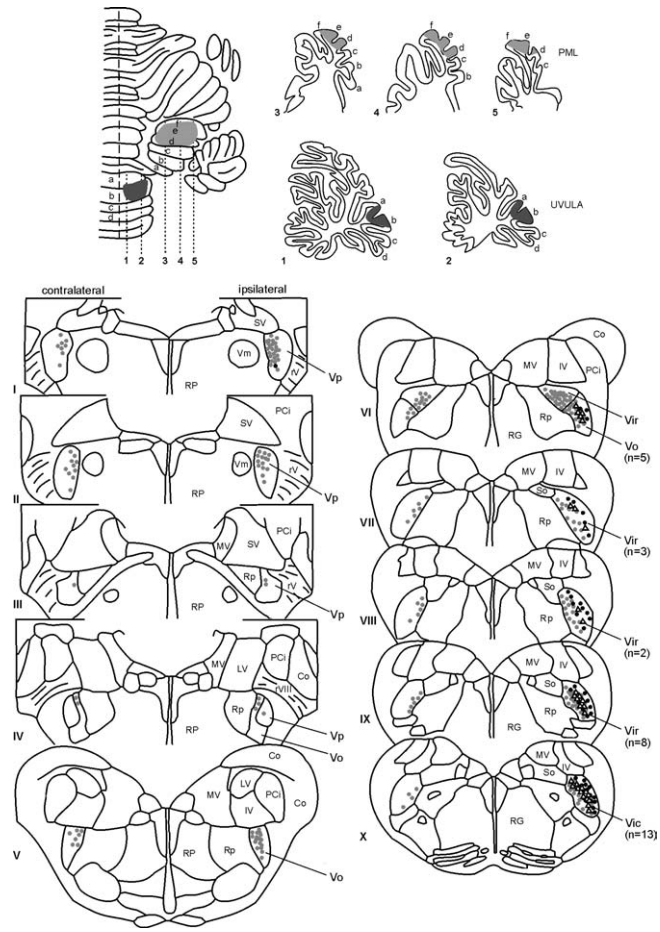

Fig. 2. Diagrams of the injection sites in the cerebellum and distribution pattern of labeled neurons in TSN, in case 20. (Upper) The cerebellar surface unfolded in one plane of the right side (left panel) and the five sagittal sections through the uvula (1 and 2) and PML (3, 4, and 5) (right panel) illustrating the extent of DY (heavy gray) and FB (light gray) injections. (Below) Transverse sections through the pons and medulla illustrating localization of FB (light circle), DY (heavy circle) and FB+DY (triangle) labeled neurons. Each circle corresponds to 3–6 single labeled cells at levels I–V, 10–25 at levels VI–IX and 35 at level X. Each triangle corresponds to one double-labeled cell (number in parenthesis). (Co) cochlear nuclei; (IV) inferior vestibular nucleus; (MV) medial vestibular nucleus; (PCi) inferior cerebellar peduncle; (RG) gigantocellular reticular nucleus; (RP) pontine reticular nucleus; (Rp) parvocellular reticular nucleus; (SV) superior vestibular nucleus; (rV) root of trigeminal nerve; (rVIII) root of vestibular nerve; (So) nucleus of solitary tract; (Vic) caudal subnucleus interpolaris; (Vir) rostral subnucleus interpolaris; (Vm) motor trigeminal nucleus; (Vo) subnucleus oralis; (Vp) principal trigeminal nucleus.

Table I

Total number of single FB or DY and double FB+DY labeled neurons in the ipsilateral (i) and contralateral (c) TSN subdivisions as a result of unilateral injections of FB into PML and DY into the uvula

	Vp			Vo				Vir				Vic			
Case	FB		DY	FB		DY	FB+DY	FB		DY	FB+DY	FB		DY	FB+DY
no.	i	c	i	i	c	i	i	i	c	i	i	i	c	i	i
44	227	110		78	27	17		829	188	345	4	276	66	496	2
42	458	277	6	86	22	40	3	1073	567	1032	22	153	55	1174	4
33	38	12	8	58	21	7		447	123	129	3	468	27	644	14
23	462	122		272	40	42		2411	853	537	1	395	87	525	3
22	455	170		185	11	4		2464	848	610	1	337	83	527	2
20	233	105	3	329	112	35	5	1445	519	379	13	400	132	533	13
6	15	8	3	79	6	40	3	467	81	583	30	181	30	653	15
Total	1888	804	20	1087	239	185	11	9136	3179	3615	74	2210	453	4552	53

ally in Vp (n=20). With regard to apparent clustering of FB or DY labeled cells, a clear-cut topical relationship could be disclosed between the tracer injection sites and distribution of labeled neurons in the above nuclei (Fig. 1A).

One advantage of using the fluorescent dyes is the possibility of detecting double-labeled cells, i.e. cells that project to both injection sites. In our material, few double FB+DY labeled neurons (n=138) were observed in Vo, Vir and Vic, exclusively ipsilaterally (Fig. 1B, C). Vp was avoided of double labeling. Double-labeled neurons were found in the transitional area between FB and DY labeling, and rarely they were present among either FB or DY labeled cells.

Figure 2 (bottom) illustrates distribution pattern of single FB and DY labeled neurons as well as localization of double FB+DY labeled cells in representative case 20, and Fig. 3 shows pattern of single labeling for all cases.

In Vp, FB labeled neurons occupied mainly the medial three quarters of the nucleus with exception of the most ventral region where only small number of labeled cells could be seen. In addition, in some sections few labeled cells were scattered laterally. The most numerous labeled neurons were observed in the rostral half (levels I–II) and more caudally labeling became apparently weaker. As regards DY, few labeled neurons were present scattered in the lateral part of this nucleus.

In the rostral pole of Vo (levels IV) only FB labeled cells in small number were found mainly in the dorsal part. More caudally (levels V–VI) they were apparently numerous and occupied the medial three quarters of the nucleus, but in the ventral region labeling was sparse. Moreover, in these levels DY labeled neurons appeared, mainly in the lateral half. In a narrow band of overlap double-labeled cells (n=11) were recognized.

Throughout the entire rostrocaudal extent of Vir (levels VI–IX) two populations of FB and DY labeled cells were clearly distinguishable. The former population lay in the medial two thirds and the latter one in the lateral two thirds of this nucleus. Double-labeled neurons (n=75) were found mainly at levels between VII and IX in a common area of single labeling, although in individual sections they could be seen among either FB or DY labeled neurons.

As concerns Vic, both single and double-labeled neurons were present only in the rostral half. Caudally from level X, Vic was avoided of labeling. The mediolateral distribution pattern of FB and DY single-labeled neurons and localization of double-labeled cells (n=53) were very similar to those in Vir.

Summarizing this work, above results provide evidence that mossy fiber projections from defined TSN subdivisions to the rostral parts of PML and of the uvula have different intensity and laterality, and they

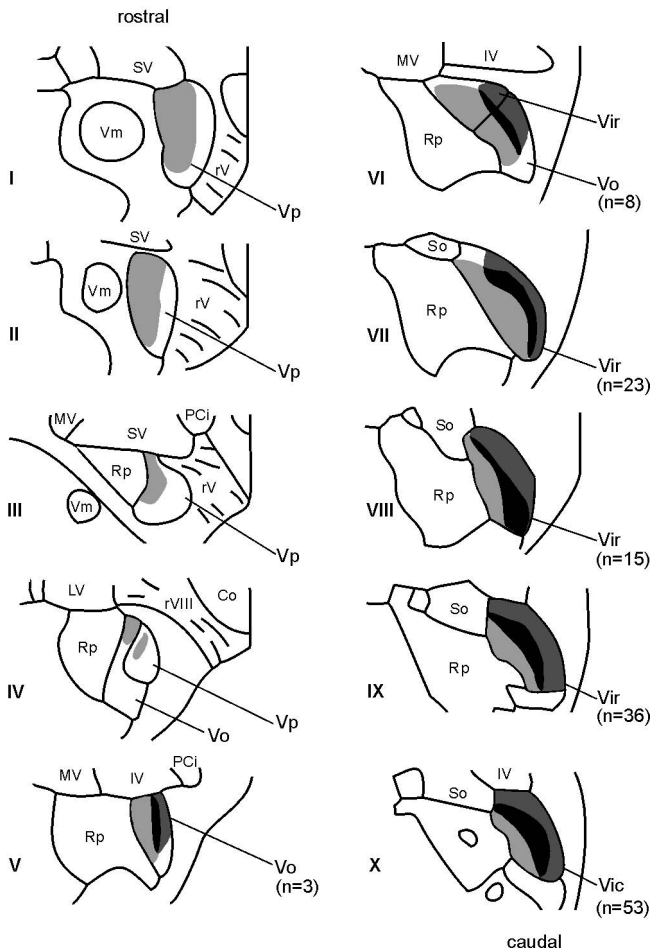

Fig. 3. Summarizing diagram illustrating approximately areas of distribution of labeled neurons with single FB (light gray) or DY (heavy gray) tracers in TSN subdivisions of the right side for all cases. The common areas for these neurons are black and they contain double-labeled cells. All these areas correspond to the highest density of labeling, but not to its largest extent. Localization of cells is shown only on the injection side where distribution pattern of both tracers can be observed. Sparse DY labeling in Vp (n=20) is not shown (see text and Table I). Level X represents the middle transection of the rostrocaudal extent of Vic. The more caudal level is not shown because of absence of labeling. In parenthesis the number of double-labeled neurons is indicated. For abbreviations see Fig. 2.

are spatially separated. Projection to PML is over two times greater than that to the uvula. PML receives fibers from bilateral TSN (with ipsilateral preponderance from two to five times as regards individual nuclei) whereas the uvula is influenced exclusively by ipsilateral TSN. Projections to PML arise from Vp, Vo, Vir and Vic, and these to the uvula from Vo, Vir and Vic, and only sporadically from Vp. No projection found from Vme and Vc confirms findings in other species but contradicts strong connections from Vc to the uvula in the rat (Yatim et al. 1996).

Projection under study is topographically organized in such a way that the medial regions of TSN send fibers to PML and its lateral regions to the uvula. Topography within these connections was not shown in previous reports. It is widely accepted that PML receives somatosensory information from the spinal cord and is involved in the control of limbs movement and that the uvula is interconnected with the vestibular system (Ito 1984). Different function of the two cerebellar lobules allows to assume that spatially separated TSN-PML and TSN-uvula projecting neurons indicated herein may also received different information. However, a functional meaning of the findings is difficult to explain, because electrophysiological and immunocytochemical procedures are required to identify the types of inputs on these neurons and neurotransmitters used. In spite of this, it is possible, that some of neurons in the lateral region of TSN showed in this study as projecting to the uvula may be involved in coordination of the eye and head movements because extraocular muscle and upper cervical afferents terminate in the ventrolateral and lateral regions of Vi and Vo (Porter and Donaldson 1991, Xiong and Matsushita 2000). Moreover, TSN neurons demonstrated to receive secondary vestibular fibers (Buisseret-Delmas et al. 1999) would indirectly complement the well-known direct vestibulo-uvular projection. It is proved also that the red nucleus projects to the ventromedial region of Vp (Godefroy et al. 1998) and it may modulate transmission of somatosensory information through neurons in the medial Vo (Davis and Dostrovsky 1986). Therefore, it is not unlikely that the medial population of TSN neurons sending axons to PML in the current material may be influenced by an activity of the rubral descending fibers. The present findings in addition show that the narrow region in TSN common for independent projections contains neurons which send divergent axons to supply ipsilateral PML and the uvula, simultaneously. Projection by way of axon collaterals arises mainly from Vir and Vic, and it is weaker from Vo and absent from Vp. Collateralization in the trigeminocerebellar pathways has been for the first time described in our laboratory with respect to intra- and interlobular connections of PML (Bukowska et al. 1998, 2003) as well as in this paper to PML and the

uvula. However, TSN may also contain neurons which axonal ramifications reach the cochlear nucleus and inferior colliculus (Li and Mizuno 1997), inferior olive and superior colliculus (Huerta et al. 1983), facial motor nuclei of both sides (Dauvergne et al. 2002), thalamus and cerebellum (Patrick and Robinson 1987) in the rat apart from the two targets of the cerebellar cortex in the current study in the rabbit.

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