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The Projection from the Lateral Reticular Nucleus to the Cerebellar Vermal Lobule VI in the Rat: A Retrograde Labelling Study Using Horseradish Peroxidase

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The projection from the lateral reticular nucleus (LRN) to three subdivisions of the cerebellar vermal lobule VI was studied in the rat by utilizing the retrograde transport of wheatgerm agglutinin-conjugated horseradish peroxidase. Labelled neurons were located bilaterally throughout the LRN, but with ipsilateral predominance. There seemed to be a dorsal-to-ventral transition in the ipsilateral magnocellular neurons projecting to the cerebellar vermal lobules VIa-to-VIc. In the contralateral side, cells projecting to vermal lobule VIa were observed at more rostral sections, whereas those projecting to lobule VIc were located at caudal sections. There were relatively few labelled neurons in parvocellular or subtrigeminal divisions at either side. Computer-aided three dimensional reconstruction of LRN projections to lobules VIa/VIb or lobules VIb/VIc exhibited extensive oberlap within each combination of injection cases.

KEY WORDS: Lateral Reticular Nucleus, Cerebellar Vermis, WGA-HRP, Topographic Organization

The lateral reticular nucleus (LRN) in the rat consists of three cytologically distinct subdivisions including magnocellular, parvocellular, and subtrigeminal regions (Kapogianis *et al.*, 1982). It receives inputs from a variety of sources including the spinal cord, the cerebral cortex, the red nucleus, the fastigial nucleus, and parts of the vestibular nuclei (Brodal *et al.*, 1967). Among these afferent systems, the projection from the spinal cord onto the LRN has been systematically and extensively investigated and it proven to be somatotopically organized (Künzle, 1973; Shokunbi *et al.*, 1985). The medial aspect of the LRN was the site of preferential termination of the cervical spinal projection. Lumbar spinal projections terminated preferentially in the rostrolateral region of the nucleus.

The efferent projection from the LRN is massive and is exclusively to the cerebellum via the inferior cerebellar peduncle (Brodal, 1975). Previous electrophysiological evidence in the cat suggested that there is little LRN input to the cerebellar cortex outside the classical spinal receiving areas of the anterior lobe and the paramedian lobule (Clendenin *et al.*, 1974). Anatomical study in the rat, however, reported that the projection to the anterior part of the posterior lobe hemisphere considerably exceeds that to the paramedian lobule (Payne, 1987). Extensive labelling was also reported in the LRN projection onto the vermal lobule VIII (Eisenman, 1982). Using auto-radiography and retrograde transport of horseradish peroxidase, authors reported that the projection to the vermis is greater than that to the hemisphere and there is, in general, a greater LRN input to the anterior vermis than to the posterior vermis, and to the anterior part of the posterior lobe hemisphere than the posterior part

(Chan-Palay *et al.*, 1977; Dietrichs and Walberg, 1979; Eisenman, 1982; Hrycyshyn *et al.*, 1982; Payne, 1987).

Although the projection pattern of LRN neurons onto each lobule of the cerebellar cortex has been rather precisely reported, there is some controversy about LRN projection to the vermal lobules VI and VII in the rat. Some investigators reported a prominent LRN projection onto these lobules (Chan-Palay *et al.*, 1977), others indicated only a scant projection to the lobules (Hrycyshyn *et al.*, 1982). Previous studies involved a relatively large injection site including both right and left sides of posterior vermis. It seemed necessary to investigate the projections under the condition that an injection site was localized within either right or left side of a specific subdivision of each lobule in the posterior lobe vermis. The aim of the present study was, therefore, to determine the anatomical organization of the LRN projection to each subdivision of vermal lobule VI, with special attention to the topographic organization. The LRN projection to each subdivision of vermal lobule VII will be separately reported in a subsequent paper. The data provided should complement the information available on the pattern of lateral reticulo-cerebellar projection and its topographic organization in the rat.

Materials and Methods

Thirty-two albino rats ranging in weight from 300 to 320g were used. During operation, animals were anesthetized using 1.0 ml of 3.6% chloral hydrate per 100g of body weight.

Tracer injection

An aliquot of 2% wheatgerm agglutinin-conjugated horseradish peroxidase (WGA-HRP) dissolved in bacteriostatic water was drawn up into a glass micropipette (tip diameter 30-50 μm) attached by the bone wax to a 2 μl Hamilton syringe. The micropipette with the syringe was then mounted in a Stölting stereotaxic apparatus. The animal was properly positioned in the apparatus using ear and incisor bars and the scalp over the dorsal surface of the head was incised using a scalpel blade. An electric drill was used to make an opening in the skull covering the cerebellar vermis. The loaded micropipette was positioned over each subdivision of vermal lobule VI including VIa, VIb, and VIc, at a precise location 1.0 mm lateral to the midline. Subsequently it was lowered through a small slitlike defect in the dura produced by a syringe needle. The micropipette was always aligned perpendicular to the surface of the vermal lobule and maintained at the depth of approximately 500 μm from the surface. A total of 0.2 μl WGA-HRP was deposited at each injection site over 30 min period. The above-mentioned amount of tracer over the specific subdivision of vermal lobule VI was consistently utilized, because it produced a circumscribed injection site which was confined only in one side (usually left) and one specific subdivision of vermal lobule VI.

Perfusion and fixation

After the survival period of approximately 48 hrs, animals were artificially ventilated using a respirator and perfused through the left ventricle with a brief flush of saline (100-200 ml) followed by 500 ml of a cold (4°C) fixative containing 1% paraformaldehyde and 1.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.4). One hundred milliliter of 10% sucrose in the same buffer was subsequently used to prevent excessive depression of WGA-HRP enzyme activity, which might be caused by aldehydes remaining in the brain tissue. The brain was removed immediately after perfusion and stored in 30% sucrose in the buffer at 4°C.

WGA-HRP histochemistry

On the following day, the medullary region containing the LRN was serially sectioned in the transverse plane and the injection site including the cerebellum sectioned in the sagittal plane at the thickness of 40 μm on a cryostat. Every fifth section through the medulla and every tenth section through the injection site were collected in a plate of tissue-culture wells and processed according to the combined method of nitroprusside-stabilized TMB reaction and cobalt/nickel-intensified DAB reaction as

described by Rye *et al.* (1984). After the histochemical reaction, sections were washed with saline and subsequently mounted, dried, and counterstained with 1% neutral red solution in 0.05 M sodium acetate buffer (pH 4.8).

Computer-aided three-dimensional reconstruction

The location of labelled cell bodies within the LRN was carefully mapped under both dark and bright field illumination with the aid of a camera lucida. Transverse and antero-posterior axes of each tissue section were determined based on the atlas of Paxinos and Watson (1986). For the three-dimensional reconstruction, overall morphology of tissue section and the locations of the inferior olivary nucleus (ION) and the LRN within the section were, first of all, traced using a scanner. The area occupied by labelled neurons in the ipsi- and contralateral LRN was drawn in each section and represented as a specific color for each injection case. Three-dimensional reconstruction representing a rostro-caudal series of diagrams in either combined VIa/VIb cases or combined VIb/VIc cases was executed using a 3-D studio program produced by the Autodesk Co., U. S.A. The image was then printed out using the Mitsubishi electric color video copy processor.

Results

The specified amount of 2% WGA-HRP injected into each subdivision of the vermal lobule VI

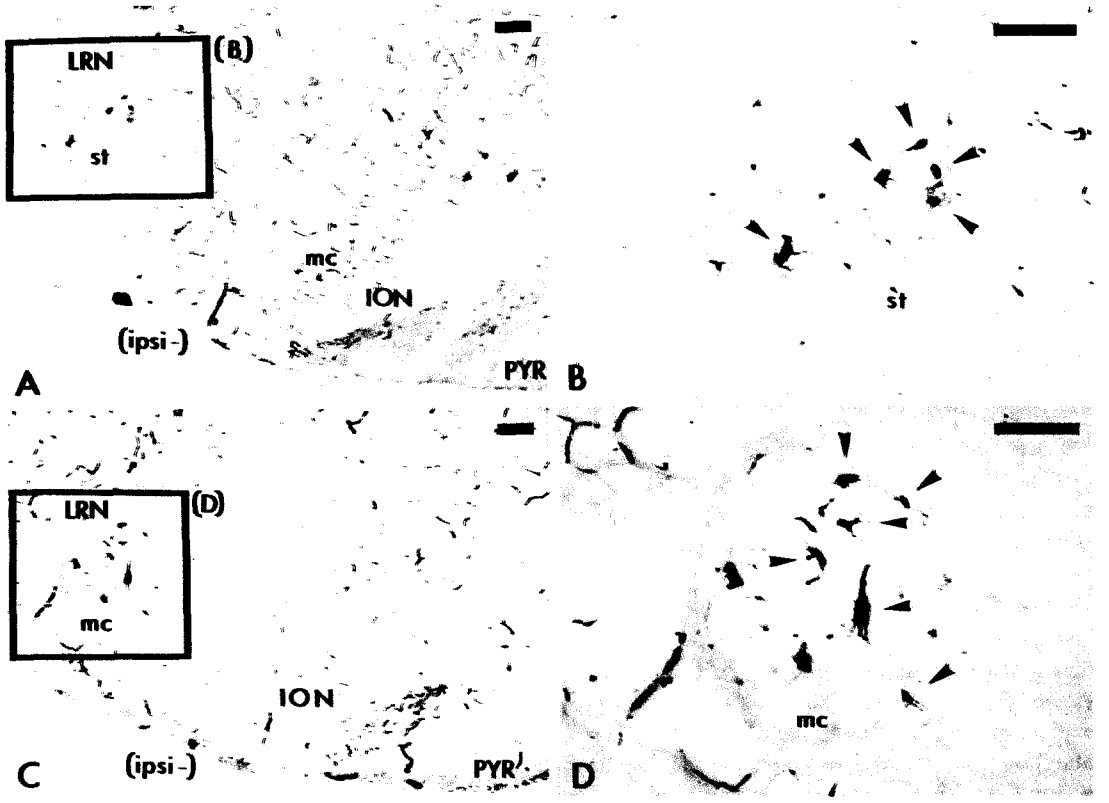

Fig. 1. Low and high magnification views of labelled neurons (arrowheads) at the rostral (A and B) and caudal (C and D) LRN, ipsilateral to the injection site in the vermal lobule VIa. Note that few cells were labelled in the ipsilateral ION. Bars represent 100 μm. ION, inferior olivary nucleus; LRN, lateral reticular nucleus; mc, magnocellular division of the LRN; PYR, pyramid; st, subtrigeminal division.

consistently produced a stained sphere involving most of the granular layer within the specific lobule. The LRN of the rat extended rostro-caudally for a distance of approximately 1,800 μm within the ventrolateral medulla oblongata. Retrogradely-labelled neurons in the LRN as a result of cerebellar injections were distinctively filled with black reaction product after the cobalt/nickel-intensified DAB reaction. Labelled neurons were multipolar or bipolar in morphology and various in size, i.e., ranging from 10 to 25 μm in diameter, depending on their specific locations within the LRN (Figs. 1, 3, and 5).

LRN projections to vermal lobule VIa

A total of twelve cases involved injection of WGA-HRP into the vermal lobule VIa (Fig. 2A). Retrogradely labelled neurons in subtrigeminal and magnocellular divisions of the ipsilateral LRN were illustrated in Fig. 1. In addition, labelled LRN neurons in a representative case (Rat No. 8) were mapped at rostro-caudal series of sections (Fig. 2B). Labelled cells were located bilaterally throughout the LRN, but with ipsilateral predominance. At rostral levels of the ipsilateral LRN, labelled cells were located mostly at the dorsal surface of the magnocellular division and a few cells found in the medial portion of the subtrigeminal division (Fig. 2B, sections 1-4). At caudal levels, labelled cells were extended throughout the entire magnocellular division, with somewhat dense distribution in the dorsolateral region (Fig. 2B, sections 5-8). A few labelled cells were also located in the medial portion of the parvocellular division (Fig. 2B, sections 4-6).

At the contralateral LRN, most of the labelled cells were located in the medial region of the magnocellular division (Fig. 2B, sections 1-7). Few cells were labelled in the subtrigeminal and parvocellular divisions (Fig. 2B, sections 4 and 6).

LRN projections to vermal lobule VIb

Ten cases included injection of WGA-HRP into the vermal lobule VIb (Fig. 4A). A large number of cells were labelled in each subdivision of the ipsilateral and contralateral LRN (Fig. 3). Retrogradely labelled neurons in a representative

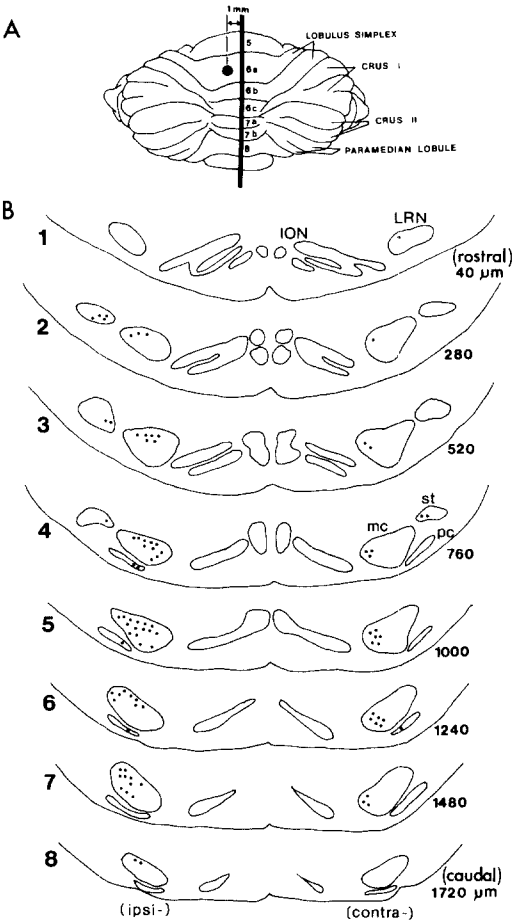

Fig. 2. WGA-HRP injection site (A) in the cerebellar vermal lobule VIa and a rostro-caudal series (B) of transverse sections illustrating labelled neurons (filled circles) at the LRN in a representative case (Rat No. 8). ION, inferior olivary nucleus; LRN, lateral reticular nucleus; mc, magnocellular division of the LRN; pc, parvocellular division; st, subtrigeminal division.

case (Rat No. 14) were illustrated at a rostro-caudal series of sections in Fig. 4B. Cells were bilaterally located, with ipsilateral predominance. At the ipsilateral side, a large number of labelled cells were located in the central to medial regions of the magnocellular division (Fig. 4B, sections 1-8). A few cells were also labelled in the medial region of the subtrigeminal division (Fig. 4B, sections 3 and 4) and the parvocellular division (Fig. 4B, sections 4-6).

At the contralateral side, labelled cells were

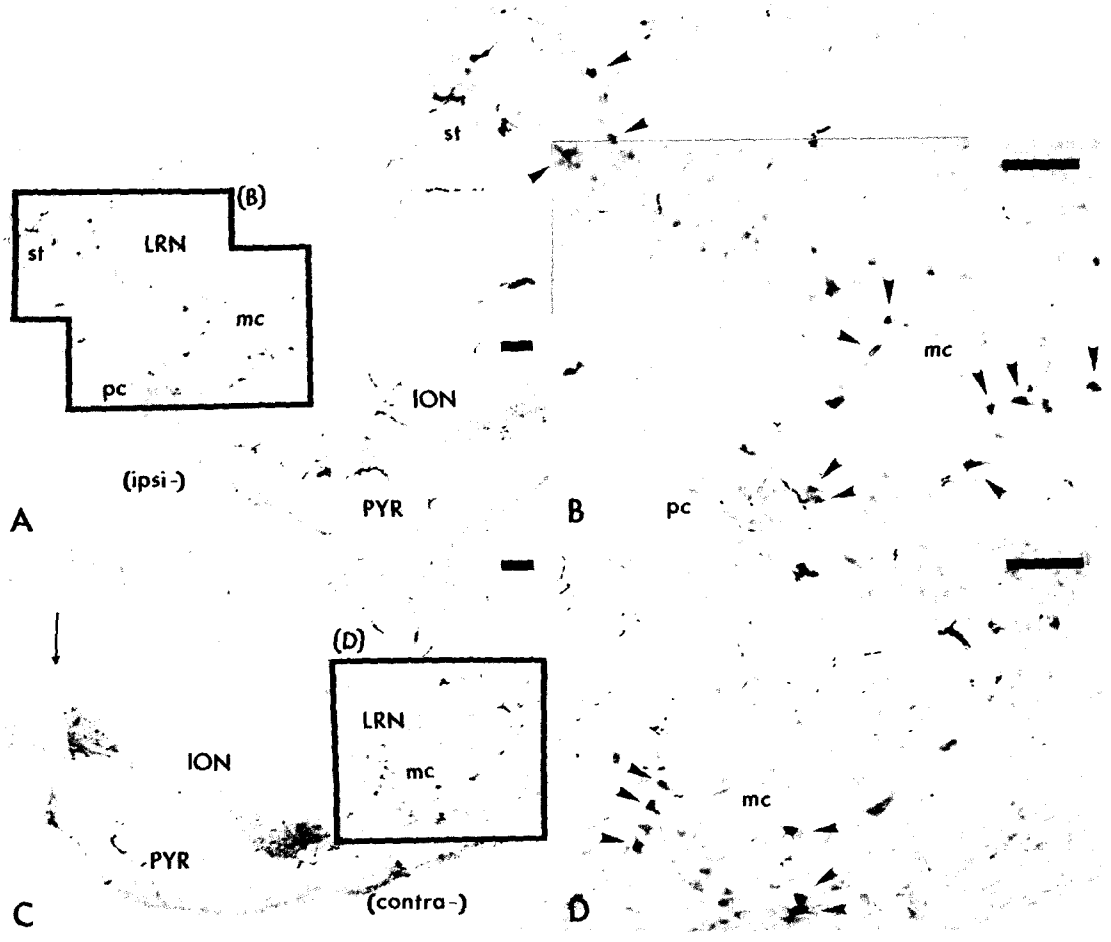

Fig. 3. Low and high magnification views of labelled cells (arrowheads) in the LRN, ipsilateral (A and B) and contralateral (C and D) to the injection site in the vermal lobule VIb. Note that the neurons in all three(magnocellular, parvocellular, and subtrigeminal) divisions of the ipsilateral LRN (A and B) were labelled. Bars represent 100 μm. Arrow, midline; ION, inferior olivary nucleus; LRN, lateral reticular nucleus; mc, magnocellular division of the LRN; pc, parvocellular division; PYR, pyramid; st, subtrigeminal division.

located in the ventromedial region of the magnocellular division (Fig. 4B, sections 2-7). Few cells were labelled in the parvocellular division (Fig. 4B, sections 4 and 5).

LRN projections to vermal lobule VIc

A total of ten cases involved WGA-HRP injections at cerebellar vermal lobule VIc (Fig. 6A). Labelled neurons in the magnocellular and subtrigeminal divisions of ipsilateral LRN were demonstrated in Fig. 5. Labelled cells in a representative case (Rat No. 26) were mapped at a rostro-caudal series of LRN sections (Fig. 6B). At the ipsilateral side, labelled neurons were located in the ventromedial region of the magnocellular division (Fig. 6B, sections 1-8). A few cells were located in the medial portion of the subtrigeminal division (Fig. 6B, sections 2-4) and in the parvocellular division (Fig. 6B, section 7).

At the contralateral side, cells were found in the ventromedial location of the magnocellular division, with somewhat caudal predominance (Fig. 6B, sections 2-8). Few cells existed in the medial portion of the subtrigeminal division (Fig.

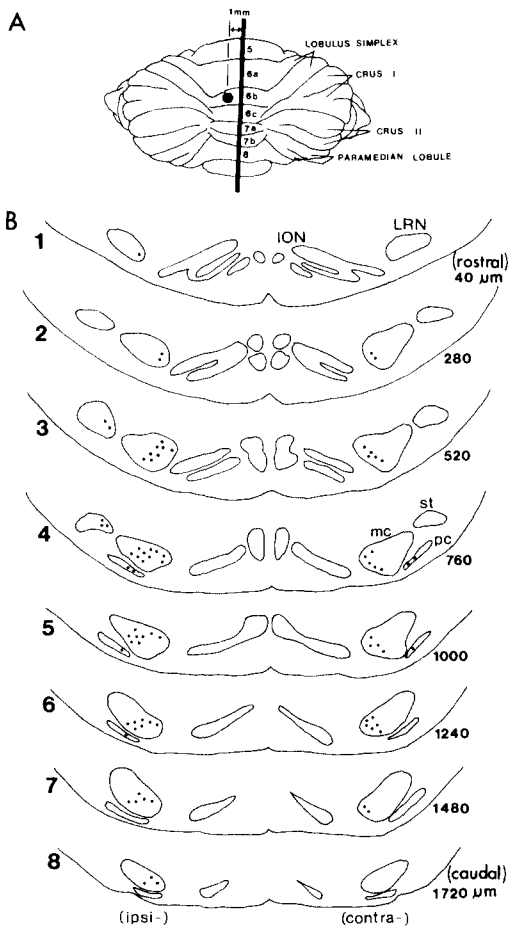

Fig. 4. An injection site (A) in the vermal lobule VIb and a rostro-caudal series (B) of transverse sections illustrating labelled neurons (filled circles) at the LRN in a representative case (Rat No. 14). ION, inferior olivary nucleus; LRN, lateral reticular nucleus; mc, magnocellular division of the LRN; pc, parvocellular division; st, subtrigeminal division.

6B, section 4).

Three-dimensional reconstruction of labelled LRN neurons

A three-dimensional image combining results of all three injection cases was not executed because of technical difficulties. Instead, either combined VIa/VIb or combined VIb/VIc injection cases were represented in Fig. 7 and Fig. 8, respectively. Each VIa, VIb, and VIc injection case was represented as blue, scarlet, and green, whereas overlapping zones of either combined VIa/VIb or combined VIb/VIc injection cases were represented as purple and yellow, respectively (Figs. 7 and 8). As described, labelling was the most pronounced in the ipsilateral and contralateral magnocellular division in all three injection cases. Extensive overlap was also observed either between VIa and VIb or between VIb and VIc injection cases.

Discussion

The lateral reticular nucleus has long been considered as one of the reticular relay nuclei in a spino-cerebellar pathway. All cells of the LRN give rise to fibers which pass to specific parts of the cerebellum. Previous anatomical and electrophysiological data support the thesis that exteroceptive impulses may be relayed to the cerebellum via this route (Combs, 1956).

The present study suggested that a relatively large number of neurons were labelled in the LRN, following injections into each subdivision of vermal lobule VI (Figs., 2, 4, and 6). Each subdivision of the cerebellar vermal lobule VI received LRN input primarily from the ipsilateral magnocellular division. It also received a modest input from the medial region of the contralateral magnocellular division. Ipsilateral or contralateral projection from the parvocellular and subtrigeminal division was scant. These results coincide with the previous report which suggested that unilateral injections of radioactive amino acid into the entire LRN resulted in bilateral terminal field labelling within the granular cell layer of lobules I through VIII (Chan-Palay *et al.*, 1977). Most of the previous studies employed a large injection site involving both sides of the lobules VI-IX and thus were unable to describe a discrete LRN projection to each subdivision of the cerebellar lobule VI (Hrycyshyn *et al.*, 1982; Payne, 1987).

The second aspect of the present results was related to the issue of topographic organization. Although projection pattern from the LRN onto each subdivision of cerebellar vermal lobule VI was not so sharply organized as that from the ION,

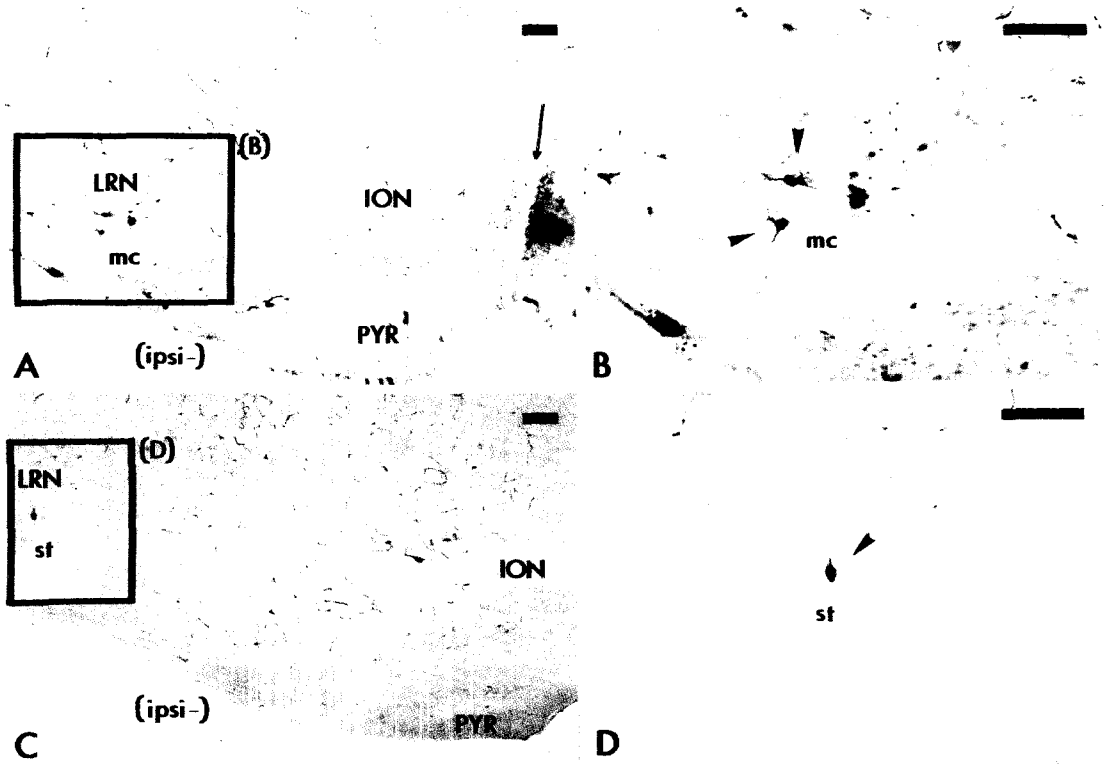

Fig. 5. Labelled neurons (arrowheads) were found in the magnocellular (A) and subtrigeminal (C) divisions of the LRN, ipsilateral to the injection site in the vermal lobule VIc. Higher magnification views of each division were shown in B and D, respectively. Note also that few cells were labelled in the ipsilateral ION, whereas a large number of cells labelled in the contralateral ION (A). Bars represent 100 μm. Arrow, midline; ION, inferior olivary nucleus; LRN, lateral reticular nucleus; mc, magnocellular division of the LRN; PYR, pyramid; st, subtrigeminal division.

some topography has been observed in the ipsilateral and contralateral magnocellular divisions of the LRN (Figs. 7 and 8). In the ipsilateral side, cells projecting to vermal lobule VIa occupied dorsal surface at rostral levels and dorsolateral region at caudal levels. In lobule VIb cases, neurons were observed in the central to medial regions of the magnocellular division as a dorsoventral band, whereas cells projecting to lobule VIc localized in the ventromedial portion of the LRN. Thus there seemed to be a dorsal-to-ventral transition in the ipsilateral magnocellular neurons projecting to the cerebellar vermal lobules VIa-to-VIc. In the contralateral side, cells projecting to vermal lobule VIa were observed at more rostral sections, whereas those projecting to lobule VIc were located at caudal sections.

There were relatively few labelled neurons in parvocellular and subtrigeminal divisions at either side (Figs. 2, 4, and 6). It has been described that the vermal lobule VIIIb is amply supplied with fibers from the LRN, especially from its parvicellular portion. Brodal (1975) has previously shown that the subtrigeminal part is connected with the paramedian lobule and the anterior lobe (chiefly its forelimb area). From these observations it has been speculated whether the subtrigeminal part was particularly active in transmission of impulses from the face.

Parasagittal zonation is a major feature of the organization of the cerebellum for certain afferent pathways including the olivocerebellar and reticulocerebellar systems (Chan-Palay *et al.*, 1977). By contrast, the pontocerebellar projection

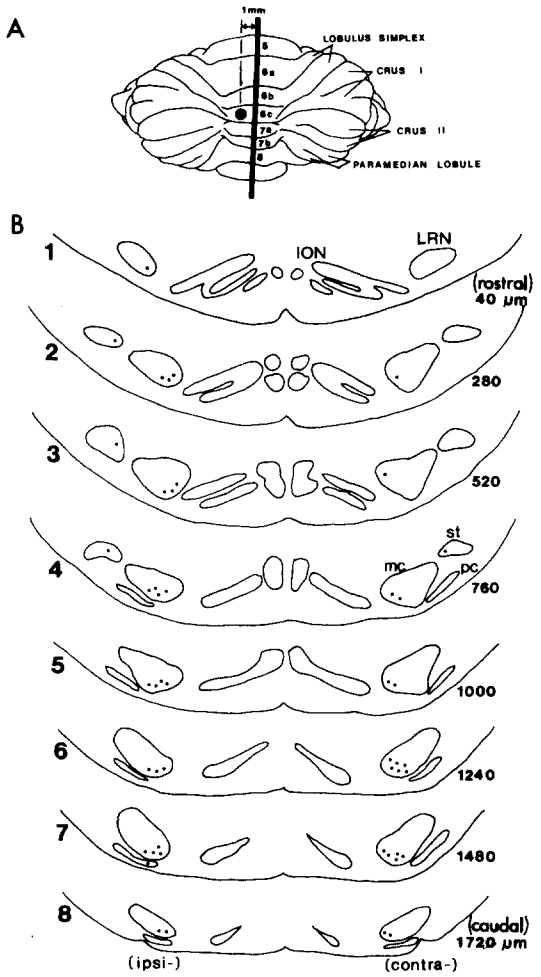

Fig. 6. An injection site (A) in the vermal lobule VIc and a rostro-caudal series (B) of transverse sections illustrating labelled neurons (filled circles) at the LRN in a representative case (Rat No. 26). ION, inferior olivary nucleus; LRN, lateral reticular nucleus; mc, magnocellular division of the LRN; pc, parvocellular division; st, subtrigeminal division.

exhibited little, if any, comparable parasagittal organization (Gould, 1980). Unilateral injections of radioactive amino acid in the LRN produced heavy, uncrossed, ipsilateral and light, crossed, contralateral projections (Chan-Palay *et al.*, 1977). The authors indicated that terminal arborizations in the cerebellar cortex were dense fields of mossy fiber rosettes forming narrow sagittal bands restricted to the granular layer of the cerebellar cortex. They specifically mentioned that the LRN representation in the entire vermis consists of eight sagittal bands in lobules V through VIII; the band widths are approximately 300-400 μm and the interspaces slightly narrower, approximately 250-300 μm wide. Using the retrograde fluorescent double labelling method, Payne (1987) indicated that LRN neurons exhibited axonal branching both within and between parasagittal zones of the cerebellar cortex, but that branching within zones is more common. As described in the previous section, animals within relatively small range of weight (300-320g) had been used and injection sites were precisely localized (1.0 mm lateral to the midline) in the present study. Overlapping zones observed in Figs. 7 and 8 might, therefore, represent axonal branching of LRN neurons within parasagittal zones of lobules VIa and VIb or lobules VIb and VIc. In order to describe axonal branching into the two adjacent subdivisions of lobule VI, one might inject two different fluorescent dyes (e.g. true blue and nuclear yellow) into each subdivision within lobule VI. However, the artifact caused by diffusion or transfer of dyes into the adjacent subdivision was suspected and had not been used in the present study.

Based on the three-dimensional reconstruction study, it can be concluded in the rat that there exists a substantial LRN projection to each subdivision of the cerebellar vermal lobule VI, including VIa, VIb, and VIc (Figs. 2, 4, and 6). Projections to vermal lobule VII including VIIa and VIIb subdivisions will be subsequently investigated in our laboratory. These results combined with previous autoradiographic and retrograde labelling studies might provide an overall information about the pattern of reticulocerebellar projection and its topographic organization in the rat. Computer-aided three-dimensional reconstruction of projections from the LRN onto the cerebellar cortex might also be, in the future, utilized to describe the projections from other precerebellar nuclei in the brainstem onto the cerebellum and the degree of overlap within the projections into each lobule of the cerebellar cortex.

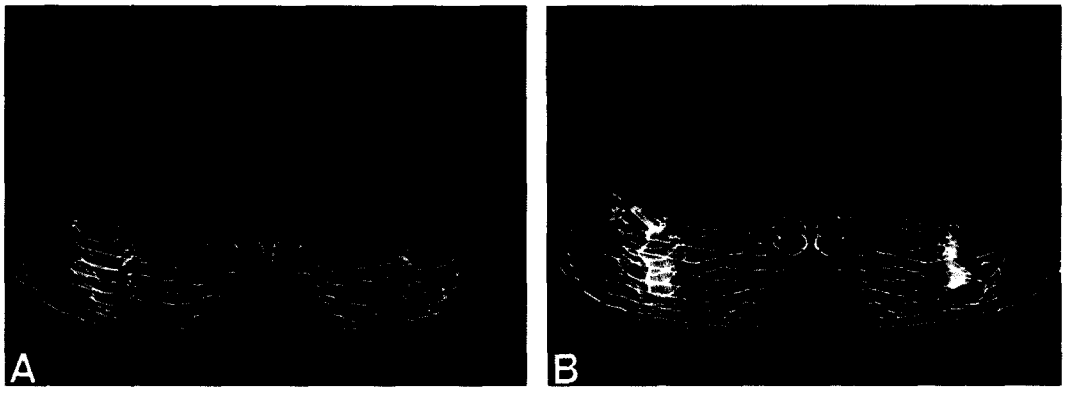

Fig. 7. Cells projecting to cerebellar vermal lobule VIa and VIb were represented within a rostral (drawn at upper position) to caudal (drawn at lower position) series of LRN sections (A). Regions occupied by neurons projecting to VIa were represented as blue, whereas areas taken by neurons projecting to VIb represented as scarlet. Overlapping zones were purple-colored. Regions occupied by neurons projecting to cerebellar vermal lobule VIb and VIc were shown in B. Cells projecting to VIb were scarlet-colored, whereas those projecting to VIc green-colored. Overlapping zones were represented as yellow.

Acknowledgements

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쥐의 외측 망상핵으로부터 소뇌충부 제6엽으로의 신경 경로에 관한 연구

이현숙(건국대학교 의과대학 의예과)

쥐의 외측 망상핵으로부터 소뇌충부 제6엽 내의 각 소엽으로의 신경경로를 WGA-HRP를 이용한 역행수송법을 써서 조사하였다. 표지된 신경세포는 양측의 외측 망상핵에 모두 존재하였으나, 동측의 경우에 편중되어 있었다. 동측 또는 대측의 외측 망상핵의 큰세포구획(magnocellular division)에서 국소순서적 배열이 관찰되었는데, a소엽에서 c소엽으로의 투사가 동측의 큰세포구획에서 등쪽에서 배쪽으로의 분포 양상을 보였으며, 대측의 큰세포구획에서는 다소 머리측에서 꼬리측 절편으로의 분포 양상을 보였다. 그 외 동측 또는 대측의 작은세포구획(parvocellular division) 및 삼차밑구획(subtrigeminal division)에서의 표지된 신경세포의 수는 극히 적었다. 한편 외측 망상핵으로부터 소뇌충부의 제6엽의 a소엽/b소엽 또는 b소엽/c소엽으로의 투사에 관한 컴퓨터를 이용한 삼차원 재구성은 각 경우에 있어서 상당량의 투사의 중첩이 존재함을 보여주고 있다.