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SHORT COMMUNICATION
Connections of the Lateral Reticular Nucleus to the Lateral Vestibular Nucleus in the Rat. An Anterograde Tracing Study with *Phaseolus vulgaris* Leucoagglutinin

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Abstract

Efferent projections from the lateral reticular nucleus in the rat were investigated with anterograde transport of *Phaseolus vulgaris* leucoagglutinin. Besides the well known mossy fibre connections to the cerebellar cortex and collaterals to the cerebellar nuclei, a substantial bilateral projection to the lateral vestibular nucleus was found. Terminal arborizations found within this nucleus appeared to detach from the reticulocerebellar fibres in the cerebellar white matter and enter the lateral vestibular nucleus from dorsally. This projection may have functional relevance for the control, by ascending spinal pathways, of the descending lateral vestibulospinal tract.

The lateral reticular nucleus (LRt) is an important relay station for ascending information destined for the cerebellum. Spinal afferents to the LRt have been described in various animals including the cat (Brodal, 1949; Morin *et al.*, 1966; Künzle, 1973; Corvaja *et al.*, 1977) and rat (Menétrey *et al.*, 1983; Shokunbi *et al.*, 1985; Rajakumar *et al.*, 1992; for review see Ruigrok and Cella, 1995). Mossy fibre projections arising from the LRt terminate in large areas of the cerebellar cortex and in addition terminal arborizations have been found within the cerebellar nuclei, especially in the interposed and fastigial nuclei (cat: Künzle, 1975; Dietrichs and Walberg, 1979; Dietrichs, 1983; rat: Hrycyshyn *et al.*, 1982; Ghazi *et al.*, 1987; Payne, 1987). Most of these studies have been performed with the aid of retrograde tracing studies or with relatively large LRt injections with anterograde tracers such as tritiated leucine or wheatgerm agglutinin coupled to horseradish peroxidase.

In order to study the mossy fibre projections arising from the LRt in increasing detail we used iontophoretic injections with the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L). Upon examination of the material, we noted a prominent, but as yet not described, bilateral projection to the lateral vestibular nucleus. This projection may be engaged in the control of the locomotion-related modulation of activity observed in lateral vestibulospinal tract neurons (Orlovsky, 1972; Marlinsky, 1992).

Eight Wistar rats, anaesthetized with pentobarbital (120 mg/kg i.p.), were mounted in a stereotactic device according to Paxinos and Watson (1986). The squamosal part of the occipital bone was freed of neck musculature and the foramen magnum was slightly enlarged dorsalwards. A glass micropipette, filled with PHA-L solution (Vector Laboratories, Burlingame, CA; 2.5% in 0.05 M Tris-buffered saline, pH 8.0) and a tip of 12–15 μm, was inserted in the caudal brainstem at 45° to the vertical plane. Neuronal activity was monitored conventionally by way of a silver wire introduced into the PHA-L solution. The approximate position of the LRt was established by way of stereotactic coordinates and was verified by recording spike activity that could be modulated by inducing limb movements (Marini and Wiesendanger, 1987). When appropriate responses were obtained, PHA-L was injected by applying positive current (4 μA; 7 s on, 7 s off) for 15–30 min. Afterwards the wounds were sutured and the animal survived for 7 or 8 days, during which they were checked daily. Subsequently, under deep pentobarbital anaesthesia, they were perfused transcardially with 400 ml 0.05 M phosphate buffer, pH 7.4 (PB) containing 0.8% saline, 0.8 % sucrose and 0.4% glucose, which was followed by 1000 ml fixative (2.5% glutaraldehyde and 0.05% paraformaldehyde in PB). The brain was extracted, postfixed for 2–4 h in the same fixative and stored overnight in 10% sucrose in PB. After embedding in gelatine (12%) the brains were stored in 30% sucrose in PB until they sank. Transverse sections (40 μm) were made on a freezing microtome and were collected in glass vials in PB. Selected vials were incubated with goat anti-PHA-L (Vector Laboratories) for 24 h, biotinylated rabbit anti-goat (2 h; Vector Laboratories) and ABC elite (2 h; Vector Laboratories). All solutions were made in 0.05 M Tris-buffered saline containing 0.05% Triton X-100 (TBS^+; pH 8.6), and between incubations the sections were thoroughly rinsed in TBS^+. Finally, the sections were reacted with diaminobenzidine/H_2O_2 or diaminobenzidine/cobalt/H_2O_2, mounted and counterstained with thionine.

The location and spread of the PHA-L injection sites were carefully screened. Injections that incorporated the adjacent inferior olivary complex or overlying reticular formation were discarded. Four cases were selected with injections that were confined to the LRt. Since all these cases showed essentially similar projection patterns, the description will be based on case p1 (Figs 1 and 2). Here, the injection site was centred on the medial part of the LRt at the level of the caudal pole of the inferior olive (Fig. 1A). Most fibres leaving the

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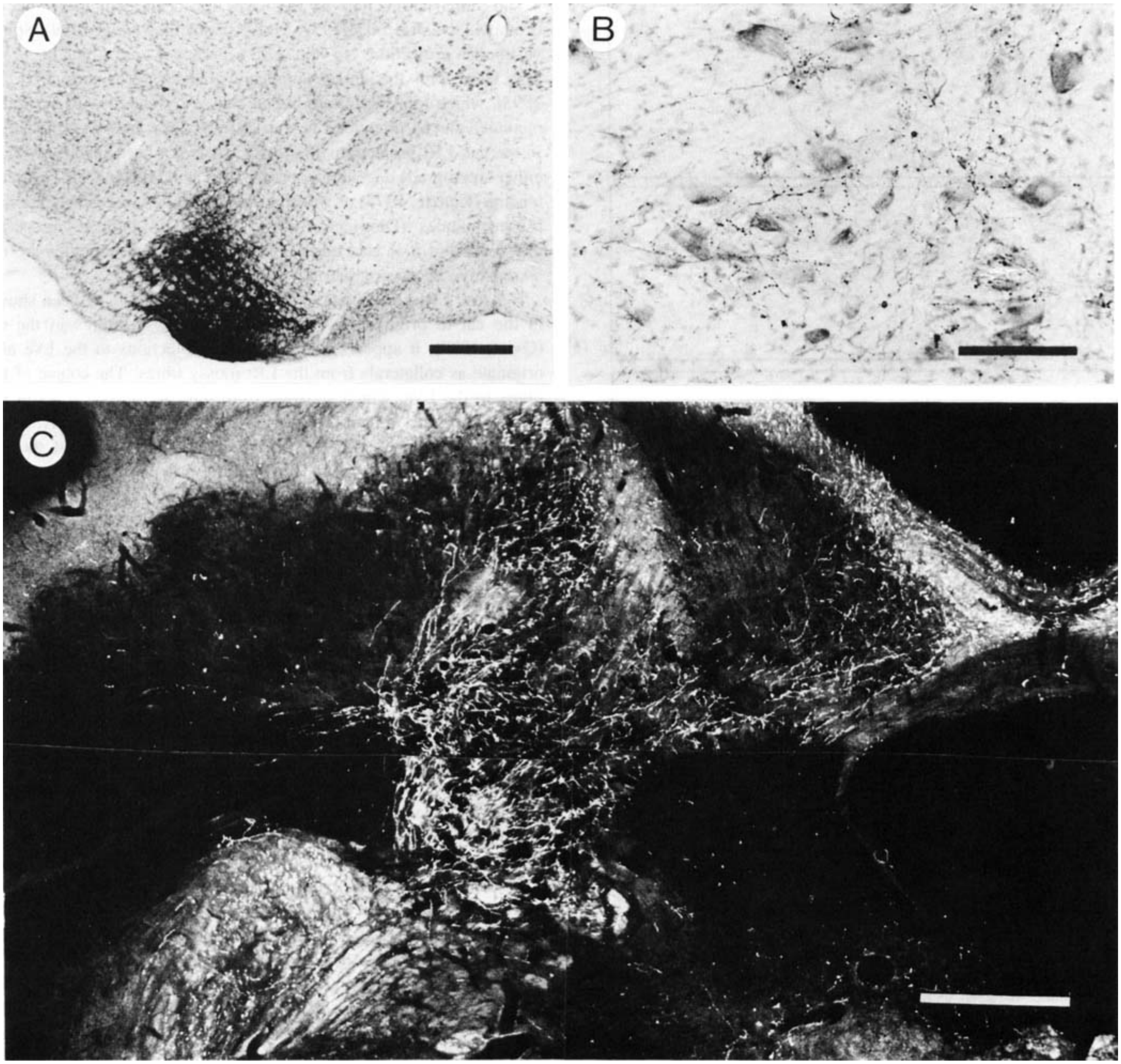

FIG. 1. (A) Microphotograph of the PHA-L injection site in animal p1. Note that the injection is centred on the medial part of the LRt and does not incorporate either the inferior olive or the overlying reticular formation. (B) Detail of labelled terminal arborizations within the ipsilateral LVe. Thionine-counterstained section. (C) Dark-field microphotograph showing terminal PHA-L labelling within the ventromedial part of the medial cerebellar nucleus, medial part of the anterior interposed nucleus and within the LVe, ipsilateral to the injection site. Same section as depicted in middle panel of Figure 2. Bar = 500 μm in A and C, 100 μm in B.

injection site could be seen to enter the ipsilateral inferior cerebellar peduncle (icp), although some crossed the midline and joined the contralateral icp. Labelled fibres entered the cerebellum, by way of the icp, immediately rostral and dorsal to the anterior interposed nucleus, from where they dispersed to terminate as mossy fibres in various areas of the cerebellar cortex, especially within the anterior lobe, the simple lobule, vermal lobule VIII, crus I and the paramedian lobule. Some fibres crossed the cerebellar midline. Numerous fibres, presumed to be collaterals (Qvist, 1989), coursed caudally and ventrally to enter the cerebellar nuclei, where terminal arborizations with varicosities could be found, especially within the medial parts of the anterior and posterior interposed nuclei (IntA and IntP). Additional projections were observed in the ventral and medial parts of the medial cerebellar nucleus (Med) and ventrally within the rostral part of the lateral cerebellar nucleus (LatC). Ongoing fibres descend through the interposed nuclei and the superior cerebellar peduncle (scp) to enter the lateral vestibular nucleus (LVe) where, again, many terminal arborizations were found (Figs 1B, C and 2). The

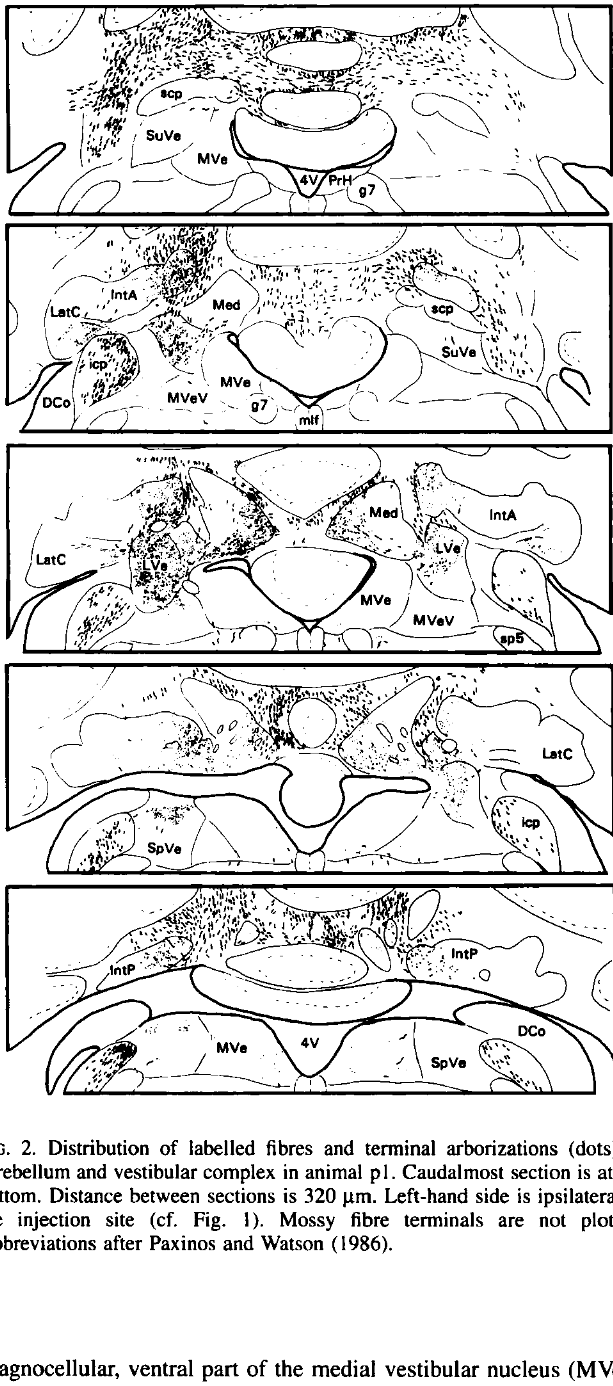

FIG. 2. Distribution of labelled fibres and terminal arborizations (dots) in cerebellum and vestibular complex in animal p1. Caudalmost section is at the bottom. Distance between sections is 320 μm. Left-hand side is ipsilateral to the injection site (cf. Fig. 1). Mossy fibre terminals are not plotted. Abbreviations after Paxinos and Watson (1986).

magnocellular, ventral part of the medial vestibular nucleus (MVeV) (Rubertone *et al.*, 1994) remained free of labelled fibres as did the medial vestibular nucleus proper (MVe). Some terminal labelling was found in the dorsal part of the spinal vestibular nucleus (SpVe). All projections were found bilaterally with ipsilateral preponderance (Fig. 2).

To the best of our knowledge, projections from the LRt to the LVe have not yet been described with either anterograde or retrograde techniques. Therefore, although PHA-L is not liable to be taken up by passing fibres (Gerfen and Sawchenko, 1984) one should consider this possibility. In particular, passing spinovestibular fibres (Hazlett *et al.*, 1972) may have been labelled inadvertently. However, spinovestibular fibres labelled at the level of the LRt are not likely to terminate bilaterally. Moreover, they have been described to terminate predominantly within the medial and inferior vestibular nuclei, while a substantial projection to the LVe has been denied in the cat (Pompeiano and Brodal, 1957; McKelvey-Briggs *et al.*, 1989). Spinovestibular projections to the LVe, moreover, are thought to be associated with the dorsal spinocerebellar tract (Rubertone *et al.*, 1995), which follows a more lateral course compared with our rather medially placed injections in the LRt (Paxinos and Watson, 1986). In the cat, LRt projections to the LVe have not been mentioned with either anterograde degeneration (Matsushita and Ikeda, 1976), tritiated leucine (Künzle, 1975) or wheatgerm agglutinin–horseradish peroxidase techniques (Dietrichs, 1983). This could indicate a species difference but it is also quite possible that these techniques are not as sensitive as anterograde tracing with PHA-L.

Because LRt projections to the cerebellar nuclei have been shown in the cat to originate as collaterals from mossy fibres in the cat (Qvist, 1989), it appears likely that the projections to the LVe also originate as collaterals from the LRt mossy fibres. The course of the fibres to the LVe, which separate from the LRt mossy fibres in the cerebellar white matter and enter the LVe from dorsally, is in accordance with a collateral origin. Labelled fibres from the icp entering the LVe from laterally were rarely observed.

The projection from the LRt to the LVe may reflect involvement of the spino-reticulo-cerebellar system in the control of descending motor pathways. In the cat, the cells of origin of one of the major pathways from the spinal cord to the LRt, the bilateral ventral flexor reflex tract (bVFRT; Clendenin *et al.*, 1974), are monosynaptically activated by the lateral vestibulospinal tract (Holmqvist *et al.*, 1960). Thus, our results suggest that a closed loop may exist, connecting bVFRT neurons to LRt to LVe and back to bVFRT neurons. Furthermore, Arshavsky *et al.* (1978) have shown in the cat that LRt neurons may show locomotion-related activity, even during fictive walking. Locomotion-related activity is also found in the LVe, and has been thought to be induced by a combination of excitatory actions by spinovestibular projections (Wilson *et al.*, 1966; ten Bruggencate *et al.*, 1972a) and inhibitory actions of Purkinje neurons in the lateral vermis of the anterior lobe (Ito and Yoshida, 1966; Wilson *et al.*, 1966; ten Bruggencate *et al.*, 1972b; Orlovsky, 1972). The latter mechanism would imply a disinhibition of the lateral vestibulospinal neurons by an 'out of phase' modulation of the Purkinje cells in relation to the LVe modulation (Orlovsky, 1972). However, Udo *et al.* (1981, 1982) have found indications that the Purkinje cells in the b zone of the lateral vermis, which project to the LVe (Voogd, 1964; Voogd *et al.*, 1991), are modulated 'in phase' with related LVe neurons. This would imply that the inhibitory actions of Purkinje cells counteract rather than induce the modulation of LVe neurons. Similar conclusions were reached for locomotion-related activity in the anterior interposed nucleus (Armstrong and Edgley, 1984a, b). It follows that other afferent sources may be responsible for inducing the locomotion-related modulation of LVe activity. It is tempting to speculate that the projection from the LRt to the LVe that is reported here may contribute in producing this modulation.

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Abbreviations

icp	inferior cerebellar peduncle
IntA	anterior interposed nucleus
IntP	posterior interposed nucleus
LatC	lateral cerebellar nucleus
LRt	lateral reticular nucleus

LVe	lateral vestibular nucleus
Med	medial cerebellar nucleus
MVe	medial vestibular nucleus
MVeV	ventral part of the Mve
PB	phosphate buffer
PHA-L	*Phaseolus vulgaris* leucoagglutinin
scp	superior cerebellar peduncle
SpVe	spinal vestibular nucleus

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