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# ORGANIZATION OF BILATERAL SPINAL PROJECTIONS TO THE LATERAL RETICULAR NUCLEUS OF THE RAT

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## INTRODUCTION

Recent works on patients with unilateral cerebellar lesions show changes in kinematics of contralateral limbs with reduction of the peak speed and slowing of pointing movements (7). Recently, Greger *et al.* (3) observed that the activity of neurons of the lateral cerebellum are dependent on variation of movement parameters, independently of the used limb. On the basis of their results, these Authors suggested the possibility of a gradient for the effects elicited by contralateral inputs, with a minimal action in the more medial part of the cerebellum and increasing influences on the more lateral parts. Concerning the possible pathways mediating these bilateral inputs, the lateral reticular nucleus (LRN) could represent a solid candidate. As matter of fact, data available from investigations in different mammals indicate that the main afferent inputs to the LRN are a bilateral, topographically organized projection from the spinal cord (for review, see 18). Furthermore, the projections from LRN to the cerebellum are bilateral, with an ipsilateral predominance (P14), and they involve both anterior and posterior cerebellar lobes (23,25).

Menetrey *et al.* (11), on the basis of neuroanatomical experiments with horseradish peroxidase (HRP) injections in the LRN, have found that labeled neurons were present at all spinal levels and in particular large numbers in the cervical and lumbar enlargements. Labeled cells were located, with contralateral predominance, in all segments of the spinal cord, mainly within laminae VI, VIII, and X of Rexed (17). These results were only partly confirmed by Shokunbi *et al.* (20) and Cella *et al.* (2), who noticed that small injections of WGA-HRP in the dorsal LRN regions (involving the magnocellular portion of the nucleus, LRN-m) mainly resulted in retrogradely labeled cells within the ipsilateral cervical cord. Whereas, injections placed in the ventral LRN (involving the parvicellular portion of the nucleus, LRN-p) predominantly labeled neurons located bilaterally within the lumbar spinal cord. Injections involving the subtrigeminal part of the structure (LRN-s) are characterized by a combination of bilateral cervical and lumbar labeling. However, Rajakumar *et al.* (16), by using anterograde transport of WGA-HRP, failed to note an ipsilateral spinoreticular projection arising from lumbar levels; furthermore, they were unable to differentiate between dorsal and ventral horn projections. Finally,

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Koekkoek and Ruigrok (8), in a retrograde double labeling study in the rat, did not find spinoreticular cells with bilateral projections.

The present investigation examines this organization further for bilateral spinoreticular projections from the entire spinal cord in the rat by using two different protocols. In the first group of experiments, we used orthograde transport of small amount of Phaseolus vulgaris-leucoagglutinin (PHA-L) ionophoretically (22) injected in the spinal gray matter of one side to identify ipsi- as well as contralateral LRN areas reached by spinoreticular fibers. In a second group of experiments, we used the retrograde transport of two different fluorescent tracers (9), i.e. "cytoplasmatic" tracers Fast Blue (FB) and "nuclear" tracer Diamidino Yellow dihydrochloride (DY); one of the tracer was injected in the LRN of one side and the other in the contralateral one for identifying bilateral spinoreticular projections.

## MATERIAL AND METHODS

*Animals and treatment.*

Experiments were carried out on young adult male rats (Wistar, 200-280 g, n = 25) housed under a 12 hr light-12 hr dark cycle, given food and water ad libitum and deeply anesthetized with urethane (1.2 g/kg i.p.). Animal care and handling were carried out in accordance with guidelines issued by the Committee of Experimental Animals of University of Catania; all efforts were made to minimise animal suffering and to reduce the number of animal used.

*Anterograde experiments.*

Fifteen rats were submitted to small injection of PHA-L (2.5% in 0.01 mol/liter sodium phosphate buffer, pH 8.0, Vector Laboratories Inc.) into the spinal cord (21) ionophoretically (22). The tip of a glass micropipette (tip diameter, 10-20 m) was inserted into the spinal cord under microscopic guidance. Then, a positive current (5 μA, 7 seconds on/7 seconds off) was applied for 20 minutes. Injections were made unilaterally into several cord levels: cervical (5 cases; C1-C2, C2-C3, C2-C4, C4-C5 and C6-C7), cervico-thoracic (1 case; C6-T1), thoracic (3 cases; T1-T2, T2-T4 and T6-T10), thoraco-lumbar (2 cases; T10-L1 and T11-L3), lumbar (2 cases; L1-L2 and L3-L4), lumbo-sacral (1 case; L5-S2) and sacral (1 case; S1-S3). Each area was exposed by laminectomy of the two adjacent vertebrae and particular care was taken to control a possible spread of tracer contralaterally or in the subarachnoid space.

After a 8-12 day survival period, the rats were reanesthetized with urethane and perfused sequentially with 500 ml of 0.9% saline at 42 °C, 500 ml of 1.25% gluteraldehyde-1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature and 500 ml of an ice-cold 10% sucrose solution in 0.1 M phosphate buffer (pH 7.4). The brains and injected spinal cord sections were removed and postfixed for 1 hour at 4 °C. Then, the tissue was embedded in gelatin. Frontal as well as sagittal frozen sections (20 μm) were cut and collected in 0.1 M pH 7.4 phosphate buffer.

Sections were processed for immunohistochemistry by the avidin-biotin complex (ABC) method (4). Immunoprocessing steps include 1) incubations in primary antibody solution containing biotinylated goat anti-PHA-L (BA-0224, Vector; dilution 1:200), 2) two hours incubation at room temperature in avidin-biotin complex solution (ABC-kit PK-400; Vector Vecstain, dilution 1:500), 3) 10 min incubation at room temperature with 0.05% 3,3-diaminobenzidine (DAB) in rinse in 0.05 M Tris Buffer, pH 7.6 (TB), 4) 15 min incubation at room temperature with 0.05% DAB in TB containing 0.002% $H_2O_2$.

The immunostained sections were mounted onto gelatin-coated slides and coverslipped with Entellan (Merck) to be examined by microscopy. Some of immunostained sections were stained with Neutral Red for study of cytoarchitecture.

The slides were examined with both bright- and dark-field illumination. Crossed polarizers were placed in the light path to produce an intensified image of reaction products.

To locate the PHA-L injection sites, labeled cell bodies were plotted from all sections in which they were present. Anatomic boundaries were outlined from adjacent Neutral Red-stained sections by using a camera lucida, and the outlines were superimposed on PHA-L-stained sections. PHA-L-labeled fibers were considered to constitute a terminal plexus if the fibers were thin, branching, had varicosities, and were found in successive sections. If the labeled fibers were thick, straight, and had no varicosities, they were considered to be passing fibers. The density of PHA-L terminal labeling was determined semiquantitatively and assigned one of three density grades of terminal labeling: light, moderate and heavy. Photographs were take of salient features.

*Retrograde double-labeling experiments.*

Nine animals were submitted to injections of fluorescent tracers FB (Sigma; 2% w/v in 0.1 M cacodylate buffer) and DY (Sigma; 2% w/v in saline); the FB was injected by ionophoresis (5-10 min of 5-10 μA pulsed positive current) (19), whereas DY was hydraulically delivered (30-50 nl).

The ionophoretic injections were carried out by using glass micropipettes with tips broken at approximately 20 μm diameter. The hydraulic injections were made by pressure through glass micropipettes (tips 20-30 μm) cemented to a 1 μl Hamilton syringe.

The micropipettes were inserted in the LRN according to the stereotaxic coordinates of Paxinos and Watson's atlas (15). To avoid that, during the penetration, the micropipettes will cross through the spinocerebellum and the external cuneate nucleus, they were inclined in latero-medial direction, with an angle of about 10° with respect to the vertical axis.

After a 5-7 day survival period the rats were reanesthetized with urethane and perfused sequentially with 500 ml of 0.9% saline at 42 °C, 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature and 500 ml of an ice-cold 10% sucrose solution in 0.1 M phosphate buffer (pH 7.4).

The brains and the spinal cords were removed and placed overnight in the same ice-cold 10% sucrose in phosphate buffer solution and then for 3-4 days in a 30% sucrose in phosphate buffer solution at 4 °C. Coronal frozen sections (30 μm) were cut and mounted on glass slides coated with chrome alum. Half the slides were counterstained with 1% neutral red and all were dehydrated, cleared and covered as described above.

The sections were analyzed using an Axioplan microscope (Zeiss) with bright- and dark-field illumination as well as fluorescent emission. Labeled cells in the spinal cord were drawn on camera lucida outlines and their number in each lamina was counted in four adjacent sections of seven different spinal levels (C1, C7, T2, T11, L2, L5 and S2). Borders of the spinal laminae were identified on the basis of the cytoarchitectonic studies of Molander *et al.* (12, 13). The boundaries of the injection sites in the LRN were traced using a 2.5x objective. The fluorescent injection areas had two concentric fluorescent zones around the needle track. The uptake of tracers by terminals and damaged fibers appears to occur only in the inner zone (5, 6). Injected areas were quantitatively evaluated by adapting the method of Wolf and Gollob (24).

## RESULTS

*Anterograde experiments.*

Injections of PHA-L solution in the spinal cords of 15 rats were distributed from the upper cervical to lower sacral levels.

Figure 1 shows three experiments (rats A4, A5 and A7) in which the tracer injection involved nearly all of laminae III-VII in cervical segments (rat A4: C1-C2; rat A5: C4-C5; rat A7: C6-C7). Heavy terminal labeling was present in the dorsal parts

of LRN-m in rats A4 and A5 while the terminal labeling in the same nuclear area was less dense in rat A7. In these experiment very few labeled terminals were observed in LRN-p and no terminals were found in LRN-s. When the injection included only the upper thoracic levels (Figure 1; rat A9: T1-T2), the labeling was still restricted to LRN-p. However, injections involving the lumbar levels (Figure 1; rat A14: L1-L2; rats A15: L3-L4) produced a very strong labeling in the parvicellular division of LRN, with few labeled terminals in LRN-p and very few labeled ter-

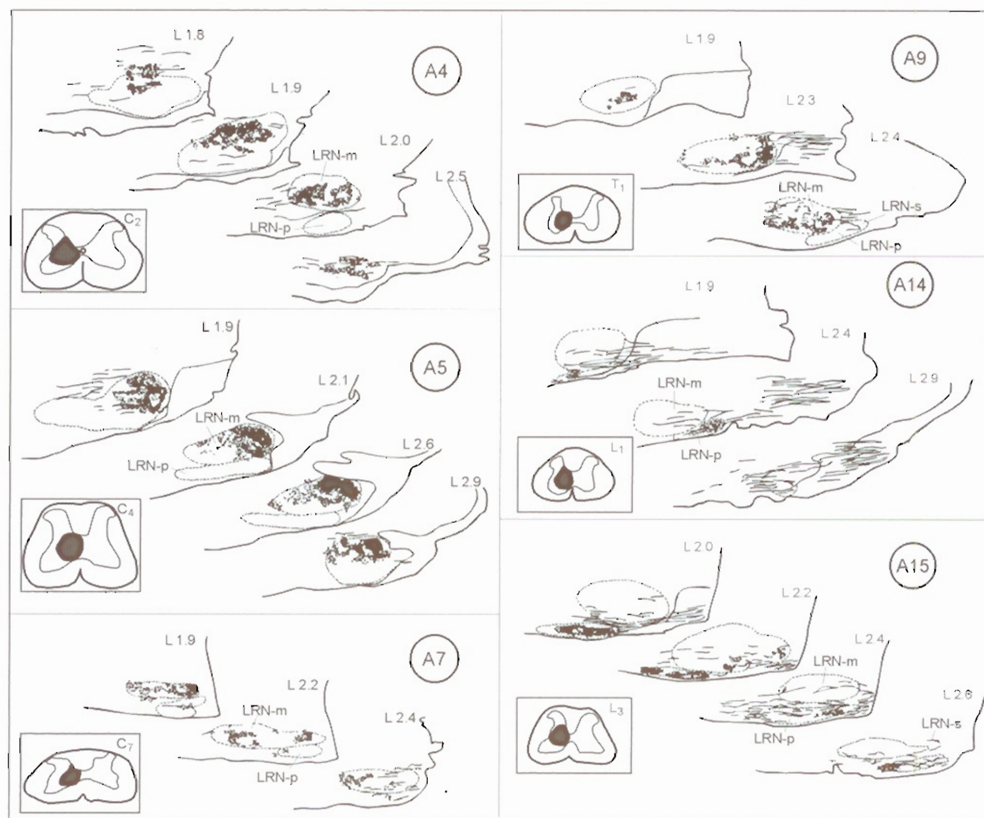


Fig. 1. - *Examples of labeled terminals distribution within the lateral reticular nucleus (LRN) following Phaseolus vulgaris-leucoagglutinin (PHA-L) injections into different segments of the spinal cord.*
For each experiment there are shown camera lucida drawings of sagittal sections of the brainstem, contralateral with respect to the injected spinal segment, whereas the lower inset illustrates the injection site (the black area indicates the effective tracer uptake area). Drawings on the left show three experiments (rats A4, A5 and A7) in which the tracer injection involved nearly all of laminae 3-7 in cervical segments (rat A4: C1-C2; rat A5: C4-C5; rat A7: C6-C7). Heavy terminal labeling was present in the magnocellular part of LRN (LRN-m) in rats A4 and A5 while the terminal labeling in the same nuclear area was slightly less dense in rat 7A. In these experiments very few labeled terminals were observed in parvocellular part of LRN (LRN-p). Drawings on the right illustrate experiments where the injections involved the lower portions of the spinal cord. It can be seen then, when the injection included only the upper thoracic levels (rat A9: T1-T2), the labeling was still restricted to LRN-m. On the other hand, when the injections involved the lumbar levels (rat A14: L1-L2; rats A15: L3-L4) produced a very strong labeling in LRNp, with few labeled terminals in LRN-m.

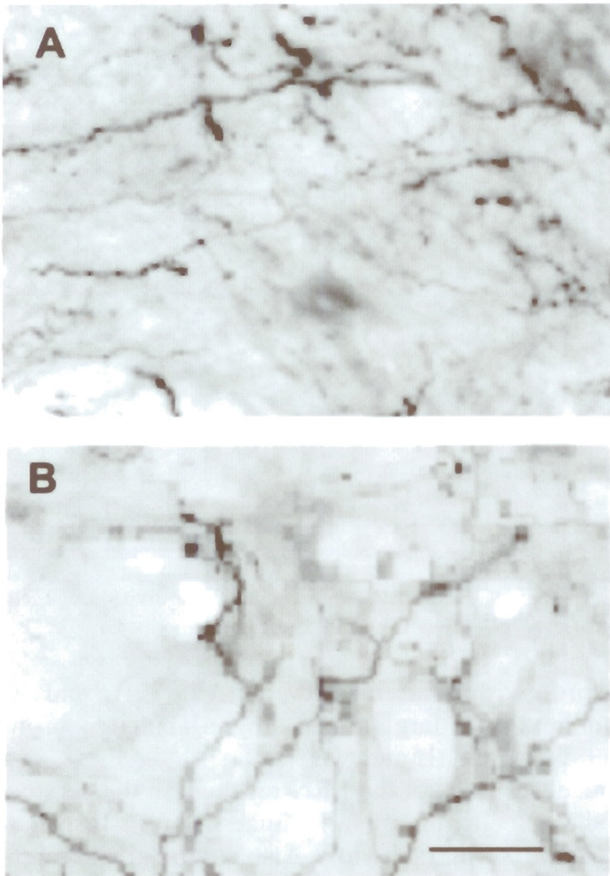


Fig. 2. - *Photomicrographs of spinoreticular labeled terminals within the LRN.*
Calibration bar: 10 μm.

minals in LRN-s. Examples of anterogradely labeled spinoreticular terminals are showed in Figure 2.

As summarized by Table 1, we found that LRN-m receives spinoreticular fibers coming from cervical and upper thoracic segments, while the parvicellular division is reached by labeled fibers arising in the lower thoracic, lumbar and sacral segments. Very few labeled fibers were observed in the subtrigeminal division of LRN. Table 1 also shows that, in all the experiments, spinoreticular projections from a certain spinal segment involve bilaterally the same LRN divisions with a more intense terminal labeling in the contralateral one.

*Retrograde experiments.*

Table 2 shows the involvement of different subdivisions of right and left LRN by injections of two different fluorescent tracers in nine experiments.

As can be seen in Table 3, single-labeled neurons were present at all spinal levels and in particular large numbers in the cervical and lumbar enlargements. Retrogradely single-labeled cells were located, with contralateral predominance,

Table 1. - *Anterograde labeling within the different divisions of ipsilateral as well as contralateral lateral reticular nucleus (LRN) following small injections of Phaseolus vulgaris-leucoagglutinin into the gray matter of the spinal cord of one side.*

| Rat | Uptake Area | Ipsi LRN-m | Ipsi LRN-p | Ipsi LRN-s | Contra LRN-m | Contra LRN-p | Contra LRN-s |
|---|---|---|---|---|---|---|---|
| A1 | C2-C3 | + + | + | - | + + + | + | - |
| A2 | C2-C4 | + + | + | - | + + + | + | + |
| A3 | C6-T1 | + + | - | - | + + | + | - |
| A4 | C1-C2 | + + | + | + | + + + | + | + |
| A5 | C4-C5 | + + + | + | - | + + + | + | - |
| A6 | T2-T4 | + | + | - | + | + + | - |
| A7 | C6-C7 | + | + | - | + + | + | - |
| A8 | T6-T10 | - | + | - | + | + + | - |
| A9 | T1-T2 | - | + | - | + | + + | - |
| A10 | T10-L1 | + | + + | - | + | + + + | - |
| A11 | T11-L3 | + | + + | - | + | + + + | + |
| A12 | L5-S2 | + | + + | + | + | + + + | - |
| A13 | S1-S3 | - | + | - | + | + + | - |
| A14 | L1-L2 | + | + + | - | + | + + | - |
| A15 | L3-L4 | + | + + + | - | + | + + + | + |

Incidence of PHA-L-reactive terminals: + + +, large; + +, moderate; +, few; -, none. Uptake area indicates the spinal segments involved by tracer injection.
Abbreviations: C, cervical; T, thoracic; L, lumbar; S, sacral; LRN-m, magnocellular division of LRN; LRN-p, parvocellular division of LRN; LRN-s, subtrigeminal division of LRN.

in all segments of the spinal cord, within laminae IV, V, VI, VIII, and X. Furthermore, in the cervical and lumbar enlargements, single-labeled neurons were also observed in the ipsilateral laminae III and VII.

Tracer placements in the magnocellular part of the LRN resulted, in the cervical and upper thoracic segments, in labeled cells mainly in laminae III and VII ipsilaterally, and in laminae IV, V, VI, VIII and X contralaterally. Also at lumbar and sacral levels, the few labeled cells were located ipsilaterally in laminae III and VII and in the contralateral laminae IV, V, VI, VIII and X. Tracer injections in the parvocellular part of the LRN, instead, resulted in retrograde labeling of few cervical and upper

Table 2. - *Involvement of different subdivisions of right and left LRN by injections of fluorescent tracers Fast Blue (FB) and Diamidino Yellow (DY).*

| Rat | Right Tracer | LRN -m right | LRN -p right | LRN -s right | Left Tracer | LRN -m Left | LRN -p left | LRN -s left |
|---|---|---|---|---|---|---|---|---|
| R1 | FB | 0 | 15% | 70% | DY | 10% | 10% | 70% |
| R2 | DY | 60% | 10% | 0 | FB | 50% | 15% | 0 |
| R3 | DY | 10% | 50% | 40% | FB | 15% | 40% | 50% |
| R4 | FB | 60% | 20% | 5% | DY | 65% | 30% | 0 |
| R5 | FB | 35% | 65% | 0 | DY | 25% | 50% | 0 |
| R6 | DY | 30% | 40% | 0 | FB | 25% | 50% | 0 |
| R7 | FB | 50% | 50% | 10% | DY | 60% | 35% | 15% |
| R8 | DY | 15% | 55% | 0 | FB | 70% | 15% | 5% |
| R9 | FB | 10% | 60% | 0 | DY | 60% | 25% | 0 |

Numbers are the percentage of the area of each LRN subdivision involved in the injection site. Abbreviation as in Table 1.

Table 3. - *Number of cells retrogradely labeled by fluorescent tracers Fast Blue (FB) or Diamidino Yellow (DY) in the different laminae of ipsi-and contralateral spinal segments with respect to the injected LRN.*

| | | ipsi | | | | | | | contra | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C1 | C7 | T2 | T11 | L2 | L5 | S2 | C1 | C7 | T2 | T11 | L2 | L5 | S2 | Total | Total |
| I | FB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | DY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| II | FB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | |
| | DY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| III | FB | 4 | 22 | 20 | 19 | 27 | 19 | 4 | 2 | 9 | 8 | 11 | 14 | 10 | 0 | 169 | |
| | DY | 1 | 11 | 15 | 14 | 13 | 12 | 2 | 1 | 4 | 8 | 6 | 6 | 5 | 0 | | 98 |
| IV | FB | 0 | 9 | 10 | 17 | 23 | 8 | 2 | 5 | 19 | 21 | 24 | 32 | 20 | 5 | 195 | |
| | DY | 0 | 7 | 10 | 6 | 5 | 3 | 2 | 4 | 18 | 19 | 14 | 13 | 9 | 0 | | 110 |
| V | FB | 1 | 3 | 6 | 9 | 10 | 11 | 0 | 13 | 12 | 18 | 16 | 26 | 27 | 3 | 155 | |
| | DY | 0 | 5 | 5 | 3 | 2 | 2 | 1 | 4 | 14 | 13 | 7 | 7 | 6 | 0 | | 69 |
| VI | FB | 0 | 2 | 2 | 11 | 3 | 7 | 1 | 6 | 10 | 15 | 18 | 13 | 14 | 1 | 103 | |
| | DY | 0 | 4 | 4 | 1 | 4 | 2 | 1 | 1 | 9 | 10 | 9 | 7 | 6 | 0 | | 58 |
| VII | FB | 3 | 15 | 17 | 27 | 33 | 24 | 6 | 3 | 9 | 10 | 15 | 16 | 10 | 2 | 190 | |
| | DY | 1 | 18 | 18 | 18 | 21 | 15 | 4 | 4 | 11 | 9 | 7 | 11 | 5 | 2 | | 144 |
| VIII | FB | 0 | 3 | 4 | 3 | 13 | 8 | 0 | 4 | 14 | 18 | 21 | 19 | 12 | 0 | 119 | |
| | DY | 0 | 3 | 1 | 2 | 6 | 2 | 1 | 0 | 13 | 10 | 10 | 9 | 5 | 0 | | 62 |
| IX | FB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | |
| | DY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| X | FB | 0 | 4 | 4 | 5 | 5 | 10 | 0 | 4 | 11 | 14 | 13 | 14 | 13 | 0 | 97 | |
| | DY | 0 | 3 | 3 | 5 | 5 | 3 | 0 | 1 | 9 | 11 | 4 | 6 | 5 | 0 | | 55 |
| Total | | | | | | | | | | | | | | | | 1030 | 596 |

Numbers are the total number of labeled cells from all section of all animals. Abbreviation as in Table 1.

Table 3. - *Number of cells retrogradely labeled by fluorescent tracers Fast Blue (FB) or Diamidino Yellow (DY) in the different laminae of ipsi-and contralateral spinal segments with respect to the injected LRN.*

| | | ipsi | | | | | | | contra | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C1 | C7 | T2 | T11 | L2 | L5 | S2 | C1 | C7 | T2 | T11 | L2 | L5 | S2 | Total | Total |
| I | FB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | DY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| II | FB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | |
| | DY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| III | FB | 4 | 22 | 20 | 19 | 27 | 19 | 4 | 2 | 9 | 8 | 11 | 14 | 10 | 0 | 169 | |
| | DY | 1 | 11 | 15 | 14 | 13 | 12 | 2 | 1 | 4 | 8 | 6 | 6 | 5 | 0 | | 98 |
| IV | FB | 0 | 9 | 10 | 17 | 23 | 8 | 2 | 5 | 19 | 21 | 24 | 32 | 20 | 5 | 195 | |
| | DY | 0 | 7 | 10 | 6 | 5 | 3 | 2 | 4 | 18 | 19 | 14 | 13 | 9 | 0 | | 110 |
| V | FB | 1 | 3 | 6 | 9 | 10 | 11 | 0 | 13 | 12 | 18 | 16 | 26 | 27 | 3 | 155 | |
| | DY | 0 | 5 | 5 | 3 | 2 | 2 | 1 | 4 | 14 | 13 | 7 | 7 | 6 | 0 | | 69 |
| VI | FB | 0 | 2 | 2 | 11 | 3 | 7 | 1 | 6 | 10 | 15 | 18 | 13 | 14 | 1 | 103 | |
| | DY | 0 | 4 | 4 | 1 | 4 | 2 | 1 | 1 | 9 | 10 | 9 | 7 | 6 | 0 | | 58 |
| VII | FB | 3 | 15 | 17 | 27 | 33 | 24 | 6 | 3 | 9 | 10 | 15 | 16 | 10 | 2 | 190 | |
| | DY | 1 | 18 | 18 | 18 | 21 | 15 | 4 | 4 | 11 | 9 | 7 | 11 | 5 | 2 | | 144 |
| VIII | FB | 0 | 3 | 4 | 3 | 13 | 8 | 0 | 4 | 14 | 18 | 21 | 19 | 12 | 0 | 119 | |
| | DY | 0 | 3 | 1 | 2 | 6 | 2 | 1 | 0 | 13 | 10 | 10 | 9 | 5 | 0 | | 62 |
| IX | FB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | |
| | DY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| X | FB | 0 | 4 | 4 | 5 | 5 | 10 | 0 | 4 | 11 | 14 | 13 | 14 | 13 | 0 | 97 | |
| | DY | 0 | 3 | 3 | 5 | 5 | 3 | 0 | 1 | 9 | 11 | 4 | 6 | 5 | 0 | | 55 |
| Total | | | | | | | | | | | | | | | | 1030 | 596 |

Numbers are the total number of labeled cells from all section of all animals. Abbreviation as in Table 1.

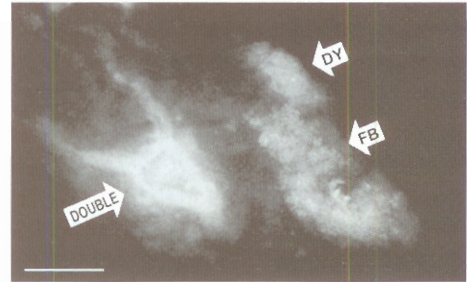


Fig. 3. - *Photomicrographs of spinoreticular neurons belonging to lamina 7 of cervical spinal cord.*
Cells were retrogradely labeled following injection of two different retrograde fluorescent tracers, "cytoplasmatic" tracer Fast Blue (FB) and "nuclear" tracer Diamidino Yellow dihydrochloride (DY). In this experiment (rat R4), FB was injected in the right LRN, and DY in the left LRN. Note a neuron labeled only by FB, a cell labeled only by DY and a double-labeled neuron, i.e. a cell displaying the two fluorescent tracers. Calibration bar = 20 µm.

thoracic neurons located in ipsilateral laminae III and VII and contralateral laminae IV, V, VI, VIII and X. Conversely, a high number of single-labeled cells were observed at lumbar and sacral levels ipsilaterally in laminae III and VII and contralaterally in laminae IV, V, VI, VIII and X. Finally, injections involving the subtrigeminal part of LRN are characterized by the bilateral labeling of very few cells in cervical and lumbar segments.

A very small amount of retrogradely double-labeled cells was found in all segments of the spinal cord (Fig. 3), mainly within laminae III, IV, VII and VIII as well as, but less heavily, in laminae V. As a matter of fact, in the 28 selected section of all nine rats, a total of 1,756 labeled neurons was counted, 1,030 of them were FB labeled and 596 were DY labeled; only 130 neurons (7,4%) contained both tracers (Table IV). In particular, in experiments where LRN-m of both sides were injected (rats R2, R4 and R7), a mean value of 19 double-labeled cells for rat was observed, mainly in cervical and upper thoracic segments, whereas in those experiments were injections involved on both sides the LRN-p (rats R3, R5 and R6), a mean value of 17.7 double-labeled cells for rat was observed, almost exclusively in lumbar and sacral segments. In the experiment (rat R1) were injections involved almost exclusively the LRN-s of both sides, 11 double-labeled cells were observed, in both lumbar and sacral segments. Finally, in the two experiments (rats R8 and R9), where in

Table 4. - *Number of retrogradely double-labeled cells in the different laminae of right and left spinal segments.*

| lamina | C1 right | left | C7 right | left | T2 right | Left | T11 right | left | L2 right | left | L5 right | left | S2 right | left | total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| II | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| III | 0 | 0 | 2 | 5 | 3 | 1 | 3 | 3 | 1 | 1 | 3 | 3 | 0 | 0 | 25 |
| IV | 0 | 0 | 2 | 1 | 3 | 3 | 1 | 0 | 1 | 4 | 2 | 3 | 0 | 0 | 20 |
| V | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 5 | 2 | 7 | 0 | 0 | 0 | 0 | 17 |
| VI | 0 | 0 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 6 |
| VII | 0 | 0 | 0 | 2 | 2 | 1 | 1 | 1 | 5 | 6 | 6 | 5 | 0 | 0 | 29 |
| VIII | 0 | 0 | 2 | 4 | 1 | 1 | 1 | 3 | 2 | 3 | 5 | 4 | 0 | 0 | 26 |
| IX | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 6 |
| Total | 0 | 0 | 9 | 15 | 11 | 7 | 9 | 13 | 13 | 22 | 16 | 15 | 0 | 0 | 130 |

Numbers are the total number of labeled cells from all section of all animals. Abbreviation as in Table 1.

one side was injected LRN-m and in the other LRN-p, only a mean value of 4.5 double-labeled cells for rat was detected.

## DISCUSSION

Data available from investigations in different mammals indicate that the main afferent inputs to the LRN are a bilateral, topographically organized projection from the spinal cord (for review, see 18).

Previous anterograde labeling experiments (2) showed that the injections of PHA-L in the spinal cord resulted in four different labeling patterns in LRN, depending on the segmental level and laminae in which the injection was made. Injections in the cervical enlargement demonstrated, when made in the dorsal horn, an essentially ipsilateral projection to the LRN, in the caudal dorsolateral magnocellular area of the nucleus, and, when made in the ventral horn, a bilateral projection to the LRN, in a more dorsomedial magnocellular area. Instead, injections in the lumbar enlargement demonstrated a bilateral projection to the LRN in more ventral, parvicellular areas. Termination patterns varied only slightly with injections made in either the dorsal or the ventral horn. Labeled terminals were occasionally found in the rostromedial and rostrolateral regions of the LRN. These results are somewhat at variance with those obtained by Rajakumar *et al.* (16), who, using anterograde transport of WGA-HRP, failed to note an ipsilateral spinoreticular projection arising from lumbar levels. Furthermore, they were unable to differentiate between dorsal and ventral horn projections.

The results of the anterograde experiments in the present study are largely confirmatory of data described by Cella *et al.* (2), with some differences. In fact, we found that LRN-m receives spinoreticular fibers coming from cervical and upper thoracic segments while the parvocellular division is reached by labeled fibers arising in the lower thoracic, lumbar and sacral segments. Very few labeled fibers were observed in the subtrigeminal division of LRN. However, we observed that in all the anterograde experiments, spinoreticular projections from a certain spinal segment involve bilaterally the same LRN divisions, with a more intense terminal labeling in the contralateral one.

By summarizing the data of the present study, obtained following injections of retrograde fluorescent tracers into the LRN, we conclude that:

1) spinal afferents to LRN-m arise mainly from cervical and upper-thoracic segments, whereas those to LRN-p came from the lower-thoracic and lumbosacral levels; the few spinal afferents for the LRN-s originate from neurons scattered throughout the spinal cord;
2) in all the spinal segments, retrogradely labeled cells were observed within all the laminae, with the exclusion of laminae I, II and IX;
3) within all the spinal laminae containing LRN-projecting neurons, we found cells sending their axons either to the ipsi- or to the contralateral LRN;
4) in the laminae IV, V, VI, VIII and X predominate the cells projecting to the contralateral LRN, whereas in the laminae III and VII are mainly concentrated neurons sending axons to the ipsilateral LRN;

5) In all the experiments, within a specific lamina of a spinal segment, we found cells projecting to the same LRN subdivision of both sides.

The present results only partly confirm previous researches with retrograde labeling techniques. In fact, Menetrey *et al.* (11), following HRP injections in the LRN, found that retrogradely labeled neurons were present at all spinal levels and in particular large numbers in the cervical and lumbar enlargements. Labeled cells were found, in all segments of the spinal cord, within laminae VI, VIII, and X, mainly contralaterally. In addition, a specific labeling pattern was exclusively observed in the cervical and lumbar enlargements, which contained labeled neurons in the ipsilateral lamina VII and in the contralateral laminae III and IV, respectively. On the other hand, Shokunbi *et al.* (20), by using the same tracer, showed that small HRP injections in the caudomedial part of the LRN labeled, in the cervical segments, neurons in laminae V and VII and, less heavily, in laminae III and IV ipsilaterally, in laminae VII and VIII contralaterally, and in lamina X. At thoracic levels, few neurons were labeled bilaterally in lamina VII, whereas the lumbar segments showed a certain amount of labeled neurons in the contralateral lamina VIII and adjacent lamina VII. In the same study, it has been observed that HRP injections in the caudolateral part of the LRN, instead, retrogradely labeled few neurons located in ipsilateral cervical lamina VII and several neurons in laminae IV and V of the contralateral lumbar segments. No labeled neurons were encountered within the most superficial laminae of the dorsal horn. These studies concluded that spinal neurons projecting to the LRN are mainly located within cervical and lumbar spinal segments, with a specific ipsilateral pathway originating in the cervical cord. The studies of Menetrey *et al.* (11) and Cella *et al.* (2), moreover, show that lumbar and cervical neurons project bilaterally to the LRN.

Concerning the existence of spinoreticular neurons with branching axons reaching LRN of both sides, Kockkoek and Ruigrok (8), on the basis of a retrograde nonfluorescent double labeling study, concluded that most, if not all spinal neurons with projections to the LRN direct their terminals either ipsi- or contralaterally. They attributed the few (~ 2%) double-labeled neurons observed to tracer spread to structures adjacent to the LRN, as ventrolateral reticular formation and dorsal reticular nucleus, which receive projections from the spinal cord (1, 10).

In the present study, double-labeled neurons were a small percentage (7.4%) of the retrogradely labeled cells. However, although tracer spread outside the LRN cannot be excluded, the present results support the view that at least a fraction of double-labeled cells are specific spinoreticular neurons with bilateral branching axons. First of all, retrogradely double-labeled cells were found not only within laminae III and IV, where are located neurons projecting to the ventrolateral reticular formation and dorsal reticular nucleus, very close to LRN (1, 10), but also within laminae VII and VIII. Moreover, in experiments where the same LRN subdivision was injected on both sides, we found a number of double-labeled cells 4 times higher than when the two injections involved different LRN parts. Finally, in experiments where the LRN-m was injected on both sides, double-labeled cells were found mainly in cervical and upper-thoracic segments, whereas when the two injections involved the

LRN-p of both sides, the double-labeled neurons were found mainly in the lower-thoracic and lumbar levels.

The present study confirmed that spinoreticular projections coming from cervical and upper-thoracic segments terminate bilaterally in the LRN-m, while those coming from the lower-thoracic, lumbar and sacral segments end in the LRN-p of both sides; only a sparse spinal input has been observed in the LRN-s. Furthermore, in all the spinal segments, retrogradely labeled cells were observed within all the laminae, with the exclusion of laminae I, II and IX; in the laminae IV, V, VI, VIII and X predominate the cells projecting to the contralateral LRN, whereas in the laminae III and VII are mainly concentrated neurons sending axons to the ipsilateral LRN. Finally, although the double-labeled neurons found in the present study were a small fraction (7,4%) of the retrogradely labeled cells, they seem to be specific spinoreticular neurons with bilateral branching axons projecting to the same LRN subdivision of both sides.

## SUMMARY

The present study was carried out to analyze the topography of bilateral spinal projections to the lateral reticular nucleus (LRN). We used retrograde transport of fluorescent tracers Fast Blue and Diamidino Yellow to identify spinal neurons projecting to the ipsilateral and/or contralateral LRN, as well as orthograde transport of Phaseolus vulgaris leucoagglutinin to identify the LRN areas where spinoreticular axons terminate. Orthograde labeling confirmed that bilateral spinoreticular projections coming from cervical and upper-thoracic segments terminate in the magnocellular division of LRN, while those coming from the lower-thoracic, lumbar and sacral segments end in the parvocellular division of the nucleus; only a sparse spinal input has been observed in the subtrigeminal division of LRN. Retrograde labeling showed that labeled neurons were present at all spinal levels and in particular large numbers in the cervical and lumbar enlargements. Retrogradely single-labeled cells were located, with contralateral predominance, in all segments of the spinal cord, within laminae IV, V, VI, VIII, and X, whereas in laminae III and VII labeled neurons were mainly observed ipsilaterally. Furthermore, a small fraction of double-labeled cells (7.4%) was observed throughout the spinal cord, mainly in laminae III, IV, VII and VIII.

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