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CELLS OF ORIGIN OF PROPRIOSPINAL AND ASCENDING SUPRASPINAL FIBERS IN A LIZARD *(LACERTA GALLOTI)*

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SUMMARY

The location of cells of origin of propriospinal and ascending supraspinal fibers has been determined by injecting horseradish peroxidase (HRP) unilaterally into various parts of the spinal cord of the lizard *Lacerta galloti*. The distribution of retrogradely labeled cells after unilateral high spinal cord injections suggests that ascending supraspinal fibers are derived from neurons in the following areas: in the cervical intumescence in most areas of the spinal gray, particularly ipsilaterally, in thoracic and lumbar segments, in deeper situated areas, especially contralaterally. The presence of cells of origin of long descending propriospinal pathways has been demonstrated following HRP-injections into the lumbar intumescence.

In various mammals the cells of origin of spinothalamic [2,13,32,33], spinocerebellar [3,5,22,26,27,30,34] and spinoreticular [11,15,25] pathways have been demonstrated with the horseradish peroxidase (HRP) retrograde axonal transport technique. Cells of origin of propriospinal pathways have been studied in the cat and monkey [24,25,29]. So far in non-mammalian vertebrates no experimental data concerning the cells of origin of the above-mentioned pathways are available. In the present study the HRP-technique has been used in the lizard *Lacerta galloti* in order to determine the location of cells of origin of fibers ascending to supraspinal levels and of ascending and descending propriospinal fibers.

In 18 lizards (*Lacerta galloti*) 3—6 unilateral injections were made of 0.1 μl HRP (a 20% solution) each into cervical, thoracic and lumbar segments of the spinal cord following a technique applied in mammals by Kuypers and Maisky [18] and Molenaar and Kuypers [24,25]: the series of injections damaged many axons, and damaged fibers as well as terminals taken up HRP and transport this enzyme retrogradely to their cell bodies. The lizards survived the operation for 4—5 days and were then perfused transcardially with a mixture of

1% formaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain, injected segment as well as some selected segments of the spinal cord were stored overnight in 0.1 M phosphate buffer (pH 7.4) with 30% sucrose at 4°C and subsequently cut into 40 μm transverse sections on a freezing microtome. The sections were further processed according to the classical HRP-visualizing technique [16] as well as with some recent modifications [1,12]. The brains have also been used in a study on the cells of origin of descending pathways to the spinal cord in the lizard [6].

The distribution of labeled neurons in the spinal cord in two representative cases, viz., after a series of injections into the second spinal segment as well as into the 24th segment is shown in Fig. 1. The spinal gray has been subdivided into 10 areas according to recent data by Kusuma et al. [17]. Their subdivision of the gray matter in various reptiles clearly reflects the influence of Rexed's [28] laminar approach, especially as regards the dorsal horn. The neutral term area has been used since not all cell groups are distinguishable as laminae. To facilitate comparison with Rexed's parcellation and to avoid confusion by introducing a different subdivision of the reptilian spinal gray Rexed's approach and numbering convention has been followed as close as possible.

Following HRP-injections into the second spinal segment (case 6033, Fig. 1) labeled neurons were present particularly in the adjacent spinal segments. In more caudal segments the number of labeled cells is rapidly declining. In the cervical intumescence, i.e. spinal segments 5—9 (see Fig. 1, segments 5—7) labeled neurons were predominantly ipsilaterally situated, whereas in the 14th (thoracic) segment and in more caudal segments (the lumbar intumescence, i.e. segments 24—28, and the sacral segment 30) labeled cells were particularly contralaterally situated. As regards the laminar distribution of labeled cells in the cervical intumescence ipsilaterally retrogradely labeled neurons were found in almost all areas. In particular, labeled cells were present in area V—VI and area VII—VIII. Contralaterally, most retrogradely labeled neurons were also present in the latter two areas. In thoracic segments (Fig. 1, 14th segment) ipsilaterally only a few labeled cells were found in areas V—VI, VII—VIII and X. Contralaterally labeled neurons were found especially in the medial part of area VII—VIII. In cervical and thoracic segments the contralateral area IV contains a few very large retrogradely labeled neurons. In the lumar intumescence and in more caudal segments (e.g. segment 30, Fig. 1) labeled cells were situated, predominantly contralateral to the injection side, in area VII—VIII, a few in areas IV, V—VI and X. In the contralateral lateral motoneuron column (area IX) a few large cells resembling motoneurons were labeled. In addition a few labeled cells were found bordering the spinal white matter (Fig. 1, see segment 24). The large motoneuron-like cells are not entirely restricted to area IX, some are also found in the medial part of area VII—VIII.

HRP-injections into the 24th spinal segment (case 6017), i.e. the rostral part of the lumbar intumescence, resulted in the presence of retrogradely labeled neurons caudal as well as rostral to the injected segment. In the lumbar intumescence (Fig. 1, segments 26 and 28) labeled cells were found particularly contralaterally in area VII—VIII. Part of the labeled cells in the lumbar intumescence are probably propriospinal. Rostral to the injection labeled neurons

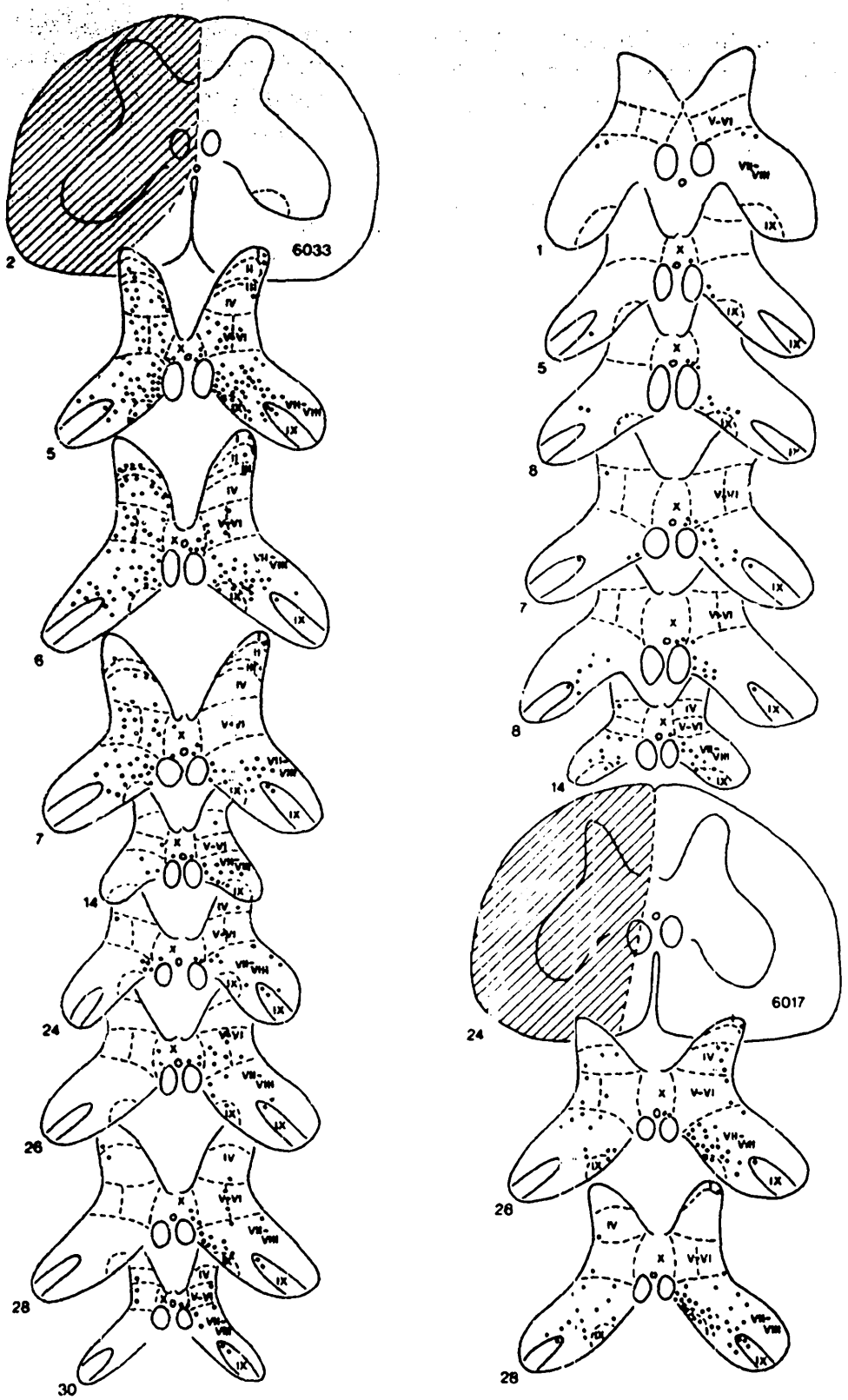

Fig. 1. Distribution of retrogradely labeled neurons throughout the spinal cord after HRP-injections into the second spinal segment (case 6033) and the 24th segment (case 6017) respectively. Each level represents the composite of plots of 10 consecutive sections. The shaded areas indicate the extent of the injections. The spinal gray has been subdivided into 10 areas according to recent data by Kusuma et al. [17]. These areas are indicated by broken lines, the lateral motoneuron column by a continuous line.

were present bilaterally in thoracic segments (e.g. segment 14, Fig. 1) as well as in the cervical intumescence (Fig. 1, segments 5—8). Even in the first spinal segment a few labeled cells were observed. In the cervical intumescence retrogradely labeled neurons were found particularly contralaterally in the medial part of area VII—VIII.

The distribution of retrogradely labeled cells after unilateral high cervical cord injections strongly suggests that the long spinal fibers which pass through the injection portion of the various segments are derived from neurons in the following areas: in the cervical intumescence from cells in most areas of the spinal gray, particularly ipsilaterally, whereas in thoracic and lumbar segments from cells which were present in deeper situated areas, especially area VII—VIII, predominantly contralaterally. The labeled cells in the cervical intumescence probably include cells of origin of short propriospinal fibers. Most of the labeled neurons, however, represent tract cells, i.e. cells of origin of long spinal fibers ascending to the brain stem and diencephalon. In various reptiles [7,8] the presence of spinoreticular, spinocerebellar and small spinomesencephalic and spinothalamic pathways have been demonstrated with anterograde degeneration techniques. Anterograde degeneration studies following hemicordotomies in *Lacerta galloti* have confirmed the existence of the above-mentioned ascending supraspinal pathways for that lizard (Ten Donkelaar, unpublished observations). Although no direct evidence is available yet as regards the cells of origin of spinothalamic, spinocerebellar or spinoreticular tracts in reptiles, it seems likely that an important part of the retrogradely labeled tract cells are spinoreticular in nature, since the overwhelming amount of long ascending spinal fibers appears to terminate in the brain stem reticular formation [7,8]. Moreover, in mammals [9,11] the cells of origin of spinal fibers projecting to the medullary reticular formation are predominantly situated in laminae VII and VIII, comparable to area VII—VIII in reptiles [17].

It should be emphasized, however, that in mammals ascending tracts may give collaterals within the cord itself [10,21,22] and so function in addition as interneurons. Spinothalamic tract axons may have collaterals to the brain stem [23], whereas some elements coursing with spinocerebellar tracts may also project to bulbar nuclei [19,23].

In the present study no evidence for the existence of a column of Clarke has been found. Cytoarchitectonically [17] such a structure is not observable in reptiles. The presence of a central cervical nucleus, which in mammals [5,22, 26,30,34] projects to the cerebellum, could not be confirmed in *Lacerta galloti*.

As regards the tract cells it should be stressed that only a few labeled cells were found in area I. The comparable lamina I (the marginal zone) in mammals appears to be an important location for cells of origin of the nociceptive component of the spinothalamic pathway [14,32].

The most intriguing finding in the lumbar intumescence following HRP-injections into the first spinal segments is the presence of retrogradely labeled large motoneuron-like cells in the contralateral area IX. Comparison with data in mammals [3,4,10,31] renders it likely that these cells are comparable to cells of origin of the ventral spinocerebellar tract in mammals. At the lateral border of area VII—VIII some large labeled cells were observed reminiscent of

the so-called spinal border cells in mammals [4,24,26,30,31].

The presence of cells of origin of long descending propriospinal pathways has been demonstrated by injecting HRP into the lumbar intumescence: labeled neurons in the cervical intumescence were present in the medial part of area VII—VIII, particularly contralateral to the injection side. These results are in keeping with recent data in mammals [24,25,29]. The cells of origin of long ascending propriospinal fibers are difficult to demonstrate with the present technique. It should be mentioned, however, that unilateral HRP-injections just caudal to the cervical intumescence resulted in labeled cells in the lumbar intumescence ipsilateral to the injection side in medial part of area VII—VIII. These cells were not observed following high spinal HRP-injections. Contralaterally, cells of origin of long ascending propriospinal fibers are impossible to distinguish from the tract cells which are located in the same area. The presence of long propriospinal fibers interconnecting the cervical and lumbar intumescences has also been demonstrated with anterograde degeneration techniques in the lizard *Tupinambis nigropunctatus* and in the turtle *Testudo hemanni* [17]. These long propriospinal fibers terminate in the same part of the spinal gray where the cells of origin of such fibers are situated, i.e. the medial part of area VII—VIII.

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