ASCENDING AND DESCENDING AXON-COLLATERALS FROM CERVICAL SPINAL NEURONS
A RETROGRADE FLUORESCENT DOUBLE-LABELING STUDY IN THE RAT

Opstijgende en afdalende axoncollateralen van neuronen gelegen in het cervicale ruggemerg
Een onderzoek in de rat met behulp van de retrograde fluorescente dubbel-labeling methode

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CONTENTS

CHAPTER 1.
GENERAL INTRODUCTION

The spinal cord. The spinal cord represents the most caudal part of the central nervous system. It is located within the vertebral canal of the vertebral column. Rostrally the spinal cord is continuous with the intracranial part of the central nervous system, consisting of the cerebral hemispheres, diencephalon, brainstem and cerebellum. Peripheral spinal nerves enter and leave the spinal cord, connecting this part of the central nervous system to the trunk, limbs and internal organs. The spinal cord, considered the lowest level in the central nervous system hierarchy, displays the same basic anatomical organization in all vertebrate classes [67, 68, 96, 199]. Along the rostro-caudal extent of the spinal cord, its structure reflects early embryonic segmentation, its peripheral nerve fibers being grouped into segmental spinal nerves leaving the vertebral canal at corresponding levels. In the rat, the spinal cord can thus be subdivided into 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 3 coccygeal segments [117, 394]. On cross-section the spinal cord consists of a centrally located grey matter, containing the spinal nerve cell bodies, surrounded by white matter consisting of nerve fibers.

The spinal grey matter is more or less butterfly-shaped, composed of two symmetrical left and right halves. Each half consists of a dorsal horn and a ventral horn with the intermediate zone located in between. On the basis of their cytoarchitectonics these areas can be further subdivided into different cell layers (or laminae) as will be discussed in Chapter 2.1. Generally speaking, the dorsal horn is considered the main sensory area of the spinal grey matter. Sensory information from receptors in the skin, joints and muscles of the trunk and limbs enters the spinal cord by way of the dorsal divisions (the dorsal roots) of the spinal nerves. Most of these fibers connect to neurons in the spinal dorsal horn which in turn convey this information to "higher", supraspinal, levels involved in the processing and perception of sensory stimuli. The ventral horn, the motor area of the spinal grey, contains somatic motoneuronal cell-groups which distribute fibers, via the ventral roots of the spinal nerves, to striated muscles of the trunk and limbs. Motor commands for voluntary movement reach the ventral horn via descending pathways from supraspinal levels; reflex motor activity is effectuated by segmental relay of sensory information via interneurons within the intermediate zone. The intermediate zone contains various types of neurons which relay sensory and motor information, and also contains many neurons which distribute their fibers within the spinal cord, interconnecting different spinal segments (propriospinal neurons). Autonomic neurons located within the intermediate zone distribute presynaptic sympathetic and parasympathetic nerve fibers via the ventral roots to the autonomic ganglia, which in

turn innervate visceral organs, endocrine and exocrine glands and smooth muscles within the organs.

The white matter surrounding the spinal grey can be subdivided into different fiber areas or funiculi. The dorsal funiculi are located dorsal to the dorsal root entry zone. The left and right dorsal funiculi are separated from each other by the dorsal septum. The ventral funiculi are located ventral to the area where the ventral roots traverse the white matter. In the midline they are interconnected by the crossing fibers of the ventral commissure. The lateral funiculi, which can be subdivided into dorsolateral and ventrolateral funiculi, are located in the lateral part of the cord, between the dorsal and ventral funiculi. The various funiculi not only contain the different sets of (ascending and descending) fibers connecting the spinal cord to supraspinal levels, but also many propriospinal fibers, i.e. fibers which interconnect different spinal levels.

From the above it may be apparent that the spinal cord, representing the interface between "higher" levels and the periphery of the body, enables the organism to interact with its environment. In conveying sensory or motor information the spinal cord serves not just as a passive go-between. Instead it constitutes an important center for the integration of information from supraspinal, spinal and peripheral levels, providing the organism with a vast repertoire of behavioral responses.

Aims and scope of the present study. In order to gain insight into the function of the central nervous system it is of importance to know the lay-out of fiber connections between its constituent cells and cell-groups. Much is already known of the anatomy of the spinal cord and its afferent and efferent fibers; this will be reviewed in Chapter 2. A large part of the spinal neuronal network consists of propriospinal connections representing the anatomical basis for integration of information within the spinal cord itself. The present study was undertaken to investigate propriospinal neurons in more detail. Specifically, a search was made for neurons which project to more than one target area by way of divergent side-branches, or collaterals, of their axon. Earlier anatomical findings already suggested that such "branching" neurons[1] might be present within the spinal cord. Thus retrograde HRP studies in the cat [254, 286, 288, 359, 416] identified the spinal neurons which give rise to *descending* propriospinal fibers. The distribution of these neurons largely overlaps that of the neurons giving rise to *ascending* propriospinal and supraspinal fibers [286, 288]. Therefore some of these neurons may be "branching" neurons which give rise to ascending and descending collaterals. In fact, such neurons were already noted in several Golgi studies [68, 242, 344], but their collaterals could be traced only over

Footnote 1. For convenience the term "branching" neurons, though actually incorrect (it is the axon which branches, not the neuron itself), will be used throughout this study.

short distances. Electrophysiological studies [9, 146, 173, 360] supported these Golgi findings, indicating that some cervical spinal neurons projecting to lumbar levels also give rise to ascending supraspinal collaterals.

In the present series of experiments in the rat, an attempt was made:

a) to determine the location and relative numbers of such branching neurons in the cervical cord, and

b) to approximate the rostral and caudal extent of projection of their collaterals.

To this purpose we used the retrograde fluorescent double-labeling method (described in Chapter 3).

The results of the *first set of experiments* (Chapter 4), in which tracer injections were made at various spinal levels, indicated that many neurons in the cervical grey matter in the rat are branching neurons, distributing ascending collaterals to upper cervical or supraspinal levels, and descending collaterals to thoracic or lumbosacral levels. Most descending collaterals of the branching cervical neurons were found to terminate at short distances, i.e. in the upper thoracic cord. On the other hand, the majority of the ascending collaterals were distributed to supraspinal levels. Although the findings suggested that many of these collaterals projected to the caudal brainstem, it remained unsettled whether the supraspinally ascending fibers specifically belonged to any of the various ascending tracts.

Therefore, *further sets of experiments* were designed to clarify this issue, investigating the presence and relative numbers of supraspinal collaterals to the cerebellum (Chapter 5), the thalamus and tectum (Chapter 6) and the dorsal medulla (Chapter 7). Summarizing, the results of the latter series of experiments indicate that relatively many of the supraspinally ascending collaterals are distributed to the medulla, only few to the thalamus and tectum and extremely few to the cerebellum.

CHAPTER 2.
ANATOMY OF THE SPINAL CORD AND ITS FIBER CONNECTIONS

2.1 Cytoarchitecture of the rat spinal cord

The grey matter of the spinal cord contains a great variety of cells of different shapes, types and sizes. Only some of these can be classified as belonging to more or less well-described nuclei, though many attempts have been made to fit all spinal neurons into nuclei or territories [reviewed in 68, 328, 329]. In 1952 a cytoarchitectonic division of the spinal grey was proposed by Rexed, who noted that spinal neurons "are arranged with an extraordinary regularity into several zones or regions" [328]. These zones could be seen in longitudinal sections as a number of cell layers, henceforth called laminae. The laminae, which could also be observed in transverse sections, were numbered I to IX from dorsal to ventral, with a tenth "lamina" located around the central canal [328, 329, 330]. Although proposed as a scheme and based primarily on the cytological characteristics and cytoarchitectonic arrangement of the neurons, not taking into account the dendritic extensions of the individual neurons, this lamination has been adapted ever since by authors studying the spinal cord. The great merit of Rexed's laminar scheme is that it serves as an universal reference in locating neurons or terminals in the spinal grey; therefore it will be used throughout this study. Rexed's original description applies in its details only to the cat, yet the intimate structure of the spinal cord is similar in all mammals [328]. In our studies in the rat, the laminae were first studied in 100 μm thick (as in Rexed's studies) cresyl-violet stained sections, followed by their identification in 40 μm sections which were counterstained with a fluorescent substance. The cytoarchitectural organization as observed in the rat (Fig. 2.1) is basically similar to that in the cat [cf. 52, 264, 269, 282, 313, 349], although certain differences can be noted in the extensions of the laminae. These differences will be commented on in describing the individual laminae.

Lamina I (the "marginal layer") constitutes a thin cell layer covering the top of the dorsal horn, consisting of loosely distributed small, medium-sized and fairly large cells (up to 15 μm in the rat [282]), which are triangular or spindle-shaped, flattened along the surface of the dorsal horn. In our fluorescent material this lamina could not be identified as a distinct layer, but labeled lamina I cells were easily recognized by their position at the outer rim of the dorsal horn. Some (displaced) lamina I neurons were also observed in the white matter covering the dorsal horn (cf. 328).

Lamina II, which is markedly thicker than lamina I, forms a well defined band across the dorsal horn. It consists of tightly packed, small rounded cells (about 5 μm in the rat [282]). As in the cat, lamina II in the rat can be subdivided into a

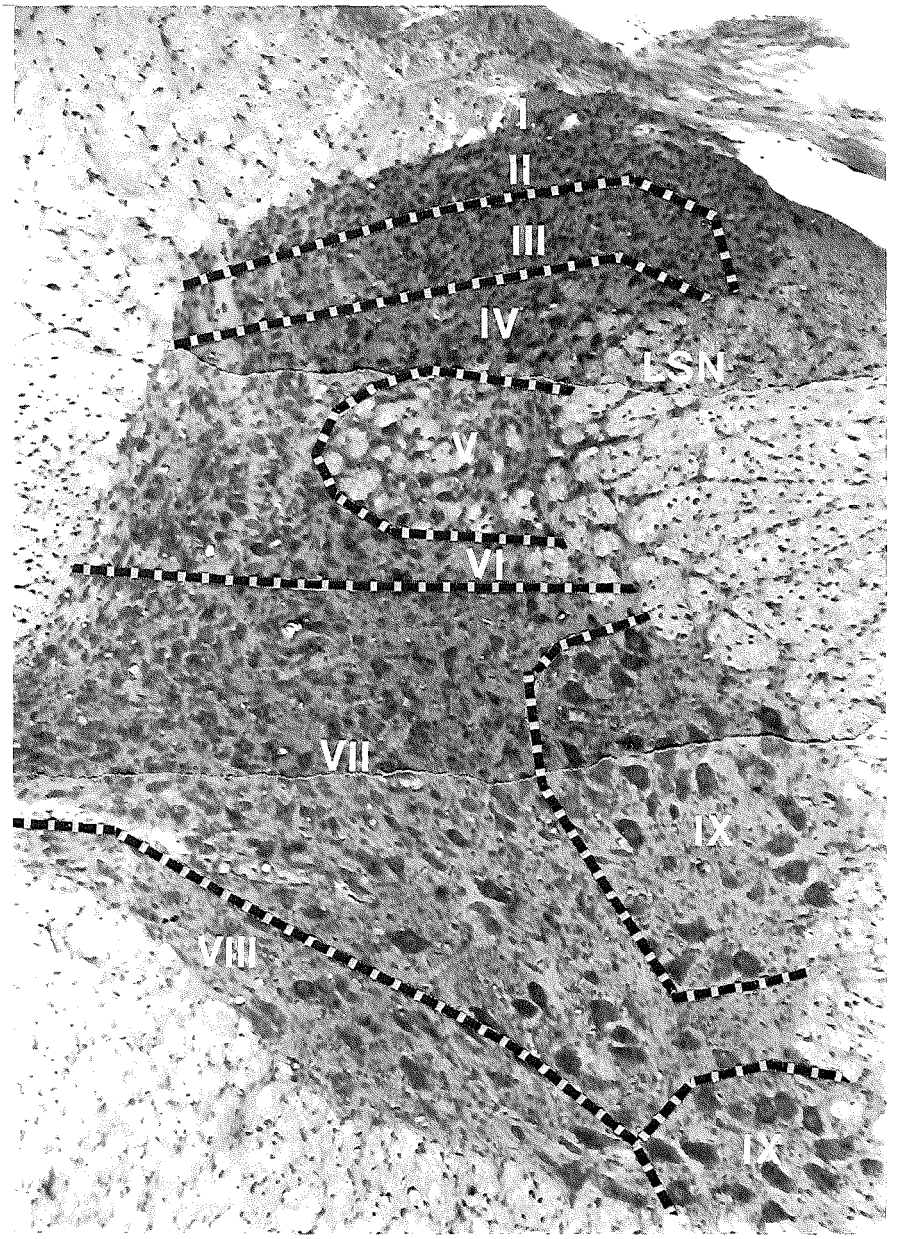

Fig. 2.1 Composite photomicrograph of a transverse section of the segment C8 in the rat, illustrating the cytoarchitectonic laminar division of the spinal grey matter. Note that boundaries between laminae are zones of transition instead of exact lines of demarcation (see text for details). *I-IX*: laminae I to IX (lamina X, surrounding the central canal, is not indicated). *LSN*: neurons within the dorsolateral funiculus, constituting the lateral spinal nucleus (cf. text).

densely packed outer zone (IIo) and a less compact innner zone (IIi) [282, 353]; this distinction was not made in the present study.

Lamina III also contains uniformly rounded or slightly elongated small cells, which, however, are a little larger and less closely packed than in lamina II, giving lamina III a lighter appearance. In the cat, the laminae I to III display a characteristic lateral bend around the apex of the dorsal horn, covering the lateral side of lamina IV (cf. Fig. 5.4). In the rat, however, this lateral bend of the superficial laminae is not very pronounced [Figs. 2.1 and 5.1; cf. 52, 264, 282, 313]. This may be related to the fact that the dorsal horn is more slender and pointed at the apex in the cat than in the rat, in which species it is broader and partly follows the contours of the pial surface to which it is in close proximity. Together, laminae II and III correspond to the substantia gelatinosa.

Lamina IV extends straight across the dorsal horn, containing a mixture of rounded, triangular and star-shaped cells of all sizes, including some fairly large ones (about 15 μm in the rat [282]). In our material it could easily be distinguished from lamina III by its heterogeneity in cell sizes, the larger neurons being especially conspicuous. Lamina IV corresponds to the nucleus proprius of the dorsal horn.

Lamina V, occupying the neck of the dorsal horn, is fairly similar in characteristics to lamina IV, containing triangular and star-shaped cells of all sizes. It can be subdivided into a medial zone, in which small and medium-sized cells are preponderant, and a lateral zone containing a number of larger neurons. The lateral zone is traversed by numerous thick, longitudinally directed, fiber bundles, giving this zone a characteristic reticulated appearance in cross-section. This 'reticulation' was the main criterion to delimit the lateral lamina V from the adjacent laminae in the present study. The lateral zone constitutes about one-third of lamina V in the cat, but up to about two-thirds in the rat [Figs. 2.1 and 5.1; cf 264, 282, 394].

Lamina VI is composed of a relatively compact group of small and medium-sized cells in its medial third, whereas the lateral two-thirds contain larger, star-shaped cells which are more loosely distributed. In our material many neurons in the lateral part appeared to be oriented transversely, which served as an additional criterion in drawing the boundary with lamina VII (the main criterion being the homogeneous and "loose" appearance of lamina VII). According to Rexed, lamina VI exists in its typical form and extent only at the level of the cervical and lumbar enlargements [328, 329]. Correspondingly, in our fluorescent material it was impossible to identify lamina VI with any certainty at upper cervical levels. In the medial part of the dorsal horn the distinction between laminae IV, V and VI was never very clear. Consistent borders, therefore, cannot be drawn in this region. Many authors seem to have met the same problem and state that the medial part of lamina IV continues ventrally towards the grey commissure in the rat [52, 313, 368; cf. however, 264].

Lamina VII constitutes a large area in the central and ventral part of the grey matter, comprising a major part of the "intermediate zone", which also includes the lateral laminae V and VI and lamina VIII. Its neurons are rather uniform, being medium-sized to fairly large (15-20 μm) star-shaped cells, and are evenly distributed and loosely packed. As a whole, therefore, lamina VII has a homogeneous appearance. Some well-recognized nuclei are present within lamina VII such as Clarke's nucleus and the presynaptic autonomic nuclei. With respect to our studies on the cervical cord the central cervical nucleus, CCN, deserves mention. This nucleus is present on both sides of the central canal at C1-C4. The CCN is rather ill-defined in Nissl-stained sections, surrounded as it is by many "displaced" neurons, but stands out as a distinct nucleus after retrograde labeling from the cerebellum (cf. Chapter 5).

Lamina VIII. According to Rexed, lamina VIII is restricted to the medial half of the ventral horn in the cervical and lumbosacral enlargements, whereas in other segments it occupies a larger part of the ventral horn. It consists of triangular and star-shaped cells of all sizes. Some of its cells are very large indeed, only slightly smaller than motoneurons. The main criterion in delineating lamina VIII is its heterogeneous appearance; no particular cell-size predominates in this lamina. In our material it was not always possible to delimit lamina VIII on this basis. In the cervical enlargement a more obvious feature of lamina VIII appeared to be the preferred orientation of its neurons [cf 328, 329]. Therefore, lamina VIII was defined in our studies as the area in the medial part of the ventral horn containing medium-sized and large neurons, the cell bodies and dendrites of which are oriented parallel to the ventromedial border of the grey matter. When lamina VIII was defined in this way it corresponded closely to Rexed's definition of lamina VIII in the cervical enlargement, but its borders clearly differed in the upper cervical segments.

Lamina IX contains the cell columns of the motor nuclei. In the upper cervical segments it consists mainly of a medial group innervating the axial muscles, whereas in the cervical enlargement a pronounced lateral motoneuronal cell group is found in addition, innervating the muscles of the extremities. The spinal motoneurons in the lateral cell group are somatotopically organized. The motoneurons projecting to individual limb muscles are distributed in long slender columns parallel to the longitudinal axis of the cord [cf. refs. 115, 116 and 370, which studies have been performed in the cat], the different motor nuclei occupying specific positions. Thus in the lateral motoneuronal cell group the motoneurons innervating physiological flexor muscles are located dorsally, the motoneurons to extensor muscles are located more ventrally and laterally, and those supplying the muscles of the girdle and the proximal part of the upper limbs are located ventromedially. In addition the motoneurons innervating proximal limb muscles are located more rostrally than those supplying distal muscles; thus the motor nuclei of the intrinsic hand muscles are located dorsally at C8 and T1 [115, 116, 370].

Lamina X consists of the small to medium-sized cells surrounding the central canal, containing the dorsal and ventral grey commissures. Its border with lamina VII and VIII is often ambiguous.

As stated by Rexed, the boundaries between laminae are zones of transition instead of exact lines of demarcation, the laminar characteristics changing sometimes abruptly, sometimes more gradually (cf Fig. 2.1). Therefore the delineation of the different laminae is open to considerable subjective interpretation as evidenced by the many small or larger differences in the laminar schemes proposed for the rat [cf. present study and 264 vs. 52, 269, 313, 368]. Moreover, as the configuration of the various laminae is somewhat different between segments, regional variations in the cytoarchitectonic organization of the spinal cord can be noted [329; cf. Fig. 5.1 on page 53 which illustrates the cytoarchitectonic organization of the different cervical segments in the rat].

Finally, it should be mentioned that two additional cell groups are present in the spinal cord of the rat. Both are located in the white matter of the *dorsolateral funiculus*. In the upper cervical segments (C1-C3) a distinct group of small rounded neurons is observed close to the pial surface, constituting the lateral cervical nucleus, LCN, which is also present in other species. A second group of neurons, the lateral spinal nucleus, LSN, is located next to the dorsal horn (laminae I to V) at all spinal levels. The LSN is found in the rat and the guinea-pig, but not in the cat and the monkey [136, 137]. These two nuclei will be commented on in more detail in Chapter 6.

2.2 Primary afferent input to the spinal cord

The primary afferent fibers reaching the spinal cord from the dorsal root ganglion cells convey information from various types of receptors in skin, subcutaneous tissue, muscle and joint capsules, as well as from the viscera. The spectrum of afferent fibers contains elements of different sizes, ranging from 0,2 μm to 20 μm in diameter. The different fibers have been classified as groups Aα-δ and C, representing large- and small-diameter myelinated fibers and unmyelinated fibers, respectively. (Afferent fibers from muscle have similarly been classified as groups I to IV, group I constituting the largest myelinated fibers). The primary afferent fibers enter the spinal cord through the dorsal roots of the spinal nerves (some fibers, a small number of which apparently originate from "aberrant ganglion cells" [83[a], 415], also enter through the ventral roots). Some of them continue rostrally in the white matter to the dorsal column nuclei. The majority of afferent fibers, however, upon entering the cord bifurcate into short ascending and descending branches which give off collaterals to the grey matter [55, 68, 232, 345].

The terminal distribution of the primary afferents in the spinal grey has been the subject of many studies, mainly in the cat and the monkey, using different

methods including Golgi-staining, anterograde degeneration studies after section of (discrete parts of) the dorsal roots and anterograde transganglionic transport of HRP or WGA-HRP applied to cut peripheral nerves or injected into identified muscles or small skin areas. Most authors [131, 200, 283, 284, 365, 369, 378, 418] have described an exclusively ipsilateral projection of primary afferents to the spinal grey. However, some have noted collaterals which project to the contralateral dorsal or ventral horn [92, 231, 232, 233, 277, 345, 363]. Yet, this contralateral projection is never prominent, except perhaps at sacral and coccygeal levels [92].

As a whole, the dorsal root distributes collaterals and terminals throughout the ipsilateral grey matter [222, 277, 326, 346, 369]. However, different types of fibers were observed to display differential termination patterns. Thus cutaneous afferent fibers were found to project to the "dorsal horn", i.e. laminae I to V, but not to deeper laminae [2, 62, 200, 283, 284, 305, 363, 378, 411, 418]. This cutaneous projection appears to be particularly dense in lamina II [2, 62, 77, 200, 222, 277, 283, 284, 305, 365, 378, 418]. The majority of the afferent fibers from muscle, on the other hand, are distributed to deeper laminae, i.e. lamina V/VI and below, including lamina IX [2, 55, 62, 90, 277, 284, 363, 378, 411]. Many of the terminations of muscle afferents are found in areas containing cells known to project to the cerebellum, such as Clarke's column [62, 90, 284] and the central cervical nucleus [16, 294].

In addition to the differential distribution of cutaneous and muscle afferents, some differences were also noted in the termination patterns of fine-caliber and larger afferents to the dorsal horn. Large fibers (corresponding to myelinated Aα fibers conveying "non-noxious" information) project to the deeper dorsal horn, i.e. laminae III to V [55, 131, 222, 231, 232, 296, 323, 363, 411], whereas fine-caliber fibers project to laminae I and II [131, 176, 222, 231, 232, 296, 363, 411]. The latter fibers correspond to small myelinated Aδ and unmyelinated C fibers, conveying information about "noxious" stimuli. Several authors have tried to further differentiate the laminar terminations of Aδ and C fibers. Although complete agreement between them is lacking, Aδ fibers seem to distribute mainly to lamina I, while C fibers preferentially project to lamina II [176, 222, 232, 234, 296, 411]. Small afferent fibers, however, have also been observed to project to laminae other than I and II. Thus, identified high-threshold mechanoreceptive fibers have been traced not only to lamina I, but also to (the lateral part of) lamina V and lamina X [233, 270, 411], and visceral Aδ and C afferents were found to terminate in lamina I as well as laminae III-V and X [77, 83, 295]. Similarly, fine-caliber (group III and IV) muscle-afferents are distributed not only to the superficial dorsal horn [2, 90, 294] but also to the lateral lamina V [90] [2]. Thus the segregation between laminae I and II receiving

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Footnote 2. It is of interest to note that the above termination areas of fine-caliber afferents (lamina I, lateral lamina V, and lamina X) contain neurons projecting to the thalamus and the midbrain (cf. Chapter 6).

small afferents, laminae III to V receiving larger cutaneous afferents, and the deeper laminae receiving muscle afferents only holds true in general terms, representing the resultant distribution of afferents from many different receptor types. Staining individual afferent fibers from identified receptors, Brown [55], who reviewed the different types of afferent units, showed that each has its own specific morphology and branching pattern of collaterals, laminar distribution and orientation of terminal arborizations and arrangement of boutons, indicating great specificity and complexity in the organization of connections between afferent units and their "target neurons" in the cord.

Superimposed on the laminar arrangement of primary afferent input is a rostrocaudal and mediolateral arrangement of afferents related to their receptive fields, i.e. a somatotopic organization of afferent input. This somatotopy can be most clearly observed in laminae II to IV, being less discernable or absent in other laminae [200, 283, 284, 305, 369, 378]. The rostrocaudal arrangement reflects the dermatomal organization of the skin; thus fibers from the medial foot are distributed more rostrally than those from the lateral foot [283] and the radial forelimb projects more rostrally than its ulnar counterpart [305]. The mediolateral distribution of afferent fibers corresponds to the ventrodorsal or distal-proximal location of their receptive fields. Fibers from distal parts of the limb project more medially than those from more proximal parts [200, 305, 369, 378, 411] and fibers from the ventral (palmar, plantar) skin project more medially than those from the dorsal skin [200, 283, 284, 305, 378, 411, 418]. The mediolateral (i.e. distal-proximal) gradient in the somatotopic organization of the dorsal horn is reversed with respect to lamina IX, where distal muscles are represented lateral to the more proximal ones.

The somatotopic organization and differential laminar projection of the presynaptic afferent input constitute the basis of the organization and properties of the postsynaptic spinal neurons. Modifications and refinements of this basic organization are brought about by the intrinsic spinal circuitry, such as the dendritic extensions and geometry of spinal neurons and local interneuronal connections [cf. 55], but the general pattern laid out by the primary afferents can still be discerned. Thus, neurons in laminae II-V display a somatotopic arrangement quite similar to that of the primary afferents [54, 61, 63, 64, 270, 366, 411] and neurons in different laminae of the dorsal horn are often differentially excited by "noxious" or "non-noxious" stimuli (cf. Chapter 2.6).

2.3 Descending fibers to the spinal cord

The intrinsic spinal circuitry provides the connections which subserve the generation of automatic and stereotyped reflex responses to stimuli. More complex responses such as postural adjustments and movements require that the spinal neuronal network receives additional afferent information both from peripheral spinal nerves and "higher" levels of the nervous system.

Descending fibers to the spinal cord are not only derived from the "highest level", i.e. the cerebral cortex, but from many areas of the brainstem as well [30, 215, 230, cf. 219, 384]. Much attention has been focussed on descending supraspinal fibers to motoneurons and interneurons projecting to motoneurons, which fiber systems apparently influence motor control, i.e. "descending motor pathways". It was noted by Kuijpers that these various fiber systems which descend to the spinal intermediate zone and ventral horn can be grouped into separate pathways displaying differential patterns of termination [213[a], 219, 220]. He suggested that this differential distribution to spinal interneurons and motoneurons would reflect functional differences in the roles these pathways play in motor control. Subsequent lesion experiments in the rhesus monkey [225, 226] confirmed this suggestion.

Thus Kuijpers subdivided the *descending motor pathways from the brainstem* into two distinct systems according to their spinal termination pattern [cf. 219, 220]. The *ventromedial* system of descending brainstem motor pathways (consisting of vestibulospinal, interstitiospinal and tectospinal fibers as well as of reticulospinal fibers derived from the mesencephalic, pontine and medullary medial reticular formation) distributes its fibers mainly to lamina VIII and the ventromedial part of lamina VII on both sides of the cord. These spinal areas contain interneurons projecting preferentially to motoneurons supplying girdle and axial muscles. The brainstem projection to the ventromedial intermediate zone represents a "non-focussed" system in the sense that many fibers appear to provide collateral projections to many segments of the spinal cord [161, 162], whereas their target interneurons project bilaterally to ventromedial motoneurons in many segments of the cord. The ventromedial brainstem pathway thus is mainly important in the maintenance of erect posture, the integration of movements of body and limbs and in synergistic whole-limb movements. In contrast, the *dorsolateral* system of descending brainstem motor pathways (consisting of the rubrospinal tract and the crossed pontospinal tract derived from the ventrolateral pontine tegmentum) distributes its fibers, by way of the dorsolateral funiculus, unilaterally to the dorsolateral intermediate zone (lateral laminae V and VI, dorsolateral lamina VII [cf. 60 in rat]). This part of the intermediate zone contains interneurons which preferentially project to ipsilateral motoneurons supplying the intrinsic flexor muscles of the forelimb. The dorsolateral brainstem projection, in contrast to the ventromedial system, represents a "focussed" system in the sense that its fibers are distributed to interneurons within a

restricted set of spinal segments [161, 162], which neurons project ipsilaterally and over only few spinal segments [287]. The dorsolateral brainstem pathway thus is involved in the steering of movements of the individual limbs, especially of their distal parts.

One step up in motor hierarchy, the cerebral cortex provides *corticospinal fibers* to the intermediate zone. When comparing their distribution in lower mammals such as the rat and the cat with that in the monkey and man, it is found that in the "higher species" progressively more corticospinal fibers project not only to both the medial and lateral intermediate zones but also directly to motoneurons. The direct cortico-motoneuronal connections enable primate species to perform highly fractionated distal extremity movements such as independent finger movements. In lower species, such as the rat [59] and opossum [247], corticospinal fibers terminals are only sparsely observed within laminae VII and VIII, being distributed mainly to the dorsal horn, i.e. lamina IV and the medial parts of laminae V and VI. In such species the (sensorimotor) cortex thus appears to be mainly involved in sensory control. Yet, also in higher species such as the cat [306, 319, 371] and the monkey [212] a sizeable proportion of the corticospinal fibers are distributed to the dorsal horn, such fibers originating from cells in the somatosensory cortex.

Consequently the descending pathways to the spinal cord are not only involved in motor control but also influence the processing of *sensory* information at spinal levels. In fact, several studies have shown that brainstem nuclei considered to be "sensory" in nature project to the spinal cord. Thus neurons within the spinal trigeminal nuclei, the periaqueductal grey, and within the gracile and cuneate nuclei [30, 66, 215, 230, 334, 399] distribute descending fibers to the spinal cord, the latter thus apparently exerting descending control over their own afferent spinal input.

Additional descending pathways to the spinal cord appear to be involved in *autonomic and limbic control* mechanisms. Thus the hypothalamus has been shown to project directly to the spinal cord [30, 215, 230, 399], distributing fibers to the thoracolumbar and sacral autonomic motoneurons and to laminae I and X [155]. Much interest has been evoked by projections to the dorsal horn from the nucleus raphe magnus and the adjacent gigantocellular reticular nucleus [30, 229, 398], part of which are serotoninergic, while some display enkephalin-immunoreactivity [151]. The fibers from these nuclei mainly distribute to laminae I, II and the lateral lamina V [153]. It has been shown in many experiments [e.g. 31, 106, 125; cf. 107] that this pathway is capable of inhibiting pain-transmission by dorsal horn neurons, including spinothalamic tract neurons. Projections to the dorsal horn from the area of the locus coeruleus and subcoeruleus [152, 153], which are partly noradrenergic, might subserve similar functions. Recent anatomical studies using sensitive anterograde tracing techniques have demonstrated the existence of projections to the intermediate zone and lamina IX from the locus coeruleus and subcoeruleus and the caudal raphe

nuclei [152, 153]. Electronmicroscopic studies in the rat [156, 157] demonstrated that these fibers to the lateral and medial lamina IX establish direct monosynaptic connections with motoneurons. These, partly serotoninergic or noradrenergic, direct motoneuronal connections are presumed to represent a special feature in motor control: functioning as "level-setting systems", inducing changes in excitability within the motoneuronal cell groups, they might provide an animal with a motivational drive in the execution of movements [158, 220]. In this respect it is of interest to note that the raphe nuclei and locus coeruleus/subcoeruleus are under the afferent control of components of the limbic system such as the hypothalamus, amygdala, bed nucleus of the stria terminalis and periaqueductal grey matter [154, 155]. Thus the "emotional" brain, by way of its projections to the dorsal horn and motoneuronal cell groups via the locus (sub)coeruleus and raphe nuclei, exerts control over both sensory input and motor output of the spinal cord, facilitating appropriate behavior in response to environmental stimuli.

2.4 Spinal efferents to supraspinal levels

The relay of information from various somatic or visceral receptors to higher levels is mediated by way of dorsal root fibers which ascend in the dorsal funiculus, and thus bypass the spinal cord, or by the ascending axons or axon-collaterals from intercalated spinal neurons. The ascending fibers from the cord to the cerebellum and the brain stem convey different sensory modalities and are segregated to some extent into tracts which terminate in different supraspinal regions (e.g. spinocerebellar and spinothalamic tracts).

The supraspinal distribution of the **fibers ascending from the spinal cord** has been investigated in anterograde degeneration studies in several species [19, 50, 144, 177, 195, 267, 268, 302, 322, 419]. Following high cervical hemisection or section of the lateral and ventral funiculi, many degenerated fibers were observed to enter the brain stem. Some of these ascending fibers project to the cerebellum, terminating somatotopically in the anterior and posterior lobes [18, 130, 259, 332, 393, 406], while the majority terminate throughout the brain stem, up to hypothalamic levels. In the brain stem, the largest amount of degenerated terminals was found in the central medullary and pontine tegmentum. Profuse termination was observed in the ventral part of the nucleus medullae oblongatae centralis and the nucleus gigantocellularis, while a smaller number of degenerated fibers were present in the lateral part of the medullary reticular formation. Similarly, spinoreticular fibers to the pons were mainly concentrated medially. At rostral pontine levels, a gradual lateral shift of this "diffuse tegmental pathway" [302] was noted, terminations in the mesencephalic reticular formation being present in the area cuneiformis and subcuneiformis [331, 332].

In addition to the rather diffuse projection to the reticular formation, spinal fibers were also observed to terminate in several circumscribed brain stem nuclei.

Dense spinal projections were found in the parvocellular and magnocellular parts of the lateral reticular nucleus [cf. 113, 210] and in the inferior olivary complex, mainly in the dorsal and medial accessory olives [cf. 377]. The dorsal column nuclei receive a major spinal input, both from the dorsal and dorsolateral funiculi [336, 337, 338], while ascending fibers are also distributed to the external cuneate nucleus and the nuclei intercalatus, commissuralis, solitarius and prepositus hypoglossi [367]. Spinal afferents were further observed in the ventromedial facial nucleus (these afferents originate at high cervical levels [cf. 144, 267, 268, 299, 382]), in the lateral division of the parabrachial nuclei [73, 312] and in the vestibular nuclei (nuclei x and z and the caudal parts of the lateral, medial and inferior vestibular nuclei [53]). In several studies the spinal cord was reported to project to the nucleus of Darkschewitsch [44, 91, 194, 195, 332, 400, 401], while some fibers in the spinal ventral funiculus seem to project to the red nucleus and the Edinger-Westphal nucleus [195].

Spinal fibers were also noted to terminate in the mesencephalic tectum [cf. 22, 44, 91, 331, 414]. Spinotectal fibers project most densely to the intercollicular nucleus, lateral to the rostral pole of the inferior colliculus [267, 268, 400], but also terminate in the lateral periaqueductal grey and the deep layers of the superior colliculus.

Spinal afferents to the thalamus constitute only a relatively small part of all ascending spinal fibers [177, 267, 268]. Yet, in view of their proposed importance in the transmission of sensory information to the cortex, they have been studied specifically by many authors [cf. 47, 48, 89, 91, 239, 244, 316, 318, 332]. The results of these studies agree in many respects, but also show some marked differences. These differences might be partly accounted for by "atlas semantics", i.e. the cytoarchitectonic delineation of the various thalamic nuclei is open to subjective interpretation, and therefore similar spinothalamic targets may be denominated differently by different authors [268]. On the whole, the following picture emerges. Two spinothalamic tracts may be distinguished. The "classical" lateral spinothalamic tract consists of fibers which ascend uninterrruptedly to the ventrobasal complex of the thalamus. The medial spinothalamic tract is part of the system which terminates in the reticular formation at lower levels, and which distributes to the intralaminar nuclei of the thalamus (which nuclei are sometimes considered as the rostral extension of the reticular formation). The lateral spinothalamic tract projects to the medial magnocellular portion of the medial geniculate body, to the medial part of the posterior thalamic nucleus and to the specific somatosensory thalamic nucleus. The latter nucleus (nucleus ventralis posterior lateralis, VPL, in the monkey; nucleus ventralis lateralis, VL, in the cat; and nucleus ventrobasalis, VB, in the rat) projects to the primary somatosensory cortex. Its spinal afferents are organized somatotopically [239, 244, 316], while in primates the afferent terminals are arranged in patches or clusters [48, 50, 195, 244, 267, 268]. Therefore this nucleus is thought to

be involved in the discrimination and localization of painful stimuli. Spinal afferents to the medial thalamus, on the other hand, are believed to be involved mainly in the emotional and motivational aspects of pain. The "medial spinothalamic tract" terminates in various intralaminar and adjacent nuclei, this projection being partly bilateral [48, 89, 91, 195, 239, 316, 419]. The densest intralaminar termination was observed in the central lateral nucleus, more modest projections being found in the parafascicular and central medial nuclei, and in the adjacent medial dorsal nucleus. In some studies, spinal afferents to the paracentral nucleus were described [89, 91, 244]. A small, but significant, spinothalamic projection was observed in the submedius nucleus [89, 91, 244, 316, 318, 332]. Some authors further reported spinothalamic terminations in the pretectal nuclei [91, 239, 332], in the ventromedial, paraventricular, reuniens and reticular nuclei [89, 91] and in the posterolateral nucleus [239]. Finally, ascending spinal fibers were noted to terminate in the subthalamic zona incerta [19, 47, 48, 91, 239, 302, 332, 419] and in the posterior hypothalamus [19, 194, 195].

The **cells of origin** of the different tracts ascending to supraspinal levels have been studied in the rat, the cat and the monkey using retrograde transport of HRP or fluorescent tracers.

Spinothalamic neurons in the rat are located mainly in lamina I, the LSN, the lateral part of lamina V and the medial part of the ventral horn, i.e. laminae X and VIII and the medial lamina VII [120, 121, 133, 192, 196, 198]. At lumbar levels, an additional group is found in the ventromedial dorsal horn [78, 133, 192, 274], while at upper cervical levels spinothalamic neurons are additionally present in large numbers in the lateral cervical nucleus (LCN), the spinal extensions of the dorsal column nuclei (sDCN) and in the lateral part of lamina VII. On the whole, the great majority project to the contralateral thalamus. Spinothalamic neurons in the cat [69, 70, 84, 186, 187, 385, 387, 388] and the monkey [8, 143, 193, 386, 387, 388, 408] show approximately the same distributions as those in the rat. The spinothalamic neurons projecting to the "lateral" thalamus (ventrobasal complex) are distributed somewhat differently from those projecting to the "medial" thalamus (intralaminar nuclei), the former being located preferentially in the DCN, LCN and laminae I and V, while the latter are most numerous in laminae VII and VIII [7, 8, 69, 121, 123, 124, 198, 408]. However, the populations of "medial" and "lateral" spinothalamic tract neurons are not strictly segregated, 15% to 20% of their neurons even projecting to both the medial and lateral thalamus [198]. Such branching neurons are mainly located in the lateral lamina V. Finally, it is of interest to note that the small spinothalamic projection to the submedius nucleus seems to originate exclusively from lamina I neurons [89].

Recently, direct *spinohypothalamic* projections to both the lateral and medial hypothalamus have been described in the rat. Its neurons are located mainly in

lamina I, the LSN, the lateral lamina V and lamina X [65]. Most cell groups project bilaterally to the hypothalamus.

The neurons which project to the dorsal mesencephalon, i.e. the superior colliculus, intercollicular nucleus and periaqueductal grey (*"spinotectal neurons"*) are distributed over the same areas as the spinothalamic neurons. Thus, they are located in lamina I, the lateral lamina V and laminae VII, VIII and X at all levels, while at high cervical levels they are additionally present in the LCN, spinal DCN and lateral lamina VII [44, 243, 400]. In the rat, the LSN also contains many spinotectal neurons [78, 235, 236, 272, 379]. Most cell groups project to the midbrain bilaterally, but with a strong contralateral predominance. Spinal neurons projecting to the (lateral) parabrachial nuclei show the same distribution as the spinotectal ones, but appear to be preferentially located in lamina I [73, 168, 169, 312].

Spinoreticular neurons, as could be expected from anterograde degeneration studies, are more numerous than those projecting to the thalamus [cf. 196, 273]. Otherwise they show the same distribution. Thus the neurons projecting to the medial bulbopontine reticular formation are mainly located in the lateral part of lamina V and in laminae VII, VIII and X [20, 78, 197, 198, 273, 314, 317]. Most neurons project contralaterally, but those in lamina VII project mainly ipsilaterally. Spinal neurons which distribute fibers to the lateral reticular nucleus are distributed similarly, but are also present in fairly large numbers in lamina I and in the LSN [78, 85, 273, 358]. Neurons projecting to the inferior olive are mainly located in lamina VIII and in laminae IV and V [27, 273, 290, 291, 377].

The above findings show that the distributions of the spinothalamic, spinotectal, and spinoreticular neurons overlap extensively. In fact, some neurons have been shown to project both to the thalamus and to the tectum or the reticular formation in the rat. These neurons are located in the lateral lamina V, the medial ventral horn and the LSN, but not in lamina I. On the whole, such branching neurons constitute only about 10% of each population [196, 198, 236], indicating that spinothalamic, spinotectal and spinoreticular neurons are nevertheless largely separate populations in the rat.

The distributions of spinocerebellar neurons and of neurons projecting to the dorsal column nuclei follow a different pattern. *Spinocerebellar neurons* are located mainly in laminae V to VIII [253, 255, 320, 364]. They are not only present in "classical" spinocerebellar cell groups such as Clarke's column at T1-L4 [cf. 321] and the group of spinal border cells in the lateral ventral horn at T12-L5 [cf. 138], but in other areas of the spinal grey as well [cf. 364]. Thus spinocerebellar projections also take origin from neurons in laminae V and VIII at all levels of the cord and from the medial part of lamina VII at sacral and caudal levels. At cervical levels, a prominent spinocerebellar cell group is found next to the central canal at C1-C4, constituting the central cervical nucleus [93, 402, 403], while the central part of lamina VII and

medial part of lamina VI of the cervical enlargement also contain many spinocerebellar neurons [cf. 321, 405].

The *spinal projection to the dorsal column nuclei* is largely composed of primary afferent fibers which travel directly from the dorsal root ganglia to the medulla. A smaller proportion (about 30-40%) of the spinal input to the gracile and cuneate nuclei [126] is constituted by non-primary afferents originating from spinal neurons, the so-called postsynaptic dorsal column (PSDC) neurons. PSDC neurons are concentrated in lamina IV and the medial parts of laminae V and VI [33, 126, 143, 288, 339, 340, 341, 342]. They project almost exclusively ipsilaterally. The PSDC neurons are at the origin of a somatosensory projection to the thalamus, having a relay in the DCN. It is of interest to note the presence of another somatosensory system, the *spinocervical tract* (SCT), which also projects to the thalamus but has a relay in the LCN. SCT neurons, like PSDC neurons, are heavily concentrated in lamina IV [28, 54, 63, 64, 74, 87, 160]. Some lamina IV neurons distribute axon-collaterals both to the LCN and to the DCN [88, 185].

2.5 Propriospinal connections

One of the most obvious functions of the spinal cord is the relay of supraspinal input to muscles and internal organs and the transmission of peripheral sensory information to "higher" centres by way of the various ascending tracts. Yet, only about 3% of its neurons [81, 82] are involved in these "relay" functions of the cord. The majority of the spinal neurons with their cell bodies and their, often extensive, axonal and dendritic processes are confined to the spinal cord itself [118] and thus constitute propriospinal neurons[3]. The propriospinal connections make up the intrinsic neuronal circuitry of the spinal cord, integrating the various types of input and conveying them to the "output" neurons. In general, the axonal trajectory of the propriospinal neurons, especially of those in laminae V to VIII, is complex and richly collateralized, each neuron establishing synaptic connections with many other neurons in widely different parts of the grey matter [344, 371, 380, 381], both ipsilaterally and contralaterally. Almost all propriospinal neurons project to the white matter, their fibers reentering the spinal cord at shorter or longer distances [68, 242, 249, 250, 344]. Classical anatomical studies in the monkey [383] and the human [300] indicated that the propriospinal fibers are concentrated in the deep parts of the funiculi, immediately surrounding the grey matter, and that many of them are unmyelinated and of small diameter. However, quantitative electronmicroscopical studies in the rat suggest that the propriospinal fibers contain relatively more large-diameter and myelinated elements than the other fiber systems in the white matter, and that

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Footnote 3. The term interneurons, used by some authors for these neurons, should be reserved for spinal neurons which are physiologically defined as being interposed in reflex pathways.

propriospinal fibers are intermingled with other fibers in all parts of the white matter [81]. The majority of the propriospinal fibers extend over only short distances (up to 3 or 4 segments; [cf. 380, 381]), while fewer fibers are of intermediate length (extending over 6 to 8 segments) or very long, interconnecting the enlargements [129, 371].

The *long propriospinal fibers*, interconnecting the cervical and lumbosacral cord, have their origin and termination mainly in laminae VII and VIII. Following unilateral section of either the long ascending or the long descending propriospinal fibers, the largest number of degenerating terminals were observed bilaterally in lamina VIII and the adjoining part of lamina VII, while some additional fibers were observed to distribute ipsilaterally to the lateral parts of laminae V to VII. Only few degenerated fibers were observed in lamina IX; such degeneration was only present in the medial motoneural cell groups [129].

The cells of origin of the long propriospinal fibers have been studied using retrograde transport of HRP. Cervical neurons projecting to the lumbosacral cord (long *descending* propriospinal neurons) were present in large numbers in lamina VIII and the adjoining part of lamina VII, while less were observed in the lateral laminae V and VI and in lamina I [254, 275, 286, 288, 359, 416]. The lumbar neurons projecting to the cervical cord (long *ascending* propriospinal neurons) are less easily identified by way of retrograde transport methods, as tracer injections in the upper spinal cord are liable to interrupt fibers ascending to supraspinal levels, thus labeling neurons projecting to supraspinal levels as well. Yet, it seems likely that neurons giving rise to long ascending propriospinal fibers are concentrated ventrally in lamina VIII and the adjacent part of lamina VII [99, 288]. In addition, many of such neurons appear to be present in laminae IV and V [99].

The *short propriospinal fibers* (extending over 2 to 5 segments) distribute their ascending and descending axons mainly to laminae IV to VIII ipsilaterally, and to lamina VIII and the adjoining part of lamina VII contralaterally. Moreover, the short propriospinal fibers project heavily to both the medial and lateral motoneuronal cell groups [129, 251]. Neurons which give rise to short propriospinal fibers are present throughout the spinal grey, being concentrated in lamina I and laminae IV to VIII [275, 286, 288, 416]. As stated, some of the short propriospinal fibers project directly to the motoneuronal cell groups. Anatomical studies in the brachial cord of the cat [287, 371] have revealed a clear pattern in the organization of those projections. Neurons in the lateral parts of laminae V to VII preferentially project to the ipsilateral motoneurons supplying the intrinsic flexor muscles of the forelimb, while the neurons located in the central part of lamina VII mainly project to the ipsilateral motoneurons supplying the intrinsic extensor muscles. The neurons in the medial part of lamina VII and in lamina VIII project, to some degree bilaterally, to the motoneurons supplying the girdle- and axial muscles. The latter projection is partly

subserved by intermediate and long propriospinal fibers as well [287, 371]. Similar findings were obtained in the rat [150]. It has to be noted that the different propriospinal-motoneuronal projections are not discrete systems, but overlapping ones. Nevertheless, the above arrangement, which also holds true in general terms for the lumbosacral cord of the cat [285, 335], correlates well with the differential distribution of the various descending brainstem pathways to the spinal cord and their contribution to motor control [cf. 219 for review].

The *short propriospinal neurons*, projecting heavily to all motoneuronal cell groups, play an important role in the (mono- or polysynaptic) relay of descending activity onto motoneurons. Thus short propriospinal neurons in the lumbar cord have been shown to be intercalated in cortico-, vestibulo- and reticulospinal pathways [205, 392]. Lundberg and coworkers extensively studied the role of propriospinal neurons at C3-C4, which project to forelimb motoneurons, in motor control. These C3-C4 propriospinal neurons ("PN's") were found to receive monosynaptic input from corticospinal, rubrospinal and tectospinal tracts, which descending inputs often converge on the same neuron. These cells also receive input from cutaneous (low threshold) afferents and group I muscle afferents [170, 171, 174] and monosynaptic excitatory or inhibitory reticulospinal input [175]. The C3-C4 PN's project monosynaptically to all groups of forelimb motoneurons, evoking EPSP's [12, 171, 174] or IPSP's (mainly in extensors [cf. 12]). In addition, they project monosynaptically to the IA inhibitory interneurons [12, 170, 172] and to other neurons in laminae VI to VIII in C6-T1 [14], and distribute ascending axon-collaterals to the lateral reticular nucleus [9, 173]. The findings indicate that these propriospinal neurons subserve other functions than just the transmission of effects from higher centres. They occupy an appropriate position for the conjoint control, by descending motor systems and afferent systems of the forelimb, of propriospinal influences on motoneurons [cf. 171]. Therefore the group of C3-C4 PN's, which also receive feed-forward and feed-back inhibition from neighbouring neurons [11] can be regarded as a premotor neuronal integrative center [9]. The existence of subgroups of C3-C4 PN's with different convergence patterns of their afferents and different patterns of axonal termination [14] would provide for flexibility in motor control. Obviously, this group of PN's are not the only neurons relaying descending activity to the forelimb; rather they serve as an example how groups of short propriospinal neurons may serve as premotor integrative centers. The C3-C4 PN's seem to be maximally involved in target-reaching movements, but not in other types of movement such as walking [10, 13]. Other groups of PN's may be active during these other types of centrally initiated movements [cf. 183].

The *long propriospinal fibers*, interconnecting spinal segments throughout the length of the cord, enable the cord to function as a whole [119]. In accordance with anatomical findings, only few of them establish direct monosynaptic connections with

motoneurons [179]. Rather, most of them terminate on short propriospinal neurons or interneurons [178, 179, 181, 391], including IA inhibitory and IB inhibitory or excitatory interneurons and interneurons interposed in flexion reflex pathways [178, 179]. Their main role can be viewed as the control of interneuronal transmission, which is of importance in interlimb coordination. For example: a postural change in the forelimb would, by way of long descending propriospinal fibers, lead to a modulation of the segmental reflexes in the hindlimb. This interlimb coordination is most obvious in locomotion. In "high spinal" cats, i.e. cats which have sustained a transection of the spinal cord at C1, the spinal cord is still able to generate locomotor patterns, in which the movements of the forelimb are in register with those of the hindlimbs [281]. The locomotor movements, i.e. the alternate contractions of different groups of extensor and flexor muscles, in each of the limbs seem to result from the intrinsic activity of the segmental neuronal circuitry controlling the limbs, the so-called spinal locomotor generators [cf. 134 for review). The coupling of fore- and hindlimb movements in different types of gait would be effected by the long propriospinal connections, which serve as the links between the fore- and hindlimb locomotor centers [119, 279, 280, 351, 352]. Thus long propriospinal pathways from the lumbosacral cord can enhance or decrease segmental reflexes to brachial motoneurons, and vice versa [1, 23, 119, 248, 280, 293, 333, 350, 351, 352].

2.6 Physiology of spinal neurons

Spinal neurons, whether "projecting neurons" or "interneurons", are subject to influences from many different fiber systems impinging upon them. Many show background discharges in the absence of external stimuli, which might be due to the convergence of many synapses onto these neurons [164, 165]. They display a variety of convergence patterns of excitatory and inhibitory inputs from different sources, including low- and high-threshold cutaneous, muscular and visceral primary afferents [cf. 159, 165]. Spinal neurons can be classified into four different groups according to their responses to mechanical peripheral stimuli [cf. 42, 270, 366, 407]:

-low-threshold (LT) neurons are only driven by non-noxious stimuli such as movement of body hairs and light touch or pressure on a skin area.

-wide-dynamic-range (WDR) neurons display graded responses to both non-noxious and noxious stimuli; they are driven by the same stimuli as the LT neurons, but in addition they increase their firing rate in response to noxious stimuli such as pinch, pin-prick and thermal stimuli in the noxious range.

-high-threshold (HT) neurons respond exclusively to noxious stimuli.

-finally, "deep" (D) neurons respond to movements in joints and to pressure applied to "deep" tissues (muscle, subcutaneous tissue).

These four groups of cells are distributed more or less differentially over the grey matter [25, 110, 120, 124, 270, 324, 325, 327, 366, 407]; LT neurons are preferentially

located in laminae III to V, WDR and HT neurons in lamina I and laminae IV to VI, while D neurons are mainly present in laminae V to VIII. The neurons in laminae II to IV appear to be driven only by somatic stimuli, while those in laminae I and V-VIII display convergent visceral and somatic inputs [76]. As stated earlier, these distributions to some extent reflect the differential distributions of the various primary afferent fibers to the spinal grey. The properties of the neurons in different laminae will be described briefly.

Lamina I neurons generally display little or no background activity [75, 80, 266, 410]. In most cases their cutaneous receptive fields are small [25, 75, 166, 167, 266, 270, 325, 327, 407, 410]. The great majority of lamina I neurons, including those giving rise to spinothalamic and spinomesencephalic projections, appear to be nociception-specific, HT, cells [75, 89, 166, 167, 234, 271, 409], although a varying proportion of these neurons have also been classified as WDR cells [80, 266, 270, 410, 411] and even some LT and D cells have been identified in this lamina [26].

Lamina II contains both LT, WDR and HT neurons [57, 209], the majority of these being WDR and HT neurons [111]. Their responses to peripheral stimulation suggest that the neurons in this lamina may be more concerned with long-term changes of excitability, which might modify the output of projecting cells in adjacent lamina, than with the transmission of incoming information to other centers [111].

Laminae III and IV contain many PSDC and SCT neurons. The majority of these neurons exhibit a spontaneous resting discharge [21, 58, 74, 389, 395], and have rather small and circumscript cutaneous receptive fields [21, 54, 56, 58, 64, 74, 127, 160, 237, 270, 389, 395]. Many - PSDC and SCT - neurons respond to movement of (even single) hairs or gentle tactile stimuli [63, 64, 139, 395], but a major proportion (35% to 85%) also respond to noxious stimuli (WDR) [21, 34, 54, 58, 127, 188, 237, 238, 263, 303, 389]. In the cat, some HT neurons have been found in lamina IV [21, 188].

Lamina V (laminae IV to VI in most studies) contains all four classes of neurons [95, 103, 120, 270, 271, 366]. Most of them, including those identified as spinothalamic neurons, are WDR neurons [76, 95, 103, 114, 124, 139, 271, 395, 407, 411]. In some studies, up to 30% of the spinothalamic neurons in these laminae were shown to be HT cells [124, 407]. About 55% of the identified spinoreticular neurons were LT cells [105]. Lamina V neurons also receive small-diameter muscle [114] and visceral input [76, 139, 265]. The neurons in laminae IV to VI are driven from small [6, 7, 17, 103, 105, 124] or fairly large [95, 270, 271, 327, 407] receptive fields.

The neurons in *laminae VII and VIII*, which include spinothalamic, spinomesencephalic and spinoreticular neurons, seem to be characterized by the fact that they have large and complex receptive fields, which are often bilateral and have both excitatory and inhibitory components [6, 17, 70, 104, 105, 124, 139, 262, 274, 278,

289, 417]. Most of them display spontaneous activity [278]. All four classes (LT, WDR, HT and D) of neurons have been identified in these laminae, their relative numbers differing widely between studies [6, 26, 70, 104, 105, 120, 124, 262, 278, 417]. In some studies, many units (20-40%) were unresponsive to cutaneous or "deep" stimuli [70, 417]. In lamina VII different groups of spinocerebellar neurons are also present; such neurons are generally excited by group I muscle afferents [147, 149, 240, 241], as well as by cutaneous and deep afferents [145, 147]. They may also be excited or inhibited by high-threshold flexor reflex afferents [145].

The neurons in *lamina X* respond exclusively to noxious stimuli applied to small circumscript receptive fields [297].

Neurons in the *LCN* mainly respond to hair movement, but also to noxious stimuli [88, 122], while those in the *LSN* do not display cutaneous sensitivity, responding only to "deep" stimuli [122, 271].

It is clear that the different laminae, and the ascending pathways which originate from them, contain a heterogeneous population of neurons excited by different types of stimuli (noxious, non-noxious and proprioceptive, cutaneous and visceral). Only in the laminae I and X a single type (HT) of neuron prevails. It is of interest to note that lamina I neurons, which are mainly excited by nociceptive stimuli, do not send their ascending fibers via the "classical pain-pathway", i.e. the ventrolateral funiculus, but via the dorsolateral funiculus [24, 168, 186, 187, 266, 288]. Many studies have focussed on the nature of the information signalled by the various ascending somatosensory pathways, especially with regard to the perception of pain [reviewed in 42, 57, 94, 395, 409]. The various anatomical, physiological, pharmacological and behavioral data suggest that "nociception does not exclusively rely on a single system of pathways, relays or projection sites [42] and that " no system can be claimed as having the exclusive ability to mediate either nociception or light touch [94]. Clinical data indicate likewise [300[a], 304, 396].

CHAPTER 3.
THE RETROGRADE FLUORESCENT DOUBLE-LABELING METHOD

3.1 General introduction

The nervous system contains a stunning amount of neurons and cell groups which are interconnected by axonal pathways. In order to gain more insight in the function of the nervous system it is essential to study these fiber connections in great detail. For this purpose many different anatomical methods are available, each of them with its own merits and limitations [cf. 219 for review]. By way of the *Golgi-method* and its modifications the cell bodies, dendrites, axons and axon-collaterals of single cells are impregnated. Earlier investigators using this method, such as Cajal [68], have obtained a wealth of information concerning the morphology and connectivity of individual neurons. The axonal projections can be visualized over only short distances, however. Several *anterograde labeling methods* visualize the trajectory of axons over long distances, and make it possible to identify their terminals under light- or electronmicroscopic observation. Thus, axons and terminals which undergo anterograde degeneration following lesions of cell groups or fiber bundles can be selectively labeled and studied [108, 301]. Another method is based on the uptake of injected amino-acids by the cell bodies; the subsequent anterograde axonal transport of these -tritiated- substances can be demonstrated autoradiographically [86]. Similar anterograde labeling can be obtained with lectins such as wheat germ agglutinin, WGA, [101], which may be conjugated with horseradish peroxidase, and phaseolus-leucoagglutinin, PHA-L, [118[a], 412]. The anterograde tracing methods provide much information and detail about the sites of termination of fiber projections from a given cell-group. However, it is impossible to accurately define the cells of origin of a given projection. For this purpose, *retrograde labeling methods* are available. The current methods make use of the uptake of several substances by axon terminals and damaged fibers, and the subsequent transport by retrograde axonal flow to the parent cell bodies. Such retrogradely transported substances include aminoacids, such as proline [211], proteins, such as bovine serum albumin, BSA, [372] and endogeneous proteins [109], but also potentially pathogenic substances such as the herpes simplex virus [207, 390], tetanustoxin [100, 354, 374, 375] and choleratoxin [375], and lectins such as wheat germ agglutinin [355, 375]. Some substances, such as aspartate and nerve growth factor, appear to be selectively transported by neurons which are provided with specific uptake systems for these substances [342, 356]. The enzyme horseradish peroxidase, HRP, has proven to be a suitable tracer for retrograde transport over long distances [208, 214, 223, 224], and has therefore been used in many studies. Its presence in the cell body can be demonstrated histochemically; of

the several methods to demonstrate HRP the reaction using tetramethylbenzidine (TMB) appears to be the most sensitive [276].

By combining the above anterograde and retrograde tracing methods the fiber connections in the brain can be studied in great detail. Yet, these methods are unable to demonstrate whether single neurons project to more than one target by way of divergent collaterals. Such axonal collaterals, which appear to be numerous in the central nervous system [68], can be visualized with the Golgi method, but only over short distances. Collateralized projections can also be demonstrated electrophysiologically by antidromic activation of single neurons from different areas [3, 4]. However, such studies cannot supply accurate anatomical and quantitative data due to the difficulties in localization of the recording site, the relatively small numbers of neurons sampled and the possibility of a sampling bias in recording from these cells. Therefore anatomical methods were developed to obtain "double-labeling" of neurons by different retrograde tracers which can be demonstrated independently in the parent cell body. When two such tracers are injected at two different sites, only neurons projecting to both sites (by way of divergent axon-collaterals) will contain both tracers after retrograde transport (cf. Fig. 3.1). Such double-labeling methods were obtained by combining the retrograde transport of HRP with that of iron-dextran [309], tritiated BSA [373] or tritiated, enzymatically inactive, apo-HRP [142]. A series of fluorescent retrograde tracers was tested by Kuijpers and coworkers [reviewed in 221]. These tracers are visualized independently by means of fluorescence microscopy and can therefore be effectively applied in double-labeling experiments. The earliest combinations, e.g. Evans Blue and Dapi-Primuline [36, 201, 202, 216] or Propidium Iodide and Bisbenzimide [217] required illumination with a different excitation wavelength for the visualization of each tracer. A "newer generation" of fluorescent tracers has the advantage of displaying different colors of fluorescence under the same excitation wavelength. Moreover, the two tracers are located in different compartments of the cell body. Thus the tracers True Blue, Granular Blue and Fast Blue [35, 38] display a blue fluorescence under 360 nm, and are localized in the cytoplasm and nucleolus. The tracers Bisbenzimide, Nuclear Yellow and Diamidino Yellow [38, 190, 217] produce a yellow fluorescence under 360 nm, which is mainly localized in the nucleus. The combination of a "yellow" and a "blue" tracer therefore is ideally suited for double-labeling experiments [218] and has been used in many studies to display the topographical relationships of neighbouring groups of neurons projecting to different areas and to demonstrate and quantify neurons giving rise to branching axons [43, 71, 72, 161, 162, 163, 184, 191]. Using fluorescent tracers it has even been possible to obtain triple-labeling of neurons [39, 308]. The tracers can also be used in combination with histofluorescence [5, 45, 203] and immunofluorescence [204, 343, 362] methods to demonstrate the neurotransmitter present in identified projection-neurons.

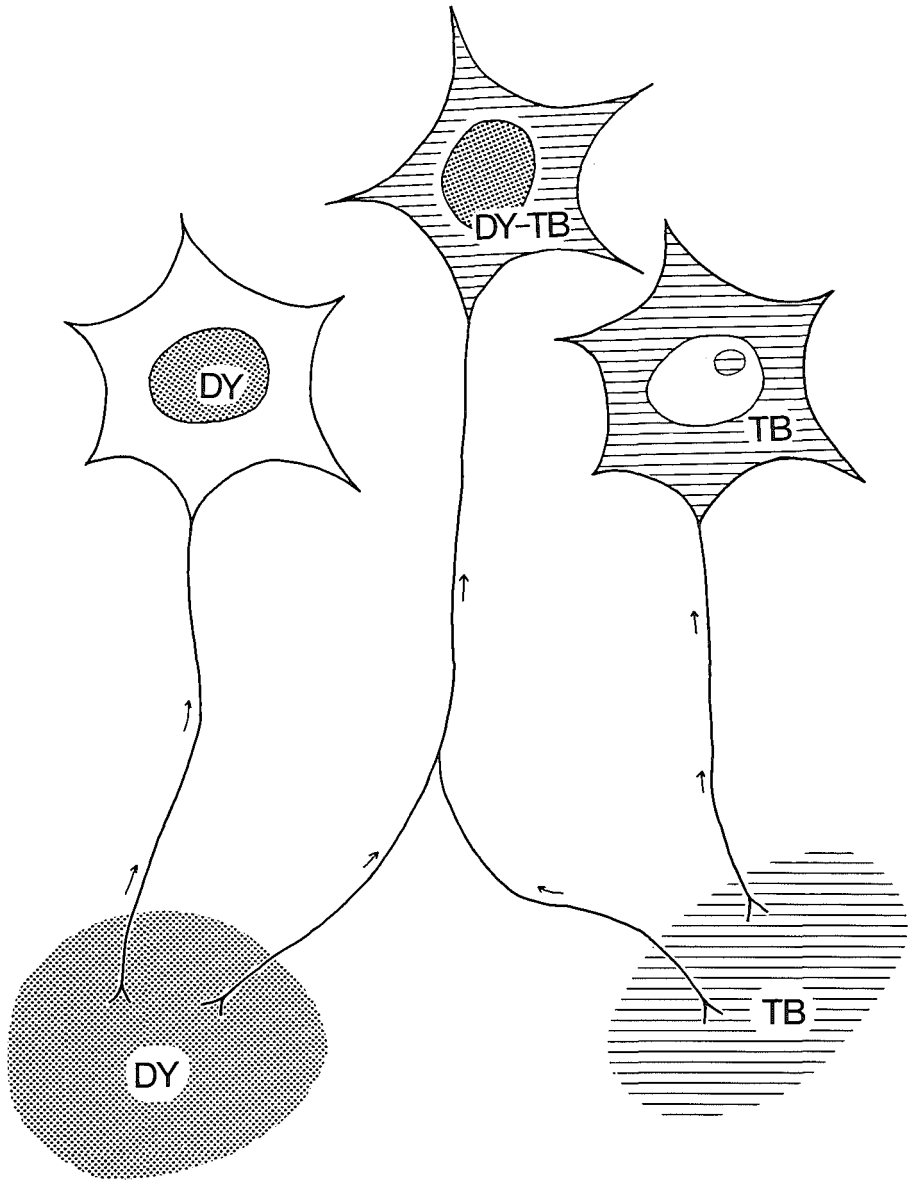


Fig. 3.1 Diagram depicting the principle of fluorescent double-labeling of neurons. The fluorescent tracers are taken up from fiber terminals and are transported retrogradely to their parent cell bodies. Cells projecting to only one of the two injection-sites or to both injection-sites can be demonstrated independently and simultaneously, as they contain one or both of the fluorescent tracers. In the present experiments the yellow "nuclear" tracer DY and the blue "cytoplasmic" tracer TB are visualized simultaneously under illumination with excitation light of 360 nm wavelength (see text).

When choosing between the fluorescent and other available double-labeling methods, their respective properties should be considered. The fluorescent tracers are liable to fading under prolonged microscopic illumination, while the cytoarchitecture of the tissue is most difficult to judge in the fluorescence microscope. The HRP/iron-dextran, HRP/^{3}H-BSA and HRP/^{3}H-HRP methods do not have these drawbacks. On the other hand, the combined histochemical and autoradiographic procedures make their application laborious, in contrast to the fluorescent tracers which can be visualized without any processing. The HRP/iron-dextran method has the disadvantage that the different types of label can obscure each other [309]. The tritiated compounds used in the two other methods only give rise to detectable amounts of silver grains in the emulsion layer when they are contained in the upper 2 μm of the section, due to the limited penetration of the emitted ß-particles. Therefore the fluorescence method probably gives the best approximation of the actual fraction of branching neurons within a given population [cf. 41]. New fluorescent tracers, such as Fluoro-Gold and rhodamine-labeled latex microspheres, have been reported to be more resistent to fading [189, 348], but experience with their use in double-labeling experiments is still limited.

3.2 Application of the method in the present study

The present experiments were all carried out in rats. In one part of this study (Chapter 5) additional experiments were carried out in 3 cats. The two retrograde tracers True Blue (TB) and Diamidino Yellow Dihydrochloride (DY) were applied in the rat. In the cat, Fast Blue (FB) was used instead of TB, as FB is more effectively transported over long distances in this species [38]. Data concerning the location and extent of the different tracer injections, as well as details on the amount of tracer injected and the survival times, are presented in the chapters dealing with the different sets of experiments.

All injections were made under Nembutal anesthesia (intrahepatic, 6%). When the animals suffered discomfort, morphine was administered postoperatively. After appropriate survival times, all animals were deeply anesthetized with Nembutal and perfused transcardially with 0.5 l of 0.9% saline followed by 1 l of 10% phosphate-buffered formalin (pH 7.2). The brain and spinal cord were removed. The dorsal rootlets were left attached to the spinal cord for identification of the segments. The material was stored overnight in a solution of 30% sucrose and 10% formalin in a 0.2 M phosphate buffer (pH 7.2) at 4°C.[4] The injected segments were stored separately and, before being cut, were coated with 3% carboxymethylcellulose in

Footnote 4. A slightly different procedure was used in the cats: they were transcardially perfused with 2 l of 0.9% saline, followed by 3 l of 20 % citrate-buffered formalin (pH 7.4), followed by 1.5 l of 10% citrate-buffered sucrose (pH 7.4). The material was not stored, but sectioned immediately after perfusion instead.

order to keep the sections containing the needle-tracks intact. The brain and spinal cord were cut into 30 μm sections on a freezing microtome; the sections were immediately mounted from distilled water. After air-drying, they were stained at pH 7.4 for 50 seconds with a 0.00002% solution of acridine orange (Gurr) and air-dried again at room temperature. They were not cover-slipped. The sections were studied using a Leitz Ploemopack fluorescence microscope, equipped with filter-mirror systems A and I2, providing excitation light of 360 nm and 450-490 nm wavelength, respectively.

Under 360 nm excitation wavelength, the fluorescent injection areas and the retrograde labeling of neurons could be visualized.

The *injection areas* displayed two or three concentric zones of different intensity around each needle-track.

The *TB* injection-sites [72, 161] displayed a bright blue zone immediately surrounding the needle-tracks, containing fluorescent cellular debris and a dense accumulation of fluorescent glial nuclei. A second zone, surrounding the first zone, contained fluorescent glial nuclei which progressively decreased in number and brightness towards the periphery of this zone, fading into an area of normal tissue with little or no fluorescence.

The *FB* injection-sites [72, 162] displayed three zones; the inner one was brilliantly white-blue with orange fluorescent granules, the second area contained a dense accumulation of blue fluorescent glial cells and the outer zone contained some fluorescent cellular elements and fibers.

The *DY* injection-sites [190] contained a relatively wide central zone of brown-yellow fluorescent necrotic material, surrounded by a narrow zone with yellow-blueish tissue fluorescence and many fluorescent glial and neuronal nuclei. The subsequent narrow third zone contained fluorescent glial and neuronal nuclei without tissue fluorescence.

Earlier studies have demonstrated that the tracers are taken up by terminals and damaged fibers from zone 1 as well as from zone 2, but not from zone 3 [162, 163]. Our findings confirmed uptake from these zones. The spinal and medullary hemi-injections in the present study were made as a row of needle-penetrations in order to damage as many fibers of passage as possible. The neurons labeled from such injections can therefore be considered to project to the injected levels or beyond.

Retrogradely labeled neurons were studied under 360 nm (filter-mirror system A). TB-labeled neurons displayed a deep blue fluorescence of the cytoplasm and proximal dendrites as well as of the nucleolus [35]. FB-labeling produced a similar type of fluorescence, but of a lighter shade of blue with silvery granules in the cytoplasm [38]. DY-labeled neurons displayed an intensely yellow fluorescent nucleus with a fluorescent ring around the nucleolus; the cytoplasm occasionally contained a dull yellow fluorescence, sometimes extending into the proximal dendrites [190].

Double-labeled neurons were recognized by their yellow nuclear fluorescence and a blue fluorescent cytoplasm with sometimes some yellow admixture. The photomicrographs in Chapters 4 to 7 give examples of the different types of labeling.

Retrogradely labeled neurons were studied in the segments C3-C8 in the first set of experiments (Chapter 4), while in the other experiments the segments C1-C8/T1 were included (Chapters 5, 6 and 7). The distribution of labeled neurons was charted with the aid of an X-Y plotter connected with transducers to the microscope stage. The TB-labeled, DY-labeled and DY-TB double-labeled neurons were counted in each ninth or eighteenth section. The percentage of double-labeling of the DY-labeled neurons was calculated in each case by dividing the total number of double-labeled neurons by the total number of DY-labeled neurons (i.e. DY single-labeled plus DY double-labeled neurons): DY-TB / (DY + DY-TB) x 100%. The percentages of double-labeling of the TB (or FB) neurons were calculated in the same way: % double-labeling = DY-TB / (TB + DY-TB) x 100%.

The *cytoarchitectural features* of the tissue are hard, if not impossible, to judge under the fluorescence microscope. Therefore we applied a fluorescent counterstain with acridine orange, slightly modified from that originally described by Schmued et al. [347]. Acridine orange results in a green Nissl-like staining of neurons when studied under 450-490 nm excitation wavelength, enabling the cytoarchitectonic laminar division of the spinal cord to be recognized. Rather than using standard diagrams of spinal segments, the outlines of the different laminae were first determined under 450-490 nm in each section before charting the distribution of labeled neurons under 360 nm excitation wavelength (a description of the cytoarchitecture of the rat spinal cord, as observed in the present study, is supplied in Chapter 2.1).

In applying the fluorescent tracers some precautions were taken. Leakage from the needle-tracks was limited as much as possible by leaving the micropipette in the tissue for several minutes after each injection. Following perfusion and sectioning, the regions surrounding the injection areas were always checked for pial staining, as intact fiber systems passing near the pial surface appear to be capable of incorporating the tracers [98]. The "early" tracers Bisbenzimide (Bb) and Nuclear Yellow (NY) have been found to diffuse from retrogradely labeled neurons into the surrounding tissues after long survival times [37], giving rise to labeling of surrounding glial cells and, eventually, to false labeling of neighbouring neurons. Although the more recently developed tracer DY, used in the present study, displays only slight leakage from intensely labeled neurons [190], the precautions recommended in using Bb and NY [37] were taken. Thus survival times after DY-injections were kept as short as possible to obtain sufficient labeling of neurons, and DY-labeling was only considered reliable in the absence of labeled glial nuclei. It was found that prominent DY-labeling was obtained at long distances from the injection-

sites, whereas "false labeling" which may have been due to leakage was limited to the immediate vicinity (1 or 2 mm) of the DY-deposit. (It has to be noted that the DY as used in this study was supplied by Dr. Illing, Gross Umstadt, Germany. Commercially available DY from other sources may have different transport- or diffusion characteristics).

CHAPTER 4.
BRANCHING NEURONS IN THE CERVICAL SPINAL CORD OF THE RAT

Introduction

In the present set of experiments, an attempt was made to demonstrate the possible existence of "branching" cervical spinal neurons which give rise to ascending and descending collaterals and to determine their location and relative numbers (experiments of group A: tracer injections at C1 and T2). Following the demonstration of such branching neurons, further experiments were carried out to approximate the rostral and caudal extent of projection of their collaterals (experiments of groups B-D).

The results indicate that many neurons in the cervical grey matter are branching neurons which distribute ascending collaterals to upper cervical or supraspinal levels and descending collaterals to thoracic or lumbosacral levels. Most *descending* collaterals terminate at upper thoracic levels, whereas the majority of the *ascending* collaterals are distributed to supraspinal levels.

Material and methods

In 39 rats, the two fluorescent tracers - 2% True Blue (TB), dissolved in H_2O, and 2% Diamidino Yellow Dihydrochloride (DY), suspended in a 0.2 M phosphate buffer (pH 7.2) - were injected unilaterally either at two different spinal levels or at a spinal and a lower brain stem level. Injections were made by means of a glass micropipette (tip diameter 100 -200 μm).

Group A (10 rats)

In 8 rats laminectomies were made in the C1 and T1 vertebrae. At C1 0.6-1.0 μl of 2% TB was injected unilaterally into the spinal grey and white matter by making a mediolateral row of micropipette penetrations. Similarly, 0.6-0.9 μl of 2% DY was injected ipsilaterally at T2. In 2 rats the tracers were reversed, i.e. TB at T2 and DY at C1. All animals were sacrificed 4 to 5 days after the injections.

Group B (17 rats)

In 17 rats TB was injected unilaterally at C1, and DY was injected ipsilaterally at progressively more caudal levels; i.e. at T6 (1 rat), T8 (3 rats), T9 (3 rats), T13 (4 rats), L2 (1 rat), L3 (3 rats) and S1/S2 (2 rats). The survival times after the DY-injections ranged from 5 days (T6) to 8 days (S1/S2). In order to minimize diffusion of TB from the C1 injection-site (see results) the survival times after the TB-injections were kept at 3 to 5 days. In most animals the two tracers were therefore injected in separate sessions.

Group C (4 rats)

In 4 rats, 1.0 μl of 2% TB was injected unilaterally at C3/C4 while 0.9 μl of 2% DY was injected ipsilaterally at T2. These animals were sacrificed 3 days later.

Group D (8 rats)

TB-injections were made unilaterally in one half of the lower brain stem at three levels, i.e. at the levels of the caudal part of the lateral reticular nucleus (3 rats), the rostral part of the inferior olive (4 rats) and the facial nucleus (1 rat); in all cases DY was injected ipsilaterally either at T2 or at T3. In total 0.8-1.2 μl of 2% TB was injected into the brain stem by means of multiple micropipette penetrations which were made after part of the occipital bone had been removed. The surface of the brain stem was penetrated at the level of the obex, with the tip of the pipette directed ventrally or rostroventrally. These animals were sacrificed after 5 days.

Postoperatively, the animals recovered rather slowly. In most cases, however, they only exhibited a paresis of one hindlimb apparently without suffering general discomfort. The animals were sacrificed and perfused as described in Chapter 3.2.

In the present set of experiments, the TB-labeled, DY-labeled and DY-TB double-labeled neurons were each counted in every eighteenth section.

Results

At the TB and DY injection-sites, the fluorescent zones around adjoining needle-tracks fused with one another. In almost all cases, the maximum uptake area extended over the width of one half of the spinal cord or medulla, without involving the other half.

As stated before, DY migrates only very slowly out of the retrogradely labeled cells [190]. Correspondingly, 3-5 days after DY-injection at T2, pronounced migration of DY out of retrogradely labeled neurons, as indicated by prominent DY-labeling of glial nuclei, was present only in the caudal part of T1 .

Distributions of the labeled cells in C3 to C8

Group A

After injection of TB at C1 and of DY at T2, many thousands of labeled neurons were counted in C3-C8, most of which were single-labeled. However, also many DY-TB double-labeled neurons were found. The TB neurons must project to C1 or above, while the DY neurons must project to T2 or below. The DY-TB neurons must represent branching neurons, the collaterals of which project to C1 or above and to T2 or below.

The distributions of these three populations of neurons were largely the same (Fig. 4.1). *Ipsilateral* to the injections, several labeled neurons were present in

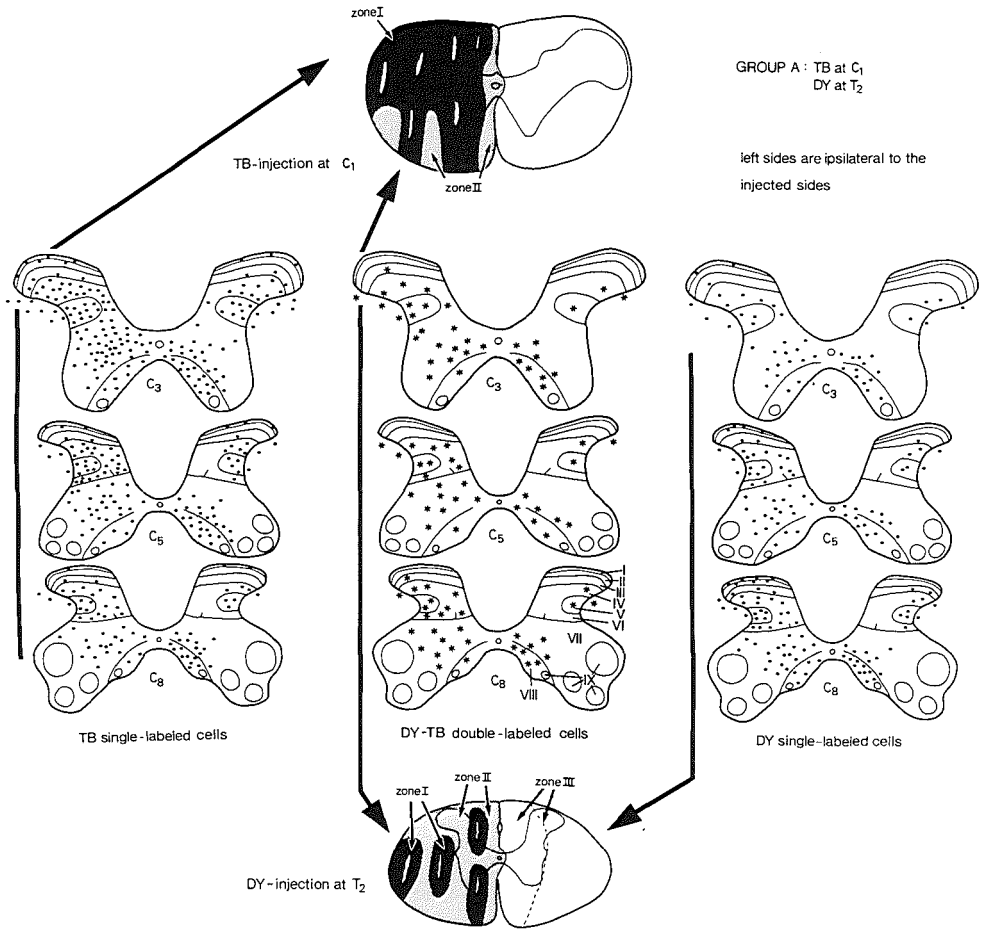


Fig. 4.1 Distributions of the retrogradely labeled neurons (TB single-labeled, DY-TB double-labeled, and DY single-labeled) at C3, C5 and C8 after TB-injections at C1 and DY-injections at T2 (group A). Each drawing represents the distribution of the labeled neurons in one section.

laminae I and II (see below); relatively few were present in lamina III. Many labeled neurons were found in laminae IV to VII. In lamina VII they tended to be concentrated in its central and ventromedial parts. Fewer labeled neurons were present in lamina VIII. *Contralaterally* some labeled neurons were present in laminae I, II and III. Several were found laterally in laminae IV and V, but only few in lamina VI. The highest densities of labeled neurons on this side, i.e. contralateral to the injections, were found in lamina VIII and the adjoining ventromedial part of lamina VII. Throughout C3 - C8, a separate group of labeled neurons was present *bilaterally* in the dorsolateral funiculus. This group extended from the tip of the dorsal horn to its base. The neurons were elongated in shape, with their long axes

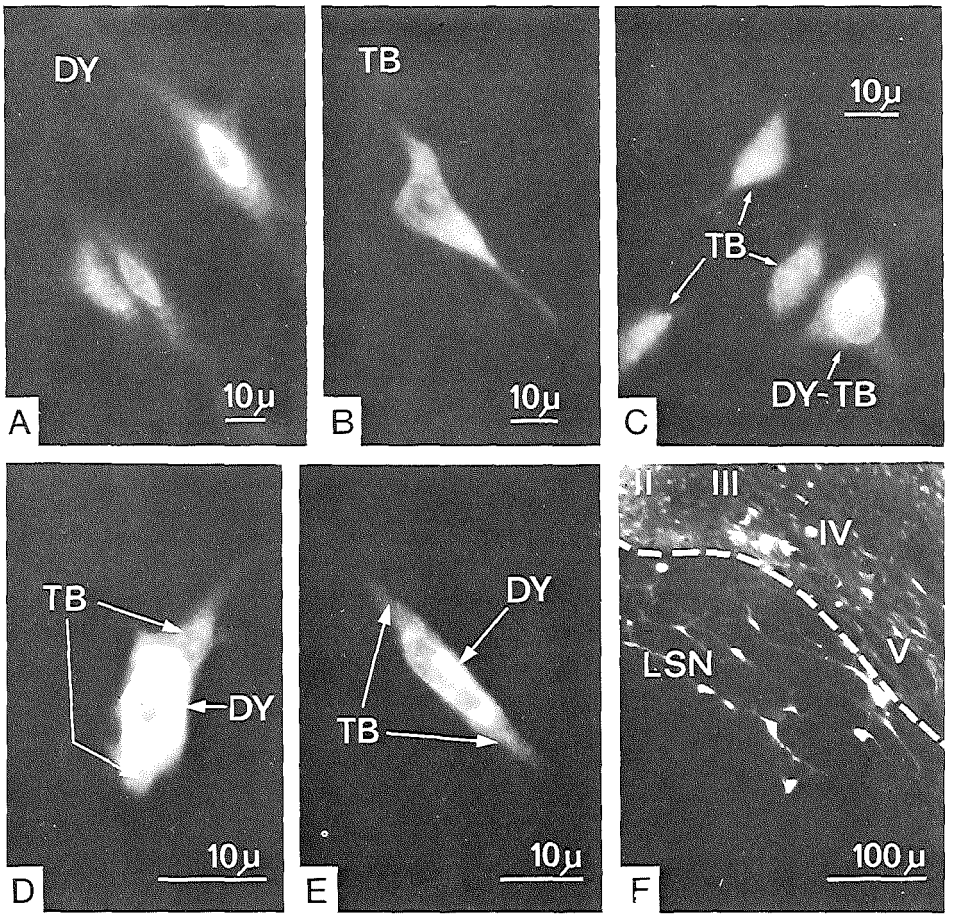


Fig. 4.2 Photomicrographs of neurons labeled with DY and with TB after TB-injection at C1 and DY-injection at T2.

A. DY single-labeled neurons in contralateral lamina VIII at C4. Note predominantly nuclear labeling with DY

B. TB single-labeled neuron in contralateral lamina V (lateral part) at C2. Note absence of nuclear labeling with TB, except for the nucleolus

C. DY-TB double-labeled neuron and TB single-labeled neurons in ipsilateral lamina VII at C4

D. DY-TB double-labeled neuron in ipsilateral lamina VII at C4

E. DY-TB double-labeled neuron in contralateral lamina V (lateral part) at C7

F. Labeled neurons in the ipsilateral LSN at C7. Note that dendrites and somata are oriented parallel to the lateral border of the dorsal horn.

parallelling the ventrolateral border of the dorsal horn (cf. Fig. 4.2F). This group of neurons will be referred to as the lateral spinal nucleus, LSN [cf. 128], which is distinct from the lateral cervical nucleus, LCN, at C1 and C2.

It is of importance to emphasize that the labeled neurons in laminae I and II on both sides were single-labeled. In lamina I, the TB neurons, projecting rostrally, were present bilaterally while the DY neurons, projecting caudally, were present almost exclusively ipsilaterally. In lamina II, both the DY-labeled and the TB-labeled neurons were present mainly ipsilaterally. They decreased in numbers at increasing distances from the injected segments.

In the segments adjoining the injections some pial staining occurred, presumably due to leakage of the tracers from the needle-tracks. This pial staining was frequently accompanied by staining of neuronal cell bodies and neuropil in the immediately subjacent part of the dorsal horn. In these segments some of the labeled neurons in the upper parts of the dorsal horn could therefore have been stained by diffusion of the tracers. In three control experiments up to 0.8 μl of TB and of DY was deposited on the pial surfaces of C1 and T2. In these cases some neurons in the dorsal horn were labeled, but they were always surrounded by stained neuropil. In the experiments with the tracer injections labeled laminae I and II cells without staining of the neuropil were present close to the injection site. Therefore, these neurons were regarded to be truely retrogradely labeled.

At all levels, the total number of labeled neurons was about twice as high ipsilaterally than contralaterally. The neurons labeled from C1 and those labeled from T2 were much more numerous than those observed in earlier retrograde HRP experiments. Thus, in each case a total of about 4000 to 8000 TB single-labeled (ascending) neurons were counted in the sections of C3 to C8, about 2000 to 4000 DY single-labeled (descending) neurons and approximately 1000 to 2000 DY-TB double-labeled ones. Thus, a total of 7000 to 14000 labeled neurons were counted in each case. Since the cells in every eightheenth section were counted, the actual numbers of the labeled neurons must have been approximately eighteen times higher. In the two cases in which the tracers were reversed, the numbers of the labeled ascending and descending neurons per case fell within the above ranges.

The total numbers of labeled neurons per section ranged from 140 to 300. In all cases the general distribution of the labeled neurons in the grey matter remained the same when proceeding caudally from C3 to C8. However, the total number per section decreased in this direction. This must, at least in part, be related to the fact that when proceeding from C3 to C8 the TB cells labeled from C1 showed a reduction of about 50%, while the DY cells labeled from T2 showed an increase of only about 25% (see Table 4.1A on next page).

Many cells, of all types and sizes, were double-labeled; they were not concentrated in specific areas, except perhaps in the LSN. About 20% of all TB cells in C3-C8 were also DY-labeled and about 30% of all the DY cells were also TB-labeled. Therefore, 20% of the cells projecting to C1 (TB-injection) or above possessed descending collaterals to T2 or below, and 30% of the cells projecting to

Table 4.1 Numbers of labeled cells and the percentages of double-labeling in different segments in the different groups of experiments. Values are mean values (and SD) for all cases within each group. TB+DL, DY+DL, DL: numbers of TB-labeled, DY-labeled and TB-DY double-labeled cells *per section*. %DL/TB+DL, %DL/DY+DL : percentages of double-labeling of the TB-labeled cells and of the DY-labeled cells, respectively.

A	Group A : TB at C1, DY at T2		10 cases[1]		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	139 (38)	98 (32)	29 (11)	20,8 (6,2)	30,1 (7,3)
C3	190 (65)	76 (26)	28 (10)	15,0 (5,2)	37,9 (11,7)
C5	143 (45)	96 (33)	31 (17)	21,8 (9,8)	30,0 (10,9)
C8	94 (28)	102 (31)	27 (10)	28,7 (10,2)	25,4 (7,2)
B	Group B : TB at C1, DY at T8/9		6 cases		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	166 (40)	38 (7)	12 (3)	7,5 (1,8)	28,2 (4,6)
C3	228 (30)	35 (7)	12 (3)	5,4 (1,2)	34,2 (6,1)
C5	164 (52)	39 (8)	12 (3)	7,2 (2,1)	29,6 (5,9)
C8	117 (31)	43 (7)	11 (3)	9,8 (2,7)	26,4 (6,5)
	Group B : TB at C1, DY at T13		4 cases		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	149 (27)	18 (5)	6 (3)	3,7 (1,3)	31,3 (7,9)
C3	213 (36)	17 (3)	5 (3)	2,4 (1,3)	31,3 (18,0)
C5	147 (33)	20 (5)	5 (2)	3,8 (1,2)	30,8 (6,2)
C8	109 (13)	21 (5)	7 (3)	6,0 (2,4)	31,0 (9,5)
	Group B : TB at C1, DY at L2/3		4 cases		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	170 (50)	14 (3)	5 (1)	3,3 (1,1)	35,2 (7,8)
C3	225 (72)	15 (5)	5 (1)	2,2 (0,7)	37,5 (7,8)
C5	174 (55)	14 (3)	5 (1)	3,5 (1,6)	37,4 (2,8)
C8	126 (30)	18 (5)	6 (2)	4,5 (2,1)	30,4 (3,2)
	Group B : TB at C1, DY at S1/2		2 cases		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	146 (48)	7 (1)	1 (1)	1,1 (0,1)	24,1 (8,1)
C3	214 (66)	6 (1)	1 (1)	0,4 (0,4)	17,0 (17,0)
C5	140 (40)	8 (1)	2 (1)	1,2 (0,4)	25,3 (14,2)
C8	109 (37)	8 (1)	2 (0)	1,8 (0,3)	25,8 (5,3)
C	Group C : TB at C3/4, DY at T2		4 cases		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	195 (37)	125 (14)	52 (1)	27,2 (4,9)	41,7 (3,7)
C5	202 (23)	119 (11)	52 (5)	25,5 (1,7)	43,6 (4,5)
C8	171 (37)	131 (9)	51 (4)	30,8 (5,0)	39,6 (4,6)
D	Group D : TB at cLRN, DY at T2		3 cases		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	132 (17)	100 (13)	26 (7)	19,8 (5,9)	26,7 (3,8)
C3	167 (18)	78 (6)	24 (6)	14,6 (3,1)	29,8 (6,3)
C5	122 (23)	96 (23)	23 (9)	20,1 (10,2)	23,4 (4,8)
C8	110 (24)	115 (18)	25 (0)	23,4 (4,9)	21,0 (2,0)
	Group D : TB at rIO/N.VII, DY at T3		5 cases		
	TB+DL	DY+DL	DL	%DL/TB+DL	%DL/DY+DL
C3-C8	121 (21)	70 (12)	10 (2)	8,2 (0,8)	14,2 (2,2)
C3	193 (39)	60 (10)	11 (1)	5,8 (0,6)	18,7 (2,0)
C5	117 (16)	70 (16)	11 (3)	9,4 (1,5)	15,8 (3,5)
C8	86 (9)	84 (10)	8 (2)	10,0 (2,5)	9.9 (1,7)

[1] The two cases in which the tracers were reversed (see text) are included in these results as if in these cases TB was also injected at C1 and DY at T2

T2 (DY-injection) or below possessed collaterals to C1 or above. These percentages differed in the different segments (cf. Table 4.1A).

Group B

In the various cases with TB-injections at C1 and DY-injections at progressively more caudal levels (i.e. T6, T8/9, T13, L2/3 and S1/2), the *TB cells* (including the double-labeled ones), as could be expected, were approximately of the same numbers and showed the same distribution as in group A. However, the *DY cells*, including the double-labeled ones, showed a progressive decrease in numbers (Table 4.1B, Fig. 4.4-2) and a gradual change in their distribution (cf. Fig. 4.3).

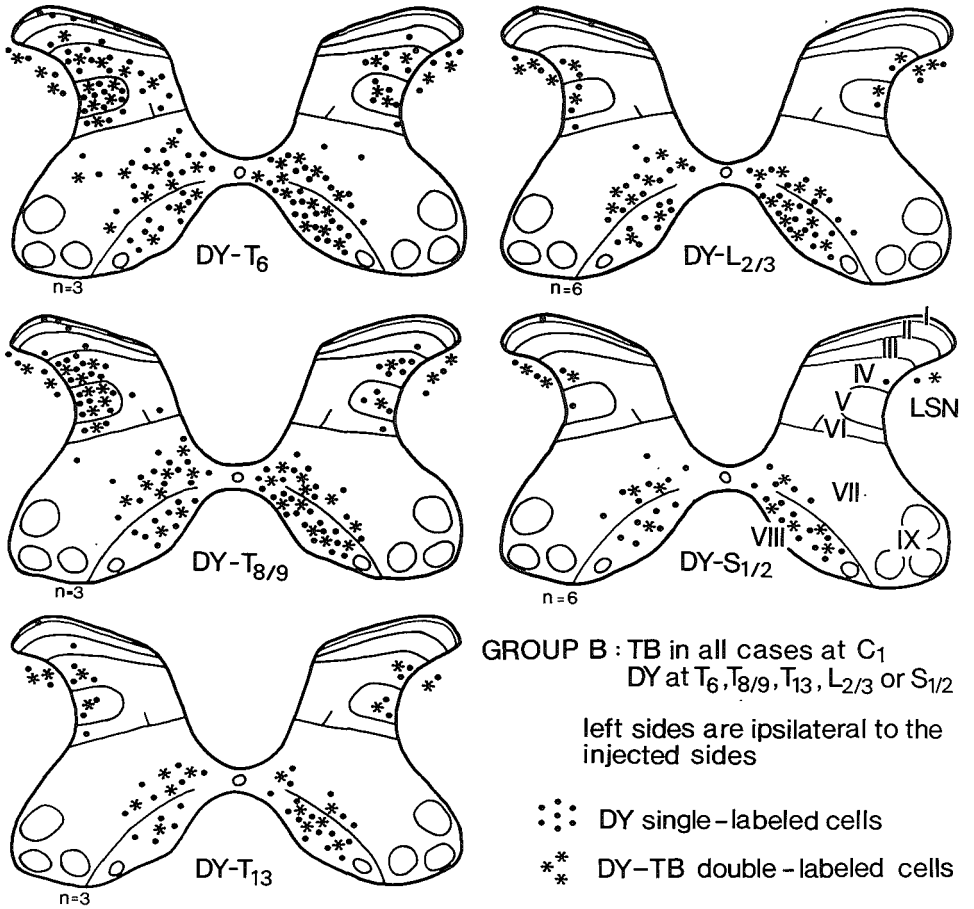


Fig. 4.3 Distributions of the retrogradely DY-labeled neurons and of the DY-TB double-labeled neurons at C5 after TB-injections at C1 and DY-injections at T6, T8/9, T13, L2/3 or S1/2. Each drawing represents the superimposed chartings in 3 (n=3) or 6 (n=6) sections. The distributions of the TB single-labeled neurons in these cases are not shown; they were the same as in Figure 4.1.

Thus, after DY-injections at L2/3 or S1/2, most of the DY cells in C3-C8, including the double-labeled ones, were present in lamina VIII and the adjoining ventromedial part of lamina VII on both sides. Some were also present bilaterally in the lateral parts of laminae IV to VI, as well as in the LSN, and a few DY single-labeled cells were present ipsilaterally in laminae I and II. No DY cells were present in the medial parts of laminae IV to VI and in the central and lateral parts of lamina VII, which areas contained many DY cells ipsilaterally after DY-injections at T2 (cf. Figs 4.1 and 4.3).

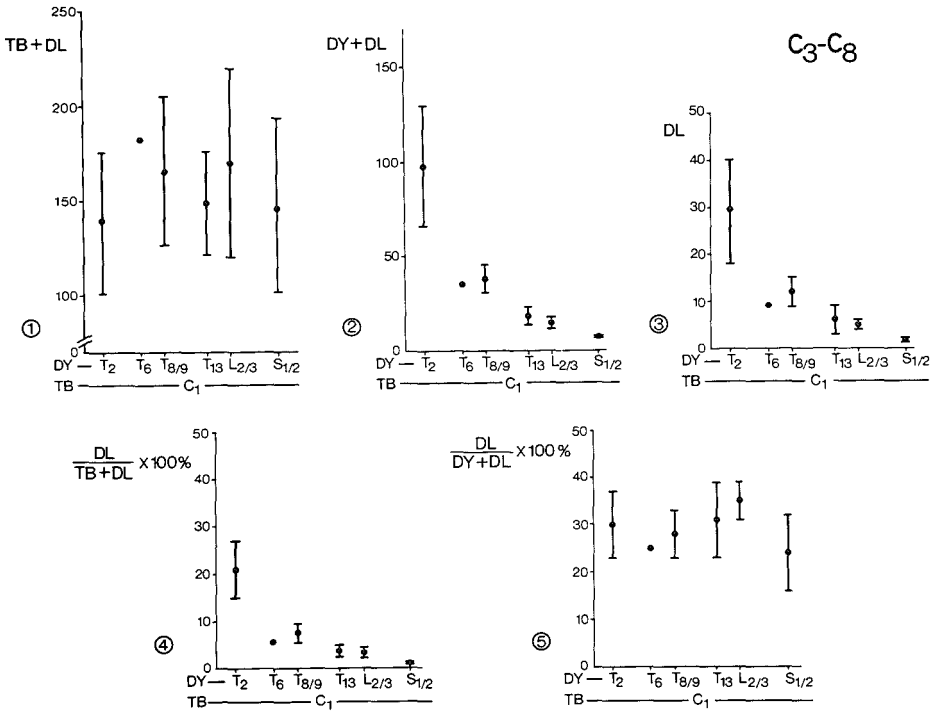


Fig. 4.4 Comparison of the results obtained in the segments C3 to C8 in the cases of groups A and B. TB-injections in all cases at C1, DY-injections at different levels ranging from T2 to S1/2. The graphs show the numbers of labeled neurons per section and the percentages of double-labeling relative to the level of the DY-injection.

Graphs 1,2,3: TB+DL, DY+DL, DL: numbers of labeled neurons per section which were TB-labeled, DY-labeled, or DY-TB double-labeled, respectively.

Graphs 4,5: (DL/TB+DL)X100%, (DL/DY+DL)X100% : percentages of double-labeling of the TB-labeled neurons and of the DY-labeled neurons, respectively.

The reduction in the number of *DY cells* after more caudal injections was accompanied by a similar reduction in the number of DY-TB double-labeled cells (Fig. 4.4-3). Therefore, independent of the level of the DY-injections, the percentages of the DY cells which were double-labeled remained about the same, i.e. about 30% (cf. Fig. 4.4-5). However, since the number of *TB cells* remained roughly the same (Fig. 4.4-1), their percentages of double-labeling showed a progressive decrease after more caudal injections (Table 4.1B on page 38, Fig. 4.4-4).

In the LSN, the percentages of double-labeling were generally higher than in the grey matter. For example, after DY-injections at L2/L3, about 10-15% of the TB cells in the LSN on both sides were double-labeled, whereas in the grey matter, only 3%. In all cases of group B, about 40-50% of the DY cells in the LSN were double-labeled, as compared to 30% in the grey matter.

Group C

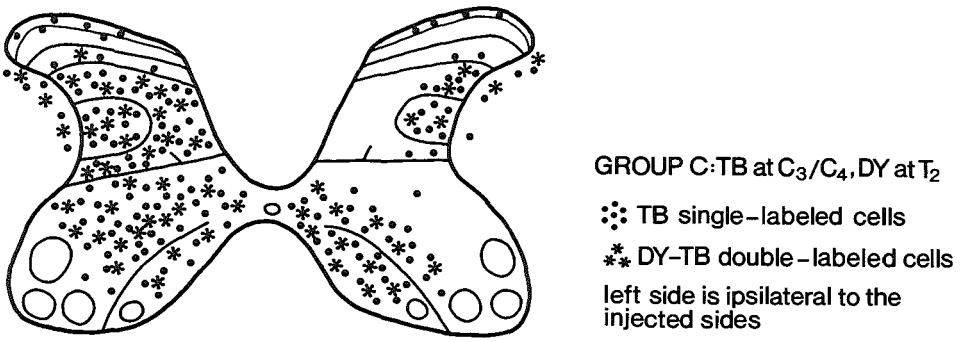


Fig. 4.5 Distributions of the retrogradely TB-labeled neurons and of the DY-TB double-labeled neurons at C5 after TB-injections at C3/4 and DY-injections at T2 (group C). The drawing represents the findings in one section. The distribution of the DY single-labeled neurons is not shown; it was the same as in Figure 4.1.

After TB-injections at C3/C4 (instead of at C1) and DY-injections at T2, the *DY cells* in C5-C8, including the DY-TB double-labeled ones, showed the same distribution as in group A and were of roughly the same number (Fig. 4.6-2). However, as could be expected on the basis of the proximity of the injection, the mean number of *TB cells* per section was much higher than in group A[5] (Fig. 4.6-1); yet, their distribution was roughly the same as in group A (cf. Figs. 4.1 and 4.5). In C5-C8 also many DY-TB double-labeled cells were found. About 27% of the TB cells (projecting to C3/C4 or above) were also DY-labeled from T2, which is comparable to the 25% in group A (Fig. 4.6-4). However, about 42% of the *DY cells* (projecting to T2 or below) were also TB-labeled from C3/C4, which is much higher than the 29% in group A (Fig. 4.6-5).

Footnote 5. In all cases, the numbers of TB-labeled and DY-labeled neurons, and the percentages of double-labeling differed between segments (cf. Table 4.1, page 38). In group C only the segments C5-C8 were studied; therefore the comparison with group A was restricted to the data pertaining to these segments. Data of group A concerning C5-C8 are found in Fig. 4.6 only; Table 4.1 and Figs. 4.4 and 4.8 contain data concerning the segments C3-C8.

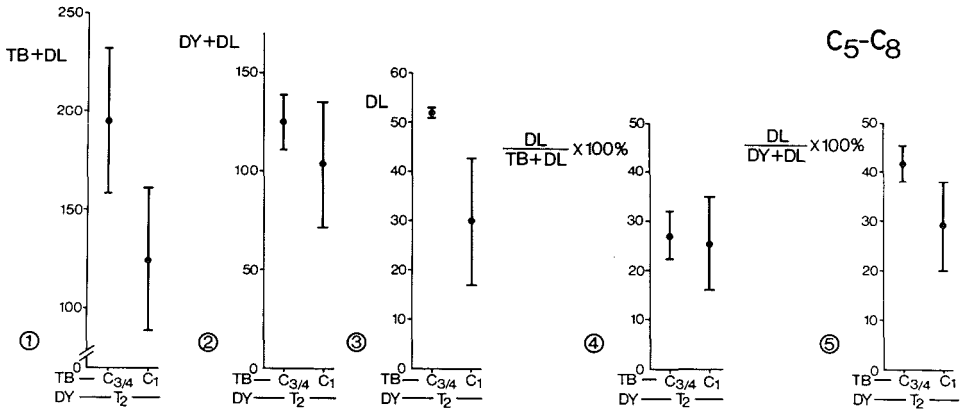


Fig. 4.6 Comparison of the results obtained in the segments C5 to C8 in groups A and C. DY-injections in all cases at T2, TB-injections at C1 and C3/C4 respectively. The graphs show the numbers of labeled neurons and the percentages of double-labeling relative to the level of the TB-injection.
TB+DL, DY+DL, DL, (DL/TB+DL)X100%, (DL/DY+DL)X100% : as in Fig. 4.4.

Group D

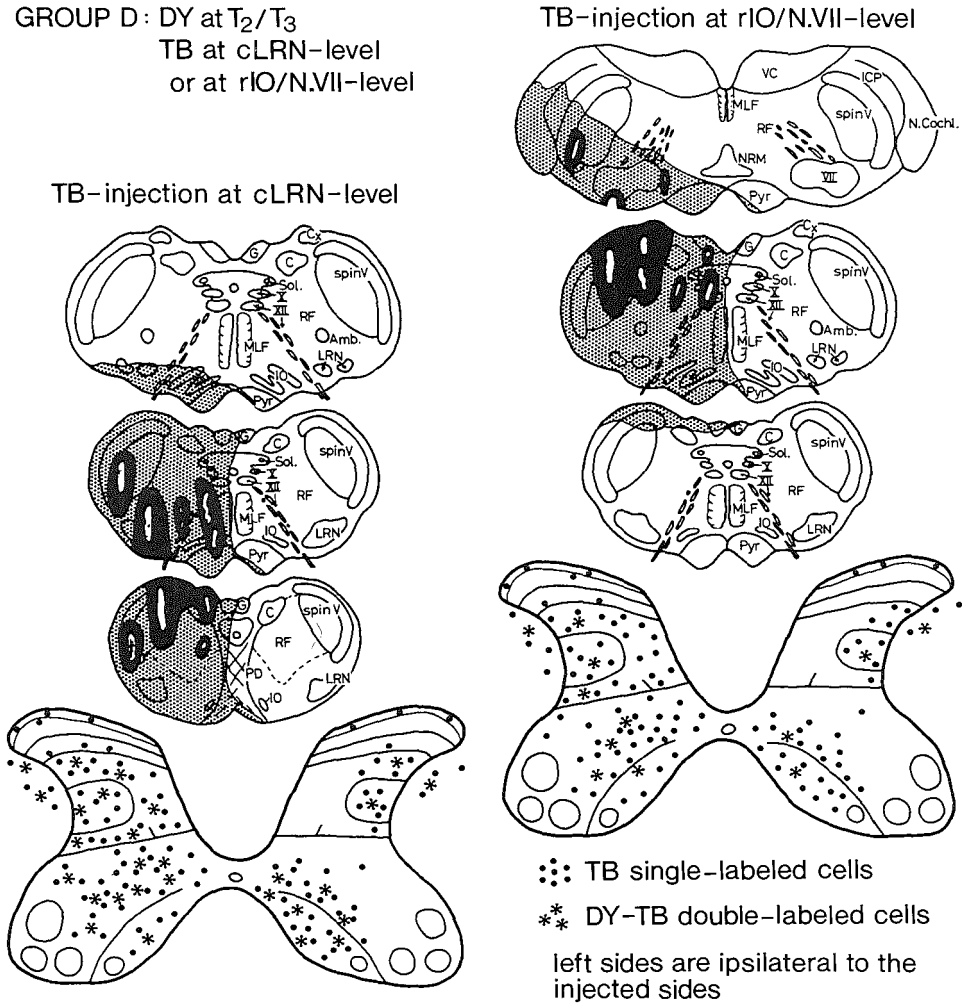


Fig. 4.7 Distributions of the retrogradely TB-labeled neurons and of the DY-TB double-labeled neurons at C5 after TB-injections at the cLRN-level and DY-injections at T2 (left column), and after TB-injections at rIO/N.VII-levels and DY-injections at T3 (right column) (group D). Each drawing represents the findings in one section. The distributions of the DY single-labeled neurons are not shown; they were the same as in Figure 4.1. Abbreviations: Amb.-ambiguus nucleus; C.-cuneate nucleus; Cx-external cuneate nucleus; G.-gracile nucleus; ICP-inferior cerebellar peduncle; IO-inferior olive; LRN-lateral reticular nucleus; MLF-medial longitudinal fasciculus; N.cochl.-cochlear nuclei; NRM-nucleus raphe magnus; PD-pyramidal decussation; Pyr-pyramidal tract; RF-reticular formation; spin.V-spinal trigeminal tract and nucleus; Sol.-solitary tract and nucleus; VC-vestibular complex; VII-facial nucleus; X-dorsal motor nucleus of vagus; XII-hypoglossal nucleus.

In the animals of this group the TB-injections were made in the lower brain stem at progressively more rostral levels, i.e. 1) at the level of the caudal part of the lateral reticular nucleus (cLRN), 2) at the level of the rostral part of the inferior olive (rIO) and 3) at the level of the facial nucleus (N.VII). Most of these TB-injections involved the entire width of one half of the brain stem, but in some cases they spared its most lateral rim. As the various TB-injections were made through the area of the obex, in all cases the dorsal column nuclei and the inferior cerebellar peduncle were involved to some extent. The cLRN-injections were combined with ipsilateral DY-injections at T2, while the rIO- and N.VII-injections were combined with DY-injections at T3 (Fig. 4.8).

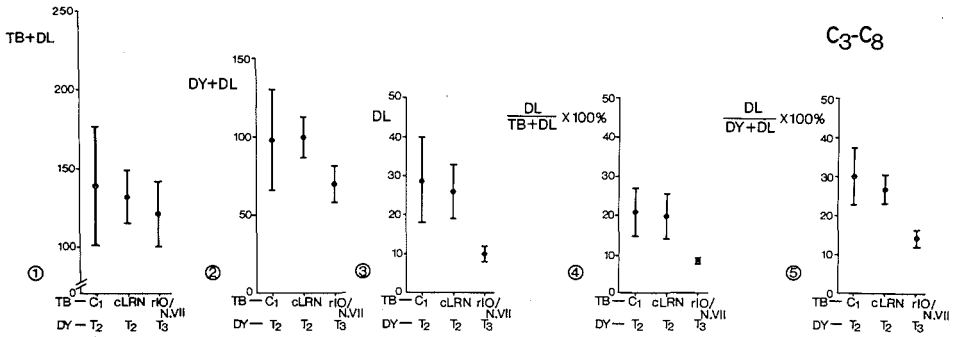


Fig. 4.8 Comparison of the results obtained in the segments C3 to C8 in groups A and D, after TB-injections at C1 and at different brain stem levels, respectively, combined with DY-injections at T2 or T3. The graphs show the numbers of labeled neurons per section and the percentages of double-labeling relative to the level of the TB-injections. Note that in the cases with TB-injections at the level of the rostral part of the inferior olive (rIO) and of the facial nucleus (N.VII) DY was injected at T3, whereas in the other cases, with TB-injections at C1 and at the level of the caudal part of the lateral reticular nucleus (cLRN), DY was injected at T2.
TB+DL, DY+DL, DL, (DL/TB+DL)X100%, (DL/DY+DL)X100% : as in Fig. 4.4.

In the cases with the TB-injections at the different brain stem levels the *TB cells* in C3-C8 showed roughly the same distribution and were about as numerous as those in the cases of group A (cf. Figs. 4.1 and 4.7). Thus, after the progressively more rostral injections the numbers of TB-labeled cells per section in C3-C8 showed only a slight, insignificant, decrease (Tables 4.1A and 4.1D on page 38, Fig. 4.8-1). In these cases the cells *DY-labeled* from T3 were about 30% fewer than those which were DY-labeled from T2 (Table 4.1D on page 38, Fig. 4.8-2).

DY-TB double-labeled cells were distributed according to the same pattern as those in group A. After TB-injections at the level of the cLRN, the percentages of double-labeling were about the same as in group A (Figs. 4.8-4 and 4.8-5). On the

other hand, after TB-injections at the rIO or N.VII level, the percentages were smaller (Tables 4.1A and 4.1D on page 38, Figs. 4.8-4 and 4.8-5). However, it should be noted that in these latter cases DY was injected at T3 instead of at T2, which probably influenced the percentages of double-labeling to some extent (see above).

Discussion

The present findings in the rat cervical cord provide information concerning:

a) the location, in the same section, of neurons with ascending fibers and of neurons with descending fibers, and

b) the presence of branching neurons with both ascending and descending fibers.

TB and DY are transported retrogradely not only from fiber termination areas but also from damaged axons [35, 162, 190, 221]. As a consequence the neurons which are retrogradely labeled after injection of these tracers can be regarded to distribute fibers to the injected area or through it to more distant areas.

Neurons with descending propriospinal fibers

The findings in group A show that the neurons with *descending fibers to T2 or below* are located in all laminae, except in lamina IX. However, they are mainly present in lamina I, laminae IV to VIII, and in the LSN, being less numerous in laminae II and III. This population of neurons comprises elements which give rise to long descending fibers as well as elements which give rise to short descending fibers.

The *long descending propriospinal fibers* (experiments of group B) may reach the lumbosacral cord (Fig. 4.3). They are mainly derived from neurons in lamina VIII and the adjoining part of lamina VII, in the lateral parts of laminae IV to VI, in the LSN and in lamina I. These various sets of neurons distribute their long descending fibers bilaterally, except the neurons in lamina I which distribute them only ipsilaterally. No "long descending propriospinal" neurons (projecting to levels below T8) are present in the medial parts of laminae IV to VI and in the central and lateral parts of lamina VII (Fig. 4.3), which is in keeping with HRP findings in the rat [275], the cat [254, 286, 288, 359, 416] and the monkey [288, 359].

The neurons which give rise to *short descending propriospinal fibers* (projecting to T2-T8) are much more numerous than those giving rise to long descending propriospinal fibers (cf. Tables 4.1A and 4.1B on page 38). These "short propriospinal neurons" are located both in the areas containing the "long descending propriospinal" neurons, and in the remaining areas, i.e. the medial parts of laminae IV to VI and the central and lateral parts of lamina VII (cf. Figs. 4.1 and 4.3). The neurons in these parts of laminae IV to VI and of lamina VII distribute their fibers almost exclusively ipsilaterally (Fig. 4.1). This is in keeping with earlier HRP-findings in the cat [286, 288].

Neurons giving rise to ascending propriospinal and supraspinal fibers

The neurons which give rise to *fibers ascending to C1 or above* (experiments of group A) are present in all laminae, except for lamina IX. They are concentrated, however, in lamina I, in laminae IV to VIII, and in the LSN, being less numerous in laminae II and III. Most of the sets of neurons in these laminae distribute their ascending fibers bilaterally, except those in the central and lateral parts of lamina VII and the medial parts of laminae IV to VI, which distribute them ipsilaterally (Fig. 4.1). This is in keeping with earlier HRP-findings in the cat [286, 288]. However, neurons projecting from lamina VIII to C1 or above showed a more bilateral distribution than in the HRP-experiments [286, 288], probably because the fluorescent tracer labeled a larger number of neurons.

In all cases the numbers of TB- and DY-labeled neurons per section were larger close to the TB- and DY-injections than at more distant levels (cf. Table 4.1 on page 38), indicating the additional labeling of neurons with short axons. Correspondingly the neurons in C5-C8 labeled from C3/C4 were more numerous than those labeled from C1 (cf. Fig. 4.6-1). The cells which make up the difference (about 70 cells per section) must represent neurons which give rise to *short ascending propriospinal fibers* to C2-C4. These neurons are located in the same areas as the neurons projecting to C1 or above (cf. Figs. 4.1 and 4.5). Moreover, the distribution of the neurons with short ascending fibers to C2-C4 resembles that of the neurons with short descending fibers to T2-T6 (see group B, cf. Figs. 4.3 and 4.5).

The neurons which give rise to *ascending supraspinal fibers* (experiments of group D) are almost as numerous as those projecting to C1 or above (group A) and are located in the same areas (cf. Figs. 4.1 and 4.7). Thus, the vast majority of the axons reaching C1 from neurons in C3-C8 continue to supraspinal levels. This population of "ascending supraspinal" neurons must be heterogeneous comprising e.g. spinothalamic tract cells, spinomesencephalic, spinoreticular and spinocerebellar tract cells, as well as cells projecting to the dorsal column nuclei. With the exception of some of the postsynaptic dorsal column neurons, the distributions of the various types of "tract cells" overlap extensively (cf Chapter 2.4), precluding their separate identification in the present experiments.

The labeled ascending neurons in C3-C8 were more numerous than the descending ones, especially in the upper cervical segments. This suggests a concentration of ascending supraspinal neurons at these upper cervical levels.

Branching neurons giving rise to ascending and descending collaterals

The finding of a large number of double-labeled neurons in all cases indicates the presence of many branching neurons. They were found to be intermingled with the purely "ascending" and "descending" neurons. However, no such branching neurons were found in laminae I and II. A comparison of the percentages of double-labeling observed in the cases of groups A, B, C and D (summarized in Fig. 4.9) suggests the following.

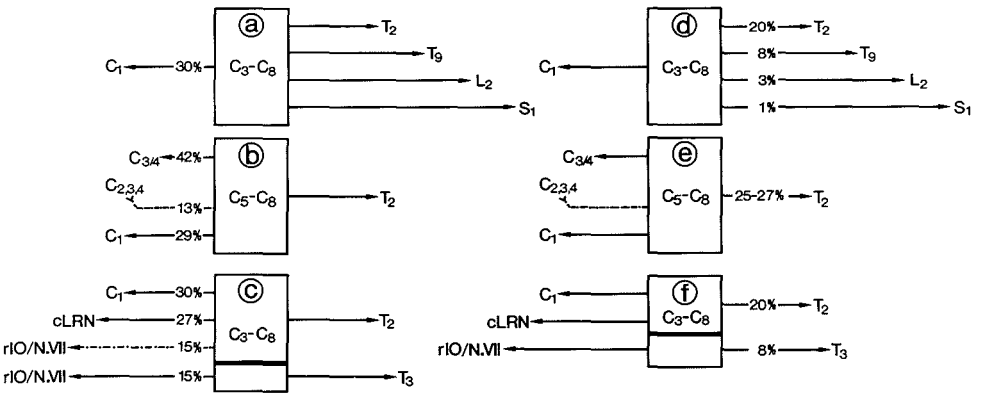


Fig. 4.9 Schematic summary of the double-labeling results obtained in the present study.
Left row: neurons giving rise to descending fibers, and the percentage of them which also gives rise to an ascending collateral reaching the indicated levels.
Right row: neurons giving rise to ascending fibers, and the percentage of them which also gives rise to a descending collateral reaching the indicated levels.
uninterrupted lines: percentages directly obtained in the different experiments
interrupted lines: percentages derived from those obtained in the experiments

About 20% of the C3-C8 neurons projecting to C1 or above give rise to a *descending propriospinal collateral* reaching T2, whereas 8%, 3% and 1% give rise to longer descending collaterals reaching T9, L2 and S1 (Fig. 4.9d). In other words, the descending propriospinal collaterals of branching neurons extend to various levels throughout the length of the spinal cord, but the majority are relatively short and terminate in the upper half of the thoracic cord.

About 30% of the C3-C8 neurons projecting to T2 or below are branching neurons which give rise to an *ascending collateral* reaching C1, and about 27% of the C3-C8 neurons projecting to T2 or below have an ascending collateral reaching the cLRN-level in the brain stem (Fig. 4.9c). Thus, the vast majority of the collaterals reaching C1 continue into the lower brain stem. In all likelihood, about half of the

ascending collaterals to the cLRN-level end between this level and the rIO-level (cf. Fig. 4.9c). This may be related to the electrophysiological findings that in the cat some cervical neurons project to both the lower spinal levels and the LRN [9, 173]. In the segments C5-C8, 13% of the neurons which project to T2 or below give rise to a short ascending collateral which is restricted to C2-C4 (Fig. 4.9b). This is only about half as many as the C5-C8 neurons which distribute an ascending collateral to C1 or above (29%, cf. Fig. 4.9b). Thus only some of the ascending collaterals of branching cervical neurons end at upper cervical levels (C2-C4), while the majority continue into the lower brain stem. Obviously, the fibers to the brain stem still might provide collaterals to the upper cervical segments.

Further data were obtained regarding the different sets of branching neurons projecting to different levels. Ascending collaterals reaching C1 are derived from short as well as from long *descending* propriospinal neurons in C3-C8 (Fig. 4.9a). Although the short descending propriospinal neurons are much more numerous than the long descending ones, an equal proportion of both cell populations, i.e. 30%, were found to give rise to ascending collaterals reaching C1 (cf. Tables 4.1A and 4.1B, Fig. 4.9a). Similarly, roughly equal proportions of the short and long *ascending* neurons in C5 to C8 give rise to descending collaterals reaching T2 (Fig. 4.9e).

CHAPTER 5.
SPINOCEREBELLAR NEURONS AND PROPRIOSPINAL NEURONS IN THE CERVICAL SPINAL CORD OF THE RAT AND THE CAT

Introduction

The present chapter deals with a series of experiments which were carried out to investigate, in the cervical cord of the rat and the cat, whether descending propriospinal neurons give rise to ascending collaterals to the cerebellum, and to determine the location and relative numbers of such neurons. To that purpose Diamidino Yellow was injected unilaterally in the spinal cord, whereas True Blue (in rat) or Fast Blue (in cat) was injected ipsilaterally in the cerebellum.

Following the electrophysiological demonstration of a rostral spinocerebellar tract from the cervical cord [310, 311], various groups of spinocerebellar neurons have been identified in the cervical cord [cf. 253, 255, 403, 405]. The present study shows that in many areas those spinocerebellar neurons are located amidst the descending propriospinal neurons; yet only extremely few cervical neurons project both to the cerebellum and to lower spinal levels. In the cervical cord, such branching neurons are present only in the central part of lamina VII of the enlargement.

Material and methods

In 10 rats and 3 cats, tracer injections were made in the spinal cord (T2) and in the cerebellum by means of a glass micropipette (tip diameter 100-200 μm). The cerebellar injections were centered on the vermis (in which spinocerebellar terminals appear to be concentrated - cf. refs. 260, 261, 393, 406 -), but were also made into more lateral parts of the cerebellum.

In the rats, 2% True Blue (TB), dissolved in H_2O, was injected unilaterally into the cerebellum by making two parasagittal rows of needle-penetrations from the superior cerebellar surface. The total amount of TB which was injected ranged from 0.9 to 3.0 μl in 6 cases while in 2 other cases 6.1 and 6.8 μl was injected. Ipsilateral to the cerebellar TB injections, 1.0-1.2 μl of 2% Diamidino Yellow Dihydrochloride (DY), suspended in a 0.2M phosphate buffer (pH 7.2), was deposited unilaterally at T2 in a mediolateral row of micropipette penetrations. The survival times after TB-injections ranged from 5 to 7 days, while the survival time after DY-injections was kept at 2 days. In all rats the two tracers therefore were injected in separate sessions.

In the cats, 7.6-7.8 μl of 7% Fast Blue (FB), dissolved in H_2O, was injected in the cerebellum. FB was used instead of TB since in the cat FB is more effectively

transported over long distances [38, 218]. Parasagittal rows of micropipette penetrations were made unilaterally into the cerebellum: in the anterior lobe via a transtentorial approach and in the posterior lobe from the superior cerebellar surface. During the same session, 7.5-8.5 μl of 2% DY was injected at T2, ipsilateral to the FB-injections. The cats were sacrificed 18 or 19 days later.

All rats recovered quickly from both operations and showed remarkably little discomfort. The cats, on the other hand, took longer time to recover and exhibited a more pronounced hindlimb paresis. When necessary, they were given morphine and antibiotics. The animals were sacrificed with an overdose of Nembutal and perfused as described in Chapter 3.2. The brain and spinal cord were cut into 30 μm frontal sections on a freezing microtome, whereas the cerebellum was cut sagittally. In two of the rats, the spinal cord was cut horizontally.

Retrogradely labeled neurons were studied in the segments C1 to T1. The TB/FB-labeled, the DY-labeled, and the DY-TB/FB double-labeled neurons were each counted in every 9th (rat) or 6th (cat) section.

Results

DY-labeled propriospinal neurons in the rat

The DY-injections (zones 1 and 2) in most cases completely involved one half of the T2 segment without involving the other half. Two days after DY-injection at T2, DY-labeled neurons were present throughout the spinal cord as well as in the brainstem and the cerebral cortex. Yet, DY migration out of retrogradely labeled neurons was limited [cf. 190], being observed in the caudal part of T1 only.

In all cases, many DY-labeled neurons were found on both sides of the cervical cord following unilateral DY-injections at T2 (Fig. 5.1). Their distribution was similar to that observed in the first set of experiments (Chapter 4). Thus, *ipsilateral* to the DY-injection, the DY neurons were present in all laminae, except lamina IX. They were most numerous in lamina I and laminae IV to VIII. The labeled neurons in lamina VII displayed a slight preferential distribution in the central part of this lamina (in C1 and C2 they tended to be located mainly in its ventral part, cf. Fig. 5.1). *Contralaterally*, the DY neurons were concentrated in lamina VIII and the adjoining ventromedial part of lamina VII, while some were also found in the lateral laminae IV to VI. Throughout C1-T1 DY-labeled neurons were present *bilaterally* in the lateral spinal nucleus, LSN, ventrolateral to the dorsal horn.

In each case, about 6600 (± 1400) DY neurons were counted in C1-T1, cell-counts being made in every ninth section. The mean number of DY neurons per section was roughly the same as in the experiments of Chapter 4 (cf. Table 5.1A, page 56).

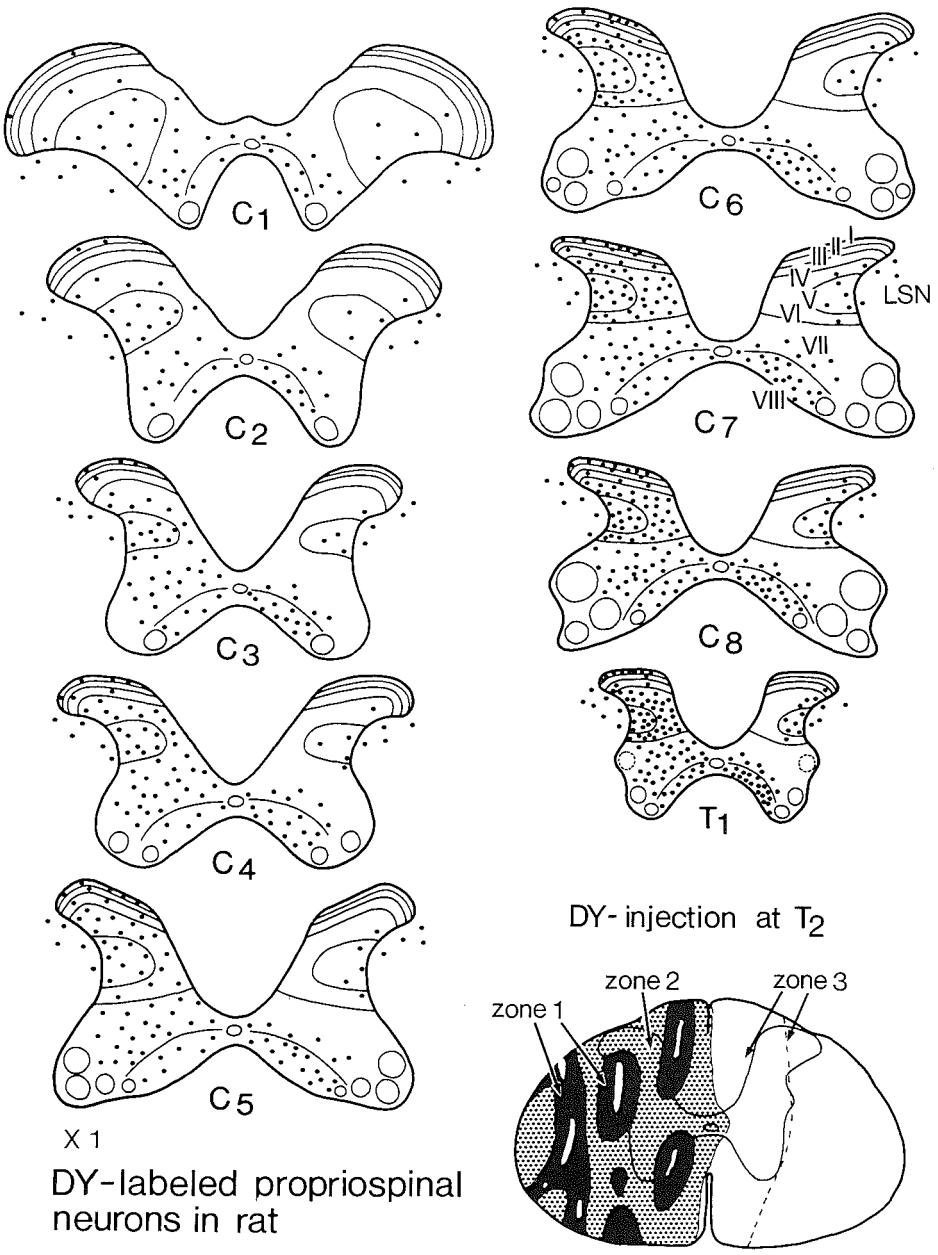


Fig. 5.1 Distribution, in the rat, of the DY-labeled descending propriospinal neurons after DY-injection at T2. Every drawing represents the findings in one section, while each dot represents one labeled cell.

TB-labeled spinocerebellar neurons in the rat

In all rats, the TB-injections were entirely restricted to the cerebellum. The injection areas in all cases were very similar in extent. TB was deposited in the lobules IV to VII and in the basal part of the lobules VIII and IX. The injections to some extent also involved the underlying white matter and central nuclei, and the caudal part of the cerebellar commissure in the medullary ray of the posterior lobe (Fig. 5.2). In all cases, the injections were located mainly in the vermis and adjacent intermediate zone. Although the largest quantity of TB was deposited ipsilateral to the spinal DY-injection, TB deposit was also observed contralaterally.

TB-labeled neurons were found throughout C1-T1 (Fig. 5.2). They were distributed more or less symmetrically on both sides of the cord. In the *segments C1 to C4*, most TB neurons were located within the central cervical nucleus (CCN), lateral to the central canal, being most numerous contralaterally. They were all single-labeled, medium-sized, rounded neurons (cf. Fig. 5.3D). The distribution of these neurons was discontinuous; they were present in groups of 3 to 8 in one transverse section, while none were observed in other sections. In two additional cases, the segments C1 to C4 were cut horizontally. In these cases the labeled CCN neurons were found to be grouped in horizontally aligned clusters (Fig. 5.3E). In the upper cervical segments scattered TB neurons were also found outside of the CCN. Some of them seemed displaced CCN neurons, while others were found throughout lamina VII and VIII, some of them being located very close to the motoneurons in lamina IX. TB-labeled neurons were further present in lamina V on both sides, but mainly ipsilaterally at C1 (Fig. 5.2). In the *segments C5 to T1*, TB neurons were concentrated in two groups; one in the central part of lamina VII and the other in the medial part of lamina VI and the adjoining dorsomedial part of lamina VII. The latter cell group was present also in the upper cervical segments, i.e. dorsolateral to the CCN (cf. Fig. 5.2). TB-labeled neurons were also found in lamina VIII, predominantly contralaterally, in the lateral part of lamina V, particularly in C8 and T1, and in Clarke's column at T1. Throughout *C1 to T1*, a few TB neurons were found ventrally in lamina IV. However, none were observed in laminae I to III, in the lateral cervical nucleus (LCN) and in the LSN.

The TB-labeled spinocerebellar neurons were of all types and sizes. Those located centrally in lamina VII and medially in lamina VI were in general fairly large cells, often conspicuously larger than the neighbouring DY-labeled propriospinal neurons (cf. Fig. 5.3B).

The TB-labeled spinocerebellar neurons were much fewer in number than the DY-labeled propriospinal neurons, i.e. about 3 per section. The highest numbers of TB neurons were found at C1 (cf. Table 5.1B on page 56). In each case, about 260 ($\pm$ 80) TB neurons were counted in C1-T1, cell-counts being made in every ninth section.

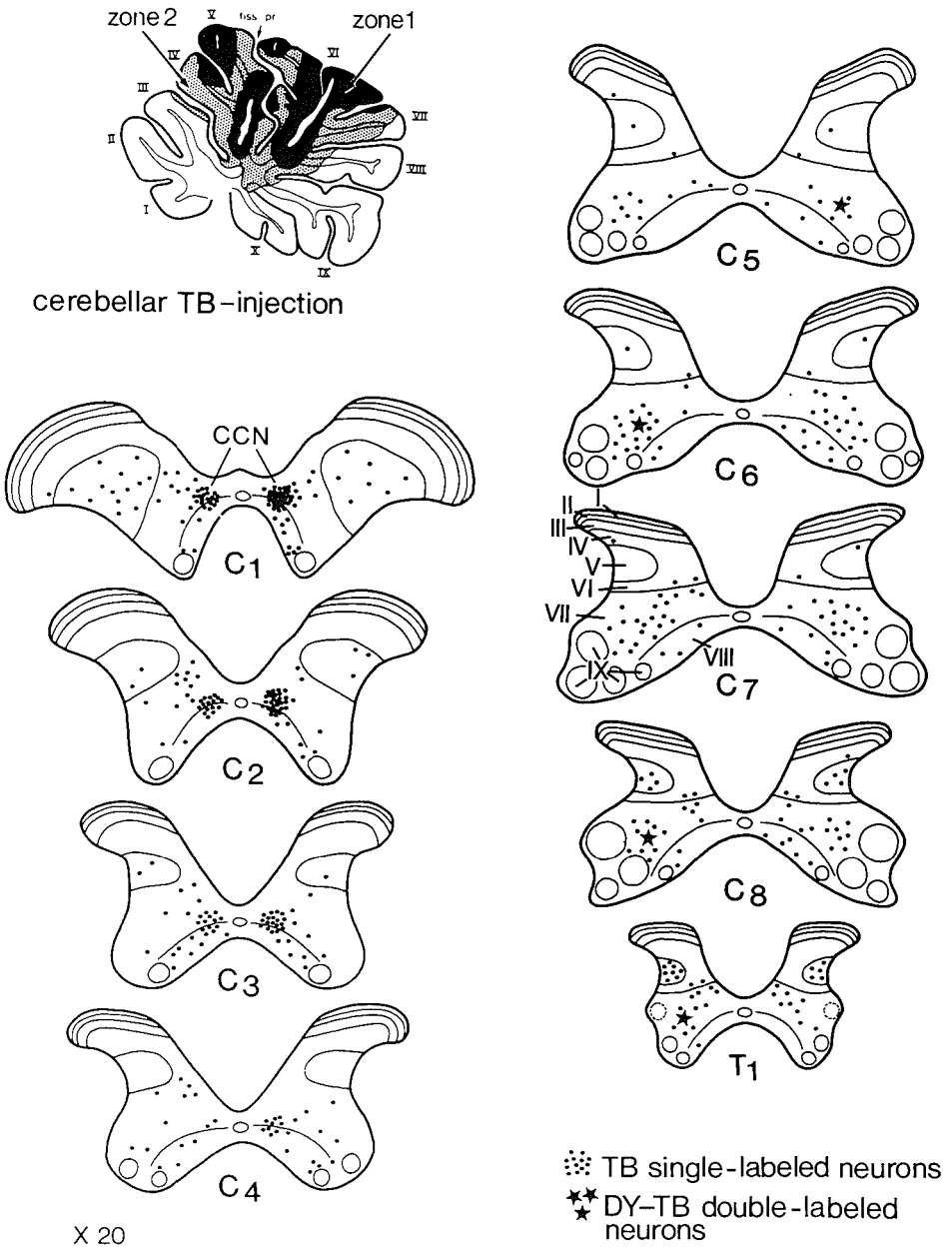


Fig. 5.2 Distributions, in the rat, of the TB-labeled spinocerebellar neurons and of the DY-TB double-labeled neurons after TB-injection in the cerebellum and DY-injection at T2. *Left sides are ipsilateral to the injections*. Note that every drawing represents the superimposed chartings of 20 sections; each dot represents one labeled cell. The extent of the cerebellar injection is indicated in a parasagittal section passing through the vermis.

Table 5.1 Numbers of labeled neurons per section in different segments in the rat and the cat. In the rat, the values represent mean numbers (and SD) for all 8 cases. In the cat, the numbers in cases A, B and C are shown separately. Note that in cat B fewer neurons were labeled than in cats A and C.
DY+DL, TB+DL/FB+DL : numbers of DY-labeled and TB/FB-labeled neurons, respectively.

		C1-T1	C1	C3	C5	C8
1A. rat	DY+DL	81 (23)	47 (26)	59 (27)	84 (28)	122 (25)
mean (SD)	TB+DL	2,9 (1,3)	5,6 (2,2)	3,6 (2,1)	1,4 (0,6)	2,5 (1,0)
1B. cat	DY+DL	53 25 48	28 9 18	36 10 23	73 28 54	125 61 93
A B C	FB+DL	5,0 2,0 3,9	10,9 4,5 9,0	6,0 2,3 4,9	2,3 0,5 2,4	3,6 1,2 3,4

DY-TB double-labeled neurons in the rat

The majority of the TB-labeled spinocerebellar neurons were intermingled with DY-labeled propriospinal neurons, except in the CCN and Clarke's column, which contained only spinocerebellar neurons. Yet, only very few DY-TB double-labeled neurons were observed. Such neurons were all located in the central part of lamina VII at C5-T1, mainly ipsilateral to the tracer injections (cf. Fig. 5.2). The double-labeled neurons were of the same size as, or somewhat smaller than, the TB single-labeled ones in this lamina. The DY-TB double-labeled neurons constituted only about 0.5% ($\pm$ 0.8%) of all the TB-labeled spinocerebellar neurons in C1-T1. Of the subset of TB neurons in the central lamina VII in C5-T1, about 1.5% ($\pm$ 2.2%) were double-labeled; 2.5% ($\pm$ 3.9%) ipsilaterally and 0.6% ($\pm$ 1.8%) contralaterally. Conversely, only extremely few, i.e. about 0.03% ($\pm$ 0.05%), of the DY-labeled descending propriospinal neurons in C1-T1 were also TB-labeled from the cerebellum.

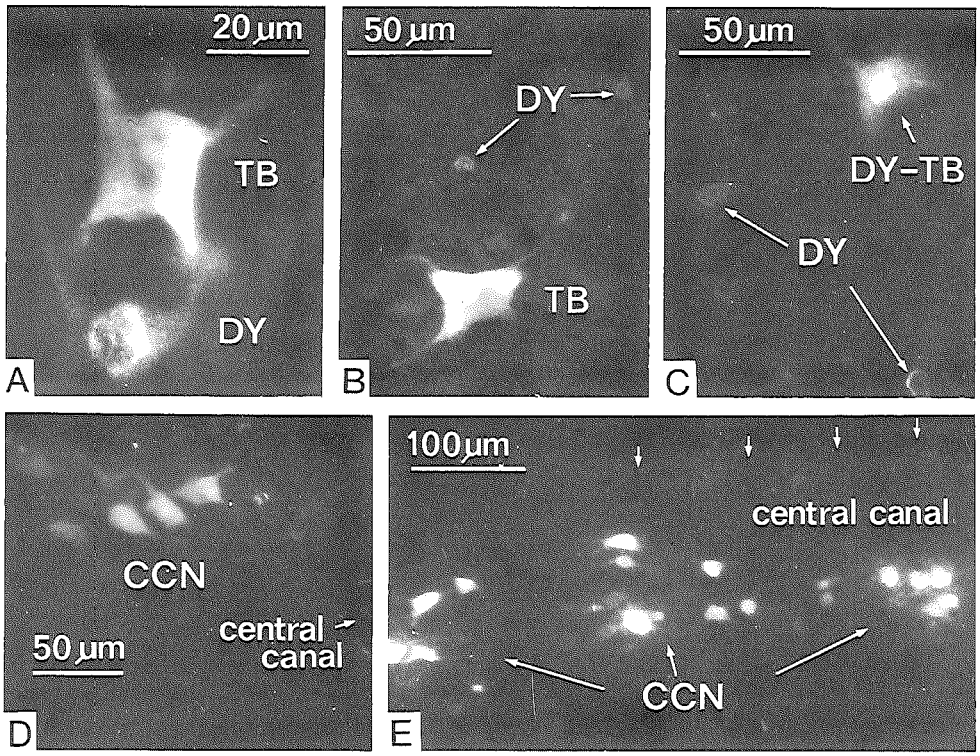


Fig. 5.3 Photomicrographs of retrogradely labeled neurons in the rat after TB-injection in the cerebellum and DY-injection at T2.

A. TB single-labeled neuron and DY single-labeled neuron in ipsilateral lamina VII at C8. Note predominantly cytoplasmic labeling with TB and predominantly nuclear labeling with DY

B. TB single-labeled neuron and DY single-labeled neurons in ipsilateral lamina VII at C6. Note that, although the cytoplasm of the DY neurons is not visible, the TB spinocerebellar neuron is clearly larger than the DY propriospinal neurons

C. DY-TB double-labeled neuron and DY single- labeled neurons in ipsilateral lamina VII at C7

D. Group of TB single-labeled neurons in contralateral CCN at C1, transverse section

E. Horizontal section passing through the CCN at C2. Note that the TB single-labeled neurons in the CCN are clustered in rostrocaudally aligned groups

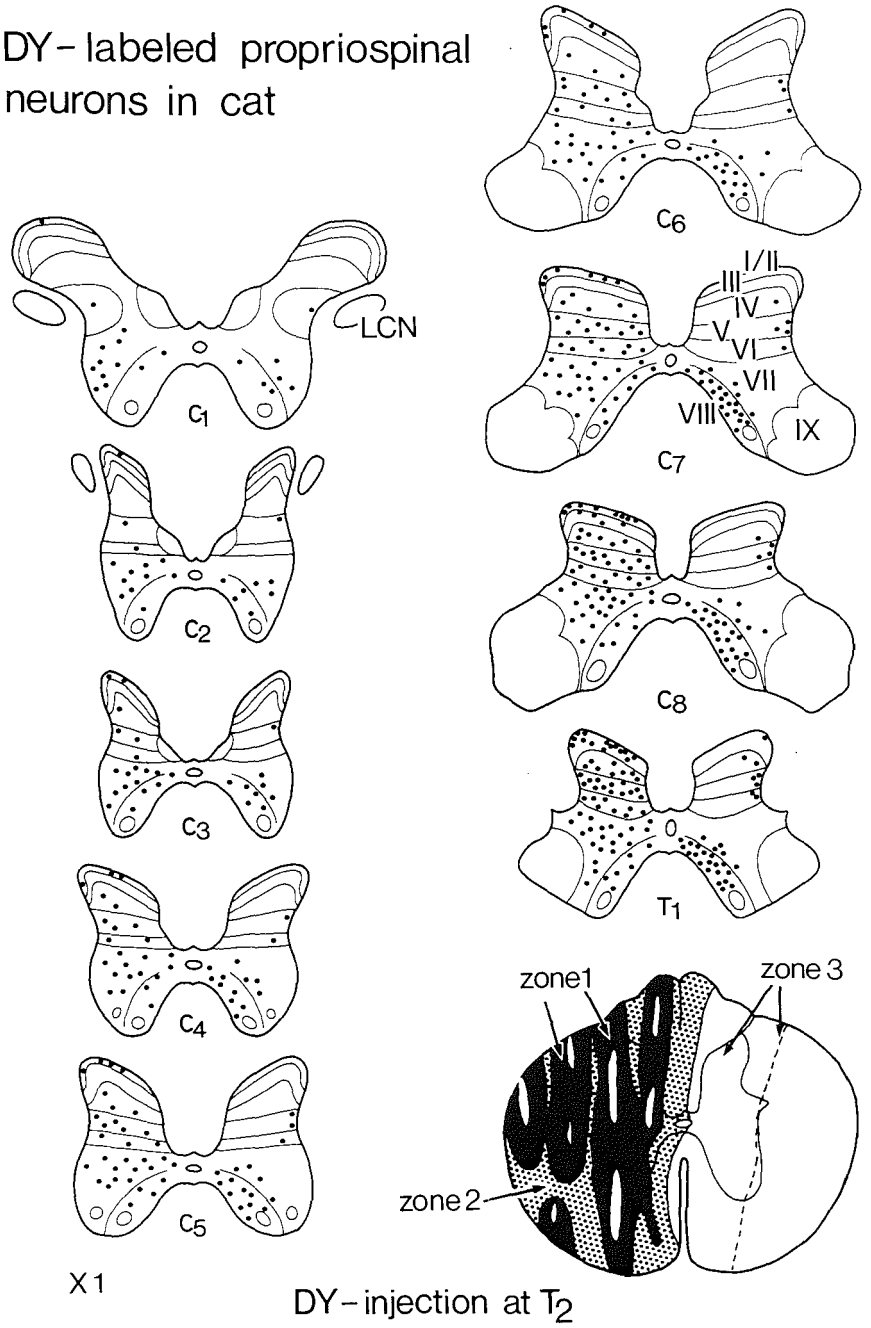


Fig. 5.4 Distribution, in the cat, of the DY-labeled descending propriospinal neurons after DY-injection at T2. Every drawing represents the findings in one section; each dot represents one labeled cell.

DY-labeled descending propriospinal neurons in the cat

After DY-injection at T2 followed by 19 days survival, DY-labeled neurons were observed in the cervical cord as well as in the brainstem. Migration of DY out of retrogradely labeled neurons was only observed in the caudal part of T1.

In C1-T1, DY neurons were present on both sides (Fig. 5.4). Their distribution was very similar to that in the rat (cf. Figs 5.1 and 5.4). *Ipsilaterally*, DY neurons were mainly present in lamina I and laminae IV to VIII. *Contralaterally*, they were concentrated in lamina VIII but were also present in the adjoining part of lamina VII and in the extreme lateral parts of laminae V and VI. In contrast to the rat, no labeled neurons were observed in the dorsolateral funiculus. In the lateral cervical nucleus, LCN, a total of only 8 DY neurons were found in the 3 cases.

Per case, about 7000 to 20000 DY neurons were counted in C1-T1, cell-counts being made in every sixth section[6]. The average number of DY neurons per section in the cat was smaller than in the rat. As in the rat, this number increased when proceeding from C1 to T1 (cf. Table 5.1B on page 56).

FB-labeled spinocerebellar neurons in the cat

The extent of the cerebellar FB-injections was somewhat different in the three cases, as was the degree of brainstem involvement. In all cases the injections involved large parts of the cerebellar anterior lobe and the underlying white matter containing the cerebellar commissure, whereas the posterior lobe was involved to variable extents (cf. Fig. 5.5). Further, in only one case (case B) the FB deposit seemed to be

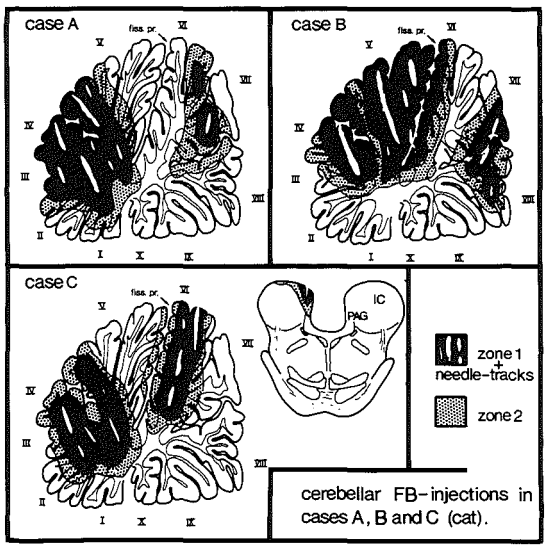


Fig. 5.5 Extent of the cerebellar FB-injections in cats A, B, and C; parasagittal section passing through the vermis. Note that in cat C the tip of the inferior colliculus was involved by the injection.

Footnote 6. In cat B the numbers of both the DY and the FB neurons were about half of those in cats A and C (Table 5.1B, page 56), which might be related to the poorer postoperative recovery in this animal.

restricted to one side of the cerebellum.

In case A, the brain stem was free from tracer deposit, while in case B some FB staining was present on the outer surface of the inferior colliculus and in the cells lining the aqueduct. In case C one of the needle-tracks extended through the inferior colliculus into the periaqueductal grey (cf. Fig. 5.5).

In all 3 cases retrogradely FB-labeled neurons were found throughout C1-T1. Their distributions were largely the same in all cases and were very similar to that observed in the rat (Fig. 5.6). Thus, many FB neurons were present in clusters in the CCN on both sides, with some neurons scattered outside this nucleus. FB neurons were further observed in the lateral lamina V, mainly at C1 and C7-T1, in the medial lamina VI and dorsomedial lamina VII at C3-T1, and in Clarke's column. A prominent group of FB neurons was present in the central and ventral lamina VII at C5-T1, being most numerous at C7-T1.

Throughout C1-T1, FB neurons were present in lamina I on both sides. They were most numerous in case C, in which case the inferior colliculus and PAG were involved by the FB-injections. In this case a few labeled neurons were also present in the superior olive, in the contralateral dorsal column nuclei, and in the contralateral LCN. In the cases A and B, labeled lamina I neurons were observed likewise, although no labeled neurons were found in the superior olive, dorsal column nuclei and LCN.

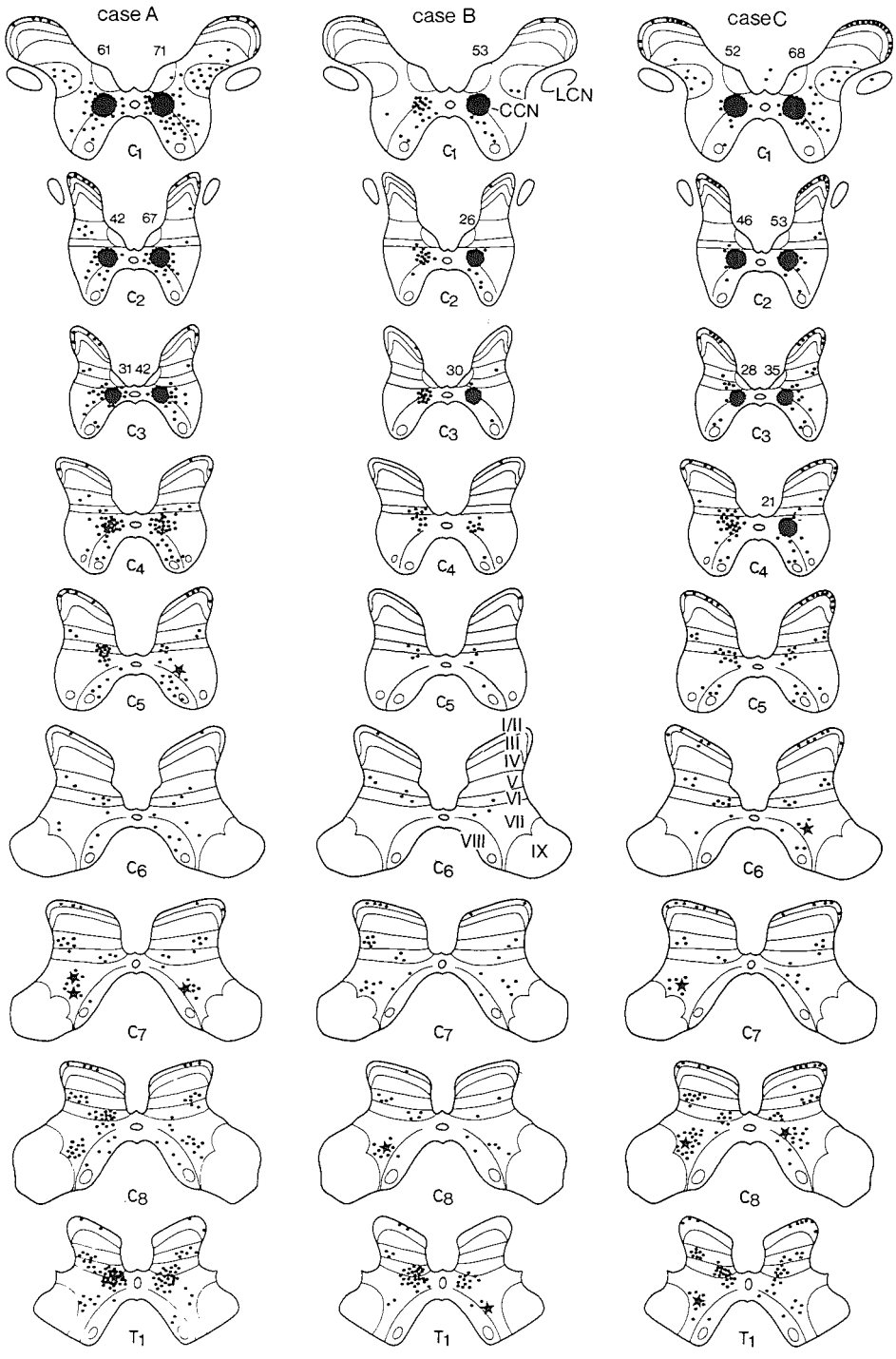
The FB neurons were distributed very similarly on both sides of the cord. However, the neurons in the CCN and in lamina VIII were slightly more numerous contralateral to the bulk of the cerebellar injections, whereas the other neurons were more numerous ipsilaterally. This differential distribution was especially noticeable in case B (with largely unilateral cerebellar tracer deposit).

As in the rat, the numbers of the labeled spinocerebellar neurons were markedly smaller than those of the labeled propriospinal neurons. In each case, from 600 to 1700 FB neurons were counted in C1-T1, cell-counts being made in every sixth section. The highest numbers were again found at C1 (cf. Table 5.1B on page 56).

DY-FB double-labeled neurons in the cat

In all three cases only few DY-FB double-labeled neurons were observed, which were all located in the central part of lamina VII at C5-T1. On an average only 0.6% ($\pm$ 1.0%) of all FB neurons in C1-T1 were double-labeled. Of the spinocerebellar FB neurons in the central lamina VII at C5-T1 about 4% ($\pm$1%) were double-labeled,

Fig. 5.6 (facing page) Distributions, in the cat, of the FB-labeled spinocerebellar neurons and of the DY-FB double-labeled neurons after FB-injections in the cerebellum and DY-injections at T2. *Left sides are ipsilateral to the injected sides.* Note that every drawing represents the superimposed chartings of 20 sections; each dot represents one labeled cell. In the drawings with very dense labeling in the CCN (blackened areas), the number of labeled neurons in the CCN is indicated.


case A
61
71
C1
42
67
C2
31 42
C3
C4
C5
C6
C7
C8
T1
case B
53
CCN
LCN
C1
26
C2
30
C3
C4
C5
I/II
III
IV
V
VI
VII
VIII
IX
C6
C7
C8
T1
caseC
52
68
C1
46
53
C2
28
35
C3
21
C4
C5
C6
C7
C8
T1

X 20

i.e. gave rise to a descending propriospinal collateral. This percentage was about the same on both sides of the cord. Conversely, only very few, i.e. about 0.05% (± 0.01%), of the DY-labeled propriospinal neurons in C1-T1 were also FB-labeled from the cerebellum.

Labeling of neurons in the lower brainstem

Both in the rat and the cat, extensive TB- or FB-labeling of neurons was observed in major cerebellar relay nuclei such as the pontine nuclei, inferior olive, lateral reticular nucleus and external cuneate nucleus. Fewer FB- or TB-labeled neurons were present in the reticular formation which, however, contained a fairly large number of DY-labeled reticulospinal neurons. No double-labeled neurons were observed in the reticular formation. In some cases double-labeled neurons were found in the locus coeruleus, ipsilateral to the injections, but such neurons were few in number.

Discussion

The distribution of cervical neurons with propriospinal fibers to T2 or below and of cervical spinocerebellar neurons follows the same pattern in the rat and the cat. Moreover, the localization of a small number of neurons with branching axons to the spinal cord and the cerebellum in the central lamina VII is the same in both species.

Distribution of descending propriospinal neurons

Cervical neurons projecting to T2 or below (descending propriospinal neurons) are present in all laminae, except lamina IX. In both species, they are concentrated in lamina I and in laminae IV to VIII. Most of these neurons project ipsilaterally. However, the sets of neurons in the lateral parts of laminae IV to VI, in lamina VIII and in the ventromedial part of lamina VII distribute their fibers bilaterally, those in lamina VIII even preferentially contralaterally.

One marked species difference was apparent. In the rat, a group of neurons is present in the white matter next to the dorsal horn at all spinal levels [136; cf. Chapter 4]. This cell group, the lateral spinal nucleus, LSN, is absent in the cat [137]. The LSN is distinct from the lateral cervical nucleus, LCN, which is present at C1-C3 both in the rat and the cat [128]. The cervical part of the LSN in the rat distributes many descending propriospinal fibers to both sides of the cord (present experiments; cf. Chapter 4). The LCN, on the other hand, contains only few descending propriospinal neurons, projecting mainly to the cervical enlargement [112, 376], in keeping with the present findings [cf. also 288].

The present distributions of descending propriospinal neurons in the rat and the cat are similar to those observed previously [Chapter 4; ref. 288], but the numbers of labeled neurons in the cat were higher than in the earlier HRP study.

Distribution of spinocerebellar neurons

In the upper cervical segments spinocerebellar neurons were found to be concentrated in the central cervical nucleus, CCN, which consists of rostrocaudally aligned cell clusters [93, 252]. The presence of labeled spinocerebellar neurons in the CCN (at C1-C4), lamina VIII (at C1-T1), the central part of lamina VII (at C5-T1 in the rat; at C7-T1 in the cat), the medial part of lamina VI and dorsomedial part of lamina VII (C3-T1), Clarke's column (T1), and the lateral part of lamina V (mainly at C1 and C7-T1) is in keeping with several HRP studies in the rat [253, 364] and the cat [255, 402, 403, 405]. Labeling of motoneurons, as described in the brainstem and the cervical cord [206, 403], was not found in the present study [cf. 413]. No spinocerebellar neurons were observed in the LCN, the LSN, and laminae II and III in both species. In the cat, but not in the rat, rather many neurons were labeled in lamina I [cf 364]. However, it is uncertain whether any of these were actually labeled from the cerebellum, as in two of the three cats the brainstem was observed to be stained by the tracer [cf. also 132, 364, 405].

It is of interest to note that spinocerebellar neurons are only small in numbers in the cervical cord. In the previous set of experiments in the rat (Chapter 4), about 140 neurons per section were found to project from C3-C8 to supraspinal levels, whereas in the present experiments about 2.4 spinocerebellar neurons per section were found in C3-C8. Spinocerebellar neurons therefore constitute only a small fraction of all supraspinally projecting cervical neurons.

Cerebellar injection sites

The cerebellar injections in both species were not restricted to one side of the cerebellum; the labeled spinocerebellar neurons therefore were distributed more or less symmetrically on both sides of the cord. However, the lamina VIII neurons and the CCN neurons were somewhat more numerous contralateral to the bulk of the cerebellar injection, while those in the central lamina VII, the medial lamina VI, the lateral lamina V and Clarke's column were slightly more numerous ipsilaterally [cf. 93, 148, 253, 255, 321, 403, 404, 405, 406].

The cerebellar injections involved large parts of the known termination areas of the spinocerebellar projections in the rat [18] and the cat [130], but did not cover them completely. Thus in the rat the fibers to lobules I and II were spared and in the 3 cats the posterior lobe was involved to variable extents. Anterograde and retrograde tracing studies (most of which were done in cats) have shown that spinocerebellar neurons in the cervical enlargement project most heavily to lobules

III to V, but less prominently to the posterior lobe and only weakly to lobules I and II [256, 257, 258, 260, 261, 332, 405, 406]. The CCN, on the other hand, distributes most of its fibers to lobules I and II, and fewer to lobules III-V and the posterior lobe [148, 257, 259, 402, 404]. Thus the limited extent of the cerebellar injections in the rats, sparing fibers to lobules I and II, probably reduced the quantity of labeled neurons in the CCN, but not in other areas[7].

The varying extent of the cerebellar injections probably had no influence on the labeling of central lamina VII neurons in the cervical enlargement, the only cell group to contain double-labeled neurons in the present study; this group seems to project only to lobules III-V [256, 257, 258], which lobules were involved by the injections in all cases.

Branching spinocerebellar-propriospinal neurons

With the exception of the CCN and Clarke's column, TB- or FB-labeled spinocerebellar neurons in the cervical cord were intermingled with the DY-labeled propriospinal neurons. Yet, only very few double-labeled neurons, representing branching neurons with both an ascending spinocerebellar collateral and a descending propriospinal collateral, were observed. Such neurons were located in the central lamina VII cell group in the cervical enlargement, in keeping with electrophysiological findings in the cat [146]. However, the proportion of the (ipsilateral) central lamina VII neurons which were found to be branching shows large differences in both studies: 43% at C7-T1 [146] versus 2.5% (rat) and 4% (cat) at C5-T1[8] in the present study. This remains difficult to explain, especially since it is unlikely that the populations of spinocerebellar and propriospinal neurons were labeled incompletely; they were labeled in large numbers as compared to other studies [253, 255, 288]. On the other hand, it is also unlikely that the branching spinocerebellar neurons were selectively recorded from by Hirai et al.; they are of the same size as the non-branching ones, as judged from their axonal conduction velocities [145, 146] and as observed in the present study. However, the studies of Hirai et al. and the present experiments are in good agreement in regard to the location of the branching neurons. In C7-T1, they are situated only in the central part of lamina VII [cf. 146] and none are present in the CCN [cf. 148]. In this respect it may be of significance that the central lamina VII cell group, containing branching

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Footnote 7. In 6 rats (not included in this study) the TB-injections in the cerebellum were quite extensive, completely involving lobules I and II. In those cases, about twice as many CCN neurons were labeled as compared to the present cases. All labeled CCN neurons were TB single-labeled. Regrettably the tracer injections in those 6 cases also involved the inferior colliculus to some extent, with resultant labeling of non-spinocerebellar neurons (e.g. in the LSN).

Footnote 8. The percentage in Hirai's study pertains to the segments C7-T1. The present percentages apply to C5-T1, but similar percentages were obtained for C7-T1 (especially in the cat, the majority of the FB-labeled lamina VII neurons being located at C7-T1).

neurons, appears to be functionally different from the neighbouring dorsomedial lamina VII cell group and from the CCN. The neurons in the latter cell groups are mono- or polysynaptically excited from primary afferents, whereas those in the central lamina VII receive predominant inhibition from flexor reflex afferents [145, 147, 149].

Recently, a brief report was published on branching propriospinal-spinocerebellar neurons in the cervical enlargement of the rat [361]. In 4 rats, TB was injected in the anterior part of the cerebellum while DY was injected in the lumbosacral enlargement. DMSO was added to both tracer solutions. The authors observed that about 45% of all labeled descending propriospinal neurons and about 37% of all labeled spinocerebellar neurons were double-labeled, in striking contrast to the present findings. Their short description does not allow for a comparison between their injection-sites and those of our study. Double-labeled neurons were heavily concentrated in lamina VIII and the dorsomedial part of lamina VII, but were virtually absent from the central part of lamina VII. Their distribution of "branching" versus "non-branching" spinocerebellar neurons, therefore, is the opposite of that observed both in the present study and in Hirai's electrophysiological study [cf. 145, 146]. In fact, their double-labeled neurons were located in the areas containing the heaviest concentrations of DY cells. This suggests that leakage of DY from heavily labeled propriospinal neurons to surrounding cells might have occurred, although survival times after DY-injections were not particularly long (30-36 hours). Glial labeling was not reported by the authors. Yet, the photomicrograph of their fig. 2 shows double-labeled neurons to be surrounded by numerous DY-labeled glial nuclei, which is a strong indication for the occurrence of false double-labeling. As a whole, these data should be considered with some reservation.

In the present experiments only about 0.5% (rat) and 0.6% (cat) of all the labeled spinocerebellar neurons in C1-T1 were found to give rise to a descending propriospinal collateral to T2 or below. Conversely, only about 0.03% (rat) and 0.05% (cat) of the DY-labeled propriospinal neurons gave rise to an ascending spinocerebellar collateral. Thus propriospinal and spinocerebellar neurons appear to be two largely separate populations in the cervical cord. Similarly, the reticulospinal and reticulocerebellar populations in the lower brainstem appear to be separate [present study; cf. 245, 397].

In the previous set of experiments (Chapter 4) many descending propriospinal neurons were found to distribute collaterals to supraspinal levels. The present experiments show that only extremely few of these ascending supraspinal collaterals project to the cerebellum.

CHAPTER 6.
PROPRIOSPINAL NEURONS IN THE RAT'S CERVICAL CORD WITH ASCENDING COLLATERALS TO THE THALAMUS OR THE DORSAL MIDBRAIN

Introduction

In the experiments of Chapter 4, the presence of "branching" neurons with descending propriospinal collaterals and ascending collaterals to supraspinal levels was demonstrated in the cervical spinal cord of the rat. The present study was designed to investigate whether any of the ascending supraspinal collaterals are distributed to the thalamus or dorsal midbrain. To that purpose, Diamidino Yellow was injected in the spinal cord at T2, whereas True Blue was injected in the thalamus or dorsal midbrain, either ipsilateral or contralateral to the spinal injections.

The findings indicate that some neurons in the rat's cervical cord give rise to both descending propriospinal collaterals and ascending collaterals to the thalamus or dorsal midbrain; such neurons are mainly present in the lateral spinal nucleus, LSN. Yet, for the largest part the ascending spinothalamic and spinotectal neurons are separate from the descending propriospinal neurons.

Material and methods

In 25 rats, 1.1 μl of 2% Diamidino Yellow Dihydrochloride (DY), suspended in a 0.2M phosphate buffer (pH 7.2), was injected unilaterally into the grey and white matter of T2 by making a mediolateral row of micropipette penetrations. At thalamic or midbrain levels, 2% True Blue (TB), dissolved in H_2O, was injected unilaterally, either ipsilateral or contralateral to the DY-injections. The various cases were divided into 6 groups, according to the location of the TB-injections:

Groups A and B (10 rats)

About 3.0 μl of 2% TB was injected into the thalamus (stereotaxic coordinates of Pellegrino et al. [315]). In group A (6 cases), the TB-injections were made ipsilateral to the DY-injections, whereas in group B (4 cases) TB was injected contralaterally.

Groups C and D (7 rats)

After removing the occipital cortex, 0.4-0.5 μl of 2% TB was injected in the inferior colliculus either ipsilateral (group C; 3 rats) or contralateral (group D; 4 rats) to the DY-injections.

Groups E and F (8 rats)

After removing the overlying cerebral cortex, 0.4-0.5 μl of 2% TB was injected in the superior colliculus, ipsilateral (group E; 4 rats) or contralateral (group F; 4 rats) to the DY-injections.

Survival after TB-injections ranged from 5 (inferior colliculus) to 9 (thalamus) days, whereas the survival time after DY-injections was 2 days. In all cases, DY- and TB-injections were therefore made in separate sessions.

All animals recovered quickly from both operations and showed no apparent discomfort, behaving normally in spite of a paresis of the hindlimb. The animals were sacrificed and perfused as described in Chapter 3.2. The sections containing the thalamic injections were counterstained with cresylviolet after the fluorescent injection areas had been charted. Thalamic nuclei were identified using the criteria of Faull and Carman [102] and Bold et al. [49]. Retrogradely labeled neurons were studied in the segments C1-C8. The TB, DY and DY-TB neurons were each counted in every ninth section.

Results

Distribution of the DY-labeled neurons

The DY-injections at T2 in most cases involved the entire grey and white matter on one side without involvement of the other side. After two days survival, DY-labeled neurons were found in the cervical cord, the brainstem and the cerebral cortex. Nevertheless, migration of DY out of retrogradely labeled neurons was limited, being observed in the caudal part of T1 only.

Throughout C1 to C8, DY-labeled neurons were present on both sides of the cord (Fig. 6.1). They were distributed as in the previous experiments, i.e.: *ipsilateral* to the DY-injections, they were found in all laminae, except lamina IX, being most numerous in lamina I, laminae IV to VII and the LSN. *Contralaterally*, most DY neurons were found in lamina VIII and the adjoining ventromedial part of lamina VII, the lateral parts of laminae IV to VI, and the LSN.

On an average, a total of 9450 $\pm$3300 (mean $\pm$ SD in the 25 cases) were counted per case in C1-C8, cell-counts being made in every ninth section. The mean number of DY neurons per section was 111 ($\pm$ 34) in the segments C1-C8 (Table 6.1 on page 75).

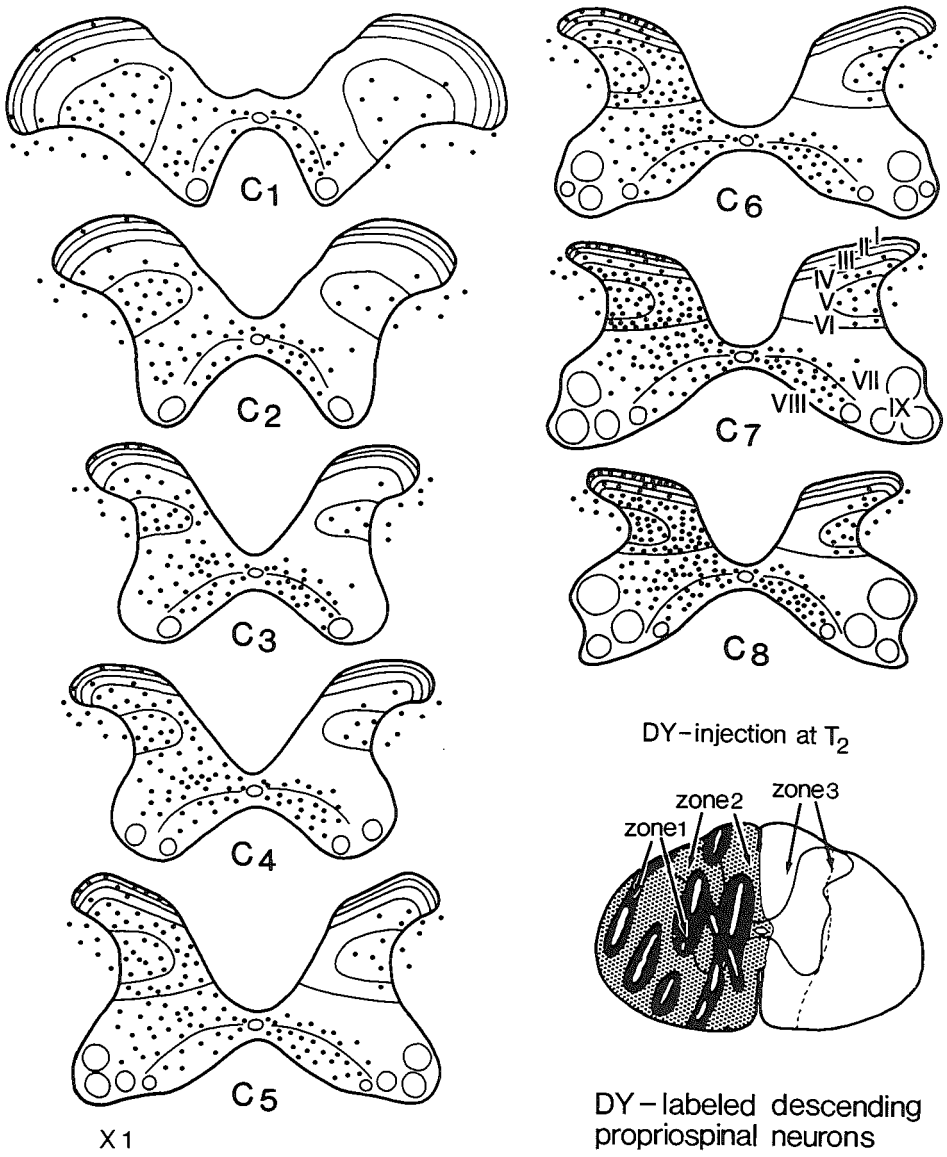

Fig. 6.1 Distribution of the retrogradely DY-labeled neurons after DY-injection at T2. Each drawing represents the findings in one section. One dot represents one labeled neuron.

Distributions of the TB-labeled and DY-TB double-labeled neurons

Thalamic and spinal injections on the same side (group A)

The thalamic TB-injections in all 6 cases of this group were very similar, covering the mediolateral and anteroposterior extent of the thalamic nuclei (Fig. 6.2).

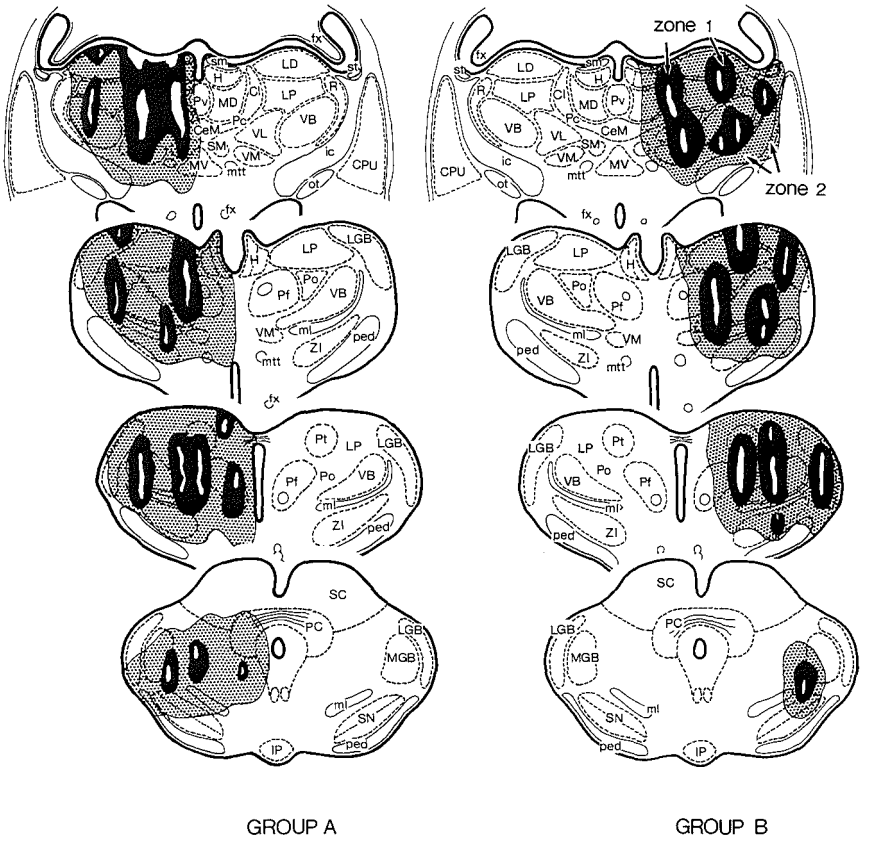

Fig. 6.2 TB-injections in the thalamus. The drawings depict the maximal extent of the tracer deposit in the 6 cases of group A and the 4 cases of group B.

Abbreviations: CeM: central medial nucleus; Cl: central lateral nucleus; CPU: caudate-putamen; Fx: fornix; H: habenular nuclei; ic: internal capsule; IP: interpeduncular nucleus; LD: lateral dorsal nucleus; LGB: lateral geniculate body; ml: medial lemniscus; mtt: mamillothalamic tract; MV: medioventral nucleus; ot: optic tract; PC: posterior commissure; PC: paracentral nucleus; ped: cerebral peduncle; Pf: parafascicular nucleus; Po: posterior thalamic nucleus; Pt: pretectal nucleus; Pv: paraventricular nucleus; R: reticular thalamic nucleus; SC: superior colliculus; SM: submedius nucleus; sm: stria medullaris; SN: substantia nigra; st: stria terminalis; VB: ventrobasal complex; VL: ventrolateral nucleus; VM: ventromedial nucleus; ZI: zona incerta

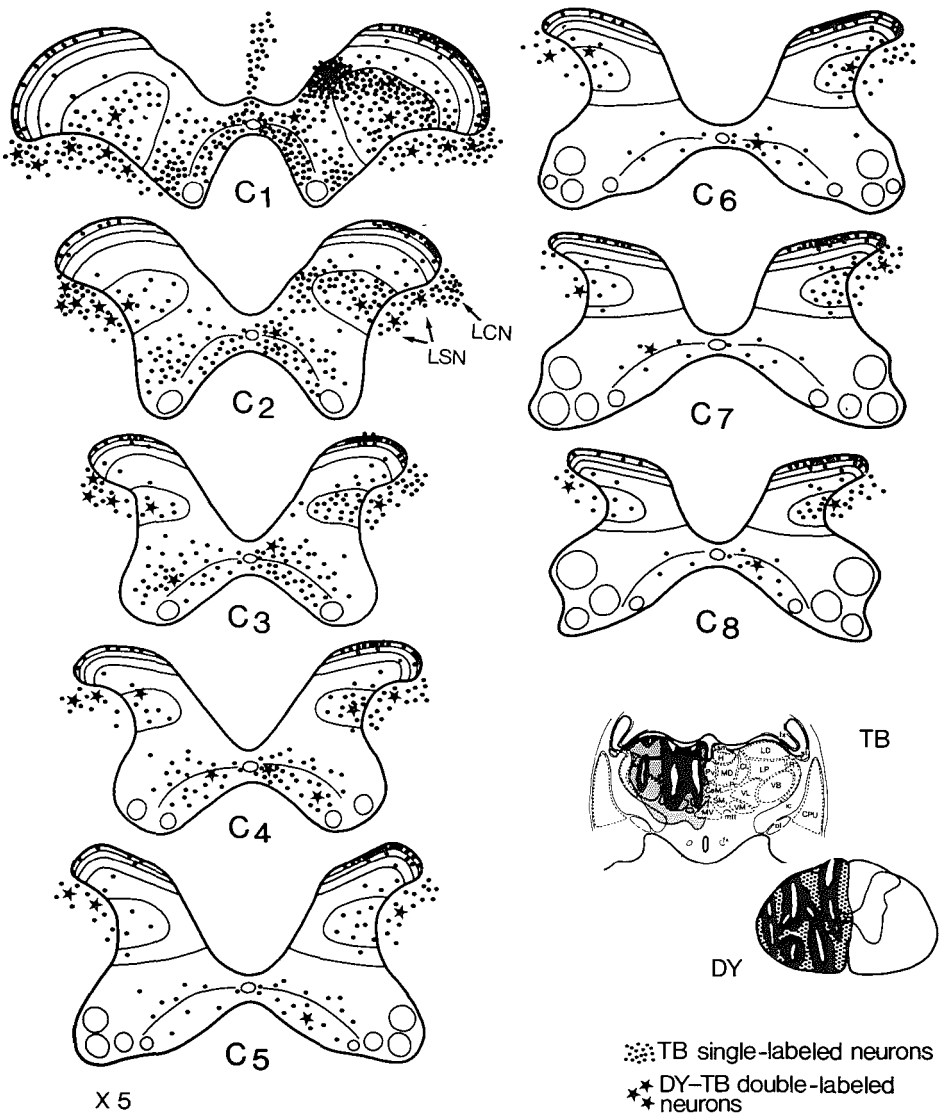

Fig. 6.3 Distributions of the retrogradely TB-labeled neurons and the DY-TB double-labeled neurons after unilateral TB-injections in the thalamus and ipsilateral DY-injections at T2 (group A). Each drawing represents the superimposed chartings of 5 sections; each dot represents one labeled neuron.

In all cases, TB neurons were present throughout the *segments C1 to C8*. They were most numerous in the upper cervical segments (cf. Fig. 6.3). Many were present in lamina I, while some were also found in the subjacent laminae II and III. In laminae IV to VI the TB neurons were located laterally, being most numerous in the lateral reticulated part of lamina V (at upper cervical levels these neurons were

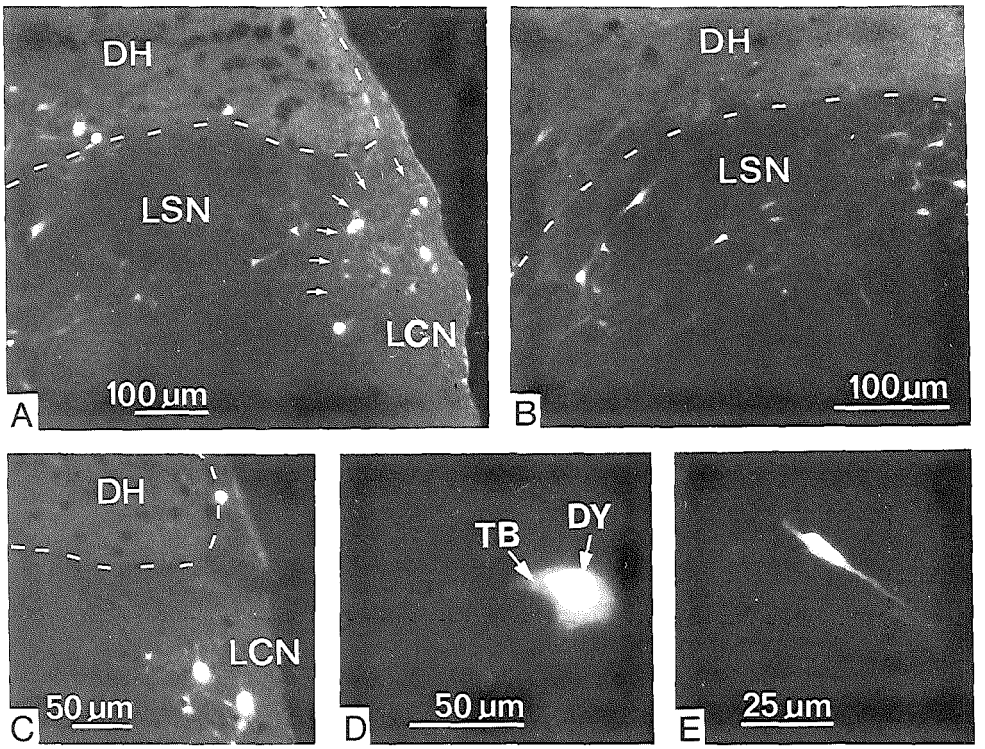

Fig. 6.4 Photomicrographs of neurons, labeled after unilateral DY-injections at T2 and ipsilateral TB-injections in the superior colliculus.

A. Dorsolateral funiculus at C2, contralateral to the injections. The outline of the lateral cervical nucleus, LCN, is indicated by arrows. Note that the neurons in the LCN are round and clustered together close to the pial surface, while the lateral spinal nucleus, LSN, contains elongated neurons which are loosely dispersed in the white matter.

B. Labeled neurons in the LSN in the dorsolateral funiculus at C3, ipsilateral to the injections.

C. TB-labeled neurons in the LCN at C2, contralateral to the injections.

D. DY-TB double-labeled neuron in the ipsilateral lamina V at C4.

E. TB-labeled neuron in the contralateral lamina I at C1.

concentrated in the dorsal part of lamina V). Many were present in lamina VIII, in the ventromedial part of lamina VII, in lamina X surrounding the central canal, and in the LSN. The TB-labeled neurons were distributed on both sides of the cervical cord, but with a clear contralateral predominance. *In the upper cervical segments* additional groups of TB neurons were observed. Thus, TB-labeled neurons were present not only in the ventromedial part of lamina VII, but also in its dorsal and lateral part. Contralateral to the thalamic injections, TB neurons were present in the dorsal funiculus, constituting the caudal continuation of the gracile nucleus, and in the medial parts of laminae V and VI, constituting the caudal continuation of the

cuneate nucleus. The latter groups of neurons will be referred to as the spinal extensions of the dorsal column nuclei (sDCN). TB neurons were also observed contralaterally in the lateral cervical nucleus, LCN. The LCN, most distinctly present at C2, is located in the dorsolateral funiculus at the tip of the dorsal horn, close to the pial surface. Its neurons are round cells, which were easily distinguished from those in the medially adjoining LSN, which are elongated, multipolar neurons, their cell bodies and dendrites being oriented parallel to the lateral border of the dorsal horn (cf. Fig. 6.4 A-C).

On an average, 4000 (± 1000) TB-labeled neurons were counted per case in C1-C8. About 40 (± 4) TB neurons per section were found in C1-C8; 13 (± 2) ipsilaterally and 27 (± 3) contralaterally. Their numbers decreased dramatically when proceeding from C1 to C8 (cf. Table 6.1 on page 75).

In all cases, some DY-TB double-labeled neurons were observed. These neurons must be branching neurons giving rise to both an ascending spinothalamic collateral and a descending propriospinal collateral to T2 or below, the thalamic termination area being ipsilateral to the spinal termination area. Such neurons were most markedly present in the LSN, but were also located in the lateral lamina V, in laminae VII and VIII, and in lamina X on both sides. Only very few double-labeled neurons were found in the ipsilateral lamina I (i.e. 2 neurons in all 6 cases), while none were found in the LCN and the sDCN.

About 3.3% (± 1.5%) of all TB neurons in C1-C8 were double-labeled (Table 6.2, page 78), i.e. gave rise to a descending propriospinal collateral. This percentage was about the same in all segments (Table 6.1, page 75). Conversely, about 1.1% (± 0.3%) of the DY-labeled descending propriospinal neurons were double-labeled, i.e. gave rise to an ascending spinothalamic collateral (Table 6.2, page 78). This percentage differed between segments, decreasing from C1 to C8 (Table 6.1, page 75). The highest percentages of double-labeling were obtained in the ipsilateral LSN (cf. Table 6.2, page 78).

Thalamic and spinal injections on opposite sides (group B)

In the 4 cases of this group, the thalamic injections were located somewhat more laterally than in the previous cases, sparing the medial part of the parafascicular and submedius nuclei (Fig. 6.2 on page 70). The TB neurons in these cases showed the same pattern of distribution as in the previous cases (Fig. 6.5).

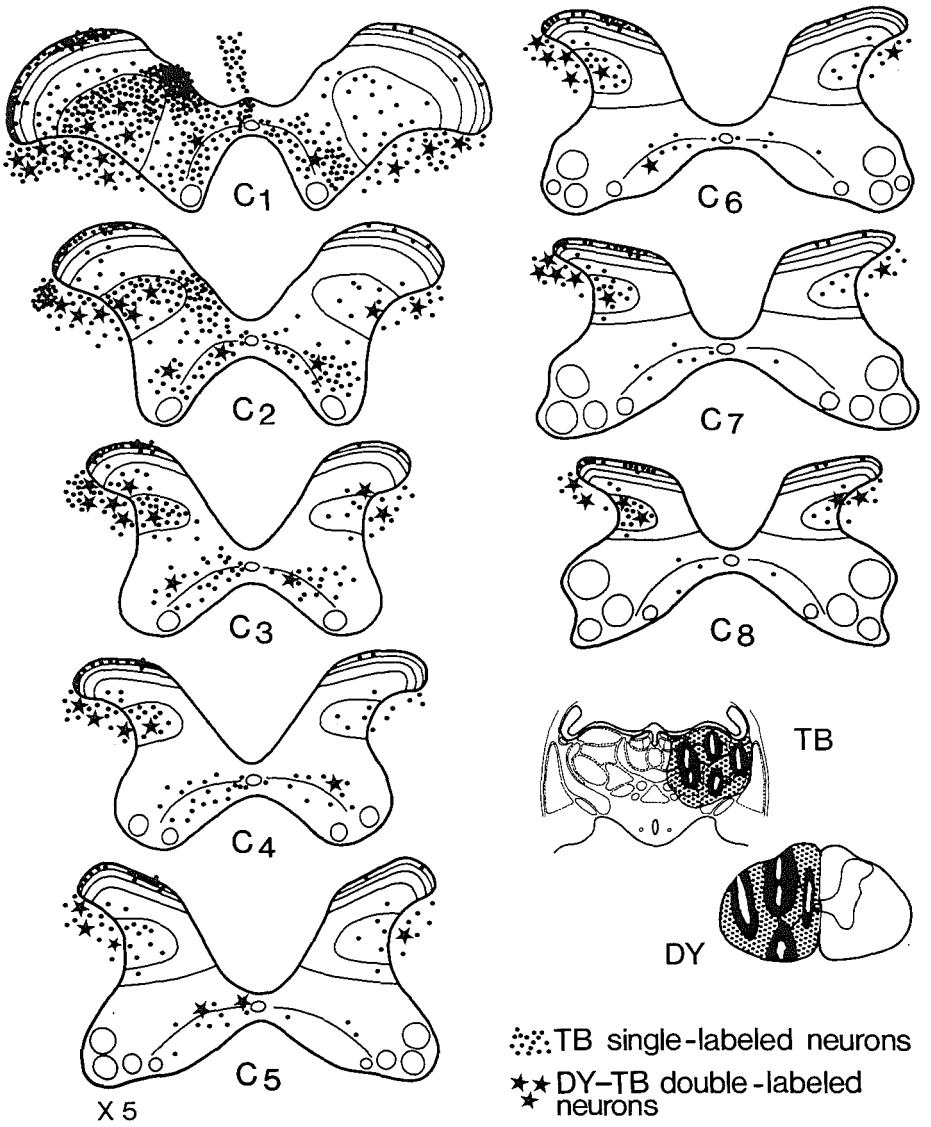

Fig. 6.5 Distributions of the retrogradely TB-labeled neurons and the DY-TB double-labeled neurons after unilateral TB-injections in the thalamus and contralateral DY-injections at T2 (group B). Each drawing represents the superimposed chartings of 5 sections; each dot represents one labeled neuron.

Table 6.1 Numbers of labeled cells per section, and percentages of double-labeling, in different segments in the different groups of experiments. All values are mean values (and SD) for all cases within the same group. Numbers of cases per group are indicated (n=...).
DY+DL, TB+DL: numbers of DY-labeled and TB-labeled neurons per section, respectively.
%DL/TB+DL, %DL/DY+DL: percentages of double-labeling of the TB-labeled and DY-labeled cells.

DY+DL		C1-C8	C1	C3	C5	C8
groups A-F	(n=25)	111 (34)	81 (30)	89 (35)	120 (35)	160 (39)
TB+DL		C1-C8	C1	C3	C5	C8
group A	(n=6)	40 (4)	122 (33)	37 (3)	19 (4)	14 (3)
group B	(n=4)	30 (6)	94 (25)	25 (5)	12 (1)	10 (4)
group C	(n=3)	2,6 (0,6)	10 (2)	3 (2)	0,5(0,5)	0,5(0,5)
group D1	(n=2)	3,1 (1,1)	11 (5)	2 (1)	0,2(0,3)	0 (0)
group D2	(n=2)	27 (4)	72 (2)	30 (11)	15 (1)	9 (3)
group E	(n=4)	16 (6)	40 (18)	21 (10)	10 (6)	7 (4)
group F1	(n=2)	4,3 (1,8)	12 (7)	4 (1)	3 (1)	2 (1)
group F2	(n=2)	20 (2)	66 (4)	20 (0)	8 (1)	6 (2)
%DL/TB+DL		C1-C8	C1	C3	C5	C8
group A	(n=6)	3,3 (1,5)	2,3 (2,2)	4,2 (2,0)	4,5 (2,7)	4,0 (2,0)
group B	(n=4)	5,2 (1,6)	3,5 (1,6)	7,2 (2,5)	8,7 (2,2)	5,0 (1,7)
group C	(n=3)	2,5 (2,3)	1,0 (1,5)	0,0 (0,0)	6,7(11,5)	0,0 (0,0)
group D1	(n=2)	1,1 (0,6)	0,6 (0,7)	0,0 (0,0)	0,0 (0,0)	0,0 (0,0)
group D2	(n=2)	5,6 (0,4)	3,5 (0,9)	5,5 (0,6)	8,7 (3,3)	8,7 (1,2)
group E	(n=4)	3,5 (0,5)	2,7 (1,5)	4,0 (1,6)	2,6 (3,7)	3,4 (2,2)
group F1	(n=2)	7,3 (0,4)	5,3 (0,8)	8,3 (0,0)	4,6 (5,5)	8,0(11,3)
group F2	(n=2)	4,1 (0,9)	3,3 (1,6)	5,3 (0,4)	4,7 (0,8)	3,3 (1,4)
%DL/DY+DL		C1-C8	C1	C3	C5	C8
group A	(n=6)	1,1 (0,3)	3,1 (1,9)	1,8 (0,8)	0,7 (0,4)	0,4 (0,2)
group B	(n=4)	1,0 (0,2)	2,6 (0,6)	1,4 (0,5)	0,7 (0,2)	0,2 (0,0)
group C	(n=3)	0,1 (0,1)	0,3 (0,5)	0,2 (0,2)	0,0 (0,0)	0,0 (0,0)
group D1	(n=2)	0,05(0,01)	0,1 (0,1)	0,1 (0,1)	0,0 (0,0)	0,0 (0,0)
group D2	(n=2)	1,2 (0,3)	3,1 (1,6)	1,7 (0,8)	1,0 (0,4)	0,5 (0,0)
group E	(n=4)	0,6 (0,3)	1,7 (1,2)	1,1 (0,6)	0,5 (0,7)	0,2 (0,2)
group F1	(n=2)	0,3 (0,0)	0,9 (0,0)	0,5 (0,2)	0,2 (0,2)	0,2 (0,2)
group F2	(n=2)	0,6 (0,0)	2,2 (0,9)	1,0 (0,2)	0,3 (0,0)	0,1 (0,1)

DY-TB double-labeled neurons were observed in all cases of this group. Such neurons must be branching neurons with ascending and descending collaterals projecting to opposite sides, e.g. the left thalamus and the right half of T2. The double-labeled neurons in group B ("crossed" injections) were distributed similarly to those in group A ("uncrossed" injections) and were present in almost the same numbers. Thus, DY-TB neurons were located in laminae V, VII, VIII and X, and in the LSN on both sides of the cord, as well as in lamina I contralateral to the thalamic injection (and ipsilateral to the T2 injection).

Per case, about 2700 (± 750) (mean ± SD in 4 cases) TB-labeled neurons were counted in C1-C8 (about 30 per section; cf. Table 6.2 on page 78). About 5.2% (± 1.6%) of all TB neurons in C1-C8 were double-labeled, with no clear differences between segments (Table 6.1). Conversely, about 1.0% (± 0.2%) of the DY-labeled neurons were double-labeled. This percentage differed between segments, decreasing from C1 to C8 (Table 6.1). Again, the LSN displayed the highest percentages of double-labeling (cf. Table 6.2 on page 78).

TB-injections in the dorsal midbrain (groups C, D, E and F)

The 15 cases with TB-injections in the dorsal midbrain were divided into different groups on the basis of the location and extent of the TB injection areas (cf. Fig. 6.6). In all groups the TB-labeled neurons were distributed according to the same pattern, but marked differences in numbers were observed among different groups (cf. Table 6.2 on page 78).

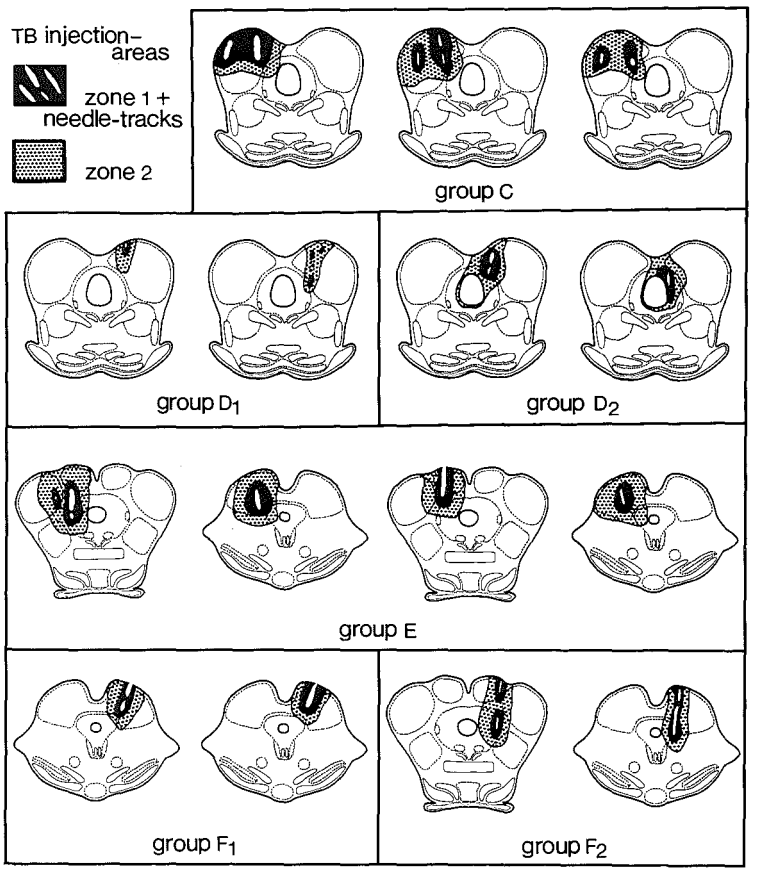

Fig. 6.6 TB-injections in the dorsal midbrain. The drawings depict the maximal extent of the tracer deposit in each case of groups C, D, E and F.

The TB- and DY-injections were made *on the same side* in groups E and C. In group E, TB-injections involved the superior colliculus and the dorsolateral part of the periaqueductal grey (PAG) and only slightly extended in the subjacent cuneiformis area (Fig. 6.6). The distribution of TB neurons in this group was similar to that observed after thalamic TB-injections (cf. Figs. 6.7 and 6.3). Some double-

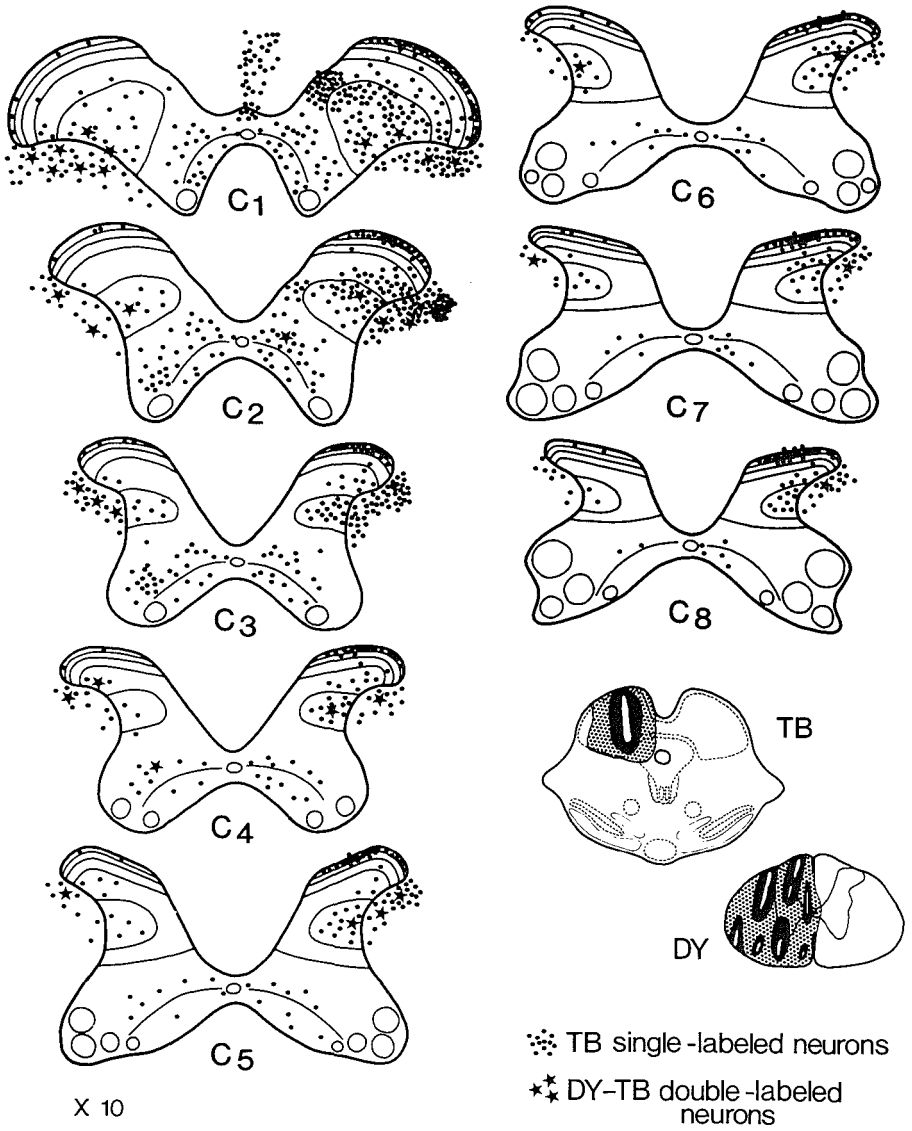

Fig. 6.7 Distributions of the retrogradely TB-labeled neurons and the DY-TB double-labeled neurons after unilateral TB-injections in the superior colliculus and ipsilateral DY-injections at T2 (group E). Note that each drawing represents the superimposed chartings of 10 sections. One dot represents one labeled neuron.

labeled neurons were observed, which were relatively most numerous in the ipsilateral LSN. No double-labeled neurons were observed in lamina I, the LCN and the sDCN.

Table 6.2 Numbers of TB-labeled neurons and percentages of double-labeling on different sides of the cord in the different groups of experiments. Values are mean values ± SD for all cases within the same group.
Total TB+DL: total numbers of TB neurons counted per case in C1-C8.
TB+DL/section: number of TB neurons per section in C1-C8. i/c: number on the side, ipsilateral/contralateral to the *TB*-injections.
%DL/TB+DL, %DL/DY+DL: percentages of double-labeling of the *TB* and *DY* neurons in C1-C8, respectively. i/c: ipsi/contralateral to the *TB*- and *DY*-injections, respectively. Percentages within the LSN are indicated between [brackets].

TB AND DY INJECTIONS ON THE SAME SIDE

	total TB+DL	TB+DL/section	%DL/TB+DL [LSN]	%DL/DY+DL [LSN]
group A (n=6) thalamus/T2	4000±1000	40±4 i:13 c:27	3,3±1,5% i:5,7% c:2,2% [15%] [5%]	1,1±0,3% i:0,9% c:1,6% [7,7%] [4,8%]
group C (n=3) inf coll/T2	260±60	2,6±0,6 i:0,4 c:2,2	2,5±2,3% i:14% c:0,4% [20%] [0,4%]	0,1±0,1% i:0,1% c:0,03% [0,8%] [0%]
group E (n=4) sup coll/T2	1300±500	16±6 i:5 c:12	3,5±0,5% i:6,8% c:2,2% [10%] [4,5%]	0,6±0,3% i:0,4% c:0,8% [2,5%] [3,4%]

TB AND DY INJECTIONS ON OPPOSITE SIDES

	total TB+DL	TB+DL/section	%DL/TB+DL [LSN]	%DL/DY+DL [LSN]
group B (n=4) thalamus/T2	2700±750	30±6 c:22 i:8	5,2±1,6% c:5,4% i:4,1% [16%] [10%]	1,0±0,2% i:1,2% c:0,8% [5,3%] [2,7%]
group D1 (n=2) inf coll/T2	210±100	3,1±1,1 c:2,7 i:0,4	1,1±0,6% c:0,8% i:3,3% [6%] [7%]	0,05±0,01% i:0,1% c:0,05% [0,3%] [0,4%]
group D2 (n=2) inf coll/T2	2040±580	26,7±3,5 c:14 i:13	5,6±0,4% c:7,2% i:3,9% [19%] [9%]	1,2±0,3% i:1,2% c:1,3% [6,5%] [4,3%]
group F1 (n=2) sup coll/T2	350±60	4,3±1,8 c:3,5 i:0,9	7,3±0,9% c:8,7% i:1,2% [20%] [2%]	0,3±0,0% i:0,4% c:0,08% [2,8%] [0%]
group F2 (n=2) sup coll/T2	1430±150	20±2 c:13,4 i:5,9	4,1±0,9% c:4,4% i:3,4% [11%] [6%]	0,6±0,0% i:0,7% c:0,4% [3,3%] [1,7%]

In group C, TB-injections were made in the inferior colliculus, only partially involving the dorsolateral PAG (Fig. 6.6 on page 76). The resulting distributions of TB single-labeled and DY-TB double-labeled neurons (Fig. 6.8) were very similar to that of group E, but the neurons were much less numerous (Table 6.2).

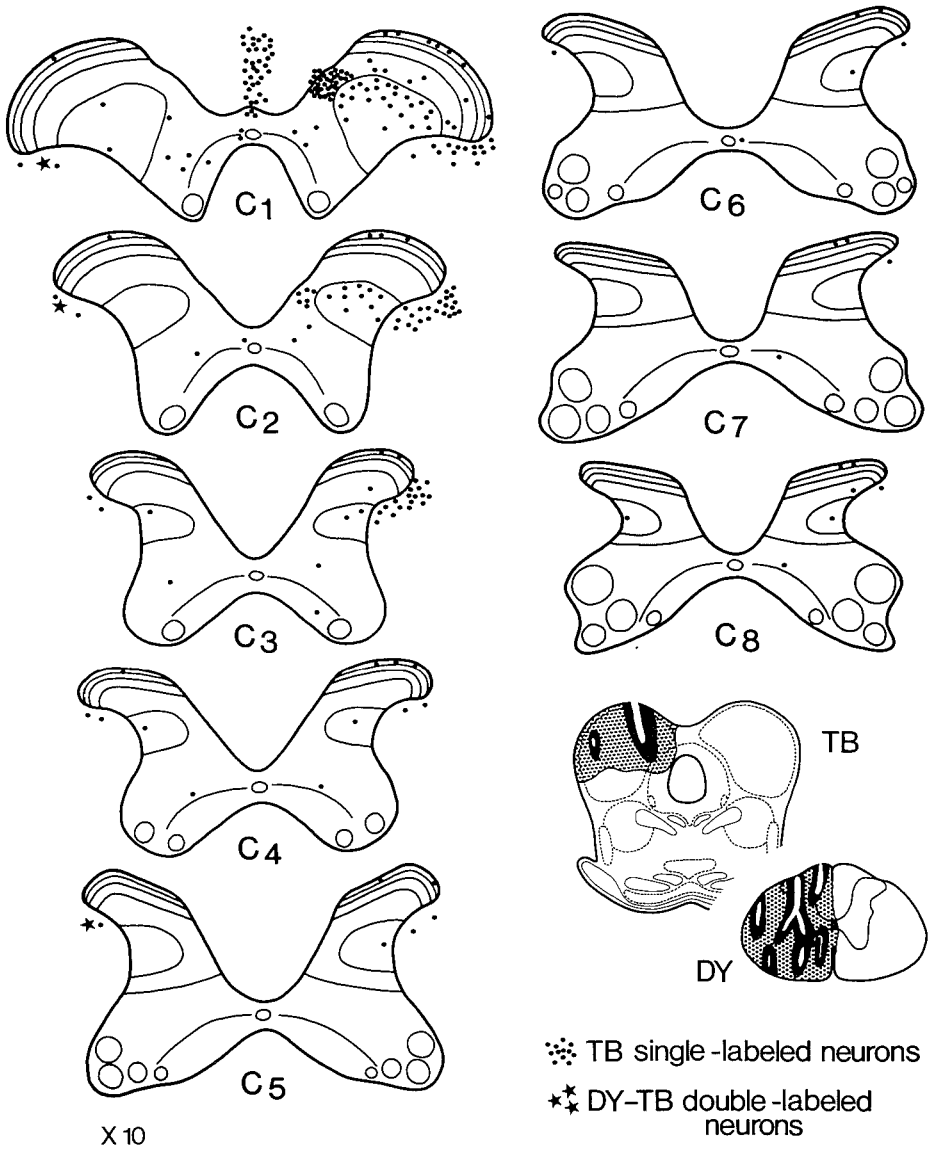

Fig. 6.8 Distributions of the retrogradely TB-labeled neurons and the DY-TB double-labeled neurons after unilateral TB-injections in the inferior colliculus and ipsilateral DY-injections at T2 (group C). Each drawing represents the superimposed chartings of 10 sections; each dot represents one labeled neuron.

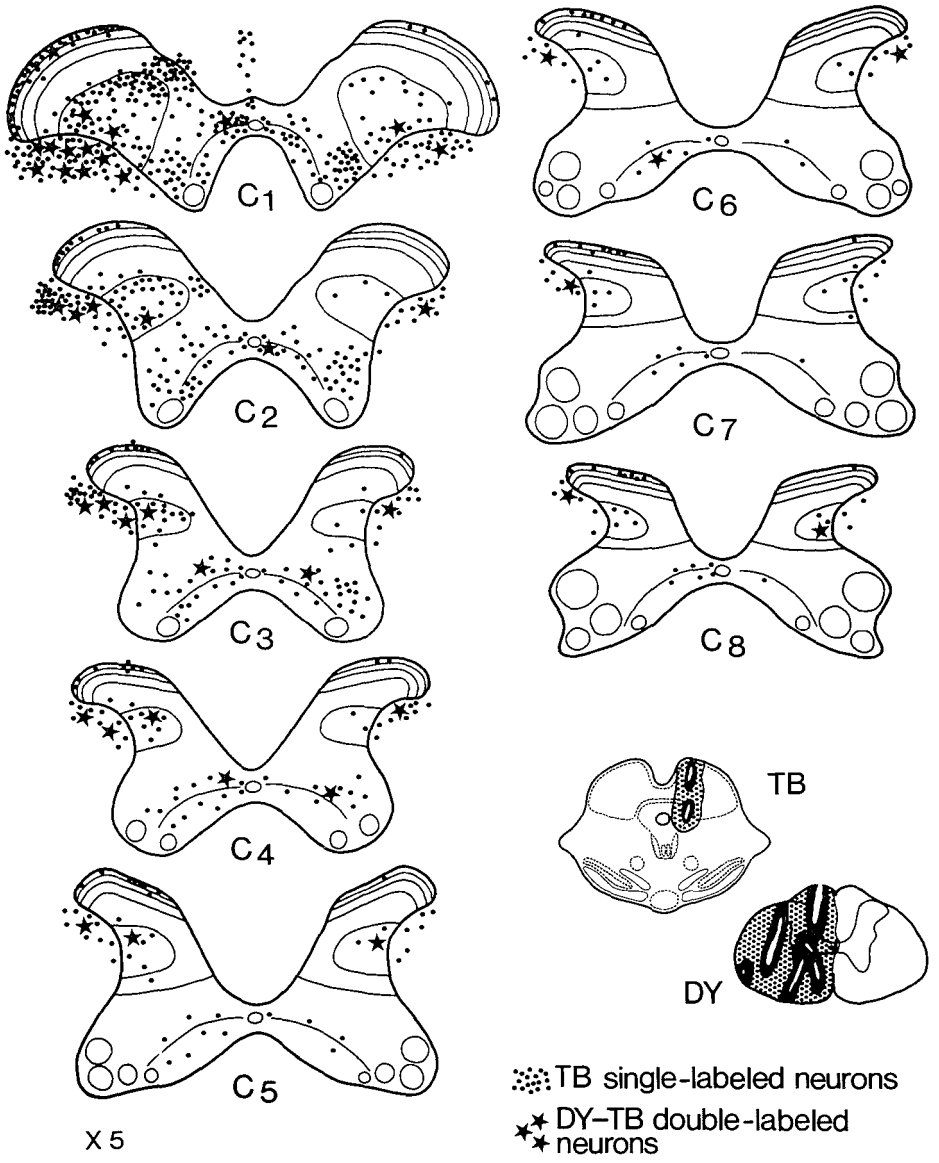

Fig. 6.9 Distributions of the retrogradely TB-labeled neurons and the DY-TB double-labeled neurons after unilateral TB-injections in the superior colliculus and contralateral DY-injections at T2 (group F2). Each drawing represents the superimposed chartings of 5 sections, while each dot represents one labeled neuron.

TB- and DY-injections were made *on opposite sides* in groups D and F. The TB-injections involved the inferior colliculus in groups D1 and D2, and the superior

colliculus in groups F1 and F2 (Fig. 6.6 on page 76). The resultant distributions were similar to those observed in the other cases (the distributions in group F2 are depicted in Fig. 6.9). Contralateral to the TB-injections, a few double-labeled neurons were present in lamina I and in the LCN. Yet, they constituted less than 0.7% and 2% of the TB neurons in these areas.

When comparing the different groups (cf. Table 6.2 on page 78), the following can be noted. In the cases with fairly superficial injections (groups C, D1 and F1; cf. Fig. 6.6 on page 76) notably fewer TB neurons were observed than in the cases with additional involvement of the PAG (groups D2, E and F2). In all cases, the numbers of TB-labeled neurons were highest on the side contralateral to the TB-injections (except in group D2, with bilateral involvement of the PAG, in which nearly equal numbers of TB neurons were found on each side of the cord; cf. Table 6.1 on page 75).

In all groups of experiments relatively few of the TB neurons were double-labeled: 1.1 to 7.2%. This percentage generally was highest on the side containing most DY neurons (i.e. ipsilateral to the DY-injections; cf. Table 6.2 on page 78). The percentages were about the same in different segments (Table 6.1 on page 75). Conversely, only very few (0.05-1.2%) of the DY-labeled descending propriospinal neurons were also TB-labeled from the dorsal midbrain. The percentages were highest in the cases with many TB neurons (Table 6.2, page 78). They were also higher on the side containing the majority of TB neurons (i.e. contralateral to the TB-injections; cf. Table 6.2, page 78) and in the segments containing a larger number of TB neurons (thus decreasing from C1 to C8; cf. Table 6.1, page 75).

In all cases, the percentages of double-labeling were higher in the LSN than in the grey matter. This was most evident in the LSN ipsilateral to the DY-injections, where up to 20% of the TB neurons and up to 7.4% of the DY neurons were double-labeled in the various groups (cf Table 6.2, page 78).

Labeling of neurons in the brain stem

In all cases many TB and DY neurons were observed in the brain stem. Only few of them were double-labeled. The majority of such neurons were located in the ventral part of the paragigantocellular nucleus, while a few were also present in the locus coeruleus, ipsilateral to the spinal injections.

Discussion

Descending propriospinal neurons

The present experiments, like the previous sets of experiments, show that the cervical spinal neurons which give rise to descending propriospinal fibers are concentrated in lamina I, laminae IV to VIII and the LSN, being less numerous in laminae II and III. Only a few descending propriospinal neurons were found in the LCN, distributing their fibers ipsilaterally [cf. 112, 376].

Spinothalamic neurons

The present results indicate that spinothalamic neurons in the rat are located at all cervical levels in lamina I, the LSN, the lateral lamina V, lamina VIII and the adjoining ventromedial lamina VII, and lamina X. These sets of neurons distribute fibers both to the ipsilateral and contralateral thalamus, the contralaterally projecting neurons, however, being most numerous. In the upper cervical segments spinothalamic neurons are also present in the dorsal and lateral parts of lamina VII (this set of neurons projects bilaterally) and in the LCN and sDCN (projecting only contralaterally). On the whole, these results are in good agreement with those obtained in other studies in the rat [121, 133, 192, 198, 236]. However, one discrepancy, which we cannot explain satisfactorily, should be mentioned. In two studies [133, 192], spinothalamic neurons were observed in the ventromedial dorsal horn (medial lamina VI) at cervical levels other than C1 and C2. Such neurons, which constitute a major spinothalamic cell group at lumbar levels [274] were not observed at cervical levels below C2 either in the present study or in other studies [121, 198, 236].

Spinal afferents to the thalamus in the rat have been shown to terminate mainly in the medial part of the medial geniculate body, the ventrobasal complex, the nucleus submedius and the intralaminar nuclei, notably the nucleus centralis lateralis [239, 268, 316, 419]. The thalamic injections in all cases largely involved these areas. Both in group A and group B, the thalamic injections extended into the zona incerta. Since degeneration studies in the rat have demonstrated a small spinal projection to the zona incerta [239, 419], the cell populations observed in the present study may include some neurons projecting to that area.

Spinal neurons projecting to the dorsal midbrain

The distribution of the C1-C8 neurons which project to the dorsal midbrain ("spinotectal" neurons), as observed in groups C to F, is in good agreement with other findings in the rat [235, 236, 272, 379]. Spinotectal neurons, though fewer in number, show the same distribution as those projecting to the thalamus (see above). This raises the question whether most spinotectal fibers constitute collaterals of

spinothalamic fibers, and also whether the present midbrain injections might have damaged spinothalamic fibers of passage.

Degeneration studies in the rat [268, 419] have demonstrated that spinothalamic fibers are located both in the central and dorsolateral mesencephalic tegmentum. In the two cases of group F2 the midbrain injections might have involved the spinal fibers coursing in the central mesencephalic tegmentum towards the medial thalamus (cf. Fig. 6.6 on page 76). However, the midbrain injections in the other 13 cases did not extend into the midbrain tegmentum. Yet, essentially similar results were obtained in all cases.

Retrograde labeling studies in the cat [41, 46] demonstrated that a major portion (up to 80%) of the tectal fibers from the LCN are collaterals of LCN-thalamic fibers. Electrophysiological studies in the cat showed that some neurons in lamina I and in laminae VII and VIII also provide collaterals both to the midbrain and the medial thalamus [168, 417]. In a double-labeling study in the rat [236], however, only 9% of the spinal neurons labeled from the medial thalamus and the PAG were found to provide collaterals to both areas. Other double-labeling findings in the rat equally showed that only few spinal neurons project to both the ventrobasal thalamus and the ventrolateral PAG [140]. Thus, some spinotectal fibers constitute collaterals of spinothalamic fibers, but many of them seem to be independent fibers, at least in the rat [cf., however, 169].

Spinotectal fibers in the rat [268, 414, 419] are distributed to the intercollicular nucleus, the deep layers of the superior colliculus and the lateral, especially the ventrolateral, part of the PAG [32, 235]. In the present experiments most neurons appeared to be labeled from the PAG, especially at superior collicular levels [cf. 292]. Injections in the superior colliculus not involving the PAG yielded quantitatively different, but qualitatively similar results. This is in keeping with findings by Menétrey et al. [272] who obtained qualitatively similar spinal labeling after HRP injections restricted to either the superior colliculus, the cuneiformis area or the PAG. In this respect it is of interest to note that these regions are supposed to function as one unit to control ventral medullary neurons which give rise to a descending pain inhibitory system [420]. Anatomical findings in the opossum [331] also showed that the area of the intercollicular nucleus, the adjacent PAG and the deep layers of the superior colliculus constitutes a common intercollicular terminal zone of the somatosensory cortical, spinal and dorsal column projections, which zone is therefore considered an integrative somatosensory center.

Branching neurons with ascending collaterals to the thalamus or dorsal midbrain

In the present experiments, double-labeled neurons were observed throughout the cervical cord. Such neurons must represent branching neurons, giving rise to both an ascending collateral (to the thalamus or dorsal midbrain) and a descending collateral (to T2 or below). The double-labeled neurons were mainly present in the LSN on either side, but were also found in the lateral lamina V, lamina VIII and the ventromedial lamina VII, and lamina X. They were only sparsely present in the LCN and lamina I and absent in the sDCN.

On the whole, the double-labeled neurons constituted only a minor proportion of all labeled cervical neurons. Thus, only about 3-5% of the spinothalamic neurons, and about 1-7% of the spinotectal neurons, were double-labeled, i.e. gave rise to a descending collateral to T2 or below. Conversely, only about 1% of the DY-labeled descending propriospinal neurons were found to give rise to an ascending spinothalamic collateral, and even fewer, 0.1 to 0.6%, gave rise to an ascending spinotectal collateral. The present findings therefore indicate that spinothalamic and spinotectal neurons on the one hand, and descending propriospinal neurons on the other hand, are largely separate sets of neurons. The same seems to be the case for reticulothalamic and reticulotectal versus reticulospinal neurons [present study; 97, 246, 397].

The lateral spinal and lateral cervical nuclei

The dorsolateral funiculus in the rat contains two different nuclei: the lateral cervical nucleus, LCN, and the lateral spinal nucleus, LSN. The LSN, also referred to as the nucleus of the dorsolateral funiculus [272, 273], extends throughout all spinal levels. It is present most conspicuously in the guinea-pig and the rat, but is absent in the cat and monkey [136, 137]. The LCN, which is present in many species, is located in the dorsolateral funiculus at C1 to C3 only (mainly at C1 and C2). In the rat, the LCN is morphologically distinct from the LSN [128; cf. Fig. 6.4 A-C on page 72]. LCN neurons are round cells, located in a discrete group near the pial surface [cf. 28]. LSN neurons, on the other hand, are multipolar or fusiform cells, dispersed loosely in the white matter alongside the dorsal horn. The LSN also appears to be functionally distinct from the LCN, as evidenced by immunohistochemical and electrophysiological studies. Thus the LSN, but not the LCN, contains many processes which can be labeled by antisera against dynorphin, substance P and met-enkephalin [29, 51, 128, 357]. LSN neurons are unresponsive to cutaneous stimuli [122, 271], in contrast to neurons in the LCN [88, 122] and in the adjacent dorsal horn [120, 270, 271, 410]. Moreover, the two nuclei show some differences in terms of their (ascending and descending) connectivity. The LCN gives rise to almost exclusively crossed projections to the thalamus and dorsal midbrain [present study; 121, 133, 192, 272]; the LSN projects to the same areas bilaterally [present study; 78, 133, 169, 235,

236, 271, 272, 379], and in addition distributes fibers bilaterally to the hypothalamus [65] and to the lateral reticular nucleus [78, 273]. The LCN gives rise to only few descending fibers reaching T2, whereas the cervical part of the LSN gives rise to many, bilaterally descending, fibers to lower spinal, including lumbosacral, levels (cf. Chapter 4). In the present study, only very few branching neurons were found in the LCN, in keeping with findings in the cat, in which none were observed [112]. In contrast, the LSN contained relatively many branching neurons; indeed the highest percentages of branching neurons were observed in this nucleus in the present experiments. Moreover, in previous experiments (Chapter 4) the LSN contained the highest percentage of branching neurons with long descending propriospinal collaterals.

CHAPTER 7.
PROPRIOSPINAL NEURONS IN THE RAT'S CERVICAL CORD WITH ASCENDING COLLATERALS TO THE DORSAL MEDULLA

Introduction

In Chapter 4, many neurons in the rat's cervical cord were found to be "branching" neurons which give rise to descending and ascending collaterals. In the present experiments a search was made for branching neurons with descending propriospinal collaterals and ascending collaterals to the dorsal medulla, in particular to the dorsal column nuclei. To that purpose, Diamidino Yellow was injected unilaterally at T2, whereas True Blue was injected ipsilaterally in the dorsal medulla.

The present study confirms that the spinal projection to the dorsal column nuclei mainly originates from lamina IV and the medial laminae V and VI, and also shows that some of its neurons distribute descending collaterals to T2 or below.

Material and methods

In 10 rats, 2% True Blue (TB), dissolved in H_2O, was injected unilaterally in the dorsal medulla. A laminectomy was made at C1 and part of the occipital bone was removed, exposing the lower medulla from C1 to the obex. The dorsal column nuclei (DCN) could be distinguished as two longitudinally oriented ridges in the upper surface of the caudal brainstem. TB was deposited in two parallel anteroposterior tracks, centered on the cuneate nucleus. The needle-tracks were made just beneath the dorsal surface of the medulla by using curved micropipettes (tip diameter 100-200 μm). In each case, about 0.3-1.0 μl of 2% TB was deposited (0.4-0.5 μl in most cases). Ipsilateral to the medullary injections, 0.7-1.2 μl (1.1 μl in most cases) of 2% Diamidino Yellow Dihydrochloride (DY), suspended in a 0.2M phosphate buffer (pH 7.2), was injected into the grey and white matter of T2 by making a mediolateral row of micropipette penetrations. The TB- and DY-injections were made in separate sessions, survival times after the injections being 5 and 2 days, respectively.

The animals recovered quickly from both operations and showed little postoperative discomfort. They were sacrificed and perfused as described in Chapter 3.2. After the fluorescent injection areas had been charted, the sections containing the medullary injections were counterstained with cresylviolet in order to more accurately define which structures were involved by the injections. Retrogradely labeled neurons were studied in the segments C1-C8. The TB, DY, and DY-TB neurons were each counted in every eighteenth section.

Results

Distribution of the DY-labeled neurons

The DY-injections in most cases involved the entire grey and white matter on one side of T2 without extending to the opposite side. After two days survival, migration

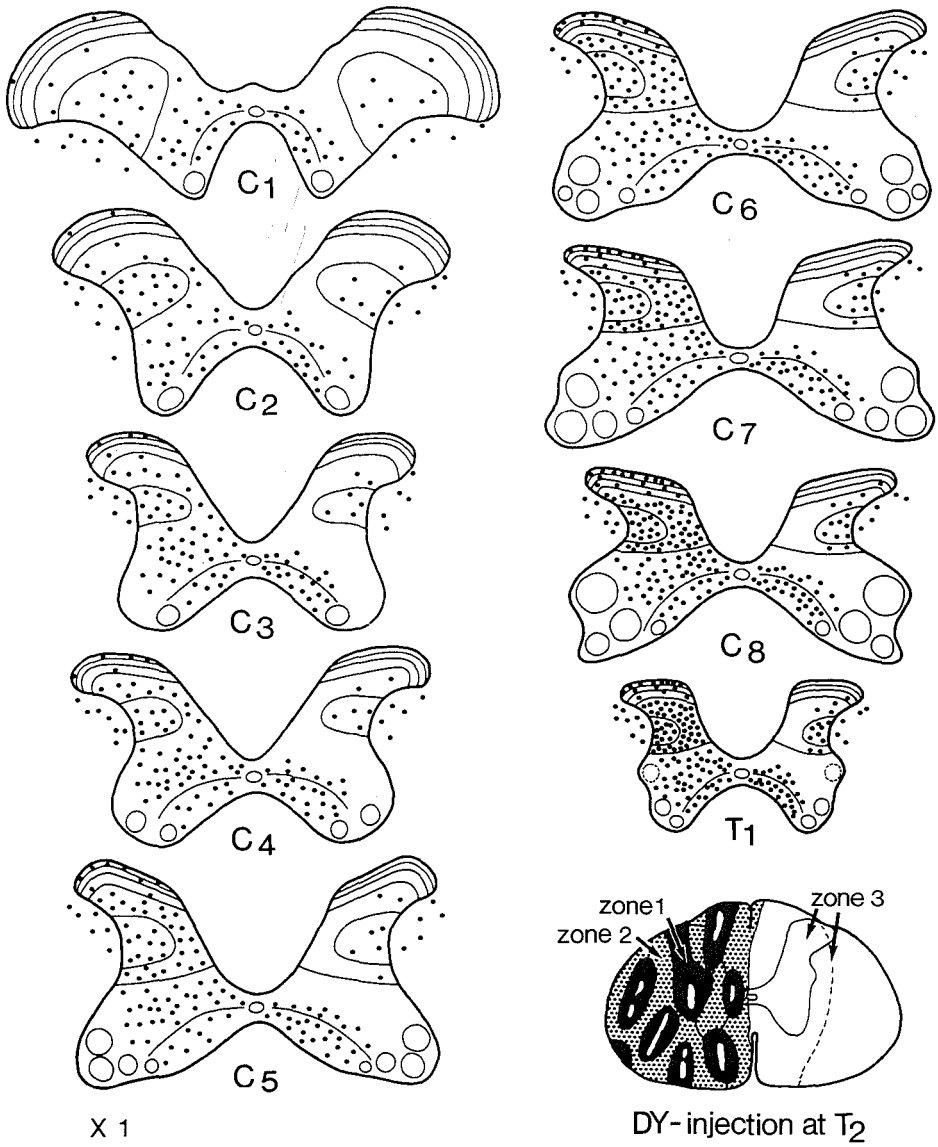

Fig. 7.1 Distribution of the retrogradely DY-labeled neurons in C1-T1 after DY-injections at T2. Each drawing represents the findings in one section. One dot represents one labeled neuron.

Table 7.1 Numbers of labeled neurons per section, and percentages of double-labeling, in different segments. All values are mean values ± SD in the 10 cases.
TB+DL, DY+DL: numbers of TB-labeled and DY-labeled neurons per section, respectively.
%DL/TB+DL, %DL/DY+DL: percentages of double-labeling of the TB-labeled and the DY-labeled neurons, respectively.
total : data pertaining to the total population of labeled neurons.
"PSDC": data pertaining to the population of labeled neurons in lamina IV, medial laminae V and VI, and dorsomedial lamina VII, ipsilateral to the injections.

total	C1-C8	C1	C3	C5	C8
TB+DL	54±18	83±23	68±20	51±17	44±18
DY+DL	115±25	67±22	93±29	117±17	160±40
%DL/TB+DL	8,4±1,8%	3,7±2,2%	6,6±4,5%	9,6±2,5%	15,9±5,5%
%DL/DY+DL	4,2±1,4%	4,5±2,1%	4,9±3,0%	4,5±2,0%	4,3±1,7%

"PSDC"	C1-C8	C1	C3	C5	C8
TB+DL	25±7	42±15	32±15	25±11	21±8
%DL/TB+DL	10±3%	4±3%	7±3%	9±5%	20±8%
%DL/DY+DL	11±5%				

of DY out of retrogradely labeled neurons was limited, being only observed in the caudal part of T1. Throughout C1-C8, DY-labeled neurons were present on both sides of the cord (Fig. 7.1). As in the previous experiments, they were about twice as numerous on the side ipsilateral to the DY-injections than contralaterally. *Ipsilaterally*, they were mainly present in lamina I, laminae IV to VIII, and the lateral spinal nucleus, LSN. *Contralaterally*, they were concentrated in lamina VIII and the adjoining ventromedial part of lamina VII, the lateral laminae IV to VI and the LSN.

In each case, a total of about 4400 DY-labeled neurons, including the DY-TB double-labeled ones, were counted in C1-C8, cell-counts being made in every eighteenth section. Their mean number per section was roughly the same as found previously, i.e. 115 ± 25 in C1-C8 and 124 ± 25 in C3-C8 (Table 7.1).

Distribution of the TB-labeled neurons

In all cases the TB-injections in the dorsal medulla were centered on the dorsal column nuclei and the adjacent fasciculi containing spinal afferents to the dorsal column nuclei, but other structures were involved as well (Fig. 7.2). Thus, in most cases the injections also involved part of the hypoglossal nucleus, dorsal motor nucleus of the vagus, solitary complex, external cuneate nucleus and the dorsal part of the subjacent reticular formation.

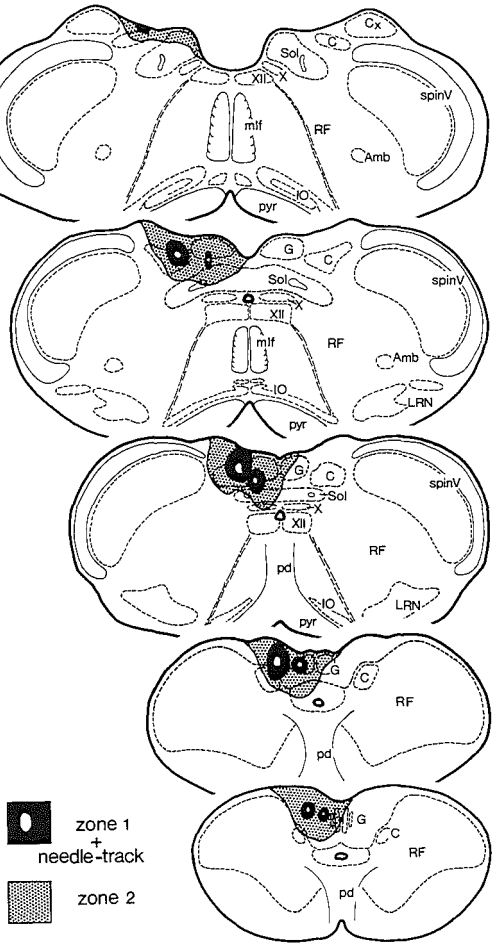

Fig. 7.2 TB-injections in the dorsal medulla. The drawings depict the maximal extent of the injections in the 4 cases with largely unilateral tracer deposit.
Abbreviations: Amb: ambiguus nucleus; C: cuneate nucleus; Cx: external cuneate nucleus; G: gracile nucleus; IO: inferior olivary complex; LRN: lateral reticular nucleus; mlf: medial longitudinal fasciculus; pd: pyramidal decussation; pyr: pyramidal tract; RF: reticular formation; Sol: solitary tract and nucleus; spinV: spinal trigeminal tract and nucleus; X: dorsal motor nucleus of vagal nerve; XII: hypoglossal nucleus

In 4 of the 10 cases the injections were largely unilateral. In these cases the following distribution of TB-labeled neurons was observed (Fig. 7.3).

TB neurons were present mainly *ipsilateral* to the injections. The great majority of them were located in lamina IV (with also some in lamina III), in the medial parts of

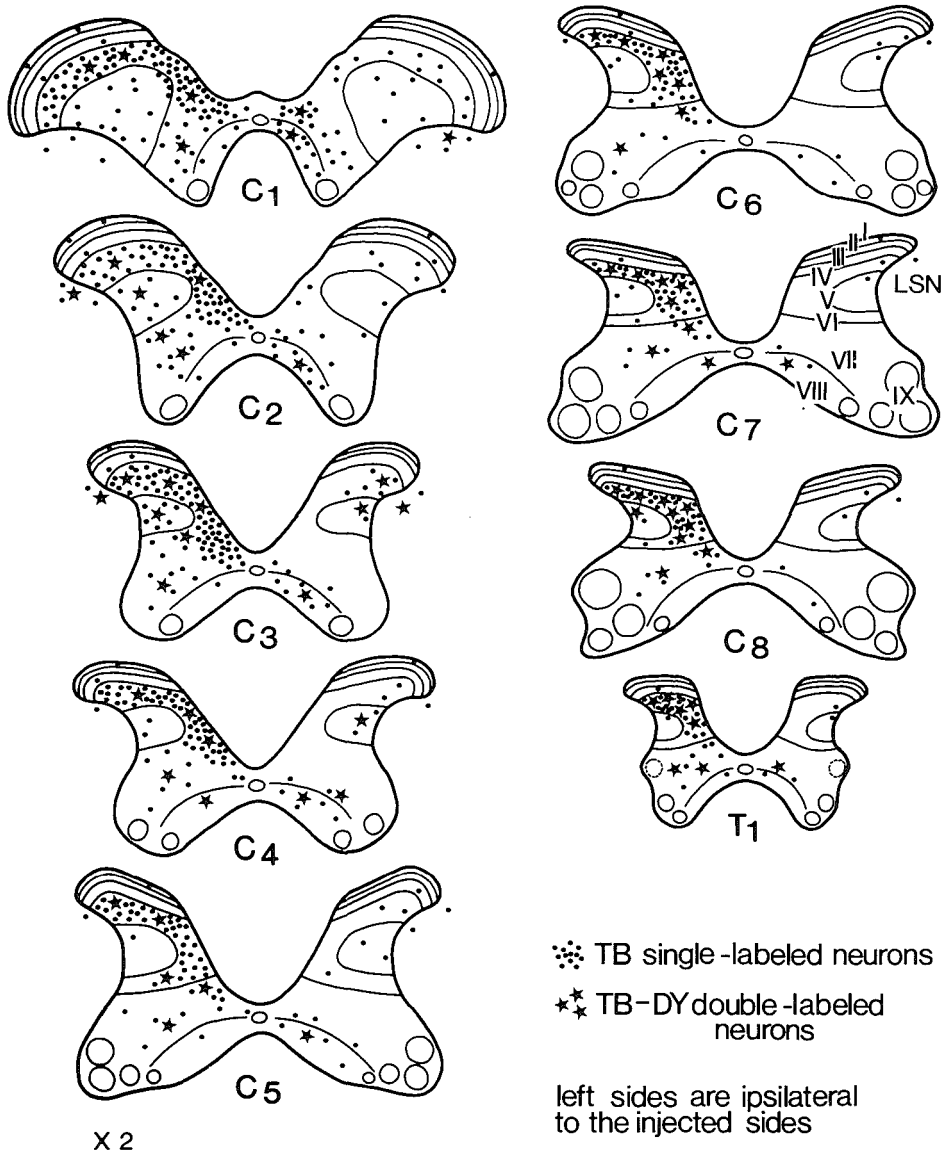

Fig. 7.3 Distributions of the retrogradely TB-labeled neurons and the TB-DY double-labeled neurons in C1-T1 after unilateral TB-injections in the dorsal medulla and ipsilateral DY-injections at T2. Each drawing represents the superimposed chartings of two sections. One dot represents one labeled neuron.

lamina V and VI and in the dorsomedial part of lamina VII. In addition to this prominent concentration of labeled neurons, some TB neurons were present in other areas of the ipsilateral spinal cord, i.e. in lamina I, in the LSN, in the lateral parts of laminae V and VI and in laminae VII and VIII. *Contralaterally*, TB neurons were

located in lamina VIII and the adjoining ventromedial part of lamina VII, the lateral parts of laminae IV to VI, the LSN and lamina I.

In 6 other cases (which are not illustrated), the TB-injections were not strictly unilateral. Although the needle-tracks and zone I were present unilaterally, the diffusion of zone II extended into the contralateral dorsal medulla. In these cases, TB neurons in lamina IV and the medial laminae V-VII were observed on both sides of the cord.

In all 10 cases, the presence of many TB neurons ipsilaterally in lamina IV and the medial laminae V, VI and VII was a consistent finding. Labeling of neurons in other areas of the spinal cord was less consistent in that the number of TB neurons in these areas, notably in lamina I, showed marked variations between cases. However, these variations could not be related to clear differences in the extent of the TB-injections. The TB neurons were of all types and sizes [cf. 126, 342]; in lamina IV both large and smaller neurons were labeled.

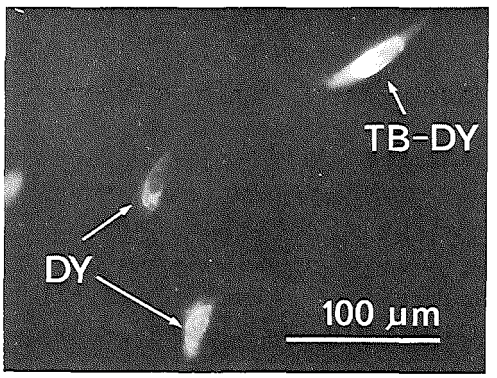

Fig. 7.4 Photomicrograph of DY single-labeled neurons and TB-DY double-labeled neuron in the ipsilateral lamina IV at C6.

In each case, a total of about 2200 TB neurons, including the double-labeled ones, were counted in C1-C8. Per section, about 35 TB neurons were observed ipsilaterally, and about 19 contralaterally[9]. In the ipsilateral lamina IV and medial laminae V to VII about 25 TB neurons were counted per section. The numbers of TB neurons were largest in the upper cervical segments, decreasing when proceeding from C1 to C8 (cf. Table 7.1 on page 89).

In all cases, fairly many DY-TB double-labeled neurons were observed. Such neurons must represent branching neurons, which give rise to a descending propriospinal collateral to T2 or below and an ascending collateral to the dorsal medulla. The double-labeled neurons were intermingled with the TB single-labeled ones, except in lamina I, in which no double-labeled neurons were observed. About

Footnote 9. Numbers reflect mean values for all 10 cases (cf. Table 7.1, page 89). In the 4 cases with unilateral TB-injections, about 8 TB neurons per section were counted contralaterally.

8.5% ± 2% of all TB neurons in C1-C8 were double-labeled, i.e. gave rise to a descending propriospinal collateral. This percentage was different for the different laminae. Thus, in the ipsilateral lamina IV and medial laminae V to VII about 10% of the TB neurons were double-labeled, versus about 1-2% of the TB neurons in the corresponding contralateral areas. In other areas of the spinal cord this percentage ranged from 10% to 18%. The percentage also differed between segments, being largest at low cervical levels (cf. Table 7.1 on page 89).

Conversely, about 4% ± 1.5% of the DY neurons in C1-C8, projecting to T2 or below, were also TB-labeled from the dorsal medulla. In the ipsilateral lamina IV and medial laminae V-VII about 11% of the DY neurons were double-labeled. These percentages were about the same in all segments (Table 7.1 on page 89).

Labeling of neurons in the brain stem

TB-labeled and DY-labeled neurons were observed in the reticular formation of the caudal brain stem. In addition, DY-TB double-labeled neurons were present in the nucleus raphe magnus and adjoining gigantocellular reticular formation, mainly ipsilateral to the tracer injections. Further, double-labeled neurons were present bilaterally in the reticular formation just dorsal to the lateral tip of the inferior olivary complex. The impression was gained that fairly many (about 20%) of the TB neurons in that area were double-labeled.

Discussion

Descending propriospinal neurons

The cervical neurons which give rise to descending propriospinal fibers to T2 or below (DY-labeled in the present experiments) are located in all laminae, except lamina IX, being most numerous in lamina I, laminae IV to VIII, and the LSN. These findings are the same as obtained in the previous experiments (Chapters 4 to 6).

Neurons projecting to the dorsal medulla

The present experiments show that the great majority of the cervical neurons projecting to the dorsal medulla in the rat (TB-labeled in the present experiments) are concentrated in lamina IV, the medial parts of lamina V and VI, and the adjacent dorsomedial part of lamina VII. These neurons seem to project exclusively ipsilaterally. As all TB-injections were centered on the dorsal column nuclei (DCN), which in addition appear to be the main recipients of ascending spinal fibers to the dorsal medulla [144, 337, 338, 341, 419], it is most likely that these neurons are cells of origin of non-primary afferents to the DCN, also referred to as postsynaptic dorsal

column (PSDC) neurons. This is in keeping with findings in the rat [126, 342], the cat [33, 288, 339] and the monkey [33, 143, 288, 340, 341]. It is of interest to note that the cytoarchitectonic distinction between the medial parts of lamina IV, V and VI is unclear in the rat (cf. Chapter 2.1). Several authors therefore proposed a cytoarchitectonic scheme for the rat in which lamina IV extends ventrally in the medial dorsal horn towards the central canal [52, 269, 313, 368]. Lamina IV, thus defined, would correspond to the bandlike area containing most PSDC neurons in the rat.

In the present study additional neurons projecting to the dorsal medulla were labeled throughout the spinal grey, notably in laminae V to VIII, but also in lamina I and the LSN. Their distribution was similar to that obtained after hemi-injections of TB in the medulla (Chapter 4, group D). Moreover, they were not consistently labeled in all cases. Therefore, although some of them might project to the DCN, they are most likely neurons projecting to other areas of the dorsal medulla, e.g. the dorsal reticular formation subjacent to the DCN. This would be consistent with HRP studies in which labeling in areas other than lamina IV and the medial laminae V and VI was more pronounced following injections which more intensely involved the medullary tegmental region [126, 339, 340, 341].

In the present fluorescent tracing study PSDC neurons were labeled in larger numbers than in the HRP study of Giesler et al. [126]. The numbers of PSDC neurons, as labeled in the present study (about 25 per section in C1-C8), were of the same order of magnitude as those of spinothalamic and spinotectal neurons (about 40 and 20 per section, respectively; cf. Chapter 6) and were much larger than those of spinocerebellar neurons (about 3 per section; cf. Chapter 5). This indicates that the non-primary afferent spinal projection to the dorsal column nuclei constitutes a major ascending pathway in the rat [cf. 126].

Branching neurons

In all cases, DY-TB double-labeled neurons were observed. Such cells must represent branching neurons which are propriospinal as well as "tract" cells, giving rise to both a descending propriospinal collateral and an ascending collateral to the dorsal medulla. These neurons were most numerous in lamina IV and the medial parts of laminae V and VI, therefore representing PSDC-propriospinal neurons. About 10% of all PSDC neurons in C1-C8 were found to give rise to a descending propriospinal collateral reaching T2. They were relatively more numerous in caudal cervical segments indicating that the descending propriospinal collaterals of PSDC neurons are preferentially distributed over short distances. This is in accordance with the fact that the medial parts of laminae IV to VI only contain short descending propriospinal neurons, which project to T2-T8 (cf. Chapter 4). Consequently only about 0.5% of

cervical PSDC neurons give rise to a collateral projecting more caudally, to T6 or beyond (unpublished observations); such neurons are located laterally in lamina IV.

In the present study, only about 1-2% of the PSDC neurons were found to give rise to a crossed descending propriospinal collateral reaching T2; thus the majority of the descending collaterals of PSDC neurons are distributed ipsilaterally.

The functional significance of the branching PSDC-propriospinal neurons remains to be elucidated. The PSDC system, as compared to the direct primary afferent-medial lemniscus system, seems to be characterized by the fact that its elements receive convergent input from different sources. Thus a large proportion (50% or more) of the PSDC neurons respond not only to stimulation of low-threshold mechanoreceptors, but also to mechanical cutaneous stimuli of high, even noxious, intensity [21, 56, 58, 127, 180, 188, 237, 389] and many also receive convergent input from muscle afferents [180]. PSDC neurons in addition receive input from corticospinal fibers, from propriospinal fibers and from collaterals of spinocervical tract fibers [180]. The PSDC neurons project mainly to multipolar neurons in the "non-cluster" areas of the gracile and cuneate nuclei [336, 337, 341]. This region of the DCN in turn projects not only to the ventrobasal thalamus, but also to the tectum, pretectum, zona incerta and parafascicular nucleus [239], as well as to the cerebellum [79] and spinal cord [66, 215]. The cerebral somatosensory cortex projects preferentially to this "PSDC relay area" of the DCN [135, 213]; this projection partly consists of collaterals of corticospinal fibers to the PSDC neurons [40].

The multiple and complex inputs on the second (PSDC) and third (DCN) order neurons of the PSDC system indicate that some integration of information from different sources takes place at these levels. The PSDC neurons themselves could add to such an integration by distributing collaterals to their immediate vicinity, even to their own dendrites [34], or to other levels [56]. The present study indicates that the majority of such collaterals are distributed ipsilaterally and over short distances.

CHAPTER 8.
GENERAL DISCUSSION

The present study supplies data on the cervical spinal cord of the rat, concerning:
a) the distribution and relative numbers of
   - different sets of propriospinal neurons (giving rise to long or short, ascending or descending fibers)
   - different sets of "tract" neurons (spinoreticular, spinocerebellar, spinotectal and spinothalamic tract neurons)
b) "branching" neurons which give rise to ascending and descending collaterals:
   - their existence, locations and relative numbers, and
   - the caudal (thoracic, lumbar or sacral cord) and rostral (upper spinal cord, medulla, tectum, thalamus or cerebellum) extent of projection of their collaterals.

The data will be discussed in this sequence, and will be preceded by a brief discussion of the method applied in the present experiments.

8.1 Retrograde fluorescent double-labeling of neurons

The method of neuronal double-labeling using multiple fluorescent retrograde tracers (cf. Chapter 3.1) offers the opportunity to study the collateralization of brain pathways and to obtain both qualitative and quantitative anatomical data on this process. Thus separate populations of neurons with either distinct, non-collateralized, projections to different "targets" or with collateralized projections to multiple targets can be visualized simultaneously and the topographical relationships of these neurons can be observed within the same section [cf. 71, 161, 162]. Moreover, the method supplies information about the relative numbers of the different types of neuron under study. The present fluorescent dyes appear to be very sensitive tracers, as judged from the large numbers of neurons labeled with these substances in comparison to other retrograde tracers such as HRP [Chapters 4 to 7; cf. 236]. Their combined use in double-labeling experiments generally yields larger numbers of double-labeled neurons than other retrograde double-labeling methods [cf. 41, 46]. The combination of a "nuclear" and a "cytoplasmic" tracer (DY vs TB and FB in the present experiments) minimizes the problem of one type of label obscuring the other and of "competition" of the tracers within the cell.

Many retrograde tracers, including TB and DY, are taken up not only from fiber termination areas but also from damaged axons of passage [35, 162, 190, 221]. In the first set of experiments (Chapter 4), the caudal and rostral extent of projection of the axon-collaterals was determined by making large injections which completely involved one half of the spinal cord or the lower brainstem. As a result, the neurons which were retrogradely labeled after such injections were considered to distribute

fibers to the injected area or, through it, to more distant areas. The more discrete injections in different supraspinal structures (Chapters 5 to 7) could generally be made from the dorsal surface of the nervous system directly into the "target area" without any risk of additional involvement of spinal fibers coursing towards other targets.

In earlier fluorescent double-labeling experiments the yellow "nuclear" tracer Nuclear Yellow (NY) was applied. The use of NY was found to be complicated by the fact that this particular substance tended to migrate, after prolonged survival times, out of retrogradely labeled neurons into the surrounding glial cells and from there even into adjacent neurons. In order to minimize the risk of obtaining "false" (single- or double-) labeling, NY survival times were restricted to a period that fluorescent labeling of glial cells was still absent or minimal [37]. In later studies, a new yellow fluorescent "nuclear" tracer, Diamidino Yellow (DY), was tested which migrates only very slowly out of retrogradely labeled neurons [190]. Yet, albeit slowly, this tracer also leaks to some extent out of labeled neurons. Appropriate measures, therefore, have to be taken to avoid the occurrence of DY-labeled glial cells adjacent to DY neurons. In most of the present experiments DY was injected at T2. Survival times after DY-injection were restricted such that glial DY-labeling was only observed within a few millimeters from the DY deposit, i.e. in the caudal part of T1. This part of the cord was discarded from further study. In the first set of experiments (Chapter 4) DY survival times ranged from 3 to 5 days. In the other sets of experiments (Chapters 5 to 7) DY survival had to be kept at 2 days to avoid excessive labeling of neurons and glial cells close to T2, whilst maintaining a high level of DY-labeling at large distances (e.g. red nucleus). Apparently DY uptake and retrograde transport were more effective in the latter experiments. This rather striking observation might be related to the fact that post-operative recovery in the first set of experiments (in which the animals sustained two spinal injections/lesions) was less complete. In the other groups of experiments the animals recovered almost completely from receiving the spinal and the supraspinal injections and displayed normal behaviour. This suggest that the post-operative "quality of life" might be an important factor in the efficacy of retrograde transport, possibly because the neuronal networks are more active [cf. 141, 182] in animals sustaining less post-operative damage.

8.2 Propriospinal neurons

Neurons which give rise to propriospinal fibers have already been studied extensively in the cat [254, 286, 288, 359, 416] and the monkey [288, 359]. Data on propriospinal neurons in the rat are limited [275]. The present experiments extend these earlier findings and provide quantitative data concerning different types of propriospinal neurons in the rat.

Descending propriospinal neurons are located in all laminae of the cervical cord, except lamina IX. They are least numerous (but nonetheless present) in laminae II and III and the LCN. The population of descending propriospinal neurons comprises elements which give rise to descending fibers of different length. The "*long* descending propriospinal" fibers extend from the cervical to the lower thoracic and lumbosacral cord. Their cells of origin are located mainly in lamina VIII and the adjoining ventromedial part of lamina VII, in the lateral parts of laminae IV to VI, in the LSN and in lamina I. Those in lamina I distribute their fibers ipsilaterally, while the other populations of long descending neurons distribute fibers to both sides of the cord. The "*short* descending propriospinal" neurons (projecting to T2-T8 from the cervical cord) are located both in the areas containg the long descending propriospinal neurons and in the remaining areas of the cord. Thus laminae II and III, the medial parts of laminae IV to VI and the central and lateral parts of lamina VII contain only descending propriospinal neurons projecting over short distances (to the ipsilateral spinal cord), whereas the other areas contain both long and short descending propriospinal neurons. The few descending propriospinal neurons in the LCN appear to project mainly over short distances. The short descending propriospinal neurons are much more numerous than the long descending ones (cf. Chapter 4). This was also reflected by the fact that in the caudal cervical segments, close to the injection at T2, more labeled neurons were encountered than at rostral cervical levels, indicating the additional labeling of neurons with short axons[10]. The present findings correspond closely to those of earlier anatomical studies [254, 275, 286, 288, 359, 416].

Ascending propriospinal neurons in the cervical cord are more difficult to study with the present method due to the fact that injections rostral to the spinal neurons under study invariably damage fibers of passage projecting to supraspinal levels. Therefore a comparison was made of the numbers and distributions of the C5-C8 neurons labeled from injections at C1 versus those labeled from C3/C4 (Chapter 4, group C). It was argued that such a comparison would indicate the numbers and locations of neurons which reach C3/C4 but not C1, i.e. neurons with "*short* ascending propriospinal fibers" projecting to C2-C4. It was found that, at C5-C8, such neurons are mainly present in the LSN, in lamina I and in laminae IV to VIII [cf. 288, 416]. The sets of neurons in lamina I, the lateral laminae IV to VI, lamina VIII and the ventromedial laminae VII distribute their fibers to both sides of the cord, whereas the other sets of neurons project mainly ipsilaterally. (Thus both the short

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Footnote 10. It could also be argued that such numerical differences would result from less effective retrograde transport from T2 to rostral than to caudal cervical segments. Yet, in the present experiments many labeled neurons were also found in the red nucleus and cerebral cortex. This indicates that, after survival times as applied in the present study, the efficacy of the retrograde transport is sufficient to ensure adequate labeling of cervical spinal neurons at all levels.

ascending and short descending neurons display largely the same distibutions). *Long* ascending propriospinal neurons were not studied in the present experiments which focussed on the cervical spinal cord.

8.3 Cervical spinal neurons with ascending projections to supraspinal levels

The distributions of spinal "tract" neurons (i.e. neurons which project their fibers into tracts ascending to various supraspinal levels) have been studied by several authors both in the rat and in other species. The present experiments generally confirm the earlier findings; in addition, they offer quantitative data regarding the cells of origin of different supraspinally ascending tracts. Thus, in the segments C3-C8 of the rat, about 140 neurons per section were observed which gave rise to fibers reaching C1 and the caudal medulla[11]. Conversely, in these segments about 2 spinocerebellar neurons, 30 spinothalamic neurons, 15 spinotectal neurons and about 18 neurons projecting to the dorsal column nuclei were counted in each section. Thus the great majority of supraspinally projecting neurons distribute fibers to the lower brainstem, whereas much less fibers reach more rostral brainstem levels, which is in accordance with anterograde degeneration studies [cf. 268, 302]. It is of interest to note that spinocerebellar neurons, the function and physiology of which have been the subject of a great many studies, quantitatively constitute only a minor fraction of all supraspinally projecting cervical neurons.

In the present experiments the numbers of cervical neurons labeled from supraspinal levels were highest in the upper cervical segments, decreasing when proceeding caudally in the cervical cord. Such findings were not only obtained after injections involving C1 or the lower medulla, but also after injections in the cerebellum, tectum and thalamus. Thus these findings do not appear to be related to the efficacy of retrograde transport, but indicate an actual concentration of supraspinally projecting neurons at upper cervical levels [cf. 65, 133, 192].

Spinocerebellar neurons in the rat's cervical cord (Chapter 5) were found to be mainly present in the central cervical nucleus, CCN, (at C1-C4), lamina VIII (C1-C8), the central lamina VII (C5-C8), the medial lamina VI and dorsomedial lamina VII (C3-C8) and the lateral lamina V (C1, C7-C8). The neurons in lamina VIII and the CCN appear to project mainly contralaterally, whereas the other sets of cervical spinocerebellar neurons distribute their fibers mainly ipsilaterally. These findings correspond closely to those of other studies [253, 364].

Spinothalamic neurons in the rat (Chapter 6) are located at all cervical levels in lamina I, the LSN, the lateral lamina V, lamina VIII and the adjoining ventromedial lamina VII, and lamina X. The majority of these neurons project

Footnote 11. By calculation it can be inferred that a total number of about 75000 to 150000 of such neurons are present in C3-C8.

contralaterally, yet some of them distribute fibers to the ipsilateral thalamus. At C1 and C2 spinothalamic neurons are additionally present in the lateral cervical nucleus and the spinal extensions of the gracile and cuneate nuclei (projecting exclusively contralaterally) and in the dorsal and lateral lamina VII (this set of neurons projects to both sides of the thalamus). The present results are in accordance with those of other studies [121, 133, 192, 198, 236]. The thalamic injections in the present experiments were large, covering the entire mediolateral and rostrocaudal extent of the thalamus in order to involve all known spinothalamic termination areas. No attempts were made to differentiate spinothalamic neurons projecting to either the "medial" or the "lateral" thalamus [198].

Spinotectal neurons in the rat's cervical cord (Chapter 6) display the same distribution as spinothalamic neurons, but are less numerous. Most spinotectal neurons appear to project to the periaqueductal grey matter at superior collicular levels [cf. 292].

"Postsynaptic dorsal column" neurons, projecting to the dorsal column nuclei (Chapter 7), are concentrated in the rat's cervical cord in lamina IV, the medial laminae V and VI and in the adjacent dorsomedial lamina VII [cf. 126, 342]. These neurons appear to project only ipsilaterally.

Spinoreticular neurons. Spinal neurons projecting to the lower brainstem or beyond (Chapter 4) are located in C3-C8 throughout the spinal grey matter. They are concentrated, however, in lamina I, laminae IV to VIII and the LSN, being less numerous in laminae II and III. Those in the central and lateral lamina VII and in the medial laminae IV to VI project ipsilaterally, whereas the other sets of neurons distribute their fibers bilaterally. The present findings correspond closely to those observed in the cat [288]. The medullary injections in the present experiments covered the entire mediolateral extent of one half of the medulla, interrupting fibers of passage projecting to more distant sites. Therefore the population of neurons labeled from such injections comprises spinothalamic, spinotectal and spinocerebellar neurons as well as spinoreticular neurons projecting to the brainstem reticular formation and adjacent nuclei. When comparing the distributions and numbers of labeled cervical neurons after thalamic, tectal, cerebellar and lower brainstem injections, it can be deduced that *spinoreticular* neurons in C3-C8 are located in lamina I, the LSN, the medial and lateral laminae IV to VI, lamina VII and lamina VIII. Such deductions, however, cannot supply accurate data on the actual distribution of spinoreticular neurons. Moreover, the resultant population of spinoreticular neurons would still be heterogeneous, including neurons projecting to the medial medullary and pontine reticular formation and to laterally located medullary nuclei such as the LRN and inferior olive.

8.4 Branching neurons

At the turn of the century, definite progress in the study of the anatomy of the nervous system was made with the advent of the Golgi method. This method makes use of the impregnation of neurons with a silver compound and results in the visualization of virtually the entire neuron, including soma, dendritic tree, axon and axon-collaterals. Fortunately, only a few neurons in each section are stained, which can be studied against a background of unstained cells. By means of this method, S. Ramón y Cajal studied and described, in great detail, the anatomy of nearly each part of the nervous system, distinguishing many types of neurons [68]. In studying axonal projections, he observed that many axons emitted side-branches or "collaterals" along their trajectories. Within the spinal cord, quite a few neurons were found to give rise to axons which divided, upon reaching the white matter, into ascending and descending branches. Such neurons will, for convenience, be referred to as "branching neurons". Branching neurons were observed both in the dorsal and ventral horn and in the intermediate zone [68]. Later studies by means of modified impregnation methods confirmed these findings [242, 344]. With the Golgi method, the ascending and descending collaterals could only be traced over relatively short distances. With the advent of retrograde double-labeling methods (cf. Chapter 3.1) it became possible to identify branching neurons both qualitatively and quantitatively and to demonstrate the extent of their collaterals over large distances. In the present study an attempt was made to demonstrate : firstly, the existence and relative numbers of branching neurons in the rat's cervical cord and, secondly, the extent of projection of their axon-collaterals.

In the first set of experiments (Chapter 4) it was demonstrated that many (up to 30%) of the neurons in C3-C8 are branching neurons which give rise to ascending and descending collaterals reaching C1 and T2, respectively. Such neurons are located in the LSN, lamina III, the medial and lateral laminae IV to VI, and in laminae VII and VIII. None were observed in laminae I and II.

Caudal extent of projection (Fig. 8.1). Of the neurons with ascending projections to C1 or beyond about 20% give rise to a descending collateral reaching T2, whereas 8%, 3% and 1% give rise to collaterals reaching T9, L2 and S1, respectively (Chapter 4). Thus the descending propriospinal collaterals in majority project over relatively short distances, terminating in the upper thoracic cord, in parallel with the extent of projection of non-branching descending propriospial neurons. Branching neurons projecting to lumbosacral levels are located mainly in lamina VIII and the adjoining ventromedial lamina VII, the lateral lamina V and the LSN. Those projecting to upper thoracic levels are present in lamina III to VIII and the LSN. It is of interest to note that the short and long descending propriospinal neurons give rise to ascending collaterals in equal proportions (about 30% of each population, cf. Chapter 4). The present data are in keeping with earlier

electrophysiological findings in the cat [360], which indicated that about 16% of the neurons in the cervical enlargement which project to lumbar levels distribute ascending collaterals reaching C1.

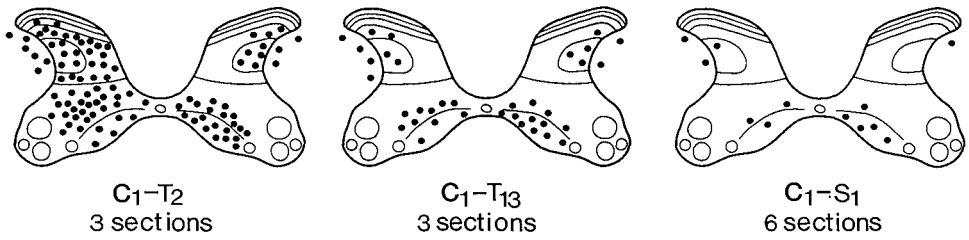

Fig. 8.1 Schematic representation of the distributions (at the level of C6) of branching neurons projecting both to C1 and to T2, T13 or S1. *Left sides are ipsilateral to the injected sides.*
Note numerical differences between different sets of branching neurons: the drawings represent the superimposed chartings of 3 or 6 sections; each dot represents one branching neuron.

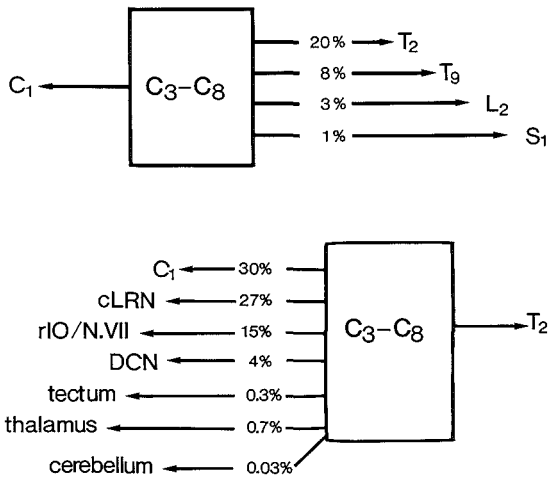

Fig. 8.2 Diagram summarizing the double-labeling results obtained in the present study.
The upper figure depicts C3-C8 neurons projecting to C1 or beyond and the percentages of them which give rise to descending collaterals reaching the indicated levels ("caudal extent of projection").
The lower figure depicts descending propriospinal neurons in C3-C8 and the percentages of them which give rise to ascending collaterals reaching the indicated levels ("rostral extent of projection").
cLRN: brainstem at the level of the caudal part of the lateral reticular nucleus
rIO/N.VII: brainstem at the level of the rostral part of the inferior olive and of the facial nucleus.
DCN: dorsal column nuclei.

Rostral extent of projection. In the segments C5-C8 (Chapter 4), about 13% of the neurons which project to T2 or below give rise to short ascending collaterals terminating at C2-C4. Such neurons are located in laminae III to VIII and the LSN. A larger proportion, about 29%, distribute collaterals reaching C1. It has to be stressed that the latter fibers also may provide collaterals to the upper cervical segments. A fairly large proportion of the C3-C8 neurons in the present study were found to be branching neurons, distributing fibers both to T2 or below and to supraspinal levels. This indicates that these spinal neurons can be considered as propriospinal cells as well as "tract" cells. Further experiments were carried out (Chapters 5 to 7) to investigate whether the ascending supraspinal collaterals specifically belonged to any of the different ascending tracts. These data can be summarized as follows (Fig. 8.2): about 30% of the descending propriospinal neurons in the cervical cord (projecting to T2 or beyond) give rise to an ascending collateral reaching the lower medulla, whereas only about 0.7% and 0.3 % give rise to an ascending spinothalamic and spinotectal collateral. Even fewer, about 0.03%, distribute ascending spinocerebellar collaterals. About 15% give rise to a collateral reaching the levels of the rostral inferior olive. Thus the majority of the ascending supraspinal collaterals are distributed to the (lower) brainstem, with only few reaching the thalamus, tectum or cerebellum.

Branching neurons projecting to the thalamus or tectum, which are relatively few in number, are mainly present in the LSN, but are also found in the lateral lamina V, lamina VIII and ventromedial lamina VII and lamina X. Only few are present in the LCN and lamina I (Chapter 6, cf. Fig. 8.3).

The sparse cervical neurons projecting both to T2 and the cerebellum are located exclusively in the central part of lamina VII at C5-T1 (Chapter 5, cf. Fig. 8.3), in keeping with electrophysiological findings in the cat [146].

The many branching neurons projecting both to T2 and to the lower brainstem appear to be mainly present in laminae IV to VII and the LSN (Chapter 4, Fig. 8.3). After injections restricted to the dorsal column nuclei branching neurons were found to be chiefly located in lamina IV and the medial laminae V and VI (Chapter 7, cf. Fig. 8.3). In these laminae about 11% of the DY-labeled descending propriospinal neurons gave rise to an (ipsilaterally) ascending collateral to the DCN. In the experiments of Chapter 4, however, up to 30% of these neurons distributed collaterals to the lower medulla. Thus branching neurons in lamina IV and the medial laminae V and VI not only project to the DCN but to other areas of the brainstem as well. Further experiments would have to be carried out to indicate the exact destination of the many ascending collaterals distributed by descending cervical propriospinal neurons to the lower brainstem. Electrophysiological and anatomical studies in the cat indicated that cervical propriospinal neurons distribute ascending collaterals to the LRN [9, 173] and to the medullary reticular formation [361].

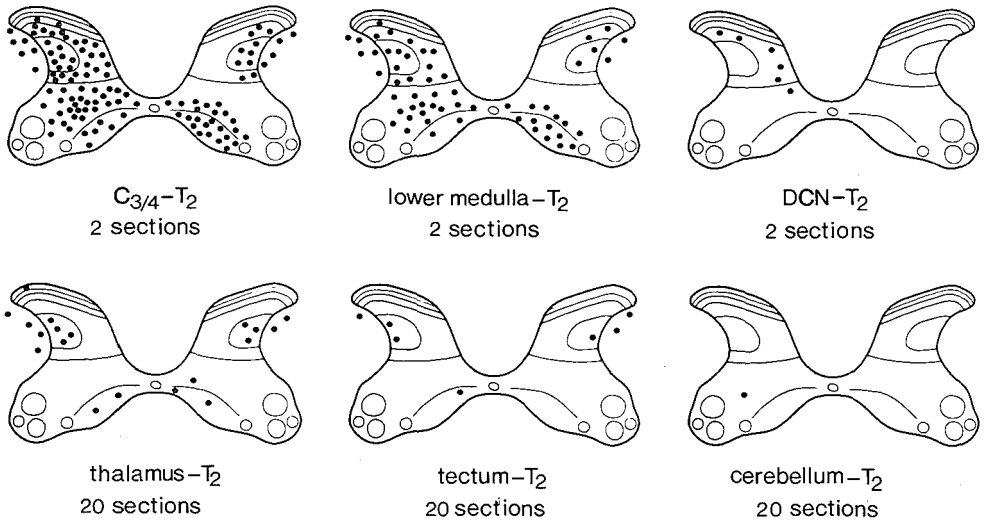

Fig. 8.3 Schematic representation of the distributions (at the level of C6) of branching neurons projecting both to T2 and to C3/4, the lower medulla (at the level of the caudal LRN), dorsal column nuclei, thalamus, tectum or cerebellum. *Left sides are ipsilateral to the injected sides.* Note numerical differences between different sets of branching neurons: the drawings represent the superimposed chartings of 2 or 20 sections; each dot represents one branching neuron.

Laminar distribution of branching neurons. The present experiments indicate that many branching neurons, projecting to T2 and to upper cervical or supraspinal levels, are present in the cervical spinal cord of the rat. They are mainly located in laminae IV to VIII and the LSN. Branching neurons in lamina I are sparse, their ascending collaterals mainly projecting to the thalamus or tectum. Lamina II does not seem to contain branching neurons. Branching neurons in lamina III are few in number and appear to project to upper cervical and medullary levels. Lamina IV and the medial laminae V and VI contain many branching neurons, many of which supply fibers to the DCN, but many also appear to project to other areas of the (lower) brainstem. The lateral lamina V, ventromedial lamina VII and lamina VIII contain a heterogeneous population of branching neurons, giving rise to some spinothalamic and spinotectal fibers but also to many spinoreticular fibers. Those in the central and lateral lamina VII mainly project to upper cervical and lower brainstem levels. A small, but perhaps important, subset of branching neurons in the central part of lamina VII gives rise to spinocerebellar fibers. A few branching neurons are present in lamina X; they project to the thalamus and tectum. In the dorsolateral funiculus, the LSN contains a large number of branching neurons, projecting both ipsilaterally and contralaterally to the thalamus, tectum and brainstem.

Possible functional significance of branching neurons. Axon-collaterals allow individual neurons to influence the activity of many other neurons, as the information transmitted via its "main axon" is also supplied to other sites via its collateral branches. These collaterals constitute anatomical pathways along which activity within various neuronal networks can be integrated. For example, collaterals can provide an "efferent copy" of the information carried by the main axon to other centers or affect their own input by providing feedback-information to "afferent" neurons. As stated by de Olmos and Heimer [307], "the axon-collateral represents a significant neuronal feature, and its importance for the integrative activities of the nervous system can hardly be overestimated". Yet, on the basis of anatomical studies it remains hard to estimate the exact functional importance of axon-collaterals; physiological studies would be needed to clarify this issue.

It is obvious from the present series of experiments that collateralization of propriospinal projections is a common feature in the rat's cervical spinal cord. Nearly all laminae contain many neurons (of many types and sizes) giving rise to such branching fibers, with the exception of laminae I and II and the LCN where they are very rare. Short and long descending propriospinal neurons, though differing in numbers and in laminar distribution, equally give rise to ascending collaterals reaching C1. Many ascending collaterals reach upper cervical levels, about two-thirds of which continue into the lower brainstem. Thus many cervical spinal neurons can be considered as propriospinal neurons as well as "tract" neurons. The great majority of the ascending collaterals does not extend beyond the lower brainstem. Propriospinal connections play an important role in the transmission of activity from descending pathways to motoneurons and in spinal intersegmental communication (cf. Chapter 2.5). Both short and long propriospinal neurons probably represent not just "passive" relay cells, but (at least in the case of laminae VII and VIII neurons) integrative neuronal centers which receive a convergent input from different sources; spinal, supraspinal and primary afferent [cf. 9, 15]. The information contained in their projections may therefore reflect the resultant of the integration of these different inputs at a given segmental level. By way of axon-collaterals intersegmental coordination of neuronal networks could be effectuated. Ascending collaterals to the lower brainstem might equally be considered to represent an extension of the "intersegmental" coordination to "supraspinal segments", as the lower brainstem reticular formation in some respects can be considered the rostral continuation of the spinal intermediate zone [219]. The ascending collaterals might also be involved in providing spinal feedback to reticulospinal neurons, or serve as a source of spinal information to such relay nuclei as the LRN or inferior olive which equally integrate afferent information from different sources. Electrophysiological findings already indicated that cervical propriospinal neurons distribute ascending collaterals to the LRN [9, 173]. The present experiments show that some ascending axon-collaterals are

distributed to the dorsal column nuclei (Chapter 7). It is of interest to note that spinal neurons projecting to the thalamus, tectum or cerebellum are largely separate from those giving rise to descending propriospinal projections. This might indicate that the spinal cord mainly provides the former areas with "straightforward" somatosensory information via its ascending spinal tracts; the "integrated" information from propriospinal neurons is not directly supplied to such areas, but might instead be relayed in "integrative" nuclei in the lower brainstem. Further experiments, employing discrete tracer injections at various sites of the lower brainstem, would be needed to determine the exact termination-areas (and therefore the possible functional significance) of the supraspinally ascending collaterals.

Finally, the *lateral spinal nucleus*, LSN, deserves special mention. Its neurons give rise to many branching fibers projecting both ipsilaterally and contralaterally to the thalamus, tectum and brainstem as well as to lower spinal, including lumbosacral, levels. Indeed the highest percentages of branching neurons were observed within this nucleus (cf. Chapters 4 and 6). The LSN (which is described in more detail in Chapter 6) is most conspicuously present in the guinea-pig and rat and appears to be functionally different from the lateral cervical nucleus, LCN, and from the adjacent dorsal horn. The LSN, therefore, constitutes a special cell group, the function of which remains to be established. Leah et al. [227] showed that peptidergic spinal neurons giving rise to ascending supraspinal fibers in the rat are located primarily in the LSN (which contained about 75% of all such neurons) and lamina X [cf. 298]. Long descending peptidergic propriospinal fibers equally originate mainly from the LSN [228]. These authors suggested that LSN neurons might not be primarily involved in conveying somatosensory information, but would rather subserve a more general role in autonomic control in target nuclei (i.e. neuropeptides contained in projection fibers may control such functions as regulation of energy metabolism and activity of enzymes involved in neurotransmitter synthesis; for references cf. 227]. The fact that the LSN, more than other areas of the cervical cord, gives rise to collateralized projections to several spinal and supraspinal levels supports such a suggestion.

SUMMARY

The spinal cord represents the interface between higher levels of the central nervous system (i.e. brainstem, diencephalon, cerebellum and cerebral hemispheres) and the peripheral nervous system. Its grey matter contains numerous nerve cells which may be involved in many different functions. Thus some spinal neurons relay peripheral sensory information, reaching the cord via afferent spinal nerves, to supraspinal levels by way of ascending fiber tracts. Other neurons transmit information in the opposite direction. The majority of spinal neurons, however, establish fiber connections within the spinal cord itself and are therefore called "propriospinal" neurons. The projections of such neurons extend over various distances between different spinal segments and enable the cord to function integratively, interconnecting local spinal neuronal networks. Much is already known about the anatomy of the different spinal projections. The literature on this subject is reviewed in *Chapter 2*. Thus various anatomical studies have indicated that spinal neurons giving rise to descending (propriospinal) projections and those giving rise to ascending (propriospinal or supraspinal) projections are located within the same areas of the spinal grey matter. This raises the question whether some of these neurons are "branching neurons" which give rise to both ascending and descending projections by way of divergent axon-collaterals.

The present study was undertaken to investigate: a) the presence, location and relative numbers of branching neurons in the rat's cervical cord and b) their rostral and caudal extent of projection.

To this purpose the retrograde fluorescent double-labeling method was applied, as described in *Chapter 3*. The cervical cord of the rat was studied after injections of two different fluorescent retrograde tracers at levels rostral and caudal to the cervical cord. Neurons giving rise to ascending projections, to descending projections, or to both ascending and descending projections could thus be studied simultaneously. In describing the location of labeled neurons Rexed's cytoarchitectonic laminar scheme [328, 329] was applied. As this scheme was originally proposed for the cat's spinal cord, a preliminary study was made of the spinal cytoarchitecture in the rat (*Chapter 2.1*).

In *Chapter 4* the first series of experiments is described. The retrograde tracer "True Blue" (TB) was injected unilaterally at C1, whereas "Diamidino Yellow" (DY) was injected at T2. The segments C3-C8 were found to contain many labeled neurons, both single- and double-labeled. Neurons with ascending projections to C1 or beyond and neurons with descending projections to T2 or beyond were mainly located in lamina I, laminae IV to VIII and in the lateral spinal nucleus, LSN, with fewer labeled neurons being present in laminae II and III. The distributions of the

"ascending" and "descending" neurons overlapped extensively. Moreover, a significant proportion (20% to 30%) of both populations were labeled by both tracers ("double-labeled"). Such double-labeled neurons constitute **branching neurons** giving rise to both ascending and descending axon-collaterals. They were mainly located in laminae IV to VIII and the LSN.

Further experiments were designed to study the extent of projection of the axon-collaterals of branching cervical neurons.

The **caudal extent of projection** was investigated by combining TB-injections at C1 with DY-injections at T6 or progressively more caudal levels (*Chapter 4*). Descending propriospinal neurons reaching low thoracic or lumbosacral levels were mainly located in lamina VIII and the ventromedial lamina VII, but also in the lateral laminae IV to VI, the LSN and lamina I. With increasingly more caudal injections the numbers of descending propriospinal neurons reaching the injected segment decreased. The number of descending collaterals reaching caudal spinal levels equally decreased. Thus 20 % of the C3-C8 neurons projecting to C1 or above had a descending collateral reaching T2, whereas 8%, 3% and 1% gave rise to a collateral reaching T9, L2 and S1, respectively. Branching neurons projecting to low spinal levels were present in laminae VII and VIII, the lateral laminae IV to VI and the LSN.

The **rostral extent of projection** was investigated by combining DY-injections at T2 with TB-injections at different cervical or brainstem levels (*Chapter 4*). Neurons projecting to upper cervical or lower brainstem levels were mainly located in lamina I, laminae IV to VIII and the LSN. Of the C5-C8 neurons projecting to T2 or below about 42% gave rise to an ascending collateral reaching C3/C4, whereas 29% had an ascending collateral reaching C1. Of the C3-C8 neurons projecting to T2 or below about 30% gave rise to an ascending collateral passing into the medulla oblongata; about half of these collaterals reached the level of the rostral part of the inferior olive. The branching neurons were located in laminae IV to VIII and the LSN. From these experiments it followed that the majority of the ascending collaterals reach supraspinal levels. Further experiments were carried out to investigate whether any of the ascending collaterals project to the cerebellum, thalamus, tectum or dorsal column nuclei.

In the experiments of *Chapter 5*, TB was injected in the cerebellum and DY-injections were made at T2. TB-labeled spinocerebellar neurons were concentrated in the central cervical nucleus (CCN) at C1-C4, in the central part of lamina VII at C5-T1, in the medial part of lamina VI and the adjoining dorsomedial part of lamina VII at C2/C3-T1, and in Clarke's column. They were also found in lamina V at C1 and C7-T1, and in lamina VIII at all levels. Only very few double-labeled neurons were observed; in C1-T1, only about 0.5% of all TB-labeled spinocerebellar neurons and about 0.05% of all DY-labeled descending propriospinal neurons were double-

labeled. The double-labeled neurons were all located centrally in lamina VII at C5-T1, but even in that area they constituted not more than 2.5% of the labeled spinocerebellar neurons. In order to allow for a comparison with electrophysiological findings in the cat [146], similar double-labeling experiments were performed in 3 cats. The results in the cat were comparable to those in the rat.

In the experiments of *Chapter 6*, TB was injected in the thalamus or dorsal midbrain, whereas DY was injected at T2. The TB-labeled spinothalamic and spinotectal neurons were located in lamina I, the LSN, the lateral lamina V, lamina VIII and the adjacent ventromedial lamina VII, and lamina X, predominantly contralateral to the TB-injections. At upper cervical levels, such neurons were also present bilaterally throughout lamina VII, and contralaterally in the spinal extensions of the dorsal column nuclei (sDCN) and the lateral cervical nucleus, LCN. Double-labeled neurons were present throughout C1-C8 in the LSN, lateral lamina V, lamina VIII, ventromedial lamina VII, and lamina X. Only very few were observed in lamina I and the LCN, and none in the sDCN. The double-labeled neurons constituted only a minor fraction of all labeled neurons. Thus, about 3-5% of the spinothalamic neurons and about 1-7% of the spinotectal neurons were double-labeled. Conversely, only about 1% of the labeled descending propriospinal neurons were found to give rise to an ascending spinothalamic collateral, and even fewer (0.1 to 0.6%) to a collateral to the dorsal midbrain.

In the experiments of *Chapter 7*, TB-injections were made in the dorsal medulla, centered on the dorsal column nuclei, and DY was again injected at T2. TB-labeled neurons were concentrated in lamina IV and the medial parts of laminae V and VI, ipsilateral to the TB-injection. These probably constitute neurons projecting to the dorsal column nuclei (postsynaptic dorsal column -PSDC- neurons). In all cases, DY-TB double-labeled neurons were found. In the ipsilateral lamina IV and the medial laminae V and VI, about 10% of the PSDC neurons were found to give rise to a descending collateral to T2 or below, whereas about 11% of the descending propriospinal neurons in these laminae gave rise to an ascending collateral to the dorsal column nuclei.

Summarizing, the present study indicates that the rat's cervical spinal cord contains many branching neurons which give rise to ascending and descending collaterals. Such branching neurons are present in all laminae, except laminae II and IX, and in the LSN. The *descending collaterals* extend to various spinal levels, including the lumbosacral cord. Yet, most of them terminate at shorter distances from their parent cell bodies, i.e. in the upper thoracic cord. The *ascending collaterals* are not only distributed to the upper cervical cord but to supraspinal levels as well. In fact, the majority of them seem to terminate in the lower brainstem, some of them projecting to the dorsal column nuclei. Only few branching neurons distribute ascending collaterals to the thalamus, tectum or cerebellum.

SAMENVATTING

De elementaire bouwstenen van het zenuwstelsel zijn de zenuwcellen, of neuronen. Ieder neuron bevat een cellichaam, met daarin de celkern, en een zenuwuitloper of axon. De axonen vormen de bedrading waarlangs de zenuwcellen informatie aan elkaar doorgeven. Sommige axonen geven zijtakken, of collateralen, af waardoor één axon contact kan maken met meerdere cellen. Het zenuwstelsel is onder te verdelen in een "centraal" gedeelte, gelegen in de schedel en het wervelkanaal, en een "perifeer" gedeelte, het deel buiten het wervelkanaal (bestaande uit zenuwvezels van en naar de huid, de spieren en de inwendige organen). Het ruggemerg[12], onderwerp van dit onderzoek, vormt de schakel tussen de zogenaamde "hogere" niveau's van het centrale zenuwstelsel (kleine hersenen, hersenstam, diencephalon of "tussenhersenen", grote hersenen) en het perifere zenuwstelsel. Het ruggemerg is onder te verdelen in een aantal hals- ("cervicale"), borst- ("thoracale"), lenden- ("lumbale"), heiligbeen- ("sacrale") en staart- ("coccygeale") segmenten. Zo zijn er bij de rat 8 cervicale segmenten (genummerd van C1 tot C8), 13 thoracale (T1-T13), 6 lumbale (L1-L6), 4 sacrale (S1-S4) en 3 coccygeale (Coc1-Coc3) segmenten te onderscheiden. Ieder segment geeft een aantal zenuwvezels af naar de spieren en de inwendige organen van een daarmee corresponderend deel van het lichaam. Op dwarsdoorsnede (zie Figuur 2.1) bestaat het ruggemerg uit een buitenste deel, de "witte stof", welke de zenuw*vezels* of axonen bevat, en een binnenste gedeelte, de "grijze stof", welke de zenuw*cellen* of neuronen bevat. In het ruggemerg bevinden zich zeer veel zenuwcellen die met elkaar meerdere verschillende functies kunnen uitoefenen. Zo zijn er spinale neuronen die de perifere informatie, welke het ruggemerg bereikt via aanvoerende spinale zenuwen, doorgeven aan hogere niveau's via opstijgende zenuwbanen. Andere neuronen geven juist informatie door in omgekeerde richting; van hogere niveau's naar de periferie. Het merendeel van de spinale neuronen, echter, onderhoudt vezelverbindingen binnen het ruggemerg zelf; zulke zenuwcellen heten daarom "proprio-spinale" (proprius = eigen) neuronen. De vezelprojecties van de propriospinale neuronen strekken zich over verschillende afstanden uit tussen verschillende ruggemergssegmenten. Deze verbindingen stellen het ruggemerg in staat om als één geheel en gecoordineerd te functioneren, doordat ze locale spinale celcircuits onderling verbinden. Er is uit vroeger onderzoek al veel bekend over de anatomie van de verschillende spinale verbindingen. Een overzicht van de betreffende literatuur wordt in *Hoofdstuk 2* gegeven. Zo blijkt uit vroegere anatomische studies dat spinale neuronen met opstijgende projecties en die met

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Voetnoot 12. officieel geheten: "medulla spinalis"; daarom worden de zenuwvezels en -cellen van het ruggemerg ook wel "spinale" zenuwen en cellen genoemd.

afdalende projecties beide gelegen zijn in dezelfde gebieden van de grijze stof. Dit roept de vraag op of misschien sommige van deze neuronen zowel opstijgende als afdalende projecties afgeven via vertakkende axoncollateralen (zulke neuronen zullen voor het gemak, hoewel feitelijk onjuist, "vertakkende neuronen" genoemd worden).

Het huidige onderzoek werd opgezet in de rat om na te gaan:

a) of er zich in het cervicale ruggemerg vertakkende neuronen bevinden en, zo ja, waar en in welke aantallen deze voorkomen

b) hoever naar rostraal[13] (d.w.z. naar het allerbovenste deel van het ruggemerg en naar hogere niveau's) of naar caudaal (d.w.z. naar de lagere delen van het ruggemerg) de axoncollateralen van vertakkende cellen zich uitstrekken.

Om dit te onderzoeken werd de "retrograde fluorescente dubbel-labeling methode", zoals beschreven in *Hoofdstuk 3* en geillustreerd in Fig. 3.1, toegepast. Er werd gebruik gemaakt van fluorescerende merkstoffen welke getransporteerd worden vanaf de zenuwuiteinden naar de bijbehorende cellichamen, d.w.z. in teruggaande ("retrograde") richting. De oorsprongscellen van de zenuwvezels worden aldus gemerkt ("gelabeld") door de fluorescerende stoffen. Twee verschillende retrograde merkstoffen werden geinjecteerd op twee niveau's, namelijk rostraal en caudaal van het cervicale ruggemerg. Vervolgens werden plakjes van het cervicale merg van de proefdieren bestudeerd onder het fluorescentiemicroscoop. Neuronen met opstijgende projecties, met afdalende projecties, en cellen met zowel opstijgende als afdalende projecties konden zo elk afzonderlijk en tegelijkertijd waargenomen worden. Bij het beschrijven van de localisatie van de gelabelde neuronen werd uitgegaan van het cytoarchitectonische schema van Rexed [328, 329]. Deze onderscheidde op basis van de vorm, grootte en rangschikking van cellen (cyto-architectuur) een tiental lagen, of "laminae", in het ruggemerg (zie Fig. 2.1). Dit schema, dat door de meeste onderzoekers gehanteerd wordt, werd indertijd door Rexed voorgesteld voor het ruggemerg van de kat. Daarom werd allereerst nagegaan of deze indeling ook toepasbaar was in de rat (wat zo blijkt te zijn, zoals beschreven in *Hoofdstuk 2.1*).

In *Hoofdstuk 4* wordt de eerste serie experimenten beschreven. De retrograde fluorescerende merkstof "True Blue" (TB) werd geinjecteerd in één helft van het segment C1, terwijl "Diamidino Yellow" (DY) ingespoten werd in T2. In de tussenliggende segmenten C3-C8 werden veel gelabelde neuronen gevonden, zowel

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Voetnoot 13. Enkele anatomische termen, welke richting of positie aangeven en in dit hoofdstuk veel gebruikt worden, zijn:
rostraal = naar de "kop" toe
caudaal = naar de "staart" toe
ventraal = naar de buikzijde toe
dorsaal = naar de rugzijde toe
lateraal = aan de "buitenzijde", van de middellijn af
mediaal = aan de "binnenzijde", naar de middellijn toe
Deze termen worden ook in samenstelling gebruikt, b.v. ventromediaal: naar de middellijn en naar de buikzijde toe gelegen.

enkel-gelabeld (één kleurstof, TB of DY, bevattend) als dubbel-gelabeld (zowel TB als DY bevatttend). TB-gelabelde neuronen met opstijgende projecties naar C1 of daar voorbij en DY-gelabelde neuronen met afdalende projecties naar T2 of daar voorbij bevonden zich voornamelijk in lamina I, laminae IV tot VIII en in de laterale spinale kern (LSN = "lateral spinal nucleus"). De verdelingen van "opstijgende" en "afdalende" neuronen over de grijze stof waren grotendeels hetzelfde. Bovendien was een aanzienlijk deel (20% tot 30%) van beide celpopulaties gekleurd door beide merkstoffen (dubbel-gelabeld). Zulke dubbel-gelabelde neuronen vertegenwoordigen **vertakkende neuronen** die zowel opstijgende als afdalende axoncollateralen afgeven. Deze cellen waren voornamelijk gelocaliseerd in laminae IV tot VIII en in de LSN.

Verdere experimenten werden verricht om na te gaan over welke afstanden de axoncollateralen van vertakkende cervicale neuronen projecteren.

De **caudale projectie van afdalende collateralen** werd onderzocht door TB-injecties in C1 te combineren met DY-injecties in T6 of op steeds lagere, meer caudaal gelegen, niveau's (*Hoofdstuk 4*). Propriospinale neuronen met afdalende vezels tot aan laag-thoracale of lumbosacrale ruggemergssegmenten waren voornamelijk gelegen in lamina VIII en het aangrenzende ventromediale deel van lamina VII, maar ook in de laterale delen van laminae IV tot VI, in de LSN en in lamina I. Wanneer de DY-injecties steeds meer caudaal gemaakt werden, nam het aantal cervicale neuronen met afdalende propriospinale projecties naar het geinjecteerde segment af. Het aantal afdalende collateralen dat caudale spinale niveau's bereikte nam gelijkelijk af. Zo gaf 20% van de neuronen in C3-C8 met een projectie naar C1 of daar voorbij een afdalende collateraal af naar T2, terwijl 8%, 3% en 1% een collateraal afgaf naar, respectievelijk, T9, L2 en S1. Vertakkende cervicale neuronen met projecties naar de onderste ruggemergssegmenten werden gevonden in laminae VII en VIII, de laterale laminae IV, V en VI, en in de LSN.

De **rostrale projectie van de opstijgende collateralen** werd nagegaan door DY-injecties in T2 te combineren met TB-injecties op verschillende cervicale- of hersenstam-niveau's (*Hoofdstuk 4*). Neuronen met projecties naar het hoog-cervicale merg of het onderste deel van de hersenstam waren voornamelijk gelegen in lamina I, laminae IV tot VIII, en de LSN. Van de neuronen in C5-C8 met projecties naar of voorbij T2 gaf ongeveer 42% een opstijgende collateraal af naar C3/C4, terwijl 29% een opstijgende collateraal naar C1 afgaf. Van de neuronen in C3-C8 met projecties naar of voorbij T2 gaf ongeveer 30% een collateraal af die opstijgt in het verlengde merg; ruwweg de helft van deze collateralen bereikt het niveau van het rostrale deel van de oliva inferior (een celgroep in het onderste deel van de hersenstam). De vertakkende neuronen met opstijgende vezelprojecties naar de hersenstam waren gelegen in laminae IV tot VIII en de LSN. Uit deze groep experimenten bleek dat de meerderheid van de opstijgende collateralen "hogere" of "supraspinale" (supra-spinaal = "boven" het ruggemerg gelegen) niveau's bereikt. Er werd verder onderzoek

verricht om na te gaan of deze opstijgende collateralen al dan niet zouden projecteren naar specifieke celgroepen in de hersenstam, te weten de thalamus, het tectum en de dorsale kolom kernen, of naar de kleine hersenen.

In de experimenten van *Hoofdstuk 5* werd TB geinjecteerd in de kleine hersenen, het cerebellum (een structuur onder meer betrokken bij het verwerken van informatie over positie en houding van het lichaam en de ledematen). DY werd ingespoten in T2. TB-gelabelde neuronen met projecties naar het cerebellum (spinocerebellaire neuronen) waren geconcentreerd in een aantal groepen, nl. de centrale cervicale kern in C1-C4, het centrale deel van lamina VII in C5-T1, het mediale deel van lamina VI en het aangrenzende dorsomediale deel van lamina VII in C2/C3-T1, en in de zogeheten kern van Clarke. Spinocerebellaire neuronen werden ook aangetroffen in lamina V in C1 en C7-T1, en in lamina VIII in C1-T1. Er werden maar heel weinig dubbel-gelabelde neuronen waargenomen: in de segmenten C1 tot T1 waren slechts 0.5% van alle TB-gelabelde spinocerebellaire neuronen en ongeveer 0.05% van alle DY-gelabelde afdalende propriospinale neuronen dubbel-gelabeld. Deze neuronen waren uitsluitend gelegen in het centrale deel van lamina VII in C5-T1, maar zelfs in dat gebied vormden zij niet meer dan een klein deel, ongeveer 2.5%, van alle gelabelde spinocerebellaire neuronen. De dubbel-labeling experimenten werden herhaald in een drietal katten, om een vergelijking te kunnen maken met een ander onderzoek [146], dat ook in de kat, maar met een andere (namelijk electrofysiologische) techniek verricht was. De dubbel-labeling resultaten in de kat waren vergelijkbaar met die in de rat.

In *Hoofdstuk 6* worden experimenten beschreven, verricht om na te gaan of opstijgende collateralen projecteren naar de thalamus of het tectum (twee structuren die betrokken zouden zijn bij het verwerken van informatie betreffende "pijn"). TB werd ingespoten in de thalamus of het tectum, terwijl DY geinjecteerd werd in T2. De TB-gelabelde spinothalamische en spinotectale neuronen waren gelegen in lamina I, de LSN, het laterale deel van lamina V, lamina VIII en de aangrenzende ventromediale lamina VII, en lamina X. Deze cellen lagen voornamelijk "gekruist" (contralateraal) ten opzichte van de injecties. In C1 en C2 werden dergelijke neuronen eveneens aangetroffen in alle delen van lamina VII aan beide zijden van het ruggemerg. Ook werden zij gevonden in de zogenaamde "spinale uitbreiding van de dorsale kolom kernen" (sDCN) en in de laterale cervicale kern (LCN), contralateraal ten opzichte van de injecties. DY-TB dubbel-gelabelde neuronen in C1-C8 bevonden zich in de LSN, laterale lamina V, lamina VIII, ventromediale lamina VII en lamina X. Slechts weinig werden waargenomen in lamina I en de LCN, en geen enkele in de sDCN. De dubbel-gelabelde neuronen omvatten slechts een klein deel van alle gelabelde cellen. Zo waren ongeveer 3-5% van de spinothalamische neuronen en ongeveer 1-7% van de spinotectale neuronen dubbel-gelabeld. Omgekeerd gaf slechts 1% van de gelabelde afdalende propriospinale

cellen een opstijgende spinothalamische collateraal af, en nog minder (0.1 tot 0.6%) cellen geven een collateraal af naar het tectum.

In de experimenten van *Hoofdstuk 7* werden TB-injecties geplaatst in het dorsale deel van het verlengde merg, gecentreerd op de dorsale kolom kernen (betrokken bij het verwerken van informatie betreffende "aanraking"). DY werd wederom ingespoten in T2. TB-gelabelde neuronen waren geconcentreerd in lamina IV en de mediale delen van laminae V en VI. Deze cellen lagen voornamelijk "ongekruist" (ipsilateraal) ten opzichte van de injecties. Het grootste deel van deze cellen bestaat waarschijnlijk uit neuronen die naar de dorsale kolom kernen projecteren, zogenaamde postsynaptische dorsale kolom (PSDC) neuronen. In alle experimenten van deze groep werden DY-TB dubbel-gelabelde neuronen gevonden. In de ipsilaterale lamina IV en mediale laminae V-VII bleek ongeveer 10% van de PSDC neuronen een afdalende collateraal naar of voorbij T2 af te geven, terwijl ongeveer 11% van de afdalende propriospinale neuronen in deze laminae een opstijgende collateraal naar de dorsale kolom kernen afgeeft.

Concluderend: het huidige onderzoek toont aan dat er zich in het cervicale ruggemerg van de rat veel "vertakkende" neuronen bevinden die opstijgende en afdalende axoncollateralen afgeven. Dergelijke neuronen zijn aanwezig in alle laminae, behalve laminae II en IX, en, met name, in de laterale spinale kern. De *afdalende collateralen* projecteren naar diverse spinale niveau's, inclusief het lumbosacrale ruggemerg. De meerderheid echter geeft hun zenuweindigingen af op korte afstand van de oorsprongscellen, d.w.z. in het bovenste thoracale ruggemerg. De *opstijgende collateralen* projecteren niet alleen naar de bovenste cervicale segmenten, maar ook naar supraspinale niveau's. De meerderheid lijkt eindigingen te hebben in het onderste deel van de hersenstam; een deel hiervan projecteert naar de dorsale kolom kernen. Slechts weinig vertakkende neuronen geven opstijgende collateralen af naar de thalamus, het tectum of het cerebellum.

REFERENCES

1. **Abrahams VC** (1971) Spino-spinal mechanisms in the chloralose anaesthetized cat. J. Physiol. 215: 755-768
2. **Abrahams VC, Swett JE** (1986) The pattern of spinal and medullary projections from a cutaneous nerve and a muscle nerve of the forelimb of the cat: a study using the transganglionic transport of HRP. J. Comp. Neurol. 246: 70-84
3. **Abzug C, Maeda M, Peterson BW, Wilson VJ** (1973) Branching of individual lateral vestibulospinal axons at different spinal cord levels. Brain Res. 56: 327-330
4. **Abzug C, Maeda M, Peterson BW, Wilson VJ** (1974) Cervical branching of lumbar vestibulospinal axons. J. Physiol. (London) 243: 495-522
5. **Albanese A, Bentivoglio M** (1982) Retrograde fluorescent neuronal tracing combined with acetylcholinesterase histochemistry. J. Neurosci. Meth. 6: 121-127
6. **Albe-Fessard D, Levante A, Lamour Y** (1974a) Origin of spinothalamic and spinoreticular pathways in cats and monkeys. Adv. Neurol. 4: 157-166
7. **Albe-Fessard D, Levante A, Lamour Y** (1974b) Origin of spino-thalamic tract in monkeys. Brain Res. 65: 503-509
8. **Albe-Fessard D, Boivie J, Grant G, Levante A** (1975) Labelling of cells in the medulla oblongata and the spinal cord of the monkey after injections of horseradish peroxidase in the thalamus. Neurosci. Lett. 1: 75-80
9. **Alstermark B, Lindström S, Lundberg A, Sybirska E** (1981a) Integration in descending motor pathways controlling the forelimb in the cat. 8. Ascending projection to the lateral reticular nucleus from C3-C4 propriospinal neurones also projecting to forelimb motoneurones. Exp. Brain Res. 42: 282-298
10. **Alstermark B, Lundberg A, Norrsell U, Sybirska E** (1981b) Integration in descending motor pathways controlling the forelimb in the cat. 9. Differential behavioural effects after spinal cord lesions interrupting defined pathways from higher centres to motoneurones. Exp. Brain Res. 42: 299-318
11. **Alstermark B, Lundberg A, Sasaki S** (1984) Integration in descending motor pathways controlling the forelimb in the cat. 12. Interneurones which may mediate descending feed-forward inhibition and feed-back inhibition from the forelimb to C3-C4 propriospinal neurones. Exp. Brain Res. 56: 308-322
12. **Alstermark B, Sasaki S** (1985) Integration in descending motor pathways controlling the forelimb in the cat. 13. Corticospinal effects in shoulder, elbow, wrist and digit motoneurones. Exp. Brain Res. 59: 353-364
13. **Alstermark B, Kümmel H** (1986) Transneuronal labelling of neurones projecting to forelimb motoneurones in cats performing different movements. Brain Res. 376: 387-391
14. **Alstermark B, Kümmel H, Pinter MJ, Tantisera B** (1987a) Branching and termination of C3-C4 propriospinal neurones in the cervical spinal cord of the cat. Neurosci. Lett. 74: 291-296
15. **Alstermark B, Lundberg A, Pinter M, Sasaki S** (1987b) Subpopulations and functions of long C3-C5 propriospinal neurons. Brain Res. 404: 395-400
16. **Ammann B, Gottschall J, Zenker W** (1983) Afferent projections from the rat longus capitis muscle studied by transganglionic transport of HRP. Anat. Embryol. 166: 275-289
17. **Ammons WS, Girardot MN, Foreman RD** (1986) Periventricular gray inhibition of thoracic spinothalamic cells projecting to medial and lateral thalamus. J. Neurophysiol. 55: 1091-1103
18. **Anderson RF** (1943) Cerebellar distribution of the dorsal and ventral spino-cerebellar tracts in the white rat. J. Comp. Neurol. 79: 415-423
19. **Anderson FD, Berry CM** (1959) Degeneration studies of long ascending fiber systems in the cat brain stem. J. Comp. Neurol. 111: 195-229
20. **Andrezik JA, Chan-Palay V, Palay SL** (1981) The nucleus paragigantocellularis lateralis in the rat. Demonstration of afferents by the retrograde transport of horseradish peroxidase. Anat. Embryol. 161: 373-390
21. **Angaut-Petit D** (1975) The dorsal column system: II. Functional properties and bulbar relay of the postsynaptic fibres of the cat's fasciculus gracilis. Exp. Brain Res. 22: 471-493
22. **Antonetty CM, Webster KE** (1975) The organisation of the spinotectal projection. An experimental study in the rat. J. Comp. Neurol. 163: 449-466
23. **Aoki M, McIntyre AK** (1975) Cortical and long spinal actions on lumbosacral motoneurones in the cat. J. Physiol. (London) 251: 569-587
24. **Apkarian AV, Stevens RT, Hodge CJ** (1985) Funicular location of ascending axons of lamina I cells in the cat spinal cord. Brain Res. 334: 160-164

25. **Applebaum AE, Beall JE, Foreman RD, Willis WD** (1975) Organization and receptive fields of primate spinothalamic tract neurons. J. Neurophysiol. 38: 572-586
26. **Applebaum AE, Leonard RB, Kenshalo DR Jr, Martin RF, Willis WD** (1979) Nuclei in which functionally identified spinothalamic tract neurons terminate. J. Comp. Neurol. 188: 575-586
27. **Armstrong DM, Child RF** (1980) Location in the spinal cord of neurons projecting directly to the inferior olive in the cat. In: Courville J (Ed.) The inferior olivary nucleus: anatomy and physiology, Raven press, New York, pp. 125-143
28. **Baker ML, Giesler GJ Jr** (1984) Anatomical studies on the spinocervical tract of the rat. Somatosens. Res. 2: 1-18
29. **Barber RP, Vaughn JE, Slemmon JR, Salvaterra PM, Roberts E, Leeman SE** (1979) The origin, distribution and synaptic relationships of substance P axons in rat spinal cord. J. Comp. Neurol. 184: 331-352
30. **Basbaum AI, Fields HL** (1979) The origin of descending pathways in the dorsolateral funiculus of the spinal cord of the cat and rat. Further studies on the anatomy of pain modulation. J. Comp. Neurol. 187: 513-532
31. **Beall JE, Martin RF, Applebaum AE, Willis WD** (1976) Inhibition of primate spinothalamic tract neurons by stimulation in the region of the nucleus raphe magnus. Brain Res. 114: 328-333
32. **Beitz AJ** (1982) The organization of afferent projections to the midbrain periaqueductal gray of the rat. Neuroscience 7: 133-159
33. **Bennett GJ, Seltzer Z, Lu GW, Nishikawa N, Dubner R** (1983) The cells of origin of the dorsal column postsynaptic projection in the lumbosacral enlargements of cats and monkeys. Somatosens. Res. 1: 131-149
34. **Bennett GJ, Nishikawa N, Lu GW, Hoffert MJ, Dubner R** (1984) The morphology of dorsal column postsynaptic spinomedullary neurons in the cat. J. Comp. Neurol. 224: 568-578
35. **Bentivoglio M, Kuijpers HGJM, Catsman-Berrevoets CE, Dann O** (1979a) Fluorescent retrograde neuronal labeling in rat by means of substances binding specifically to adenine-thymine rich DNA. Neurosci. Lett. 12: 235-240
36. **Bentivoglio M, van der Kooy D, Kuijpers HGJM** (1979b) The organization of the efferent projections of the substantia nigra in the rat. A retrograde fluorescent double labeling study. Brain Res. 174: 1-17
37. **Bentivoglio M, Kuijpers HGJM, Catsman-Berrevoets CE** (1980a) Retrograde neuronal labeling by means of bisbenzimide and nuclear yellow (Hoechst S769121). Measures to prevent diffusion of the tracers out of retrogradely labeled neurons. Neurosci. Lett. 18: 19-24
38. **Bentivoglio M, Kuijpers HGJM, Catsman-Berrevoets CE, Loewe H, Dann O** (1980b) Two new fluorescent retrograde neuronal tracers which are transported over long distances. Neurosci. Lett. 18: 25-30
39. **Bentivoglio M, Molinari M** (1982) Fluorescent retrograde triple labeling of brain stem reticular neurons with ascending and spinal projections. Neurosci. Lett. Suppl. 10: S71
40. **Bentivoglio M, Rustioni A** (1986) Corticospinal neurons with branching axons to the dorsal column nuclei in the monkey. J. Comp. Neurol. 253: 260-276
41. **Berkley KJ, Blomqvist A, Pelt A, Flink R** (1980) Differences in the collateralization of neuronal projections from the dorsal column nuclei and lateral cervical nucleus to the thalamus and tectum in the cat: an anatomical study using two different double-labeling techniques. Brain Res. 202: 273-290
42. **Besson JM, Chaouch A** (1987) Peripheral and spinal mechanisms of nociception. Physiol. Rev. 67: 67-186
43. **Bharos TB, Kuijpers HGJM, Lemon RN, Muir RB** (1981) Divergent collaterals from deep cerebellar neurons to thalamus and tectum, and to medulla oblongata and spinal cord: retrograde fluorescent and electrophysiological studies. Exp. Brain Res. 42: 399-410
44. **Björkeland M, Boivie J** (1984) The termination of spinomesencephalic fibers in cat. An experimental anatomical study. Anat. Embryol. 170: 265-277
45. **Björklund A, Skagerberg G** (1979) Simultaneous use of retrograde fluorescent tracers and fluorescence histochemistry for convenient and precise mapping of monoaminergic projections and collateral arrangements in the CNS. J. Neurosci. Meth. 1: 261-277
46. **Blomqvist A, Flink R, Bowsher D, Griph S, Westman J** (1978) Tectal and thalamic projections of dorsal column and lateral cervical nuclei: a quantitative study in the cat. Brain Res. 141: 335-341
47. **Boivie J** (1971) The termination of the spinothalamic tract in the cat. An experimental study with silver impregnation methods. Exp. Brain Res. 12: 331-353
48. **Boivie J** (1979) An anatomical reinvestigation of the termination of the spinothalamic tract in the monkey. J. Comp. Neurol. 186: 343-370

49. **Bold EJ, Castro AJ, Neafsey EJ** (1984) Cytoarchitecture of the dorsal thalamus of the rat. Brain Res. Bull. 12: 521-527
50. **Bowsher D** (1957) Termination of the central pain pathway in man: the conscious appreciation of pain. Brain 60: 606-622
51. **Bresnahan JC, Ho RH, Beattie MS** (1984) A comparison of the ultrastructure of substance P and enkephalin-immunoreactive elements in the nucleus of the dorsal lateral funiculus and laminae I and II of the rat spinal cord. J. Comp. Neurol. 229: 497-511
52. **Brichta AM, Grant G** (1985) Cytoarchitectural organization of the spinal cord. In: Paxinos G (Ed.), The rat nervous system. Academic Press Australia, pp.293-301
53. **Brodal A, Angaut P** (1967) The termination of spinovestibular fibres in the cat. Brain Res. 5: 494-500
54. **Brown AG, Fyffe REW, Noble R, Rose PK, Snow PJ** (1980) The density, distribution and topographical organization of spinocervical tract neurones in the cat. J. Physiol. (London) 300: 409-428
55. **Brown AG** (1981) Organization in the spinal cord. Springer-Verlag, Berlin Heidelberg New-York
56. **Brown AG, Fyffe REW** (1981) Form and function of dorsal horn neurons with axons ascending the dorsal columns in cat. J. Physiol. (London) 321: 31-47
57. **Brown AG** (1982) The dorsal horn of the spinal cord. Quart. J. Exp. Physiol. 67: 193-212
58. **Brown AG, Brown PB, Fyffe REW, Pubols LM** (1983) Receptive field organization and response properties of spinal neurones with axons ascending the dorsal columns in the cat. J. Physiol. (London) 337: 575-588
59. **Brown LT Jr** (1971) Projections and termination of the corticospinal tract in rodents. Exp. Brain Res. 13: 432-450
60. **Brown LT** (1974) Rubrospinal projections in the rat. J. Comp. Neurol. 154: 169-188
61. **Brown PB, Fuchs JL** (1975) Somatotopic representation of hindlimb skin in cat dorsal horn. J. Neurophysiol. 38: 1-9
62. **Brushart TM, Mesulam MM** (1980) Transganglionic demonstration of central sensory projections from skin and muscle with HRP-lectin conjugates. Neurosci. Lett. 17: 1-6
63. **Bryan RN, Trevino DL, Coulter JD, Willis WD** (1973) Location and somatotopic organization of the cells of origin of the spino-cervical tract. Exp. Brain Res. 17: 177-189
64. **Bryan RN, Coulter JD, Willis WD** (1974) Cells of origin of the spinocervical tract in the monkey. Exp. Neurol. 42: 574-586
65. **Burstein R, Cliffer KD, Giesler GJ Jr** (1990) Cells of origin of the spinohypothalamic tract in the rat. J. Comp. Neurol. 291: 329-344
66. **Burton H, Loewy AD** (1977) Projections to the spinal cord from medullary somatosensory relay nuclei. J. Comp. Neurol. 173: 773-792
67. **Cabot JB, Reiner A, Bogan N** (1982) Avian bulbospinal pathways: anterograde and retrograde studies of cells of origin, funicular trajectories and laminar terminations. In: Kuijpers HGJM, Martin GF (Eds.), Descending pathways to the spinal cord, Progress in brain research, Vol. 57, Elsevier biomedical press, pp 79-108
68. **Cajal S Ramón y** (1909) Histologie du Système Nerveux de l'Homme et des Vertébrés, Vol. I, Azoulay L (transl.), Instituto Ramón y Cajal, Madrid, 1952
69. **Carstens E, Trevino DL** (1978a) Laminar origins of spinothalamic projections in the cat as determined by the retrograde transport of horseradish peroxidase. J. Comp. Neurol. 182: 151-166
70. **Carstens E, Trevino DL** (1978b) Anatomical and physiological properties of ipsilaterally projecting spinothalamic neurons in the second cervical segment of the cat's spinal cord. J. Comp. Neurol. 182: 167-184
71. **Catsman-Berrevoets CE, Lemon RN, Verburgh CA, Bentivoglio M, Kuijpers HGJM** (1980) Absence of callosal collaterals derived from rat corticospinal neurons. A study using fluorescent retrograde tracing and electrophysiological techniques. Exp. Brain Res. 39: 433-440
72. **Catsman-Berrevoets CE, Kuijpers HGJM** (1981) A search for corticospinal collaterals to thalamus and mesencephalon by means of multiple retrograde fluorescent tracers in cat and rat. Brain Res. 218: 15-33
73. **Cechetto DF, Standaert DG, Saper CB** (1985) Spinal and trigeminal dorsal horn projections to the parabrachial nucleus in the rat. J. Comp. Neurol. 240: 153-160
74. **Cervero F, Iggo A, Molony V** (1977) Responses of spinocervical tract neurones to noxious stimulation of the skin. J. Physiol. (London) 300: 409-428
75. **Cervero F, Iggo A, Molony V** (1979) Ascending projections of nociceptor-driven lamina I neurones in the cat. Exp. Brain Res. 35: 135-149
76. **Cervero F** (1983) Supraspinal connections of neurones in the thoracic spinal cord of the cat:

ascending projections and effects of descending impulses. Brain Res. 275: 251-261
77. **Cervero F, Connell LA** (1984) Fine afferent fibers from viscera do not terminate in the substantia gelatinosa of the thoracic spinal cord. Brain Res. 294: 370-374
78. **Chaouch A, Menétrey D, Binder D, Besson JM** (1983) Neurons at the origin of the medial component of the bulbopontine spinoreticular tract in the rat: an anatomical study using horseradish peroxidase retrograde transport. J. Comp. Neurol. 214: 309-320
79. **Cheek MD, Rustioni A, Trevino DL** (1975) Dorsal column nuclei projections to the cerebellar cortex in cats as revealed by the use of the retrograde transport of horseradish peroxidase. J. Comp. Neurol. 164: 31-46
80. **Christensen BN, Perl ER** (1970) Spinal neurons specifically excited by noxious or thermal stimuli: marginal zone of the dorsal horn. J. Neurophysiol. 33: 293-307
81. **Chung K, Coggeshall RE** (1983) Propriospinal fibers in the rat. J. Comp. Neurol. 217: 47-53
82. **Chung K, Kevetter A, Willis WD, Coggeshall RE** (1984) An estimate of the ratio of propriospinal to long tract neurons in the sacral spinal cord of the rat. Neurosci. Lett. 44: 173-177
83. **Ciriello J, Calaresu FR** (1983) Central projections of afferent renal fibers in the rat: an anterograde transport study of horseradish peroxidase. J. Auton. Nerv. Syst. 8: 273-285
83[a] **Coggeshall RE, Coulter JD, Willis WD Jr** (1974) Unmyelinated axons in the ventral roots of the cat lumbosacral enlargement. J. Comp. Neurol. 153: 39-58
84. **Comans PE, Snow PJ** (1981) Rostrocaudal and laminar distribution of spinothalamic neurons in the high cervical spinal cord of the cat. Brain Res. 223: 123-127
85. **Corvaja N, Grofova I, Pompeiano O, Walberg F** (1977) The lateral reticular nucleus in the cat. I. An experimental anatomical study of its spinal and supraspinal afferent connections. Neuroscience 2: 537-553
86. **Cowan WM, Gottlieb DI, Hendrickson AE, Price JL, Woolsey TA** (1972) The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res. 37: 21-51
87. **Craig AD Jr** (1976) Spinocervical tract cells in cat and dog, labeled by the retrograde transport of horseradish peroxidase. Neurosci. Lett. 3: 173-177
88. **Craig AD Jr, Tapper DN** (1978) Lateral cervical nucleus in the cat: functional organization and characteristics. J. Neurophysiol. 41: 1511-1534
89. **Craig AD Jr, Burton H** (1981) Spinal and medullary lamina I projection to nucleus submedius in medial thalamus: a possible pain center. J. Neurophysiol. 45: 443-466
90. **Craig AD, Mense S** (1983) The distribution of afferent fibres from the gastrocnemius-soleus muscle in the dorsal horn of the cat, as revealed by the transport of horseradish peroxidase. Neurosci. Lett. 41: 233-238
91. **Craig AD, Burton H** (1985) The distribution and topographical organization in the thalamus of anterogradely-transported horseradish peroxidase after spinal injections in cat and raccoon. Exp. Brain Res. 58: 227-254
92. **Culberson JL, Haines DE, Kimmel DL, Brown PB** (1979) Contralateral projection of primary afferent fibers to mammalian spinal cord. Exp. Neurol. 64: 83-97
93. **Cummings JF, Petras JM** (1977) The origin of spinocerebellar pathways. I. The nucleus cervicalis centralis of the cranial cervical spinal cord. J. Comp. Neurol. 173: 655-692
94. **Dennis SG, Melzack R** (1977) Pain-signalling systems in the dorsal and ventral spinal cord. Pain 4: 97-132
95. **Dilly PN, Wall PD, Webster KE** (1968) Cells of origin of the spinothalamic tract in the cat and the rat. Exp. Neurol. 21: 550-562
96. **Ten Donkelaar HJ** (1982) Organization of descending pathways to the spinal cord in amphibians and reptiles. In: Kuijpers HGJM, Martin GF (Eds.), Descending pathways to the spinal cord, Progress in brain research, Vol. 57, Elsevier biomedical press, pp 25-67
97. **Ten Donkelaar HJ, de Boer - van Huizen R** (1984) Ascending and descending axoncollaterals efferent from the brainstem reticular formation. A retrograde fluorescent tracer study in the lizard, Varanus exanthematicus. Brain Res. 322: 184-188
98. **Eisenman LM** (1985) Uptake of the retrograde fluorescent tracer fast blue from the cerebrospinal fluid of the rat. Neurosci. Lett. 60: 241-246
99. **English AW, Tigges J, Lennard PR** (1985) Anatomical organization of long ascending propriospinal neurons in the cat spinal cord. J. Comp. Neurol. 240: 349-358
100. **Erdmann G, Wiegand H, Wellhöner HH** (1975) Intraaxonal and extraaxonal transport of ^{125}I-tetanus toxin in early local tetanus. Naunyn-Schmiedeberg's Arch. Pharmacol. 290: 357-373
101. **Fabian RH, Coulter JD** (1985) Transneuronal transport of lectins. Brain Res. 344: 41-48
102. **Faull RLM, Carman JB** (1978) The cerebellofugal projections in the brachium conjunctivum of the rat. I. the contralateral ascending ascending pathway. J. Comp. Neurol. 178: 495-518

103. **Ferrington DG, Sorkin LS, Willis WD Jr** (1986) Responses of spinothalamic tract cells in the cat cervical spinal cord to innocuous and graded noxious stimuli. Somatosens. Res. 3: 339-358
104. **Fields HL, Wagner GM, Anderson SD** (1975) Some properties of spinal neurons projecting to the medial brain-stem reticular formation. Exp. Neurol. 47: 118-134
105. **Fields HL, Clanton CH, Anderson SD** (1977a) Somatosensory properties of spinoreticular neurons in the cat. Brain Res. 120: 49-66
106. **Fields HL, Basbaum AI, Clanton LH, Anderson SD** (1977b) Nucleus raphe magnus inhibition of spinal dorsal horn neurons. Brain Res. 126: 441-453
107. **Fields HL, Basbaum AI** (1978) Brainstem control of spinal pain-transmission neurons. Ann. Rev. Physiol. 40: 217-248
108. **Fink RP, Heimer L** (1967) Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. 4: 369-374
109. **Fink DJ, Purkiss D, Mata M** (1985) Rapid retrograde transport of proteins in sensory neurons in rats. Brain Res. 345: 394-397
110. **Fitzgerald M, Wall PD** (1980) The laminar organization of dorsal horn cells responding to peripheral C fibre stimulation. Exp. Brain Res. 41: 36-44
111. **Fitzgerald M** (1981) A study of the cutaneous afferent input to substantia gelatinosa. Neuroscience 6: 2229-2237
112. **Flink R, Svensson BA** (1986) Fluorescent double-labelling study of ascending and descending neurones in the feline lateral cervical nucleus. Exp. Brain Res. 62: 479-485
113. **Flumerfelt BA, Hrycyshyn AW, Kapogianis EM** (1982) Spinal projections to the lateral reticular nucleus in the rat. Anat. Embryol. 165: 345-359
114. **Foreman RD, Schmidt RF, Willis WD** (1979) Effects of mechanical and chemical stimulation of fine muscle afferents upon primate spinothalamic tract cells. J. Physiol. (London) 286: 215-231
115. **Fritz N, Illert M, Saggau P** (1986a) Location of motoneurons projecting to the cat distal forelimb. I. Deep radial motornuclei. J. Comp. Neurol. 244: 286-301
116. **Fritz N, Illert M, Reeh P** (1986b) Location of motoneurons projecting to the cat distal forelimb. II. Median and ulnar motornuclei. J. Comp. Neurol. 244: 302-312
117. **Gelderd JB, Chopin SF** (1977) The vertebral level of origin of spinal nerves in the rat. Anat. Rec. 188: 45-48
118. **Gelfan S** (1964) Neuronal interdependence. In: Eccles JC, Schadé JP (Eds), Organization of the spinal cord, Progress in brain research, vol. 11. Elsevier Biomedical, Amsterdam
118[a] **Gerfen CR, Sawchenko PE** (1984) An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, Phaseolus vulgaris - leucoagglutinin. Brain Res. 290: 219-238
119. **Gernandt BE, Megirian D** (1961) Ascending propriospinal mechanisms. J. Neurophysiol. 24: 364-376
120. **Giesler GJ Jr, Menétrey D, Guilbaud G, Besson JM** (1976) Lumbar cord neurons at the origin of the spinothalamic tract in the rat. Brain Res. 118: 320-324
121. **Giesler GJ Jr, Menétrey D, Basbaum AI** (1979a) Differential origins of spinothalamic tract projections to medial and lateral thalamus in the rat. J. Comp. Neurol. 184: 107-126
122. **Giesler GJ Jr, Urca G, Cannon JT, Liebeskind JC** (1979b) Response properties of neurons in the lateral cervical nucleus in the rat. J. Comp. Neurol. 186: 65-78
123. **Giesler GJ Jr, Spiel HR, Willis WD** (1981a) Organization of spinothalamic tract axons within the rat spinal cord. J. Comp. Neurol. 195: 243-252
124. **Giesler GJ Jr, Yezierski RP, Gerhart KD, Willis WD** (1981b) Spinothalamic tract neurons that project to medial and/or lateral thalamic nuclei: evidence for a physiologically novel population of spinal cord neurons. J. Neurophysiol. 46: 1285-1308
125. **Giesler GJ Jr, Gerhart KD, Yezierski RP, Wilcox TK, Willis WD** (1981c) Postsynaptic inhibition of primate spinothalamic neurons by stimulation in nucleus raphe magnus. Brain Res. 204: 184-188
126. **Giesler GJ Jr, Nahin RL, Madsen AM** (1984) Postsynaptic dorsal column pathway of the rat. I. Anatomical studies. J. Neurophysiol. 51: 260-275
127. **Giesler GJ Jr, Cliffer KD** (1985) Postsynaptic dorsal column pathway of the rat. II. Evidence against an important role in nociception. Brain Res. 326: 347-356
128. **Giesler GJ Jr, Elde RP** (1985) Immunocytochemical studies of the peptidergic content of fibers and terminals within the lateral spinal and lateral cervical nuclei. J. Neurosci. 5: 1833-1841
129. **Giovanelli BM, Kuijpers HGJM** (1969) Propriospinal fibers interconnecting the spinal enlargements in the cat. Brain Res. 14: 321-330
130. **Grant G** (1962) Spinal course and somatotopically localized termination of the spinocerebellar tracts. An experimental study in the cat. Acta Physiol. Scand. 56: S193

131. **Grant G, Arvidsson J, Robertson B, Ygge J** (1979) Transganglionic transport of horseradish peroxidase in primary sensory neurons. Neurosci. Lett. 12: 23-28
132. **Grant G, Wiksten B, Berkley KJ, Aldskogius H** (1982) The location of cerebellar projecting neurons within the lumbosacral spinal cord in the cat. An anatomical study with HRP and retrograde chromatolysis. J. Comp. Neurol. 204: 336-348
133. **Granum SL** (1986) The spinothalamic system of the rat. I. Locations of cells of origin. J. Comp. Neurol. 247: 159-180
134. **Grillner S** (1975) Locomotion in vertebrates: central mechanisms and reflex interactions. Physiol. Rev. 55: 247-304
135. **Gulley RL** (1973) Golgi studies of the nucleus gracilis in the rat. Anat. Rec. 177: 325-342
136. **Gwyn DG, Waldron HA** (1968) A nucleus in the dorsolateral funiculus of the spinal cord of the rat. Brain Res. 10: 342-351
137. **Gwyn DG, Waldron HA** (1969) Observations on the morphology of a nucleus in the dorsolateral funiculus of the spinal cord of the guinea-pig, rabbit, ferret and cat. J. Comp. Neurol. 136: 233-236
138. **Ha H, Liu CN** (1968) Cell origin of the ventral spinocerebellar tract. J. Comp. Neurol. 133: 185-206
139. **Hancock MB, Foreman RD, Willis WD** (1975) Convergence of visceral and cutaneous input onto spinothalamic tract cells in the thoracic spinal cord of the cat. Exp. Neurol. 47: 240-248
140. **Harmann PA, Carlton SM, Willis WD** (1988) Collaterals of spinothalamic tract cells to the periaqueductal gray: a fluorescent double-labeling study in the rat. Brain Res. 441: 87-97
141. **Harrison PJ, Hultborn H, Jankowska E, Katz R, Storai B, Zytnicki D** (1984) Labelling of interneurones by retrograde transsynaptic transport of horseradish peroxidase from motoneurones in rats and cats. Neurosci. Lett. 45: 15-19
142. **Hayes NL, Rustioni A** (1979) Dual projections of single neurons are visualized simultaneously: use of enzymatically inactive [^{3}H]HRP. Brain Res. 165: 321-326
143. **Hayes NL, Rustioni A** (1980) Spinothalamic and spinomedullary neurons in macaques: a single and double retrograde tracer study. Neuroscience 5: 861-874
144. **Hazlett JC, Dom R, Martin GF** (1973) Spino-bulbar, spino-thalamic and medial lemniscal connections in the american opossum, didelphis marsupialis virginiana. J. Comp. Neurol. 146: 95-118
145. **Hirai N, Hongo T, Kudo N, Yamaguchi T** (1976) Heterogeneous composition of the spinocerebellar tract originating from the cervical enlargement in the cat. Brain Res. 109: 387-391
146. **Hirai N, Hongo T, Yamaguchi T** (1978a) Spinocerebellar tract neurones with long descending axon collaterals. Brain Res. 142: 147-151
147. **Hirai N, Hongo T, Sasaki S** (1978b) Cerebellar projection and input organizations of the spinocerebellar tract arising from the central cervical nucleus in the cat. Brain Res. 157: 341-345
148. **Hirai N, Hongo T, Sasaki S** (1984a) A physiological study of identification, axonal course and cerebellar projection of spinocerebellar tract cells in the central cervical nucleus of the cat. Exp Brain Res. 55: 272-285
149. **Hirai N, Hongo T, Sasaki S, Yamashita M, Yoshida K** (1984b) Neck muscle afferent input to spinocerebellar tract cells of the central cervical nucleus in the cat. Exp. Brain Res. 55: 286-300
150. **Hiramatsu K** (1984) Spinal afferents to lamina IX of the cervical enlargement in the rat studied by the retrograde transport of horseradish peroxidase. Brain Res. 292: 375-377
151. **Hökfelt T, Terenius L, Kuijpers HGJM, Dann O** (1979) Evidence for enkephalin immunoreactive neurons in the medulla oblongata projecting to the spinal cord. Neurosci. Lett. 14: 55-60
152. **Holstege G, Kuijpers HGJM, Boer RC** (1979) Anatomical evidence for direct brain stem projections to the somatic motoneuronal cell groups and autonomic preganglionic cell groups in cat spinal cord. Brain Res. 171: 329-333
153. **Holstege G, Kuijpers HGJM** (1982) The anatomy of brain stem pathways to the spinal cord in cat. A labeled amino acid tracing study. In: Kuijpers HGJM, Martin GF (Eds.), Descending pathways to the spinal cord, Progress in brain research, Vol. 57, Elsevier biomedical press, pp 145-176
154. **Holstege G, Meiners L, Tan K** (1985) Projections from the bed nucleus of the stria terminalis to the mesencephalon, pons and medulla oblongata in the cat. Exp. Brain Res. 58: 379-391
155. **Holstege G** (1987) Some anatomical observations on the projections from the hypothalamus to brainstem and spinal cord: an HRP and autoradiographic tracing study in the cat. J. Comp. Neurol. 260: 98-126
156. **Holstege JC** (1987) Brainstem projections to lumbar motoneurons in rat. II. An ultrastructural study by means of the anterograde transport of wheat germ agglutinin coupled to horseradish peroxidase and using the tetramethyl benzidine reaction. Neuroscience 21: 369-376

157. **Holstege JC, Kuijpers HGJM** (1987a) Brainstem projections to lumbar motoneurons in rat. I. An ultrastructural study using autoradiography and the combination of autoradiography and horseradish peroxidase histochemistry. Neuroscience 21: 345-367
158. **Holstege JC, Kuijpers HGJM** (1987b) Brainstem projections to spinal motoneurons: an update. Neuroscience 23: 809-821
159. **Hongo T, Jankowska E, Lundberg A** (1966) Convergence of excitatory and inhibitory action on interneurones in the lumbosacral cord. Exp. Brain Res. 1: 338-358
160. **Hongo T, Jankowska E, Lundberg A** (1968) Post-synaptic excitation and inhibition from primary afferents in neurones of the spinocervical tract. J. Physiol. (London) 199: 569-592
161. **Huisman AM, Kuijpers HGJM, Verburgh CA** (1981) Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with the multiple fluorescent retrograde tracer technique. Brain Res. 209: 271-286
162. **Huisman AM, Kuijpers HGJM, Verburgh CA** (1982) Differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in cat and monkey. In: Kuijpers HGJM, Martin GF (Eds.), Descending pathways to the spinal cord, Progress in brain research, vol. 57, Elsevier biomedical press, pp. 185-217
163. **Huisman AM, Kuijpers HGJM, Condé F, Keizer K** (1983) Collaterals of rubrospinal neurons to the cerebellum in rat. A retrograde fluorescent double labeling study. Brain Res. 264: 181-196
164. **Hunt CC, Kuno M** (1959a) Properties of spinal interneurones. J. Physiol. (London) 147: 346-363
165. **Hunt CC, Kuno M** (1959b) Background discharge and evoked responses of spinal interneurones. J. Physiol. (London) 147: 364-384
166. **Hylden JLK, Hayashi H, Bennett GJ, Dubner R** (1985) Spinal lamina I neurons projecting to the parabrachial area of the cat midbrain. Brain Res. 336: 195-198
167. **Hylden JLK, Hayashi H, Dubner R, Bennett GJ** (1986a) Physiology and morphology of the lamina I spinomesencephalic projection. J. Comp. Neurol. 247: 505-515
168. **Hylden JLK, Hayashi H, Bennett GJ** (1986b) Lamina I spinomesencephalic neurons in the cat ascend via the dorsolateral funiculi. Somatosens. Res. 4: 31-41
169. **Hylden JLK, Anton F, Nahin RL** (1989) Spinal lamina I projection neurons in the rat: collateral innervation of parabrachial area and thalamus. Neuroscience 28: 27-37
170. **Illert M, Lundberg A, Tanaka R** (1976) Integration in descending motor pathways controlling the forelimb in the cat. 2. Convergence on neurones mediating disynaptic cortico-motoneuronal excitation. Exp. Brain Res. 26: 521-540
171. **Illert M, Lundberg A, Tanaka R** (1977) Integration in descending motor pathways controlling the forelimb in the cat. 3. Convergence on propriospinal neurones transmitting disynaptic excitation from the corticospinal tract and other descending tracts. Exp. Brain Res. 29: 323-346
172. **Illert M, Tanaka R** (1978) Integration in descending motor pathways controlling the forelimb in the cat. 4. Corticospinal inhibition of forelimb motoneurones mediated by short propriospinal neurones. Exp. Brain Res. 31: 131-141
173. **Illert M, Lundberg A** (1978) Collateral connections to the lateral reticular nucleus from cervical propriospinal neurones projecting to forelimb motoneurones in the cat. Neurosci. Lett. 7: 167-172
174. **Illert M, Lundberg A, Padel Y, Tanaka R** (1978) Integration in descending motor pathways controlling the forelimb in the cat. 5. Properties of and monosynaptic excitatory convergence on C3-C4 propriospinal neurones. Exp. Brain Res. 33: 101-130
175. **Illert M, Jankowska E, Lundberg A, Odutola A** (1981) Integration in descending motor pathways controlling the forelimb in the cat. 7. Effects from the reticular formation on C3-C4 propriospinal neurones. Exp. Brain Res. 42: 269-281
176. **Jancsó G, Kíraly E** (1980) Distribution of chemosensitive primary afferents in the central nervous system of the rat. J. Comp. Neurol. 190: 781-792
177. **Jane JA, Schroeder DM** (1971) A comparison of dorsal column nuclei and spinal afferents in the european hedgehog (Erinaceus europeaus). Exp. Neurol. 30: 1-17
178. **Jankowska E, Lundberg A, Stuart D** (1973) Propriospinal control of last order interneurones of spinal reflex pathways in the cat. Brain Res. 53: 227-231
179. **Jankowska E, Lundberg A, Roberts WJ, Stuart D** (1974) A long propriospinal system with direct effects on motoneurones and on interneurones in the cat lumbosacral cord. Exp. Brain Res. 21: 169-194
180. **Jankowska E, Rastad J, Zarzecki P** (1979) Segmental and supraspinal input to cells of origin of non-primary afferent fibres in the feline dorsal columns. J. Physiol. (London) 290: 185-200
181. **Jankowska E, Lundberg A, Stuart D** (1983) Propriospinal control of interneurones in spinal reflex pathways from tendon organs in the cat. Brain Res. 261: 317-320
182. **Jankowska E** (1985) Further indications for enhancement of retrograde transneuronal transport of

WGA-HRP by synaptic activity. Brain Res. 341: 403-408
183. **Jankowska E, Skoog B** (1986) Labelling of midlumbar neurones projecting to cat hindlimb motoneurones by transneuronal transport of a horseradish peroxidase conjugate. Neurosci. Lett. 71: 163-168
184. **Jeffery G, Cowey A, Kuijpers HGJM** (1981) Bifurcating retinal ganglion cell axons in the rat, demonstrated by retrograde double labelling. Exp. Brain Res. 44: 34-40
185. **Jiao SS, Zhang GF, Liu YJ, Wang YS, Lu GW** (1984) Double labeling of cat spinal dorsal horn neurons with fluorescent substances. Pain Suppl. 2: S130
186. **Jones MW, Hodge CJ Jr, Apkarian AV, Stevens RT** (1985) A dorsolateral spinothalamic pathway in cat. Brain Res. 335: 188-193
187. **Jones MW, Apkarian AV, Stevens RT, Hodge CJ Jr** (1987) The spinothalamic tract: an examination of the cells of origin of the dorsolateral and ventral spinothalamic pathways in cats. J. Comp. Neurol. 260: 349-361
188. **Kamogawa H, Bennett GJ** (1986) Dorsal column postsynaptic neurons in the cat are excited by myelinated nociceptors. Brain Res. 364: 386-390
189. **Katz LC, Burkhalter A, Dreyer WJ** (1984) Fluorescent latex microspheres as a retrograde neuronal marker for in vivo and in vitro studies of visual cortex. Nature 310: 498-500
190. **Keizer K, Kuijpers HGJM, Huisman AM, Dann O** (1983) Diamidino Yellow Dihydrochloride (DY.2HCl); a new fluorescent retrograde neuronal tracer, which migrates only very slowly out of the cell. Exp. Brain Res. 51: 179-191
191. **Keizer K, Kuijpers HGJM** (1984) Distribution of corticospinal neurons with collaterals to lower brain stem reticular formation in cat. Exp. Brain Res. 54: 107-120
192. **Kemplay SK, Webster KE** (1986) A qualitative and quantitative analysis of the distributions of cells in the spinal cord and spinomedullary junction projecting to the thalamus of the rat. Neuroscience 17: 769-789
193. **Kenshalo DR Jr, Willis WD** (1978) Laminar distribution of spinothalamic tract cells in the primate lumbosacral spinal cord. Anat. Rec. 190: 443
194. **Kerr FWL, Lippman HH** (1974) The primate spinothalamic tract as demonstrated by anterolateral cordotomy and commissural myelotomy. Adv. Neurol. 4: 147-156
195. **Kerr FWL** (1975) The ventral spinothalamic tract and other ascending systems of the ventral funiculus of the spinal cord. J. Comp. Neurol. 159: 335-356
196. **Kevetter GA, Willis WD** (1982) Spinothalamic cells in the rat lumbar cord with collaterals to the medullary reticular formation. Brain Res. 238: 181-185
197. **Kevetter GA, Haber LH, Yezierski RP, Chung JM, Martin RF, Willis WD** (1982) Cells of origin of the spinoreticular tract in the monkey. J. Comp. Neurol. 207: 61-74
198. **Kevetter GA, Willis WD** (1983) Collaterals of spinothalamic cells in the rat. J. Comp. Neurol. 215: 453-464
199. **Kimmel ChB** (1982) Reticulospinal and vestibulospinal neurons in the young larva of a teleost fish, Brachydanio rerio. In: Kuijpers HGJM, Martin GF (Eds.), Descending pathways to the spinal cord, Progress in brain research, Vol. 57, Elsevier biomedical press, pp 1-23
200. **Koerber HR, Brown PB** (1980) Projections of two hindlimb cutaneous nerves to cat dorsal horn. J. Neurophysiol. 44: 259-269
201. **van der Kooy D, Kuijpers HGJM, Catsman-Berrevoets CE** (1978) Single mammillary body cells with divergent axon collaterals. Demonstration by a simple, fluorescent retrograde double labeling technique in the rat. Brain Res. 158: 189-196
202. **van der Kooy D, Kuijpers HGJM** (1979) Fluorescent retrograde double labeling: axonal branching in the ascending raphe and nigral projections. Science 204: 873-875
203. **van der Kooy D, Wise RA** (1980) Retrograde fluorescent tracing of substantia nigra neurons combined with catecholamine histofluorescence. Brain Res. 183: 447-452
204. **van der Kooy D, Sawchenko PE** (1982) Characterization of serotonergic neurons using concurrent fluorescent retrograde axonal tracing and immunohistochemistry. J. Histoch. Cytochem. 30: 794-798
205. **Kostyuk PG, Vasilenko DA, Lang E** (1971) Propriospinal pathways in the dorsolateral funiculus and their effects on lumbosacral motoneuronal pools. Brain Res. 28: 233-249
206. **Kotchabhakdi N, Walberg F** (1977) Cerebellar afferents from neurons in motor nuclei of cranial nerves demonstrated by retrograde axonal transport of horseradish peroxidase. Brain Res. 137: 158-163
207. **Kristensson K** (1970) Morphological studies of the neural spread of herpes simplex virus to the central nervous system. Acta Neuropathol. 16: 54-63
208. **Kristensson K, Olsson Y** (1971) Retrograde axonal transport of protein. Brain Res. 29: 363-365

209. **Kumazawa T, Perl ER** (1978) Excitation of marginal and substantia gelatinosa neurons in the primate spinal cord: indication of their place in dorsal horn functional organization. J. Comp. Neurol. 177: 417-434
210. **Künzle H** (1973) The topographic organization of spinal afferents to the lateral reticular nucleus of the cat. J. Comp. Neurol. 149: 103-116
211. **Künzle H** (1977) Evidence for selective axon-terminal uptake and retrograde transport of label in cortico- and rubrospinal systems after injection of ^{3}H-proline. Exp. Brain Res. 28: 125-132
212. **Kuijpers HGJM** (1960) Central cortical projections to motor and somato-sensory cell-groups. An experimental study in the rhesus-monkey. Brain 83: 161-184
213. **Kuijpers HGJM, Tuerk JD** (1964a) The distribution of the cortical fibres in the nuclei cuneatus and gracilis in the cat. J. Anat. 98: 143-162
213[a] **Kuijpers HGJM** (1964b) The descending pathways to the spinal cord, their anatomy and function. In: Eccles JC, Schadé JP (Eds.), Organization of the spinal cord, Progress in brain research, Vol. 11, Elsevier biomedical press, pp. 178-200
214. **Kuijpers HGJM, Kievit J, Groen-Klevant AC** (1974) Retrograde axonal transport of horseradish peroxidase in rat's forebrain. Brain Res. 67: 211-218
215. **Kuijpers HGJM, Maisky VA** (1975) Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. Neurosci. Lett. 1: 9-14
216. **Kuijpers HGJM, Catsman-Berrevoets CE, Padt RE** (1977) Retrograde axonal transport of fluorescent substances in the rat's forebrain. Neurosci. Lett. 6: 127-135
217. **Kuijpers HGJM, Bentivoglio M, van der Kooy D, Catsman-Berrevoets CE** (1979) Retrograde transport of bisbenzimide and propidium iodide through axons to their parent cell bodies. Neurosci. Lett. 12: 1-7
218. **Kuijpers HGJM, Bentivoglio M, Catsman-Berrevoets CE, Bharos AT** (1980) Double retrograde neuronal labeling through divergent axon collaterals, using two fluorescent tracers with the same excitation wavelength which label different features of the cell. Exp. Brain Res. 40: 383-392
219. **Kuijpers HGJM** (1981) Anatomy of the descending pathways. In: Brookhart JM, Mountcastle VB, Brooks VB, Geiger SR (Eds), Handbook of Physiology; section 1: the nervous system; vol II: motor control; ch. 13. American Physiological Society, Bethesda, Maryland
220. **Kuijpers HGJM** (1982) A new look at the organization of the motor system. In: Kuijpers HGJM, Martin GF (Eds.), Descending pathways to the spinal cord, Progress in brain research, Vol. 57, Elsevier biomedical press, pp 381-404
221. **Kuijpers HGJM, Huisman AM** (1983) Fluorescent retrograde tracers. In: Federoff S (Ed.), Labeling methods applicable to the study of neuronal pathways, Advances in cellular neurobiology, vol. 5, Academic Press, New York.
222. **LaMotte C** (1977) Distribution of the tract of Lissauer and the dorsal root fibers in the primate spinal cord. J. Comp. Neurol. 172: 529-562
223. **LaVail JH, LaVail MM** (1972) Retrograde axonal transport in the central nervous system. Science 176: 1416-1417
224. **LaVail JH, Winston KR, Tish A** (1973) A method based on retrograde intraaxonal transport of protein for identification of cell bodies of origin of axons terminating within the CNS. Brain Res. 58: 470-477
225. **Lawrence DG, Kuijpers HGJM** (1968a) The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. Brain 91: 1-14
226. **Lawrence DG, Kuijpers HGJM** (1968b) The functional organization of the motor system in the monkey. II. The effects of lesions of the descending brain-stem pathways. Brain 91: 15-36
227. **Leah J, Menétrey D, de Pommery J** (1988) Neuropeptides in long ascending spinal tract cells in the rat: evidence for parallel processing of ascending information. Neuroscience 24: 195-207
228. **Leah J, Menétrey D** (1989) Neuropeptides in propriospinal neurones in the rat. Brain Res. 495: 173-177
229. **Leichnetz GR, Watkins L, Griffin G, Murfin R, Mayer DJ** (1978) The projection from nucleus raphe magnus and other brainstem nuclei to the spinal cord in the rat: a study using the HRP blue-reaction. Neurosci. Lett. 8: 119-124
230. **Leong SK, Shieh JY, Wong WC** (1984) Localizing spinal-cord-projecting neurons in adult albino rats. J. Comp. Neurol. 228: 1-17
231. **Light AR, Perl ER** (1977) Differential distribution of large-diameter and small-diameter primary afferent fibers in the spinal dorsal gray matter as indicated by labeling with horseradish peroxidase. Neurosci. Lett. 6: 59-63
232. **Light AR, Perl ER** (1979a) Reexamination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibers. J. Comp. Neurol.

186: 117-132
233. **Light AR, Perl ER** (1979b) Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers. J. Comp. Neurol. 186: 133-150
234. **Light AR, Trevino DL, Perl ER** (1979) Morphological features of functionally defined neurons in the marginal zone and substantia gelatinosa of the spinal dorsal horn. J. Comp. Neurol. 186: 151-172
235. **Liu RPC** (1983) Laminar origins of spinal projection neurons to the periaqueductal gray of the rat. Brain Res. 264: 118-122
236. **Liu RPC** (1986) Spinal neuronal collaterals to the intralaminar thalamic nuclei and periaqueductal gray. Brain Res. 365: 145-150
237. **Lu GW, Bennett GJ, Nishikawa N, Hoffert MJ, Dubner R** (1983) Extra- and intracellular recording from dorsal column postsynaptic spinomedullary neurons in the cat. Exp. Neurol. 82: 456-477
238. **Lu GW, Bennett GJ, Nishikawa N, Dubner R** (1985) Spinal neurons with branched axons traveling in both the dorsal and dorsolateral funiculi. Exp. Neurol. 87: 571-578
239. **Lund RD, Webster KE** (1967) Thalamic afferents from the spinal cord and trigeminal nuclei. An experimental study in the rat. J. Comp. Neurol. 130: 313-328
240. **Lundberg A, Oscarsson O** (1960) Functional organization of the dorsal spino-cerebellar tract in the cat. VII. Identification of units by antidromic activation from the cerebellar cortex with recognition of five functional subdivisions. Acta Physiol. Scand. 50: 356-375
241. **Lundberg A, Oscarsson O** (1962) Functional organization of the ventral spino-cerebellar tract in the cat. IV. Identification of units by antidromic activation from the cerebellar cortex. Acta Physiol. Scand. 54: 252-269
242. **Mannen H** (1975) Reconstruction of axonal trajectory of individual neurons in the spinal cord using Golgi-stained serial sections. J. Comp. Neurol. 159: 357-374
243. **Mantyh PW** (1982) The ascending input to the midbrain periaqueductal gray of the primate. J. Comp. Neurol. 211: 50-64
244. **Mantyh PW** (1983) The spinothalamic tract in the primate: a re-examination using wheatgerm agglutinin conjugated to horseradish peroxidase. Neuroscience 9: 847-862
245. **Martin GF, Waltzer RP** (1984a) A double-labelling study of reticular collaterals to the spinal cord and cerebellum of the North American opossum. Neurosci. Lett. 47: 185-191
246. **Martin GF, Waltzer RF** (1984b) A study of overlap and collateralization of the bulbar reticular and raphe neurons which project to the spinal cord and diencephalon of the North American opossum. Brain Behav. Evol. 24: 109-123
247. **Martin GF, Cabana T** (1985) Cortical projections to superficial laminae of the dorsal horn and to the ventral horn of the spinal cord in the North American opossum. Studies using the orthograde transport of WGA-HRP. Brain Res. 337: 188-192
248. **Matsumoto A, Aoki M, Mori S** (1976) Ascending long spinal actions on forelimb motoneurons in the acute spinal cat. Exp. Brain Res. 24: 509-521
249. **Matsushita M** (1969) Some aspects of the interneuronal connections in cat's spinal gray matter. J. Comp. Neurol. 136: 57-80
250. **Matsushita M** (1970) The axonal pathways of spinal neurons in the cat. J. Comp. Neurol. 138: 391-418
251. **Matsushita M, Ueyama T** (1973) Ventral motor nucleus of the cervical enlargement in some mammals; its specific afferents from the lower cord levels and cytoarchitecture. J. Comp. Neurol. 150: 33-52
252. **Matsushita M, Ikeda M** (1975) The central cervical nucleus as cell origin of a spinocerebellar tract arising from the cervical cord: a study in the cat using horseradish peroxidase. Brain Res. 100: 412-417
253. **Matsushita M, Hosoya Y** (1979) Cells of origin of the spinocerebellar tract in the rat, studied with the method of retrograde transport of horseradish peroxidase. Brain Res. 173: 185-200
254. **Matsushita M, Ikeda M, Hosoya Y** (1979a) The location of spinal neurons with long descending axons (long descending propriospinal tract neurons) in the cat: a study with the horseradish peroxidase technique. J. Comp. Neurol. 184: 63-80
255. **Matsushita M, Hosoya Y, Ikeda M** (1979b) Anatomical organization of the spinocerebellar system in the cat, as studied by retrograde transport of horseradish peroxidase. J. Comp. Neurol. 184: 81-106
256. **Matsushita M, Ikeda M** (1980) Spinocerebellar projections to the vermis of the posterior lobe and the paramedian lobule in the cat, as studied by retrograde transport of horseradish peroxidase. J. Comp. Neurol. 192: 143-162
257. **Matsushita M, Okado N** (1981) Spinocerebellar projections to lobules I and II of the anterior lobe

in the cat, as studied by retrograde transport of horseradish peroxidase. J. Comp. Neurol. 197: 411-424
258. **Matsushita M, Hosoya Y** (1982) Spinocerebellar projections to lobules III to V of the anterior lobe in the cat, as studied by retrograde transport of horseradish peroxidase. J. Comp. Neurol. 208: 127-143
259. **Matsushita M, Tanami T, Yaginuma H** (1984) Differential distribution of spinocerebellar fiber terminals within the lobules of the cerebellar anterior lobe in the cat: an anterograde WGA-HRP study. Brain Res. 305: 157-161
260. **Matsushita M, Ikeda M, Tanami T** (1985) The vermal projection of spinocerebellar tracts arising from lower cervical segments in the cat: an anterograde WGA-HRP study. Brain Res. 360: 389-393
261. **Matsushita M, Ikeda M** (1987) Spinocerebellar projections from the cervical enlargement in the cat, as studied by anterograde transport of wheat germ agglutinin - horseradish peroxidase. J. Comp. Neurol. 263: 223-240
262. **Maunz RA, Pitts NG, Peterson BW** (1978) Cat spinoreticular neurons: locations, responses and changes in responses during repetitive stimulation. Brain Res. 148: 365-379
263. **Maxwell DJ, Koerber HR** (1986) Fine structure of collateral axons originating from feline spinocervical tract neurons. Brain Res. 363: 199-203
264. **McClung JR, Castro AJ** (1978) Rexed's laminar scheme as it applies to the rat cervical spinal cord. Exp. Neurol. 58: 145-148
265. **McMahon SB, Morrison JFB** (1982) Spinal neurones with long projections activated from the abdominal viscera of the cat. J. Physiol. (London) 322: 1-20
266. **McMahon SB, Wall PD** (1983) A system of rat spinal cord lamina I cells projecting through the contralateral dorsolateral funiculus. J. Comp. Neurol. 214: 217-223
267. **Mehler WR, Feferman ME, Nauta WJH** (1960) Ascending axon degeneration following antero-lateral cordotomy. An experimental study in the monkey. Brain 83: 718-750
268. **Mehler WR** (1969) Some neurological species differences - a posteriori. Ann. N. Y. Acad. Sci. 167: 424-468
269. **Meikle ADS, Martin AH** (1980) Rat spinal cord, a cytoarchitectonic study. Anat. Rec. 196: 241A
270. **Menétrey D, Giesler GJ Jr, Besson JM** (1977) An analysis of response properties of spinal cord dorsal horn neurones to nonnoxious and noxious stimuli in the spinal rat. Exp. Brain Res. 27: 15-33
271. **Menétrey D, Chaouch A, Besson JM** (1980) Location and properties of dorsal horn neurons at origin of spinoreticular tract in lumbar enlargement of the rat. J. Neurophysiol. 44: 862-877
272. **Menétrey D, Chaouch A, Binder D, Besson JM** (1982) The origin of the spinomesencephalic tract in the rat: an anatomical study using the retrograde transport of horseradish peroxidase. J. Comp. Neurol. 206: 193-207
273. **Menétrey D, Roudier F, Besson JM** (1983) Spinal neurons reaching the lateral reticular nucleus as studied in the rat by retrograde transport of horseradish peroxidase. J. Comp. Neurol. 220: 439-452
274. **Menétrey D, de Pommery J, Roudier F** (1984) Properties of deep spinothalamic tract cells in the rat, with special reference to ventromedial zone of lumbar dorsal horn. J. Neurophysiol. 52: 612-624
275. **Menétrey D, de Pommery J, Roudier F** (1985) Propriospinal fibers reaching the lumbar enlargement in the rat. Neurosci. Lett. 58: 257-261
276. **Mesulam MM** (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. J. Histoch. Cytochem. 26: 106-117
277. **Mesulam MM, Brushart TM** (1979) Transganglionic and anterograde transport of horseradish peroxidase across dorsal root ganglia: a tetramethylbenzidine method for tracing central sensory connections of muscles and peripheral nerves. Neuroscience 4: 1107-1117
278. **Meyers DER, Snow PJ** (1982) The responses to somatic stimuli of deep spinothalamic tract cells in the lumbar spinal cord of the cat. J. Physiol. 329: 355-371
279. **Miller S, van der Burg J** (1973) The function of long propriospinal pathways in the co-ordination of quadrupedal stepping in the cat. In: Stein RB, Pearson KB, Smith RS, Redford JB (Eds.), Control of posture and locomotion, Plenum Press, New York
280. **Miller S, Reitsma DJ, van der Meché FGA** (1973) Functional organization of long ascending propriospinal pathways linking lumbosacral and cervical segments in the cat. Brain Res. 62: 169-188
281. **Miller S, van der Meché FGA** (1976) Coordinated stepping of all four limbs in the high spinal cat.

Brain Res. 109: 395-398
282. **Molander C, Xu GA, Grant G** (1984) The cytoarchitectonic organization of the spinal cord in the rat. I. The lower thoracic and lumbosacral cord. J. Comp. Neurol. 230: 133-141
283. **Molander C, Grant G** (1985) Cutaneous projections from the rat hindlimb foot to the substantia gelatinosa of the spinal cord studied by transganglionic transport of WGA-HRP conjugate. J. Comp. Neurol. 273: 476-484
284. **Molander C, Grant G** (1986) Laminar distribution and somatotopic organization of primary afferent fibers from hindlimb nerves in the dorsal horn. A study by transganglionic transport of horseradish peroxidase in the rat. Neuroscience: 19: 297-312
285. **Molenaar I, Rustioni A, Kuijpers HGJM** (1974) The location of cells of origin of the fibers in the ventral and the lateral funiculus of the cat's lumbosacral cord. Brain Res. 78: 239-254
286. **Molenaar I, Kuijpers HGJM** (1975) Identification of cells of origin of long fiber connections in the cat's spinal cord by means of the retrograde axonal horseradish peroxidase technique. Neurosci. Lett. 1: 193-197
287. **Molenaar I** (1978) The distribution of propriospinal neurons projecting to different motoneuronal cell groups in the cat's brachial cord. Brain Res. 158: 203-206
288. **Molenaar I, Kuijpers HGJM** (1978) Cells of origin of propriospinal fibers and of fibers ascending to supraspinal levels. A HRP study in cat and rhesus monkey. Brain Res. 152: 429-450
289. **Molinari HH** (1982) The cutaneous sensitivity of units in laminae VII and VIII of the cat. Brain Res. 234: 165-169
290. **Molinari HH** (1984) Ascending somatosensory projections to the dorsal accessory olive: an anatomical study in cats. J. Comp. Neurol. 223: 110-123
291. **Molinari HH** (1985) Ascending somatosensory projections to the medial accessory portion of the inferior olive: a retrograde study in cats. J. Comp. Neurol. 232: 523-533
292. **Morrell JI, Pfaff DW** (1983) Retrograde HRP identification of neurons in the rhombencephalon and spinal cord of the rat that project to the dorsal mesencephalon. Am. J. Anat. 167: 229-240
293. **Murthy KSK, Gildenberg PL, Marchand JE** (1978) Descending long-spinal excitation of lumbar alpha and gamma motoneurons evoked by stretch of dorsal neck muscles. Brain Res. 140: 165-170
294. **Mysicka A, Zenker W** (1981) Central projections of muscle afferents from the sternomastoid nerve in the rat. Brain Res. 211: 257-265
295. **Nadelhaft I, Booth AM** (1984) The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish peroxidase study. J. Comp. Neurol. 226: 238-245
296. **Nagy JI, Hunt SP** (1983) The termination of primary afferents within the rat dorsal horn: evidence for rearrangement following capsicain treatment. J. Comp. Neurol. 218: 145-158
297. **Nahin RL, Madsen AM, Giesler GJ Jr** (1983) Anatomical and physiological studies of the gray matter surrounding the spinal cord central canal. J. Comp. Neurol. 220: 321-335
298. **Nahin RL** (1987) Immunocytochemical identification of long ascending peptidergic neurons contributing to the spinoreticular tract in the rat. Neuroscience 23: 859-869
299. **Nakano K, Tokushige A, Hasegawa Y, Kohno M** (1986) An autoradiographic study of the spinofacial projection in the monkey. Brain Res. 372: 338-344
300. **Nathan PW, Smith MC** (1959) Fasciculi proprii of the spinal cord in man. Brain 82: 610-668
300[a] **Nathan PW, Smith MC, Cook AW** (1986) Sensory effects in man of lesions of the posterior columns and of some other afferent pathways. Brain 109: 1003-1041
301. **Nauta WHJ, Gygax PA** (1954) Silver impregnation of degenerating axons in the central nervous system: a modified technique. Stain Technol. 29: 91-94
302. **Nauta WJH, Kuijpers HGJM** (1958) Some ascending pathways in the brain stem reticular formation. In: Jasper HH, Proctor LD, Knighton RS, Noshay WC, Costello RT (Eds.), "Reticular formation of the brain", Henry Ford Hospital International Symposium, Little, Brown and Co., Boston, pp. 3-20
303. **Nishikawa N, Bennett GJ, Ruda MA, Lu GW, Dubner R** (1983) Immunocytochemical evidence for a serotoninergic innervation of dorsal column postsynaptic neurons in cat and monkey; light- and electron microscopic observations. Neuroscience 10: 1333-1340
304. **Noordenbos W, Wall PD** (1976) Diverse sensory functions with an almost totally divided spinal cord. A case of spinal cord transection with preservation of part of one anterolateral quadrant. Pain 2: 185-195
305. **Nyberg G, Blomqvist A** (1985) The somatotopic organization of forelimb cutaneous nerves in the brachial dorsal horn: an anatomical study in the cat. J. Comp. Neurol. 242: 28-39
306. **Nyberg-Hansen R, Brodal A** (1963) Sites of termination of corticospinal fibers in the cat. An experimental study with silver impregnation methods. J. Comp. Neurol. 120: 369-391

307. **de Olmos J, Heimer L** (1977) Mapping of collateral projections with the HRP-method. Neurosci. Lett. 6: 107-114
308. **de Olmos J, Heimer L** (1980) Double and triple labeling of neurons with fluorescent substances; the study of collateral pathways in the ascending raphe system. Neurosci. Lett. 19: 7-12
309. **Olsson T, Kristensson K** (1978) A simple histochemical method for double labelling of neurons by retrograde axonal transport. Neurosci. Lett. 8: 265-268
310. **Oscarsson O, Uddenberg N** (1964) Identification of a spinocerebellar tract activated from forelimb afferents in the cat. Acta Physiol. Scand. 62: 125-136
311. **Oscarsson O, Uddenberg N** (1965) Properties of afferent connections to the rostral spinocerebellar tract in the cat. Acta Physiol. Scand. 64: 143-153
312. **Panneton WM, Burton H** (1985) Projections from the paratrigeminal nucleus and the medullary and spinal dorsal horns to the peribrachial area in the cat. Neuroscience 15: 779-797
313. **Paxinos G, Watson C** (1982) The rat brain in stereotaxic coordinates. Academic Press Australia
314. **Pechura CM, Liu RPC** (1986) Spinal neurons which project to the periaqueductal gray and the medullary reticular formation via axon collaterals: a double-label fluorescence study in the rat. Brain Res. 374: 357-361
315. **Pellegrino LJ, Pellegrino AS, Cushman AJ** (1979) A stereotaxic atlas of the rat brain. 2nd edition, Plenum, New York.
316. **Peschanski M, Mantyh PW, Besson JM** (1983) Spinal afferents to the ventrobasal thalamic complex in the rat: an anatomical study using wheat-germ agglutinin conjugated to horseradish peroxidase. Brain Res. 278: 240-244
317. **Peschanski M, Besson JM** (1984) A spino-reticulo-thalamic pathway in the rat: an anatomical study with reference to pain transmission. Neuroscience 12: 165-178
318. **Peschanski M, Ralston HJ III** (1985) Light and electron microscopic evidence of transneuronal labeling with WGA-RP to trace somatosensory pathways to the thalamus. J. Comp. Neurol. 236: 29-41
319. **Petras JM** (1967) Cortical, tectal and tegmental fiber connections in the spinal cord of the cat. Brain Res. 6: 275-324
320. **Petras JM** (1977) Spinocerebellar neurons in the rhesus monkey. Brain Res. 130: 146-151
321. **Petras JM, Cummings JF** (1977) The origin of spinocerebellar pathways. II. The nucleus centrobasalis of the cervical enlargement and the nucleus dorsalis of the thoracolumbar spinal cord. J. Comp. Neurol. 173: 693-716
322. **Petrovicky P** (1976) Distribution and organization of spino-reticular afferents in the brain stem of rat. J. Hirnforsch. 17: 127-135
323. **Piepmeier JM, Kauer JS, Greer CA** (1983) Laminar distributions of 2-deoxyglucose uptake in the rat spinal cord following electrical stimulation of the sciatic nerve. Brain Res. 259: 167-171
324. **Price DD, Browe AC** (1973) Responses of spinal cord neurons to graded noxious and non-noxious stimuli. Brain Res. 64: 425-429
325. **Price DD, Mayer DJ** (1974) Physiological laminar organization of the dorsal horn of M. Mulatta. Brain Res. 79: 321-325
326. **Proshansky E, Egger MD** (1977) Staining of the dorsal root projection to the cat's dorsal horn by anterograde movement of horseradish peroxidase. Neurosci. Lett. 5: 103-110
327. **Ralston HJ III** (1984) Synaptic organization of spinothalamic tract projections to the thalamus, with special reference to pain. In: Kruger L, Liebeskind JC (Eds.), Advances in pain research and therapy, vol.6, Raven press, New York, pp. 183-195
328. **Rexed B** (1952) The cytoarchitectonic organization of the spinal cord in the cat. J. Comp. Neurol. 96: 415-495
329. **Rexed B** (1954) A cytoarchitectonic atlas of the spinal cord in the cat. J. Comp. Neurol. 100: 297-379
330. **Rexed B** (1964) Some aspects of the cytoarchitectonics and synaptology of the spinal cord. In: Eccles JC, Schadé JP (Eds.) Organization of the spinal cord, Progress in brain research vol. 11. Elsevier Biomedical, Amsterdam, pp. 58-92
331. **RoBards MJ, Watkins DW III, Masterton RB** (1976) An anatomical study of some somesthetic afferents to the intercollicular terminal zone of the midbrain of the opossum. J. Comp. Neurol. 170: 499-524
332. **Robertson B, Grant G, Björkeland M** (1983) Demonstration of spinocerebellar projections in cat using anterograde transport of WGA-HRP, with some observations on spinomesencephalic and spinothalamic projections. Exp. Brain Res. 52: 99-104
333. **Ruch TC** (1936) Evidence of the non-segmental character of spinal reflexes from an analysis of the cephalad effects of spinal transection (Schiff-Sherrington phenomenon). Am. J. Physiol. 114: 457-

334. **Ruggiero DA, Ross CA, Reis DJ** (1981) Projections from the spinal trigeminal nucleus to the entire length of the spinal cord in the rat. Brain Res. 225: 225-233
335. **Rustioni A, Kuijpers HGJM, Holstege G** (1971) Propriospinal projections from the ventral and lateral funiculi to the motoneurons in the lumbosacral cord of the cat. Brain Res. 34: 255-275
336. **Rustioni A** (1973) Non-primary afferents to the nucleus gracilis from the lumbar cord of the cat. Brain Res. 51: 81-95
337. **Rustioni A** (1974) Non-primary afferents to the cuneate nucleus in the brachial dorsal funiculus of the cat. Brain Res. 75: 247-259
338. **Rustioni A, Molenaar I** (1975) Dorsal column nuclei afferents in the lateral funiculus of the cat; distribution pattern and absence of sprouting after chronic deafferentation. Exp. Brain Res. 23: 1-12
339. **Rustioni A, Kaufmann AB** (1977) Identification of cells of origin of non-primary afferents to the dorsal column nuclei of the cat. Exp. Brain Res. 27: 1-14
340. **Rustioni A** (1977) Spinal neurons project to the dorsal column nuclei of rhesus monkeys. Science 196: 656-658
341. **Rustioni A, Hayes NL, O'Neill S** (1979) Dorsal column nuclei and ascending spinal afferents in macaques. Brain 102: 95-125
342. **Rustioni A, Cuénod M** (1982) Selective retrograde transport of d-aspartate in spinal interneurons and cortical neurons of rats. Brain Res. 236: 143-155
343. **Sawchenko PE, Swanson LW** (1981) A method for tracing biochemically defined pathways in the central nervous system using combined fluorescence retrograde transport and immunohistochemical techniques. Brain Res. 210: 31-51
344. **Scheibel ME, Scheibel AB** (1966) Spinal motorneurons, interneurons and Renshaw cells. A Golgi study. Arch. Ital. Biol. 104: 328-352
345. **Scheibel ME, Scheibel AB** (1968) Terminal axonal patterns in cat spinal cord. II. The dorsal horn. Brain Res. 9: 32-58
346. **Scheibel ME, Scheibel AB** (1969) Terminal axonal patterns in cat spinal cord. III. Primary afferent collaterals. Brain Res. 13: 417-443
347. **Schmued LC, Swanson LW, Sawchenko PE** (1982) Some fluorescent counterstains for neuroanatomical studies. J. Histoch. Cytochem. 30: 123-128
348. **Schmued LC, Fallon JH** (1986) Fluoro-Gold: a new fluorescent retrograde axonal tracer with numerous unique properties. Brain Res. 177: 147-154
349. **Schoenen J** (1973) Organisation cytoarchitectonique de la moelle épinière de différents mammifères et de l'homme. Acta Neurol. Belg. 73: 348-358
350. **Schomburg ED, Meinck HM, Haustein J** (1975) A fast propriospinal inhibitory pathway from forelimb afferents to motoneurones of hindlimb flexor digitorum longus. Neurosci. Lett. 1: 311-314
351. **Schomburg ED, Roesler J, Meinck HM** (1977) Phase-dependent transmission in the excitatory propriospinal reflex pathway from forelimb afferents to lumbar motoneurones during fictive locomotion. Neurosci. Lett. 4: 249-252
352. **Schomburg ED, Meinck HM, Haustein J, Roesler J** (1978) Functional organization of the spinal reflex pathways from forelimb afferents to hindlimb motoneurones in the cat. Brain Res. 139: 21-33
353. **Schrøder HD** (1977) Sulfide silver architectonics of the rat, cat and guinea pig spinal cord. Anat. Embryol. 150: 251-267
354. **Schwab M, Agid Y, Glowinski J, Thoenen H** (1977) Retrograde axonal transport of ^{125}I-tetanus toxin as a tool for tracing fiber connections in the central nervous system; connections of the rostral part of the rat neostriatum. Brain Res. 126: 211-224
355. **Schwab ME, Javoy-Agid F, Agid Y** (1978) Labeled wheat germ agglutinin (WGA) as a new, highly sensitive retrograde tracer in the rat brain hippocampal system. Brain Res. 152: 145-150
356. **Seiler M, Schwab ME** (1984) Specific retrograde transport of nerve growth factor (NGF) from neocortex to nucleus basalis in the rat. Brain Res. 300: 33-39
357. **Seybold V, Elde RP** (1980) Immunohistochemical studies of peptidergic neurons in the dorsal horn of the spinal cord. J. Histochem. Cytochem. 28: 367-370
358. **Shokunbi MT, Hrycyshyn AW, Flumerfelt BA** (1985) Spinal projections to the lateral reticular nucleus in the rat: a retrograde labelling study using horseradish peroxidase. J. Comp. Neurol. 239: 216-226
359. **Skinner RD, Coulter JD, Adams RJ, Remmel RS** (1979) Cells of origin of long descending propriospinal fibers connecting the spinal enlargements in cat and monkey determined by

horseradish peroxidase and electrophysiological techniques. J. Comp. Neurol. 188: 443-454
360. **Skinner RD, Adams RJ, Remmel RS** (1980) Responses of long descending propriospinal neurons to natural and electrical types of stimuli in cat. Brain Res. 196: 387-403
361. **Skinner RD, Nelson R, Griebel M, Garcia-Rill E** (1989) Ascending projections of long descending propriospinal tract (LDPT) neurons. Brain Res. Bull. 22: 253-258
362. **Skirboll L, Hökfelt T, Norell G, Phillipson O, Kuijpers HGJM, Bentivoglio M, Catsman-Berrevoets CE, Visser TJ, Verhofstad A, Cuello AC, Goldstein M, Brownstein M** (1984) A method for specific transmitter identification of retrogradely labelled neurons: immunofluorescence combined with fluorescence tracing. Brain Res. Rev. 8: 99-127
363. **Smith CC** (1983) The development and postnatal organization of primary afferent projections to the rat thoracic spinal cord. J. Comp. Neurol. 220: 29-43
364. **Snyder RL, Faull RLM, Mehler WR** (1978) A comparative study of the neurons of origin of the spinocerebellar afferents in the rat, cat and squirrel monkey based on the retrograde transport of horseradish peroxidase. J. Comp. Neurol. 181: 833-852
365. **Snyder RL** (1982) Light and electron microscopic autoradiographic study of the dorsal root projections to the cat dorsal horn. Neuroscience 7: 1417-1437
366. **Sorkin LS, Ferrington DG, Willis WD Jr** (1986) Somatotopic organization and response characteristics of dorsal horn neurons in the cervical spinal cord of the cat. Somatos. Res. 3: 323-338
367. **Stechison MT, Saint-Cyr JA** (1986) Organization of spinal inputs to the perihypoglossal complex in the cat. J. Comp. Neurol. 246: 555-567
368. **Steiner TJ, Turner LM** (1972) Cytoarchitecture of the rat spinal cord. J. Physiol. 222: 123p-125p
369. **Sterling P, Kuijpers HGJM** (1967a) Anatomical organization of the brachial spinal cord of the cat. I. The distribution of dorsal root fibers. Brain Res. 4: 1-15
370. **Sterling P, Kuijpers HGJM** (1967b) Anatomical organization of the brachial spinal cord of the cat. II. The motoneuron plexus. Brain Res. 4: 16-32
371. **Sterling P, Kuijpers HGJM** (1968) Anatomical organization of the brachial spinal cord of the cat. III. The propriospinal connections. Brain Res. 7: 419-443
372. **Steward O, Scoville SA** (1976) Retrograde labeling of central nervous pathways with tritiated or evans blue-labeled bovine serum albumin. Neurosci. Lett. 3: 191-196
373. **Steward O, Scoville SA, Vinsant SL** (1977) Analysis of collateral projections with a double retrograde labeling technique. Neurosci. Lett. 5: 1-5
374. **Stöckel K, Schwab M, Thoenen H** (1975) Comparison between the retrograde axonal transport of nerve growth factor and tetanus toxin in motor, sensory and adrenergic neurons. Brain Res. 99: 1-16
375. **Stöckel K, Schwab M, Thoenen H** (1977) Role of gangliosides in the uptake and retrograde axonal transport of cholera and tetanus toxin as compared to nerve growth factor and wheat germ agglutinin. Brain Res. 132: 273-285
376. **Svensson BA, Westman J, Rastad J** (1985) Light and elctron microscopic study of neurones in the feline lateral cervical nucleus with a descending projection. Brain Res. 361: 114-124
377. **Swenson RS, Castro AJ** (1983) The afferent connections of the inferior olivary complex in rats. An anterograde study using autoradiographic and axonal degeneration techniques. Neuroscience 8: 259-275
378. **Swett JE, Woolf CJ** (1985) The somatotopic organization of primary afferent terminals in the superficial laminae of the dorsal horn of the rat spinal cord. J. Comp. Neurol. 231: 66-77
379. **Swett JE, McMahon SB, Wall PD** (1985) Long ascending projections to the midbrain from cells of lamina I and nucleus of the dorsolateral funiculus of the rat spinal cord. J. Comp. Neurol. 238: 401-416
380. **Szentágothai J** (1951) Short propriospinal neurons and intrinsic connections of the spinal gray matter. Acta Morph. Acad. Sci. Hung. 1: 81-94
381. **Szentágothai J** (1964) Propriospinal pathways and their synapses. In: Eccles JC, Schadé JP (Eds.), Organization in the spinal cord. Progress in brain research, vol.11, Elsevier Biomedical, Amsterdam
382. **Tanaka T, Takeuchi Y, Nakano K** (1978) Cells of origin of the spino-facial pathway in the cat: a horseradish peroxidase study. Brain Res. 142: 580-585
383. **Tower S, Bodian D, Howe H** (1941) Isolation of intrinsic and motor mechanisms of the monkey's spinal cord. J. Neurophysiol. 4: 388-397
384. **Tracey PJ** (1985) Ascending and descending pathways in the spinal cord. In: Paxinos G (Ed.) The rat nervous system, Academic Press Australia, pp 311-324
385. **Trevino DL, Maunz RA, Bryan RN, Willis WD** (1972) Location of cells of origin of the

spinothalamic tract in the lumbar enlargement of cat. Exp. Neurol. 34: 64-77
386. **Trevino DL, Coulter JD, Willis WD** (1973) Location of cells of origin of spinothalamic tract in lumbar enlargement of the monkey. J. Neurophysiol. 36: 750-761
387. **Trevino DL, Carstens E** (1975a) Locations of the cells of origin of the spinothalamic tract in the cat and rhesus monkey as determined by the retrograde transport of horseradish peroxidase. Anat. Rec. 181: 494-495
388. **Trevino DL, Carstens E** (1975b) Confirmation of the location of spinothalamic neurons in the cat and monkey by the retrograde transport of horseradish peroxidase. Brain Res. 98: 177-182
389. **Uddenberg N** (1968) Functional organization of long, second-order afferents in the dorsal funiculus. Exp. Brain Res. 4: 377-382
390. **Ugolini G, Kuijpers HGJM, Strick PL** (1989) Transneuronal transfer of herpes virus from peripheral nerves to cortex and brainstem. Science 243: 89-91
391. **Vasilenko DA** (1975) Propriospinal pathways in the ventral funiculus of the cat spinal cord: their effects on lumbosacral motoneurones. Brain Res. 93: 502-506
392. **Vasilenko DA, Kostyukov AI** (1976) Brain stem and primary afferent projections to the ventromedial group of propriospinal neurones in the cat. Brain Res. 117: 141-146
393. **Voogd J, Broere G, van Rossum J** (1969) The medio-lateral distribution of the spinocerebellar projection in the anterior lobe and the simple lobule in the cat and a comparison with some other afferent fibre systems. Psychiatr. Neurol. Neurochir. 72: 137-151
394. **Waibl H** (1973) Zur Topographie der Medulla spinalis der Albinoratte (Rattus norvegicus). Advances in Anatomy, Embryology and Cell-Biology, vol. 47, fasc. 6, Springer-Verlag, Berlin Heidelberg New-York, pp. 5-42
395. **Wall PD** (1967) The laminar organization of dorsal horn and effects of descending impulses. J. Physiol. 188: 403-423
396. **Wall PD, Noordenbos W** (1977) Sensory functions which remain in man after complete transection of dorsal columns. Brain 100: 641-653
397. **Waltzer R, Martin GF** (1984) Collateralization of reticulospinal axons from the nucleus reticularis gigantocellularis to the cerebellum and diencephalon. A double-labelling study in the rat. Brain Res. 293: 153-158
398. **Watkins LR, Griffin G, Leichnetz GR, Mayer DJ** (1980) The somatotopic organization of the nucleus raphe magnus and surrounding brain stem structures as revealed by HRP slow-release gels. Brain Res. 181: 1-15
399. **Watkins LR, Griffin G, Leichnetz GR, Mayer DJ** (1981) Identification and somatotopic organization of nuclei projecting via the dorsolateral funiculus in rats: a retrograde tracing study using HRP slow-release gels. Brain Res. 223: 237-255
400. **Wiberg M, Blomqvist A** (1984) The spinomesencephalic tract in the cat: its cells of origin and termination pattern as demonstrated by the intraaxonal transport method. Brain Res. 291: 1-18
401. **Wiberg M, Blomqvist A, Westman J** (1986) The midbrain projection from the dorsal column nuclei and the lateral cervical nucleus. An anatomical study in the monkey. Neurosci. Lett. Suppl. 26: S220
402. **Wiksten B** (1975) The central cervical nucleus - a source of spinocerebellar fibres, demonstrated by retrograde transport of horseradish peroxidase. Neurosci. Lett. 1: 81-84
403. **Wiksten B** (1979a) The central cervical nucleus in the cat. II. The cerebellar connections studied with retrograde transport of horseradish peroxidase. Exp. Brain Res. 36: 155-173
404. **Wiksten B** (1979b) The central cervical nucleus in the cat. III. The cerebellar connections studied with anterograde transport of ^{3}H-leucine. Exp. Brain Res. 36: 175-189
405. **Wiksten B** (1985) Retrograde HRP study of neurons in the cervical enlargement projecting to the cerebellum in the cat. Exp. Brain Res. 58: 95-101
406. **Wiksten B, Grant G** (1986) Cerebellar projections from the cervical enlargement: an experimental study with silver impregnation and autoradiographic techniques in the cat. Exp. Brain Res. 61: 513-518
407. **Willis WD, Trevino DL, Coulter JD, Maunz RA** (1974) Responses of primate spinothalamic tract neurons to natural stimulation of hindlimb. J. Neurophysiol. 37: 358-372
408. **Willis WD, Kenshalo DJ Jr, Leonard RB** (1979) The cells of origin of the primate spinothalamic tract. J. Comp. Neurol. 188: 543-574
409. **Willis WD Jr** (1984) Modulation of primate spinothalamic discharges. In: Kruger L, Liebeskind JC (Eds.) Advances in pain research and therapy, vol. 6, Raven press, New York, pp. 217-240
410. **Woolf CJ, Fitzgerald M** (1983) The properties of neurones recorded in the superficial dorsal horn of the rat spinal cord. J. Comp. Neurol. 221: 313-328
411. **Woolf CJ, Fitzgerald M** (1986) Somatotopic organization of cutaneous afferent terminals and

dorsal horn neuronal receptive fields in the superficial and deep laminae of the rat lumbar spinal cord. J. Comp. Neurol. 251: 517-531

412. **Wouterlood FG, Groenewegen HJ** (1985) Neuroanatomical tracing by use of Phaseolus vulgaris-leucoagglutinin (PHA-L): electron microscopy of PHA-L-filled neuronal somata, dendrites, axons and axon terminals. Brain Res. 326: 188-191
413. **Xu Q, Grant G** (1988) Do certain spinocerebellar neurons in lamina X at lumbosacral levels send collaterals to peripheral nerves? A retrograde fluorescent double labeling study in the cat. Arch. Ital. Biol. 126: 179-192
414. **Yamada J, Otani K** (1978) The spinoperiventricular fiber system in the rabbit, rat and cat. Exp. Neurol. 61: 395-406
415. **Yamamoto T, Takahashi K, Satomi H, Ise H** (1977) Origins of primary afferent fibers in the spinal ventral roots in the cat as demonstrated by the horseradish peroxidase method. Brain Res. 126: 350-354
416. **Yezierski RP, Culberson JL, Brown PB** (1980) Cells of origin of propriospinal connections to cat lumbosacral gray as determined with horseradish peroxidase. Exp. Neurol. 69: 493-512
417. **Yezierski RP, Schwartz RH** (1986) Response and receptive-field properties of spinomesencephalic tract cells in the cat. J. Neurophysiol. 55: 76-96
418. **Ygge J, Grant G** (1983) The organization of the thoracic spinal nerve projection in the rat dorsal horn demonstrated with transganglionic transport of horseradish peroxidase. J. Comp. Neurol. 216: 1-9
419. **Zemlan FP, Leonard CM, Kow LM, Pfaff DW** (1978) Ascending tracts of the lateral columns of the rat spinal cord: a study using the silver impregnation and horseradish peroxidase techniques. Exp. Neurol. 62: 298-334
420. **Zemlan FP, Behbehani MM** (1984) Afferent projections to the nucleus cuneiformis in the rat. Neurosci. Lett. 52: 103-109

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 7 januari 1958 te Delft geboren. Hij bezocht de Christelijke Scholengemeenschap "Johannes Calvijn" te Rotterdam, alwaar hij in 1976 het diploma Gymnasium-ß behaalde. In het zelfde jaar begon hij de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Het arts-examen werd in juni 1983 met goed gevolg afgelegd.

Van februari 1979 tot november 1981 was hij werkzaam op de afdeling neuro-anatomie van de Erasmus Universiteit Rotterdam, aanvankelijk in het kader van een keuze-practicum en later als student-assistent. Na het voltooien van de studie geneeskunde volgde een aanstelling als wetenschappelijk medewerker op dezelfde afdeling neuro-anatomie, allereerst via FUNGO/ZWO, later in dienst van de Erasmus Universiteit. In deze periode (januari 1984 tot januari 1987) werd onder leiding van Prof. Dr. H.G.J.M. Kuijpers het onderzoek verricht waarvan de resultaten zijn beschreven in dit proefschrift.

Na het vervullen van de militaire dienstplicht werd in mei 1988 aangevangen met de opleiding tot internist (opleider: Dr. P.W. de Leeuw) in het Zuiderziekenhuis te Rotterdam.