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Transplantation of Neural Tissue into the Spinal Cord

Second Edition

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We dedicate this book to our families

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PREFACE

The book gives an account of results obtained from experiments where grafts of neuronal, glial and other tissues as well as artificial materials were placed into the spinal cord. It attempts to evaluate the contributions made by these studies to our understanding of basic neurobiological questions. These include factors that regulate neuronal growth during development as well as regeneration following injury to the nervous system. The model of neural transplantation is also useful for the study of cell-to-cell interactions, and this applies to interactions between glial cells and neurones, between various populations of neuronal cells and finally between axons and skeletal muscle fibres. The mechanisms involved in the establishment of specific synaptic connections between neurones can also be investigated in this experimental paradigm. Important information regarding this issue was also obtained on systems other than the spinal cord, i.e. the cerebellum, hippocampus and striatum. Although such information of precise connections between the host and the grafted embryonic tissue is still lacking in the spinal cord, there is much information on the response of the host nervous system to the grafted embryonic tissue, and that of the graft to its new host environment.

It appears that embryonic grafts are able to induce repair processes following injury to the nervous system. Grafting specific populations of glial or neuronal cells into the damaged spinal cord can enhance the regenerative capacity of the host. To give a few examples: glial cells that lose their ability to remyelinate demyelinated axons can be induced to do so by implants of specific populations of glial cells. Grafts of embryonic cord may act as relays between disconnected parts of the spinal cord and in this way allow the re-establishment of some function. Specific populations of neurones known to release transmitters that influence the excitability of cells in the spinal cord may be grafted so as to modify the excitability of the existing circuitry of the spinal cord of the host. The mechanism by which such influences upon the damaged nervous system are exerted are discussed in the relevant chapters. The book also considers the possibility that populations of highly specialized and unique cells such as motoneurones might be replaced by homologous cells from embryonic grafts in conditions where the host cells are lost. The ability of such grafted cells to establish afferent and efferent connections is described in detail.

The book reflects the present state of the art of the subject, where there is a great body of morphological, immunocytochemical and molecular information available as to the events that occur during graft-host interactions. However, only few well documented and thorough studies on functional consequences of the various experimental paradigms used are available. Yet without these the value of these experiments to clinical medicine is difficult to see.

In addition to the contribution of grafting experiments to our understanding of basic scientific questions, an important aspect of this publication is the attempt to relate the acquired information to relevant clinical conditions, in particular spinal cord injury and diseases affecting the spinal cord. The authors

of the book feel that there is a deep rift between the interests and hence approach to the problem of basic scientists and clinicians. This lack of communication was usually considered to be due to the delay between the time new information is obtained by basic scientists and when it reaches the clinical practitioner. However, another aspect of the problem is the relative lack of understanding by basic scientists of clinical issues. This lack of understanding often encourages the uncritical belief that a particular result obtained in the laboratory is ready to be used for treatment. A much closer link to clinical practice and more direct contact with it may reduce these problems. It is hoped that the book will encourage thoughts along these lines.

The findings summarized here show that grafted tissue can survive and thrive in a host mammal, occasionally replace some lost function and re-establish a semblance of sophisticated and complex circuitries. These new insights are among the most exciting in neurobiology, for they reverse the pessimistic view about the central nervous system that claims that nothing can grow or regenerate there. This view dominated for a very long time, and results obtained with grafts give us a new vision and encourage hope that it will be possible to treat some of the incurable diseases of the CNS.

Antal Nógrádi, Urszula Slawinska and Gerta Vrbová

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CHAPTER 1

Anatomy and Physiology of the Spinal Cord

Antal Nógrádi and Gerta Vrbová

Anatomy of the Spinal Cord

Gross Anatomy

The spinal cord is part of the central nervous system (CNS), which extends caudally and is protected by the bony structures of the vertebral column. It is covered by the three membranes of the CNS, i.e., the dura mater, arachnoid and the innermost pia mater. In most adult mammals it occupies only the upper two-thirds of the vertebral canal as the growth of the bones composing the vertebral column is proportionally more rapid than that of the spinal cord. According to its rostrocaudal location the spinal cord can be divided into four parts: cervical, thoracic, lumbar and sacral, two of these are marked by an upper (cervical) and a lower (lumbar) enlargement. Alongside the median sagittal plane the anterior and the posterior median fissures divide the cord into two symmetrical portions, which are connected by the transverse anterior and posterior commissures. On either side of the cord the anterior lateral and posterior lateral fissures represent the points where the ventral and dorsal rootlets (later roots) emerge from the cord to form the spinal nerves. Unlike the brain, in the spinal cord the grey matter is surrounded by the white matter at its circumference. The white matter is conventionally divided into the dorsal, dorsolateral, lateral, ventral and ventrolateral funiculi. Each half of the spinal grey matter is crescent-shaped, although the arrangement of the grey matter and its proportion to the white matter vary at different rostrocaudal levels. The grey matter can be divided into the dorsal horn, intermediate grey, ventral horn and a centromedial region surrounding the central canal (central grey matter) The white matter gradually ceases towards the end of the spinal cord and the grey matter blends into a single mass (conus terminalis) where parallel spinal roots form the so-called cauda equina.[1]

The dorsal roots leave the dorsal horn and dorsolateral white matter, coalesce into two bundles and enter the dorsal root ganglion (DRG) in the intervertebral foramen. Immediately distal to the ganglion, the dorsal and ventral roots unite and form a trunk, the spinal nerve. The spinal nerves, which are now outside the vertebral column, converge and form plexuses and from these emerge the peripheral nerves. The number of spinal nerves and spinal segments largely corresponds to the number of vertebrae with a few exceptions: there are eight cervical, 12 thoracic, five lumbar, five sacral and one coccygeal spinal segments in humans. The number of these segments varies slightly in different species.[2]

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Fine Organization of the Spinal Cord

The fine structure of the mammalian spinal cord was studied mainly on rodents, cats and primates. The most important results were those of Rexed[2,3] and Scheibel and Scheibel[4-6] on the cat spinal cord. Although the overall organization of the human spinal cord is similar to that of other mammals, there are some differences both in the cyto- and myeloarchitecture. In the past few years several studies made an effort to describe the structure of the human spinal cord and gave a detailed account of its features. Here we give a short description of the human spinal cord and where necessary refer to the important differences between human and other mammalian species (monkey, cat, rat and mice).

Cyto- and Dendroarchitecture

The laminar distribution of spinal neurons has been widely accepted. Its main advantage is its simple and comprehensive scheme of spinal cord organization and physiological properties can also be correlated to this structural arrangement.

Cytoarchitectural laminae are characterized by the density and topography of spinal neurons in the grey matter and can usually be identified on thick cross sections (Fig. 1). In addition, each lamina has its own characteristics which are particularly distinct at the level of cervical and lumbar enlargements. Most of the information about dendritic territories has been obtained by using Golgi impregnation methods. In addition to the laminar arrangement in the coronal plain, in the ventral horn the cervical and lumbar motoneurons form rostrocaudal motor columns[7] (Fig. 1).

Lamina I is the dorsalmost lamina which covers the tip of the dorsal horn. It has a loosely packed neuropil and a low neuronal density with neurons of variable size and distribution. The most typical neuron is the so-called Waldeyer[8] cell: large, fusiform neuron with disk-shaped dendritic domain.[9-12] However, in cat and rat also small and medium-sized pyramidal neurons were identified in this lamina[3,10,11,13,14] and characterised as fusiform, pyramidal and multipolar cells.[13]

Lamina II appears as a darkly stained band in Nissl-stained sections due to its high neuronal density (substantia gelatinosa, Rolando, 1824). In cat and rodents the inner and outer zones can be distinguished[2,3] although in humans there is not a clear separation between these zones. The neuronal population consists of small fusiform neurons. There are two main cell types which form the majority of the population of lamina II: the islet cells with a rostrocaudal axis and the stalked cells with a dorsoventral dendritic tree. Other types of neurons have been described such as arboreal, curly, border, vertical, filamentous and stellate cells.[9,15-18] It is possible, however, that some of these latter neurons correspond to each other or to the two main cell types. Islet cells contain GABA therefore they are considered as the inhibitory cells of this lamina.

Lamina III can be easily distinguished from lamina II by its lower neuronal density and by the presence of intermediate size neurons. This layer has a mixed population of antenna-like and radial neurons.[19-21] These cells have a simpler dendritic morphology than those in layer II.[9,22,23] Many of the above cells contain inhibitory neurotransmitters: GABA or glycine.[24]

Lamina IV in man and cat has a variety of antenna-like cells and the so-called transverse cell.[7,9,23] Most of their dendrites originate dorsally on the cell body and spread towards lamina II and III. In animals, the axons of lamina IV neurons mainly enter the spinocervical tract, which is vestigial in humans. Most probably human lamina IV neurons project to the spinothalamic tract.[25] Laterally from this lamina there is a small group of neurons embedded in the lateral funiculus: the lateral spinal nucleus[26] (Fig. 1). Its neurons project to the midbrain and brainstem and send processes to lamina IV itself.

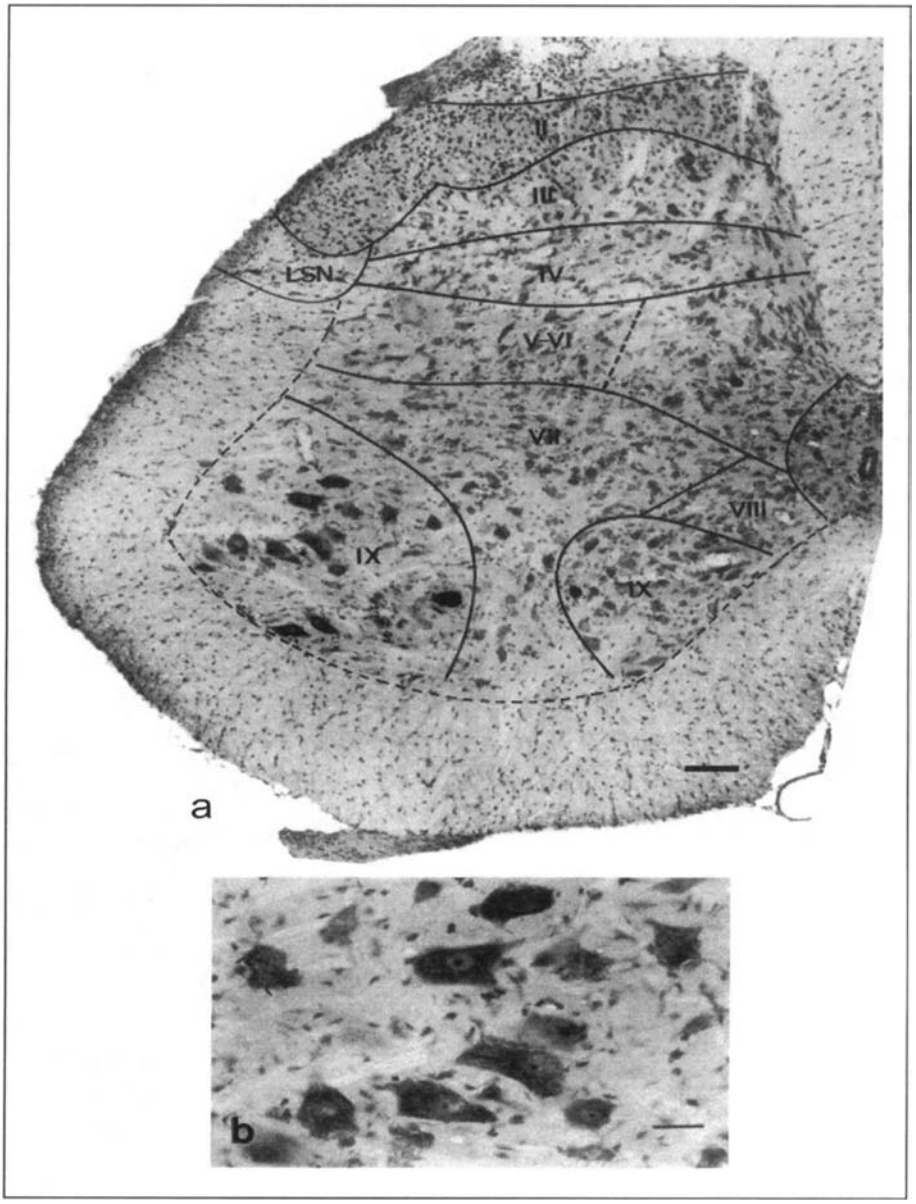

Figure 1. a) Cross section of the lumbar portion of the rat spinal cord showing the layered arrangement of the hemicord. The white matter is separated from the grey matter by a broken line. LSN= lateral spinal nucleus which takes place outside the grey matter in the dorsolateral funiculus. Cresyl violet stain. Scale bar = 50 µm. b) Higher magnification photograph shows a group of lumbar motoneurons. Note the rough and intensely stained Nissl substance which actually fills the cytoplasm. Cresyl violet stain. Scale bar = 20 µm.

Lamina V-VI have a similar cyto- and dendroarchitecture. The medial part contains fusiform and triangular neurons. The lateral part is not clearly separated from the dorsolateral funiculus. This part corresponds to the reticular formation in the brainstem and consists of medium-sized multipolar neurons.

Lamina VII occupies the intermediate zone of the grey matter and is formed by an homogeneous population of medium-sized multipolar neurons. In the appropriate segments it contains some well-defined nuclei, such as the intermediolateral nucleus (T1-L1; medially) and the dorsal nucleus of Clarke (T1-L2; laterally). The intermediolateral nucleus plays a role in the autonomic sensory and motor functions and the axons of neurons from the dorsal nucleus of Clarke form the ascending fibres of the dorsal spinocerebellar tract.

Lamina VIII has, unlike laminae I-VII a dorsoventral extension. It contains a variety of neurons with dorsoventrally polarized dendritic tree. The largest multipolar neurons can be distinguished from motoneurons only by their finer Nissl bodies by using conventional morphological techniques.[27]

Lamina IX is made up of groups of cells that form motor nuclei. Motoneurons have a unique position in this lamina, being the only spinal cord neuron which has its axon almost entirely in the peripheral nervous system. The α-motoneurons have the largest somata in the cord (50 x 70 μm) whilst the γ-motoneurons are smaller. Motoneurons can be easily recognized by the abundance of Nissl bodies in their cytoplasm and their multipolar shape (Fig. 1). Their dendrites extend for long distances, dorsally as far as lamina VI. Small neurons at the medial border of the motor nucleus are identified as the short-axoned inhibitory interneurons, the Renshaw cells. Although Rexed's classification[2,3] did not differentiate between motoneuron groups in lamina IX, these neurons can be divided into four separate columns in the human cord: the ventromedial, ventrolateral, dorsolateral and central columns[28] (Fig. 2).

Motoneurons projecting to the axial muscles are found in the ventromedial column,[28,29] those innervating proximal musculature of the limbs occupy medial and ventral position while neurons innervating distal limb muscles are located in dorsal and lateral positions. In all but one (dorsolateral) motoneuron column the dendritic polarization is longitudinal and dendritic trees overlap for a long distance (Fig. 2). Such a dendritic organization favours synchronization and synergy for axial, proximal and calf muscles.[4,5] In contrast to these columns, motoneurons in the dorsolateral column have radially oriented dendritic trees without much overlap of their dendrites (Fig. 2). This dendritic arrangement favours precise contacts with segmental afferents and may contribute to a more precise control of movements of distal muscles.

Lamina X corresponds to the substantia grisea centralis, the grey matter around the central canal. Two cell types can be recognized: (1) Bipolar cells with fan-shaped dendritic tree (dorsal portion of lamina X) and (2) bipolar cells with poorly ramified longitudinal dendrites (ventral portion).[30]

Interneurons in the Spinal Cord

Interneurons are probably the most important modulating cell types in the spinal cord. The importance of spinal interneuronal networks has only recently been acknowledged although the flexibility of these networks became apparent as early as in the 1950s.

Initially only electrophysiological approaches were used, later the precise location, morphology and immunohistochemical features helped to distinguish special interneuronal classes.

The very first morphologically and physiologically identified interneurons were the Renshaw cells and Ia interneurons (Renshaw cells project on motoneurons and thus establish the recurrent inhibition, whereas Ia interneurons are activated by Ia afferents of agonist muscles and inhibit antagonistic motoneurons).[31,32] Renshaw cells, Ia and Ib inhibitory interneurons, interneurons in disynaptic and polysynaptic reflex pathways and interneurons mediating descending commands were the "classical interneurons" and their function was thoroughly

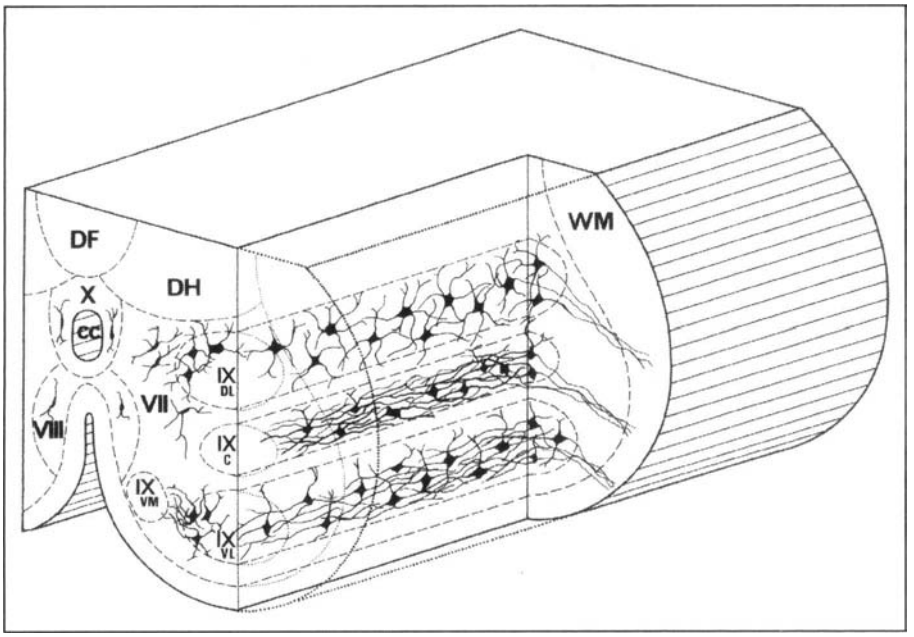

Figure 2. Schematic representation of the dendroarchitecture of spinal motoneurons in various motor columns. The ventromedial motoneurons (IX-vm) form vertical and longitudinal dendritic branches (not shown), motoneurons in the ventrolateral (IX-vl) and central (IX-c) columns tend to form dendritic bundles in the longitudinal and transverse planes. Motoneurons in these columns have long overlapping areas. On the contrary, dorsolateral motoneurons (IX-dl) have no such a dendritic bundle formation and their branches mostly branch out in the transverse plane. WM: white matter; DF= dorsal funiculus; DH= dorsal horn; CC= central canal.

analyzed in a series of studies. Recently a number of new interneurons modulating special functions were described, e.g.,interneurons involved in the clasp-knife reflex, bladder function, control of respiration and last-order premotor interneurons, etc. It is expected that the number of these highly specialized interneurons will further increase with time (for recent reviews and references see Jankowska 2001,[33] Edgley 2001[34]).

Most interneuron types have also been characterised by their neurochemical features. Renshaw cells, for example, express not only glycine, their characteristic inhibitory neurotransmitter, but they reportedly synthesize calcium binding proteins calbindin-D28k and parvalbumin.[35-37]

This short description of spinal interneurons suggests that the fine control of spinal functions mostly depends on the integrity of spinal interneuronal networks. It should be noted that interneurons named after their characteristic input (Ia, Renshaw, etc) receive a variety of multisensory inputs of different origins and these inputs together determine what the interneuron actually will do.

Glial Cells of the Spinal Cord

The central nervous system contains numerous nonneuronal, nonexcitable cells. The largest class of these cells is neuroglia or "nerve glue" a name taken from the Greek. The main glial cell types are astrocyte, oligodendrocyte, ependyma and microglia. Astrocytes together with

oligodendrocytes and ependyma develop from the neuroectoderm whilst microglia is considered to be derived from blood monocytes.

Astrocytes are large cells with a stellate morphology. These very numerous fine processes radiate in all directions and contain a specific form of cytoskeletal intermediate filament, the glial fibrillary acidic protein (GFAP, Fig. 3e). Astrocytes come in two main forms: fibrous astrocytes are primarily found in white matter and protoplasmic astrocytes in the grey matter. The latter subtype has long thin processes containing much less GFAP than the fibrous astrocytes, but can be characterized by the presence of glutamine synthase. Although these types of astrocytes differ anatomically, the developmental, functional and biochemical differences between them are not fully understood.[38]

During embryonic development astrocytes guide the migration of neurons while in the mature CNS they form a structural scaffolding for other cells. Astrocytic foot processes form perivascular cuffs around CNS capillaries thus contributing to the formation of blood-brain barrier and similar processes protect the CNS from external influences at the pial surface (glial limitans externa). Apart form many other metabolic functions astrocytes are thought to transport ions and fluid from the extracellular space to vessels and they can release a number of factors which promote axonal growth.[38] Astrocytes are able react to many deleterious effect to the CNS. Morphologically this process is characterized by the appearance and proliferation of so-called reactive astrocytes (Fig. 3). Although this astrocytic healing process is often called glial repair the proliferation of astrocytes can lead to the formation of glial scar which is considered as the impediment of axonal growth and regeneration in the CNS.

Oligodendrocytes produce myelin within the CNS. One oligodendrocyte is able to myelinate several adjacent axons (Fig. 3b). The myelin is formed by these cells wrapping spiral layers of cell membrane around the axon. The inner surfaces of the cell membranes fuse and form the so-called major dense line. The myelin contains special lipids and proteins, for example the glycolipid galactocerebroside and the myelin basic protein (MBP, Fig. 3c). The myelin in the CNS is the target of several serious diseases such as multiple sclerosis and leukodystrophies. Outside the CNS myelin is formed by Schwann cells which myelinate only a single axon. Schwann cells normally are not present in the CNS (Fig. 3g) and in the case of the spinal cord and brainstem there is a distinct junction between the PNS- and CNS-type myelin called transitional zone and characterized by a complex glial structure.[39]

The CNS has its unique set of immune cells the brain macrophages. The most important and characteristic CNS macrophages are the microglial cells[40] (Fig. 3). The phenotype of microglia suggests that they are dendritic antigen-presenting cells[41] expressing class II (I-A) major histocompatibility antigens. Under pathological circumstances microglial cells become activated, increase in size and number and are usually supplemented by blood-born monocytes.

Connections of the Spinal Cord with Other Parts of the CNS

The spinal cord has its own intrinsic pathways which are called propriospinal connections. The rest of the fibre tract system connects the spinal cord to other parts of the CNS and are described here as descending and ascending pathways. There are, of course, marked species differences, the most well known are those of the corticospinal system.

Intrinsic Pathways

These tracts not only establish connections between different neuronal groups and segments of the spinal cord but also act as relays between descending pathways and intrinsic spinal neurons. Accordingly, well defined ascending and descending white matter bundles are committed to propriospinal functions.

The Lissauer's tract can be localized between the entering dorsal roots and lamina I. It is mainly composed of unmyelinated descending and ascending fibres and both types extend a

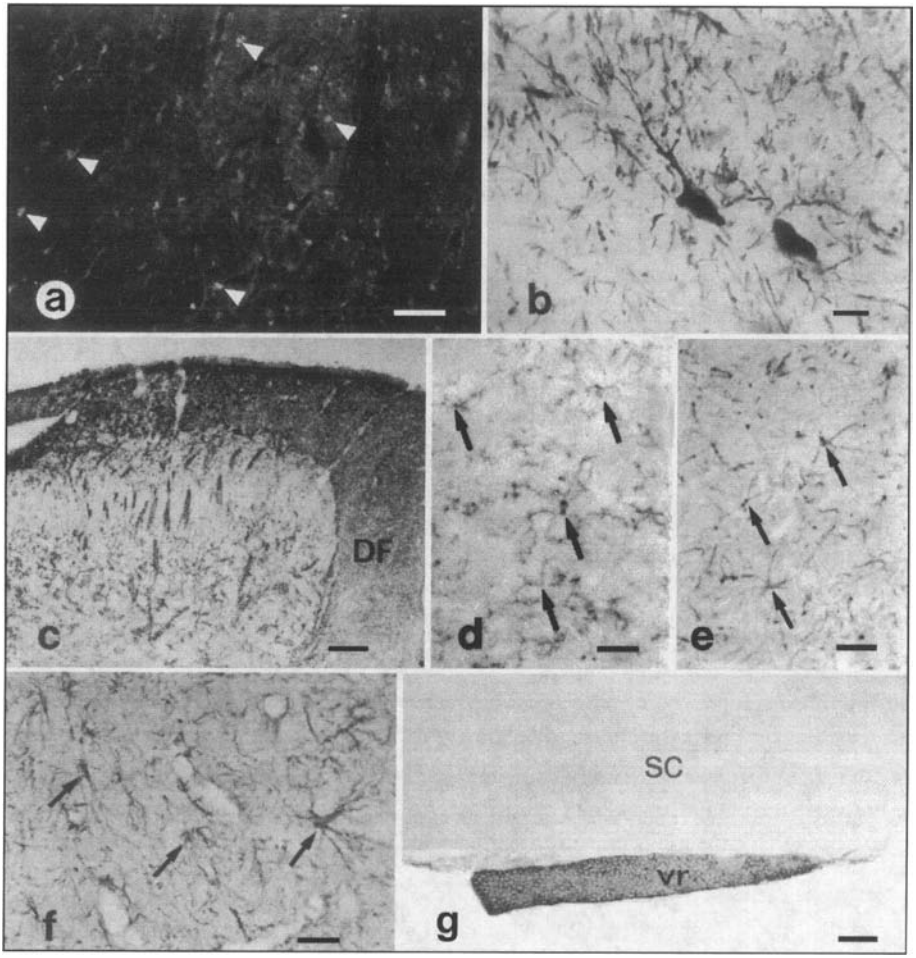

Figure 3. a) Fluorescent photograph of oligodendrocytes present in the spinal cord visualized with carbonic anhydrase II immunostaining. Scale bar = 50 µm. b) High magnification photograph shows two oligodendrocytes stained by using carbonic anhydrase enzyme histochemistry. Note the long, parallel branching processes. Scale bar = 10 µm. c) Distribution of myelinated tracts in the dorsal part of the spinal cord. Myelin sheaths are immunostained for myelin basic protein (MBP), a major protein present in normal myelin. DF: dorsal funiculus. Scale bar = 50 µm. d) Ramified microglial cells (arrows) in the grey matter of intact spinal cord. Note the faint staining and fine ramifications. Scale bar = 20 µm. Immunostaining to complement receptor type 3 (OX-42). e) Astrocytes in the intact spinal cord visualized by immunostaining to glial fibrillary acidic protein (GFAP). Scale bar = 20 µm. f) Reactive astroglial cells in an injured spinal cord (seven days after injury). Note the increased GFAP content and the thicker processes of the reactive cells (arrows). Scale bar = 20 µm. g) Low magnification photograph of the ventral part of spinal cord. The Schwann cells are immunostained with the Rat-401 antibody which is specific to Schwann cells in the adult CNS. Note that no immunostaining can be seen in the spinal cord (sc) only in the attached ventral roots (vr). Scale bar = 20 µm.

few segments. The majority of these fibres originate from the dorsal roots whilst the rest is intrinsic in nature terminating on marginal and substantia gelatinosa cells. The comma tract is a comma-shaped thin fibre bundle in between the fasciculi cuneatus and gracilis. It contains