Spinal Interneurons

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Elzbieta Jankowska

Contents

Abstract

This chapter deals with the neurons that constitute the majority of spinal neurons and are the main source of input to motoneurons, and therefore of critical importance for all motor reactions. The description of spinal interneurons focuses on the properties of their main populations and on the operation of basic interneuronal networks, both in animals and humans.

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Keywords

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Abbreviations

5-HT	Serotonin
C T and L segments	Cervical thoracic and lumbar spinal cord segments
EPSPs	Excitatory postsynaptic potentials
GABA	Gamma aminobutyric acid
HRP	Horseradish peroxidise
IPSPs	Inhibitory postsynaptic potentials
NA	Noradrenaline
VGLUT1	Vesicular glutamate transporter one
VGLUT2	Vesicular glutamate transporter two
WGA	Wheat germ agglutinin

Brief History

Progress in studies of spinal interneurons has been related to the progress in technical facilities to a much greater extent than in studies of spinal reflexes and in other fields of physiology. Each new technique has opened new possibilities of analysis of these neurons and their networks. Thus, until the 1960s the contribution of spinal interneurons to motor behavior could be deduced primarily indirectly from their actions on motoneurons. However, the situation dramatically changed when it became possible to investigate properties of single functionally identified interneurons, electrophysiological and morphological, as well as immunocytochemical and pharmacological. Recent progress in molecular biology opens further possibilities in studies of these neurons, allowing to trace the origin of their subpopulation from various embryonic neurons and to use genetically modified animals to analyze functions of various interneurons after selectively labeling, eliminating, or activating them. Taken together, the results of these studies greatly increased our knowledge on the operation of spinal interneuronal networks and provided a basis for application of this knowledge to restore motor functions in patients disabled by various central injuries.

Mammalian Spinal Interneurons

Subdivision of Spinal Neurons

Spinal interneurons constitute one of the four main categories of spinal neurons together with motoneurons, ascending tract neurons, and propriospinal neurons. All these neurons form a long column of neurons located within the core of the spinal cord,

which is surrounded by a great number of nerve fibers that interconnect different segments of the spinal cord, ascend to the brain, or descend from the brain to contact spinal neurons. Most of these fibers are myelinated and in the cross section of the spinal cord are lighter than the centrally located neurons. The outer layers of the spinal cord are therefore described as the white matter and its core as the gray matter. As illustrated in Fig. 1b, d, the areas of the gray and white matter differ in the segments that innervate limbs and in those innervating trunk; the areas of the gray matter are larger in the cervical and lumbar segments and the areas of the white matter decrease more caudally.

Motoneurons, which are the main output neurons of the spinal cord, are located in the most ventral/anterior parts of the gray matter. They are grouped in nuclei described as motor nuclei (marked with IX in Fig. 1d), motoneurons in each nucleus innervating distinct muscles (see ▶ Chap. 33, "Spinal Motoneurons"). Axons of motoneurons leave the spinal cord via ventral roots (Fig. 1c) and run within peripheral nerves before they innervate their target muscles.

Spinal interneurons, propriospinal neurons, and ascending tract neurons fill in the remaining parts of the gray matter. Their subpopulations are generally not grouped in nuclei but are intermixed with other interneurons, propriospinal neurons, and ascending neurons. However, subpopulations of these neurons, those originating from different embryonic neurons and/or characterized by similar morphology, input, and axonal projections are distributed in different areas of the gray matter. These are in roughly horizontal strips described by Rexed (1954) and are therefore known as Rexed's laminae (see Fig. 1d).

Ascending tract neurons are involved in processing information from muscle, skin, joint and any other parts of the body, as well as from intrinsic spinal neurons, and forward it to the brain for further analysis and routing. Axons of these neurons may be as long as of motoneurons but they do not leave the nervous system and ascend to the brain via lateral or ventral columns of the white matter, even though some have also local actions. Propriospinal neurons and interneurons may receive the same kinds of information as ascending tract neurons but distribute it exclusively within the spinal cord. Neurons that coordinate neuronal activity in distinct parts of the spinal cord are classified as propriospinal neurons. They project often over considerable distances, for instance, from the cervical to thoracic, lumbar, or even sacral segments, or from the lumbar to thoracic segments. In contrast, neurons that operate within only one of the enlargements, or even one segment of the spinal cord, are classified as interneurons; they are therefore referred to as "segmental interneurons" or "local interneurons." However, the borderlines between distances over which interneurons and propriospinal neurons operate are not sharp; some may be similar and the length of the axons by itself cannot be used as their differentiating feature. Only propriospinal neurons that project over longest distances can be intuitively considered as distinct from segmental interneurons and other features have to be taken into account while making distinction between them. So far, too little is known about propriospinal neurons to allow systematic comparison between their properties and properties of segmental interneurons. However, both the input to and output from the most extensively investigated propriospinal neurons considerably differ from those of the interneurons. They appear, for example,

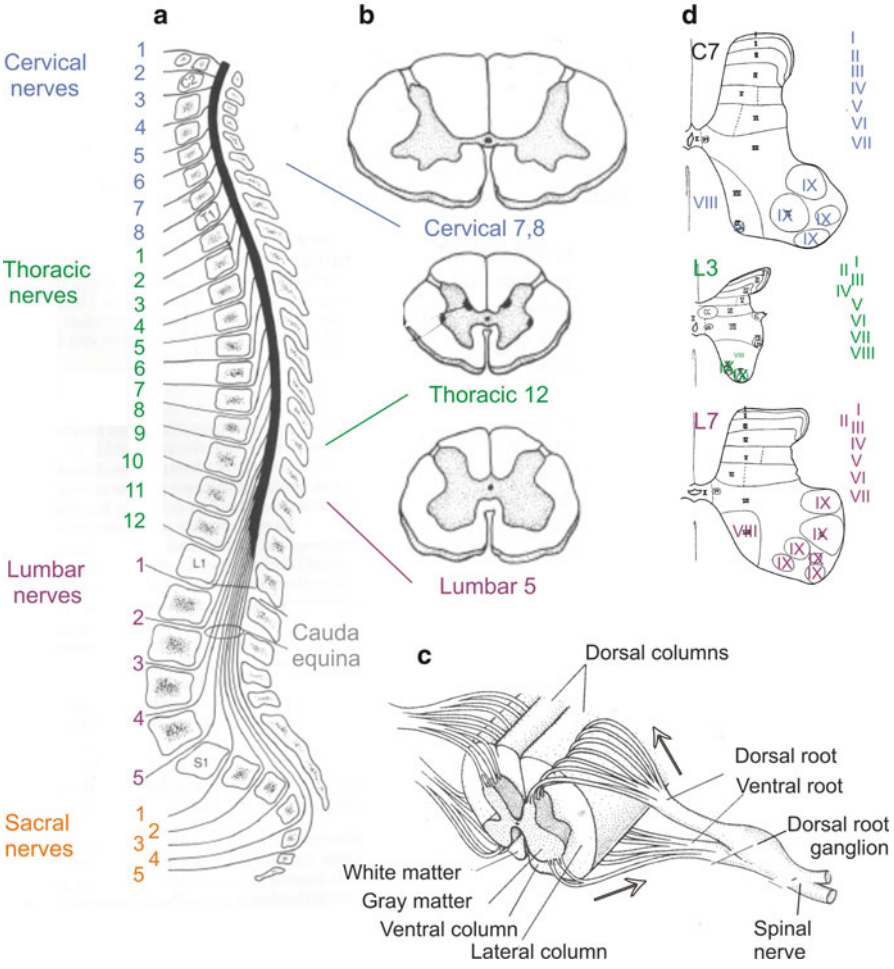


Fig. 1 Basic features of spinal cord morphology. (**a**) Location of the human spinal cord (*black*) with respect to the spinal vertebrae. Note that the spinal cord does not extend beyond thoracic vertebrae and that most of the spinal segments (defined by their connections with the successive spinal nerves) are located more rostral than the corresponding vertebrae. (**b**) Examples of relationships between the *gray* and *white* matter in different segments. Note greater volume of the *gray* matter in the segments innervating the upper and lower limbs (C7-Th2 and L1-L5 respectively) than in those innervating the trunk (Th3-Th12). (**c**) The input – output morphology in the transverse plane. The diagram illustrates that sensory fibers (axons of neurons located in the dorsal root ganglia) enter the spinal cord via the dorsal roots and that axons of motoneurons leave the spinal cord via the ventral roots. *Arrows* indicate the direction along which nerve impulses are conducted within these roots. Nerve fibers interconnecting neurons in the various segments, or neurons in the brain with those in the spinal cord, run through the ventral, lateral, and dorsal columns. (**d**) Laminar distribution of neurons in the gray matter. Laminae I–VIII include interneurons and ascending tract neurons. Motoneurons are located in several motor nuclei (labeled IX) jointly forming lamina IX. Note similar outline of the laminae in the segments innervating extremities (C7 and L7 in the cat) and a somewhat different outline in the segments innervating trunk. Similar lamination has been found in different species (**a**, **b**, and **c**, Modified from Kandel et al. 1991; **d**, Modified from Rexed (1954))

to have only minor local actions and/or not to mediate reflex actions from peripheral afferents (see below).

Both morphological and functional studies have revealed that interneurons themselves are highly nonhomogeneous with respect to their size, dendritic trees, and axonal projections in addition to their input and output connections, membrane properties, response characteristics, immunocytochemistry, and other features. Functionally, one may in particular distinguish between interneurons acting post- or presynaptically, between excitatory and inhibitory interneurons and between spiking and non-spiking interneurons. The postsynaptically acting interneurons are either excitatory or inhibitory, while the presynaptically acting ones are involved in presynaptic inhibition of synaptic transmission from peripheral afferents by modulating the amount of transmitter released from their terminals. In adult spinal cord, most of the excitatory interneurons are glutamatergic and of the postsynaptically acting inhibitory interneurons most are glycinergic, while interneurons mediating presynaptic inhibition are GABAergic (see however below). Spiking interneurons operate by generating action potentials (spikes) that are conducted without decrement along the axon and release transmitters from their axon terminals. In contrast, in non-spiking interneurons, the transmitter is released as a function of local membrane potential changes that spread electrotonically to the sites of synaptic contacts.

Another important distinction related to target cells of spinal interneurons is between the first-, second-, and later-order interneurons in neuronal pathways. The first-order interneurons are the earliest ones in reflex pathways – the interneurons with which peripheral afferents form synaptic contacts. Last-order, or premotor interneurons are those that terminate on motonerones and have direct excitatory or inhibitory actions upon them. The second, third, or later order are interneurons in interneuronal chains in polysynaptic pathways. However, depending on the coupling between particular primary afferents and motoneurons, individual interneurons may play the role of several of their categories. For example, in disynaptic pathways in which single interneurons relay actions of peripheral afferents (represented by cell A in Fig. 2), these interneurons play the role of the first as well as of the last-order interneurons. The same interneurons may also operate as the second- or third-order interneurons of pathways from other receptors in which other interneurons are first- or second-order interneurons (represented by cells B and C in Fig. 2).

Identification of Different Classes of Interneurons

For technical reasons, the easiest to recognize are the first- and last-order interneurons. The first-order interneurons may be identified by showing that they are monosynaptically excited by primary afferents (although their target cells often remain undefined) while several criteria have been used for the last-order premotor interneurons. One of the features assisting in their identification is finding the location of the interneurons projecting to one of the motor nuclei by using retrograde transport of a marker taken up by terminals of these interneurons. The most frequently used markers have been the enzyme horseradish peroxidise (HRP), alone or conjugated with wheat

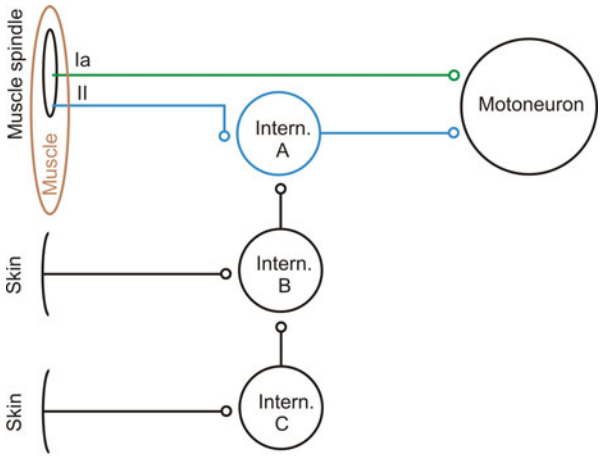


Fig. 2 Classification of spinal interneurons as first-, second-, third-, or last-order interneurons. The diagram illustrates a monosynaptic reflex arc from muscle spindle group Ia afferents, disynaptic reflex arc from muscle spindle group II afferents (via interneuron A), and reflex arcs from skin afferents via interneurons B and A or C, B, and A. It illustrates also that the same interneuron may operate as the first-order interneuron in one of the arcs (interneuron A in the arc from group II afferents) but as the second- or third-order interneuron in other arcs (e.g., from skin afferents)

germ agglutinin (WGA), various fluorescent markers, and various toxins and viruses. When the markers are introduced to an individual muscle, they are taken up by terminals of motoneuron axons, transported in the retrograde direction to the motoneuron cell bodies, released from motoneurons, taken up by terminals of interneurons which form synaptic contacts with motoneurons, and transported to the interneuron cell bodies where they can be visualized, as diagrammatically indicated in Fig. 3b. The outcome of such transneuronal labeling depends on both the uptake and the transport efficiency of the markers. In order to increase the probability of their uptake by motor axons, the markers can be applied to the cut surface of a muscle nerve (or injected into a crushed nerve), thereby shortcutting the stage of their uptake from the muscle. The markers may also be applied directly in the motor nuclei to increase the probability of their uptake by terminals of the interneurons, although in this case they can be taken up not only by interneurons terminating within the motor nuclei but also by cells located outside these nuclei and without taking into account in which of the motor nuclei they may terminate. The most recently used viral marking utilizes uptake of viruses injected into a muscle by motor axons and the multiplication of the viruses first in the motoneurons and then selectively in interneurons that contact them (Stepien et al. 2010). Despite the use of the variety of markers, the results turned out to be fairly consistent, by showing that cell bodies of the last-order interneurons are located predominantly in the ipsilateral laminae V, VI, VII, and X and in the contralateral lamina VIII. This is illustrated in Figs. 3a, d, e and 6.

Using functional criteria, last-order interneurons may be recognized by two techniques: by their antidromic activation by stimuli applied within motor nuclei

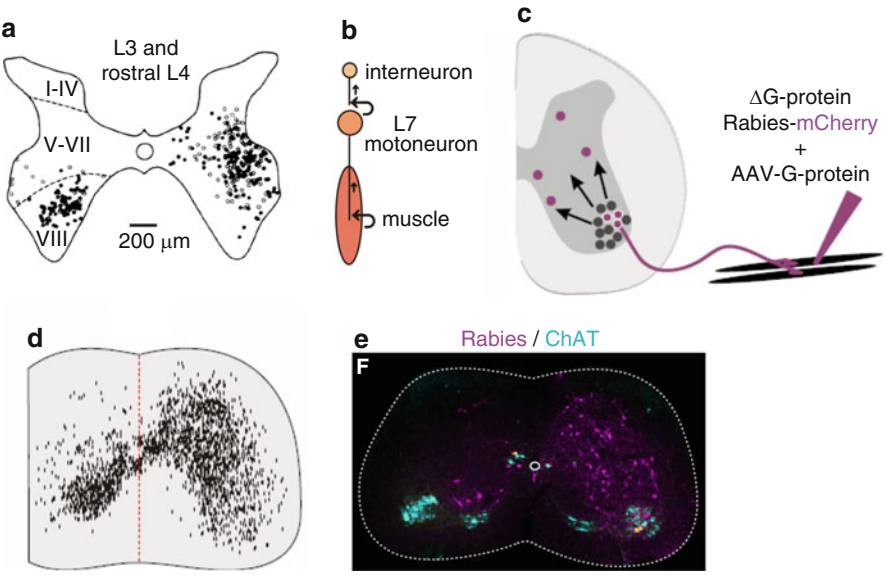


Fig. 3 Areas of location of premotor interneurons based on transneuronal transport of two retrograde markers. (**a**) Distribution of interneurons labeled in the L3 and L4 segments following application of WGA-conjugated HRP into a single muscle (semitendinosus) in two cats, followed by retrograde labeling of motoneurons in the L7 segment and transynaptic transport of the marker; the data from these cats are indicated by *open* and *filled circles*. (**b**) The experimental setup. (**c**) Experimental setup for transynaptic viral labeling. (**d**) Distribution of interneurons infected by rabies virus expressing the marker mCherry injected into the quadriceps muscle in the mouse following infection of the motoneurons. (**e**) Transverse section of the spinal cord at L3 showing noninfected motoneurons (*blue*) on both sides, an infected motoneuron (*yellow*) on the right side and the labeled interneurons (*purple*). Note generally similar pattern of labeling in the two preparations, with the majority of ipsilaterally labeled interneurons in laminae V–VII and of contralaterally labeled interneurons in lamina VIII (**a** Modified from Jankowska and Skoog 1986; **c**–**e**, Modified from Stepien et al. 2010)

(Fig. 4a, b) or within the part of the spinal cord including motor nuclei (Fig. 4i, j) or by tests showing that action potentials of the tested interneuron are followed by EPSPs or IPSPs (excitatory or inhibitory postsynaptic potentials) evoked monosynaptically in some motoneurons (Fig. 4c, g, h). The most unequivocal way of demonstrating actions of an interneuron on motoneurons would be to penetrate it, to stimulate it with intracellular current pulses and to show that it excites or inhibits its target cells ("follower neurons" according to another terminology). An easier and technically more feasible way involves simultaneous recording of extracellularly recorded spikes of an interneuron and of postsynaptic potentials from presumed target cells of this interneuron ("spike triggered averaging" by which weak potentials following the spikes are averaged in a computer to increase signal-to-noise ratio) and by showing that the latter appear at latencies compatible with monosynaptic coupling. Such latencies are of the order of <1 ms in adult feline spinal cord, as illustrated in Fig. 4c, d but may be of several ms in neonatal preparations in which

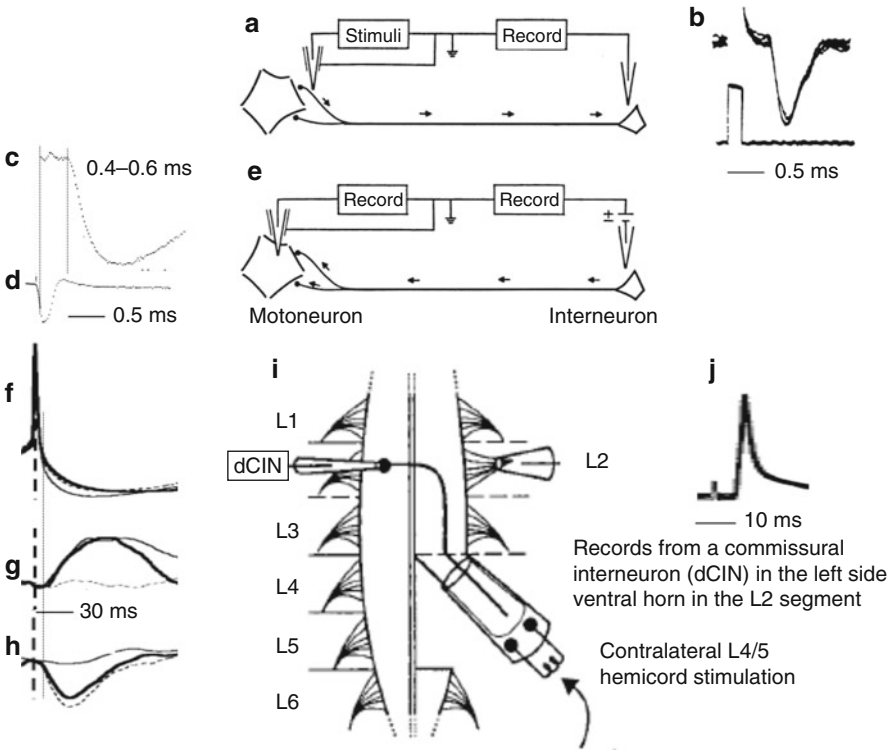


Fig. 4 Identification of last-order interneurons by antidromic activation from motor nuclei and by spike-triggered averaging. The experimental arrangement used in experiments in the cat and in the mouse are shown in (**b**), (**e**), and (**i**) respectively. (**a**) and (**b**) Stimulation in a motor nucleus and antidromically evoked spikes of an Ia inhibitory interneuron. (**c**–**e**) Simultaneous extracellular records from this interneuron and intracellular records from a motoneuron in which they were followed by IPSPs. (**i**) and (**j**) Stimulation of the hemicord including motor nuclei and antidromically evoked spikes of a caudally projecting commissural interneuron (dCIN). (**f**) and (**g**) Simultaneous intracellular records from one of such commissural interneurons and electronically spread postsynaptic EPSPs recorded from axons of motoneurons in the contralateral L2 ventral root that followed interneuronal spikes. (**h**) IPSPs following spikes of another commissural interneuron recorded in the same way (**a**–**e**, Modified from Jankowska and Roberts 1972; **f**–**j**, Modified from Butt and Kiehn 2003)

the conduction velocities along axons of interneurons are much longer (cf latencies of postsynaptic potentials following interneuronal spikes in Fig. 4c, g, h). Spikes of the interneurons may be induced either "spontaneously" or by local application of excitatory amino acids. Some interneurons or interneuron classes may also be recognized not only in mature but also in embryonic spinal cord and in neonatal genetically modified animals by using a variety of molecular techniques (see the last two sections).

By using these techniques individual interneurons of various reflex or other pathways can be recognized, their characteristic features analyzed, and organization of networks of these neurons established both in simple and more developed vertebrates. An increasing number of interneurons and interneuronal networks have been recently identified in the cat as well as in the rat and mouse, although the progress has not yet been as impressive as in the lamprey (see Grillner et al. 1998) frog tadpole (see Roberts et al. 1998) and zebrafish (see Fetcho et al. 2008).

Historically, the dominating tendency of many studies has been to try to link one particular kind of interneurons to a quite distinct function. Thus, one tried to find out which of the interneurons forward information from muscle spindles and which from tendon organs or from other muscle receptors and various joint or skin receptors, which interneurons mediate commands from the major descending tract neurons, and which are responsible for initiating complex behaviors, such as locomotion, swimming, or scratching. The results of these studies revealed that vertebrate interneurons are highly specialized, but not to the extent that any can be attributed only one narrow function. On the contrary, the function of individual interneurons appears to be to integrate information from several receptors, for example, from muscles as well as tendons, or information provided by both innocuous (e.g., tactilde) and noxious stimuli, or from several descending tract neurons (e.g., both cortico- and reticulospinal), as a rule in combination with information from muscle and skin afferents.

This reflects the situation, sometimes described in terms of "the final common interneuronal pathways" in analogy to motoneurons constituting the final common pathway of Sherrington (1906). It can also be described in terms of the *multifunctional character* of spinal interneurons. Multifunctional character of interneurons has been repeatedly revealed in various invertebrates. In vertebrates, it was first demonstrated by showing that interneurons of a particular kind can be used for a number of different purposes and that individual interneurons may be incorporated into a number of larger networks and contribute to a variety of reactions, including voluntary movements (see, e.g., Lundberg 1975; Baldissera et al. 1981; McCrea 1992). This has been most extensively documented for interneurons mediating reciprocal inhibition between flexors and extensors (see below) but the evidence for the involvement of a given class of interneuron in more than one motor behavior has also been provided for interneurons of other spinal reflex pathways, particularly with respect to their contribution to locomotion (see, e.g., McCrea 1998; Burke 1999) and in all of the species analyzed (e.g., Berkowitz et al. 2010).

The most relevant questions to ask with respect to functions of various interneuronal subpopulations is therefore: "which kinds of information do they integrate" and "how do they use this information by exciting or inhibiting other neurons." Not many kinds of spinal interneurons have so far been investigated to provide answers to these questions but everything that we have learned about them shows that they play a much more essential role in organizing motor output than originally assumed.

Examples of the Most Completely Functionally Identified Mammalian Interneurons and Their Properties

Premotor Interneurons Mediating Inhibition Between Antagonists: Ia Inhibitory Interneurons

One of the most completely identified mammalian interneuronal populations are interneurons mediating inhibition between flexor and extensor muscles in the disynaptic reflex arc in which only one interneuron is interposed between group Ia muscle spindle afferents and motoneurons as indicated in Fig. 5a. These interneurons, denoted "Ia interneurons," are located in lamina VII, just dorsal and medial of motor nuclei, as indicated by the blue circle in Fig. 6e. However, they affect motoneurons located both in the closest motor and up to one or two segments away. Axons of Ia interneurons project, therefore, over several mm distances within the white matter surrounding the ventral horn before their axon collaterals reenter the gray matter and make synaptic contacts with motoneurons innervating antagonist muscles.

Originally, these Ia interneurons were expected merely to provide means to change the type of transmitter from excitatory (in synapses made by peripheral afferents) to inhibitory (glycine), and to relay inhibition from one specific group of afferents, as indicated in Fig. 5a. It has however turned out that these interneurons function as premotoneuronal integration centers rather than as simple relays. Figure 5b shows that they operate as the last link in polysynaptic pathways from a variety of peripheral afferents (although monosynaptically they are excited exclusively by group Ia afferents) as well as from supraspinal structures. The same interneurons may thus mediate inhibition of motoneurons from several sources, not only during muscle stretches but also during crossed extensor reflexes, postural reflexes, centrally-induced fictive locomotion, and movements relayed by descending neuronal systems, including the corticospinal in primates (see Jankowska 1992). Interneurons mediating inhibition of flexors and those mediating inhibition of extensors (dark and light blue in Fig. 5b, respectively) may also mutually inhibit each other's actions to ensure that the degree of inhibition of antagonists is properly balanced.

The most characteristic feature of input to Ia interneurons is, however, that actions of these interneurons are gated by inhibitory interneurons called Renshaw cells (gray cells in Fig. 5b, see below). Renshaw cells that provide negative feedback to motoneurons following their activation and prevent their reactivation at too short intervals, prevent also Ia inhibitory interneurons from too strongly inhibiting their antagonists. Thereby, they help to ensure a proper balance between contractions of flexors and extensors and even allow that they contract simultaneously (co-contract) which is of great importance for the stability of various movements. Inhibition by Renshaw cells turned out to be a distinguishing feature of Ia inhibitory interneurons because no other interneurons were found to be under inhibitory control of Renshaw cells. This feature allows identification of Ia inhibitory interneurons even in greatly reduced preparations, for instance, in the isolated spinal cord in vitro, in slice

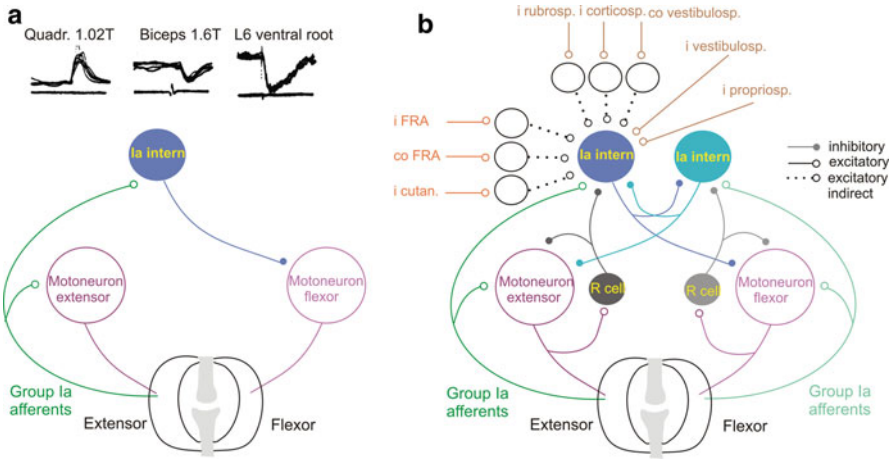


Fig. 5 Diagrams of neuronal networks of reciprocal inhibition and of recurrent inhibition. (**a**) Diagram of neuronal pathway of reciprocal inhibition between flexors and extensors as found originally. In records above the diagram, the upper ones are intracellular records from a Ia interneurons with monosynaptic EPSPs from the quadriceps nerve, disynaptic IPSPs evoked from the antagonist, the posterior biceps nerve, and disynaptic IPSPs evoked by stimulation of the L6 ventral root which includes axons of quadriceps motoneurons. The lower records are from the surface of the spinal cord and indicate timing of afferent volleys. (**b**) A more complete network of spinal neurons jointly mediating reciprocal inhibition. *Dark blue circle* represents interneurons mediating reciprocal inhibition of flexor motoneurons by extensor muscle spindle primary (group Ia) afferents. Continuous and *dashed lines* indicate monosynaptic and polysynaptic connections from group Ia muscle spindle afferents, low-threshold cutaneous afferents (cutan.), high-threshold cutaneous, muscle, and joint afferents (flexor reflex afferents, *FRA*) and from rubrospinal, corticospinal, vestibulospinal, and propriospinal tract neurons, all excitatory. *i* ipsilateral, *co* contralateral. *Gray circles* represent Renshaw cells (*R*) and *light blue circle* a population of Ia inhibitory interneurons with opposite actions (in pathways from flexor group Ia afferents to extensor motoneurons), which gate transmission through the Ia interneurons by inhibiting them. Neurons mediating presynaptic inhibition of transmission from primary afferents and neurons involved in other modulatory actions are not included in this diagram

preparations, and in neonatal or even prenatal animals in which the number of tests needed to differentiate between various functional types of interneurons is greatly limited. They can be recognized by being inhibited following stimulation of a ventral root or of single motoneurons, or by showing that neurons with features of Renshaw cells (see below) make synaptic contacts with them. Using this criterion, it became possible to label individual Ia inhibitory interneurons by intracellular markers (Fig. 6a, c) and investigate morphology of their dendritic trees and axonal projections, define the transmitter content in their axonal terminals, analyze their membrane properties, as well as determine how their activity may be modulated. Most recently, it became also possible to follow their differentiation from the V1 embryonic neurons during the development and to define their molecular characteristics (see Fig. 15; Alvarez and Fyffe 2007; Goulding 2009).

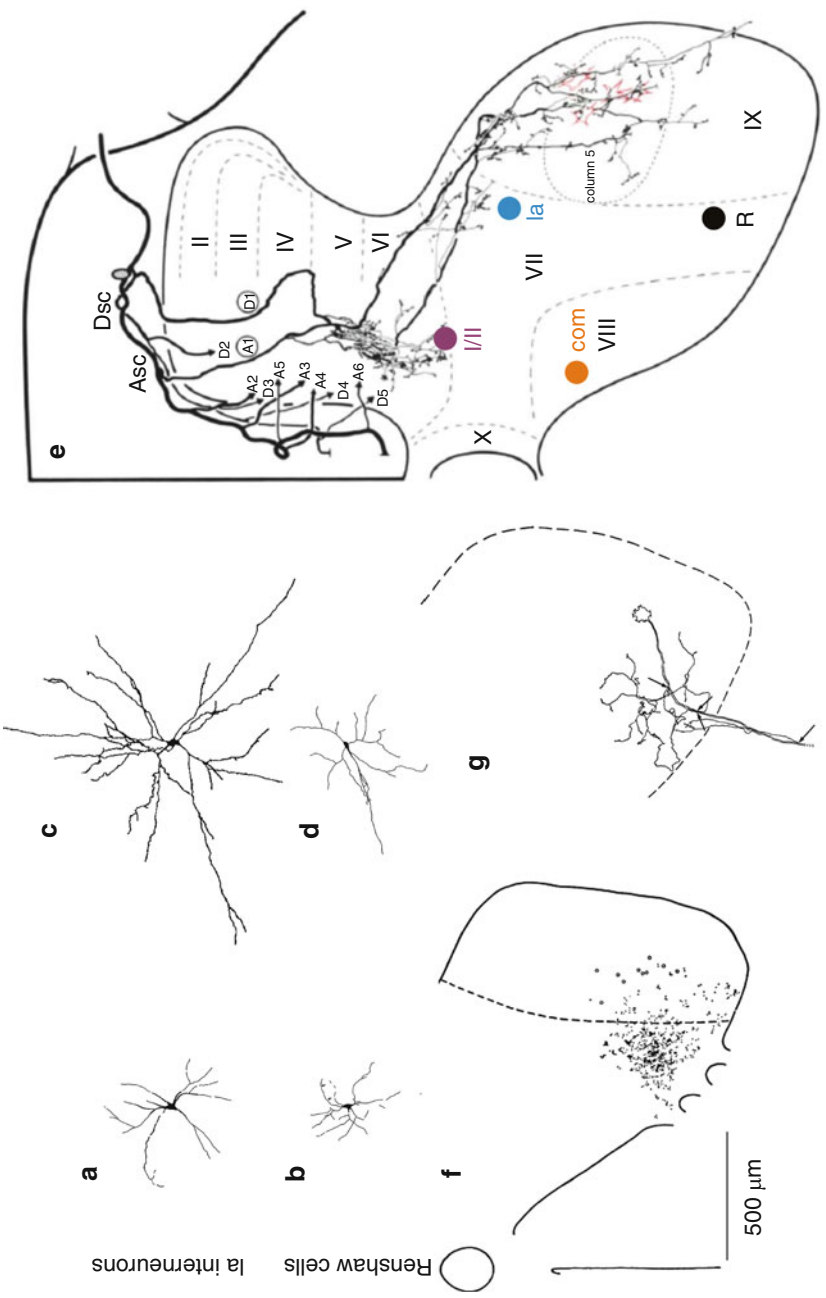


Fig. 6 (continued)

Renshaw Cells

Renshaw cells are, like Ia inhibitory interneurons, premotor inhibitory interneurons but with several distinct properties. Renshaw cells operate primarily within short distances from the location of their cell bodies in lamina VII just medial to the location of motor nuclei (indicated by R in Fig. 6e). Nevertheless, axons of some of these interneurons project over distances of several millimeters along the lateral or ventral funiculi before they reenter the gray matter. Projections of Renshaw cells are ipsilateral except in the lower sacral segments, where they are bilateral. Renshaw cells are excited by axon collaterals that are given off by motoneuron's axons before they leave the spinal cord (recurrent axon collaterals). In contrast to Ia interneurons, Renshaw cells do not receive direct input from primary afferents and are to a much smaller extent excited by other interneurons. The main function of Renshaw cells, as of Ia interneurons, is to inhibit motoneurons, but they inhibit motoneurons of synergists and not of antagonists, as indicated in Figs. 5b and 7d, e. They inhibit motoneurons that excite them as well as motoneurons that innervate a wide range of synergist muscles. However, recurrent inhibition has been found only in motoneurons of some muscles: the majority of limb muscles and of tail, back, intercostal, diaphragm, and neck muscles, but not of the most distal forelimb or hindlimb muscles, the perianal sphincters, and jaw and eye muscles.

Renshaw cells have also other functions. One of these is to adjust the degree of inhibition of antagonists to that of synergists. This is done by preventing too strong inhibition of antagonists by Ia interneurons, as indicated in Fig. 5b. They may also adjust the degree of inhibition of other motoneurons by Renshaw cells, by mutual inhibitory interactions between Renshaw cells, as indicated in Fig. 7e.

In synapses between Renshaw cells and their target neurons, the main transmitter is glycine (with strychnine as the antagonist). However, some actions of Renshaw cells, or actions of their subpopulations, have been found to depend on gamma-

Fig. 6 Examples of dendritic trees of Ia inhibitory interneurons and of Renshaw cells. (**a**, **c**) Ia interneurons. (**b**) and (**d**) Renshaw cells. The neurons were identified using criteria described in the text, on the basis of intracellular records such as in Figs. 5a and 7b in the cat. They were then injected with two different markers: *Procyon Yellow* (**a**, **b**; Jankowska and Lindstrom 1970) or Neurobiotin (**c**, **d**; Alvarez et al. 1997), visualized using light microscopy and reconstructed. Both markers revealed much larger somata and extent of dendrites of Ia interneurons than of Renshaw cells. (**e**) Projection areas of two axon collaterals of an Ia afferent fiber from the medial gastrocnemius (MG) in the L7 segment, labeled by intra-axonal injection of HRP and reconstructed in the same way as the interneurons and at the same scale (Modified from Ishizuka et al. 1979). *Blue*, *black*, *purple*, and *orange circles* indicate location of Ia inhibitory interneurons, Renshaw cells, group I and II excited interneurons and group II excited commissural interneurons, respectively. I–X, Rexed laminae. *Red contours*, motoneuron somata. Note that dendritic trees of the illustrated Ia interneurons would cover a considerable part of the region of the *gray* matter in which terminals of Ia afferents were found. (**f**) and (**g**) Projection areas of axon collaterals of motoneurons labeled by intracellular injection of HRP, reconstructed in the same scale; recurrent axon collaterals and axonal swellings of one posterior biceps motoneuron are shown in (**g**) and axonal swellings of ten such motoneurons in F (Modified from Cullheim and Kellerth 1978). Note that projection area of motoneuron axon collaterals extended more medially and more ventrally and that the dendritic trees of the illustrated Renshaw cells might have been confined within this area

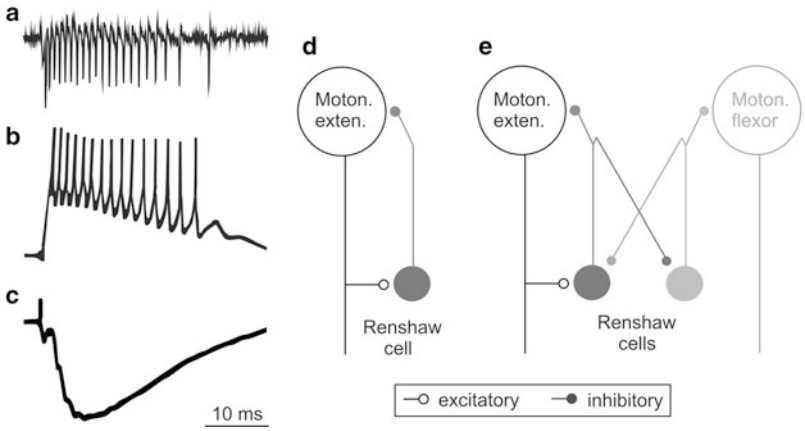


Fig. 7 Renshaw cells and their networks. (**a**) Extracellular records from a Renshaw cell illustrating a long-lasting and high frequency burst of discharges following single stimuli applied to motor axons in a ventral root. (**b**) Similar burst of discharges of a Renshaw cell but recorded intracellularly, illustrating that these discharges are initiated by large and long-lasting EPSPs evoked by nerve impulses in motoneuron axon-collaterals. (**c**) An example of long duration IPSPs evoked in motoneurons by Renshaw cells activated by a single stimulus applied to a ventral root. (**d**) The basic network of motoneurons and Renshaw cells subserving negative feedback via motoneuron recurrent axon-collaterals. (**e**) A more complete diagram of motoneuron-Renshaw cells networks, illustrating mutual inhibitory interactions between Renshaw cells. It supplements a fuller diagram of actions of Renshaw cells, on both motoneurons and Ia inhibitory interneurons, in Fig. 5b

aminobutyric acid (GABA; depressed by bicuculline or picrotoxin). The synapses made upon Renshaw cells by motor axon collaterals are cholinergic: the early components of the synaptic responses are evoked via nicotinic receptors (blocked by dihydro-beta-erythroidine), and the late responses are evoked via muscarinic receptors (blocked by atropine). Inhibitory synapses on Renshaw cells have been recently investigated in particular detail revealing that they are characterized by exceptionally large clusters of gephyrine and glycine receptors (Alvarez et al. 1997), as illustrated Fig. 8. This feature may thus be used to distinguish Renshaw cells and it has already assisted in finding their genetic markers (transcription factors, homeodomain proteins) and to reveal the process of their differentiation and development at a molecular level (from V1 embryonic neurons; see Fig. 15).

Premotor Interneurons Processing Information from Both Group I and II Muscle Afferents

Several features differentiate premotor interneurons of this population from Ia inhibitory interneurons. For example, they include both excitatory and inhibitory interneurons, are located more dorsally (in laminae V–VI), mediate reflex actions of both group Ia and Ib as well as group II afferents, have different motoneurons as their target cells, and are not under inhibitory control of Renshaw cells (Fig. 9).

Originally, the main function of these interneurons was considered to mediate reflex actions of group Ib tendon organ afferents; they were therefore referred to as

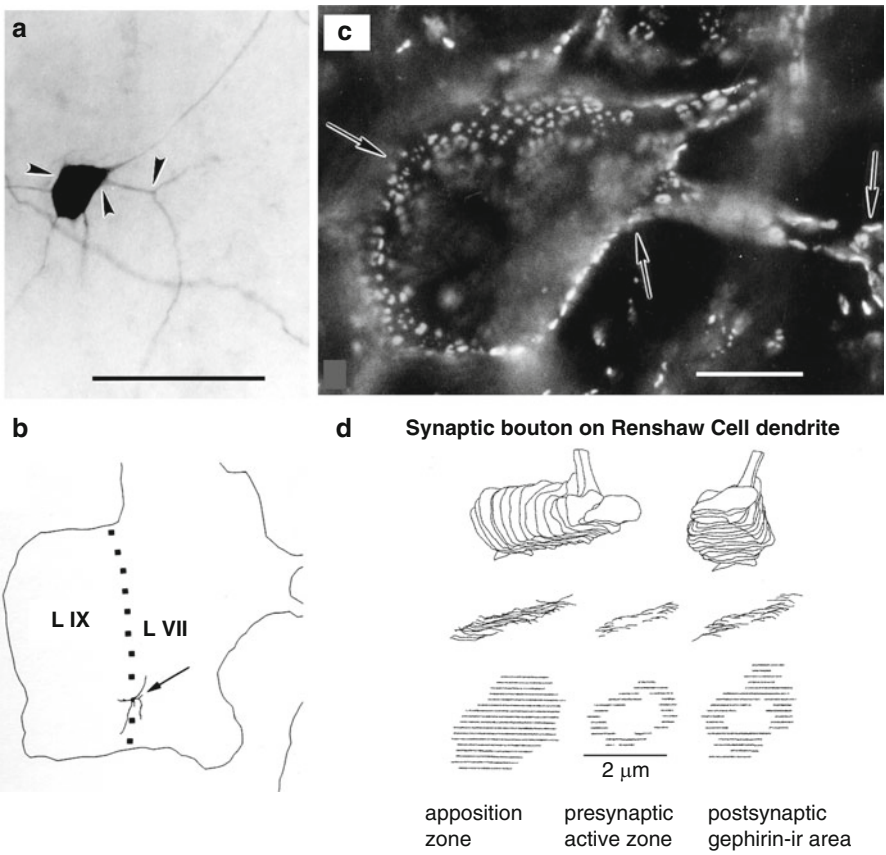


Fig. 8 Characteristic clusters of gephyrine and glycine receptors on Renshaw cells. (**a**) and (**b**) Low-magnification image of the soma and proximal dendrites of a Renshaw cell and reconstruction of its location. Scale bars 100 and 500 μm. (**c**) High-magnification micrograph of gephyrine immunoreactive clusters. Scale bar 10 μm. (**d**) Serial section reconstruction of a terminal in contact with a Renshaw cell in two different rotations. Second and third rows show the reconstructed zones of apposition between the terminal and postsynaptic element, the extension of the presynaptic active zone, and the extension of postsynaptic gephyrine immunoreactivity (Modified from Alvarez et al. (1997))

"Ib interneurons." The main function of inhibitory interneurons was primarily linked to inhibition of motoneurons innervating the contracting muscle; hence the inhibition was referred to as "autogenetic" inhibition. Excitatory interneurons were expected to excite motoneurons innervating other muscles. These two actions are represented in Fig. 9a. They are represented with inhibition of extensors and excitation of flexors because these interneurons are more effectively activated by afferents from extensors than from flexors and their overall effects are stronger inhibition of extensors than of flexors and stronger excitation of flexors than of extensors. However, to mediate reflex actions of group Ib afferents is only one of the

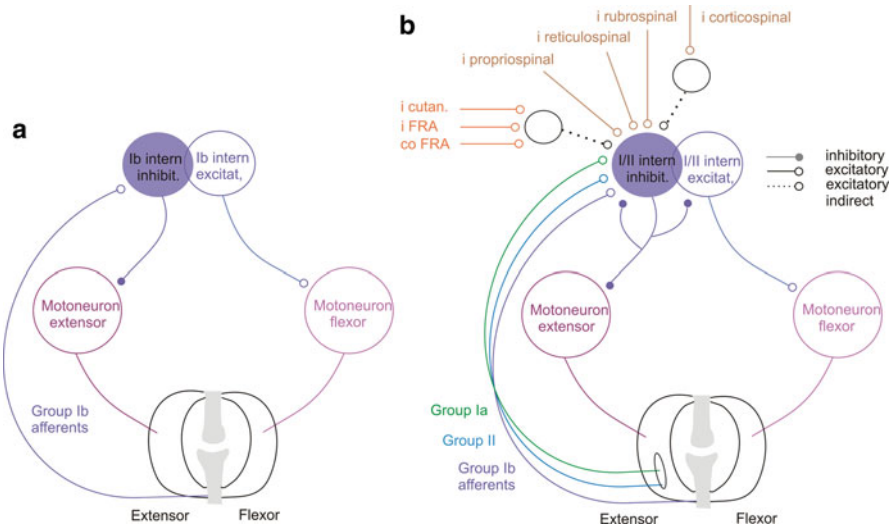


Fig. 9 Diagrams of neuronal networks of interneurons mediating reflex actions from tendon organs (group Ib afferents) as well as from muscle spindles (group Ia and II afferents). (**a**) Diagram of predominant actions evoked from tendon organs (group Ib afferents) and their reflex arcs as found originally: autogenetic inhibition of extensor motoneurons and excitation of flexor motoneurons. The indicated input from group Ib afferents is for both the inhibitory and excitatory interneurons; it can be from either the same or different tendon-muscle junctions; an example of records from such interneurons is in Fig. 10h. (**b**) A more complete network of spinal neurons that jointly mediate reflex actions of group Ia, Ib, and II afferents, low-threshold ipsilateral cutaneous afferents (i cutan.), high-threshold ipsilateral (i) and contralateral (co) cutaneous, muscle and joint afferents (flexor reflex afferents, FRA), as well as synaptic actions evoked by ipsilateral propriospinal and rubro-, cortico-, and reticulospinal neurons. As in (**a**) input from all these sources is indicated for inhibitory interneurons (represented by *filled circles*) but is much the same for the excitatory ones (represented by *open circles*). *Continuous* and *dashed lines* indicate monosynaptic and polysynaptic connections. Note inhibitory actions of inhibitory interneurons on their own population as well as on excitatory interneurons. Neurons mediating postsynaptic IPSPs from the indicated sources of input, neurons mediating presynaptic inhibition of transmission from primary afferents and neurons involved in other modulatory actions are not included in this diagram

functions of these interneurons. Their main function is to integrate information from Ib afferents activated during muscle contractions with information on the degree of stretch of the contracting muscles as well as with information on contacts with objects, or on joint movements associated with muscle contractions, as indicated in Fig. 9b. They should therefore be referred to as group I and group II excited or "group I/II interneurons." On the basis of such integrated information they select motoneurons that are best suited to react under a given situation.

Actions of the inhibitory subpopulation of these neurons on individual motoneurons are often combined with actions of their excitatory subpopulation, with the resulting mixed excitatory-inhibitory effects of the same peripheral stimuli. Inhibition of some of the motoneurons is nevertheless usually stronger than of other

motoneurons while relatively stronger excitation is evoked in the others, assisting in selection of their different combinations and the resulting different patterns of movements. The relative contribution of the excitatory and inhibitory interneurons may dramatically change in some particular movements or phases of movements. The most spectacular is the elimination of inhibition from Ib afferents during locomotion, in particular during the stance phase of the step cycle, and the replacement of actions of the inhibitory interneurons by actions of the excitatory interneurons (see, e.g., McCrea 1998). In this way the activation of extensors that is essential for supporting the body weight during the stance phase is strengthened rather than weakened by nerve impulses from Ib afferents in extensors during locomotion.

Several modulatory systems enable the flexibility of operation of this population of interneurons. For instance, selective presynaptic inhibition of transmission from group II afferents may transform them into interneurons processing in the first hand information from group I afferents. This effect may be further enhanced by noradrenaline released from terminals of coerulo- and sub-coerulospinal neurons which facilitates synaptic actions of group I afferents while it weakens synaptic actions of group II afferents. Conversely, selective presynaptic inhibition of transmission from group I afferents will favor mediation of synaptic actions of group II afferents at the expense of group I afferents. Postsynaptic excitation by reticulo- or rubrospinal neurons will facilitate activation of these interneurons by both group I and II afferents, but may be directed to only some of them, thereby assisting the selection of their subpopulations, which in turn would assist the selection of the most suitable patterns of motoneurons and muscles activation.

Further possibilities for the analysis of how these interneurons are being selected became available with the introduction of new techniques. A combination of the analysis of input to individual interneurons with their axonal projections and with new means to define their transmitter phenotype and the kind of synaptic contacts upon them (based on immunocytochemistry and confocal microscopy) should be particularly useful for finding out how they contribute to various movements and under different circumstances. Examples of some of these new data are shown in Fig. 10.

They show, for instance, that both excitatory and inhibitory interneurons in pathways from group I and II muscle afferents (Fig. 10a–d, respectively) have widely extending dendrites that overlap with the terminal projection areas of both group I (see Fig. 6e) and II afferents and of major descending tract neurons (cortico-, rubro-, reticulo-, and vestibulospinal). They project to several motor nuclei and also to the areas of the gray matter outside the motor nuclei, where they may coordinate activity of a variety of other interneurons (shaded areas in Fig. 10e, f). Some of these interneurons have a predominant input from primary afferents and only scarce contacts are made on them by other spinal or supraspinal neurons (which is indicated by the dominance of those containing vesicular glutamate transporters VGLUT1, indicated by blue triangles in Fig. 10 g, on many excitatory interneurons). In contrast, input to other interneurons is provided primarily by other spinal or supraspinal neurons, as indicated by contacts with vesicular glutamate transporters VGLUT2 (indicated by red triangles in Fig. 10i, mainly in lamina VIII commissural

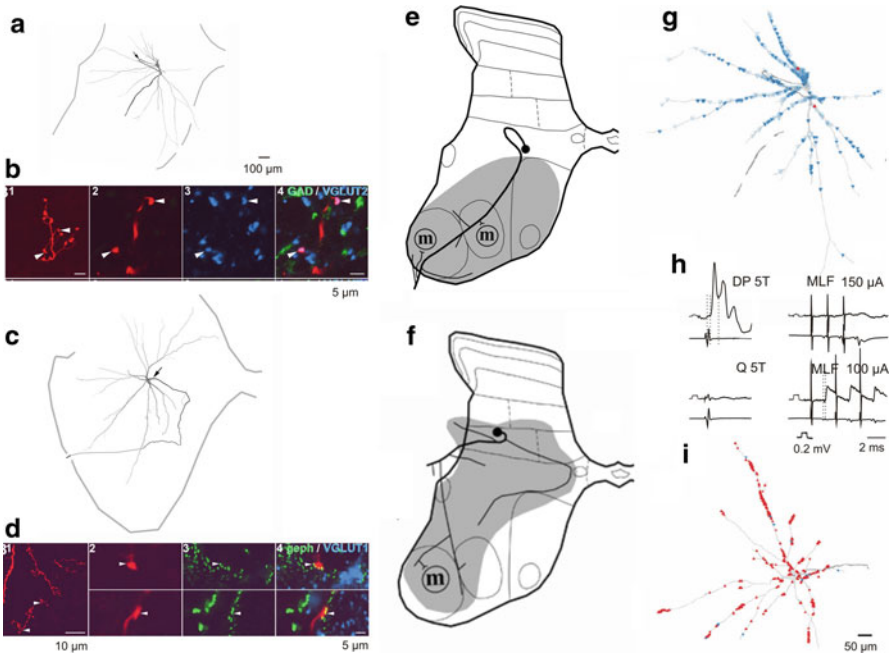


Fig. 10 Morphology, transmitter phenotypes and characteristics of input connections of interneurons mediating reflex actions of group I and II muscle afferents. (**a**) Reconstruction of the dendritic tree and of the initial part of the axon of an excitatory interneuron with input from group I and II afferents (labeled with intracellularly injected rhodamine dextran and neurobiotine). (**b**) Demonstration of glutamatergic terminals of this interneuron by overlap between products of reaction to antibodies against VGLUT2 (*blue* in **b** 3–4) and *red* labeled terminals. (**c**) As in (**a**), but for an inhibitory interneuron. (**d**) Demonstration of glycinergic terminals of this interneuron by contacts between gephyrine (*green* in **d** 3–4) and *red* labeled terminals. (**e**) and (**f**) Examples of terminal projection areas of an excitatory and an inhibitory interneuron, respectively, in the L6 segment. Contacts on motoneurons are indicated by *m*. Areas of distribution of terminals of these interneurons outside as well as inside the motor muclei are indicated by shading. (**g**) and (**i**) Distribution of terminals containg VGLUT1 (*blue*) and VGLUT2 (*red*) contacting two excitatory interneurons, interneuron in (**g**) with input from group I and II afferents in the deep peroneal (*DP*) nerve but not from the reticulospinal neurons with axons descending in the medial longitudinal fascicle (*MLF*), illustrated in top records in (**h**) and interneuron in (**i**) with monosynaptic input from the MLF but not from any of the tested peripheral nerves, as illustrated by bottom records in (**h**) (**a**–**f** Modified from Bannatyne et al. (2009); **g**–**j** Modified from Liu et al. (2010))

interneurons) while comparable proportions of VGLUT1 or VGLUT2 containing presynaptic terminals contact inhibitory interneurons in pathways from group I and II afferents.

As judged by their transmitter phenotype and axonal projections, the ipsilaterally projecting excitatory and inhibitory interneurons of this population may develop from the one class of embryonic neurons (V2; see below), while contralaterally and bilaterally projecting excitatory interneurons might develop from other embryonic neurons (from V0 or V3 and from V3 neurons, respectively).

Commissural Interneurons

The term "commissural" denotes interneurons with axons crossing via one of the commissures to the opposite side of the spinal cord. Such neurons are at present defined by only one of their morphological features, but not functionally and have cell bodies in several Rexed's laminae. Commissural interneurons constitute accordingly a greatly non-homogeneous population and only some of its subpopulations have been analyzed in detail.

The most reliably identified are premotor lamina VIII interneurons (represented by the orange circle in Fig. 6e). They include four subpopulations of premotor interneurons, two excitatory and two inhibitory, each with a characteristic source of input, target cells, and immunochemistry which might thus originate from more than one class of embryonic neurons, for example, from both V0 and V3 or Dl6 neurons. The main peripheral input to one excitatory and one inhibitory population of these neurons turned out to be from group II muscle afferents. This indicates that they mediate crossed stretch reflexes (crossed extensor and/or flexor reflexes), and that they assist in the reflex coordination of movements on both sides of the body. They may also be involved in both phasic postural adjustments and rhythmic alternating movements of left and right extremities during locomotion, swimming, or scratching. The remaining two subpopulations of lamina VIII premotor interneurons are activated monosynaptically by two descending tract neurons, reticulo- and vestibulo-spinal, and, only indirectly and to a smaller extent, by corticospinal neurons and by primary afferents. They may thus contribute to the coordination of movements on both sides of the body primarily during centrally initiated movements, or in conjunction with neck or vestibular postural reflexes.

All four subpopulations of lamina VIII commissural interneurons were found to have target cells in the contralateral motor nuclei, but also outside them, that is, to act both on motoneurons and on contralaterally located interneurons. Both excitatory and inhibitory commissural interneurons were found to contact motoneurons. This was demonstrated by disynaptic EPSPs as well as IPSPs evoked by stimulation of contralateral group II afferents or by contralaterally descending reticulospinal neurons in the cat. Direct actions of commissural interneurons in the neonatal rat were demonstrated using the technique of spike-triggered averaging, with examples of records from those located in the medial part of the ventral horn in Fig. 4f–h. In adult animals, lamina VIII commissural interneurons appeared to have practically exclusively crossed projections but in neonatal animals some commissural interneurons at a similar location were shown to project bilaterally.

The remaining commissural interneurons are less numerous but are even more diversified. They include interneurons located in laminae IV, V, VI, VII, and X. Those located most dorsally appear to draw their main input from group II and skin afferents, those located most ventrally from group II afferents and those in laminae V and VI from group I as well as group II afferents. They are co-excited by various combinations of descending tract neurons and all of them are clearly involved in integrating input from different sources. Some of these commissural interneurons were found to project only contralaterally but the other ones to project bilaterally, that is, to be able to coordinate activity of neurons on both sides. All were

found to have terminal projection areas outside motor nuclei, that is, act mainly on spinal interneurons, but some to terminate also within motor nuclei, that is, be able to add to the excitation or inhibition of motoneurons by themselves.

Other Interneurons

Much less complete information has so far been obtained on interneurons mediating other reflex actions, such as polysynaptic actions of high threshold muscle, skin, and joint afferents (jointly referred to as flexor reflex afferents, or FRA) or likewise polysynaptic actions of nociceptors, despite increasing knowledge of information that they process, their pharmacology and neuronal systems controlling their activity (see ▶ Chap. 50, "Spinal Reflexes"). It has been well established that they include both excitatory and inhibitory interneurons and that their populations are highly nonhomogeneous and may operate in conjunction with several other interneuronal populations. For instance, they may utilize information provided by interneurons with monosynaptic input from touch receptors or from group II muscle spindle afferents and act on motoneurons via premotor interneurons of other populations, for example, Ia inhibitory interneurons. They may also provide input to interneurons of other populations but details of interactions between various interneuronal populations have not yet been sorted out.

A similar situation is encountered in studies of interneurons mediating such rhythmic movements as locomotion, swimming, or scratching (see ▶ Chap. 42, "Locomotion: Circuits and Physiology"). Their distinguishing feature is that they display rhythmically occurring bursts of discharges, induced at frequencies of overground locomotion, swimming, or scratching. It is well established that rhythmic discharges of these neurons may be initiated in different ways, by peripheral stimuli, descending commands, activation of intrinsic spinal networks, or pharmacologically, most effectively by cocktails of drugs applied intravenously or to the surface of the spinal cord in preparations in vivo, or to the bath in the case of preparations analyzed in vitro. It is also known that populations of such rhythmically activated interneurons are highly heterogeneous and that their networks, often referred to as central pattern generators (CPGs), operate in conjunction with a variety of other spinal and supraspinal neurons, including Ia inhibitory intereneurons, Renshaw cells, group I/II interneurons, and commissural interneurons described above. Organization of networks of these neurons has become the subject of extensive studies and is described separately (see ▶ Chap. 42, "Locomotion: Circuits and Physiology").

Studies of interneurons involved in the control of respiration and bladder and of interneurons mediating presynaptic inhibition are at a more preliminary stage (see ▶ Chap. 49, "Respiration").

Propriospinal Neurons Mediating Descending Commands

All interneurons described above operate within spinal cord segments innervating either hindlimbs (lumbo-sacral), or forelimbs (cervico-thoracic), or trunk (thoracic)

but most often within more than one segment. In order to reach neurons in the neighboring segments their axons are usually first directed to one of the white matter funiculi whence axon-collaterals given off every 2–3 mm reenter the gray matter at successively more rostral or more caudal levels. All these interneurons operate thus as short-axoned propriospinal neurons in contrast to long-axoned propriospinal neurons that coordinate activity of fore and hindlimbs, or of the whole body, and have axons projecting well outside one of the limb enlargements. Considering their morphology, there are no sharp borderlines between the relatively short-axoned propriospinal neurons and relatively long-axoned interneurons and the subdivision between interneurons (or short-axoned propriospinal neurons) and propriospinal neurons sensu stricto (or long-axoned propriospinal neurons) is made on the basis of an arbitrary morphological criterion of whether they operate only within one or within several major subdivisions of the spinal cord. However, major functional differences have been found between the typical segmentally operating interneurons and the first population of long-axoned propriospinal neurons analyzed in detail – those located in the cervical third and fourth spinal segments and therefore referred to as the C3-4 propriospinal neurons. It is thus possible that functional differences will also be found in the case of other long-axoned propriospinal neurons (see Flynn et al. 2011).

The main difference between the C3-4 propriospinal neurons and the so far investigated interneuronal populations is that these propriospinal neurons serve to mediate descending commands (via cortico-, rubro-, tecto-, and bulbospinal systems) rather than reflex actions of primary afferents, as is schematically indicated in Fig. 11. Excitatory input from peripheral afferents was only found in their small proportion and turned out to be weak (see Alstermark et al. 2007). Another major difference is that many of these propriospinal neurons have axonal projections to not only motoneurons and interneurons but also to the lateral reticular nucleus. Thereby, they also act as ascending tract neurons and send the efference copy of all descending commands that they relay to the cerebellum (via reticulocerebellar neurons), and thus provide the basis for en route corrections of the motor commands by the cerebellum.

An important part of descending input to C3-4 propriospinal neurons comes from the tectospinal neurons, making them much better suited than spinal interneurons to organize motor reactions on the basis of visual information. This has been shown to be particularly important for visually directed reaching movements of forelimbs. However, while subpopulations of C3-4 propriospinal neurons are specialized in directing descending commands to various sets of forelimb motoneurons, other subpopulations direct them to trunk muscles and/or to hindlimb motoneurons, as judged from their projections to upper lumbar segments. They may thus select not only the visually directed forelimb movements but also postural adjustments associated with these movements. As indicated in Fig. 11b, these propriospinal neurons include both excitatory and inhibitory neurons and may therefore mediate both excitatory and inhibitory descending commands. However, they are also under inhibitory control via neurons that may adjust their excitability (represented by black circles in Fig. 11c). Cerebellar neurons that

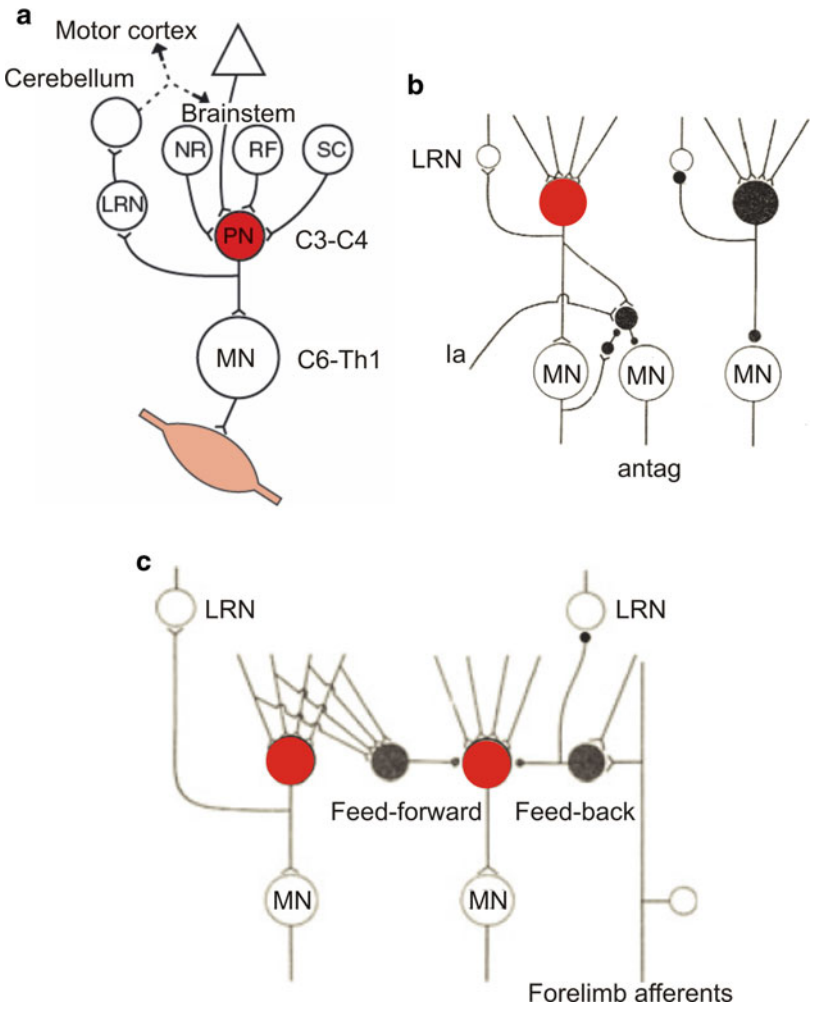


Fig. 11 Propriospinal neurons in the C3-C4 segments of the spinal cord and their networks. (**a**) Diagram showing the main input and output connections of excitatory C3-C4 propriospinal neurons (*PN*) represented by the *red circle*, with convergence of monosynaptic input (*open triangles*) from the cortical pyramidal tract neurons and from neurons in the nucleus ruber (*NR*), reticular formation (*RF*) and superior colliculus (*SC*) on the majority of the C3-C4 propriospinal neurons. The diagram shows also ipsilateral forelimb motoneurons in the C6-Th1 segments and neurons in the lateral reticular nucleus as the major target cells of these neurons. (**b**) and (**c**) Extended diagrams of networks of these neurons showing that C3-C4 propriospinal neurons include both excitatory (*red*) and inhibitory (*black*) subpopulations with similar basic input–output connections and that the target cells of the former include also Ia inhibitory interneurons. (**c**) Two sources of inhibition of C3-C4 propriospinal neurons: from inhibitory interneurons activated by the same neuronal systems that provide the excitatory input to them (feed-forward inhibition) and from peripheral afferents (feedback inhibition). Both adjust the excitability of C3-C4 propriospinal neurons and thereby their actions on motoneurons (Modified from Alstermark et al. (2007))

receive an efference copy of descending commands relayed by the C3-4 propriospinal neurons may thus adjust and correct their actions using both the excitatory and inhibitory input to them.

Interneurons Mediating Descending Commands from Supraspinal Neurons

Descending commands have long been considered as sent to motoneurons either directly or via separate sets of spinal relay neurons. The C3-4 propriospinal neurons may represent such relay neurons. However, neurons specialized in relying supraspinal commands would share this function with segmental interneurons mediating spinal reflexes and/or they might operate via these interneurons. In addition, behavioral studies revealed that such neurons play a more important role for visually guided than other centrally initiated movements and they might be more essential for forelimb than for hindlimb movements in quadrupedal animals and for hand than leg movements in humans.

Interneurons mediating all spinal reflexes have been repeatedly shown to be coactivated by peripheral afferents and by all major descending tract neurons as well as by spinal neurons. In addition to those illustrated in Figs. 5 and 9, strongest evidence to this end has been found for interneurons mediating ipsilateral flexion-extension movements and commissural interneurons coordinating muscle activity on both sides of the body. Neuronal networks mediating various muscle synergies during reflex reactions may thus be delegated to mediate the same synergies during movements induced by descending commands, without the need to double or triple the spinal output machinery. In addition, as the supraspinal neurons have direct access to the variety of neurons in spinal neuronal networks, they have also possibilities to combine the networks responsible for various synergies, adjust the degree of activation of subsets of neurons in these networks, and select their optimal activation parameters.

Records from spinal interneurons during voluntary tracking wrist movements in awake monkeys revealed that not only cervical propriospinal neurons but also a variety of segmental interneurons contribute to these movements (see Fetz et al. 2002). These were interneurons in C6-T1 segments, many of which were shown to respond to skin and/or muscle stimulation and to operate as premotor interneurons. In order to ascertain that they acted on motoneurons, the technique of spike triggering was used (see Fig. 4), but utilizing muscle electromyographic responses as the measure of interneuronal actions, as diagrammatically indicated in Fig. 12a. These are illustrated in Fig. 12b with records from three muscles. Individual interneurons were found to contribute to different patterns of muscle activities, as summarized in Fig. 12c: excitation of either agonists or antagonists, suppression of activity of either agonists or antagonists, co-excitation of agonists and antagonists or opposite actions on agonists and antagonists. The same interneurons displayed also clear-cut changes in their background activity during periods

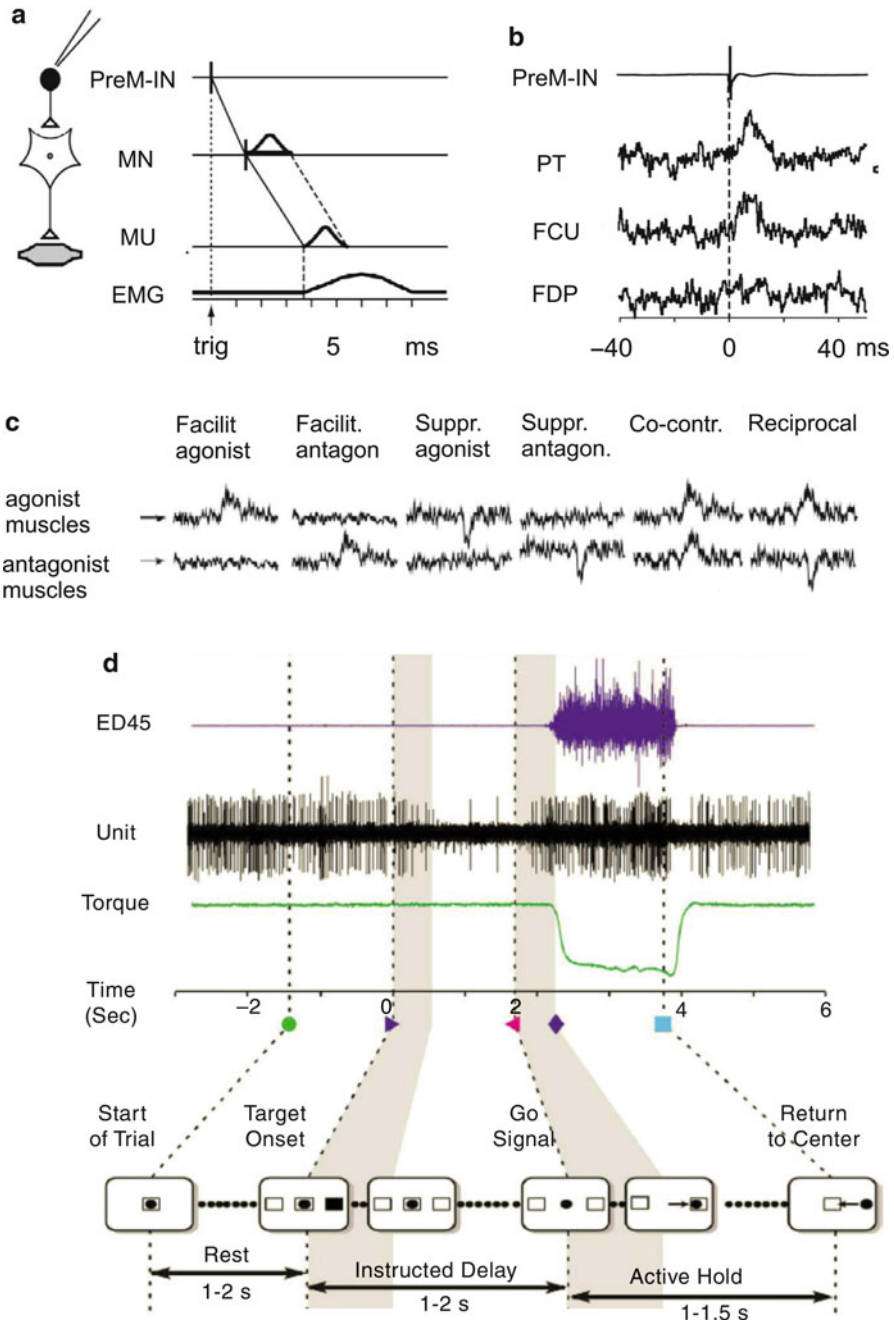


Fig. 12 Contribution of spinal interneurons to centrally initiated movements. (**a**) The diagram of tests used to identify premotor interneurons in experiments in which implanted electrodes were used to record from individual premotor interneurons (*PreM-IN*) in the cervical cord during tracking

preceding voluntary tracking movements, with, for example, less activity during the instructed delay period, and an enhancement in activity prior to muscle EMG responses and sustained activity during the involved muscle contractions, as illustrated in Fig. 12d.

Plasticity of Operation of Spinal Interneurons and Their Networks

Neuromodulators may change operation of individual interneurons as well as their networks in a very dramatic way. This has been repeatedly demonstrated in both invertebrates (e.g., Harris-Warrick et al. 1998; Selverston et al. 1998), and vertebrates (see, e.g., Lundberg 1982; Jankowska 2001). One of the earliest examples was the demonstration that the systemic application of the noradrenaline precursor L-DOPA is followed by a decrease of the early polysynaptic actions of high threshold muscle, skin and joint afferents on motoneurons and the appearance of much later and longer lasting ones ("the late DOPA responses"; see Lundberg 1982). Subsequently, ionophoretically applied noradrenaline and its alpha 2 agonists tizanidine and clonidine were shown to exert particularly strong and target-specific depressive actions on transmission from group II muscle afferents to spinal interneurons. Figure 13a illustrates these effects on responses evoked in a premotor interneuron in disynaptic pathways from both group I and II muscle afferents. It shows that noradrenaline prevented the interneuron from responding to nerve impulses from group II muscle spindle afferents, while it had either no effect on transmission from group I afferents to the same neuron, or even facilitated it. The depressive effects of the alpha 2 NA agonists tizanidine or clonidine on late components of the stretch reflex in humans illustrated in Fig. 13b may therefore be related to their actions on group II excited spinal interneurons.

Modulatory actions of ionophoretically, systemically, or topically applied 5-HT are as potent as actions of NA. Together with other drugs 5-HT and/or its agonists facilitate appearance of rhythmic activation of spinal neurons characteristic for locomotion, swimming, or scratching. They contribute to activation of different neuronal network, for example, of bilateral extension or flexion instead for ipsilateral

Fig. 12 (continued) movements in monkeys. They were identified by using spike triggered averaging technique on the basis of records from the contracting muscles (single motor units, *MU*, or from the whole muscle, *EMG*) when muscle activity followed interneuronal spikes at a critical interval (<4.5 ms), indicating direct contacts between the interneurons and motoneurorones innervating the muscle. (**b**) Averaged responses of motor units in the pronator teres (*PT*), flexor carpi ulnaris (*FCU*), and flexor digitorum profundus (*FDP*) forelimb muscles following spikes of the interneuron, detectable in the first two but probably not in the third of these muscles. (**c**) Examples of six patterns of actions of single interneurons on motoneurons innervating agonist and antagonist muscles. (**d**) An example of changes of activity of an interneuron (Unit) before, just prior to and during contractions of a muscle (extensor digitorum 4–5) used for tracking movements ("torque"). Note, an arrest of the activity during the instructed delay period and its appearance prior to muscle EMG and the sustained subsequent discharges (Modified from Fetz et al. (2002))

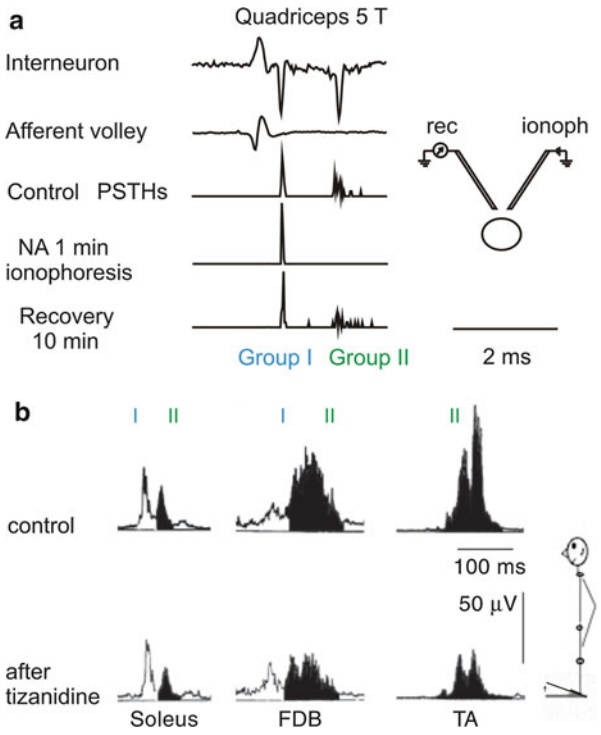


Fig. 13 Examples of input-specific modulatory actions of noradrenaline. (**a**) Effects of ionophoretically applied NA on responses of extracellularly recorded interneuron in disynaptic pathways between group I and II muscle afferents and motoneurons. The experimental setup shown to the *right* shows two micropipettes close to an interneuron: one used for the recording and another one for ejecting NA by passing positive constant current. The records are from the *top* to the *bottom*: (1) responses of the neuron to stimulation of the quadriceps nerve at intensity supramaximal for group I afferents and near maximal for group II afferents; (2) afferent volleys in the stimulated afferent fibers record from the surface of the spinal cors; (3)–(5) peristimulus time histograms of responses to 20 stimuli applied before, during, and after NA ionophoresis (Modified from Jankowska et al. 2000). (**b**) EMG responses evoked in a human subject by a stretch of the indicated muscles by displacement of a platform on which he was standing. The early (*white*) and the later (*gray shaded*) components of the responses are attributable to group I and II afferents as indicated (Modified from Pierrot-Deseilligny and Burke 2005). Note that both in (**a**) and (**b**) the later responses were depressed by tizanidine, the alpha 2 NA agonist, while the earliest ones were not

flexion and crossed extension (see ▶ Chap. 50, "Spinal Reflexes") and affect transmission in polysynaptic as well as disynaptic neuronal pathways. Actions of 5-HT have also been found to affect neurons at a molecular level (see, e.g., Hultborn 2006). Effects of noradrenaline and of serotonin on different functional types of neurons and on transmission from different categories of afferents were found to be either similar or opposite and target specific. Stimuli applied within the regions of location of the monoaminergic neurons have similar effects at both cellular and

network levels (see ▶ Chap. 50, "Spinal Reflexes"). Modulators may thus effectively change the way different interneuronal networks operate and modify their actions (see ▶ Chap. 50, "Spinal Reflexes").

Elementary Neuronal Networks

All categories of spinal interneurons investigated to date act not only on motoneurons but also on other interneurons, and several systems of interneuronal interactions have already been revealed. (1) Interactions between inhibitory interneurons of one category but with different target neurons have been found within the population of Ia inhibitory interneurons (illustrated in Fig. 5b), Renshaw cells (illustrated in Fig. 7b), interneurons in pathways from group Ib and II afferents. (2) Interactions between inhibitory and excitatory interneurons of a neuronal population have been found in neuronal networks activated by group Ib and II afferents (illustrated in Fig. 9b). (3) Recurrent inhibition (negative feedback) following excitation of the neurons is best documented for motoneurons (via their recurrent motor axon collaterals and Renshaw cells) but might also occur in the population of inhibitory interneurons in pathways from group Ib and II afferents, as indicated in Fig. 9b, in which IPSPs, following EPSPs, evoked by a stimulus suggest that these interneurons might inhibit themselves. The recurrent inhibition could contribute to the appearance of single spike responses, rather than trains of repetitive discharges to any stimuli that activate these neurons. (4) Inhibition of one category of spinal interneurons by another, as in the case of inhibition of Ia inhibitory interneurons by Renshaw cells. (5) excitation of one category of spinal interneurons by another, as indicated by disynaptic excitation of interneurons with input from group Ia, Ib, or II afferents by cutaneous afferents (see Fig. 2) or by commissural interneurons. These are the simplest interneuronal networks imagined and they are replicated in different combinations both in the spinal cord and in other parts of the nervous system, including brainstem, cerebral cortex, and cerebellum. Such interactions between interneurons have been found in various species, invertebrate as well as vertebrate. They may thus represent elementary network connections that are used in different combinations in the nervous system in addition to more specialized networks as well as specialized systems of neuromodulation (see ▶ Chap. 15, "Molecular Regulation of Synaptic Release"). More complex networks have been analyzed in the context of various specific functions, such as error corrections by inferior olive and cerebellar neurons, and during respiration or locomotion (see ▶ Chaps. 39, "Cerebellum: Anatomy and Function," ▶ 42, "Locomotion: Circuits and Physiology," and ▶ 49, "Respiration").

How to Analyze Operation of Spinal Interneurons in Humans?

Even though recording from individual human spinal interneurons is impossible, several experimental approaches have been developed to allow their studies. All of these utilize an indirect analysis, extensively used and verified in animals (see, e.g.,

Lundberg 1982) before intracellular recording from interneurones became possible. As illustrated in Fig. 14, the analysis is based on records from the muscles, taking into account that normally all action potentials generated by motoneurons evoke action potentials in muscle fibers that make part of a motor unit (see ▶ Chap. 33, "Spinal Motoneurons"). One may therefore use responses of single motor units as a measure of activation of single motoneurons. Alternatively, one may record

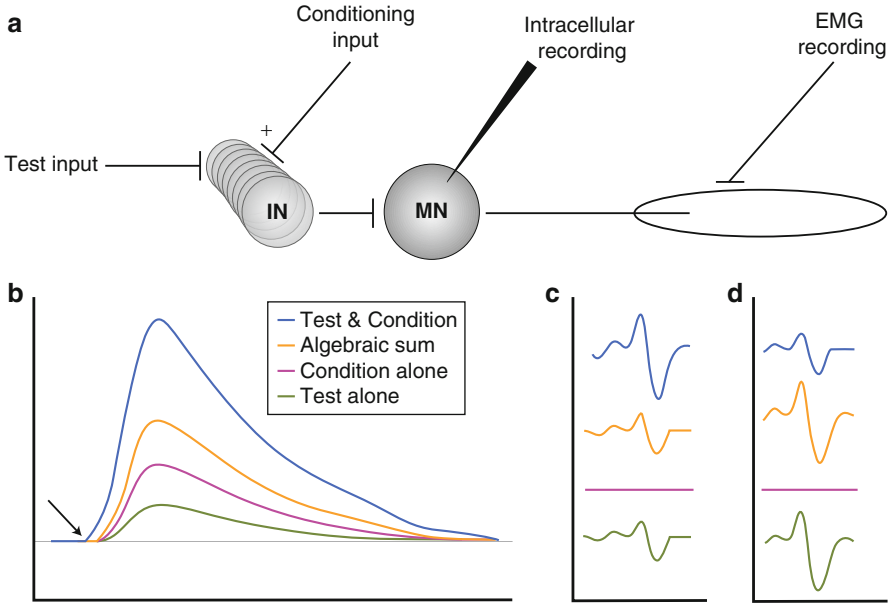
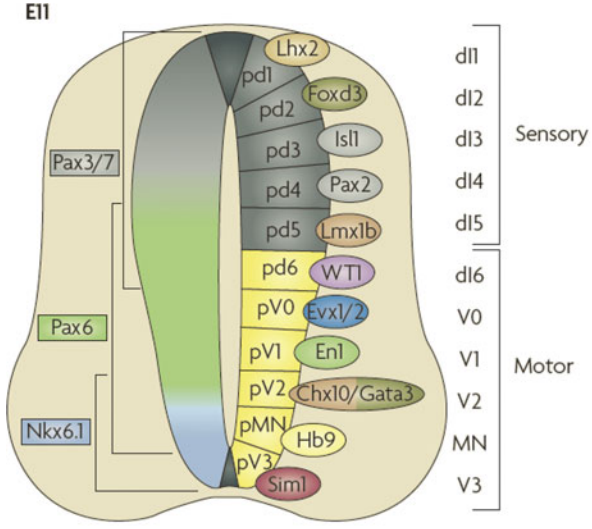


Fig. 14 Changes in EMG responses recorded from human muscles provide clues on input to motoneurons that innervate these muscles and to interneurons that affect the motoneurons. (**a**) The diagram of the relationships between interneurons and motoneurons that can explain how actions of the interneurons may be adjusted. As action potentials in motoneurons axons evoke all or none potentials in muscle fibers innervated by them, the latter can be used as a measure of activation of the motoneurons. Activation of motoneurons (when their depolarization exceeds the threshold for generating an action potential) and the size of the compound EPSPs evoked in them by a set of last-order interneurons provide in turn the measure of the proportion of the total number of interneurons providing input to the motoneurons. These EPSPs are represented by the smallest EPSP denoted test in the set of EPSPs in (**b**). As the proportion of the interneurons increases, larger EPSPs, represented by EPSPs denoted Test & Condition in (**b**), are evoked in motoneurons; for explanation see text. The number of motoneurons discharging is then increased, being reflected in the amplitudes of the resulting EMG responses. Larger EMG responses to joint actions of conditioning and test stimuli in (**c**) may thus indicate that larger numbers of these interneurons were activated by combined actions of the conditioning and test than of only test stimuli. One can then infer that the conditioning stimuli provided an additional excitatory input to the last-order interneurons. In contrast, smaller EMG responses to joint actions of conditioning and test stimuli in (**d**) may indicate that the conditioning stimuli counteracted activation of the interneurons by test stimuli by inhibiting these interneurons. However, in order to draw these conclusions, the effects of joint actions of the test and conditioning stimuli must differ from the algebraic sums of responses evoked when these stimuli are applied alone (Modified from Burke 1999)

compound EMG responses from the whole muscle and use the size of these responses to estimate the number of motor units activated. The number of motoneurons activated under different conditions may in turn be used to estimate the degree of excitation or inhibition of these motoneurons by last-order interneurons. If a test stimulus activates, for example, one half of a population of interneurons that provide input to a motoneuron this would result in a relatively small EPSP in the motoneuron, as indicated by "test alone" in Fig. 14b. Conditioning stimuli applied alone may also activate a certain number of these interneurons. However, if the test and conditioning stimuli co-excite the same interneurons, they may activate at least some that remained below the threshold for spike activation under influence of either the test or condition stimuli applied alone. The effect of joint actions of the conditioning and test stimuli on motoneurons would then exceed the algebraic sum of their separate effects (as shown by EPSPs labeled "Test & Condition" in Fig 14b). When recording from individual motoneurons, this will be evidenced by much larger amplitude of EPSPs evoked by the jointly applied stimuli, while in EMG records it will be reflected in a greater number of motoneurons discharged by the jointly applied stimuli, as reflected in records shown in C. If the conditioning stimuli counteract rather than facilitate activation of the interneurons, the effects of the joint application of the conditioning and test stimuli will be smaller, as indicated in records in D. By using different combinations of test and conditioning stimuli in humans one may thus sort out which of them co-excite the same interneurons and which have opposite effects upon them. One could further relate this to more direct records obtained using intracellular records from spinal interneurons in animal studies. The results of such studies have been summarized by Pierrot-Deseilligny and Burke (2005).

Development of Various Types of Interneurons from Spinal Progenitor Cells

Studies on neonatal spinal cord neurons distinguished several interneuron classes that are derived from transcriptionally defined progenitor domains in the ventral and dorsal parts of the spinal cord, as summarized in Fig. 15. These embryonic neurons are characterized by expression of specific transcription factors and a number of techniques have been developed to recognize them at various stages of their differentiation, with the ultimate aim to allow identification of neurons derived from them in the mature spinal cord. The main questions have been: (1) which mature neurons are derived from the various classes of embryonic neurons and (2) from which embryonic neurons are developed the classes of mature interneurons described above, as well as neurons characterized by their locomotor-like rhythmic activity. These studies are in progress and so far give only partial answers to these questions. As indicated above, both Ia inhibitory interneurons and Renshaw cells develop from V1 embryonic neurons, but according to the available evidence, some other, so far nonidentified neurons are likewise derived from these V1 neurons and Ia interneurons develop from both V1 and other embryonic neurons. Commissural interneurons



		Embryonic	Adult cells
dI6	WT1	CINs, GABA/Glycine	Disynaptic crossed inhibitory INs?
V0	Evx1/2	$V0_V$ and $V0_D$ CINs, 2–4 segments, GABA/Glycine ($V0_D$), Glutamate ($V0_V$)	Disynaptic crossed commissural INs?
V1	En1	Ipsilateral rostral INs, 1–3 segments, GABA/Glycine	Renshaw cells, Ia inhibitory INs
V2	Chx10 Gata3	Ipsilateral caudal INs, > 2 segments V2a (Glutamate), V2b (GABA/Glycine)	Unknown
MN	Hb9 Isl1	Peripheral projections, Acetylcholine	Somatic/visceral motor neurons
V3	Sim1	$V3_V$ and $V3_D$ mixed commissural/ipsilateral > 2 segments (Glutamate)	Unknown

Fig. 15 Classes of embryonic spinal neurons. In the *left half* of the diagram are indicated three early spinal cord progenitor domains (at embryonic day 9), while the *right side* shows distribution of 11 early classes of postmitotic (at embryonic day 11) cells and transcription factors that identify them. In the table, in the *middle* are indicated transmitter phenotypes and axonal projections of these 11 classes of embryonic cells and in the right column the adult cells that are most likely derived from them (Modified from Goulding 2009)

are thought to develop from Dl6, V0, and V3 embryonic neurons but no conclusive evidence appears to allow linking the four subpopulations of mature lamina VIII commissural interneurons and/or other neurons with crossing axons to particular embryonic neurons. Ipsilaterally projecting intermediate zone excitatory and inhibitory interneurons in pathways from muscle and tendon afferents apparently develop from V2 and possibly also V3 embryonic neurons but it cannot yet be finally decided from which of their subsets. The outcome of studies linking neurons involved in locomotion to the various classes and subclasses of embryonic neurons has reached higher steps of confidence (Jessell 2000; Kiehn 2006; Stepien and Arber 2008; Goulding 2009). However, the progress in the development of new markers should considerably increase the possibilities to find the so far undefined links between neurons in the early developmental stages, neurons that mediate reflex actions in adult animals, and neurons that are rhythmically active during locomotion in either adult or neonatal animals.

New Experimental Approaches to Studies of Functions of Various Types of Interneurons in Neuronal Networks In Vitro and In Vivo

Elimination of specific interneuronal populations in vitro. Lesions of various parts of the nervous system and the analysis of the resulting changes have been routinely used in an attempt to deduce the function of the eliminated ones from changes in the operation of the remaining parts. However, this approach was most meaningful when the lesions were highly selective, for example, when single muscles were denervated, when pyramidal tract fibers were transected without damaging the remaining part of the medulla, or when connections between the left and right cerebral hemispheres or between the left and right hemicords were cut, even though they included several categories of fibers. Coagulation or chemical destruction of distinct nuclei such as the vestibular nuclei or the red nucleus, and ablations of distinct parts of the cerebral cortex could also be considered as relatively well restricted. However, until recently no attempts could be made to lesion distinct classes of spinal interneurons because all of them are intermixed in the gray matter. One could only depress activation of some of these neurons pharmacologically, for example, of all glycinergic or of all GABAergic neurons, by using glycine or GABA antagonists, or of all ACh-activated neurons (including but not restricting the effect to Renshaw cells) by the nicotinic antagonist mecamylamine (Noga et al. 1987).

New possibilities have opened in this respect with respect to neurons derived from genetically well-defined embryonic cells (Zagoraiou et al. 2009) and with establishing their molecular markers which allowed subpopulations of V0, V1, V2, or V3 embryonic cells to be eliminated by genetic ablation technologies, or by decreasing their excitability and blocking output through genetic means. The effects of these ablations were in the first hand analyzed on spinal rhythmic activity and defined using changes in the pattern, frequency, and amplitudes of locomotor-like movements, both in vivo and in vitro, as described in the ► Chap. 42, "Locomotion: Circuits and Physiology."

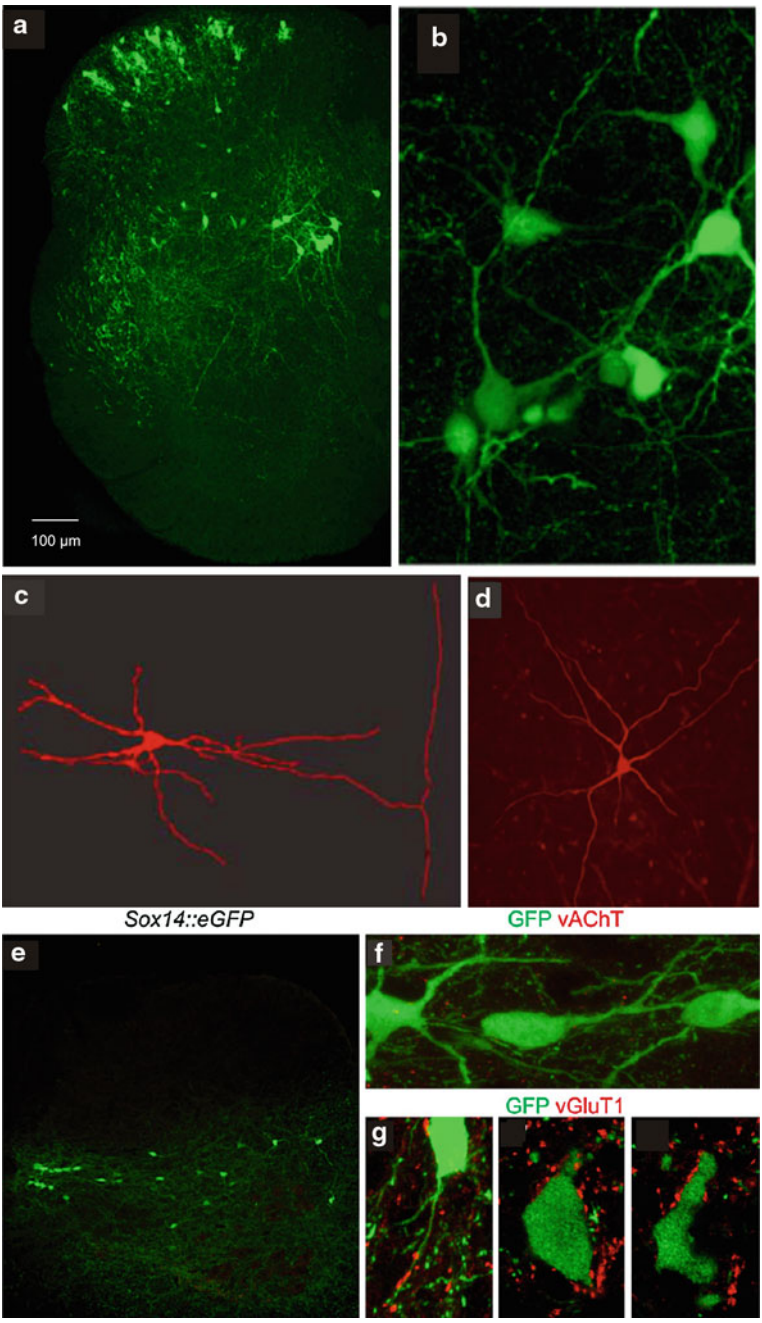


Fig. 16 Examples of marking of selected subpopulations of neurons. (**a**–**c**) Illustration of a subpopulation of GABAergic interneurons expressing GFP (*green* fluorescent protein) under the control of the promoter for GAD65 (Modified from Wilson et al. 2010). In the in vitro spinal cord

Activation of specific interneuronal populations in vitro. Using molecular markers of specific classes of spinal interneurons with light sensitive vesicular transporter proteins it has also become possible to activate selected interneurons by illuminating the spinal cord in vitro and in this way analyze their actions (Hagglund et al. 2010).

Marking of neurons derived from various embryonic neurons for their subsequent analysis in vitro. Studies of genetically altered mice may take great advantage of marking of various classes of embryonic cells, thus enabling recognition of neurons derived from particular classes of these cells at later stages of their development with a further analysis of their properties. Particularly fruitful turned out to be the use of fluorescent proteins, which are driven by the promoters of various transcription factors in preparations in which genetically modified strains are crossed with GFP (green fluorescent protein) reporter strains animals. Neurons visualized in this way, with examples in Fig. 16, may then be selected for detailed examinations in the most convenient in vitro preparations and at various stages of the development.

New neonatal preparations in vitro increase the range of studies to be carried in them. Such new preparations include for example, a fully isolated spinal cord but with attached dorsal and/or ventral roots, peripheral nerves, muscles, or even whole extremities as well as with parts of the brainstem. These preparations should thus allow examination of synaptic input to the investigated cells and of their networks using approaches similar to approaches used in in vivo preparations which are difficult or impossible to carry out in standard in vitro preparations or on slices.

Analysis of activity and properties of spinal interneurons in different behavioral situations by using implanted electrodes in vivo. This approach has been used to obtain records from awake animals by inserting recording electrodes through chronically attached recording chambers using similar techniques as for records from cortical neurons (see ► Chap. 41, "Cortical Motor Control"). Records from forelimb neurons relaying descending corticospinal commands in primates described above have been obtained in this way (see Fig. 12).

Application of immunohistochemical and morphological techniques based on confocal microscopy in studies of individual functionally identified adult spinal interneurons in vivo. The extension of studies of spinal interneurons in vivo by these techniques opens new possibilities to analyze transmitter phenotypes of functionally identified individual neurons as well as synaptic contacts formed on them and their membrane receptors, to relate these properties to the functionally defined input and output properties and thereby their role in various neuronal networks.

Fig. 16 (continued) slices, such cells were penetrated under visual control and intracellularly labeled with Neurobiotin. In (**e**–**g**) are examples of GFP labeled V2a subpopulation of Pitx2positive V2a neurons (Modified from Zagoraiou et al. 2009) showing numerous contacts with vGluT1 positive terminals made by peripheral afferents (**g**) but no contacts with AChT positive terminals (**f**)

Outlook

With all new techniques in hand, future studies should succeed in establishing what different subpopulations of spinal interneurons, both mature and embryonic, are responsible for and how their networks operate. A fuller advantage might then be taken of this new knowledge to understand the role that spinal interneurons play in motor behavior and to use this knowledge for improving the deficient spinal cord functions.

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