Cell PRESS
Neuron
Review

Assembly of Motor Circuits in the Spinal Cord: Driven to Function by Genetic and Experience-Dependent Mechanisms

David R. Ladle,[1,2,3] Eline Pecho-Vrieseling,[1,2,3] and Silvia Arber[1,2,*]
[1]Biozentrum, Department of Cell Biology, University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland
[2]Friedrich Miescher Institute, Maulbeerstrasse 66, 4058 Basel, Switzerland
[3]These authors contributed equally to this work.
*Correspondence: silvia.arber@unibas.ch
DOI 10.1016/j.neuron.2007.09.026

Motor circuits in the spinal cord integrate information from various sensory and descending pathways to control appropriate motor behavior. Recent work has revealed that target-derived retrograde signaling mechanisms act to influence sequential assembly of motor circuits through combinatorial action of genetic and experience-driven programs. These parallel activities imprint somatotopic information at the level of the spinal cord in precisely interconnected circuits and equip animals with motor circuits capable of reacting to changing demands throughout life.

Introduction

Motor behavior can be considered as the ultimate output of the nervous system. In the pathway to initiate or alter movement, motor neurons (MNs) in the ventral horn of the spinal cord represent the last leg of command and control contraction of skeletal muscles. Their assignment is to drive movement in alignment with the intentions of the nervous system at every given time and place. This demanding task is controlled by neuronal circuits upstream of MNs and by the specificity of interactions between MNs and muscles.

The computational output of local spinal circuits is conveyed to MNs and results in the appropriate sequence of muscle contractions. In addition to locally restricted interneuron networks, two different types of inputs are essential for appropriate motor behavior. One major source is sensory feedback from the body, which is channeled into the spinal cord by dorsal root ganglion (DRG) sensory neurons of different sensory modalities. Sensory input is transformed in the spinal cord and updates spinal circuits with the ongoing sensory perception of the body. A second source of inputs is generated centrally, either descending from the brain (supraspinal; e.g., voluntary movement control) or through long intraspinal projections. As a consequence, the precision of connectivity both in local spinal circuits and intersecting circuits determines functionality of motor circuits and behavior.

The coordinated developmental assembly of local spinal circuits and neuronal pathways feeding into the spinal cord provides the foundation for the control of motor behavior in the adult. This review will highlight advances in our understanding of developmental control mechanisms governing the precision with which motor circuits in the spinal cord assemble. We will discuss recent findings demonstrating the existence of dedicated genetic programs driving diversification, specification, and connectivity of neuronal subpopulations and how these genetically predetermined neuronal characteristics interact with activity-driven processes shaping circuit assembly and function. Particular emphasis will be given to circuits processing somatotopic information, as well as to discussion of physiological specificities in motor circuits and control mechanisms involved in shaping these circuits. Implicit in these questions is the issue of how local spinal motor circuits can be influenced by external signals feeding into the spinal network, both through molecular signals as well as through experience-driven feedback loops. A detailed account on recent advances on spinal interneuron networks involved in rhythm and pattern generation, commonly referred to as central pattern generator (CPG), will not be part of this review but has been covered extensively in recent reviews (Goulding and Pfaff, 2005; Grillner, 2006; Kiehn, 2006). Our review will mostly focus on information derived from work on mammalian spinal cord with an emphasis on mouse.

Sequential Assembly of Local Motor Circuits in the Spinal Cord

An important aspect to understand when considering mechanisms controlling emergence of circuit function is the role of sequential generation of defined neuronal subpopulations and their integration into neuronal circuits. The assembly of motor circuits in the mouse spans over an extended time period, from E9.5 when the first MNs are born reaching into the postnatal period when inputs descending from the brain shape spinal circuits. How does this temporally staggered sequence in the assembly of distinct neuronal elements contribute to the establishment and functionality of motor circuits?

A Sonic hedgehog (Shh)-driven signaling pathway patterns the spinal cord along the ventrodorsal axis through the generation of discrete progenitor domains marked

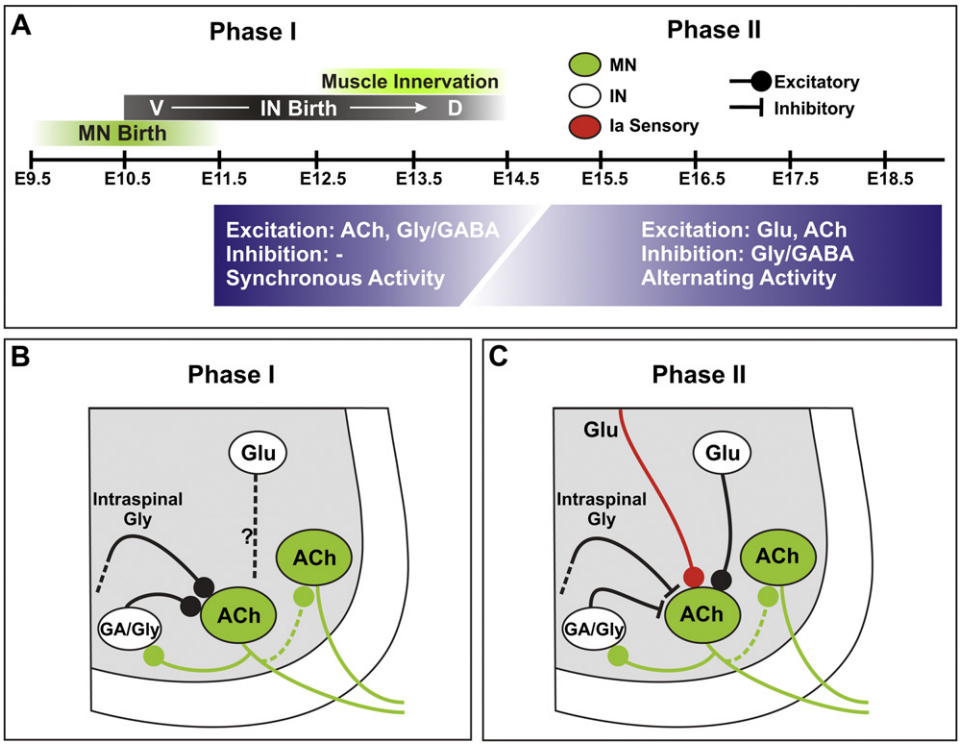

Figure 1. Sequential Assembly of Local Spinal Circuits

Time course observed for the assembly of MN, interneuron (IN), and Ia proprioceptive sensory neuron connectivity in the mouse lumbar spinal cord. (A) Approximate time line of events observed (E: embryonic day).

(B and C) Schematic illustration of two distinct phases (ventral horn shown). Dominant excitatory connections during phase I (B) are derived from MNs (neurotransmitter ACh) and GABA/Glycinergic (GA/Gly) interneurons. At these stages, the role for glutamatergic (Glu) interneuron input for circuit function and connectivity is unclear (dashed line, question mark). Phase II (C) is characterized by a gradual switch of GABA/Glycinergic inputs from excitation to inhibition and activation of glutamatergic inputs. Moreover, excitatory (Glu) Ia proprioceptive afferent connections to MNs are formed at late embryonic stages (red; see also Figure 2).

by the expression of repressive homeodomain transcription factors (Jessell, 2000). These progenitor domains in turn give rise to defined neuronal subpopulations (Jessell, 2000). Within the ventral spinal cord, MNs are among the earliest born neurons (Hollyday and Hamburger, 1977; Nornes and Carry, 1978) and are followed in short succession by four broad classes of ventral interneurons (V0, V1, V2, V3) that can be defined on the basis of transcription factor codes (Figures 1A and 2; Goulding and Pfaff, 2005; Jessell, 2000). As has been reviewed extensively elsewhere, these progenitor and early postmitotic transcriptional codes are instructive for the generation and differentiation of many key aspects of these neurons (Goulding and Pfaff, 2005; Jessell, 2000; Kiehn, 2006). For example, *Dbx1* homeobox gene mutation in the mouse eliminates V0 interneurons (Pierani et al., 2001), leading to decreased coordination in left-right alternation of rhythmic motor activity because of the absence of many contralaterally projecting inhibitory interneurons (Lanuza et al., 2004).

MNs and local spinal interneurons begin to interact functionally long before DRG sensory axons or descending inputs reach the spinal cord (Figure 1B). As early as E11.5 in the mouse, when motor axons are still on their way to muscle targets, patterned spontaneous activity can readily be observed when recording from outgrowing peripheral nerves (Hanson and Landmesser, 2003). These observations raise the question of the mechanisms and circuitry responsible for generation of these early MN bursting patterns. Throughout embryonic development, MNs grouped within pools projecting to individual muscles are coupled electrically by gap junctions important for burst generation (Chang et al., 1999; Hanson and Landmesser, 2003; Kiehn and Tresch, 2002; Milner and Landmesser, 1999).

In addition, focusing on chemical transmission, work in several species (chick, rat, and mouse) on embryonic spinal circuit formation and function provides support for a model in which motor circuit activation can be largely subdivided into two distinct phases (Kudo and Nishimaru, 1998; Milner and Landmesser, 1999; Myers et al., 2005). The first phase (E12.5–E14.5 in the mouse) begins shortly after MN generation (Figure 1A). Acetylcholine (ACh) is the major MN neurotransmitter and provides important excitatory drive for MN activation through connections to other MNs and interneurons (Figure 1B). Moreover, glycine and/or GABA provided by interneurons act as excitatory neurotransmitters during this early phase and contribute

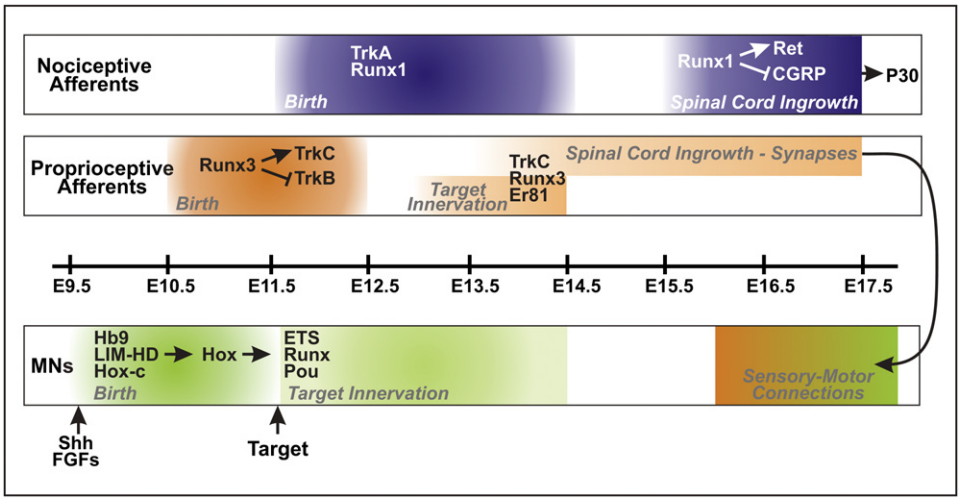

Figure 2. Time Course of MN and DRG Sensory Neuron Differentiation in the Mouse

Time course illustrating major developmental events and genes involved in differentiation of nociceptive DRG sensory neurons (blue), proprioceptive DRG sensory neurons (orange), and MNs (green). Neuronal birth, target ingrowth, and synaptogenesis are depicted by graded boxes in corresponding colors. Time line shows approximate embryonic days (E) for mouse lumbar spinal cord. Direct sensory-motor connections form between E16 and E18 (indicated by orange-green gradient below time line). Transcription factors involved in differentiation of these neuronal populations are indicated in the boxes, and regulation of neurotrophic factor receptor expression (TrkB, TrkC, Ret) through Runx transcription factors is also indicated.

to patterned activity (Figures 1A and 1B; Kudo and Nishimaru, 1998; Milner and Landmesser, 1999; Myers et al., 2005). The second phase (E15.5–E18.5 in the mouse) is dominated by upregulation of glutamatergic excitatory neurotransmission and paralleled by a gradual shift of glycinergic/GABAergic neurotransmission from excitation to inhibition (Figures 1A and 1C; Kudo and Nishimaru, 1998; Milner and Landmesser, 1999; Myers et al., 2005). This switch from excitation to inhibition can be explained by a general decrease in intracellular chloride concentration $[Cl^-]_i$ induced by upregulation of the K^+ Cl^- cotransporter KCC2 in ventral spinal neurons around these stages (Fiumelli and Woodin, 2007; Hubner et al., 2001).

From these functional observations, what can we learn about the sequence with which spinal motor circuits assemble? During the early phase, despite the fact that glutamatergic interneurons have already been generated, excitatory neurotransmission through glutamate (Glu) does not appear to be of major functional importance (Goulding and Pfaff, 2005; Jessell, 2000; Kiehn, 2006; Kudo and Nishimaru, 1998; Milner and Landmesser, 1999; Myers et al., 2005). Whether glutamatergic interneurons are already integrated in circuits but are silent because of pre- or postsynaptic mechanisms, or whether they exhibit more subtle functions not detected by the currently available assays, remains to be determined. It is clear that MNs and at least some glycinergic/GABAergic interneurons are functionally interconnected at early developmental stages. However, because glycinergic/GABAergic neurotransmission is excitatory at these stages, it is difficult to judge functionally which types of interneurons are already in place in the appropriate mature configuration. For example, spontaneous bursting activity is synchronized on both sides of the spinal cord during the early phase (Figure 1A; Branchereau et al., 2000; Hanson and Landmesser, 2003; Kudo and Nishimaru, 1998; Milner and Landmesser, 1999; Myers et al., 2005), suggesting that the feature of burst alternation may not be in place yet. However, this observation may be explained by the fact that generation of alternating activity requires inhibitory neurotransmission. Indeed in neonatal mouse spinal cord, a time point when glycine/GABA signaling is inhibitory, alternation of spontaneous activity is present, but blocking inhibition abolishes alternation (Branchereau et al., 2000; Kiehn, 2006). Glutamatergic neurotransmission during the second phase is important for rhythmic activity (Kiehn, 2006; Kudo and Nishimaru, 1998; Milner and Landmesser, 1999; Myers et al., 2005; Whelan et al., 2000). Moreover, recent work suggests that central motor axon collaterals use Glu as a neurotransmitter in addition to the well-known neurotransmitter ACh (Mentis et al., 2005; Nishimaru et al., 2005).

Is there any evidence that early neurotransmission influences later aspects of circuit assembly? A study on mice mutant in the rate-limiting ACh synthesis enzyme choline acetyltransferase (ChAT) indeed suggests that cholinergic neurotransmission plays a role during the early phase of motor circuit assembly (Myers et al., 2005). *ChAT* mutant mice exhibit premature upregulation of both glutamatergic excitatory and glycinergic/GABAergic inhibitory neurotransmission. These mice display defects in left-right and flexor-extensor alternation, which cannot be mimicked by acute blockade of ACh signaling at late embryonic stages in wild-type mice. These findings suggest that some aspects of the circuitry required for this activity are permanently changed by the absence of *ChAT* during the early embryonic phase. Whether and how absence of *ChAT* affects integration of defined interneurons into

circuits or alters the weight of existing connections is currently not known, and also, molecular correlates of this physiological phenotype have not become apparent (Myers et al., 2005). Furthermore, pharmacological reduction of overall spontaneous network activity in developing chick embryos in ovo between E8 and E10, a time point at the transition stage between the described two phases, leads to compensatory adjustments in synaptic strength of both GABAergic and glutamatergic synaptic connections (Gonzalez-Islas and Wenner, 2006). These findings suggest that altered activity patterns can scale synaptic connections through a mechanism of homeostasis within spinal circuits (Gonzalez-Islas and Wenner, 2006). Another aspect to consider is that the occurrence of spontaneous bursting episodes has been shown to transiently depress excitability of spinal networks, suggesting that the state and past history of a network has to be taken into account and can play important roles in whether and how activation can be achieved (O'Donovan, 1999).

What could be the factor(s) driving the switch from the early to late embryonic phase of spinal neurotransmission? It is intriguing to note that in all species studied, this switch occurs around the time when MN axons reach their muscle targets (Figure 1A), suggesting that perhaps target-derived signals play a role in initiating the switch (Hanson and Landmesser, 2003; Milner and Landmesser, 1999). Although direct evidence for this hypothesis is currently lacking, studies in other systems have shown that brain-derived neurotrophic factor (BDNF) can influence the expression of KCC2 (Fiumelli and Woodin, 2007). This regulation could indirectly influence the Cl^- equilibrium potential and thereby determine whether glycinergic/GABAergic neurotransmission is excitatory or inhibitory. For the motor system, an interesting model could be that target-derived signals may be at least partially responsible for the initiation of the switch to a more mature circuit configuration in a retrograde manner. Upregulation of KCC2 occurs throughout the nervous system, but distinct neuronal subpopulations undergo this switch at different developmental stages (Fiumelli and Woodin, 2007). Whether in some neurons (other than MNs) KCC2 upregulation correlates with the time when axons reach their target cells has not been investigated.

Target-Derived Signals Influencing Motor Circuit Development and Function

Local spinal circuits encompass circuit-autonomous features recapitulating many aspects of locomotor activity (Grillner, 2006; Kiehn, 2006). Nevertheless, spinal circuits in vivo rely heavily on continuous sensory feedback relayed to the spinal cord through dedicated sensory pathways intersecting with spinal circuits at distinct dorsoventral positions in the spinal cord (Brown, 1982; Brown and Fyffe, 1978; Pearson, 2004; Rossignol et al., 2006; Windhorst, 2007). In the developmental sequence of spinal circuit assembly, DRG sensory afferents enter the spinal cord after motor axons and DRG sensory axons reach their peripheral target areas (Figure 2; Davis et al., 1989; Hollyday, 1980; Landmesser, 1978a; Ozaki and Snider, 1997). Furthermore, ventrally projecting proprioceptive afferents invade the spinal cord before cutaneous afferents terminate in the dorsal horn (Figure 2; Davis et al., 1989; Ozaki and Snider, 1997). This developmental sequence of events suggests that target-derived cues in principle can influence the formation of sensory connections in the spinal cord and thus the specificity of circuit assembly in a retrograde fashion. We will now review recent work providing molecular evidence for retrograde target-derived signals exhibiting key roles in specification of spinal circuits.

The onset in the expression of several ETS transcription factors in defined MN pools and subpopulations of DRG sensory neurons correlates well with the stage when axons invade their peripheral targets (Figure 2; Arber et al., 2000; Lin et al., 1998; Livet et al., 2002). This observation raised the possibility that their expression may be initiated by peripheral signals. Indeed, early limb ablation in developing chick embryos prevents expression of *Pea3* and *Er81*, two members of the ETS transcription factor family, in MNs and DRG sensory neurons (Lin et al., 1998). This observation triggered a number of studies defining the molecular identity of the peripheral factors inducing *Pea3* and *Er81* and elucidating the role of these target-induced transcriptional programs in sensory-motor circuit assembly and function.

Intriguingly, neurotrophic factors control both the expression of *Pea3* in MNs as well as of *Er81* in proprioceptive afferents (Figures 3A and 3B). Glial cell line-derived neurotrophic factor (GDNF) is required for Pea3 expression in cutaneous maximus (CM) and latissimus dorsi (LD) MNs (Figure 3A; Haase et al., 2002), and Neurotrophin 3 (NT3) induces the expression of Er81 in proprioceptive afferents (Figure 3B; Patel et al., 2003). These findings support the notion that apart from their role in neuronal survival, neurotrophic factors also act as retrograde permissive switches to initiate transcriptional programs in defined neuronal subpopulations.

Once induced, what biological activities do Pea3 and Er81 control in MNs and DRG sensory neurons, respectively? In agreement with the regulation of Pea3 and Er81 by target-derived mechanisms, mice mutant for these genes do not exhibit defects in early neuronal specification or initiation of peripheral projections but display specific defects in late circuit assembly (Arber et al., 2000; Livet et al., 2002; Vrieseling and Arber, 2006). *Pea3* mutant MNs in the cervical spinal cord show dramatically altered elaboration of dendritic trees and a transformation in the type of sensory inputs they receive (Figure 3A; Vrieseling and Arber, 2006). Whereas wild-type CM MNs display dendrites avoiding the central gray matter almost entirely and do not receive monosynaptic sensory input, *Pea3* mutant CM MNs exhibit radial dendrites and receive direct functionally inappropriate sensory input. Furthermore, CM MN cell bodies show altered spinal cell-body positioning, and motor axons also exhibit defects in target invasion (Livet et al., 2002). In *Er81*

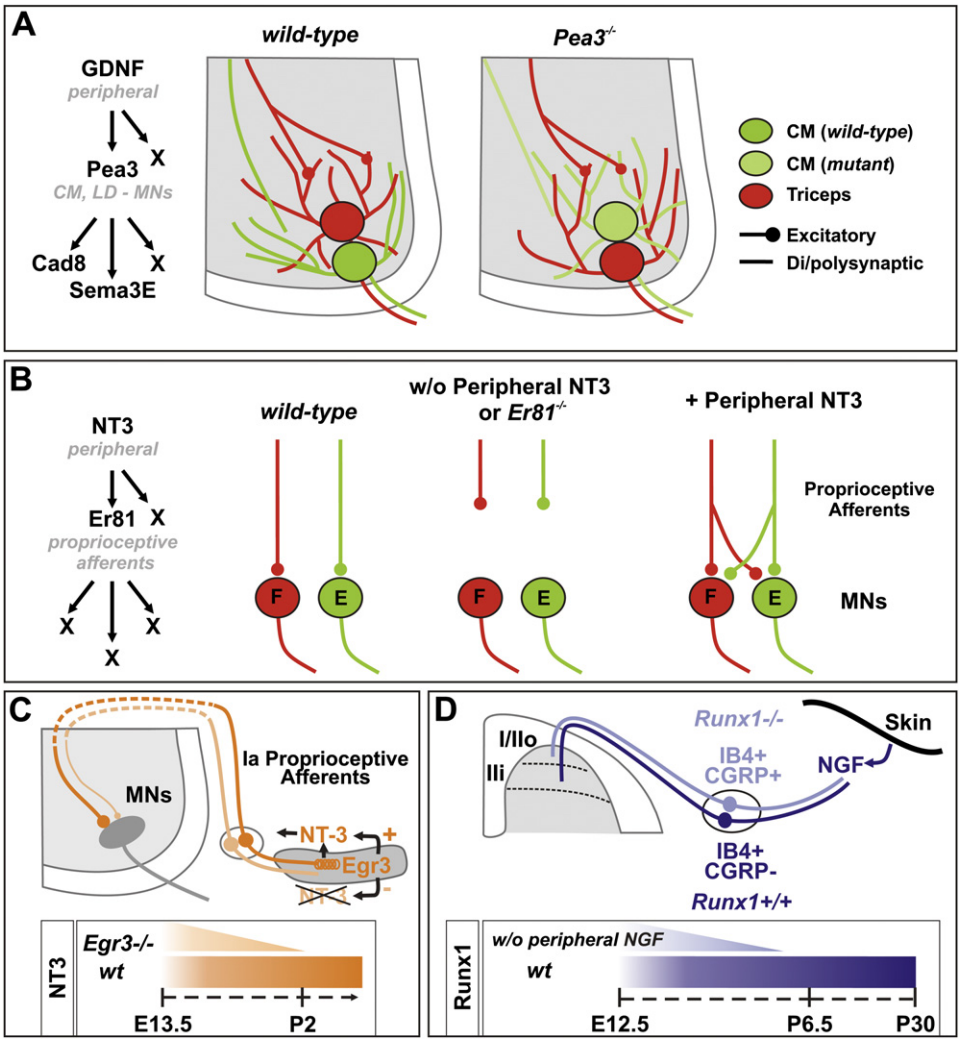

Figure 3. Assembly and Function of Sensory-Motor Connectivity by Retrograde Signals

(A) Regulation of sensory-motor connectivity by ETS transcription factor *Pea3* in CM and LD MN pools. Peripheral GDNF induces the expression of *Pea3* in CM and LD MNs, which in turn is required for activation of downstream genes (*Cad8*, *Sema3E*, X) (left). *Pea3* mutant mice exhibit defects in CM MN cell-body positioning, MN dendrite elaboration, and sensory-motor connectivity (right) when compared to wild-type (middle). Note that CM MNs in wild-type receive sensory input only at di-/polysynaptic latency (open line), and triceps (Tri) afferents in *Pea3* mutants make monosynaptic connections to Tri and CM MNs.

(B) The role of peripheral NT3 in the establishment of sensory-motor connectivity. Peripheral NT3 induces the expression of Er81 in proprioceptive afferents, which in turn regulates establishment of central projections of group Ia proprioceptive afferents through currently unknown target genes (X) (left). Peripheral NT3 regulates the precision of connections between group Ia proprioceptive afferents and MN pools. Representative examples of antagonistic flexor (F) and extensor (E) circuits are depicted. In wild-type, extensor and flexor group Ia afferents contact homonymous but not antagonistic MNs. In the absence of peripheral NT3 or Er81, group Ia afferents fail to invade the ventral horn of the spinal cord. Excess muscular NT3 during development leads to impaired specificity in sensory-motor connections.

(C) Loss of *Egr3*, a transcription factor expressed by intrafusal muscle fibers, leads to loss of muscle-spindle-derived NT3 at postnatal stages and as a consequence to severe weakening of monosynaptic sensory-motor connections. Diagram below depicts level of peripheral NT3 in wild-type and *Egr3* mutant mice.

(D) Maintenance of Runx1 expression in subset of nociceptive DRG sensory neurons (IB4) requires the presence of peripheral NGF. Absence of *Runx1* alters maturation of $IB4^+$ DRG sensory neurons and central projections in the dorsal spinal cord (laminae I and II). Diagram below depicts Runx1 expression in DRG sensory neurons of wild-type and in the absence of peripheral NGF.

mutant mice, group Ia proprioceptive afferents terminate prematurely in the intermediate spinal cord (Figure 3B; Arber et al., 2000). As a consequence, these mutant mice lack direct group Ia proprioceptive afferent input to MNs almost entirely and display severely uncoordinated limb movements, underscoring the important role of proprioceptive input for motor control. Together, these studies demonstrate that the expression of neuronal subpopulation-specific ETS transcription factors exert a potent influence on integration of neurons into spinal circuits

controlling motor behavior, both by influencing neuronal morphology as well as selectivity of synapse formation between DRG sensory neurons and MNs.

Is there evidence that target-derived signals regulate transcriptional programs other than ETS transcription factors during the assembly of sensory-motor circuits? Runx transcription factors play important roles in specification and differentiation of proprioceptive afferents (Runx3) (Chen et al., 2006b; Inoue et al., 2002; Kramer et al., 2006; Levanon et al., 2002) and Ret$^+$ nociceptive neurons (Runx1) (Figure 2; Chen et al., 2006a). Nerve growth factor (NGF) has recently been shown to be required for maintenance of Runx1 (Luo et al., 2007), but not for initial induction (Figure 3D; Kramer et al., 2006). Runx1 activity is essential in DRG sensory neuron differentiation over an extended time period. An intriguing possibility is that retrograde neurotrophic factor signaling is not only involved in initial induction of transcriptional programs but also acts to diversify and mature properties of distinct classes of sensory neurons over time. Similarly, although the initial induction of Runx3 does not depend on NT3 (Kramer et al., 2006), it remains possible that the same or other target-derived factors act at a later stage to shape diversification of proprioceptor fates, including their connectivity. Indeed, surgical experiments in the chick suggest that specificity of sensory-motor connections is controlled by peripheral signals (Frank and Wenner, 1993; Hippenmeyer et al., 2004). The generality of this principle is underscored by the recent observation that target-derived BMP4 signaling acts through the transcription factor Onecut2 to determine trigeminal sensory neuron specification and the formation of a somatosensory map (Hodge et al., 2007).

The role of target-derived NT3 reaches beyond the regulation of Er81 expression in proprioceptive afferents. Mice expressing excess NT3 from a muscle-specific promoter at late embryonic stages when sensory-motor connections form show alterations in the specificity of monosynaptic connections between group Ia proprioceptive afferents and MNs (Figure 3B; Wang et al., 2007). Whereas in wild-type, MNs projecting to a defined muscle receive strong input from their own afferents (homonymous), they are not innervated by afferents projecting to antagonistic muscle groups (Eccles et al., 1957; Mears and Frank, 1997). Surprisingly, specificity of connections is impaired upon excess developmental muscular NT3 expression, and group Ia afferents no longer distinguish between homonymous and antagonistic MNs (Wang et al., 2007). The molecular downstream pathways through which this loss of specificity arises are currently unknown. NT3 also exerts postnatal functions in sensory-motor connectivity. Mice mutant in the transcription factor *Egr3*, in which muscle spindles appear to degenerate at an early postnatal stage, and which therefore lose a major peripheral source of NT3, show a dramatic decrease in the amplitude of monosynaptic sensory responses in MNs, despite the anatomical presence of central group Ia afferent projections (Figure 3C; Chen et al., 2002; Tourtellotte and Milbrandt, 1998). However, muscular injections of NT3 at early postnatal stages prevent this decay in monosynaptic amplitude (Chen et al., 2002).

Taken together, these observations suggest that differentiation, integration, and function of DRG sensory neurons and MNs in spinal circuits are influenced by target-derived molecular signals. These retrograde signals not only regulate adjustments in traits such as the choice of a particular neurotransmitter (e.g., CGRP) (Hippenmeyer et al., 2004), but can also control transcriptional programs in defined neuronal subpopulations. It is interesting to note that different signals are responsible for regulating transcriptional programs in MNs and DRG sensory neurons (Figure 3; Haase et al., 2002; Lin et al., 1998; Patel et al., 2003). Nevertheless, retrograde programs of connectivity can act both on MNs and DRG sensory neurons, introducing flexibility in the features regulated by distinct pathways. Emerging from the findings on retrograde molecular control of circuit assembly and function is the view that these programs not only are at work during early developmental stages, but also continue to shape and adapt motor circuits throughout life. Moreover, it is currently unknown whether retrograde signals act only on MNs and DRG sensory neurons or can also influence either directly or indirectly the specificity of connections formed between defined populations of spinal interneurons and the assembly of supraspinal inputs.

Spinal motor circuits still show considerable potential for adjustment to accommodate changes in motor behavior in the adult, and many of these adjustments occur at the level of the spinal cord. For example, actions of NT3 on group Ia afferents indirectly influence strength of sensory-motor connectivity (Mendell et al., 2001), and other yet unidentified peripheral signals can also modulate these connections by directly acting on MNs (Mendell and Munson, 1999). An additional well-described example with measurable consequences at the level of motor output in the adult is the alteration of H-reflex amplitudes in the spinal monosynaptic reflex circuit (Wolpaw and Tennissen, 2001). In operant conditioning experiments of the H-reflex in several species including humans, electrical stimulation of defined muscle nerves in the periphery is coupled to a reward if the amplitude of the measured reflex is scaled in a defined direction. This conditioning protocol can lead to up- or downregulation in the amplitude of monosynaptic responses of up to 100% (Wolpaw and Tennissen, 2001). The generation of up or down states requires the presence of cortico-spinal connections, but the presence of several other tracts (rubrospinal, vestibulospinal, reticulospinal, and dorsal column ascending tract) is dispensible (Chen and Wolpaw, 1997; Wolpaw and Tennissen, 2001). Specific feedback from the brain may thus update local spinal circuits with new information to adapt output accordingly. H-reflex training persists upon removal of descending input, suggesting that the memory of the scaled H-reflex amplitude is stored in the spinal cord (Wolpaw and Tennissen, 2001). Exactly where these changes occur at the level of neuronal circuits or

molecular adjustments is currently not known. Nevertheless, these findings show that the adult spinal cord maintains considerable potential for plasticity by integrating information transferred to spinal circuits, an important consideration when thinking about strategies for regaining motor capacity upon injury.

Specificity of Connections beyond Motor Neuron Pool Topography

When reviewing the neuronal basis underlying the organization of spatial maps representing body perception and control of appropriate locomotor sequences, MNs in the ventral horn stand out as an example with an exquisite degree of organization and specificity. MN cell bodies are clustered into MN pools innervating individual muscles. These MN pools are found in highly stereotypic positions in the spinal cord (Landmesser, 1978b; Romanes, 1951), receive selective sets of group Ia proprioceptive afferent inputs (Eccles et al., 1957; Frank and Wenner, 1993; Mears and Frank, 1997), and display dendritic trees covering distinct territories in the spinal cord (Okado et al., 1990; Vrieseling and Arber, 2006). MNs can be divided into progressively smaller functionally identified subpopulations on the basis of their peripheral connectivity. Recent work on Hox transcription factor networks acting at early developmental stages has begun to offer molecular explanations for how MN pool identities are defined (Figure 2; Dasen et al., 2003, 2005). Is a similar degree of organization detected in spinal circuits feeding toward MNs or are MNs unique in their molecular, morphological, and physiological organization? And what are the mechanisms setting up specificity of spinal connections during development?

We will now turn to review specificity of connections and topographic maps in the spinal cord beyond MNs. Because neuronal subpopulations connecting within the spinal cord are more difficult to define than MNs, however, mechanisms shaping specificities of these connections are only just beginning to be uncovered. We will focus on two examples to illustrate this point: the nociceptive withdrawal reflex (NWR) and V1-derived spinal interneurons.

Topography in the Nociceptive Withdrawal Reflex

The nociceptive withdrawal reflex (NWR) is a spinal-dependent movement intended to remove an area of skin from a noxious stimulus (Schouenborg, 2002). This reaction often requires coordination of several muscle groups, because many muscles act synergistically at particular joints. Careful analysis of NWR responses in rats after application of localized noxious stimuli has revealed a modular system of WR units acting at the level of individual muscles (Figures 4A and 4B; Levinsson et al., 1999; Schouenborg et al., 1992; Schouenborg and Kalliomaki, 1990). As a consequence, the resulting WR to a given stimulus is the composite of several reflex modules acting in concert, activating the muscles required to remove the skin from the applied noxious stimulus. The underlying modular setup in somatotopic spinal columns required for accurate motor output in response to noxious stimuli exhibits similarities to the organization of cortical columns.

How is this accurate modular organization represented at the level of neuronal circuits, which process noxious information and transform it to initiate motor responses? Key neurons in the transformation of nociceptive information to motor responses are interneurons located in lamina IV and V of the spinal cord. These "reflex encoder" (RE) neurons show convergence of nociceptive (C fibers) and tactile (Aβ fibers) input matching the receptive fields of a defined NWR module (Figures 4A and 4B; Schouenborg, 2004; Schouenborg et al., 1995). Nociceptive C fiber afferents make connections to substantia gelatinosa interneurons in the superficial dorsal horn (Brown, 1982) and connect to RE neurons presumably through at least one ventrally projecting interneuron (Figure 4B; Schouenborg, 2002). Anatomical studies suggest that tactile inputs connect to neurons in lamina III-V (Brown, 1982). Direct connections to RE neurons are therefore possible, and observed synaptic latencies are consistent with this, but direct evidence is currently lacking (Figure 4B; Schouenborg, 2002). Tactile-responsive interneurons in lamina III and IV are often aligned with RE neurons with similar receptive fields located in lamina V (Levinsson et al., 2002), and receptive fields of lamina II interneurons in response to thermal noxious stimuli are spatially aligned with receptive fields evoked by mechanical stimulation in deeper dorsal horn laminae (Figure 4A; Levinsson et al., 2002). These observations suggest a general alignment of dorsal horn neurons participating in the same task. Although it remains to be determined whether RE neurons make monosynaptic connections with MNs, the appropriately weighted convergence of tactile and nociceptive inputs to RE neurons suggests that these neurons sit in a prime position to integrate all the necessary information to produce an appropriate response initiating motor output (Schouenborg, 2002, 2004). Moreover, the position of RE neurons in lamina V of the rat lumbar spinal cord follows roughly the spatial arrangement of MN pools projecting to the muscle linked through this module (Schouenborg et al., 1995), suggesting that NWR modules may even be topographically aligned between RE neurons and MNs (Figure 4A).

The observed specificity in the NWR raises the question of whether NWR modules form immediately in a mature configuration or become modified by experience-dependent mechanisms. In rats, movements generated through the WR are steadily refined during the first three postnatal weeks (Figure 4C; Holmberg and Schouenborg, 1996). From P1 to P7, noxious thermal stimulations often produce inappropriate responses including movements toward the stimulus. By P20–P25, however, the same stimulus reliably evokes an appropriate adult-like WR behavior. Whereas exposure to noxious stimuli is not required for the reflex to develop, tactile input is essential (Waldenstrom et al., 2003). The absence of innocuous tactile stimulation during the first three postnatal weeks

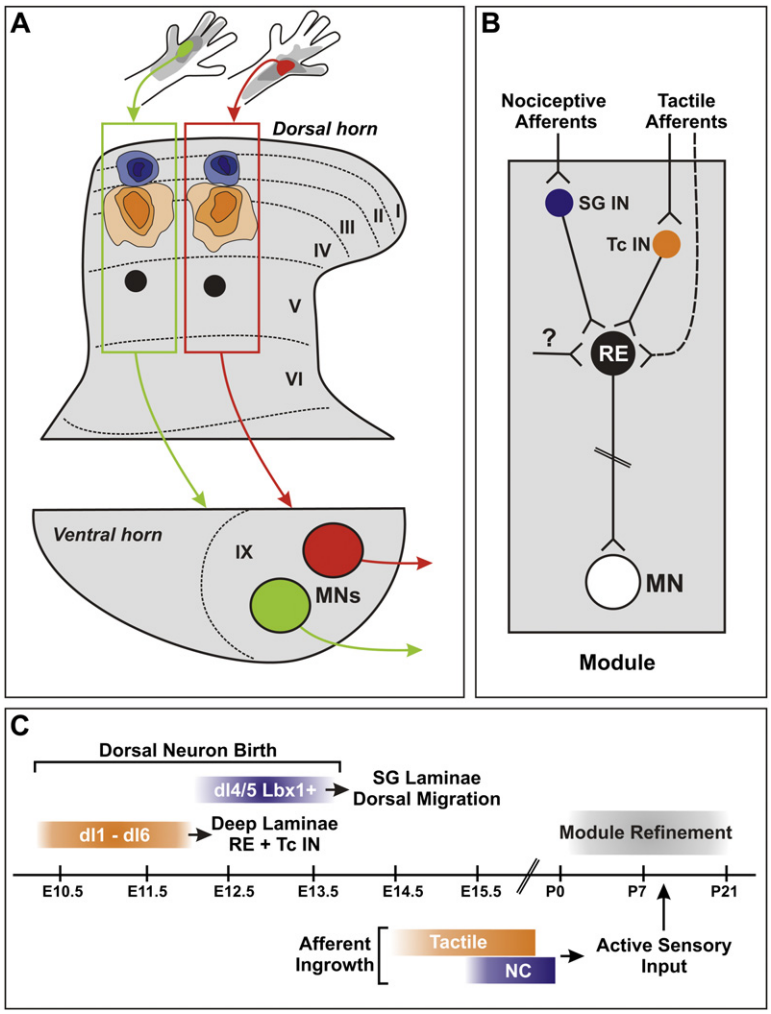

Figure 4. Modular Organization of Nociceptive Withdrawal Reflex

(A) Two NWR modules correlating with distinct peripheral stimulation sites are depicted according to observed specificities in mature connections of the NWR. Individual module is surrounded by green or red box. False color gradients indicate relative amplitude of field potentials recorded in dorsal horn laminae in response to nociceptive (blue) and tactile (orange) stimulation. Responsive areas are aligned with interneurons (black) in lamina V that have the same receptive fields. MNs activated through corresponding module are shown in lamina IX in colors corresponding to the active module. Panel adapted from Levinsson et al. (2002).

(B) Proposed neuronal circuit within one module of the NWR. Tactile and nociceptive sensory afferents contact distinct subsets of dorsal horn interneurons (SG, Substantia gelatinosa; Tc, tactile). Signals from these two modalities and most likely other input (question mark) converge on putative last-order reflex encoder (RE) interneurons to activate MNs. Direct tactile inputs to RE interneurons are also possible (dashed connection).

(C) Time line of dorsal horn neuron birth, migration, and sensory afferent ingrowth to dorsal horn in the lumbar spinal cord of mice. Time line shows embryonic (E) and postnatal (P) days. NWR module refinement occurs during a critical period between P1 and P21.

results in inappropriate WR responses, similar to the ones observed in very young animals (Waldenstrom et al., 2003). How does tactile input train the WR? Evidence suggests that spontaneous muscle twitches evoked during sleep in neonatal rats provide sufficient tactile stimulation to correctly train the tail WR (Petersson et al., 2003). Together, these findings show the existence of a critical period (P1–P20), during which tactile sensory feedback acts to organize spinal circuits and to prime them to elicit appropriate motor output in response to noxious stimuli in the adult.

It is currently unknown at which levels of circuitry developmental adjustments occur in the NWR. Changes could occur at the level of sensory inputs to spinal circuits but also within spinal circuits themselves. Modules with synergistic actions are located in close proximity to each other throughout the circuit, and the relative weight of tactile inputs to different synergist modules could be adjusted by postnatal experience (Schouenborg et al., 1995). Detailed answers to these questions await identification of individual components of interconnected NWR modules. Given the key role of RE neurons in the NWR, future anatomical and molecular identification of RE neurons will certainly be instructive.

Early postmitotic neurons generated in the dorsal spinal cord can be divided into six classes (dI1–dI6) (Figure 4C) on the basis of combinatorial expression of several transcription factors (Gross et al., 2002; Helms and Johnson, 2003; Muller et al., 2002). Many early-born dorsal neurons (E10.5–E11.5) migrate to occupy deep dorsal horn regions in the mature spinal cord, whereas later born (E11.5–E14.5) Lbx1$^+$ dI4/5 neurons migrate superficially to generate the substantia gelatinosa (Figure 4C; Gross et al., 2002; Helms and Johnson, 2003; Muller et al., 2002). These studies suggest that RE interneurons are generated early, and neurons processing nociceptive information belong to the Lbx1$^+$ dI4/5 class of late-born neurons and migrate through the early-born neuron class. In principle, this migration strategy could provide a rough context for topographical alignment of superficial and deep dorsal horn neurons by a mechanism of cell-cell interactions during migration.

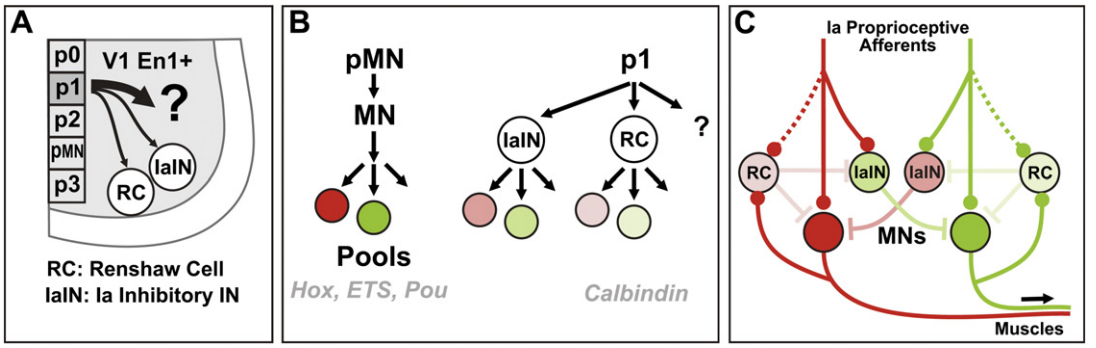

Figure 5. Specificity in Interneuron Connectivity

Genetic determination and connectivity of Renshaw cells (RC) and Ia inhibitory interneurons (IaIN) in the ventral spinal cord.

(A) These and many unassigned (big arrow) interneurons are derived from the ventral progenitor domain p1 and are included in the En1$^+$ V1 interneuron population.

(B) MNs can be distinguished molecularly at the level of individual MN pools (two of many are shown) on the basis of transcription factor expression (Hox, ETS, Pou), but no molecular distinctions are currently known for subtypes of IaINs and RCs, with the exception of Calbindin expressed by all RCs.

(C) Mature connectivity for one example of antagonistic MN pools and associated IaINs and RCs to illustrate specificity of observed connections. Transient neonatal group Ia afferent sensory neuron input to RCs is shown by dotted lines.

Specificity of Renshaw Cell and Ia Inhibitory Interneuron Connectivity

Whether interneurons in the ventral spinal cord exhibit specificity of interconnected neuronal circuits correlating with the high degree of organization observed at the level of MNs is more difficult to approach. From studies on the function of local interneuron circuits involved in locomotor activity, it is known that rhythmic and alternating activity can be more easily induced at rostral than caudal lumbar spinal levels in neonatal spinal cord preparations (Kiehn, 2006). However, the cellular basis explaining these differences is unknown. Analysis of differences in connectivity of interneurons directly contacting MNs has been more fruitful and we will therefore focus on these connections. In particular, Renshaw cells and Ia inhibitory interneurons provide an entry point to study selective connectivity for which molecular pathways involved in generation and differentiation are beginning to be discovered.

Renshaw cells and Ia inhibitory interneurons are developmentally derived from En1$^+$ V1 interneurons, and both are ipsilaterally projecting spinal interneurons (Figures 5A and 5B; Alvarez et al., 2005; Goulding and Pfaff, 2005; Kiehn, 2006; Wenner et al., 2000). Their common developmental origin may also be reflected in the recent observation that both Renshaw cells and Ia inhibitory interneurons in mice receive direct group Ia proprioceptive afferent input during development (Mentis et al., 2006). However, whereas direct sensory inputs to Ia inhibitory interneurons remain strong in the adult (Jankowska, 1992; Windhorst, 2007), inputs to Renshaw cells weaken significantly after P15 (Figure 5C; Mentis et al., 2006). The significance of these transient sensory inputs to Renshaw cells, however, remains unclear.

Both Ia inhibitory interneurons and Renshaw cells show a high degree of selectivity in their connections to MNs in adult animals (Figure 5C). The most striking feature observed for the connectivity pattern of Ia inhibitory interneurons is that they get direct group Ia afferent input and connect in turn directly to antagonist MN pools (Jankowska, 1992; Windhorst, 2007). Moreover, in addition to receiving direct sensory input, they serve to integrate pre- and postmotor signals such as direct corticospinal inputs and Renshaw cell inputs indirectly derived from MNs receiving the same sensory input (Hultborn, 2006; Rossignol et al., 2006; Windhorst, 2007). Ia inhibitory interneurons therefore serve as a convergent integrator of specific sets of inputs and forward this information directly to MNs (Hultborn, 2006; Windhorst, 2007).

In the adult, Renshaw cells receive direct cholinergic input from MNs and form recurrent inhibitory synapses with homonymous and synergistic, but not with antagonistic, MNs (Figure 5C; Alvarez and Fyffe, 2007; Windhorst, 1990). This specificity pattern in connections seems to generally match the pattern observed for connectivity between group Ia afferents and MNs (Figures 3B and 5C; Eccles et al., 1957; Mears and Frank, 1997). Moreover, convergence of Renshaw cell and group Ia afferent synapse positioning along MN dendrites has been described. Both inputs are found mostly on dendrites with only a small fraction observed on MN somata (Fyffe, 1991, 2001). Whether specificity and positioning of Renshaw cell and group Ia afferent input to MNs are regulated by common mechanisms is currently unknown.

The observations on the specificity of connections to and from Renshaw cells and Ia inhibitory interneurons in the mature spinal cord raise the question of how specificity arises during development. Preliminary evidence suggests that at least for one pair of antagonistic MN pools in the lumbar spinal cord of mice, reciprocal Ia inhibitory input is established correctly from the outset, indicating that the establishment of these connections might be regulated through molecular cues (Z. Wang and E. Frank,

2006, Soc. Neurosci., abstract). Nevertheless, these findings do not exclude the possibility that spinal interneuron connectivity is shaped by activity-dependent mechanisms driven by intrinsic spinal cord activity even before the arrival of group Ia sensory neuron input to the ventral spinal cord. Physiological analysis of spinal interneurons recurrently connected to MNs (R-interneurons) at different developmental stages in the chick embryo indeed suggests that GABAergic interneuron connectivity may be adjusted during development (Xu et al., 2007). Moreover, anatomical observations derived from cat triceps surae MNs over early postnatal stages provide evidence for a substantial reduction in the number of MN axon collateral swellings within the projection field, suggesting that also recurrent inhibition from MNs may be subject to developmental adjustments (Cullheim and Ulfhak, 1985).

Is there evidence that genetic programs act to specify functionally distinct interneuron populations? Ia inhibitory interneurons and Renshaw cells are included within the $En1^+$ V1 interneuron population (Figure 5A; Alvarez and Fyffe, 2007; Alvarez et al., 2005), and Renshaw cells can be identified by the expression of the calcium-binding protein Calbindin (Figure 5B; Carr et al., 1998), but no further distinction at the level of molecular markers is currently known that would explain the observed specificity of connections. Nevertheless, several recent studies suggest that the four cardinal ventral interneuron classes can give rise to distinct neuronal populations also at the molecular level. For example, p2 interneuron progenitors generate two distinct neuronal classes (excitatory V2a and inhibitory V2b) through a Notch receptor-mediated signaling mechanism at early postmitotic stages (Peng et al., 2007). In addition, a small population of segmentally restricted interneurons with defined physiological properties and located in medial lamina VIII has recently been linked to the expression of the homeodomain protein Hb9 (Hinckley et al., 2005; Wilson et al., 2005). It is also intriguing to note that Hox genes, which are instrumental in determination of MN pool properties, are expressed in distinct patterns in spinal interneurons (Dasen et al., 2003, 2005) and could therefore contribute to specification of defined interneuron subpopulations as well.

From these studies, it seems likely that these molecular observations provide just the tip of the iceberg in the identification of genetic programs controlling interneuron subtype diversification and connectivity in the spinal cord. Recently developed approaches with *trans*-synaptic viral tracing technology (Boldogkoi et al., 2004; Wickersham et al., 2007) should prove to be very helpful in the morphological identification of defined sets of premotor interneurons after viral injections into defined muscles and can also assist the identification of further molecular markers. Hopefully, in analogy to MN pools, more will soon be known about whether "Renshaw cell pools" and "Ia inhibitory interneuron pools" exist at a molecular level (Figure 5B) and how their connectivity patterns are determined during development.

Dendritic Aspects of Selectivity for MN Function

Irrespective of the pathways acting to integrate information upstream of MNs, this information is ultimately read and interpreted by MNs. Therefore, an important aspect when considering the control of MN activation is the question of whether and how differential dendrite patterning and compartmentalization of inputs on soma and dendrites contribute to shape MN responses. There is indeed evidence for both possibilities: MN subpopulations exhibiting distinct dendrite patterns and selective locations of inputs on MNs.

MN dendrites exhibit a high degree of heterogeneity in projection patterns. Although many studies have focused on MNs with radially projecting dendrites, recent evidence underscores the fact that MN pools exhibit distinct morphologies in their dendritic trees and cover different areas of gray and white matter (Vrieseling and Arber, 2006). As a consequence of the observed diversity in MN morphologies, it is plausible to assume that correlating with the covered territories, different potential inputs are accessible to MN pools. This possibility is particularly appealing because defined interneuron populations as well as descending tracts show highly stereotypic positions in the spinal cord (Brown, 1981). MNs innervating dorsal neck muscles provide one example in which such preferential inputs have been observed (Grande et al., 2005; Rose et al., 1995). Vestibulospinal inputs to MNs innervating the splenius neck muscle show highly preferential inputs to medial MN dendrites (Grande et al., 2005). This clustering of inputs can increase the local density of synapses that are synchronously activated, which could help to optimize the sensitivity to particular inputs channeled exclusively through distinct dendritic branches.

What is known about differential location of inputs to MN compartments? Whereas group Ia sensory afferent inputs and Renshaw cell inputs are primarily located on dendrites (Burke and Glenn, 1996; Fyffe, 1991), inputs from Ia inhibitory interneurons are found in significant numbers connecting to both dendritic and somatic MN compartments (Fyffe, 2001). Despite the fairly widespread overall placement of group Ia afferent synapses, detailed anatomical analysis of connectivity between individual group Ia sensory neurons and MNs in the cat showed that contacts to a particular MN form exclusively through one of the group Ia afferent collaterals and are clustered at the same distance from the cell body (Brown and Fyffe, 1981). A particularly striking example of compartmentalized inputs to MN somata is derived from spinal cholinergic neurons whose cell bodies are located in lamina X and medial part of lamina VII (Conradi et al., 1979; Hellstrom et al., 2003; Miles et al., 2007). These cholinergic inputs (C boutons) are located on the soma and very proximal dendrites of MNs (Conradi et al., 1979; Hellstrom et al., 2003), activate muscarinic ACh receptors (Hellstrom et al., 2003; Miles et al., 2007), and increase MN firing rate by decreasing the afterhyperpolarization that follows an action potential (Miles et al., 2007). C boutons on MNs are restricted to similar locations already at early

postnatal stages in mice but mature considerably over the first two postnatal weeks (Wilson et al., 2004). These findings suggest that at least some MN inputs are highly compartmentalized at early stages, but mechanisms controlling this selectivity are currently unknown. In the cerebellum, recruitment of basket cell interneuron synapses to the axon initial segment of Purkinje cells is controlled by selective recruitment of immunoglobulin L1 proteins through Ankyrin G (Huang, 2006). It is feasible to speculate that specification of subcellular synapse location can also be determined by genetic mechanisms in the spinal cord.

Evolution of Motor Circuits and Implications for Changing Functions

Motor circuits adjust throughout evolution to accommodate to the need for different motor behaviors. Despite the differing repertoires of motor behaviors such as swimming, flying, and walking on two or four legs, there is surprising conservation at the level of MN pools and basic local spinal circuit organization. Homologous MN pools can be assigned across species even between MNs innervating chick wing muscles and cat forelimb muscles (Ryan et al., 1998; Straznicky and Tay, 1983). Moreover, rhythmic behavior leading to flexor-extensor and left-right alternation is also a conserved feature among different vertebrate species' locomotor behaviors (Grillner, 2006; Kiehn, 2006). These findings suggest that a robust central spinal system, onto which modules of input converge and integrate, may be flexible enough to cope with changes required in motor behavior throughout evolution. For example, during evolution of the WR, somatosensory transformations within the spinal cord can adjust to different peripheral inputs. Whereas cats touch ground with their digits, rats do so with their entire plantar surface. These peripheral differences lead to somatosensory transformations with similar receptive fields but different weights represented in the spinal cord when comparing the two species (Schouenborg, 2002). Similarly, behavioral differences between species can also be attributed to altered patterns in descending connectivity. In particular, it is thought that direct connections between layer V motor cortex neurons and MNs in the spinal cord occur preferentially to MNs controlling fine movement of fingers in humans and certain species of monkey (Katz and Harris-Warrick, 1999; Lemon and Griffiths, 2005). In contrast, mostly indirect connections to MNs exist in other species (Yang and Lemon, 2003), suggesting that adjustments of circuitry at the level of descending projections can influence motor behavior in a pronounced way. In summary, local spinal circuits allow dedicated modules to plug into a basic spinal circuit setup and thereby permit adjustment of motor responses not only throughout life within one animal but also across evolution in different species.

Concluding Remarks

This review set out to explore developmental control mechanisms acting to specify the assembly of motor circuits in the spinal cord, classically considered as a major output system with little flexibility. In this review, we discuss the high degree of specificity that can be observed in the connections of spinal neuronal circuits. These dedicated circuit modules are generated during development by a tight interaction between programs of genetic predetermination and experience-driven processes, equipping motor circuits for function. Sequential temporal assembly of circuit elements in the spinal cord and different phases of excitability throughout this process provide an important backbone for the control of motor behavior in the adult. In addition to mechanisms acting within the spinal cord, information from outside the spinal cord also plays an important role in motor circuit assembly and function. A series of peripheral pathways, acting both through the induction of transcriptional programs as well as experience shape circuits in a retrograde manner. The combination of these strategies allows the establishment of functional motor circuits capable of reading, modifying, and activating motor output in accordance with the ongoing activities impinging on an animal throughout life, making the motor system highly flexible. The unique property of motor circuits of providing a direct link between fine details of dedicated circuits and an immediate behavioral output promises exceptional opportunities for future research to understand how dedicated neuronal circuits control defined function in the nervous system.

ACKNOWLEDGMENTS

We are very grateful to Tom Jessell, Ole Kiehn, David Maxwell, Andrew Todd, and members of the Arber laboratory for helpful comments on the manuscript and discussions. D.R.L., E.P.-V., and S.A. were supported by a grant from the Swiss National Science Foundation, by the Kanton of Basel-Stadt, and by the Novartis Research Foundation.

REFERENCES

Alvarez, F.J., and Fyffe, R.E. (2007). The continuing case for the Renshaw cell. J. Physiol., in press. Published online July 19, 2007. jphysiol.2007.136200v1.

Alvarez, F.J., Jonas, P.C., Sapir, T., Hartley, R., Berrocal, M.C., Geiman, E.J., Todd, A.J., and Goulding, M. (2005). Postnatal phenotype and localization of spinal cord V1 derived interneurons. J. Comp. Neurol. *493*, 177–192.

Arber, S., Ladle, D.R., Lin, J.H., Frank, E., and Jessell, T.M. (2000). ETS gene Er81 controls the formation of functional connections between group Ia sensory afferents and motor neurons. Cell *101*, 485–498.

Boldogkoi, Z., Sik, A., Denes, A., Reichart, A., Toldi, J., Gerendai, I., Kovacs, K.J., and Palkovits, M. (2004). Novel tracing paradigms-genetically engineered herpesviruses as tools for mapping functional circuits within the CNS: present status and future prospects. Prog. Neurobiol. *72*, 417–445.

Branchereau, P., Morin, D., Bonnot, A., Ballion, B., Chapron, J., and Viala, D. (2000). Development of lumbar rhythmic networks: from embryonic to neonate locomotor-like patterns in the mouse. Brain Res. Bull. *53*, 711–718.

Brown, A.G. (1981). Organization in the Spinal Cord (Berlin: Springer).

Brown, A.G. (1982). The dorsal horn of the spinal cord. Q. J. Exp. Physiol. *67*, 193–212.

Brown, A.G., and Fyffe, R.E. (1978). The morphology of group Ia afferent fibre collaterals in the spinal cord of the cat. J. Physiol. *274*, 111–127.

Brown, A.G., and Fyffe, R.E. (1981). Direct observations on the contacts made between Ia afferent fibres and alpha-motoneurones in the cat's lumbosacral spinal cord. J. Physiol. *313*, 121–140.

Burke, R.E., and Glenn, L.L. (1996). Horseradish peroxidase study of the spatial and electrotonic distribution of group Ia synapses on type-identified ankle extensor motoneurons in the cat. J. Comp. Neurol. *372*, 465–485.

Carr, P.A., Alvarez, F.J., Leman, E.A., and Fyffe, R.E. (1998). Calbindin D28k expression in immunohistochemically identified Renshaw cells. Neuroreport *9*, 2657–2661.

Chang, Q., Gonzalez, M., Pinter, M.J., and Balice-Gordon, R.J. (1999). Gap junctional coupling and patterns of connexin expression among neonatal rat lumbar spinal motor neurons. J. Neurosci. *19*, 10813–10828.

Chen, X.Y., and Wolpaw, J.R. (1997). Dorsal column but not lateral column transection prevents down-conditioning of H reflex in rats. J. Neurophysiol. *78*, 1730–1734.

Chen, H.H., Tourtellotte, W.G., and Frank, E. (2002). Muscle spindle-derived neurotrophin 3 regulates synaptic connectivity between muscle sensory and motor neurons. J. Neurosci. *22*, 3512–3519.

Chen, C.L., Broom, D.C., Liu, Y., de Nooij, J.C., Li, Z., Cen, C., Samad, O.A., Jessell, T.M., Woolf, C.J., and Ma, Q. (2006a). Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. Neuron *49*, 365–377.

Chen, A.I., de Nooij, J.C., and Jessell, T.M. (2006b). Graded activity of transcription factor Runx3 specifies the laminar termination pattern of sensory axons in the developing spinal cord. Neuron *49*, 395–408.

Conradi, S., Kellerth, J.O., and Berthold, C.H. (1979). Electron microscopic studies of serially sectioned cat spinal alpha-motoneurons. II. A method for the description of architecture and synaptology of the cell body and proximal dendritic segments. J. Comp. Neurol. *184*, 741–754.

Cullheim, S., and Ulfhak, B. (1985). Postnatal changes in the termination pattern of recurrent axon collaterals of triceps surae alpha-motoneurons in the cat. Brain Res. *349*, 63–73.

Dasen, J.S., Liu, J.P., and Jessell, T.M. (2003). Motor neuron columnar fate imposed by sequential phases of Hox-c activity. Nature *425*, 926–933.

Dasen, J.S., Tice, B.C., Brenner-Morton, S., and Jessell, T.M. (2005). A Hox regulatory network establishes motor neuron pool identity and target-muscle connectivity. Cell *123*, 477–491.

Davis, B.M., Frank, E., Johnson, F.A., and Scott, S.A. (1989). Development of central projections of lumbosacral sensory neurons in the chick. J. Comp. Neurol. *279*, 556–566.

Eccles, J.C., Eccles, R.M., and Lundberg, A. (1957). The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. J. Physiol. *137*, 22–50.

Fiumelli, H., and Woodin, M.A. (2007). Role of activity-dependent regulation of neuronal chloride homeostasis in development. Curr. Opin. Neurobiol. *17*, 81–86.

Frank, E., and Wenner, P. (1993). Environmental specification of neuronal connectivity. Neuron *10*, 779–785.

Fyffe, R.E. (1991). Spatial distribution of recurrent inhibitory synapses on spinal motoneurons in the cat. J. Neurophysiol. *65*, 1134–1149.

Fyffe, R.E. (2001). Spinal motoneurons: synaptic inputs and receptor organization. In Motor Neurobiology of the Spinal Cord, T.C. Cope, ed. (New York: CRC Press), pp. 21–46.

Gonzalez-Islas, C., and Wenner, P. (2006). Spontaneous network activity in the embryonic spinal cord regulates AMPAergic and GABAergic synaptic strength. Neuron *49*, 563–575.

Goulding, M., and Pfaff, S.L. (2005). Development of circuits that generate simple rhythmic behaviors in vertebrates. Curr. Opin. Neurobiol. *15*, 14–20.

Grande, G., Armstrong, S., Neuber-Hess, M., and Rose, P.K. (2005). Distribution of contacts from vestibulospinal axons on the dendrites of splenius motoneurons. J. Comp. Neurol. *491*, 339–351.

Grillner, S. (2006). Biological pattern generation: the cellular and computational logic of networks in motion. Neuron *52*, 751–766.

Gross, M.K., Dottori, M., and Goulding, M. (2002). Lbx1 specifies somatosensory association interneurons in the dorsal spinal cord. Neuron *34*, 535–549.

Haase, G., Dessaud, E., Garces, A., de Bovis, B., Birling, M., Filippi, P., Schmalbruch, H., Arber, S., and deLapeyriere, O. (2002). GDNF acts through PEA3 to regulate cell body positioning and muscle innervation of specific motor neuron pools. Neuron *35*, 893–905.

Hanson, M.G., and Landmesser, L.T. (2003). Characterization of the circuits that generate spontaneous episodes of activity in the early embryonic mouse spinal cord. J. Neurosci. *23*, 587–600.

Hellstrom, J., Oliveira, A.L., Meister, B., and Cullheim, S. (2003). Large cholinergic nerve terminals on subsets of motoneurons and their relation to muscarinic receptor type 2. J. Comp. Neurol. *460*, 476–486.

Helms, A.W., and Johnson, J.E. (2003). Specification of dorsal spinal cord interneurons. Curr. Opin. Neurobiol. *13*, 42–49.

Hinckley, C.A., Hartley, R., Wu, L., Todd, A., and Ziskind-Conhaim, L. (2005). Locomotor-like rhythms in a genetically distinct cluster of interneurons in the mammalian spinal cord. J. Neurophysiol. *93*, 1439–1449.

Hippenmeyer, S., Kramer, I., and Arber, S. (2004). Control of neuronal phenotype: what targets tell the cell bodies. Trends Neurosci. *27*, 482–488.

Hodge, L.K., Klassen, M.P., Han, B.X., Yiu, G., Hurrell, J., Howell, A., Rousseau, G., Lemaigre, F., Tessier-Lavigne, M., and Wang, F. (2007). Retrograde BMP signaling regulates trigeminal sensory neuron identities and the formation of precise face maps. Neuron *55*, 572–586.

Hollyday, M. (1980). Motoneuron histogenesis and the development of limb innervation. Curr. Top. Dev. Biol. *15*, 181–215.

Hollyday, M., and Hamburger, V. (1977). An autoradiographic study of the formation of the lateral motor column in the chick embryo. Brain Res. *132*, 197–208.

Holmberg, H., and Schouenborg, J. (1996). Postnatal development of the nociceptive withdrawal reflexes in the rat: a behavioural and electromyographic study. J. Physiol. *493*, 239–252.

Huang, Z.J. (2006). Subcellular organization of GABAergic synapses: role of ankyrins and L1 cell adhesion molecules. Nat. Neurosci. *9*, 163–166.

Hubner, C.A., Stein, V., Hermans-Borgmeyer, I., Meyer, T., Ballanyi, K., and Jentsch, T.J. (2001). Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. Neuron *30*, 515–524.

Hultborn, H. (2006). Spinal reflexes, mechanisms and concepts: from Eccles to Lundberg and beyond. Prog. Neurobiol. *78*, 215–232.

Inoue, K., Ozaki, S., Shiga, T., Ito, K., Masuda, T., Okado, N., Iseda, T., Kawaguchi, S., Ogawa, M., Bae, S.C., et al. (2002). Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. Nat. Neurosci. *5*, 946–954.

Jankowska, E. (1992). Interneuronal relay in spinal pathways from proprioceptors. Prog. Neurobiol. *38*, 335–378.

Jessell, T.M. (2000). Neuronal specification in the spinal cord: inductive signals and transcriptional codes. Nat. Rev. Genet. *1*, 20–29.

Katz, P.S., and Harris-Warrick, R.M. (1999). The evolution of neuronal circuits underlying species-specific behavior. Curr. Opin. Neurobiol. *9*, 628–633.

Kiehn, O. (2006). Locomotor circuits in the mammalian spinal cord. Annu. Rev. Neurosci. *29*, 279–306.

Kiehn, O., and Tresch, M.C. (2002). Gap junctions and motor behavior. Trends Neurosci. *25*, 108–115.

Kramer, I., Sigrist, M., de Nooij, J.C., Taniuchi, I., Jessell, T.M., and Arber, S. (2006). A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. Neuron *49*, 379–393.

Kudo, N., and Nishimaru, H. (1998). Reorganization of locomotor activity during development in the prenatal rat. Ann. N Y Acad. Sci. *860*, 306–317.

Landmesser, L. (1978a). The development of motor projection patterns in the chick hind limb. J. Physiol. *284*, 391–414.

Landmesser, L. (1978b). The distribution of motoneurones supplying chick hind limb muscles. J. Physiol. *284*, 371–389.

Lanuza, G.M., Gosgnach, S., Pierani, A., Jessell, T.M., and Goulding, M. (2004). Genetic identification of spinal interneurons that coordinate left-right locomotor activity necessary for walking movements. Neuron *42*, 375–386.

Lemon, R.N., and Griffiths, J. (2005). Comparing the function of the corticospinal system in different species: organizational differences for motor specialization? Muscle Nerve *32*, 261–279.

Levanon, D., Bettoun, D., Harris-Cerruti, C., Woolf, E., Negreanu, V., Eilam, R., Bernstein, Y., Goldenberg, D., Xiao, C., Fliegauf, M., et al. (2002). The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. EMBO J. *21*, 3454–3463.

Levinsson, A., Luo, X.L., Holmberg, H., and Schouenborg, J. (1999). Developmental tuning in a spinal nociceptive system: effects of neonatal spinalization. J. Neurosci. *19*, 10397–10403.

Levinsson, A., Holmberg, H., Broman, J., Zhang, M., and Schouenborg, J. (2002). Spinal sensorimotor transformation: relation between cutaneous somatotopy and a reflex network. J. Neurosci. *22*, 8170–8182.

Lin, J.H., Saito, T., Anderson, D.J., Lance-Jones, C., Jessell, T.M., and Arber, S. (1998). Functionally related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. Cell *95*, 393–407.

Livet, J., Sigrist, M., Stroebel, S., De Paola, V., Price, S.R., Henderson, C.E., Jessell, T.M., and Arber, S. (2002). ETS gene Pea3 controls the central position and terminal arborization of specific motor neuron pools. Neuron *35*, 877–892.

Luo, W., Wickramasinghe, S.R., Savitt, J.M., Griffin, J.W., Dawson, T.M., and Ginty, D.D. (2007). A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. Neuron *54*, 739–754.

Mears, S.C., and Frank, E. (1997). Formation of specific monosynaptic connections between muscle spindle afferents and motoneurons in the mouse. J. Neurosci. *17*, 3128–3135.

Mendell, L.M., and Munson, J.B. (1999). Retrograde effects on synaptic transmission at the Ia/motoneuron connection. J. Physiol. (Paris) *93*, 297–304.

Mendell, L.M., Munson, J.B., and Arvanian, V.L. (2001). Neurotrophins and synaptic plasticity in the mammalian spinal cord. J. Physiol. *533*, 91–97.

Mentis, G.Z., Alvarez, F.J., Bonnot, A., Richards, D.S., Gonzalez-Forero, D., Zerda, R., and O'Donovan, M.J. (2005). Noncholinergic excitatory actions of motoneurons in the neonatal mammalian spinal cord. Proc. Natl. Acad. Sci. USA *102*, 7344–7349.

Mentis, G.Z., Siembab, V.C., Zerda, R., O'Donovan, M.J., and Alvarez, F.J. (2006). Primary afferent synapses on developing and adult Renshaw cells. J. Neurosci. *26*, 13297–13310.

Miles, G.B., Hartley, R., Todd, A.J., and Brownstone, R.M. (2007). Spinal cholinergic interneurons regulate the excitability of motoneurons during locomotion. Proc. Natl. Acad. Sci. USA *104*, 2448–2453.

Milner, L.D., and Landmesser, L.T. (1999). Cholinergic and GABAergic inputs drive patterned spontaneous motoneuron activity before target contact. J. Neurosci. *19*, 3007–3022.

Muller, T., Brohmann, H., Pierani, A., Heppenstall, P.A., Lewin, G.R., Jessell, T.M., and Birchmeier, C. (2002). The homeodomain factor lbx1 distinguishes two major programs of neuronal differentiation in the dorsal spinal cord. Neuron *34*, 551–562.

Myers, C.P., Lewcock, J.W., Hanson, M.G., Gosgnach, S., Aimone, J.B., Gage, F.H., Lee, K.F., Landmesser, L.T., and Pfaff, S.L. (2005). Cholinergic input is required during embryonic development to mediate proper assembly of spinal locomotor circuits. Neuron *46*, 37–49.

Nishimaru, H., Restrepo, C.E., Ryge, J., Yanagawa, Y., and Kiehn, O. (2005). Mammalian motor neurons corelease glutamate and acetylcholine at central synapses. Proc. Natl. Acad. Sci. USA *102*, 5245–5249.

Nornes, H.O., and Carry, M. (1978). Neurogenesis in spinal cord of mouse: an autoradiographic analysis. Brain Res. *159*, 1–6.

O'Donovan, M.J. (1999). The origin of spontaneous activity in developing networks of the vertebrate nervous system. Curr. Opin. Neurobiol. *9*, 94–104.

Okado, N., Homma, S., Ishihara, R., and Kohno, K. (1990). Distribution patterns of dendrites in motor neuron pools of lumbosacral spinal cord of the chicken. Anat. Embryol. (Berl.) *182*, 113–121.

Ozaki, S., and Snider, W.D. (1997). Initial trajectories of sensory axons toward laminar targets in the developing mouse spinal cord. J. Comp. Neurol. *380*, 215–229.

Patel, T.D., Kramer, I., Kucera, J., Niederkofler, V., Jessell, T.M., Arber, S., and Snider, W.D. (2003). Peripheral NT3 signaling is required for ETS protein expression and central patterning of proprioceptive sensory afferents. Neuron *38*, 403–416.

Pearson, K.G. (2004). Generating the walking gait: role of sensory feedback. Prog. Brain Res. *143*, 123–129.

Peng, C.Y., Yajima, H., Burns, C.E., Zon, L.I., Sisodia, S.S., Pfaff, S.L., and Sharma, K. (2007). Notch and MAML signaling drives Scl-dependent interneuron diversity in the spinal cord. Neuron *53*, 813–827.

Petersson, P., Waldenstrom, A., Fahraeus, C., and Schouenborg, J. (2003). Spontaneous muscle twitches during sleep guide spinal self-organization. Nature *424*, 72–75.

Pierani, A., Moran-Rivard, L., Sunshine, M.J., Littman, D.R., Goulding, M., and Jessell, T.M. (2001). Control of interneuron fate in the developing spinal cord by the progenitor homeodomain protein Dbx1. Neuron *29*, 367–384.

Romanes, G.J. (1951). The motor cell columns of the lumbo-sacral spinal cord of the cat. J. Comp. Neurol. *94*, 313–363.

Rose, P.K., Jones, T., Nirula, R., and Corneil, T. (1995). Innervation of motoneurons based on dendritic orientation. J. Neurophysiol. *73*, 1319–1322.

Rossignol, S., Dubuc, R., and Gossard, J.P. (2006). Dynamic sensorimotor interactions in locomotion. Physiol. Rev. *86*, 89–154.

Ryan, J.M., Cushman, J., Jordan, B., Samuels, A., Frazer, H., and Baier, C. (1998). Topographic position of forelimb motoneuron pools is conserved in vertebrate evolution. Brain Behav. Evol. *51*, 90–99.

Schouenborg, J. (2002). Modular organisation and spinal somatosensory imprinting. Brain Res. Brain Res. Rev. *40*, 80–91.

Schouenborg, J. (2004). Learning in sensorimotor circuits. Curr. Opin. Neurobiol. *14*, 693–697.

Schouenborg, J., and Kalliomaki, J. (1990). Functional organization of the nociceptive withdrawal reflexes. I. Activation of hindlimb muscles in the rat. Exp. Brain Res. *83*, 67–78.

Schouenborg, J., Holmberg, H., and Weng, H.R. (1992). Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. Exp. Brain Res. *90*, 469–478.

Schouenborg, J., Weng, H.R., Kalliomaki, J., and Holmberg, H. (1995). A survey of spinal dorsal horn neurones encoding the spatial organization of withdrawal reflexes in the rat. Exp. Brain Res. *106*, 19–27.

Straznicky, C., and Tay, D. (1983). The localization of motoneuron pools innervating wing muscles in the chick. Anat. Embryol. (Berl.) *166*, 209–218.

Tourtellotte, W.G., and Milbrandt, J. (1998). Sensory ataxia and muscle spindle agenesis in mice lacking the transcription factor Egr3. Nat. Genet. *20*, 87–91.

Vrieseling, E., and Arber, S. (2006). Target-induced transcriptional control of dendritic patterning and connectivity in motor neurons by the ETS gene Pea3. Cell *127*, 1439–1452.

Waldenstrom, A., Thelin, J., Thimansson, E., Levinsson, A., and Schouenborg, J. (2003). Developmental learning in a pain-related system: evidence for a cross-modality mechanism. J. Neurosci. *23*, 7719–7725.

Wang, Z., Li, L.Y., Taylor, M.D., Wright, D.E., and Frank, E. (2007). Prenatal exposure to elevated NT3 disrupts synaptic selectivity in the spinal cord. J. Neurosci. *27*, 3686–3694.

Wenner, P., O'Donovan, M.J., and Matise, M.P. (2000). Topographical and physiological characterization of interneurons that express engrailed-1 in the embryonic chick spinal cord. J. Neurophysiol. *84*, 2651–2657.

Whelan, P., Bonnot, A., and O'Donovan, M.J. (2000). Properties of rhythmic activity generated by the isolated spinal cord of the neonatal mouse. J. Neurophysiol. *84*, 2821–2833.

Wickersham, I.R., Lyon, D.C., Barnard, R.J., Mori, T., Finke, S., Conzelmann, K.K., Young, J.A., and Callaway, E.M. (2007). Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. Neuron *53*, 639–647.

Wilson, J.M., Rempel, J., and Brownstone, R.M. (2004). Postnatal development of cholinergic synapses on mouse spinal motoneurons. J. Comp. Neurol. *474*, 13–23.

Wilson, J.M., Hartley, R., Maxwell, D.J., Todd, A.J., Lieberam, I., Kaltschmidt, J.A., Yoshida, Y., Jessell, T.M., and Brownstone, R.M. (2005). Conditional rhythmicity of ventral spinal interneurons defined by expression of the Hb9 homeodomain protein. J. Neurosci. *25*, 5710–5719.

Windhorst, U. (1990). Activation of Renshaw cells. Prog. Neurobiol. *35*, 135–179.

Windhorst, U. (2007). Muscle proprioceptive feedback and spinal networks. Brain Res. Bull. *73*, 155–202.

Wolpaw, J.R., and Tennissen, A.M. (2001). Activity-dependent spinal cord plasticity in health and disease. Annu. Rev. Neurosci. *24*, 807–843.

Xu, H., Clement, A., Wright, T.M., and Wenner, P. (2007). Developmental reorganization of the output of a GABAergic interneuronal circuit. J. Neurophysiol. *97*, 2769–2779.

Yang, H.W., and Lemon, R.N. (2003). An electron microscopic examination of the corticospinal projection to the cervical spinal cord in the rat: lack of evidence for cortico-motoneuronal synapses. Exp. Brain Res. *149*, 458–469.