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Review

Motor Circuits in Action: Specification, Connectivity, and Function

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Mammalian motor behavior is enabled by a hierarchy of interleaved circuit modules constructed by interneurons in the spinal cord, sensory feedback loops, and bilateral communication with supraspinal centers. Neuronal subpopulations are specified through a process of precisely timed neurogenesis, acquisition of transcriptional programs, and migration to spatially confined domains. Developmental and genetic programs instruct stereotyped and highly specific connectivity patterns, binding functionally distinct neuronal subpopulations into motor circuit modules at all hierarchical levels. Recent work demonstrates that spatial organization of motor circuits relates to precise connectivity patterns and that these patterns frequently correlate with specific behavioral functions of motor output. This Review highlights key examples of how developmental specification dictates organization of motor circuit connectivity and thereby controls movement.

Introduction

Movement is generated by the activity of neuronal circuits collecting and integrating information, ultimately leading to precisely timed skeletal muscle contractions. Work over many years has demonstrated that the motor control system exhibits a multitude of interleaved layers of organization. It produces an enormous repertoire of behaviors including routine actions such as walking, as well as sophisticated movements like playing a violin or dancing. Independent of the action type performed, the interplay of three main components is important and adds modularity and flexibility to the system. First, neurons with projections confined to the spinal cord are essential to produce rhythmic and patterned motor activity as well as to support many other activities (Jankowska, 2001; Kiehn, 2011; Orlovsky et al., 1999). These include highly diverse neuronal populations globally referred to as spinal interneurons. Second, spinal circuits are dependent on interactions with supraspinal centers in brainstem and higher brain areas (Grillner et al., 2005; Orlovsky et al., 1999). Communication is bidirectional and includes many descending and ascending channels intersecting with local spinal circuits. Third, sensory feedback systems constantly monitor consequences of motor action (Brown, 1981; Rossignol et al., 2006; Windhorst, 2007). The modular and interconnected nature of these three systems lies at the core of motor behavioral repertoire diversification but, at the same time, also makes understanding connectivity and function of the motor system a challenging task.

The combined efforts of studies on motor circuits using functional approaches, anatomical morphological analysis, as well as more recent developmental and genetic entry points, now allow for a synthesized look at the overall logic of motor circuit organization at multiple hierarchical levels. This Review will focus on emerging understanding of developmental and genetic programs that regulate neuronal diversification and in turn anatomical and functional connectivity in the motor system. Through specific perturbations of functional or genetic differentiation programs in defined neuronal populations, recent studies have successfully probed models of motor circuit organization and output. Studies on spinal interneurons, sensory-motor connectivity, descending motor control through cortical and basal ganglia circuits, as well as ascending pathways from the spinal cord to the cerebellum, provide evidence that common organizational and mechanistic principles guide connectivity and function across diverse neuronal circuits controlling motor behavior.

Genetic, Developmental, and Functional Diversification of Spinal Interneurons

Diversification of spinal neurons has its origin at early developmental stages. This process establishes functional spinal circuits that are needed to generate and maintain rhythmic motor output, including repetitive alternation of left-right and extensor-flexor muscle contractions as key motor output behaviors. Recent studies have begun to address the important question of how diversification programs established during development control the emergence of functionally distinct neuronal subpopulations required to support these tasks. They highlight the importance of genetic programs and time of neurogenesis in setting up a spatial matrix in which terminally differentiated neuronal subpopulations are interconnected in highly precise patterns.

Progenitor Domain Origin Contributes to Spinal Neuron Diversification

Neurons with cell bodies positioned in the spinal cord are derived from local progenitors. Spinal progenitor cells are arrayed at conserved dorsoventral positions along the midline and proliferate to give rise to postmitotic neurons during temporally restricted periods. Early action of ventral sonic hedgehog (shh) and dorsal bone morphogenetic protein (BMP) signaling sources leads to spatial subdivision of progenitor domain territory along

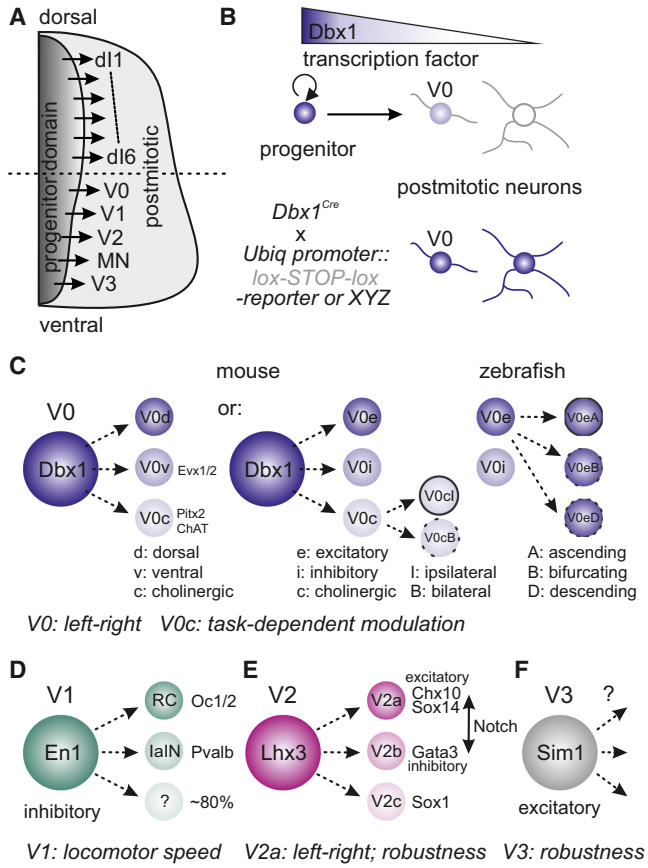

Figure 1. Progenitor Domain Origin and Transcriptional Programs Control Neuronal Diversification

(A) Division of spinal neurons into 11 cardinal populations based on developmental progenitor domain origin. These include six populations with dorsal origin (dI1–dI6; d, dorsal; I, interneurons) and five populations with ventral origin (MN, motor neurons; V0–V3, four ventral interneuron populations).

(B) Permanent labeling of spinal subpopulations in the mouse using Cre expression driven from a genomic locus transiently expressed in a defined progenitor domain. The cross between *Dbx1*^{Cre} mice and a ubiquitous reporter mouse strain leads to the expression of a reporter or XYZ protein upon Cre recombination. This strategy initiates Cre recombination in *Dbx1*-expressing progenitor cells and permanently labels V0 postmitotic neurons as they differentiate.

(C–F) Diversification of V0 (C): mouse and zebrafish; dark and different dotted borders indicate further diversification of subtypes; V1 (D), V2 (E), and V3 (F) neuronal subpopulations in the spinal cord. Further subtype diversification is likely but not shown for reasons of clarity. Assigned function by genetic perturbation experiments is shown below.

the dorsoventral axis (Jessell, 2000). This process is accompanied by the acquisition of a combinatorial transcription factor code allowing distinction of 11 progenitor domains based on molecular and genetic criteria (Jessell, 2000). Developmental progenitor domain origin can therefore be used as an entry point to divide postmitotic neuronal descendants into six dorsal and five ventral cardinal populations (Alaynick et al., 2011; Goulding, 2009; Jessell, 2000; Kiehn, 2011) (Figure 1A).

Is the identity of progenitor domain origin linked to the generation of distinct and functionally diverse neuronal populations? This question has been addressed rigorously for neurons of ventral origin mostly by genetic lineage-tracing analysis to fate map neurons derived from a progenitor domain transiently expressing a defined transcription factor (Figure 1B). With the exception of the progenitor domain-generating motor neurons (pMNs), the other domains probably give rise to more than one generic neuronal type, as several well-documented examples illustrate (Figures 1C–1F). V0 interneurons are derived from Dbx1-expressing progenitors and make up a diverse set of mostly commissural neurons including excitatory (V0e) and inhibitory (V0i) populations (Lanuza et al., 2004), as well as the minor fraction of V0c neurons of cholinergic partition cells in mice (Zagoraiou et al., 2009) (Figure 1C). A recent study in zebrafish demonstrates diversification of V0e neurons into ascending (V0eA), descending (V0eD), and bifurcating (V0eB) populations based on projection patterns (Satou et al., 2012) (Figure 1C). V1 interneurons are defined by the expression of the transcription factor Engrailed-1. They are inhibitory and contain Renshaw cells, Ia inhibitory interneurons (Alvarez et al., 2005), and several as-yet-uncharacterized subpopulations (Figure 1D). The case of Ia inhibitory interneurons illustrates that not all functionally defined neuronal subpopulations derive from a single progenitor domain. Mice lacking V1 interneurons still show functional Ia inhibitory interneurons, suggesting that at least one additional progenitor domain contributes to their generation (Wang et al., 2008). V2 interneurons (Lhx3 labeled, excluding motor neurons) include ipsilaterally projecting excitatory V2a neurons (Chx10 labeled) (Crone et al., 2008) and inhibitory V2b (GATA3 labeled) and V2c (Sox1 labeled) neurons (Panayi et al., 2010) (Figure 1E), each with likely additional subtype diversification. Notch signaling through the regulation of the transcriptional cofactor Lmo4 tilts the balance between V2a-V2b subtypes and contributes to diversification (Del Barrio et al., 2007; Joshi et al., 2009; Lee et al., 2008). Similar V2 neuron diversification occurs in zebrafish (Batista et al., 2008; Kimura et al., 2008). Finally, little is known about diversification of excitatory and predominantly commissural V3 interneurons (Sim1 labeled) (Zhang et al., 2008). In summary, subtype diversification for neurons derived from most of the 11 cardinal progenitor domains is likely. The extent of neuronal diversification still remains to be fully elucidated and is likely to vary for different progenitor domains. Caution should be taken since very few examples exist with firm links between developmental and/or molecular identity and functional subtype as assessed by electrophysiology and/or connectivity patterns.

Several studies have assessed the developmental significance of spinal neuron diversification by determining the functional consequences of ablating or silencing neurons derived from single progenitor domains or subpopulations (for detailed review, see Goulding, 2009; Kiehn, 2011; Stepien and Arber, 2008). Briefly, perturbation of V0 interneurons leads to defects in left-right alternation (Lanuza et al., 2004), V1 interneurons are required to regulate locomotor speed (Gosgnach et al., 2006), V2a interneurons are involved in left-right alternation and are required for robust locomotor patterns (Crone et al., 2008), and V3 interneurons are also needed to maintain a stable locomotor pattern (Zhang et al., 2008) (Figures 1C–1F).

These experiments raise several open issues for future research. First, individual spinal progenitor domains are the

source of many functionally diverse neuronal subpopulations. Perturbations therefore affect multiple descendant populations en bloc and may lead to defects that are difficult to interpret. More targeted genetic interference at the level of individual populations will be possible as soon as developmental maps are more closely aligned to subpopulation maps defined by electrophysiology and connectivity. For example, silencing of V1 interneurons using Engrailed-1 expression as an entry point affects locomotor speed (Gosgnach et al., 2006) (Figure 1D), but it is difficult to predict how coincident elimination of Renshaw cells, a fraction of Ia inhibitory interneurons, and a handful of other populations compares to unique perturbation of any one V1 subpopulation alone. Neuron population-specific perturbations are beginning to be feasible. Cholinergic partition cells make up a minor fraction of V0 neurons (Zagoraiou et al., 2009). This allows for selective perturbation of cholinergic neurotransmission in V0c neurons by eliminating choline acetyl transferase (*ChAT*) using *Dbx1*^{Cre} mice (Zagoraiou et al., 2009). V0c *ChAT* conditional mutant mice exhibit selective behavioral defects in task-dependent motor performance during swimming but not basic locomotion (Zagoraiou et al., 2009) (Figure 1C). These subtle defects clearly would have been masked in an analysis perturbing the entire V0 cohort, a manipulation leading to massive overall defects in left-right alternation (Lanuza et al., 2004). Elucidation of connectivity patterns between V0c neurons and motor neurons using transsynaptic rabies viruses revealed a further fractionation into an exclusively ipsilaterally projecting population and a bilaterally projecting population (Figure 1C) with motor neuron subtype-specific connectivity (Stepien et al., 2010). These findings illustrate that even a seemingly uniform population can diversify further, at least anatomically speaking, as it remains to be determined whether these V0c subpopulations also exhibit different functional profiles.

Second, mice with genetic perturbation of neuronal subpopulations have frequently been analyzed using a fictive locomotion assay at neonatal stages to assess possible defects in left-right and/or extensor-flexor motor burst alternation. These assays employ extracellular electrophysiological recordings to monitor activity from ventral roots at different segmental levels to determine the generation of motor bursts in the form of compound action potentials (Kudo and Yamada, 1987; Smith et al., 1988). Bilateral recording at one segmental level is considered to be a proxy for properties of left-right alternation, whereas coincident ipsilateral recording from ventral roots at lumbar segmental levels L2 and L5 is used to measure properties of flexor-extensor alternation. This widely used assay has been extremely valuable since it allows a first assessment of motor defects in an isolated preparation by activation of local spinal circuits through the application of combinatorial drug cocktails mimicking descending input (5-HT, dopamine, NMDA) or by electrical stimulation of descending tracts or sensory fibers. It also allows for the tractable interrogation of circuit-level effects of genetic perturbations that are pre- or postnatally lethal. However, while left-right alternation assessed by ventral root recordings can be considered to be a straightforward readout, credible parameters for extensor-flexor alternation may be more difficult to acquire. L2 ventral roots burst in alignment with flexor muscle contractions and L5 bursts align with extensor muscle activity, but the significance of this coincidence is unclear since L2 and L5 roots both contain axons innervating extensor and flexor muscles. This may also explain the conspicuous rarity of extensor-flexor phenotypes in neonatal fictive locomotion assays of mutant mice when scoring for L2-L5 burst alternation defects, and more refined in vitro assays may be needed to extract information. In summary, to get definitive answers on the functional role of defined spinal subpopulations in movement, it is essential to combine in vitro with in vivo assays, in which neural pathways feed spatially, temporally, and quantitatively accurate information into the system.

Timing of Neurogenesis as an Additional Determinant of Neuronal Diversification

The high degree and complexity of neuronal diversification in the spinal cord suggests that developmental mechanisms in addition to progenitor domain origin are probably involved in subpopulation specification. Early findings have demonstrated that temporal gradients of neurogenesis progress along the ventrodorsal and rostrocaudal axis in the spinal cord (Nornes and Carry, 1978). As such, it is interesting to ask whether this neurogenic gradient may influence neuronal diversification in spinal circuits. Pulse-chase labeling experiments can track neurons born during defined developmental time windows to later stages to assess molecular markers, connectivity, and function. One of the earliest observations of differences in birthdating according to progenitor domain territory in the mouse spinal cord was described for Lbx1^{on} dI4–dI6 neurons that separate into two waves of early- and late-born neurons (Gross et al., 2002) (Figure 2A, above timeline). Birthdating analysis was also carried out for several single ventral progenitor domains. Renshaw cells make up the earliest-born Engrailed-1-labeled V1 interneuron subpopulation in mice, sharply separated in birthdate from later-born populations giving rise to other V1 interneuron subclasses (Benito-Gonzalez and Alvarez, 2012; Stam et al., 2012) (Figure 2A, below timeline).

Without clonal analysis, the cellular mechanisms for dI4–dI6 and V1 diversification are currently unclear. Recently, some studies have shed light on the question of clonal lineage within individual spinal progenitor domains for V0 and V2 interneuron populations in zebrafish. For V2 populations, excitatory V2a and inhibitory V2b populations originate from a single pair-producing progenitor cell at the final cell division (Kimura et al., 2008) (Figure 2B). For commissural V0 neurons, inhibitory V0i neurons derive from distinct progenitors than excitatory V0e neurons (Satou et al., 2012) (Figure 2B). Within the V0e category, V0eA and V0eB/V0eD subtypes also originate from different progenitor cells (Satou et al., 2012) (Figure 2B). Birthdating analysis demonstrates an orderly sequence in generation time (Figure 2B) that can be shifted to preferentially early-born subtypes by reducing Notch signaling (Satou et al., 2012). These studies suggest that strategies for neuronal subtype diversification are distinct for different progenitor domains. Some generate very diverse cell types still at the last cell division (e.g., V2), and others make use of more elaborate schemes of progenitors and birthdating (e.g., V0). It will be interesting to compare strategies between species and progenitor domains to have a more complete picture of the developmental mechanisms involved in spinal neuron diversification.

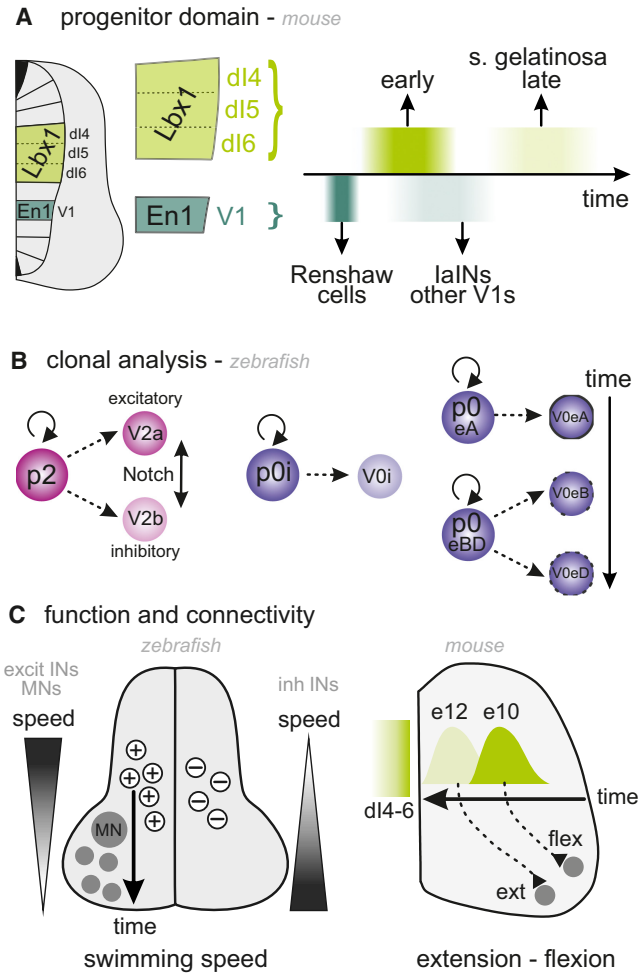

Figure 2. Time of Neurogenesis as Axis for Neuronal Diversification

(A) Lbx1 (dI4–dI6) and En1 (V1) spinal neurons in the mouse diversify by birthdate, despite deriving from the same transcriptionally marked progenitor domain territory. Along the axis of developmental time, Renshaw cells are the earliest V1 interneurons to differentiate, before IaINs and other subtypes follow. Similarly, Lbx1-derived interneurons are generated in two waves, the latter populating substantia gelatinosa in the mature spinal cord.

(B) Clonal analysis of p2- and p0-derived neurons in zebrafish. Strategies of neuronal diversification differ across progenitor domains, with generation of V2a and V2b neurons at the final cell division from one progenitor type and different progenitors generating V0 subtypes.

(C) Left: in zebrafish, motor neurons (gray circles) and spinal interneurons (plus circles, excitatory; minus circles, inhibitory) exhibit diversification according to time of birth correlating with speed of movement and dorsoventral position. Right: in mouse, dorsally positioned extensor and flexor premotor interneurons arise from the same progenitor domain territory (Lbx1-labeled dI4–dI6) at different times (peak of generation embryonic day [e] 10 for flexor and e12 for extensor premotor interneurons) and settle along the mediolateral axis.

How does time of neurogenesis translate into neuron identity as it relates to differential connectivity and function? Tight links between developmental time and transcriptional cascades instructing cell fate were observed for *Drosophila* neuroblast lineages through a mechanism involving inheritance of transcriptional identity from neuroblast to postmitotic offspring (Isshiki et al., 2001). Whether similar mechanisms exist for vertebrate neuron diversification remains to be determined, but it is likely that emerging neuronal subpopulations at least exhibit distinct transcriptional profiles correlating with time of neurogenesis. Supporting this model, different Engrailed-1-labeled V1 subpopulations in mice express unique transcriptional profiles (Renshaw cells: Engrailed-1/MafB; IaINs: Engrailed-1/Foxp2) (Benito-Gonzalez and Alvarez, 2012; Stam et al., 2012).

Postmitotic neurons integrate into circuits through the action of developmental programs established at early stages. Correlation between time of neurogenesis and (connectivity related to) function has been described in both zebrafish and mouse spinal cord. In larval zebrafish, motor neurons and excitatory interneurons located dorsally are born early and recruited during fast swimming, whereas ventral counterparts are born later and activated during slow swimming (McLean et al., 2007, 2008; McLean and Fetcho, 2009) (Figure 2C). In the dorsal lumbar spinal cord of mice, interneurons with direct synaptic connections to extensor motor neurons are positioned medially and born later than populations positioned laterally and connected to flexor motor neurons (Tripodi et al., 2011) (Figure 2C). Both dorsal extensor and flexor premotor interneuron populations are well represented among Lbx1^{on} dI4–dI6 neurons (Figure 2C), each containing glutamatergic and inhibitory (GABAergic/glycinergic) interneurons (Tripodi et al., 2011). Taken together, these studies support a model in which birthdate correlates with differential functional properties. In future work, it will be interesting to assess whether such subdivisions based on time of neurogenesis, connectivity, and function can also be revealed at the level of clonally related subpopulations. Correlation between time of neurogenesis and connectivity is not restricted to spinal circuits. Transcriptionally distinct dentate granule cells in the mouse hippocampus are born at different times, and synapse maturation with CA3 pyramidal neurons follows a population-specific temporally matched schedule (Deguchi et al., 2011). Moreover, in the cerebellar molecular layer, granule cell parallel fiber axons line up according to a clear temporal order (Espinosa and Luo, 2008).

Spatial Distribution of Spinal Neurons Influences Circuit Function

The spatial overlap between axons entering the spinal cord and dendritic territory of spinal neurons represents an important parameter in defining possible synaptic connections. The migratory routes taken by neurons derived from different spinal progenitor domains are highly stereotyped such that the final target destination of each neuronal subpopulation is spatially confined and, especially in the dorsal spinal cord, follows a laminar organization pattern (referred to as Rexed's laminae) (Figure 3A). In the mammalian spinal cord, axons of spinal origin, or descending axons, project along the surrounding white matter and enter the cell body-rich gray matter area at subpopulation-specific sites. Axons derived from dorsal root ganglia (DRG) sensory neurons enter the spinal cord dorsally. The observed spatial stereotypy in spinal neuronal subtype positioning and axonal trajectories has important consequences for how neuronal circuits connect and function, as illustrated by the following two examples.

First, Renshaw cells are located in an extreme ventral position near the ventral root exit point of motor axons. Renshaw cells

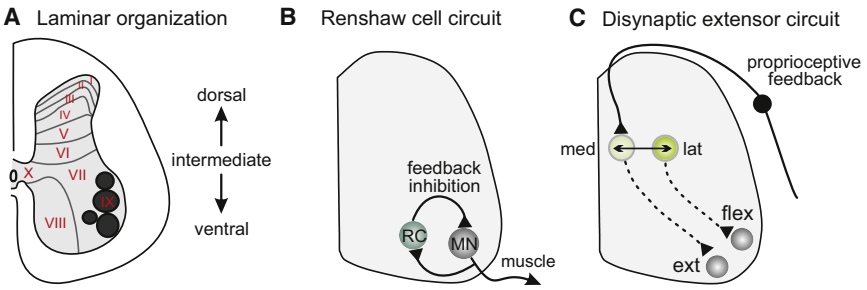

Figure 3. Spatial Organization in the Spinal Cord Shapes Circuit Modules

(A) Spatial organization by lamination (position of Rexed's laminae I–X is shown; black circles in lamina IX represent motor neurons).

(B) Renshaw cell feedback inhibitory microcircuit is spatially confined to the ventral-most spinal cord.

(C) Mediolateral spatial segregation of extensor (light green) and flexor (darker green) premotor interneurons in the intermediate spinal cord. Proprioceptor cell bodies reside in DRG outside the spinal cord and axons enter the spinal cord medially, with preferential connections to extensor premotor interneurons in this domain. Light gray circles indicate motor neurons.

receive direct synaptic input from locally projecting motor axon collaterals providing a main source of synaptic input and in turn connect to motor neurons through a spatially confined feedback inhibitory loop (Alvarez and Fyffe, 2007; Renshaw, 1941; Windhorst, 1990) (Figure 3B). Second, recent experiments using monosynaptic transsynaptic rabies viruses to map premotor interneurons connected to functionally antagonistic extensor and flexor motor neurons reveal a high density of medially located interneurons in the dorsal spinal cord connected to extensor but not flexor motor neurons (Tripodi et al., 2011) (Figure 3C). Dorsal extensor premotor interneurons receive a high density of synaptic input by proprioceptive sensory neurons in contrast to the more laterally located flexor premotor interneurons (Tripodi et al., 2011) (Figure 3C). These findings provide anatomical evidence for the electrophysiologically well-studied disynaptic pathway from proprioceptors to extensor motor neurons (Angel et al., 2005; Conway et al., 1987; McCrea, 1998; Pearson et al., 1998) and offer another example of a correlative link between the spatial distribution and synaptic connectivity and/or function of spinal populations.

Neurotransmitter Identity and Control of Motor Output Patterns

An additional important factor for the acquisition of neuronal identity is the choice of neurotransmitter expressed by a given neuronal population. The majority of spinal interneurons signal through either the excitatory neurotransmitter glutamate or the inhibitory neurotransmitter(s) GABA and/or glycine. Neurotransmitter identity is tightly linked to neuronal subpopulation fate. The transcriptional specification of neuronal subpopulations in the dorsal spinal cord provides an impressive illustration of this fact since transcriptional fate is tightly linked to neurotransmitter choice (Glasgow et al., 2005; Mizuguchi et al., 2006). Acquisition of inhibitory fate in the dorsal spinal cord is in large part dictated by the transcription factor Ptf1a, and *Ptf1a* mutant mice exhibit a complete absence of dorsal spinal inhibition (Glasgow et al., 2005). The balance between excitation and inhibition mediated by a variety of different interneuron populations controls many of the functional properties and parameters of motor output bursting behavior. Several recent approaches using mouse genetics provide evidence that interfering with excitatory and inhibitory connectivity can have profound effects on motor behavior.

One of the most striking behavioral consequences was reported for mice with mutations in components of the EphrinB3-EphA4 signaling pathway, including the downstream Rac-GAP effector molecule alpha2-Chimerin (Beg et al., 2007; Iwasato et al., 2007; Kullander et al., 2003; Wegmeyer et al., 2007) (Figure 4A). Mutations in any of these signaling molecules lead to aberrant axonal midline crossing by yet-to-be-identified spinal interneuron subpopulations (Beg et al., 2007; Iwasato et al., 2007; Kullander et al., 2003; Restrepo et al., 2011; Wegmeyer et al., 2007). This conversion from normally ipsilaterally projecting to "pseudocommissural" interneurons (Figure 4A) is a likely reason for the hopping gait that deviates from the rodent-typical alternating gait. Future work will determine the precise circuit mechanism(s) at the level of neuronal subpopulations responsible for this species-aberrant behavior.

Several studies describe a much more dramatic genetic intervention by functionally muting the majority of glutamatergic spinal interneurons through mutation of the vesicular glutamate transporter *vGlut2* in mice (Gezelius et al., 2006; Talpalar et al., 2011; Wallén-Mackenzie et al., 2006) (Figure 4B). As expected, *vGlut2* mutant mice are lethal at birth due to defects in respiratory circuits (Wallén-Mackenzie et al., 2006), but, as mentioned, spinal circuitry and function can be assayed at late embryonic stages using in vitro preparations. It came as a surprise that motor burst alternation under conditions of fictive locomotion by the exogenous application of a neurotransmitter cocktail revealed close-to-normal patterns (Gezelius et al., 2006; Wallén-Mackenzie et al., 2006). However, more careful analysis of *vGlut2* mutant mice (Talpalar et al., 2011) revealed two important functional ramifications of glutamatergic interneurons in spinal motor circuits. First, these glutamatergic spinal interneurons are absolutely essential to generate and maintain locomotor bursting, since descending or sensory neuron stimulation cannot induce rhythmic motor bursting in *vGlut2* mutant spinal cords. Nevertheless, exogenous application of a neurotransmitter cocktail promotes *vGlut2*-deficient spinal circuits to surprisingly normal functionality. These findings suggest that local drug action on motor neurons and connected interneurons, collaborating with a local inhibitory network directly connected to motor neurons (Figure 4B), is sufficient for rhythmic motor bursting in the spinal cord. Second, these findings have direct implications for the interpretation of results from the analysis of mouse mutants using fictive locomotion assays. Since near-to-normal motor bursts can be produced in *vGlut2* mutant spinal cords using this assay, it can be expected that other mutant spinal cords with actual circuit defects may

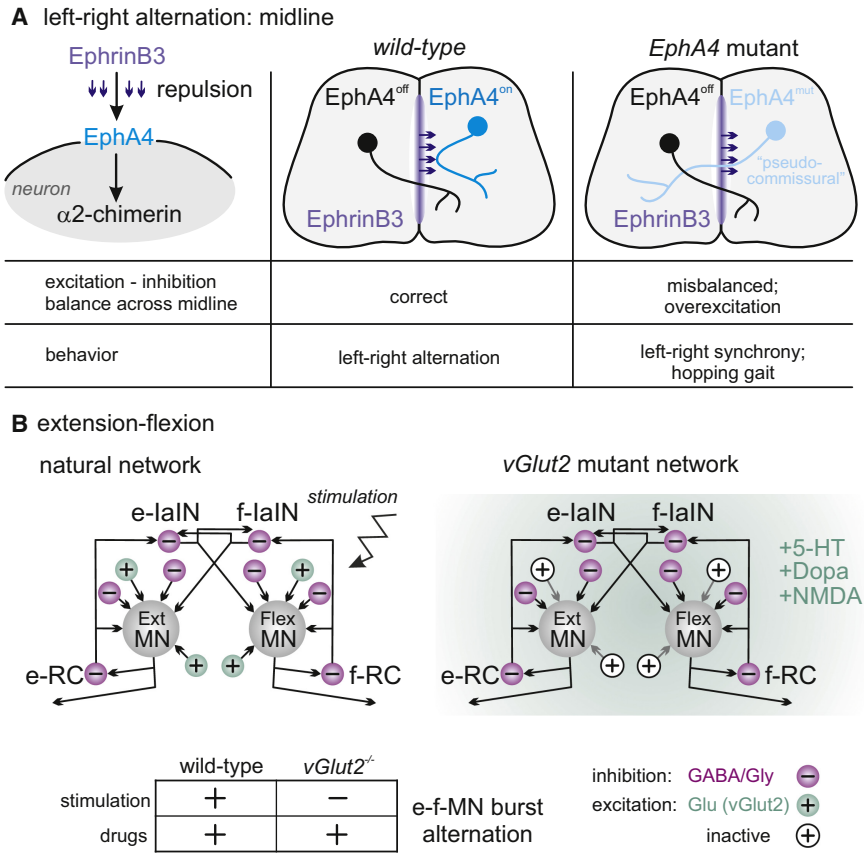

Figure 4. Balancing Excitation and Inhibition in Spinal Motor Circuits

(A) Scheme illustrating the role of EphrinB3-EphA4-α2-Chimerin signaling in controlling inhibition-excitation balance across the midline in the spinal cord. Aberrant midline crossing is observed in mice mutant in components of this signaling pathway, a phenotype correlating with hopping gait detected in these mice.

(B) Glutamatergic transmission mediated by vGlut2^{on} spinal interneurons is essential for locomotion, but in its absence, spinal circuits produce almost normal motor bursts by the addition of 5-HT, dopamine, and NMDA. A local inhibitory network connecting to extensor and flexor motor neurons on which locomotor drug cocktails act is likely to explain this phenomenon.

show similarly obscured motor phenotypes. This particular feature of fictive locomotion assays may also explain why genetic approaches have so far failed to decipher the core elements involved in rhythm generation. Consequently, while these assays have the potential to point to circuit malfunction, some defects may be masked or compensated. Complementary assays including in vivo assessment of neuronal function can assign conclusive roles to circuit elements in the spinal cord.

Premotor Organization of Spinal Circuits

Most studies on spinal interneurons have focused on overall network function or properties of individual neurons. Progress in developing transsynaptic virus tools has made it possible to begin to take a global view at the anatomical organization of connectivity matrices of spinal networks. Upon injection into skeletal muscles, rabies viruses are transmitted through the motor system retrogradely, a tremendously useful feature for the visualization of interconnected motor pathways (Ugolini, 2010). While the ability of rabies viruses to spread transsynaptically serves as a valuable tool in tracing neural networks, it also makes the results of such retrograde labeling experiments inherently difficult to interpret. For example, to distinguish direct from indirect synaptic connections, the uncertain parameter of "time after injection" was often used as a determinant for this critical distinction (Jovanovic et al., 2010; Rathelot and Strick, 2006; Ugolini, 2010). A recently introduced modification to this technology now allows for an unambiguous assignment of synaptic connectivity in the central nervous system (CNS) (Callaway, 2008; Wickersham et al., 2007). In this strategy, genomic deletion of the gene encoding a glycoprotein (Gly) essential for transsynaptic spread renders the rabies virus spreading incompetent. Introduction of Gly expression by genetic or viral tools to selectively complement Gly-deficient rabies in primarily infected neurons reestablishes the ability for transsynaptic spread to label neurons presynaptic to primary infection but prohibits subsequent spread due to absence of Gly in presynaptic neurons (Callaway, 2008; Wickersham et al., 2007).

This monosynaptically restricted transsynaptic rabies virus system was used in two recent studies to map the three-dimensional distribution of spinal interneurons with direct synaptic connections to motor neurons in mice (Stepien et al., 2010; Tripodi et al., 2011). Using retrograde motor axonal coinfection strategy from specific muscles, transsynaptic spread is initiated from functionally defined motor neuron pools (Figures 5A and 5B). Analysis of the overall distribution patterns of spinal premotor interneuron connectivity to an individual motor neuron pool demonstrates a high degree of reproducibility across animals (Stepien et al., 2010). In contrast, analysis of premotor interneurons connecting to motor neuron pools with distinct function in motor behavior reveals striking differences in overall distribution (Stepien et al., 2010; Tripodi et al., 2011). These observations uncover the existence of anatomical or structural engrams at the premotor circuit level that correlate with motor function.

The results raise a number of interesting and currently unresolved questions. Premotor neurons encompass a diverse array of neuronal subpopulations, including distinct neurotransmitter phenotypes, synaptic input driving their activation, and additional synaptic partners contacted. It will be interesting to determine the relationship between connectivity-based anatomical maps and functional maps assessing activity patterns in relation to locomotor output. At present, it is unclear which of the many

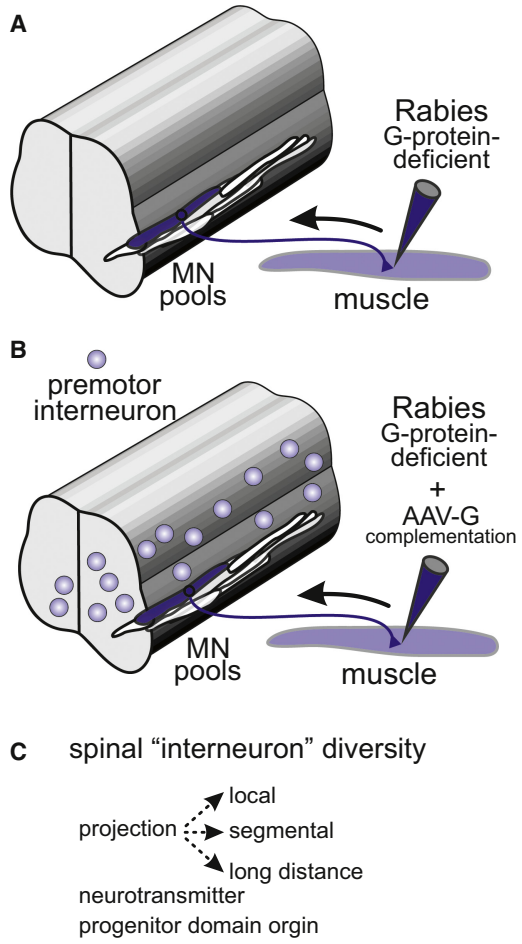

Figure 5. Anatomical Organization of Premotor Spinal "Interneurons"

(A and B) Schemes illustrating the use of monosynaptically restricted rabies viruses to visualize premotor interneurons in the spinal cord. In the absence of complementary G protein in motor neurons, G protein-deficient rabies virus infects motor neurons retrogradely but does not spread to premotor neurons (A). Coinjection of G protein-deficient rabies virus with AAV-G for complementation induces labeling of premotor neurons, revealing their wide segmental distribution in the spinal cord (B).

(C) Summary of spinal "interneuron" diversification based on projections, connectivity, neurotransmitter phenotype, and developmental origin.

premotor interneurons are required for or involved in the core components of interneuron circuits that give rise to rhythm generation and perpetuation. In addition, motor neuron pools may tap into connections from distinct possible premotor interneuron populations differentially. This in turn may contribute to the observed overall differences in interneuron distribution for motor neuron pools of distinct function. Combined spatial analysis of genetically and functionally defined interneuron populations with an assessment of quantitative contributions to the synaptic regulation of different motor neuron pools will provide answers to these questions.

Spinal "Interneurons" Are Multifaceted and Differ from Cortical Interneurons

As has become apparent, spinal interneurons cannot be considered to be simply a limited group of local neurons shaping and modulating motor circuit function in recurrent modules. Spinal interneuron diversification is evident at the developmental level by progenitor domain origin, time of neurogenesis, migratory path, and acquisition of distinct transcriptional profiles. These early features translate to diversification in the mature spinal cord, in which neuronal subpopulations exhibit differential spatial distribution patterns, neurotransmitter profiles, connectivity matrices including synaptic in- and output, and functional properties (Figure 5C). Although interneuron populations are often loosely categorized along a single dimension (e.g., transcriptional, neurotransmitter, or spatial profile), these same interneurons may in fact be functionally multifaceted (Edgley, 2001; Jankowska, 2008), which complicates classification criteria.

Analysis of connectivity profiles provides ample evidence that many spinal interneurons establish connections over many segments, and individual motor neuron pools receive direct input from segmentally widely distributed interneuron populations (Stepien et al., 2010; Tripodi et al., 2011). Consequently, many spinal "interneurons" exhibit properties analogous to long-distance projection neurons not unlike pyramidal neurons in the cerebral cortex and therefore cannot be strictly considered to function as local interneurons. Neurons in the spinal cord of this category exhibit fundamentally different connectivity profiles and functions, including excitatory and inhibitory subtypes. On the other end of the spectrum, Renshaw cells or spinal interneuron populations in the substantia gelatinosa (Brown, 1981; Todd, 2010) can be considered more similar to locally projecting cortical interneurons such as fast-spiking Parvalbumin interneurons (Isaacson and Scanziani, 2011), both contributing exclusively to local circuit computations. In the cortex, one defining arbiter for the use of the term "interneuron" is based on the fact that these neurons migrate into the cortex from distant sites (i.e., ganglionic eminence) and many of them project locally (Fishell and Rudy, 2011; Gelman and Marín, 2010; Klausberger and Somogyi, 2008). In contrast, spinal neurons are generated locally, eliminating this distinguishing parameter. For future reference, it will be important to consider that the commonly used terminology "spinal interneurons" embraces a bewildering array of functionally distinct neuronal subtypes in sum charged with local as well as long-distance computations in the spinal cord (Figure 5C).

Sensory-Motor Connectivity and Integration in the Spinal Cord

Sensory feedback circuits provide important external input to the spinal cord by transmitting functionally different flavors of sensory information from distinct body parts to spinal neurons (Brown, 1981; Scott, 1992; Windhorst, 2007). A unique and conserved feature of all DRG sensory neurons is the establishment of two distinct axonal processes, extending from DRG cell bodies toward peripheral and central targets. Sensory neuron subtypes differ in identity of these targets, thereby channeling functionally distinct primary sensory information to dedicated spinal subcircuits for integration and processing.

Spatial and Molecular Determinants Regulate Direct Sensory-Motor Connections

Group Ia proprioceptors account perhaps for the most studied DRG sensory neuron subtype, owing to their unique wiring

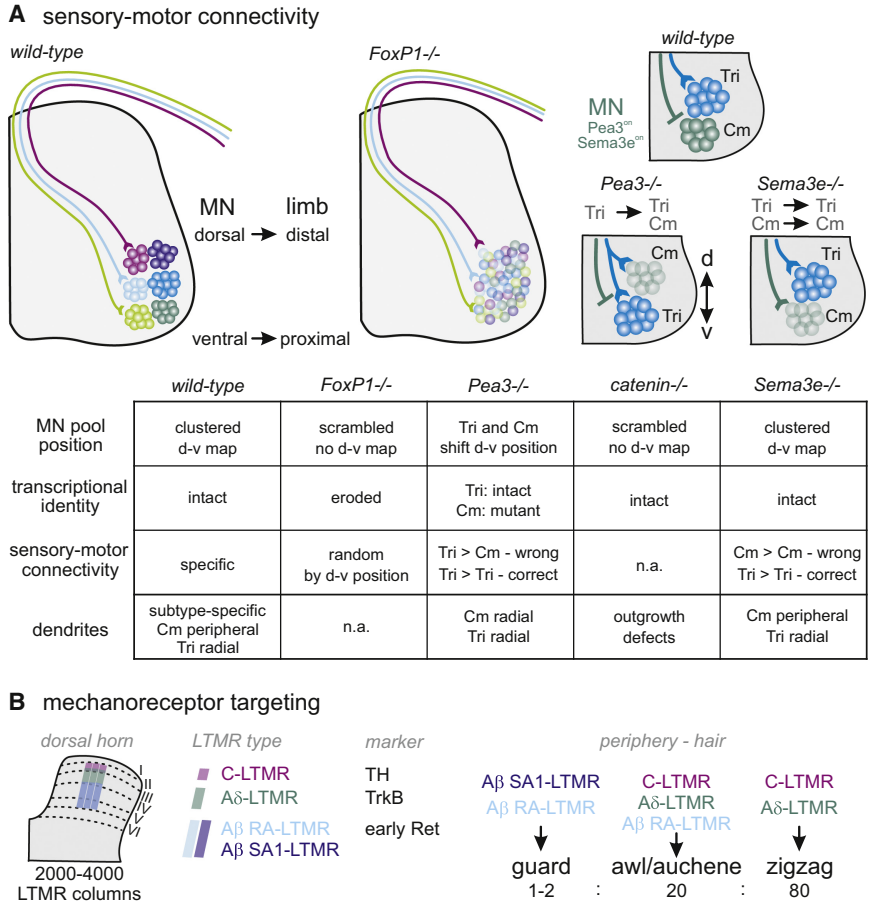

Figure 6. Mechanisms Regulating Organization and Connectivity of Sensory Input Channels

(A) Group Ia proprioceptive afferents target specific motor neuron pools with high accuracy. Left: the process of sensory-motor targeting is disrupted in *FoxP1* mutant mice in which both motor neuron identity and cell body position are eroded. Right: *Pea3* and *Sema3e* mutant mice exhibit specific defects in sensory-motor connectivity. In wild-type, Cm motor neurons do not receive direct sensory input (indicated by T-arrow), but in the absence of the repulsive cue *Sema3e*, Cm proprioceptors establish synaptic contacts with Cm motor neurons (indicated by V-arrow). In *Pea3* mutant mice, Cm motor neurons receive direct synaptic input from Tri but not Cm sensory afferents. Mutant motor neurons are shown in light color compared to wild-type throughout all panels. Summary of observed phenotypes in different mouse mutants is shown in table below.

(B) Left: targeting of functionally distinct mechanoreceptor afferents to dorsal horn LTMR columns (REXED's laminae I–VI indicated by dashed lines). Different LTMR types express distinct molecular markers (C-LTMR, TH^{on}; Aδ−LTMR, $TrkB^{on}$; Aβ−LTMR, $Npy2R^{on}$/$earlyRet^{on}$) that can be used to genetically label these neurons and trace their peripheral and central projections. Right: peripheral innervation ratios of three hair follicle types (guard, awl/auchenne, and zigzag) by different LTMR subtypes are shown.

properties into monosynaptic reflex circuits directly connecting sensory feedback to motor output. Their peripheral projections target muscle spindles, sensors embedded within skeletal muscles and endowed with detecting changes in muscle contraction (Brown, 1981; Scott, 1992). Their central projections dive deep into the spinal cord to establish direct synaptic connections with motor neurons (Brown, 1981; Burke and Glenn, 1996; Eccles et al., 1957). The monosynaptic reflex arc is highly suitable to understand mechanisms driving synaptic specificity programs. Direct sensory-motor connections exhibit a high degree of synaptic specificity, as assessed extensively by electrophysiological methods in several species (Eccles et al., 1957; Mears and Frank, 1997). These studies demonstrate the existence of numerous and strong connections between homonymous sensory-motor pairs projecting to the same peripheral target muscle and a lower degree of connectivity between synergistic or functionally related pairs. In contrast, synaptic connections between antagonistic or functionally unrelated sensory-motor pairs are negligible.

Transcriptional programs expressed in motor neuron column- and pool-specific patterns are tightly and causally linked to the establishment of accurate motor axonal trajectories to target muscles. Combinatorial expression of Hox and Lim-homeobox transcription factors by motor neuron subpopulations at early postmitotic stages instructs axonal outgrowth to target muscles by control of downstream signaling molecules (Dalla Torre di Sanguinetto et al., 2008; Dasen et al., 2005; Jessell, 2000; Kania and Jessell, 2003; Shirasaki and Pfaff, 2002). At later stages, target-derived cues act to control additional aspects of motor neuron differentiation in part by regulation of ETS transcription factors (Dalla Torre di Sanguinetto et al., 2008; Haase et al., 2002; Livet et al., 2002; Vrieseling and Arber, 2006).

These collective observations on peripheral targeting mechanisms raise the question of whether and how motor neuron pool-specific genetic programs are also instrumental in controlling the establishment of central connectivity, including sensory-motor specificity. One approach to address this question has been the analysis of *FoxP1* Hox cofactor mutant mice, which almost completely lack motor neuron subtype diversification programs as a result of muted Hox signaling (Dasen et al., 2008; Sürmeli et al., 2011) (Figure 6A). In *FoxP1* mutant mice, motor neurons establish muscular projections, but retrograde labeling from defined muscles reveals randomly dispersed spinal motor neurons (Dasen et al., 2008; Sürmeli et al., 2011) instead of the normally observed clustered and topographically arranged motor neuron pools (McHanwell and Biscoe, 1981; Romanes, 1964) (Figure 6A). Conditional elimination of *FoxP1* in motor neurons was used to assess sensory-motor connectivity profiles at postnatal stages by an anatomy-based tracing assay in an otherwise wild-type background (Sürmeli et al., 2011). These experiments demonstrate that when cell bodies of motor

neurons that share a common muscle target are stripped of *FoxP1* identity, they no longer obey the tight specificity rules observed in wild-type and receive randomized sensory input instead (Figure 6A). A much more stunning observation was made when sensory-motor specificity profiles were analyzed according to dorsoventral position of motor neuron cell bodies. In *FoxP1* mutant mice, only motor neurons with dorsoventral position similar to the respective wild-type motor pool receive direct sensory input from corresponding sensory afferents, whereas aberrantly positioned motor neurons escape this source of input. These findings suggest that group Ia proprioceptive afferents target dorsoventral spinal positions independent of molecular cues provided by motor neurons and point to motor neuron cell body position in a virtual spatial grid as an important factor for the regulation of specific sensory-motor connections (Figure 6A). A spatial grid also operates to establish sensory targeting domains in the *Drosophila* nerve cord, implemented by gradients of signaling molecules but with fundamental differences relative to the mouse (Tripodi and Arber, 2012).

To separately assess respective contributions of molecular identity and cell body position to the control of sensory-motor specificity, mutations in molecular programs exclusively affecting either motor neuron pool identity or cell body position are needed. The ETS transcription factor *Pea3* is expressed in two caudal cervical motor neuron pools with ventral cell body position, innervating cutaneous maximus (Cm) and latissimus dorsi (Ld) muscles, but not in a neighboring dorsal pool innervating the triceps (Tri) muscle (Livet et al., 2002; Vrieseling and Arber, 2006) (Figure 6A). Cm and Tri motor neuron pools switch dorsoventral position in *Pea3* mutant mice, leading to a configuration shifting the Tri pool to an aberrant ventral position secondary to *Pea3* mutation in Cm motor neurons (Figure 6A). But despite ventral cell body shift, electrophysiological analysis demonstrated that Tri proprioceptors still contact most Tri motor neurons with high accuracy (Vrieseling and Arber, 2006). These data provide evidence that cell body position is not a sufficient determinant to program sensory-motor connectivity and may be accounted for by the fact that Tri dendrites in both *Pea3* mutants and wild-type extend into similar territory and can thereby connect to Tri proprioceptors. A more dramatic scrambling of motor neuron cell body position without coincident change in molecular programs involved in the establishment of peripheral projections was observed in mice mutant in catenin signaling (Demireva et al., 2011). Columnar cell body position was also affected by catenin perturbation experiments in chick embryos (Bello et al., 2012). Since catenin mutant mice die early, it was not possible to assess specificity of sensory-motor connectivity (Demireva et al., 2011) (Figure 6A). Finally, transcription factors control cell surface signaling molecules in expression patterns that label specific motor neuron pools or subtypes of sensory neurons. For example, the semaphorin family member *Sema3e* is expressed by Cm motor neurons and its receptor *PlxnD1* by subpopulations of proprioceptors (Pecho-Vrieseling et al., 2009). Selective genetic perturbation of the Sema3e-PlxnD1 signaling system in mice rewires specificity of sensory-motor connections in the Cm reflex arc as assessed by electrophysiological and anatomical assays (Pecho-Vrieseling et al., 2009) (Figure 6A). These findings demonstrate that subpopulation-specific molecular interactions between possible future pre- and postsynaptic partners are important to regulate this process.

In summary, currently available experimental evidence supports a model in which the combinatorial actions of several most likely intertwined programs instruct the synaptic precision of direct sensory-motor connections. Presynaptic sensory afferents and postsynaptic motor neuron dendrites target to spatially stereotyped and conserved spinal domains, leading to the emergence of confined zones of anatomical overlap. Genetic programs involved in neuronal subtype specification probably control the generation of these common targeting domains. In addition, pre- and postsynaptic partners depend on the presence of cell surface signaling cues allowing recognition to occur and synaptic connections to consolidate. In this model, some of the same programs involved in controlling the establishment of spatial order may also act to control precision of synaptic connections within these domains. Intriguingly, synaptic specificity of sensory-motor connections is under the influence of yet-to-be-identified retrograde signals from muscle targets (Smith and Frank, 1988), and it will be interesting to unravel the pathways controlled through these signals. Finally, the establishment of differential-synaptic weights may also be influenced by circuit activity.

Precision of Sensory Targeting to the Spinal Dorsal Horn

The precision of spatial targeting to defined domains in the spinal cord is a feature prominently observed for sensory subpopulations terminating in the superficial-to-deep spinal dorsal horn (Brown, 1981; Todd, 2010). Functionally distinct sensory afferents innervate dorsoventrally confined laminar territories spatially subdividing the dorsal horn into dedicated receiver subcircuits for different sensations including pain, temperature, and touch. Sensory inputs are processed and relayed to ascending pathways for perception, but many of them also influence motor output indirectly through polysynaptic pathways in the spinal cord (Rossignol et al., 2006). Elucidating the organization and molecular underpinnings of spinal targeting domains including connecting subcircuits is essential to understand how sensory information in the dorsal horn is processed.

Recent work sheds light on the high degree of spatial organization of primary mechanoreceptive touch sensory information in the dorsal horn (Li et al., 2011). Low-threshold mechanoreceptors (LTMRs) diversify into functionally distinct sensory neurons relaying different touch-related sensations from the skin to the spinal dorsal horn. Using mouse genetics to selectively mark different LTMR subtypes, the analysis reveals the precise stoichiometry in peripheral innervation at three main hair follicle types, each receiving highly stereotyped innervation by functionally distinct LTMRs (Figure 6B). Touch-related sensory information derived from one such peripheral LTMR unit is probably bound together and processed in one central LTMR column in the dorsal spinal cord (Figure 6B). From the observed volume of individual LTMR columns in the adult mouse, it can be estimated that the dorsal horn combines 2,000–4,000 such LTMR units in three-dimensional space (Li et al., 2011), probably reflecting peripheral receptive fields from the skin in exquisite order.

These observed LTMR columns are similar in concept to the previously described nociceptive withdrawal reflex (NWR) modules in the dorsal horn (Ladle et al., 2007; Petersson et al., 2003; Schouenborg, 2008). The developmental crystallization of NWR modules to reach adult configuration is thought to arise by activity-driven mechanisms (Granmo et al., 2008; Petersson et al., 2003), raising the question of whether and how LTMR columns overlap and align with NWR modules during development. In summary, the topographically arranged and spatially confined organization of functionally distinct sensory channels contacting spinal subcircuits probably represents an important principle for the formation of dedicated circuit units in the spinal cord. The observed organization contributes to processing of sensory information, bundling of ascending information, and sensory-motor transformation.

Initiation and Monitoring of Action by Descending and Ascending Pathways

Spinal circuits communicate bidirectionally with supraspinal centers through many pathways (Grillner et al., 2005; Lemon, 2008). Supraspinal centers are involved in initiation and activation of action programs. Many substructures of descending pathways are evolutionarily conserved and their contribution to action program diversification can be assessed in different species (Grillner et al., 2005). Supraspinal centers are also the target for diverse information channels from the spinal cord, reporting on action programs to the brain. Aspects featured here will include a handful of specific examples for which defined subcircuits are implicated in certain behavioral aspects and/or molecular entry points have been elucidated.

Cortical Control of Motor Behavior

Cross-regulatory transcription factor networks are involved in developmental specification of cortical pyramidal neurons. They instruct the establishment of subcortical projections to pons, tectum, and spinal cord and distinguish this cortical population from callosal projection neurons with trajectories to contralateral cortical territory. In this transcriptional network, Fezf2 acts through Ctip2 to program corticospinal axonal trajectories (Arlotta et al., 2005; Chen et al., 2008; Molyneaux et al., 2005), whereas SatB2 represses Ctip2 and promotes callosal projections (Alcamo et al., 2008; Britanova et al., 2008) (Figure 7A).

Subcortical projection neurons establish synaptic connections with many different postsynaptic targets. Direct connections between cortical neurons and motor neurons are subject to evolutionary adaptation, and their existence and weight correlate with the degree of skilled motor performance involving distal forelimb muscles used during object manipulation tasks (Lemon, 2008). Cortical neurons also exhibit pronounced indirect influence on motor neurons through connections to brainstem centers and spinal interneurons (Lemon, 2008; Orlovsky et al., 1999), but it is difficult to assess the relative contributions of these diverse connections to motor behavior.

Recent work has put forward the provocative idea that descending cortical control of motor behavior may not be restricted to motor cortex but, at least in the whisker system, is in part mediated by somatosensory cortical territory (Matyas et al., 2010). In this system, pyramidal neurons in motor cortex M1

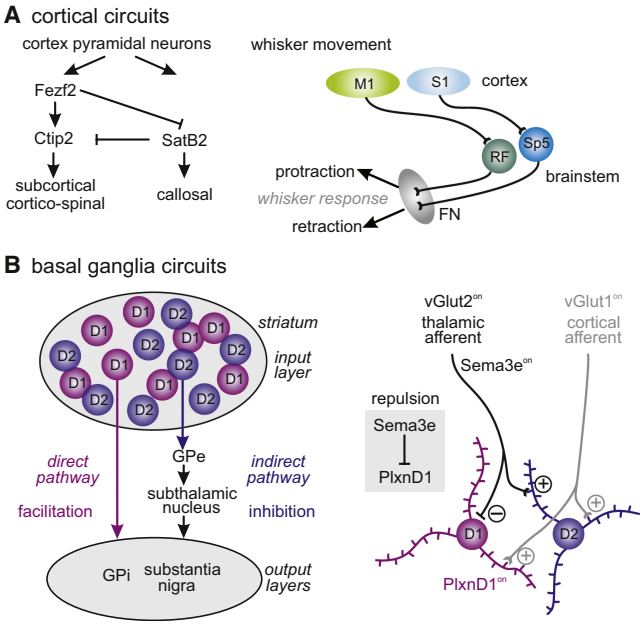

Figure 7. Organization and Connectivity of Descending Motor Circuits

(A) Specification of cortical pyramidal neurons by transcription factor network (left) and access of different brain stem structures from M1 and S1 cortex controlling antagonistic whisker movement (right; RF, reticular formation; Sp5, spinal trigeminal nucleus; FN, facial nucleus). (B) Main layers of basal ganglia circuits (left) illustrating differences between direct (mediated via D1-expressing MSNs) and indirect (mediated via D2-expressing MSNs) pathway. D1-expressing MSNs (right) coexpress the receptor *PlxnD1*, while thalamostriatal afferents express its ligand *Sema3e*-mediating repulsion (indicated by minus sign), thereby ensuring selective thalamic-D2 MSN connectivity.

connect to the reticular formation in the brainstem, which in turn controls the activity of facial motor neurons regulating whisker protraction (Figure 7A). The antagonistic movement of whisker retraction is initiated by descending input from somatosensory cortex S1 connecting to motor neurons via the spinal trigeminal nucleus (SPV), without M1 involvement in this pathway (Figure 7A). These findings suggest that fundamentally different descending cortical pathways influence specific motor behaviors. Given that both motor- and somatosensory cortex project to spinal levels, these observations raise the possibility that spinal motor circuits may also be differentially regulated by similar mechanisms. Whether distinct molecular programs of the kind observed for different cortical projection neurons (Arlotta et al., 2005) instruct differences between M1 and S1 projection neurons and perhaps also control the finer degree of connection specificity to distinct subcortical targets in the brainstem and spinal cord will be an interesting avenue to pursue.

Basal Ganglia Circuits in Action Initiation and Termination

Basal ganglia circuits play key roles in the control of motor behavior including action selection, and perturbations lead to movement disorders such as Parkinson's disease or chorea (Gerfen and Surmeier, 2011; Grillner et al., 2005; Kreitzer and Malenka, 2008). Basal ganglia output only accesses circuits in the spinal cord indirectly through nuclei in the brainstem, which

in turn establish connections to spinal interneurons and motor neurons (Grillner et al., 2005). To define the role of basal ganglia circuits in motor behavior, the activity of individual neurons can be monitored in behaving animals to determine patterns and changes as the animal learns to perform a task (Jog et al., 1999). Using such methods, a subset of nigrostriatal circuits was recently shown to play a highly specific role in initiation and termination of learned action sequences, a property blocked by selective elimination of striatal NMDAR1 (Jin and Costa, 2010).

The function of basal ganglia circuits highlights the importance of precise synaptic input-output regulation and recent work begins to unravel the mechanisms regulating synaptic specificity. The striatum is the basal ganglia input layer and combines many different presynaptic sources, including glutamatergic cortical and thalamic afferents and substantia nigra (SN)-derived dopaminergic input (Gerfen and Surmeier, 2011; Grillner et al., 2005; Kreitzer and Malenka, 2008) (Figure 7B). GABAergic medium spiny neurons (MSNs) make up ca. 95% of all striatal neurons and can be divided into two main subpopulations based on expression of molecular markers (most notably distinct dopamine receptors [Drds]), connectivity, and function. Direct-pathway MSNs express Drd1a (D1) and project directly to basal ganglia output layers (GPi, internal segment of globus pallidus; SNr, substantia nigra pars reticularis), whereas indirect-pathway MSNs express Drd2 (D2) and have access to output layers only through intermediate relays (GPe, external segment of globus pallidus; subthalamic nucleus). These two distinct pathways have been implicated in functionally opposing motor behaviors, movement facilitation for the D1-direct pathway, and movement inhibition for the D2-indirect pathway (Figure 7B). Making use of the striking molecular distinction between MSN subpopulations, this model was recently directly tested and essentially confirmed by the combination of MSN neuron subtype-specific Cre expression and conditional light-mediated activation of channelrhodopsin-2 in striatal neurons (Kravitz et al., 2010).

Pathway divergence in the striatum raises the question of how the selection of synaptically appropriate input to D1- and D2-MSN subpopulations is regulated during development. A recent study provides evidence that Sema3e-PlxnD1 signaling between thalamic afferents and MSNs plays an important role in this process (Ding et al., 2012). Within the striatum, the receptor PlxnD1 exhibits highly selective expression in D1-MSNs (Figure 7B). Its ligand Sema3e is expressed by vGlut2^{on} thalamic but not vGlut1^{on} cortical afferents (Figure 7B). Genetic elimination of either presynaptic Sema3e or postsynaptic PlxnD1 leads to increased thalamostriatal input specifically to D1-MSNs but not D2-MSNs assessed by electrophysiology and anatomy. This work highlights that at the mechanistic level, the same molecular pathway is employed for the regulation of synaptic specificity in basal ganglia circuits and sensory-motor connectivity in the spinal cord. Whereas in the spinal cord, presynaptic PlxnD1 expression in proprioceptors prevents the establishment of direct synaptic contacts with postsynaptic Sema3e-expressing Cm motor neurons (Pecho-Vrieseling et al., 2009) (Figure 6A), thalamostriatal synapses use the same ligand-receptor pair but with switched pre- and postsynaptic localization to regulate synaptic specificity.

Dopaminergic input from the SN to the striatum gates the shift of MSNs between active up and inactive down states (Gerfen and Surmeier, 2011; Grillner et al., 2005; Kreitzer and Malenka, 2008). Dopaminergic neurons in the midbrain exhibit functional heterogeneity, at least in part originating from differential synaptic input to these neurons mediated by dendritic arborization (Henny et al., 2012). Analysis of anatomical and functional properties of dopaminergic neurons with cell bodies positioned in SN pars compacta (SNc) differentiates two main types. Neurons with dendrites extending into the neighboring SN pars reticulata (SNr) exhibit a higher proportion of GABAergic inputs than the ones with dendrites confined to SNc, a feature tightly correlating with in vivo responses to aversive stimuli (Henny et al., 2012). These findings provide additional support for the notion that the elaboration of dendritic arbors during development profoundly influences assembly of presynaptic input and neuronal function.

Action Program Monitoring by Ascending Pathways to the Cerebellum

Ascending spinal pathways concerned with motor control are involved in reporting predicted future action and past events assessed through sensory feedback. Internal monitoring of motor behavior exists at a multitude of hierarchical levels and was studied in many species (Poulet and Hedwig, 2007; Sommer and Wurtz, 2008). While the briefly summarized studies on pathways carrying ascending information to the cerebellum are based on work carried out over many years, they clearly illustrate the existence of spatially confined and task-related reporting channels of spinal origin. They also highlight the lack of knowledge about genetic and developmental pathways involved in specification and connectivity of these important neuronal populations.

In the cervical spinal cord, a specialized group of C3-C4 propriospinal neurons was characterized using a combination of electrophysiological, anatomical, and behavioral approaches in cat and monkey (Alstermark et al., 2007; Pettersson et al., 2007). Segmentally restricted neurons at cervical levels C3-C4 (hence the name) receive input from many descending sources. An important hallmark of C3-C4 propriospinal neurons is the establishment of a bifurcating axonal trajectory (Figure 8A). Descending collaterals establish synaptic connections to motor neurons at C6-T1 and interneurons, whereas ascending axon collaterals extend to the lateral reticular nucleus (LRN), which in turn gives rise to mossy fiber inputs to the cerebellum (Figure 8A). A series of lesion studies in the cat proposes an essential role of these relay neurons in target reaching of the forelimb. Both excitatory and inhibitory neurons are contained within C3-C4 propriospinal neurons, but genetic identity of this specialized premotor action reporting system is currently unknown.

The spinal cord is the origin of a diverse set of spinocerebellar projection neurons, establishing direct mossy fiber input to cerebellar granule cells (Orlovsky et al., 1999; Oscarsson, 1965). Details regarding the anatomical and functional diversification of spinocerebellar projection neurons extend beyond the scope of this Review; however, in considering these issues more broadly, a few important points can be made. Functionally distinct populations of spinocerebellar neurons are generally located at defined rostrocaudal segments in conserved laminar

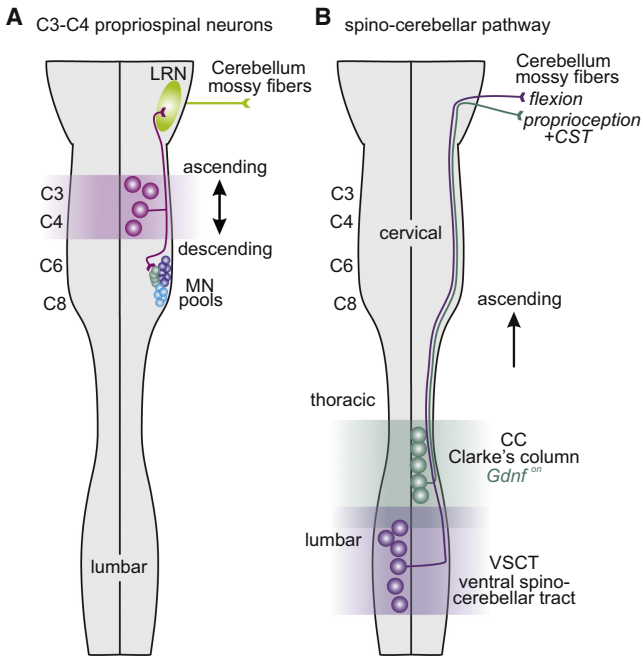

Figure 8. Ascending Spinal Pathways to the Cerebellum

(A) C3-C4 propriospinal neurons (magenta circles) establish bifurcating axons, ascending to the lateral reticular nucleus (LRN) and descending to C6-T1 motor neurons.

(B) Direct ascending projections from the spinal cord to the cerebellum are formed by a variety of distinct spinocerebellar populations, including Clarke's column (CC, green) or ventral spinocerebellar tract (VSCT, violet), each initiating at segmentally restricted spinal levels and channeling specific information to the cerebellum by mossy fibers (CST, corticospinal tract).

positions and establish projections to stereotyped cerebellar lobules. Ventral spinocerebellar tract (VSCT) neurons reside at lumbar levels and are active preferentially during the flexion phase of stepping, monitoring intrinsic spinal network activity in the cat (Arshavsky et al., 1978) (Figure 8B). In contrast, Clarke's column (CC) neurons are located more rostrally, receive direct sensory feedback (Walmsley, 1991), integrate this information with descending corticospinal input, and express the neurotrophic factor *GDNF* (Hantman and Jessell, 2010) (Figure 8B).

Spatial and functional diversification of ascending spinal projection neurons highlights the need to understand the developmental and genetic cascades involved in their specification. At the mechanistic level, neuron diversification along the rostrocaudal axis has been studied most extensively for motor neurons, in which combinatorial expression of different Hox transcription factors plays important roles (Dalla Torre di Sanguinetto et al., 2008; Dasen et al., 2005, 2008). Since all spinal neurons arise locally, these include long-distance projection neurons to supraspinal targets. A possible mechanism for generation of required diversity at the molecular level may therefore be an intersection between dorsoventral and Hox transcriptional networks.

Outlook

Recent studies have begun to address how early developmental diversification relates to connectivity and function and have added an important facet to our understanding of motor circuits. But at the same time, these studies also reveal the difficulty of integrating the enormous repertoire of existing data on motor circuits across different mammalian species. A challenging task for the future will be to bridge the gap in knowledge between development and function. This includes a deeper understanding of how developmental programs align with functional circuit units and behavior, a problem that can now be tackled from many different angles. This Review demonstrates that a similar logic applies to multiple levels in the hierarchical organization of motor circuits and outlines some of the open questions and opportunities for further experimental investigation. Since motor behavior is the final common output of most nervous system activity and also influences circuits not directly concerned with movement, understanding organizational principles of motor circuits will have an impact far beyond the direct control of motor behavior.

ACKNOWLEDGMENTS

The broad coverage of topics in this review required a citation strategy mainly focusing on original recent literature described in more detail here. I would like to apologize to authors of the many additional important original studies for citing Review articles instead. I am grateful to Rui Costa and Ole Kiehn for discussions and comments on the manuscript and to Ole Kiehn for pointing out the term "pseudocommissural" to me. S.A. was supported by an ERC Advanced Grant, the Swiss National Science Foundation, the Kanton Basel-Stadt, EU Framework Program 7, and the Novartis Research Foundation.

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