Assembly and Function of Spinal Circuits for Motor Control

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Abstract

Control of movement is a fundamental and complex task of the vertebrate nervous system, which relies on communication between circuits distributed throughout the brain and spinal cord. Many of the networks essential for the execution of basic locomotor behaviors are composed of discrete neuronal populations residing within the spinal cord. The organization and connectivity of these circuits is established through programs that generate functionally diverse neuronal subtypes, each contributing to a specific facet of motor output. Significant progress has been made in deciphering how neuronal subtypes are specified and in delineating the guidance and synaptic specificity determinants at the core of motor circuit assembly. Recent studies have shed light on the basic principles linking locomotor circuit connectivity with function, and they are beginning to reveal how more sophisticated motor behaviors are encoded. In this review, we discuss the impact of developmental programs in specifying motor behaviors governed by spinal circuits.

Contents

INTRODUCTION

The spectrum of motor behaviors expressed by animals is defined by genetic programs that shape the organization and function of neuronal circuits. Neuronal networks residing within the spinal cord have a significant role in defining species-specific motor behaviors. Many basic features of these networks are governed by patterning systems operating in the early phases of neural development. Spinal circuits contain the basic instructions for coordinating the sequence of muscle activation during locomotor activities such as walking, and are engaged by descending and ascending supraspinal systems necessary for volitional tasks such as object manipulation (Arber 2012, Miri et al. 2013). Modulation in spinal circuit connectivity programs was also key during vertebrate evolution, enabling certain lineages to achieve novel and increasingly complex motor skills (Fetcho

1992, Jung & Dasen 2015). A major focus in recent years has been linking the organization and function of spinal and supraspinal neural networks with specific types of behaviors.

Our current understanding of how motor circuits are assembled derives from classical studies on development and neuroanatomy in experimentally accessible systems such as chick embryos (Bekoff 2001, Landmesser 2001), as well as characterization of the basic properties of spinal circuits in several vertebrate model systems (Grillner 2006, Jankowska 2001, Jankowska & Edgley 1993, Sherrington 1906). With the emergence of molecular and genetic tools, significant progress has been made in deciphering the mechanisms through which major classes of neurons within the spinal cord are specified (Jessell 2000, Philippidou & Dasen 2013, Shirasaki & Pfaff 2002). Studies on the pathways controlling axonal guidance and synaptic specificity have also defined many of the factors involved in the selection of synaptic targets during motor circuit assembly (Bonanomi & Pfaff 2010, Butler & Tear 2007). A complete understanding of how spinal circuits impact motor behaviors has been challenging, owing in part to our limited knowledge of the basic wiring diagrams of most circuits within the spinal cord.

Recent advances in methods to selectively map circuit connectivity with viral-based transsynaptic labeling techniques, as well as the ability to manipulate and monitor the activity of defined neuronal classes, has revolutionized the field. The idea of bridging our relatively advanced understanding of neural tube patterning with functional attributes of the motor system now appears to be a tenable goal. In this review, we discuss how early specification and connectivity programs contribute to the types of motor behaviors expressed by vertebrates, highlighting some examples in which relatively small changes in circuit features have a significant impact on motor output. These examples illustrate the modular nature of spinal circuits and the profound roles that individual cellular and genetic components can exert on the overall function of the motor system.

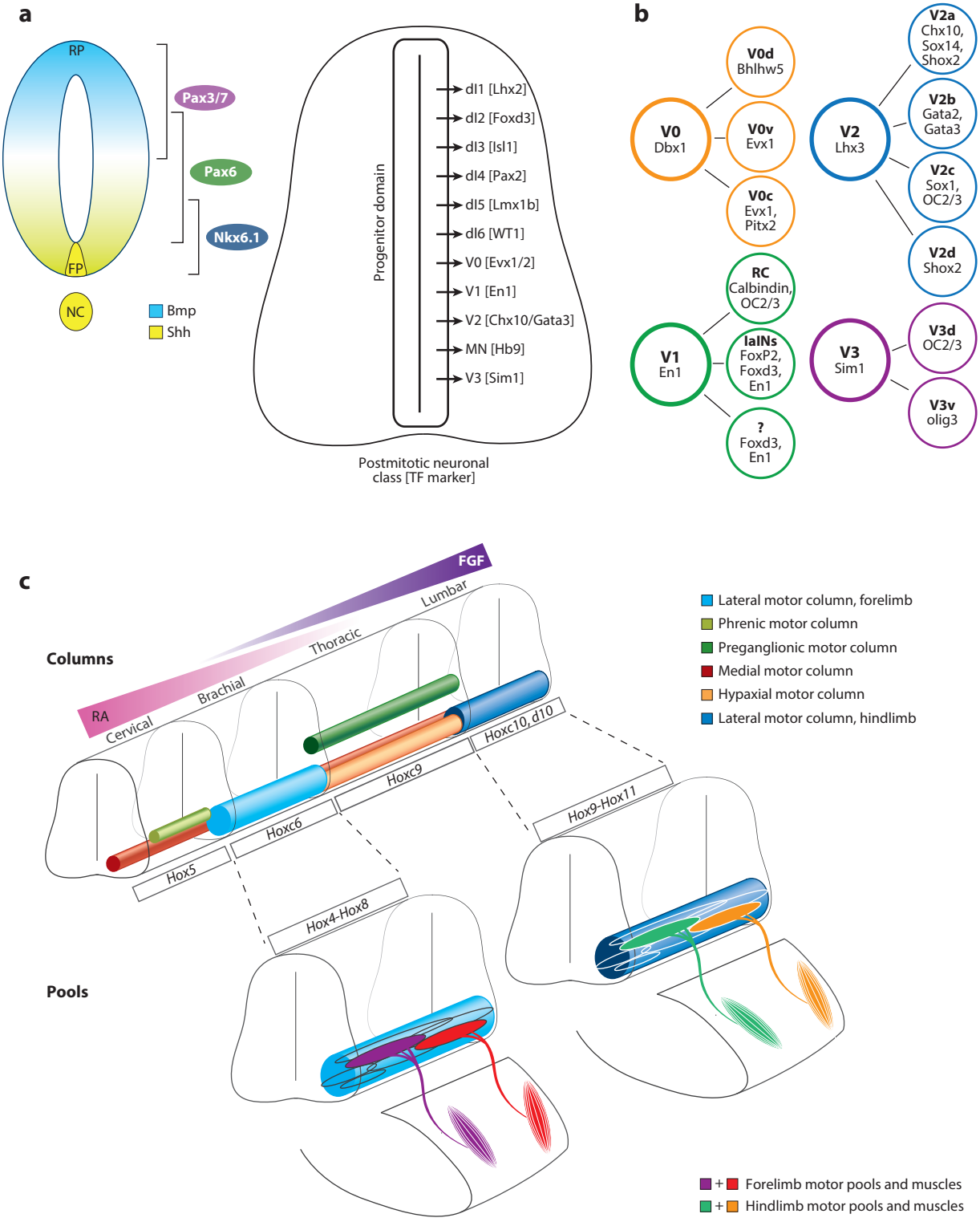
ESTABLISHING THE EARLY ARCHITECTURE OF LOCOMOTOR CIRCUITS

Spinal circuits establish their early architecture by means of inductive cues that lay the preliminary framework for neuronal specification. Depending on their relative distance from extrinsic signaling hubs, neural progenitors give rise to one of several classes of excitatory and inhibitory interneurons, as well as motor neurons (MNs). Around the time of cell cycle exit, spinal neurons further differentiate and acquire subtype identities that define their core physiological properties and basic pattern of connectivity. In this section, we briefly outline the strategies through which spinal neurons differentiate, with emphasis on the contribution of early signaling programs to the overall organization of motor circuits.

Dorsoventral Patterning Confers Neuronal Class Identity

Patterning along the dorsoventral axis of the neural tube is governed largely through ventrally derived Sonic hedgehog (Shh), secreted from the notochord and floor plate, and dorsal bone morphogenetic proteins (Bmps) emanating from the roof plate and surface ectoderm (Jessell 2000). Shh activity yields neural progenitor populations fated to become the principal postmitotic neurons of locomotor circuits, including four interneuron classes (V0, V1, V2, V3) and MNs. Bmps are responsible for producing dorsal cell types (dI1–dI6), which are typically, but not exclusively, associated with sensory relay circuits (**Figure 1*a***).

Bmps and Shh establish concentration gradients along the dorsoventral axis; these gradients induce patterns of transcription factor expression in spinal progenitors. How progenitors interpret morphogen gradients has been worked out in greatest detail for Shh signaling. Shh acts via the Gli



family of transcription factors to regulate the expression profile of two classes of homeodomain (HD) transcription factors (Class I/Class II) in the ventral spinal cord (Shirasaki & Pfaff 2002). Shh induces expression of the more ventrally expressed Class II protein genes, whereas genes encoding Class I proteins are expressed more dorsally and are repressed by Shh (Briscoe et al. 2000). These initial patterns are further fine-tuned by selective cross-repressive interactions between pairs of transcription factors. Recent evidence indicates that repressive interactions among these factors lead to a stable memory of Shh signaling (Balaskas et al. 2012). These intrinsic repressive networks establish and maintain sharp boundaries between progenitor domains and ensure the production of defined classes of postmitotic cells (**Figure 1*a***).

Commissural interneuron (CIN): an interneuron that projects an axon across the midline of the nervous system

Like spinal progenitors, postmitotic neuronal classes are defined by the specific transcription factors they express, and they can be further categorized on the basis of their connectivity patterns, neurotransmitter systems, and physiological properties (Jessell 2000). All spinal MNs, for example, arise from a single progenitor domain, express a set of core early postmitotic determinants, extend axons outside the central nervous system (CNS), and are cholinergic. MNs diversify into hundreds of subtypes, each tasked with targeting a specific region or muscle in the periphery (Dasen & Jessell 2009). Recent efforts to classify ventral interneurons have begun to descry a similar degree of subtype diversity (**Figure 1*b*,*c***). V0 interneurons derive from progenitors expressing the Dbx1 HD protein and can be further subdivided into excitatory V0 interneurons (V0v), inhibitory V0 interneurons (V0d), and a relatively underrepresented group of cholinergic interneurons (V0c) (Lanuza et al. 2004, Zagoraiou et al. 2009). Included in the V1 interneuron population are Renshaw cells and Ia inhibitory interneurons, marked by expression of En1 (Alvarez et al. 2005, Gosgnach et al. 2006). The Lhx3-expressing V2 interneuron group gives rise to $Chx10^+$ excitatory V2a interneurons, $Gata3^+$ inhibitory V2b interneurons, and $Sox1^+$ V2c interneurons (Crone et al. 2008, Panayi et al. 2010). V3 interneurons comprise an excitatory class of commissural interneurons (CINs) that encompasses two subgroups (V3d and V3v) demarcated by their differential intrinsic properties and spatial distribution (Zhang et al. 2008). A more extensive categorization of interneuron subtypes and identity markers demonstrates the multifaceted nature of ventral interneurons, in terms of expression profile, function, and distribution within the spinal cord (Francius et al. 2013), suggesting each class may exhibit diversity similar to that observed in MN subtypes.

Rostrocaudal Signaling and Neuronal Subtype Diversification

The specialization of nascent neuronal cells in the spinal cord involves not only dorsoventral but also rostrocaudal patterning, which establishes the initial blueprint for positioning spinal motor

Figure 1

Patterning of the neural tube. (*a*) The notochord and floor plate secrete Shh, whereas the roof plate secretes Bmps, resulting in the differentiation of progenitor cells into five ventral neuron types (V0–V3 interneurons and motor neurons) and six dorsal neuron types (dI1–dI6). Each stage of differentiation is associated with the expression of a specific TF or set of TFs. The progenitor domain can be divided into a dorsal Pax3- and Pax7-expressing region, an intermediate Pax6-expressing region, and a ventral Nkx6.1-expressing region. Unique TF markers, some of which are illustrated, further distinguish the 11 postmitotic groups. (*b*) The four principal ventral interneuron classes further differentiate into more diverse subclasses, each expressing unique TFs. (*c*) Opposing concentration gradients of RA and FGF elicit expression of specific *Hox* genes along the rostrocaudal axis of the spinal cord. Cross-repressive interactions between different Hox proteins shape regional character (e.g., cervical, brachial, thoracic, or lumbar), compartmentalize MNs into specialized columns innervating different muscle groups, and create motor pools targeting specific muscles. Abbreviations: Bmps, bone morphogenetic proteins; FGF, fibroblast growth factor; FP, floor plate; IaINs, Type Ia inhibitory interneurons; MNs, motor neurons; NC, notochord; RA, retinoic acid; RC, Renshaw cell; RP, roof plate; Shh, Sonic hedgehog; TF, transcription factor.

HOX GENES, ROSTROCAUDAL PATTERNING, AND COLINEARITY

The chromosomally arrayed *Hox* genes are instrumental in patterning along the rostrocaudal axis of most animal species (Alexander et al. 2009, Krumlauf 1994, McGinnis & Krumlauf 1992). In vertebrates, they comprise 39 genes organized in four clusters. Expression of a gene within a cluster is related to its position, such that genes at the 3′ end are expressed more rostrally, whereas genes at the 5′ end are expressed more caudally. This relationship between chromosomal location and spatial expression is termed spatial colinearity and characterizes the pattern of *Hox* genes in both neuronal and non-neuronal tissues (Duboule 1998). In the vertebrate nervous system, *Hox* genes are essential for the subtype diversification of motor neurons within the hindbrain and spinal cord (Philippidou & Dasen 2013). In addition to determining MN subtype identity along the rostrocaudal axis, *Hox* genes contribute to MN diversification within a specific segment. *Hox* expression patterns are controlled through a complex interplay between morphogen signals, epigenetic modifications within the clusters, and cross-regulatory interactions between Hox proteins and *Hox* genes. Whereas signals acting on spinal progenitors pattern *Hox* gene expression, Hox activities are deployed largely in postmitotic cells, where they influence guidance decisions by regulating downstream transcription factors and other synaptic specificity determinants.

circuits in relation to their future peripheral targets. The appropriate functionality of central pattern generators (CPGs), which orchestrate limb-based motor behaviors such as walking, depends upon their proper positioning along the rostrocaudal axis in register with their eventual targets (Kiehn & Butt 2003). Because the molecular strategies of rostrocaudal axis diversification are best understood for spinal MNs, this section focuses on their organization and subtype specification. As interneurons are subject to the same rostrocaudal patterning cues, analysis of MN diversification may provide a paradigm for understanding the subtype differentiation of multiple neuronal classes within the spinal cord.

Central pattern generator (CPG): a neural network that generates a rhythmic pattern of motor neuron activation

***Hox* genes:** a large family of chromosomally arrayed genes encoding transcription factors that are involved in patterning along the head-to-tail axis of most animal species

Lateral motor column (LMC): a cluster of motor neurons found at the forelimb and hindlimb levels of the spinal cord that innervates limb muscle

Similar to the relationship between Shh/Bmps, secreted fibroblast growth factor (FGF) and retinoic acid (RA) form opposing concentration gradients along the rostrocaudal axis (**Figure 1*c***). Within the neural tube, RA secreted from the paraxial mesoderm acts as a rostralizing signal, whereas FGF acts as a caudalizing signal. FGFs and RA confer positional identity by controlling the expression of the chromosomally arrayed *Hox* genes (see sidebar, *Hox* Genes, Rostrocaudal Patterning, and Colinearity) (Bel-Vialar et al. 2002, Dasen et al. 2003, Liu et al. 2001). The neural tube undergoes temporally and spatially progressive RA/FGF-mediated patterning, so that the cervical, brachial, thoracic, and lumbar regions are molecularly distinct according to the particular *Hox* genes they express (Dasen et al. 2003, 2005). The relatively obtuse regionalization established by RA/FGF gradients is subsequently sharpened by the cross-repressive action of neighboring Hox proteins.

Hox-mediated patterning has a key role in establishing the diversity of MNs. A typical mammal contains hundreds of anatomically and functionally distinct muscle groups, each innervated by a dedicated MN subtype. Shortly after birth, MNs segregate into longitudinally arrayed columns that are positioned in registry with the target tissues they will eventually project toward (Landmesser 1978, 2001). At the most rostral level of the spinal cord, Hox5 proteins specify the identity of phrenic column MNs targeting the diaphragm muscle (Philippidou et al. 2012). At the brachial (forelimb) and lumbar (hindlimb) levels, MNs that express Hox6 and Hox10 proteins, respectively, acquire a lateral motor columnar (LMC) identity, fating those cells to become part of the neural networks assigned to control limb musculature (Dasen et al. 2003, 2008; Lacombe et al. 2013; Rousso et al. 2008; Shah et al. 2004; Wu et al. 2008). At thoracic levels, the *Hoxc9* gene

is essential for the assignment of MN fates, including the subtypes targeting body wall muscles [hypaxial motor column (HMC) neurons] and sympathetic chain ganglia [preganglionic motor column (PGC) neurons] (Jung et al. 2010). A network of an additional two dozen *Hox* genes operates within these columnar groups to define the molecular identity and connectivity of MN pools targeting individual limb muscles (Dasen et al. 2005) (**Figure 1*c***).

Examining differences in Hox-dependent MN specification programs between vertebrate species has provided an entry point to analyze the evolution of motor networks and how MN topography varies in species with distinct motor behaviors. Comparison of Hox expression patterns between limb-bearing and limbless vertebrates has revealed that an extended expression domain of the thoracic *Hoxc9* gene is associated with the absence of limb-innervating LMC neurons in snake embryos (Jung et al. 2014). Hoxc9 acts in part by inhibiting expression of the *Foxp1* gene, a key determinant of LMC identity (Dasen et al. 2008, Rousso et al. 2008). Interestingly, this repressive activity is present in Hoxc9 proteins of the most primitive vertebrate species, indicating that Hox-dependent patterning is an ancestral strategy for specifying and organizing spinal MNs. Evolutionary modification of Hox signaling has also been demonstrated within sensory circuits of the spinal cord (Guo et al. 2011), suggesting *Hox* genes have a key role in governing adaptive changes within the CNS.

Motor neuron (MN) pool: a cluster of motor neurons that targets a single muscle, typically in the limb

Lim homeodomain (HD) proteins: a family of related transcription factors, including Lhx1, Lhx3, Isl1, and Isl2, characterized by a conserved protein-protein interaction domain

Local Signaling and Neuronal Specification

At later stages of neuronal specification, there appears to be a transition from broadly acting positional cues to more local signals provided by newly born neurons. In addition to its role in early rostrocaudal patterning, MN-derived retinoid signaling can impact the segregation of LMC neurons and influence how motor axons select limb trajectories. LMC neurons partition into medial and lateral subdivisions (LMCm and LMCl) characterized by expression of specific Lim HD proteins (Tsuchida et al. 1994). Earlier-born LMCm neurons express Isl1, as well as the RA-synthesizing enzyme retinaldehyde dehydrogenase 2 (Raldh2). MN-derived RA signaling has been proposed to induce LMCl character in later-born LMC neurons as they migrate through RA-producing LMCm neurons (Kania et al. 2000, Sockanathan & Jessell 1998). This trek through LMCm neurons triggers expression of *Lhx1* in the younger LMCl neurons, fating those cells to project into the dorsal limb compartment.

Spinal interneuron diversification also relies on local cell-cell communication. Notch signaling appears to be important for generating physiologically distinct V2 subtypes (Peng et al. 2007). Expression of the Notch ligand Delta4 within progenitor domain p2 triggers a program that evokes an inhibitory V2b identity. Perturbing Notch signaling through deletion of the Presenilin1 gene results in the absence of V2b interneurons and the generation of supernumerary V2a subtypes. Notch signaling has additionally been implicated in the late maturation of MNs, where suppression of Notch signaling through Delta-like homolog 1 (Dlk1) promotes expression of genes that define fast MN subtypes (Muller et al. 2014).

Collectively, these studies indicate that dorsoventral signaling has a key role in establishing neuronal class identities within the spinal cord, whereas both rostrocaudal and local cell-cell signaling contribute to neuronal subtype diversification.

GUIDANCE AND ADHESION SYSTEMS CONFERRING SYNAPTIC SPECIFICITY WITHIN MOTOR CIRCUITS

The fidelity with which motor circuits are assembled relies on guidance systems that determine the trajectories of neurons and define the vicinity in which axons and dendrites establish synapses. Target selection is dependent on sets of guidance proteins expressed by neuronal subtypes

(Bonanomi & Pfaff 2010, Landmesser 2001). An extensive body of work has dissected the signaling mechanisms through which specific molecules impact guidance decisions (Dudanova & Klein 2013); however, much remains unknown concerning the regulation of these molecules' activities during development. Nevertheless, it appears that combinations of transcription factors are pivotal in influencing axonal pathfinding, orchestrating the expression of guidance receptors and ligands (Thor et al. 1999, Tsuchida et al. 1994, Zarin et al. 2014).

Medial motor column (MMC): a cluster of motor neurons present throughout the spinal cord tasked with innervating dorsal axial muscles

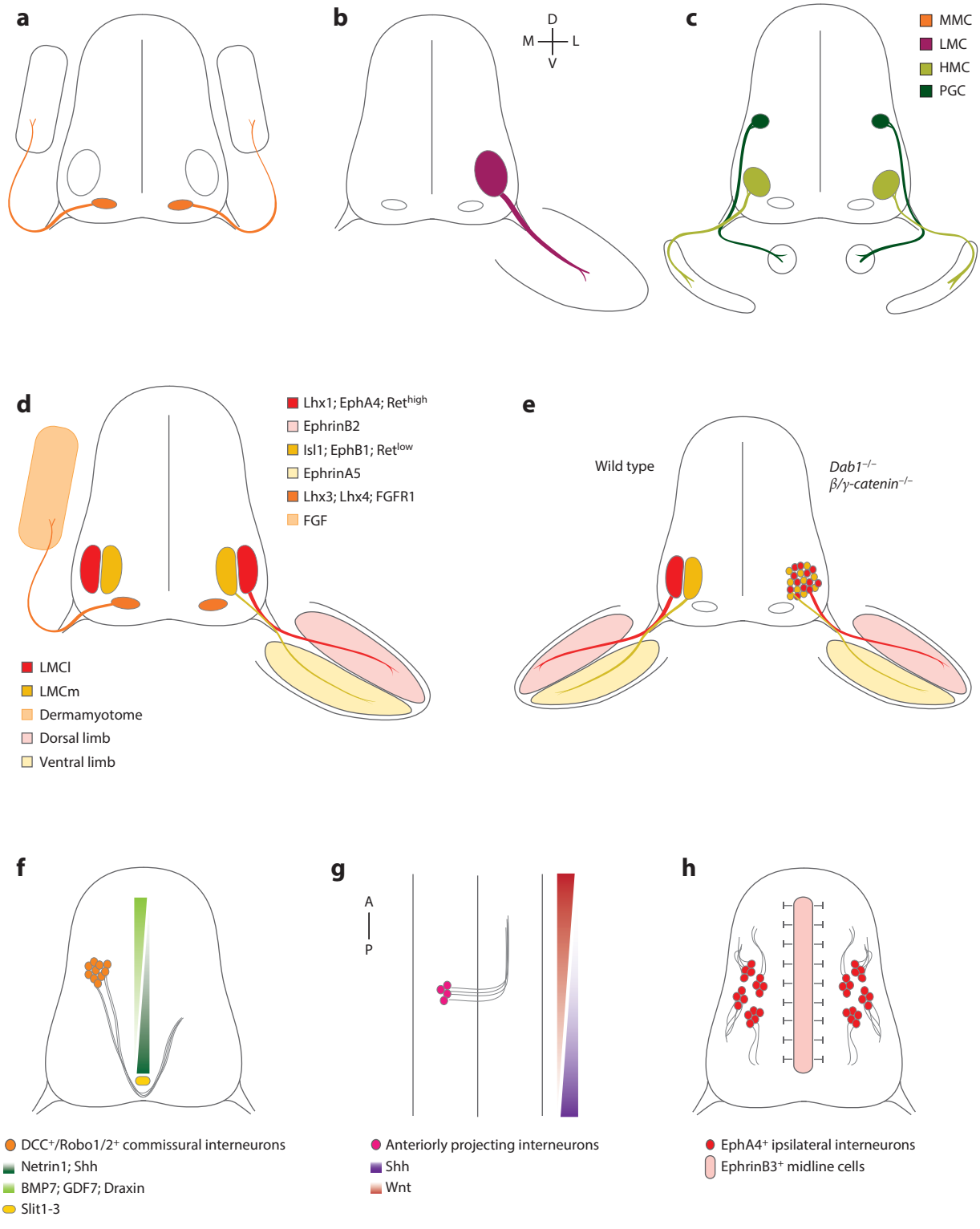
Early Steps in Locomotor Circuit Assembly: Wiring the Periphery

Soon after spinal MNs are generated, they begin to extend axons into the periphery, pursuing one of three trajectories depending on the rostrocaudal level: a dorsal route toward axial muscles (**Figure 2*a***), a ventrolateral route toward the limbs or hypaxial muscle derivatives (**Figure 2*b***), or a ventromedial route toward the sympathetic chain ganglia (**Figure 2*c***). Each motor column expresses a code composed of Lim HD factors, which contributes to these early guidance decisions (Thor et al. 1999, Tsuchida et al. 1994). For example, the activities of Lhx3 and Lhx4 in medial motor column (MMC) neurons are necessary for correct innervation of axial muscles. If *Lhx3* expression is artificially maintained in non-MMC neurons, their axons are redirected to axial muscles (Sharma et al. 2000, Shirasaki et al. 2006). Lhx3 regulates the expression of the Fibroblast Growth Factor Receptor 1 gene (*Fgfr1*) in MMC neurons and sensitizes their axons to attractive sources of FGFs, such as the dermamyotome (**Figure 2*d***). The MMC neurons of mice lacking *Fgfr1* show abnormal projections to the limbs and dorsal root ganglia (DRG) (Shirasaki et al. 2006, Soundararajan et al. 2010).

Lim HD proteins are also critical in defining the trajectory of limb-innervating axons. Once LMC axons reach the base of the limb, they are confronted with a binary choice of either projecting dorsally or ventrally. The simplicity of this choice has made the initial innervation of the limb an attractive model for understanding how axons select one path over another. In LMC neurons, Lim HD proteins regulate the expression of genes belonging to the Eph/ephrin signaling system (Kania et al. 2000). Lhx1, present in LMCl neurons, induces expression of the receptor EphA4. Expression of EphA4 in LMCl neurons drives axons to project dorsally, avoiding ephrin-a5 repulsive signals originating from the ventral limb mesenchyme (Helmbacher et al. 2000, Kania & Jessell 2003). Conversely, EphB1 receptor expression, induced by Isl1, directs LMCm axons to the ventral limb mesenchyme by sensitizing them to repulsive signals from dorsal cells containing ephrinB2 (Luria et al. 2008) (**Figure 2*d***).

Figure 2

Axon guidance in the spinal cord. (*a–c*) Medial motor column (MMC) neurons project dorsally to axial muscles, lateral motor column (LMC) and hypaxial motor column (HMC) motor neurons (MNs) adopt a ventrolateral route toward limb and hypaxial muscles, and preganglionic motor column (PGC) neurons project ventromedially to sympathetic chain ganglia. (*d*) MMC neurons expressing *Fgfr1* are directed toward the dermamyotome, which expresses fibroblast growth factors (FGFs). In LMC neurons, Lhx1^{+} LMCl neurons express *EphA4* and high levels of *Ret* and extend their axons toward the dorsal limb compartment, owing to repellent ephrinA5 signals present in the ventral mesenchyme. LMCm neurons express *EphB1* and project to the ventral limb, avoiding repulsive ephrinB2 signals located in the dorsal mesenchyme. (*e*) Comparison between wild-type, *Dab1*$^{-/-}$, and *β/γ-catenin*$^{-/-}$ mice, illustrating that even though MN organization is disrupted, the projection pattern to the limb is preserved. (*f*) Commissural interneurons expressing the Deleted in Colorectal Carcinoma (DCC) receptor respond to high levels of Netrin1 and Shh (Sonic hedgehog) and are attracted to the ventral region of the spinal cord. These neurons are also repelled by Bmp7, Gdf7, and Draxin signals emanating from the dorsal spinal cord. After crossing the midline, commissural axons expressing Roundabout homolog (Robo) 1/2 are repelled by Slit signals present in the floor plate, promoting growth away from the midline. (*g*) Commissural interneurons project anteriorly upon crossing the midline in response to attractive Wnt or repellent Shh morphogen gradients. (*h*) Ipsilateral interneurons expressing *EphA4* do not cross the spinal cord midline, owing to repulsive signals from midline cells expressing *ephrinB3*.



Eph/ephrin signaling is mediated through interactions that can occur both in *cis* and *trans*. Expression of ephrins within LMC neurons acts in *cis* to attenuate the responses mediated through coexpressed Eph receptors (Dudanova et al. 2012, Kao & Kania 2011). Ephrins also have a growth-promoting effect by activating reverse signaling (Dudanova et al. 2012). Furthermore, the Ret tyrosine kinase receptor functions as a coreceptor for both ephrinAs and Glial cell line–derived neurotrophic factor (GDNF), modulating responses to these two mesenchymal signals (Bonanomi et al. 2012, Kramer et al. 2006). High Ret levels in the LMCl promote dorsal projection through interaction with EphA4 (**Figure 2*d***). In mice lacking *Ret*, LMCl axons are rerouted toward the ventral limb, recapitulating the EphA4 loss-of-function phenotype (Kramer et al. 2006). These examples illustrate that correct guidance of axons is achieved by the coordinated function of a combination of different guidance molecules, either acting at different choice points along the trajectory or interacting with each other to create a robust mechanism to precisely direct axons to their ultimate destination.

Neuronal Topographic Maps and Peripheral Target Selection

MN subtypes occupy highly stereotyped positions within the spinal cord, and this topographical arrangement is predictive of their peripheral projection patterns (Kania 2014, Landmesser 2001). Transcription factors involved in MN subtype identity and connectivity, such as Hox proteins (Philippidou & Dasen 2013) and Lim HD factors, contribute to the formation of these topographic maps (Kania & Jessell 2003, Shirasaki et al. 2006). Genetic manipulation of these factors results in both aberrant cell body location and altered target selection. For example, misexpression of *Lhx1* or *Isl1* leads to the abnormal location of LMC neurons and anomalous axon trajectories (Kania & Jessell 2003). In mice mutant for *Foxp1*, an essential gene target of Hox proteins in MNs, molecular features of LMC subtype identities are lost (Dasen et al. 2008, Rousso et al. 2008). Although MNs lacking *Foxp1* successfully project to the limb, neurons targeting a specific muscle are scattered within the ventral spinal cord.

These observations raise the question of whether MN position and axon guidance are coupled events or independently regulated by common groups of transcription factors. Studies of the role of reelin and cadherin signaling in MNs support the latter hypothesis. Reelin is expressed in dorsomedial LMC neurons, whereas its signaling mediator Dab1 is expressed at high levels in LMCl neurons. Both *Reelin* and *Dab1* knockout mice display impaired LMCl and LMCm positioning; however, the selection of appropriate targets by LMC neurons is preserved (Palmesino et al. 2010). Similarly, disruption of cadherin signaling leads to the disorganization of MN topography without affecting peripheral target selection. Cadherins mediate adhesive cell-cell interactions, important for MN pool clustering (Bello et al. 2012, Price et al. 2002), and signal through catenins, which are linked to the cytoskeleton. Loss of β- and γ-catenins results in MN pool scrambling. However, peripheral connectivity is unaffected (Demireva et al. 2011, Price et al. 2002) (**Figure 2*e***). Collectively, these studies indicate that genes acting in parallel pathways regulate MN location and axon projection.

Mechanisms of Axonal Guidance Within Central Spinal Circuits

Although significant progress has been made in deciphering the mechanisms of motor axon guidance, the determinants driving synaptic specificity within central spinal circuits have proved more elusive. Studies of CINs, which cross the midline and connect with contralateral populations, have provided basic insights into how diverse guidance cues contribute to target selection. CINs initially extend their axons ventrally and then cross the midline below the floor plate. Ventral

growth is driven by attractive signals emanating from the ventral spinal cord and repulsive cues from the roof plate. Netrin1 and Shh, which display graded expression in ventral midline cells, provide attractive cues (Charron et al. 2003, Keino-Masu et al. 1996, Serafini et al. 1996). Commissural axons expressing the netrin receptor DCC (Deleted in Colorectal Carcinoma) and the Shh mediator Smoothened respond to high levels of Netrin1 and Shh, respectively, in the ventral region. At the same time, repulsive signals from the roof plate, such as Bmp7, Growth differentiation factor 7 (GDF7), and Draxin, contribute to the ventral growth of commissural axons (Augsburger et al. 1999, Butler & Dodd 2003, Islam et al. 2009) (**Figure 2*f***).

An additional mechanism prevents commissural axons from stalling at the midline, where the concentration of attractant cues is highest. Commissural axons express Roundabout homolog 1 (Robo1) and Robo2 receptors, which inhibit netrin1-DCC intracellular signaling upon activation by floor plate–derived Slit ligands (Stein & Tessier-Lavigne 2001). Interestingly, before axons are exposed to Slits, this mechanism is inhibited by Robo3.1 (an alternative splice form of Robo3), which silences Robo1, avoiding premature crossing of commissural axons (Sabatier et al. 2004). Furthermore, Robo3.2 is expressed only upon midline crossing in commissural axons, preventing them from recrossing the midline (Chen et al. 2008). Recently, it has been shown that Slits can exert their repellent effect by binding to the Plxna1 receptor (Delloye-Bourgeois et al. 2015). The response of commissural axons to Slits depends on the deubiquitinating enzyme USP33, which interacts with Robo1, promoting midline crossing (Yuasa-Kawada et al. 2009). In addition, *Sema3B* expression in the floor plate reinforces repellent Slit/Robo signaling in postcrossing axons, mediated by the receptor Neuropilin-2, which is present in commissural axons (Nawabi et al. 2010, Zou et al. 2000). The repulsive response of commissural axons to semaphorin signals is triggered by Shh (Parra & Zou 2010) and GDNF and is mediated through NCAM/GFRa1 signaling (Charoy et al. 2012). Finally, stem cell factor (SCF) has a role in promoting the growth of axons expressing the SCF receptor Kit away from the midline (Gore et al. 2008).

Most CINs project rostrally after crossing the midline. This behavior is triggered by postcrossing attraction to high levels of Wnt signaling in the rostral region of the spinal cord (Lyuksyutova et al. 2003). In chicks, this rostral to caudal gradient of Wnt activity is established by an opposing Shh gradient, which induces expression of the gene encoding the Wnt antagonist Secreted frizzled-related protein 1 (Sfrp1) (Domanitskaya et al. 2010) (**Figure 2*g***). Furthermore, Shh has repulsive effects in commissural axons that are mediated through Hedgehog interacting protein (Hip) and 14-3-3 proteins (Bourikas et al. 2005, Yam et al. 2012). If Wnt signaling is abrogated, commissural axons adopt an aberrant longitudinal direction upon crossing the midline (Lyuksyutova et al. 2003).

Spinal interneurons that project ipsilaterally rely on repulsive signals generated in the midline. EphrinB3 expression in midline cells functions as a barrier to EphA4-expressing ipsilateral interneurons, impeding them from projecting axons to the contralateral side of the spinal cord. In mice lacking *Epha4* or *ephrinb3*, interneurons that would normally project ipsilaterally project across the midline, presumably owing to the influence of attractive cues such as Netrin and Shh (Butt et al. 2005, Kullander et al. 2003) (**Figure 2*h***). Signaling through EphA4 and ephrinb3 are also essential for the wiring of ascending and descending pathways that ensure communication between spinal circuits and higher brain centers (Paixão et al. 2013).

Taken together, the examples described above illustrate that connectivity within motor circuits relies on the ability of neurons to integrate cues from multiple guidance systems. A major challenge in the future will be to resolve how neuronal subtype identity determinants orchestrate the expression of factors involved in guidance and synaptic specificity decisions. With a handful of exceptions, the downstream effectors of most intrinsic determinants are unknown.

CONSTRUCTION OF LOCAL SENSORIMOTOR CIRCUITS

Proprioceptive sensory neuron (pSN): a class of sensory neuron located in the dorsal root ganglia that relays muscle status information to the central nervous system

Coordination of motor output depends on sensory feedback to inform the CNS of both external stimuli and muscle status in real time. All sensory neurons (SNs) extend projections to peripheral targets and relay with neurons within the CNS, whereas their cell bodies coalesce within DRG adjacent to the spinal cord. Similar to MNs, the molecular profile of SNs endows them with specific identities and functions. However, the organization of MNs and SNs diverges greatly. Whereas MNs merge into discrete columnar and pool subtypes, a single DRG accommodates SNs with varying peripheral targets as well as wholly unique modalities (Abraira & Ginty 2013, Dasen 2009).

Owing to the sheer complexity of the sensory microcircuits involved in facilitating motor output, attention has been placed on parsing the assembly of the comparatively simple monosynaptic reflex arc. Proprioceptive SNs (pSNs), which convey information about muscle position and contractile status, share expression of the TrkC receptor but otherwise deviate in firing rate patterns and the location at which their axons terminate within the spinal cord (Brown 1981, Snider 1994). Group Ia and II proprioceptive neurons synapse peripherally at spindle structures embedded in skeletal muscle and transmit information pertaining to changes in muscle length or stretch (Brown 1981, Brown & Fyffe 1978, Hoheisel et al. 1989, Scott 1992) (**Figure 3*a***). Group Ib proprioceptive neurons extend to Golgi tendon organs within muscle and synapse within the intermediate spinal cord, while Group II pSNs only project sparsely in the ventral spinal cord (Brown & Fyffe 1979). Group Ia central projections pervade the ventral spinal cord, where they synapse directly onto α-MNs innervating the same muscle at which the Ia afferents terminate in the periphery (Brown 1981, Burke & Glenn 1996, Eccles et al. 1957, Mears & Frank 1997). This Ia pSN/α-MN/muscle trifecta forms a feedback loop—the crux of the monosynaptic spinal reflex arc and the core of homeostatic muscle activity maintenance (Windhorst 2007). Group Ia pSNs also synapse onto MNs innervating synergistic muscles and inhibitory interneurons involved in the control of antagonistic muscle activity (Brown 1981, Brown & Fyffe 1978).

Guiding Sensory Neurons to Peripheral Muscle Targets

Studies of the ontogeny of pSN-MN connectivity have provided insights regarding the choreographic events of locomotor circuit assembly. Upon exiting the ventral spinal cord, motor axons converge with sensory axons emanating from the neighboring DRG (Honig et al. 1998). Although motor and sensory axons seemingly take the same journey, the axons of the MNs precede those of SNs (Landmesser & Honig 1986, Tosney & Landmesser 1985). The conventional notion was that MNs break ground as they actively seek out their peripheral targets, and sensory axons are then passively ushered toward the appropriate target by way of established MN tracks (Matise & Lance-Jones 1996). Early studies in which MNs were ablated prior to axon outgrowth corroborated this hypothesis, demonstrating that in such instances sensory projections to muscle are almost completely abolished (Landmesser & Honig 1986, Scott 1988, Swanson & Lewis 1986, Tosney & Hageman 1989). Subsequent studies, however, suggested that correct sensory-muscle targeting remains generally intact and is perhaps less dependent on motor axon pathfinding than previously thought (Wang & Scott 1999). Previous experiments involved ablation of MNs prior to SN differentiation, explaining the disparity between these findings: Resultant observations reflected changes in unspecified neural crest cells rather than differentiated SNs.

Experiments using genetic ablation techniques validate and modify aspects of both hypotheses. In SNs, loss of the axon guidance receptor, Neuropilin-1 (Npn-1), elicits increased defasciculation of both motor and sensory axon bundles and also results in leading sensory axons relative to motor

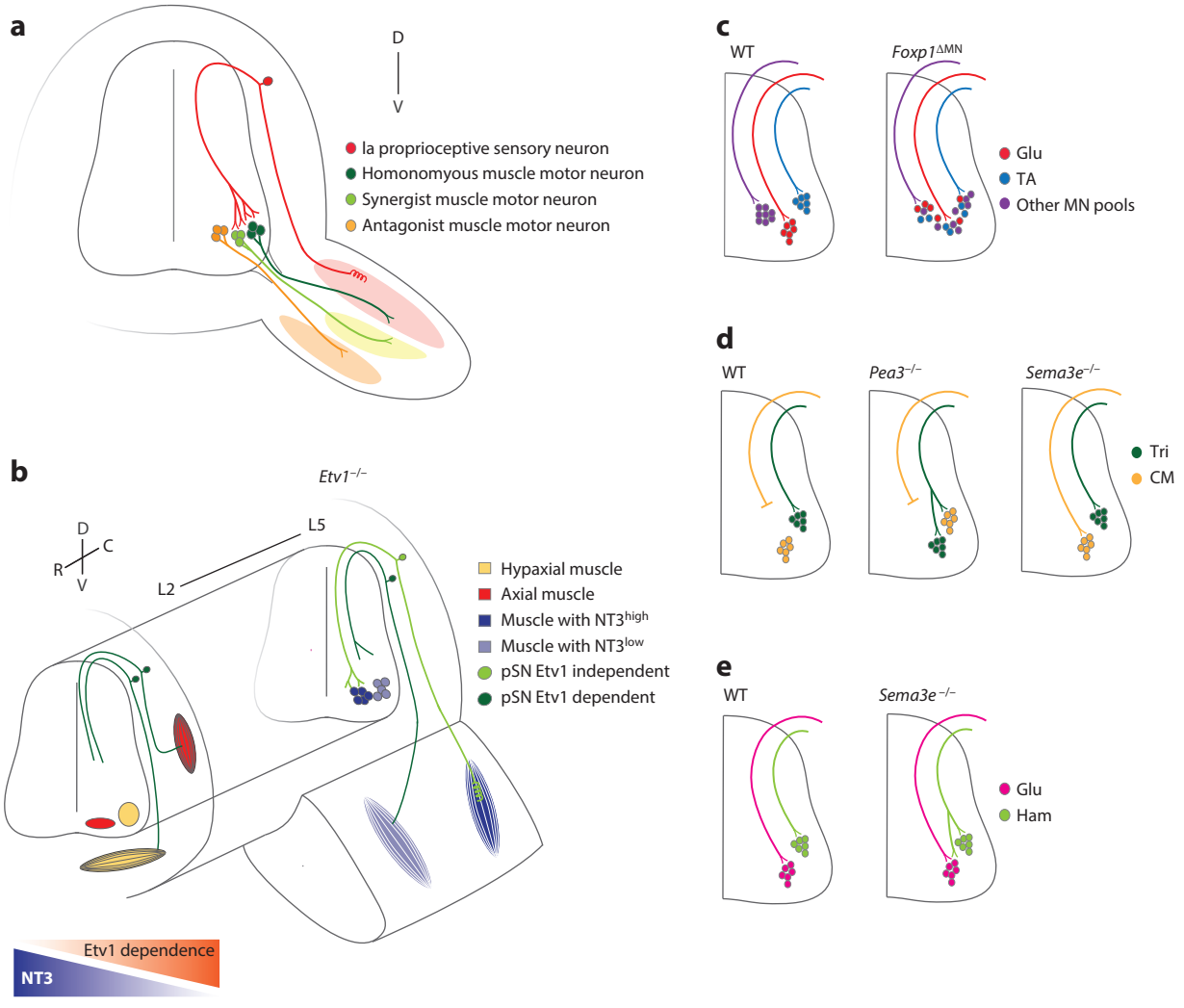


Figure 3

Sensorimotor circuits. (*a*) Simplified diagram of the monosynaptic reflex circuit. Proprioceptive sensory neurons (pSNs) relay information to the spinal cord, where they synapse onto motor neurons (MNs) innervating the same muscle peripherally. A pSN targeting a specific muscle also establishes secondary connections to spinal MNs innervating muscle synergists but avoids making connections to MNs targeting antagonistic muscles. (*b*) pSNs express the transcription factor Etv1, which is induced by TrkC/NT3 signaling. In *Etv1* mutant mice, most pSNs targeting hypaxial and axial muscles fail to establish appropriate central and peripheral connections. In the hindlimb, SNs targeting muscles with high NT3 expression levels form normal connections, whereas those targeting muscles with low NT3 expression do not. (*c*) In *Foxp1* mutant mice, MNs target limb muscles, but their position is randomized within the spinal cord. However, as in wild-type (WT) mice, hindlimb Ia afferents target their stereotypic dorsoventral zones in the spinal cord. (*d*) In WT mice, the Pea3^{+} cutaneous maximus (CM) motor pool does not receive monosynaptic input from pSNs. In *Pea3* mutants, the relative position of CM and triceps MNs are inverted. Triceps (Tri) Ia afferents form normal connections with triceps MNs but also ectopically target CM and latissimus dorsi (LD) MNs. In *Sema3e* mutants, CM MNs receive ectopic inputs from CM pSNs, with no change in triceps sensory input. (*e*) At the hindlimb level, gluteus (Glu) MNs express *Sema3e*, whereas hamstring (Ham) MNs lack *Sema3e*. In *Sema3e* mutants, Glu MNs receive ectopic input from Ham pSNs.

axons (Huettl et al. 2011). Depletion of Npn-1 in MNs leads to defasciculation only in motor axons. In either case, the general trajectory of MN and SN axons remains similar to wild type. Genetic ablation of MNs has revealed that sensory axons need only a minimal scaffold to project into the distal limb. From these analyses, the authors argue that SNs require MNs to project toward and into the plexus at early stages, but after reaching the plexus, sensory target navigation is MN independent (Huettl et al. 2011). This implies that rather than piggybacking onto MN axons, SNs participate in interaxon communication with MNs and that, although initially dependent on motor axons to enter the plexus, extrinsic guidance cues direct sensory target specificity beyond the plexus. Evidence of heterotypic axon-axon interaction has been demonstrated, showing that the loss of contact-dependent repulsion by ephrinA-EphA signaling between MNs and SNs results in (*a*) invasion of axial muscle–targeting MN axons into the DRG and (*b*) abnormal SN burst activity mirroring the rhythmicity of MN activity (Gallarda et al. 2008, Wang et al. 2011).

Peripheral Signaling in Proprioceptive Sensory Neuron–Motor Neuron Connectivity

A major roadblock in understanding the mechanisms underlying pSN subtype identity and connectivity has been the lack of intrinsic determinants comparable to those in MN pools. Similar to MNs, basic features of pSN class identity are determined by a core set of transcription factors, including Runx3 and Etv1 (Dasen 2009). Although intrinsic pSN target-matching determinants may very well exist, evidence suggests that graded peripheral signaling may be at least one alternative mechanism responsible for pSN target selectivity (de Nooij et al. 2013). pSNs require TrkC activation by neurotrophin 3 (NT3) to survive during early development, and TrkC/NT3 signaling promotes expression of *Etv1* in pSNs (Arber et al. 2000, Farinas et al. 1994, Klein et al. 1994, Patel et al. 2003). Deletion of *Etv1* redirects pSNs to terminate in an uncharacteristic intermediate position within the spinal cord (Arber et al. 2000), suggesting that activation of the *Etv1* gene by NT3 is a key step in the central targeting of pSN axons to MNs.

Further analysis has revealed marked variability in the requirement for Etv1 in pSN survival and connectivity (de Nooij et al. 2013). Although pSNs targeting hypaxial/axial muscles depend on Etv1, limb pSNs display specific requirements related to the level of NT-3 that a specific muscle expresses. The pSNs that survive in the absence of Etv1 target hindlimb muscles that express higher levels of NT3 than other hindlimb muscles. By increasing muscle NT3 levels, and thereby bypassing the necessity of Etv1, the number of pSNs in *Etv1* mutants can be restored to wild-type levels. These findings follow the logic of a previous study by Li et al. (2006), which stated that Etv1 and NT3 trigger similar molecular pathways and therefore pSNs targeting NT3-expressing muscles can survive in the absence of Etv1. The variable degree of reliance of pSNs on Etv1, which is ultimately a function of the differential expression of NT3 by pSN peripheral targets, serves as a means of pSN diversification (**Figure 3*b***). This paradigm may also afford peripheral targets a considerable amount of influence in shaping the central projections of pSNs within the spinal cord.

Role of Synaptic Specificity Determinants and Motor Neuron Position in Circuit Assembly

Within the Ia stretch-reflex circuit, pSNs and MNs projecting to the same peripheral muscle target establish selective central connections with an impressive degree of fidelity (Eccles et al. 1957, Mears & Frank 1997). Even in the absence of activity, homonymous SN-MN pairs persist, suggesting that predisposing molecular determinants drive sensory and motor complements to find one another (Frank 1990, Mendelson & Frank 1991). Remarkably, each pSN targeting a single

muscle in the periphery establishes monosynaptic connections with each of the ~50–200 MNs targeting the same muscle (Mendell & Henneman 1968). How this remarkable example of synaptic specificity is achieved is not well understood, although recent studies have provided insights into the potential underlying mechanisms.

One surface recognition system that has been suggested to drive wiring specificity between sensory afferents and MNs is semaphorin-plexin signaling (Pecho-Vrieseling et al. 2009). At the brachial levels of the spinal cord, *Sema3e* is selectively expressed within the MN pool targeting the cutaneous maximus (CM) muscle, with corollary expression of the semaphorin-binding receptor, Plxnd1, in CM-associated pSNs (Pecho-Vrieseling et al. 2009). CM proprioceptive axons are repelled by *Sema3e* expression in CM MNs, and this repulsion accounts for the absence of monosynaptic input from pSNs to this particular motor pool. Removal of *Sema3e* gene function permits monosynaptic input from CM proprioceptors onto CM MNs (**Figure 3*c***). The connectivity of SNs to MNs at the hindlimb level is also dependent on Sema3e- Plxnd1 signaling. Loss of either *Sema3e* in gluteus-targeting MNs or *Plxnd1* in hamstring-related pSNs leads to ectopic hamstring pSN input onto gluteus MNs, indicating that sema-plexin signaling contributes to MN-pSN specificity (Fukuhara et al. 2013).

Because different cell types in the spinal cord have stereotypic settling positions related to their identity, the dorsoventral and mediolateral coordinates of MN pools may be a key factor in directing SNs to match MN targets. The possible role of neuronal positioning in sensorimotor connectivity is supported by analysis of *Foxp1* mutant mice. An initial assessment of a constitutive *Foxp1* mutant mouse revealed a random distribution of MN pools targeting specific muscles rather than the stereotypic clusters (Dasen et al. 2008). In mice with a conditional deletion of *Foxp1* from MNs, pSN afferents target their characteristic dorsoventral zones within the spinal cord (Surmeli et al. 2011) (**Figure 3*c***). These SNs indiscriminately establish synapses onto MNs, despite their lack of pool identity (**Figure 3*c***). In contrast to these studies, deletion of the gene encoding the pool-restricted factor Pea3 leads to an inversion of the positions of the triceps and CM pools but does not alter the specificity of triceps pSN input onto triceps MNs (Vrieseling & Arber 2006). These observations suggest that both MN position–dependent and –independent programs contribute to pSN-MN specificity.

DEVELOPMENT OF SPINAL CIRCUITS FOR LOCOMOTION

A major task of circuits within the spinal cord is to coordinate the activation of muscle groups during locomotor behaviors. Spinal circuits govern the basic pattern of muscle activation through the connections established between highly diverse interneuron and MN populations (Goulding 2009, Grillner 2006, Kiehn 2011). Genetic and electrophysiological studies have defined functional roles for a significant proportion of interneuron classes within mammalian locomotor CPGs. Recent studies have elucidated the modular nature of CPG microcircuits and revealed how specific interneuron subtypes contribute to the diversity of locomotor behaviors.

Mammalian CPG networks consist of interconnected circuits that govern two distinct types of motor pattern. One circuit facilitates communication between the left and right sides of the spinal cord (L-R CPG) and ensures alternate activation of MN pools across the midline during walking. A second governs the coordination between muscle antagonists residing on one side of the spinal cord, such as extensors and flexors (E-F CPG). Both CPG circuits are characterized by regular bursts of MN firing, and local excitatory and inhibitory interneurons are thought to control this rhythmic pattern. The major components of CPG circuits derive from the four classes of ventral interneurons (V0, V1, V2, and V3) but also appear to include several dorsally derived populations. Most V0 and V3 interneurons are commissural, whereas V1 and V2 interneurons

project ipsilaterally. In this section, we discuss the role of these interneuron populations in CPG networks, emphasizing their roles in establishing the rhythmicity and pattern of output from locomotor circuits.

Excitatory Interneurons Contribute to Central Pattern Generator Rhythm Generation

A hallmark feature of CPGs is the ability to produce a motor pattern independently of external inputs from other regions of the nervous system. The basic physiological properties and composition of vertebrate CPG networks has been most thoroughly investigated in species with relatively simple nervous systems, such as the lamprey (Grillner 2006) (**Figure 4*a***). In lamprey, the locomotor CPG controls the pattern of MN firing along the spinal cord, but each segment is capable of independently generating appropriate rhythmic patterns of output. Pharmacological and electrophysiological studies indicate this rhythm is established by ipsilaterally projecting excitatory interneurons that burst at regular intervals.

Studies attempting to define the rhythm-generating neurons in the mammalian locomotor CPG have focused on defining populations of ipsilateral excitatory interneurons. The most prominent of such neurons is the V2a subtype, which establishes direct connections with MNs. In mice ablated for V2a neurons, however, the spinal cord is still capable of generating a locomotor rhythm (Crone et al. 2008). Additional, smaller populations of excitatory interneurons, such as those expressing the transcription factors Hb9 and Shox2, fire in an oscillatory manner during drug-induced locomotor activity (Dougherty et al. 2013, Wilson et al. 2005). Silencing the output of Shox2 interneurons significantly reduces the frequency of locomotor bursts, with no effect on the pattern of L-R or E-F CPG output (Dougherty et al. 2013). In the absence of Shox2 interneuron function, motor bursting remains, suggesting that multiple excitatory populations contribute to rhythm generation.

Commissural Interneurons Facilitate Motor Coordination Across the Midline

Communication between the left and right sides of the spinal cord is essential for limb alternation in animals that walk and for synchronous activation of limbs in species that fly or hop. The pattern of MN bursting across both sides of the spinal cord is governed by CINs that project across the midline (**Figure 4*b***). Disruption of commissural communication in rodents via hemisection of the spinal cord leads to discoordination in the L-R CPG. Moreover, pharmacological manipulations that block inhibitory transmission can lead to synchronous activity of both left and right sides of the spinal cord (Cowley & Schmidt 1997), suggesting a critical role for inhibitory interneurons.

Early genetic studies hinted that coordination of the L-R CPG depends on the relative number of excitatory and inhibitory interneurons crossing the midline. Mutations that cause ipsilaterally projecting excitatory interneuron populations to ectopically cross the midline can switch locomotor gaits from alternation to a hopping-like behavior. In mice mutant for the *EphA4* or *ephrinB3* gene, the activity between left and right sides of the spinal cord switches from alternation to synchronous activity (Borgius et al. 2014, Kullander et al. 2003). EphrinB3 is expressed at the midline, repelling the axons of EphA4^{+} interneurons and preventing them from crossing. EphA4 is expressed by several classes of excitatory interneurons, as well as cortical spinal tract (CST) neurons that project from the cortex to MNs, suggesting that synchrony results from ectopic projections from multiple populations (**Figure 4*d***). Interestingly, deletion of *EphA4* selectively from CST neurons does not affect basic locomotor output but leads to bilateral voluntary movement in the limbs, due to CST misprojections (Serradj et al. 2014). Moreover, in mice mutant for the

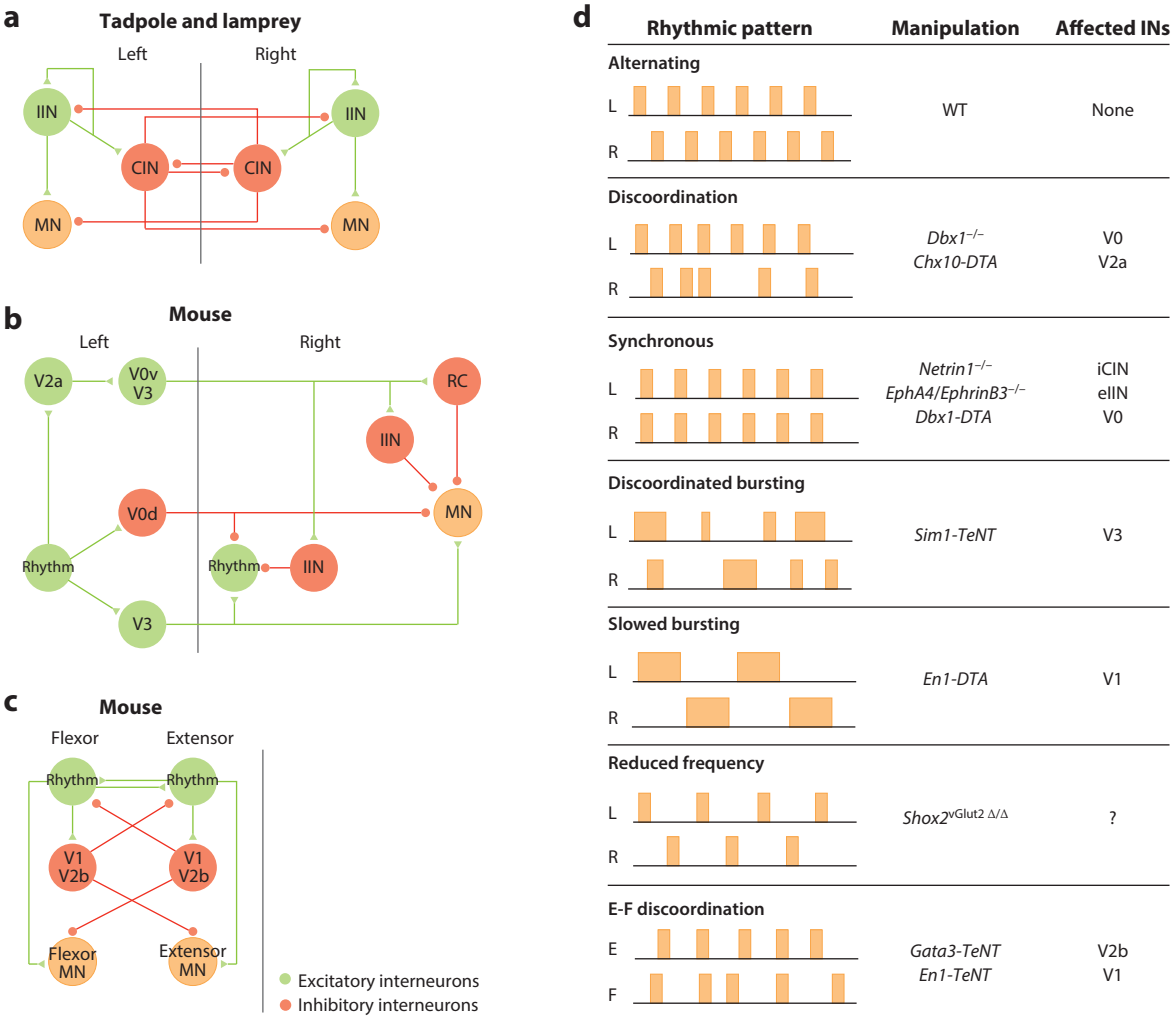


Figure 4

Locomotor central pattern generators (CPGs). (*a*) Simplified circuit model for undulatory locomotion in lamprey and tadpoles. Excitatory ipsilateral interneurons (IINs) drive rhythmic activation of motor neurons (MNs) to produce muscle contractions within a segment. Inhibitory commissural interneurons (CINs) ensure alternation between both sides of the spinal cord by inhibiting excitatory IINs and MNs on the contralateral side. Excitatory neurons are shown in green and inhibitory neurons in red. (*b*) Model of the mammalian left-right (L-R) CPG (Talpalar et al. 2013). Inhibitory commissural V0d interneurons (INs) play a key role in L-R alternation by inhibiting rhythm generators and MNs on the contralateral side. Excitatory V2a and V3 INs also have important roles in securing and stabilizing the L-R pattern. (*c*) The extensor-flexor (E-F) CPG. Ipsilateral inhibitory INs derived from the V1 and V2b classes ensure alternation of extensor and flexor muscles. (*d*) Summary of the effects of gene manipulation on the pattern of locomotor output from the spinal cord. Bursts of MN firing in wild-type (WT) and mutant cells are indicated by rectangles, and the INs affected are indicated. Defects are typically measured through ventral root recordings at lumbar levels of the spinal cord in fictive locomotor assays. Abbreviation: RC, Renshaw cell.

genes encoding the midline attractant Ntn1 or its receptor DCC, an imbalance in the number of inhibitory and excitatory spinal interneurons crossing the midline leads to L-R discoordination (Rabe Bernhardt et al. 2012, Rabe et al. 2009).

Interneuron Subtypes Involved in Left-Right Coordination Within Central Pattern Generator Networks

Although analysis of *EphA4* and *ephrinB3* mutants has revealed the importance of restricting the crossing of excitatory interneurons, L-R motor coordination during normal development is orchestrated primarily through inhibitory CINs. In mice, CINs derived from progenitor domain p0 play a major role in governing L-R CPG output. Mutation in the gene encoding the transcription factor Dbx1, which is expressed by p0 progenitors, causes discoordination between the left and right halves of the spinal cord (Lanuza et al. 2004). By contrast, ablation of Dbx1 lineages leads to synchronous activation of MNs on both sides of the spinal cord (Talpalar et al. 2013). Why this difference in phenotypes? In *Dbx1* mutants, interneurons acquire the fates of the adjacent dI6 and V1 interneuron populations (Gosgnach et al. 2006), whereas in ablation experiments, the distribution of other interneurons is unchanged.

Excitatory interneurons also have key roles in securing the pattern of alternation between both sides of the spinal cord. Ablation of ipsilaterally projecting V2a interneurons leads to a discoordination of L-R CPGs without affecting E-F alternation (Crone et al. 2008). V2a neurons form direct contacts onto V0 interneurons, indicating that they participate in an indirect pathway to facilitate L-R alternation (**Figure 4*b***). V3 excitatory CINs also play an accessory role in coordinating L-R alternation, as genetic silencing of their output erodes the normal coordination between both sides of the spinal cord. V3 interneurons are also required for the appropriate balancing of burst duration in the locomotor CPG, as manipulating V3 firing leads to increased variability in the duration of locomotor bursts (Zhang et al. 2008) (**Figure 4*d***).

Central Pattern Generators for Extensor-Flexor Coordination

The neuronal constituents of the E-F CPG have proved more elusive. Much of our understanding of E-F CPGs derives from fictive locomotor assays at the lumbar level of the spinal cord, where alternations between ventral root bursts recorded at L2 and L5 provide a rough approximation of extensor and flexor activities, respectively. In manipulations that affect L-R CPGs, the output of E-F CPGs is typically preserved. Because reciprocal activation of E-F muscles relies on connections established on one side of the spinal cord, attention has focused on ipsilaterally projecting interneuron populations that target MNs. Pharmacological manipulations that block inhibitory transmission disrupt the alternating patterns of bursting between L2 and L5 (Cowley & Schmidt 1995), whereas E-F alternation is preserved after genetic ablation of excitatory glutamatergic transmission in the ventral spinal cord (Talpalar et al. 2011). These observations suggest that local inhibitory interneurons are critical for ensuring reciprocal activation of extensors and flexors during locomotion.

Because V1 neurons represent one of the largest classes of inhibitory ipsilateral interneurons, studies have focused on these subtypes as mediators of E-F CPGs. In genetic manipulations that silence the output of V1 interneurons, however, E-F alternation is largely preserved, as assessed by fictive locomotor assays (Gosgnach et al. 2006). Instead, E-F CPGs appear to rely on the combined action of V1 and V2b interneurons, as silencing of both populations leads to E-F discoordination (Zhang et al. 2014) (**Figure 4*c***). Given that standard assays for E-F CPGs rely on ventral root

recordings that sample large populations of MN pools, it is likely that V1 and V2b interneurons have independent roles in E-F coordination that are obscured by the currently available methods.

Premotor: any of the various classes of neurons that synapse directly onto motor neurons

Modularity of Central Pattern Generator Composition and Locomotor Gaits

Recent studies have also provided basic insights into how specific interneuron populations are recruited during changes in gait patterns and locomotor speeds. The classically defined interneuron classes can be divided into many subtypes, each of which contributes to a distinct aspect of locomotor output. The V0 class segregates into both excitatory (V0v) and inhibitory (V0d) subtypes. Whereas initial studies suggested that excitatory Evx1$^+$ V0v neurons are dispensable for L-R alternation (Lanuza et al. 2004), further studies indicated that they are essential when rodents change locomotor speeds (Talpalar et al. 2013). Genetic silencing of V0v switches the gait pattern to hopping at high and medium velocities, whereas ablation of V0d function affects L-R coordination at low and medium frequencies. Similar speed-dependent changes in locomotor output are observed after genetic manipulation of V2a interneurons (Crone et al. 2009, Zhong et al. 2011). Collectively, these studies indicate that an essential parameter of gait control involves the recruitment of specific interneuron subtypes at different locomotor velocities.

Genetic analysis of horse breeds has also demonstrated that the composition of CPG circuits has a key role in defining animal gait. These studies have revealed that interneurons derived from dorsal lineages constrain the types of gaits displayed by specific breeds (Andersson et al. 2012). A natural mutation in the *Dmrt3* gene allows for a specific type of gait—pacing—observed in Icelandic horse species. In mice, Dmrt3 is expressed within the dI6 lineage, a relatively understudied interneuron class that has been shown to be rhythmically active during fictive locomotion (Dyck et al. 2012). Mutation in the *Dmrt3* gene leads to a discoordination in the output of L-R CPG networks, consistent with an important role for dI6 neurons in facilitating locomotor output. This research reveals that changes to a single interneuron subtype can have a significant impact on the pattern of locomotor output, and hence motor behavior.

Comparison of Mammalian Central Pattern Generators with Those of Other Vertebrate Species

In contrast to most land vertebrates, aquatic species typically generate propulsive locomotor forces through undulation of the tail fin. Although the muscles recruited during swimming are fundamentally different from those of limbed animals (i.e., axial versus limb muscles), much of the logic of the premotor CPG circuit organization is conserved across vertebrate species. Many of the basic interneuron classes are present in both zebrafish and rodents (Grillner & Manira 2015), and it has been argued that elements of the mammalian CPG network evolved from cooption of axial circuits used for postural correction in fish (Bagnall & McLean 2014).

A significant difference between fish and mammals is how MN subtypes are recruited at different locomotor speeds. In zebrafish, MNs are recruited in a dorsal to ventral order that is scaled to the speed of locomotion (Fetcho & McLean 2010). MNs driving slow-speed swimming are located ventrally, and fast MNs are located dorsally. A recent study indicates that excitatory V2a interneuron subclasses are also differentially recruited to drive swimming at slow, intermediate, and fast speeds (Ampatzis et al. 2014). Thus, although zebrafish and mammals utilize common neuronal elements within CPG circuits, they appear to organize these circuits in very different ways to achieve specific types of motor outputs.

COMMUNICATION BETWEEN THE BRAIN AND SPINAL CORD

Coordinate movement relies on the continuous flow of information between the periphery, the spinal cord, and the brain. Communication across these regions involves multiple circuits that integrate peripherally and centrally generated motor information. We describe some of the general features of ascending and descending motor pathways as well as more recent molecular characterization of the neuronal populations involved in generating motor behaviors.

Mapping Novel Circuits from the Spinal Cord to the Brain

The onset of locomotion is elicited by motor commands originating from higher brain centers, which are transmitted to the spinal cord to achieve a specific action. This process recruits discrete circuits within the cortex, midbrain, cerebellum, brainstem, and spinal cord. Owing to the complex nature of motor circuits, dissecting the circuits responsible for a particular action seems daunting. However, recently developed transsynaptic viral tracing techniques have begun to unravel the basic anatomical locations of premotor populations. Furthermore, by combining this technique with genetic tools, it is now feasible to characterize the molecular profiles of these networks (Stepien et al. 2010, Wall et al. 2010).

Comparative analysis of premotor populations has revealed that the descending and local spinal circuits controlling specific muscles groups are quite distinct. For example, the spinal interneurons connecting to antagonistic extensor or flexor muscles are segregated spatially along the mediolateral axis (Tripodi et al. 2011) (**Figure 5*a***). Moreover, comparison of the distribution of premotor neurons targeting LMC and MMC neurons reveals strikingly distinct anatomical organization. Whereas MMC premotor interneurons are evenly distributed across both sides of the spinal cord, LMC premotor interneurons reside predominantly on the ipsilateral side (**Figure 5*b***). This may reflect a dependency on bilateral coordination of axial muscles during postural stabilization, whereas control of the limbs can occur in a more independent fashion (Goetz et al. 2015). Furthermore, strikingly different premotor brainstem nuclei target forelimb and hindlimb LMC neurons (Esposito et al. 2014) (**Figure 5*c***). These observations suggest that distinct muscle groups engage almost nonoverlapping descending pathways depending on the type of motor task, such as walking or grasping.

A similar approach using transsynaptic tracing methods has demonstrated that hindlimb-innervating MNs receive direct inputs from interneurons located in the deep dorsal horn of the spinal cord. These interneurons, termed motor synergy encoder (MSE) neurons, receive both corticospinal and proprioceptor inputs and are suggested to activate groups of synergist muscles during movement (Levine et al. 2014). MSE neurons are hypothesized to simplify the task of the nervous system, such that instead of relying on a large number of interneuron subtypes to independently control individual limb muscles, motor networks can manage a more limited number of motor units with a smaller number of variables.

Relay of Locomotor Sensory Information

Sensory signals relayed by proprioceptor neurons convey information about muscle contractile status and position. In addition to relaying peripheral events to spinal circuits, proprioceptor neurons transmit the same information to the brainstem and cerebellar regions by extending their axons along specific ascending tracts. SNs innervating forelimb muscles project centrally to the external cuneate nucleus within the brainstem via the cuneocerebellar tract, whereas those innervating the hindlimb primarily synapse on Clarke's column neurons in the spinal cord. Clarke's

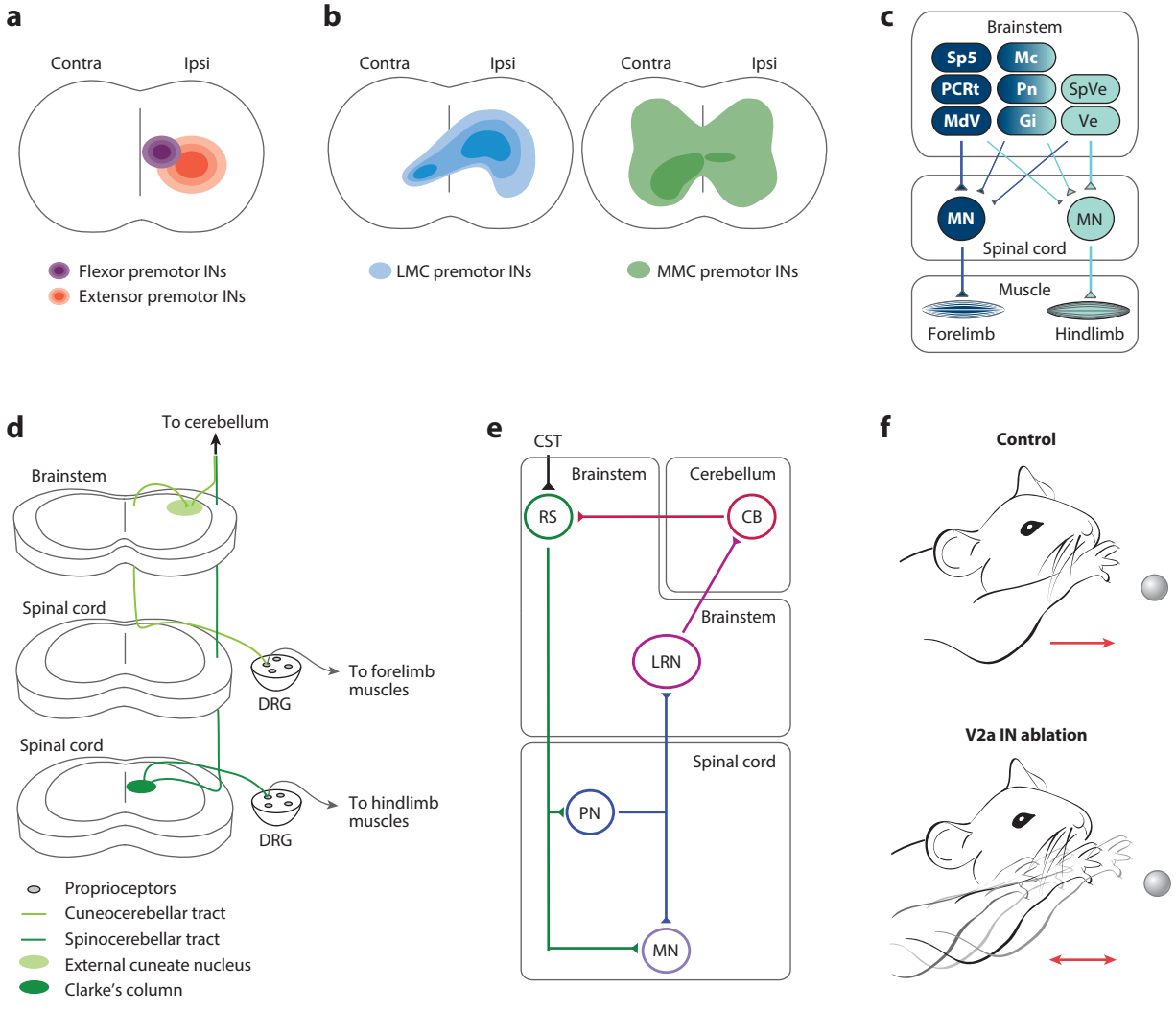


Figure 5

Circuits facilitating communication between the spinal cord and the brain. (*a*) Diagram illustrating the distribution of extensor and flexor premotor IN populations within the postnatal spinal cord. Color shades reflect cell densities. (*b*) LMC premotor INs are localized predominantly on the Ipsi side of the spinal cord, whereas those synapsing onto MMC neurons are equally distributed. (*c*) Differences between brainstem nuclei connecting with motor neurons of the forelimb (*dark blue*) or hindlimb (*light blue*). (*d*) pSNs projecting to forelimb muscles relay information to the external cuneate nucleus through the cuneocerebellar tract. By contrast, hindlimb pSNs synapse on Clarke's column neurons, which in turn project to the cerebellum via the spinocerebellar tract. (*e*) Diagram illustrating the circuitry involved in forelimb reaching. For simplicity, direct corticospinal projections to the spinal cord have been omitted. (*f*) In mice in which V2a interneurons are ablated, reaching tasks are performed at a slower velocity with an increased failure rate. The arrow illustrates forelimb position, which alternates between forward and reverse directions after V2a ablation. Abbreviations: CB, cerebellum; Contra, contralateral; CST, corticospinal tract; DRG, dorsal root ganglia; IN, interneuron; Ipsi, ipsilateral; LMC, lateral motor column; LRN, lateral reticular nucleus; MMC, medial motor column; MN, motor neuron; PN, pontine nucleus; pSN, proprioceptive sensory neuron; RS, reticulospinal nucleus.

column neurons, in turn, project to the cerebellum via the dorsal spinocerebellar tract (Bosco & Poppele 2001, Windhorst 2007) (**Figure 5*d***).

The information conveyed by proprioceptors is vital not only for movement perception but also for necessary adjustments to trajectory or velocity during locomotion. The fine-tuning of motor behaviors occurs faster than sensory feedback from the periphery can be relayed between the spinal cord and higher brain centers. This conundrum led to the idea of internal efferent copies of motor commands, which allow rapid motion correction without the need to rely solely on peripheral feedback (Wolpert & Miall 1996). Efferent copies encode predictive information about both motor output and sensory feedback, allowing constant update and fast adjustment of the planned action. Different variations of efferent copies permit organisms to distinguish self-generated movement from environmental influences on the body, estimate kinematic variables (e.g., trajectory and velocity), plan movements, and overcome time delays that lead to erroneous motor tasks. The source of these predictive models is not completely clear (Crapse & Sommer 2008, Poulet & Hedwig 2007, Todorov 2004). Most likely, different types of models are generated within the cortex, the brainstem, or the cerebellum and then transmitted to spinal centers. Interestingly, Clarke's column has been suggested to work as a convergent site for corticospinal and proprioceptor inputs, functioning as a local spinal hub capable of modulating sensory information (Hantman & Jessell 2010). One might speculate, therefore, that other spinal or brain stem nuclei important for somatosensory relay work similarly in planning and fine-tuning body motion.

Spinal Circuits for Skilled Limb Control

Skilled forelimb movement is a complex voluntary action involving precise movements necessary for object manipulation, such as reaching and grasping. This motor skill relies on engagement of descending pathways, including the corticomotor and corticospinal tracts (Isa et al. 2007). In primates, the main pathway involved in reaching and grasping is the direct monosynaptic corticomotor pathway, whose axons project directly from the cortex to spinal MNs. Studies in primates have revealed that lesions to the spinal cord result in defects in both reaching and grasping abilities (Lawrence & Kuypers 1968). Interestingly, the corticomotor tract is exclusive to primates, suggesting that the precision of hand dexterity observed in higher mammals evolved with its onset (Lemon 2008). By contrast, in mammals such as cats and rodents, forelimb actions require connections from the cortex to spinal interneurons (i.e., the corticospinal tract); these spinal interneurons include propriospinal interneurons (PNs). Electrophysiological and lesion studies have identified PNs at the C3–C4 segments of the spinal cord. The axons of these neurons bifurcate, establishing connections with both MNs and lateral reticular nucleus (LRN) neurons in the brainstem, which, in turn, relay information to the cerebellum (**Figure 5*e***). PNs also receive convergent inputs from rubrospinal, reticulospinal, and tectospinal tracts originating in the midbrain and brain stem (Isa et al. 2007, Lemon 2008). Interestingly, PNs are also present in primates and have the capacity to compensate for loss of corticomotor function during forelimb movement (Alstermark et al. 1999). Silencing PNs in monkeys results in difficulties performing reaching tasks (Kinoshita et al. 2012).

Recently, optogenetic, electrophysiological, genetic, and transsynaptic labeling techniques have unraveled the molecular identity of PNs. Azim et al. (2014) have identified two criteria for rodent PNs: neurons that (*a*) have dual projections to forelimb innervating MNs and the LRN and (*b*) simultaneously receive inputs from reticulospinal neurons. Neurons that meet these conditions are not confined to C3–C4 segments but extend to other segments of the brachial spinal cord, up to segment T1. Mouse PNs comprise a subpopulation of excitatory interneurons belonging to the

V2a class. Ablation of these cells causes defects in reaching tasks, but grasping ability is preserved (**Figure 5*f***).

An independent study revealed that PNs derive not only from the V2a class but also from the V1, V3, and dI3 populations (Pivetta et al. 2014). Azim et al. (2014) and Pivetta et al. (2014) have speculated that the circuit involving PN and LRN neurons functions as a fast-adjusting system for motor output, bypassing higher motor brain centers. By relaying information from the cortex directly to the LRN, and from there to the cerebellum, PNs send an internal copy of the planned movement. The cerebellum communicates the copy to reticulospinal neurons, which in turn synapse on MNs. This feedback loop allows constant adjustment of movement, ensuring correct performance of the planned task (Azim et al. 2014). The smooth execution of the reaching movement also relies on presynaptic inhibition of proprioceptor terminals by GABAergic interneurons expressing Gad2 (Fink et al. 2014).

Specific spinal interneuron populations involved in grasping abilities have also been identified. dI3 interneurons have been implicated in grasping movement by relaying cutaneous sensory information that is necessary for handgrip directly to MNs (Bui et al. 2013). Moreover, both grasping and reaching ability appear to involve the brainstem nucleus medullary reticular formation ventral part (MdV) (Esposito et al. 2014). This brainstem population synapses directly on MNs, as well as excitatory and inhibitory interneurons, and receives inputs from higher centers in the brain. Ablation of glutamatergic neurons in the MdV nucleus results in aberrant skilled motor movement. Finally, a recent study has identified a population of dorsal horn interneurons essential for corrective limb movements through integrating peripheral cutaneous inputs with descending motor commands (Bourane et al. 2015).

Collectively, these studies have defined the basic organization of spinal circuits required for fine motor skills and have begun to assess the function of specific neuronal classes in limb control.

CONCLUSIONS

Studies on the development of spinal circuits have provided fundamental insights into the principles governing neuronal subtype identity and connectivity within the CNS. Our comprehensive knowledge of the origins of most spinal neurons has enabled the generation of genetic tools that allow for precise manipulation of individual elements within spinal circuits. It is now feasible to not only understand how basic locomotor patterns are generated within the spinal cord but to resolve how sensory and supraspinal inputs modulate motor output. These studies should allow us to assess the roles of specific circuit modules in shaping the diverse motor behaviors expressed by animals.

It will also be important to define the intermediate pathways that link neuronal identity and connectivity with motor function. Although progress has been made in delineating many of the factors involved in the target specificity of MNs, very little is known regarding how interneurons establish specific connections with their synaptic partners. Modern tracing methods have advanced our understanding of the basic anatomy of spinal circuits and will ultimately provide a means to assess the requirements for specific genes and neuronal subtypes within spinal circuits. However, the sheer complexity of mammalian locomotor networks may stymy attempts to define the specificity determinants that contribute to their assembly. As relatively simple model systems, such as zebrafish and *Drosophila*, emerge as powerful genetic systems to study locomotor circuit assembly, new insights into the basic principles driving synaptic specificity will likely emerge. These studies could provide fundamental insights into conserved connectivity rules governing spinal circuit assembly and resolve how these programs evolved in various animal lineages.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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