Symposium/Mini-Symposium

Spinal Interneurons as Gatekeepers to Neuroplasticity after Injury or Disease

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Spinal interneurons are important facilitators and modulators of motor, sensory, and autonomic functions in the intact CNS. This heterogeneous population of neurons is now widely appreciated to be a key component of plasticity and recovery. This review highlights our current understanding of spinal interneuron heterogeneity, their contribution to control and modulation of motor and sensory functions, and how this role might change after traumatic spinal cord injury. We also offer a perspective for how treatments can optimize the contribution of interneurons to functional improvement.

Key words: **interneuron; plasticity; propriospinal; spinal cord**

Introduction

Some of the earliest documentation of cellular heterogeneity in the nervous system dates back to the 19th century when the pioneering studies by Ramon y Cajal characterized the vast diversity, complexity, and morphologic heterogeneity of the nervous system (Ramon y Cajal et al., 1995). Building on these data, Sir Charles Sherrington contributed to the classification of spinal cellular phenotypes with his work in canines, describing populations of spinal cells connecting multiple spinal cord segments that play a role in essential motor reflexes (Sherrington and Laslett, 1903a,b). These propriospinal neurons were further characterized via classical electrophysiology experiments, which provided evidence of long descending projections connecting cervical propriospinal neurons to lumbosacral motor pools (Lloyd, 1942; Lloyd and McIntyre, 1948). The overall structural organization and morphologic heterogeneity of the spinal cord were further elaborated by Rexed (1952, 1954), who used classical histologic methods to describe the anatomic distribution of spinal neurons classified by their size and shape and delineated the anatomic structure into dorsoventral "laminae" in the cat. With some effort over time, these laminae were associated with identifiable functions.

The spinal cord is comprised of two main types of neuronal populations: spinal interneurons (SpINs) and projection neurons. Projection neurons are those with the cell body within the spinal cord, but projections outside the spinal cord (either to other parts of CNS or periphery). SpINs are cells within the spinal cord that project to other cells within the spinal cord. SpINs comprise a vast range of neuronal types with unique properties and connectivity. These include (1) long and short propriospinal neurons, with ascending and descending projections; and (2) local SpINs with projections on the same side (ipsilateral) and/or that cross the spinal midline (commissural).

While anatomic studies were initially limited to general histologic or ultrastructural assessment, the development of transneuronal tracing (e.g., with pseudorabies virus) led to the identification of vast populations of SpINs integrated with motor, sensory, and autonomic networks. In addition, these approaches revealed neuroplastic changes within the connectivity of SpINs, and identified phenotypic subsets that may contribute to plasticity after traumatic spinal cord injury (SCI). Transgenic models have enabled developmental neurobiologists to better define populations of SpINs and their contribution to network function.

This review focuses on some of the recent discoveries that have propelled our understanding of the spinal cord as a site of convergence, divergence, and processing of multiple avenues of information, as well as revealing the rich anatomic and functional diversity of SpINs and their neuroplastic and therapeutic potential.

Molecular characterization of SpINs: from development to function

In the past few decades, developmental neurobiology has offered unique and important insights into the identity of progenitor cell

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types that contribute to SpIN classification. Genetic manipulations have enabled scientists to establish "cardinal classes" of spinal neurons, visualize their migration and connectivity, and define their specific roles in motor and sensory networks (for review, see (Goulding, 2009; Kiehn, 2016; Lai et al., 2016). The use of transcription factors expressed during development has helped define spinal neuron populations, but linking embryonic lineage with mature progeny has proved more difficult. Thus, genetic tools are being combined with other complementary approaches, including morphology, electrophysiology, and connectivity, to define cell phenotype (Fig. 1). These tremendous efforts have increased our understanding of the general diversity of interneurons, particularly within the ventrally (V0-V3) and dorsally (dIs) derived classes. Detailed overviews of the interneuronal classes and subclasses were provided previously (Dougherty and Kiehn, 2010b; Alaynick et al., 2011; Lu et al., 2015; Rybak et al., 2015; Flynn et al., 2017; Zholudeva et al., 2018a; Dobrott et al., 2019). Here, we draw on a few select populations of ventrally derived neurons to provide examples of heterogeneity and of the contributions SpINs make to spinal circuit function.

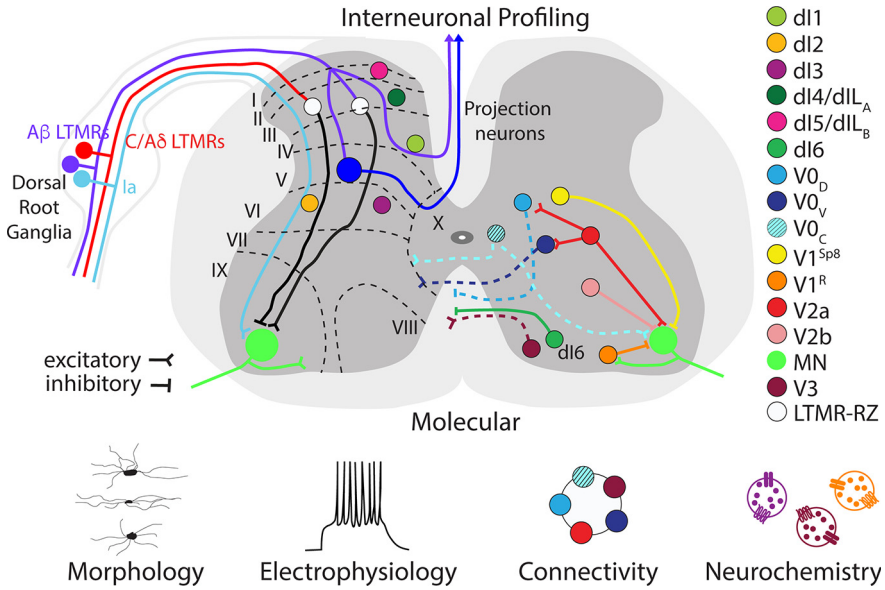

Figure 1. A multivariate approach for interneuronal profiling includes molecular (transcription factor expression), morphologic, electrophysiological, neuroanatomical (connectivity), and neurochemical (receptor phenotype) classification. Interneurons can be molecularly classified as part of dorsal and ventral "cardinal classes" (dI1-6, V0-3; see key), laminae location, and efferent innervation. The subclasses depicted here are not exhaustive, and the complexity of SpIN diversity is beyond the scope of this schematic. LTMR, Low-threshold mechanoreceptors; LTMR-RZ, low-threshold mechanoreceptor recipient zone; I-X, Rexed laminae I-X. Color scheme reflects neuronal classes described in the key.

The integration of multidisciplinary approaches has allowed researchers to divide major classes of interneurons into finer subtypes and to investigate their roles in disease. For example, the V0 class of interneurons is now divided into at least four subpopulations (Fig. 1) (Lanuza et al., 2004; Zagoraiou et al., 2009; Talpalar et al., 2013). A recent study demonstrated that large cholinergic synapses on motoneurons (most likely the V0c interneurons) (Rozani et al., 2019) could contribute to aberrant excitation during amyotrophic lateral sclerosis progression (Konsolaki et al., 2020).

The inhibitory (GABA/glycine) V1 class of SpINs, which express the homeodomain transcription factor Engrailed-1, comprise more than a dozen distinct neuronal subpopulations, categorized into four major groups based on the expression of 19 transcription factors (Bikoff et al., 2016; Gabitto et al., 2016; Sweeney et al., 2018). Transcriptionally distinct groups of V1 interneurons diverge in their physiological properties, display neuronal input specificity (e.g., from sensory vs motor neurons), and form inhibitory microcircuits that are tailored to individual limb muscles. V1 subpopulations are also divergent in their rostrocaudal positioning along the neural axis, corresponding with limb and thoracic motor output, and segmentally specified by their Hox gene expression profile (Sweeney et al., 2018). Studies by the Goulding research team have used mouse genetics to explore the role of V1 interneurons in locomotion. In addition to altering the speed of the step cycle (Gosgnach et al., 2006), deletion of V1 interneurons results in prolonged activation (disinhibition) of motoneurons innervating flexor muscles, disrupting the stereotypical flexor-extensor phases of contraction during locomotion (Britz et al., 2015). In addition, a recent study using the SOD^{G93A} mouse model of amyotrophic lateral sclerosis detailed motoneuron innervation by V1 subtypes and demonstrated the susceptibility of these connections to degeneration over time. The work included an elaborate model of progressive upregulation (e.g., compensatory plasticity) in V1 synaptic connectivity before breakdown of interneuronal circuits (Salamatina et al., 2020).

The V2 class of SpINs, located within the intermediate-ventral gray matter, is divided into excitatory (V2a) and inhibitory (V2b) subclasses. Much like the V1 neurons, V2b interneurons have been associated with inhibition of motoneuron output, primarily in the context of flexor-extensor coordination during locomotion. Whereas the loss of V1 interneurons results in prolonged flexion, the loss of the V2b subclass results in prolonged hyperextension (Britz et al., 2015). More recently, the V2b interneurons have been shown to play a role in speed of locomotion (Callahan et al., 2019). In contrast, the V2a interneurons are an ipsilaterally projecting, excitatory population of rhythmically active (Dougherty and Kiehn, 2010a,b; Zhong et al., 2010) premotor cells, implicated in left-right coordination. In addition to their role in locomotion, the V2a SpINs have recently been shown to be integrated with spinal respiratory networks, and to contribute to plasticity in models of SCI (Zholudeva et al., 2017; Jensen et al., 2019b) and amyotrophic lateral sclerosis (Romer et al., 2016).

While much of what we have learned about SpINs has come from studying lumbar spinal networks and locomotor function, ongoing research is now highlighting roles for interneuronal subtypes in other motor (e.g., skilled reaching, respiratory) and sensory (e.g., pain, itch) functions. These diverse roles are being explored in both the intact spinal cord (for review, see Zholudeva et al., 2018a; Dobrott et al., 2019) and in models of injury (Zholudeva et al., 2017; Jensen et al., 2019a) and disease (Romer et al., 2016). While in general terms, the ventrally derived neurons can be thought of as premotor, and more dorsally derived cells as sensory-related, this is an oversimplification, and motor and sensory functions are closely interlinked.

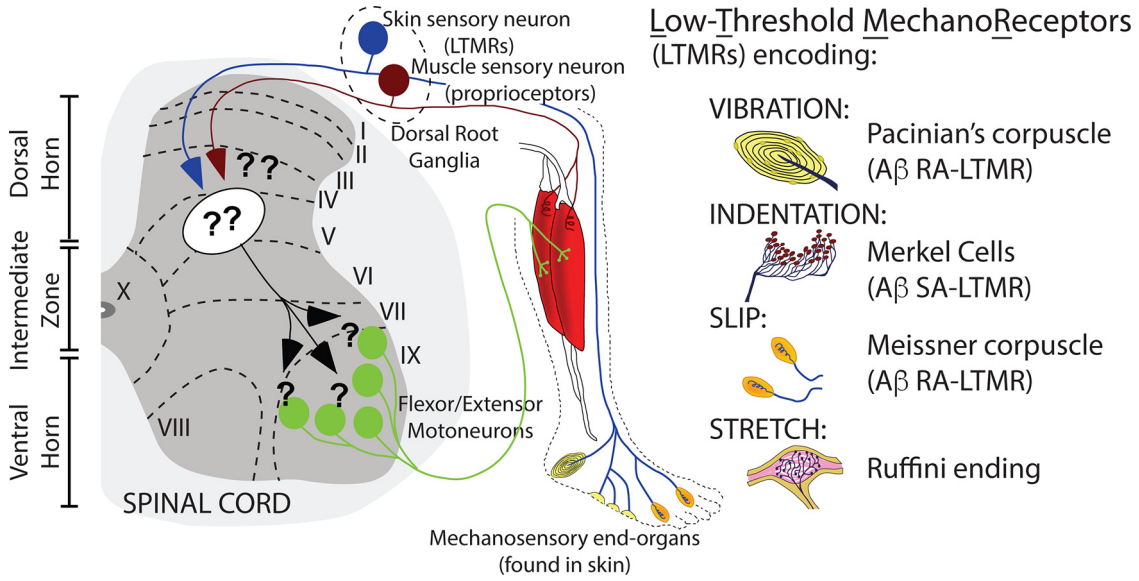

Figure 2. The intermediate spinal cord receives convergent information from proprioceptors (innervating muscles) and cutaneous receptors located on the soles of feet and joints (also known as low-threshold mechanoreceptors [LTMRs]). Current efforts are centered on understanding the functional logic of the spinal cord networks that bridge cutaneous/proprioceptive sensory information with motor centers of the spinal cord ventral horn and how these contribute to SCI recovery. RA, rapidly adapting; SA, slowly adapting; I-X, Rexed laminae I-X.

The "cardinal class" description of interneurons has been useful for broadly describing neuronal subtypes. However, identifying the diversity that exists within each class requires a battery of measures, including genetic marker expression, morphologic characteristics, electrophysiological signatures, and network connectivity. For example, a combination of morphology, electrophysiology, and functional analysis has been used to show that the V1 interneuron population is composed of multiple cell types, including Renshaw cells and Ia inhibitory interneurons (Sapir et al., 2004; Alvarez et al., 2005; J. Zhang et al., 2014). Likewise, the recent study by Abraira et al. (2017) used genetic tools to label and probe 11 dorsal horn neuronal populations, describing their location, morphology, connectivity, and electrophysiological properties. This study aligned multiple cellular features, thereby providing an in-depth understanding of interneuronal diversity within the dorsal horn.

Spinal interneuron diversity: beyond the cardinal classes

Defining each progenitor domain as a unique entity is organizationally simplistic, but recent single-cell RNA sequencing studies have begun to challenge this categorization of cell populations (Haring et al., 2018; Rosenberg et al., 2018; Sathyamurthy et al., 2018; Delile et al., 2019). These studies highlighted the difficulty in limiting classification to the cardinal classes. For instance, while cardinal classifications may be appropriate for some dorsal interneurons, the ventral populations of SpINs have been found to have overlapping gene expression profiles as the animal matures. Greater attention to SpIN heterogeneity across the rostrocaudal axis has also shed light on these differences. For instance, differences have been detected in both the V1 (Sweeney et al., 2018) and V2a (Hayashi et al., 2018) populations along the neural axis. At present, the cardinal class categorization also excludes some of the locomotor rhythm-generating neurons. Two examples include the Shox2 expressing non-V2a SpINs (Dougherty et al., 2013) and the Hb9-expressing Vx SpINs (Brownstone and Wilson, 2008; Caldeira et al., 2017). Combining RNA sequencing with a range of other assessments (e.g., behavioral activity) (Sathyamurthy et al., 2018) or spinal stimulation (Skinnider et al., 2020) will continue to provide the most rigorous assessment of interneuronal phenotype classification.

Ongoing research perspectives

Interpreting the functional consequences of SCI (and compromised spinal networks) and the progressive neuroplastic changes that follow requires a better understanding of how SpINs facilitate and modulate function normally, how they adapt to changing functional requirements (context dependency), and how networks of interneurons spontaneously adapt to SCI. The following sections highlight work being done by the Goulding, Abraira, Satkunendrarajah, and Magnuson research teams, then describe how SpINs have been therapeutically targeted, offering examples of how the field of spinal cord neurobiology is advancing to further appreciate the diversity, functional importance, and therapeutic relevance of SpINs.

Touching on locomotion: an anatomical and functional analysis of spinal cord neurons that shape the way we move

The spinal cord integrates proprioceptive, touch and nociceptive input to shape motor output. Our increased understanding of how spinal cord circuits integrate multiple sensory modalities has helped to improve rehabilitative therapies for SCI. Since Sherrington's pioneering work on the proprioceptive reflex pathway (Burke, 2007), great progress has been made in understanding how proprioceptors (i.e., muscle sensory neurons) shape motor activity, in particular locomotion (Grillner and Rossignol, 1978; Pearson and Rossignol, 1991; Hiebert et al., 1996; Lam and Pearson, 2001; Akay et al., 2014). Touch receptors in skin that encode sensory modalities, such as vibration, indentation, and slip, are also critical for adapting locomotion to changes in our environment, and they have begun to be recognized as an important component for rehabilitation-based recovery after SCI (Fig. 2) (Sławińska et al., 2012; Bui and Brownstone, 2015; Bui et al., 2015). How the spinal cord integrates touch information to sculpt motor activity is poorly understood. Nonetheless, progress is now being made by drawing on an ever-expanding mouse

genetic toolbox to visualize, quantify, and manipulate touch-specific spinal cord circuits (Li et al., 2011; Abraira and Ginty, 2013; Bourane et al., 2015; Rutlin et al., 2015; Abraira et al., 2017; Gatto et al., 2020). Leveraging these powerful genetic tools with motor assays that use high-speed cameras, muscle recordings (Mayer and Akay, 2018), and machine learning (Wiltschko et al., 2015; Nath et al., 2019) will enable a deeper understanding of how specific spinal cord touch networks are wired to shape movement, and how sensory information sculpts the recovery process after injury.

Using these tools, ongoing work by Abraira, Goulding, and colleagues have begun to identify touch-specific premotor networks important for sensorimotor function and recovery from injury. SCI studies in the cat and epidural stimulation protocols indicate the recruitment of cutaneous afferent pathways can facilitate locomotion. Interneurons interposed in these cutaneous pathways contribute to corrective motor behaviors (Rossignol et al., 2006; Bourane et al., 2015; Abraira et al., 2017; Paixão et al., 2019; Gatto et al., 2020), in part via networks in the intermediate spinal cord that integrate touch and proprioceptive information to influence specific patterns of muscle groups that facilitate both corrective movements during locomotion and motor "switching" during naturalistic behaviors (Fig. 2). Current work looking at the role of spinal cord intermediate zone interneurons in facilitating recovery from injury points to this spinal cord region as a critical therapeutic locus for SCI research. Although most current studies focus on sensory modalities in isolation, because the process of motor control and recovery encompasses several sensory modalities, the long-term goal of this type of work is to understand how spinal cord circuits process multimodal sensory information to shape movement. This will lay a foundation for novel ways of thinking about improving motor function after injury or disease.

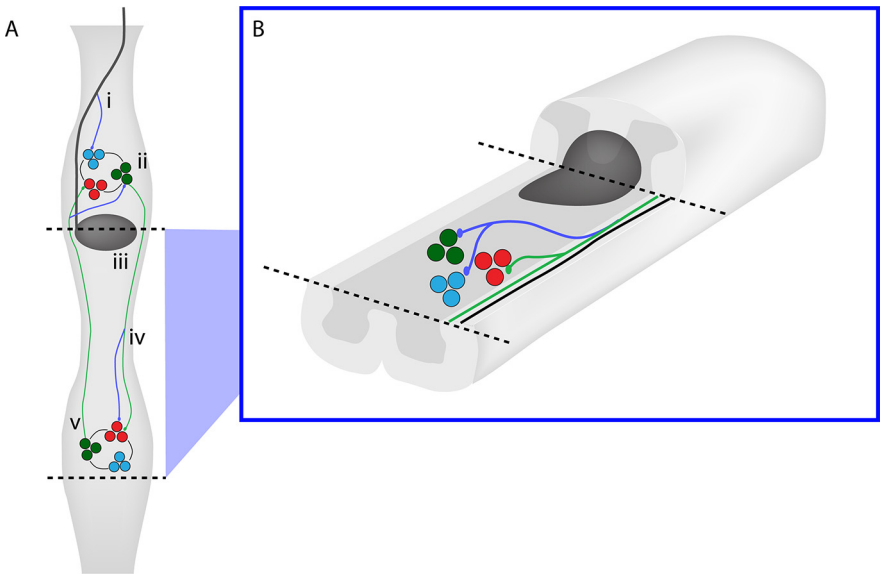

Figure 3. ***A***, Plasticity of descending (i) and spinal interneuronal (ii) networks after SCI (iii). Axons from both descending (iv) and ascending (v) interneuronal pathways can undergo sprouting and form new connections, establishing a new anatomy after SCI. ***B***, A schematic representation of anatomic reorganization of spinal networks caudal to injury. Black, green, and blue represent supraspinal, spinal, and sprouting axons, respectively. Colored circles represent diversity of SpIN subtypes. Adapted from Zholudeva et al. (2018a).

Cervical interneurons: critical role in movement control from supraspinal regions

Approaches for restoring motor function after SCI have focused on either coaxing cortical and subcortical axons across the lesion to their synaptic partners (Raineteau and Schwab, 2001; Courtine et al., 2008), or activating cortical neurons using stimulation protocols to reestablish motor commands to spinal motor circuits below the lesion, either via rewiring or strengthening of spared connections. Within the field of SCI, it has long been appreciated that SpINs may contribute to the plasticity and functional improvement following injury, either via the formation of novel neuronal pathways (e.g., "bypass" pathways) or synapse remodeling (e.g., strengthening of existing pathways) (Stelzner, 2008; Zholudeva et al., 2018a) (Fig. 3). Work by Bareyre et al. (2004) was among the first to anatomically demonstrate this, showing that, after a partial thoracic SCI, cervical interneurons relayed supraspinal input to otherwise denervated lumbar locomotor networks. Subsequent studies by the Edgerton group showed that interneurons not only contributed spontaneously to plasticity after injury, but they could also be therapeutically harnessed to improve outcome after partial SCI. This was also more recently shown within respiratory networks (Darlot et al., 2012), where chemogenetic (Satkunendrarajah et al., 2018) or neurochemical (Streeter et al., 2020) stimulation of SpINs can modulate and even improve functional output after SCI. Engineering and transplantation of subsets of SpINs have also been shown to enhance plasticity and functional recovery after SCI (White et al., 2010; Brock et al., 2018; Dulin et al., 2018; Kumamaru et al., 2018; Zholudeva et al., 2018b). Notably, excitatory interneuron subtypes, particularly V2a SpINs, have been shown to be crucial for anatomic and functional plasticity (Satkunendrarajah et al., 2018) of respiratory function (Zholudeva et al., 2017).

Current strategies to restore walking after SCI stem from the classical view that locomotion is a simple behavior, consisting of rhythmic alternation of flexor and extensor motor pools on the left and right sides of the body. The basic rhythm and walking pattern are generated by a spinal neural network known as the locomotor central pattern generator (CPG) (Kiehn, 2006, 2016). While the component neurons of the locomotor CPG are distributed across numerous spinal cord segments, the SpINs with the greatest rhythmogenic potential are harbored in the ventral (laminae VII, VIII, X) lower thoracic, and upper lumbar spinal cord (Kjaerulff and Kiehn, 1996). To execute purposeful locomotion, however, activity in the locomotor CPG must be initiated based on internal goals and then modulated based on environmental signals. Therefore, the production of locomotion requires supraspinal and sensory inputs originating outside the spinal cord (Steeves and Jordan, 1980; Shefchyk et al., 1984; Garcia-Rill and Skinner, 1987; Drew, 1993; Matsuyama et al., 2004; Hagglund et al., 2010; Jankowska et al., 2011; Bouvier et al., 2015).

In mammals, lesion and stimulation studies have demonstrated that the motor cortex is the main area responsible for volitional movement control (Choi and Bastian, 2007; Rossignol et al., 2007; Grillner et al., 2008). However, to navigate the complex environment in which we live, the spinal cord's locomotor

neural network relies heavily on feedback control from sensory fibers and feedforward control from higher centers, such as the visual cortex (Rossignol et al., 2006; Grillner et al., 2008; Drew and Marigold, 2015). A recent study demonstrated that the primary somatosensory cortex (SI), which receives and integrates information about the ever-changing internal and external environment, governs voluntary walking (Karadimas et al., 2020). Electrophysiological evidence suggests that a portion of the neural activity generated in SI before and during movement is a motor signal (Karadimas et al., 2020). Anatomical and electrophysiological investigations demonstrated that this SI motor signal bypasses the motor cortex and other brain or brainstem locomotor areas and modulates the lumbar locomotor CPG (L1-L2) via cervical excitatory interneurons (C4-C5) (SI locomotor pathway). Furthermore, chemogenetic stimulation of this lumbar-projecting cervical pathway leads to enhanced locomotor speed, whereas chemogenetic silencing impairs ongoing locomotion and speed. However, numerous questions remain about the functional significance of the cervical SpINs that integrate descending cortical and bulbar signals to modulate spinal locomotor networks.

A critical challenge is to unravel how SpIN networks integrate descending inputs and sensory feedback to program locomotion. Long descending propriospinal neurons within the cervical spinal cord are ideally situated to integrate descending and ascending commands and to play key roles in speed, directionality, and postural stability of movement (Ruder et al., 2016). Lumbar-projecting cervical interneurons and lumbar SpINs play a key role in postural balance and forelimb and hindlimb coordination during high-speed treadmill locomotion (Ruder et al., 2016). These lumbar-projecting cervical neurons are mainly excitatory with unique developmental origin and projection patterns. Moreover, neurotransmitter-specific monosynaptic retrograde tracing experiments demonstrated that SI pyramidal neurons' efferent connectivity to the lumbar locomotor region is via cervical excitatory interneurons. These long descending cervical interneurons receive synaptic inputs from many cortical and bulbar regions involved in the regulation of locomotion, and thus provide a neuronal substrate for integrating and disseminating supraspinal information throughout the spinal circuitry to produce locomotion. Further knowledge of SpIN networks' function during mammalian locomotion will facilitate delineation of the neural mechanisms of locomotion and the development of novel targeted treatment strategies to restore walking in SCI patients.

Synaptic silencing of SpINs reveals context-dependent locomotor hierarchies

Work by McCrea et al. has investigated a phenomenon, known as "deletions," which was well known from studies of fictive locomotion in the decerebrate cat preparation (Lafreniere-Roula and McCrea, 2005). During these functional deletions, which occur when anatomic networks remain intact, the bursting activity of motoneurons decreases and disappears for a step or two within an otherwise robust period of fictive locomotion coordinated by the locomotor CPG. Importantly, when the deletions occur, the underlying rhythm of the fictive stepping does not change.

A collaborative effort between the McCrea and Rybak research teams to determine how and why deletions occur revealed that that the amplitude of motoneuron output during fictive locomotion is functionally independent of the locomotor rhythm (timing). This suggests that locomotor circuitry controlling each limb is arranged in levels with a functional hierarchy (Rybak et al., 2006; McCrea and Rybak, 2007). Over the past 15 years, this concept has led to the suggestion that the mammalian locomotor circuitry is arranged with a top "rhythmogenic module," or rhythm generator, responsible for the timing or tempo of movements, overlying a pattern formation network, that is responsible for intralimb (hip, knee, and ankle) coordination (Rybak et al., 2006, 2015; McCrea and Rybak, 2008). Specifically, the most recent computer models contain a "half-center" rhythm generator consisting of reciprocally coupled flexor and extensor related excitatory interneurons controlling each limb, with a pattern formation layer controlling motor synergies and the output of muscle activity patterns within the limb. Interlimb coordination is determined by networks of local commissural interneurons and long ascending and descending propriospinal neurons that interconnect the circuits in each quadrant (Rybak et al., 2015; Danner et al., 2017). Interlimb coordination has been attributed to SpINs derived from V-class progenitor cells in the mouse; but, as discussed earlier, their contributions to the rhythm generator and pattern formation circuits remain poorly understood.

A recent set of experiments by Magnuson et al. began to explore the contribution of SpINs within the proposed sites of rhythm generation, and the long ascending and descending proprio-SpINs that integrate cervical and lumbar networks, to locomotion. Using excitotoxic (Magnuson et al., 1999; Hadi et al., 2000) and contusive (Magnuson et al., 2005) spinal cord injuries revealed that the greatest impact on locomotor function occurs when the L2 spinal segment is targeted, rather than the L3/4 or T9/10 levels. This result supports the notion that the rhythm generator circuitry is centered in the upper lumbar cord. Accordingly, the Magnuson team began exploring the roles of SpINs with cell bodies at L2 using a two-virus synaptic silencing system (Kinoshita et al., 2012). These experiments revealed that, of the L2 neurons that project to L5, 50:50 were ipsilateral and contralateral, and most were excitatory. Based on the simple concept that L2 motor output is more flexor-related while L5 output is more extensor-related, the researchers hypothesized that silencing these neurons would disrupt intralimb (flexor-extensor) coordination. However, when L2-L5 interneurons were silenced, the right and left hindlimbs were partially decoupled, and could adopt any right-left phase from pure alternation to synchrony (hopping) at speeds normally associated with pure alternation. None of the other fundamental characteristics of locomotion were disrupted (Pocratsky et al., 2017). This, together with other functional studies targeting ventral interneurons (Lanuza et al., 2004; Y. Zhang et al., 2008; Talpalar et al., 2013; J. Zhang et al., 2014), demonstrates that, for the hindlimbs at least, interlimb coordination circuits can be functionally separated from rhythm generation and intralimb pattern formation. Ongoing research, some described below, is focused on further elucidating the specific roles of SpINs in rhythm generation, interlimb coordination, and pattern formation.

Subsequent studies focused on long propriospinal neurons that interconnect the two hindlimb quadrants (containing rhythm generators for each hindlimb) to the forelimb circuitry with rhythm generators for both forelimbs. Specific attention was given to a subpopulation of long-ascending proprio-SpINs with cell bodies residing in the intermediate gray matter at lumbar segments (L1-L3), and with projections to the intermediate gray matter at C6-C7, regions essential for locomotor pattern

generation for the hindlimbs and forelimbs, respectively. This population is composed of ~50:50 ipsilaterally and contralaterally projecting interneurons, with decussating axons that cross at or near the level of the cell bodies. Given their role in interenlargement (lumbar to cervical) connectivity, the expectation was that silencing long-ascending propriospinal neurons would lead to disruption in hindlimb-forelimb coordination. Instead, overt disruptions in right-left coordination were observed in both forelimbs and hindlimbs. In addition, each limb pair, normally coupled strongly to maintain strict alternation at walking and trotting speeds, was partially decoupled. This disruption affected both limb girdles equally (approximately the same proportion of disrupted steps), despite the fact that only ascending information was silenced (L2 to C6). These disruptions were independent of walking speed, and surprisingly, did not alter intralimb (flexor-extensor) coordination. Hindlimb-forelimb coordination was only modestly influenced, and at no time was the 1:1 ratio of limb involvement in stepping disrupted. These disruptions in interlimb coordination were also seen to be context-dependent, and occurred only when the animals were walking from point A to B, nose-up (nonexploratory) on a surface with good grip. They did not occur on a treadmill, when the animals were exploring (nose-down) or when the animals were walking on a slick surface (Pocratsky et al., 2020). A further point of interest is that silencing these long-ascending propriospinal neurons had no influence on another locomotor activity, swimming. In rats, swimming is a bipedal activity where only the hindlimbs are used for propulsion. Animals retained strict hindlimb alternation during swimming when the long-ascending propriospinal neurons were silenced arguing that sensory input modulates or gates the roles played by these interneurons during different modes of locomotion (Pocratsky et al., 2020).

These observations support the concept that there are quasi-independent modules comprised of commissural interneurons, long-ascending and descending propriospinal neurons that mediate the coordination of each pair of limbs, and that the balance of their activity defines the locomotor gait, allowing for great flexibility in interlimb coordination while preserving stability (Danner et al., 2016, 2017).

In addition, the results lead to speculation that there exists a dynamic relationship between spinal autonomy and supraspinal oversight. When walking on a smooth surface with good grip, silencing long-ascending proprio-SpINs results in partial decoupling of the right and left limbs at each girdle by reducing the efficacy of the right-left phase module. However, when stepping on the treadmill, on a slick surface, or during exploratory, nose-down locomotion, supra-spinal oversight ensures the functional stability of right-left alternation at each girdle. Thus, it could be that there is a state-dependent hierarchy where right-left phase control of both limb pairs is driven by lumbar circuitry in some contexts and is supraspinally secured in others (Pocratsky et al., 2017, 2020). While the interconnectivity between long proprio-SpINs has been explored for many years (Giovanelli and Crotti, 1972; English et al., 1985; Rybak et al., 2015), demonstration of the functional consequences of disconnection between cervical and lumbar populations offers a unique insight into flexibility and how locomotion can persist after SCI, albeit with altered kinematics.

Therapeutically targeting SpINs

As highlighted here, SpINs play diverse roles in shaping motor and sensory information. What these roles are normally, and how they might change following traumatic SCI, becomes important in understanding postinjury function (adaptive or maladaptive plasticity). Indeed, SpINs are now recognized as a key component of plasticity and function post-SCI, by contributing to neural circuit remodeling and modulation of motoneuron excitability (Stelzner and Cullen, 1991; Bareyre et al., 2004; Courtine et al., 2008; Harkema, 2008; Lane et al., 2008, 2009; Sandhu et al., 2009; Alilain et al., 2011; Flynn et al., 2011; Zholudeva et al., 2017). With a growing appreciation of the neuroplastic potential of the injured spinal cord, and of the contribution of SpINs to this plasticity (Takeoka et al., 2014; Zholudeva et al., 2017; Satkunendrarajah et al., 2018; Jensen et al., 2019a), has come a greater focus on how plasticity can be therapeutically enhanced.

Exciting spinal networks to promote recovery: targeting glutamatergic SpINs

Two key areas of development of therapeutics that enhance plasticity and recovery following incomplete SCI are as follows: (1) activity-based therapies and rehabilitative approaches and (2) neural interfacing to activate neural networks with exogenous stimuli (for review, see Houle and Côté, 2013; Behrman et al., 2017; Hormigo et al., 2017). With the use of intraspinal electrophysiology, advanced neuroanatomical methods (viral tracing, immunohistochemistry) and/or genetic sequencing, the contribution of SpINs to plasticity has become more evident in preclinical models of SCI (Skup et al., 2012; van den Brand et al., 2012; Gajewska-Woźniak et al., 2016; Streeter et al., 2017; Skinnider et al., 2020). Although defining similar roles for SpINs within the injured human spinal cord has been more challenging, there is some evidence to support their role in patient recovery.

With increasing clinical use of operative neuromodulation and translational interest in electrical stimulation (e.g., functional electrical stimulation) (Johnston et al., 2005; Dimarco and Kowalski, 2013; DiMarco and Kowalski, 2019; Bourbeau et al., 2020), transmagnetic stimulation (Hou et al., 2020), epidural stimulation (Edgerton and Harkema, 2011; Sayenko et al., 2014), and intraspinal stimulation (Kasten et al., 2013; Mondello et al., 2014; Mercier et al., 2017; Toossi et al., 2017), there has been increased effort to identify the contribution that SpINs make to functional outcomes. While the substrates affected by stimulation have remained elusive, several studies have now confirmed that SpINs are activated by epidural (van den Brand et al., 2012; Skinnider et al., 2020) and intraspinal stimulation (Mushahwar et al., 2004; Sunshine et al., 2018). Similarly, histologic analyses of SpINs following activity-based therapies has shown that they increase their activity with treatment (Houle and Côté, 2013; Streeter et al., 2017). The phenotype of interneurons targeted by each treatment strategy, however, and whether different treatments might target different subpopulations, remains unclear.

Another more recent advance in therapeutics that harness SpINs has been the use of cellular engineering to create populations of specific interneuronal subtypes for transplantation. While transplantation of neural precursor cells has long been shown to improve outcomes following SCI (Fischer et al., 2020), recent attention has been given to the origin of neuronal progenitors and the SpIN component of these transplantable cells (White et al., 2010; Dulin et al., 2018). As developmental biology has provided markers for identifying subsets of SpINs, research has begun to tailor donor cell populations to contain therapeutically appropriate subpopulations (e.g., engineering SpINs for transplantation that are known to contribute to plasticity and recovery) (Iyer et al., 2017; Butts et al., 2019). For instance, the delivery after cervical SCI of excitatory V2a premotor interneurons,

which are known to contribute to respiratory plasticity (Romer et al., 2016; Zholudeva et al., 2017; Satkunendrarajah et al., 2018; Jensen et al., 2019a), enhances the degree of respiratory recovery (Zholudeva et al., 2018b). These more refined and tailored transplants will likely improve on prior transplantation methods, providing greater and more consistent functional recovery. It is hoped that, as we learn which subtypes of interneurons are involved in adaptive and/or maladaptive plasticity, more appropriately targeted therapies can be developed.

To inhibit or disinhibit: targeting the complex roles of inhibitory SpINs

While there has been mounting interest in the contribution of excitatory SpINs to the restoration of motor function, an essential consideration is that excessive excitation could exacerbate maladaptive plasticity, thus, for example, increasing pain, spasticity, or dysreflexia. These functional deficits, which are generally considered to be a result of overexcitation, can be attenuated by suppressing overexcitation directly (with glutamatergic antagonists) or indirectly (by targeting inhibitory networks, e.g., with GABA agonists or analogs). As shown with transplantation of excitatory neuronal progenitors, delivery of inhibitory interneurons may also provide a means for attenuating dysfunctional overexcitability in spinal networks (Jergova et al., 2012; Fandel et al., 2016).

Although treating pain and spasticity may require attenuation of excitatory neurons and/or inhibition of overactive excitatory networks, excessive inhibition is also deleterious for recovery of motor function (Chen et al., 2018). Indeed, silencing inhibitory SpINs has been shown to (re)activate dormant/latent spinal pathways, thus amplifying restorative plasticity and recovery. This disinhibition or "unsilencing" of spinal networks by targeting inhibitory SpINs has been achieved by targeting neurotransmitter receptors pharmacologically. Local delivery of GABAergic or glycinergic antagonists can even enhance the efficacy of other treatments (Bezdudnaya et al., 2020). Perhaps more selective approaches, such as the delivery of a KCC2 agonist, as described by Chen et al. (2018), will allow efficacious yet targeted treatment. As our understanding of the specific populations involved in premotor inhibitory spinal networks improves, treatments can be developed to better target those neurons selectively.

In conclusion, there is a growing effort to identify SpINs that contribute to motor, sensory, and autonomic functions. Research in developmental neurobiology and spinal cord biology, neurotrauma, and disease is revealing a highly diverse population of neurons that not only shape many neural functions, but also act as gatekeepers to many restorative and maladaptive forms of plasticity. Collaborative efforts among multidisciplinary teams are providing a wealth of information, and the identity of specific interneurons associated with spontaneous or therapeutically driven plasticity can now be more effectively assessed. As ongoing work continues to identify SpIN subtypes and improve our understanding of their contribution to function following injury, treatments can be tailored to minimize maladaptive consequences and better harness their restorative neuroplastic potential.

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