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Interneuron diversity and function in the spinal motor system

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Neural circuits in the spinal cord are the ultimate arbiters of movement, serving as the conduit through which the rest of the nervous system controls muscle contraction to implement behavior. Over the last two decades, the genetic identity of neurons in the spinal cord has come into view, revealing the richness of cellular diversity that underlies motor control. Despite this progress, our understanding of how discrete types of spinal interneurons contribute to motor output remains tenuous. Here, I review the landscape of interneuron diversity in the spinal motor system, and highlight key challenges and novel approaches to linking the molecular and genetic taxonomy of spinal interneurons to functional aspects of movement.

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Introduction

I move, therefore I am.

Haruki Murakami, 1Q84

How do we move? At a basic level, movement emerges from the activity of motor neurons in the spinal cord that innervate muscles in the periphery, causing them to contract. This motor neuron-centric view has profoundly influenced our understanding of neural circuitry and cellular diversity in the spinal cord, providing a detailed mechanistic description of how morphogen gradients and the combinatorial expression of transcription factors specify the hundreds of genetically distinct motor pools, each dedicated to the control of an individual muscle [1–3]. Yet motor neurons are just the final actors in the spinal circuits that control movement. Their activity is directed by networks of spinal interneurons that are capable of generating patterned motor output, while integrating sensory input and supraspinal information arriving directly or indirectly from cortical, basal ganglia, brainstem, and cerebellar systems to enable animals to move in a manner that meets their behavioral needs [4–6]. Revealing the identity, organization, and function of spinal interneurons is, therefore, essential to understanding the neural control of movement.

Early studies of spinal interneuron organization largely focused on locomotion, one of the most fundamental motor functions in an animal's behavioral repertoire. Over a century ago, Thomas Graham Brown showed that neurons intrinsic to the spinal cord can generate rhythmic patterns of locomotor activity, establishing the concept of central pattern generators and setting the stage for decades of work dissecting the components of these networks [7]. Subsequent efforts by Eccles, Lundberg, Jankowska, Hultborn, and others provided insight into the anatomical and physiological organization of interneurons in the cat spinal cord, taking advantage of the ability to selectively stimulate sensory afferents or ventral roots and record intracellular neuronal responses [8–10]. In this manner, several canonical interneurons were characterized, including muscle spindle-activated group Ia interneurons involved in reciprocal inhibition of antagonistic motor neurons [11], group Ib interneurons activated by Golgi tendon organs [12], and Renshaw interneurons mediating recurrent inhibition of motor neurons [13].

More recently, the advent of molecular and genetic approaches to studying neural circuit organization has revolutionized our understanding of spinal circuits controlling movement [14–16]. We now have insight into the basic logic through which cardinal interneuron classes are formed, and the functional contribution of these neurons to locomotion is beginning to become clear. Nevertheless, substantial challenges remain, both in terms of understanding the genetic diversity of interneurons in the spinal cord, and how such diversity relates to functional aspects of movement. Here I review the current state of efforts to dissect spinal interneuron diversity and function, focusing on the ventral spinal cord that is primarily involved in controlling movement, and highlight novel approaches that promise to dramatically improve our understanding of how spinal circuits contribute to behavior.

Genetic dissection of interneuron diversity: from one, many

One of the major challenges in modern neuroscience is the systematic identification of cell type diversity within

the nervous system. More than just a 'parts list', classification schemes provide a way to interrogate complex neural systems in a manner that enables reproducible identification of neurons across experiments, and can be used to define the relation of one type of neuron to another [17]. Though the conceptual definition of how exactly to distinguish cell types remains contentious, classification schemes have generally relied on differences in anatomical (morphology and connectivity), physiological (patterns of neuronal firing), and molecular (patterns of gene expression) properties, the latter of which is particularly useful because it enables genetic access to manipulate specific cell types [18,19]. Ideally, multiple orthogonal criteria would be used to define cell type identity, as shown for classification studies of ganglion cells and bipolar cells in the retina, perhaps the gold-standard for correlation of molecular, anatomical, and functional definitions of cell type diversity [20•,21•]

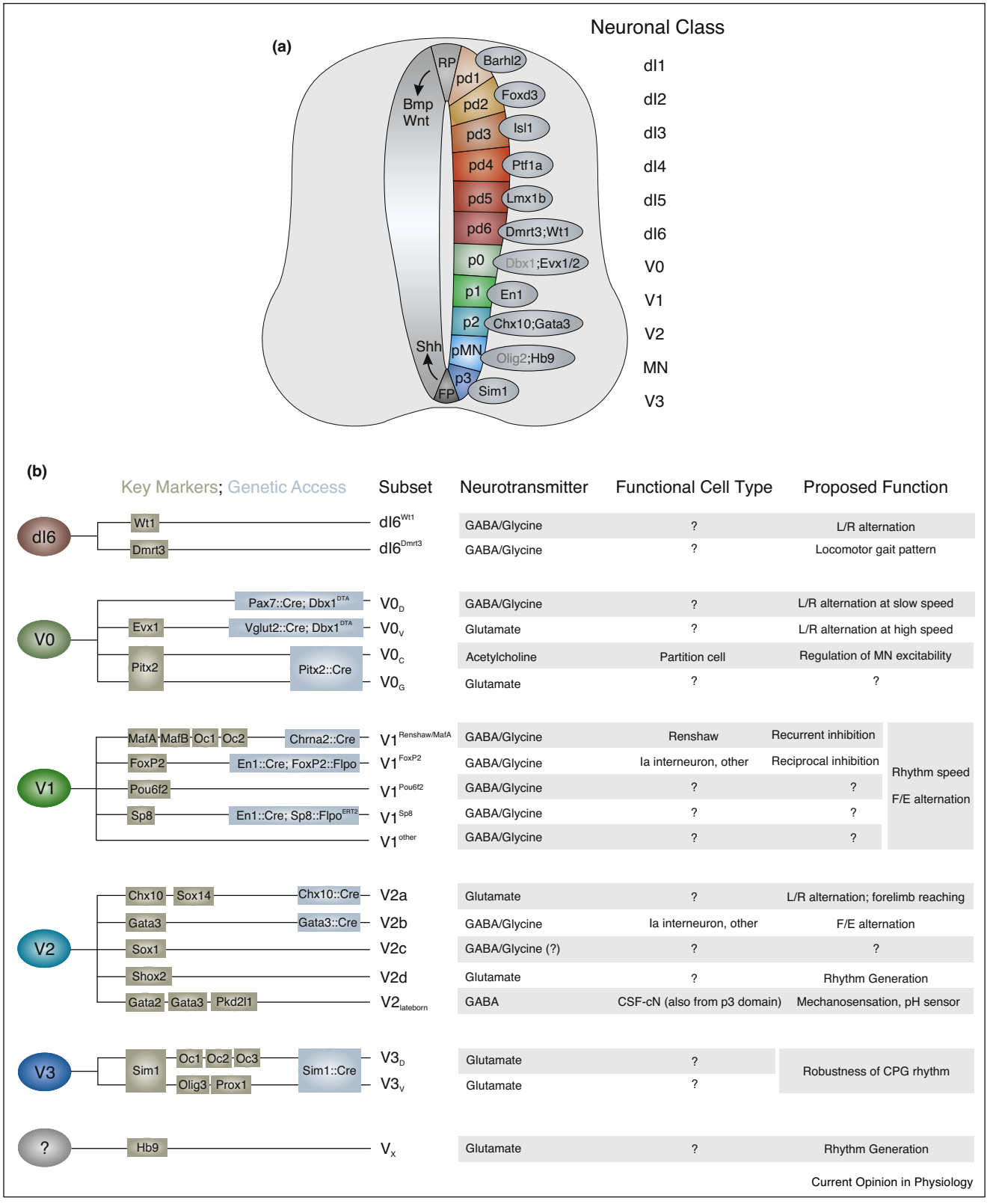
The spinal cord has proven to be a powerful system for the genetic dissection of neuronal diversity, in large part because the developmental provenance of different classes of neurons has been delineated. Neuronal identity in the spinal cord emerges during embryonic development, where opposing dorsal and ventral morphogen gradients impart positional information to progenitor cells in the ventricular zone, thereby establishing six dorsal progenitor domains (pd1–pd6) and five ventral progenitor domains (p0–p3, as well as the motor neuron progenitor domain pMN) (Figure 1a). These domains in turn give rise to motor neurons, the dI1–dI6 cardinal classes of dorsal interneurons, and the V0–V3 cardinal classes of ventral interneurons, each defined by the expression of key transcription factors [22–24].

The ventral half of the spinal cord contains the core circuits driving motor output [25], and harbors at least five of these cardinal interneuron classes: V0 (Dbx1-derived), V1 ($En1^+$), V2 ($Chx10^+$/$Gata3^+$), V3 ($Sim1^+$), and dI6 ($Dmrt3^+$/$Wt1^+$) interneurons [26]. By ablating or silencing these interneurons, a series of elegant genetic experiments have provided insight into their contribution to movement, primarily in the context of locomotor output. Thus, V0 interneurons coordinate left/right alternation [27,28], V1 interneurons affect locomotor speed [29] and together with V2b interneurons coordinate flexor/extensor limb activity [30•,31], V2a interneurons help orchestrate left/right alternation at high speeds [32,33], V3 interneurons influence the robustness of rhythmic output [34], and dI6 interneurons contribute to normal gait and left/right coordination [35,36]. Each of these manipulations almost certainly affects a large number of unknown cell types with different connectivity and function, complicating the interpretation of the exact role played by these neuronal populations. Indeed, it is now clear that just as the single pMN domain gives rise to hundreds of molecularly and functionally distinct motor pools, an analogous principle applies for interneurons, where each cardinal interneuron class is composed of molecularly distinct subsets (Figure 1b).

Several recent studies have greatly expanded our view of the landscape of spinal interneuron diversity, providing glimpses into the promise and challenges of cell type classification. V1 interneurons, for example, can be fractionated into four mutually exclusive clades and ~50 candidate cell types on the basis of the combinatorial expression of nineteen transcription factors, revealing subsets with distinct physiological signatures, spatial distributions, and patterns of connectivity in the lumbar spinal cord [37••,38•]. Much of this subtype diversity can be recapitulated *in vitro* in embryonic stem cell (ESC)-derived V1 interneurons, which have been used to investigate the mechanisms underlying neuronal subtype specification [39•]. Moreover, just as motor neurons are organized along the rostrocaudal axis into molecularly and functionally distinct pools, V1 interneurons exhibit limb and thoracic-enriched molecular signatures that are specified in a Hox-dependent manner, illustrating how terrestrial vertebrates may use interneurons with different segmental identities to control limb and axial-based movement [40••]. V2a interneurons also exhibit pronounced differences in identity along the rostrocaudal axis, where type I ($Chx10^+$, local projecting, lumbar-enriched) and type II ($Chx10^-$, local/supraspinal projecting, brachial-enriched) V2a interneurons are arrayed in counter-gradients, and can be fractionated into 11 molecularly distinct subsets [41••]. It seems likely that other interneuron classes will exhibit similar levels of diversity, suggesting that the number of ventral interneuron cell types may enter into the hundreds [42]. Striking the right balance in how finely to subdivide neuronal types by molecular criteria so as to avoid biologically insignificant distinctions will continue to be challenging, but will benefit from efforts to correlate molecular signatures with anatomical and functional differences.

The field of cell type classification is currently undergoing a revolution, in large part due to technological advances in single-cell and single-nucleus transcriptomics that have facilitated molecular classification of cells throughout the nervous system [43,44•,45•]. Focusing specifically on the spinal cord, single-cell transcriptomic approaches have identified 30 molecularly distinct subtypes of neurons in the dorsal horn of adolescent mice [46••] and 43 subpopulations in the adult spinal cord [47••]. The level of interneuron diversity detected in these studies is lower than what might have been expected based on the more focused analysis of V1 and V2a classes, which were performed in neonatal animals. This may in part reflect genuine developmental differences similar to what has been observed in the *Drosophila* olfactory system, where the diversity of projection neurons appears to be maximal during development, with

Figure 1



Genetic dissection of neuronal diversity in the mammalian spinal cord.
(a) Progenitor domains and neuronal classes in the developing spinal cord. During embryonic development, Shh secreted by the floor plate (FP) and Bmp and Wnt-family proteins secreted by the roof plate (RP) generate dorsal (pd1–pd6) and ventral (p0–p3 and pMN) progenitor domains.

molecularly distinguishable cell types converging to more common transcriptomic signatures in the adult [48•]. Even with the increased molecular resolution enabled by single-cell transcriptomics, an emerging theme is that genetic access to discrete cell types may routinely require multiple markers rather than single genes [38•,48•,49]. The refinement of intersectional genetic approaches, along with viral tracing based on distinctions in axonal projection patterns [50], will continue to play an important role in accessing cell types at finer and finer resolution.

The seemingly bewildering array of molecular diversity begs the question of how gene expression relates to prior anatomical and functional (physiological) classifications of interneurons. In most cases, this remains unclear, but there are several instances where we can be confident that a distinct genetic identity maps reasonably well onto a functionally coherent cell type. Cholinergic partition cells, whose modulatory input to motor neurons increases excitability by reducing the action potential afterhyperpolarization [51], appear to arise entirely from the small $V0_C$ subset marked by the transcription factor Pitx2 [52•]. Renshaw interneurons, the classic mediator of recurrent inhibition of spinal motor neurons, correspond to a subset of V1 interneurons distinguished by expression of Maf and Onecut-family transcription factors and the nicotinic cholinergic receptor Chrna2 [53–55]. Other cases are more complicated. An intriguing group of mechanosensory cerebrospinal fluid-contacting neurons (CSF-cNs) that regulate locomotion can be identified by Gata2/3 and Pkd2l1 expression, but appears to arise from multiple progenitor domains that generate CSF-cNs with different positions and electrophysiological properties [56,57•]. Moreover, Ia inhibitory interneurons mediating reciprocal inhibition of antagonistic muscles are found in both V1 and V2b populations, representing another example of a cell type with a dual developmental origin [30•]. Presumably such molecular heterogeneity reflects variations in connectivity or function, which will require further characterization to resolve.

Linking interneuron identity to behavior

The molecular classification of interneurons in the spinal motor system has helped transform our understanding of their functional role in controlling movement, most notably in the context of locomotion. Beyond identifying core roles for different interneurons in rhythm generation, and left-right and flexor-extensor coordination, these studies have revealed a modular organization to spinal circuits. For example, experiments in zebrafish have shown that during swimming, dorsal and ventral motor pools appear to be controlled by distinct premotor networks [58], and that three separate V2a interneuron microcircuits are recruited in a modular fashion as a function of increasing locomotor speed [59]. In mice, detailed characterization of walk, trot, and bound gaits have similarly suggested that premotor circuits are recruited in a modular fashion as a function of speed [60], with inhibitory $V0_D$ interneurons securing alternation at slower speeds, and excitatory $V0_V$ interneurons mediating alternation at faster speeds [28]. So where does this leave us in the broader context of using interneuron identity as a lens to understanding behavior?

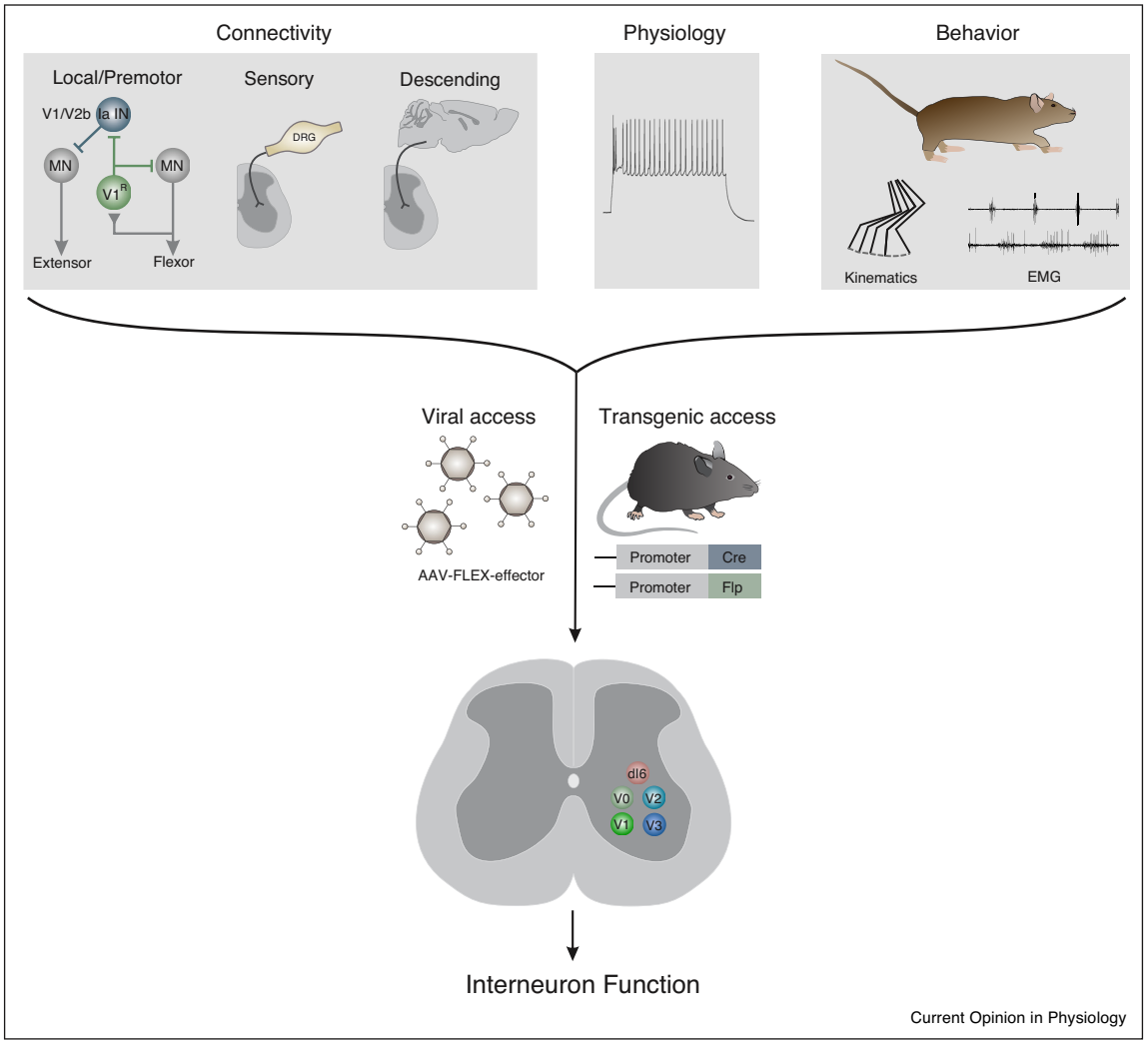
A number of thoughts come to mind. First, linking neuronal identity to function ideally involves more than performing an intervention to demonstrate neuron 'x' perturbs a particular behavior. Careful consideration should be given to the goals of the behavior and the development of a conceptual framework that enables behavioral tasks to inform specific hypotheses about how and why the motor system activates muscles in a particular pattern [61]. Second, the spinal cord does not control movement in isolation. Examining how sensory input and supraspinal systems interact with spinal circuits will be crucial to provide an integrated view of motor control. This is an area of rapid progress, as illustrated by recent studies identifying neuronal populations in the midbrain and caudal brainstem that influence specific functional aspects of locomotion, including the speed and termination of ongoing movement [62–64]. Third, there is an increasing appreciation for the importance of studying behaviors other than locomotion. This includes skilled forelimb tasks such as those used to demonstrate a role for V2a interneurons in reaching [65], and ethologically relevant, naturalistic types of movement that provide greater insight into the range of behaviors implemented by neural circuits [66]. Defining how specific interneurons contribute to context-dependent motor output should reveal the extent to which genetically defined cell types have restricted roles in mediating subsets of behaviors, or alternately function during multiple behaviors depending on the behavioral state of the animal.

(Figure 1 Legend Continued) These in turn give rise to eleven cardinal classes of spinal neurons: dI1–dI6 interneurons dorsally, and V0–V3 interneurons and motor neurons (MN) ventrally. Key transcription factors that serve as genetic markers for these cardinal classes are highlighted, with progenitor markers in gray and postmitotic markers in black. Not shown, the pdIL progenitor domain which gives rise to late-born dILA and dILB interneurons. Schematic adapted from Goulding [14].

(b) Subtype diversity of interneurons in the ventral spinal cord involved in motor control, highlighting the major subsets derived from each cardinal interneuron domain. Key markers are shown in brown. Cre, Flp, and other transgenic lines that enable genetic access for selective manipulation of a given interneuron subset are shown in blue. With the exception of $V0_C$ interneurons that represent partition cells and $V1^{Renshaw}$ interneurons, the subsets outlined here generally do not reflect single cell types, and can be further divided based on molecular, anatomical, and physiological criteria. Cerebrospinal fluid contacting neurons (CSF-cNs) are late-born neurons that primarily arise from the p2 progenitor domain, but have a smaller cohort that arises from p3 progenitors bordering the floor plate. Note that many dorsal interneuron populations are also involved in regulating movement, for example, by processing cutaneous and proprioceptive sensory information.

The above points highlight the fundamental reality that multidisciplinary approaches involving circuit tracing, physiology, and behavioral analysis will be needed to provide a cohesive picture of interneuron function (Figure 2). For example, novel viral technologies (e.g. monosynaptic rabies tracing) have been applied to great effect in mapping the connectivity of premotor interneurons [67], and will continue to play an important role in revealing neural circuit architecture in the spinal cord and elsewhere. Single-cell transcriptomic approaches can now be combined with patch-clamp recording to correlate physiological aspects of neuronal function to the expression of ion channels and neurotransmitter receptors [68,69]. Moreover, rapid advances in machine learning and computer vision are transforming how we obtain quantitative measures of movement not just for whole body postures, but for limbs, joints, and even individual digits, which should dramatically change our understanding of complex behaviors [70,71]. Each of these approaches to studying behavior stands to benefit significantly from ongoing efforts to map spinal interneuron identity and develop more refined genetic approaches to access discrete cell types.

Figure 2



Assigning function to spinal interneurons.
A multidisciplinary approach will be required to understand how genetically defined subtypes of spinal interneurons contribute to motor control. At an anatomical level, circuit tracing will be crucial to identify how information is conveyed between different neuronal types in the spinal cord, as well as from sensory and supraspinal systems. Physiological characterization of spinal interneurons provides insight into their intrinsic and active properties (e.g. Renshaw cells exhibit an initial burst in response to a depolarizing current), and input-output relationships between defined circuit elements. Behavioral studies have largely focused on locomotion, assaying limb kinematics and muscle (EMG) activity under conditions in which genetically defined populations of spinal interneurons have been manipulated. Consideration of other types of movement (e.g. reaching and grasping), along with developments in machine learning to characterize naturalistic behavior, will provide important insight into different strategies and underlying computations used by the nervous system to control movement. When combined with viral and transgenic access, these methods will significantly advance our understanding of how spinal circuits control movement.

Conclusion

The delineation of molecular diversity in the spinal motor system is a reminder that even seemingly simple movements emerge from neural circuits composed of extremely heterogenous cell types. And if the diversity observed in the ventral spinal cord is not daunting enough, equally impressive arrays of cell types are likely to constitute cardinal interneuron classes in the dorsal spinal cord. Yet for the first time, a comprehensive atlas of spinal interneurons seems within reach, enabling the identification of genes that can be used to genetically target neurons for visualization, chemogenetic or optogenetic manipulation, and circuit mapping. When viewed as one characteristic in a wider space of biological traits (e.g. morphology, physiology, connectivity) that collectively define neuronal identity, it is clear that continued efforts to molecularly dissect interneuron identity offer tremendous promise in clarifying how spinal circuits implement behavior.

Conflict of interest statement

Nothing declared.

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