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Inhibition downunder: an update from the spinal cord

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Abstract

Inhibitory neurons in the spinal cord perform dedicated roles in processing somatosensory information and shaping motor behaviors that range from simple protective reflexes to more complex motor tasks such as locomotion, reaching and grasping. Recent efforts examining inhibition in the spinal cord have been directed toward determining how inhibitory cell types are specified and incorporated into the sensorimotor circuitry, identifying and characterizing molecularly-defined cohorts of inhibitory neurons and interrogating the functional contribution these cells make to sensory processing and motor behaviors. Rapid progress is being made on all these fronts, driven in large part by molecular genetic and optogenetic approaches that are being creatively combined with neuroanatomical, electrophysiological and behavioral techniques.

Introduction

The role of inhibition in the working of the nervous system has proved to be more extensive, and more and more fundamental as experiment has advanced in examining it.

CS Sherrington, Nobel Lecture, 1932.

The importance of inhibition for shaping neural activity was first demonstrated by Charles Sherrington 130 years ago [1,2]. Sherrington observed that reflexes such as the nociceptive withdrawal reflex required both the excitation of motor neurons innervating the flexor muscles and the concomitant inhibition of opposing limb extensor muscles and their associated motor neurons. He argued that a similar neural mechanism must operate during bouts of scratching or locomotion, thus emphasizing the importance of reciprocal inhibition for all limb movements [2,3]. Sherrington concluded that the neurons responsible for reciprocal inhibition were likely to be a type of Schalt-Zellen, or switching cell, that was located centrally in the grey matter of the spinal cord [3]. The discovery of reciprocal inhibition marked the beginning of efforts to understand both the cellular and physiological basis of inhibition, together with the role that inhibition plays in controlling neuronal

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activity. For much of this last century, these efforts were heavily centered on sensorimotor pathways in the spinal cord that control movement. More recently, the focus has moved to inhibitory circuits in forebrain and cortex. Nonetheless, the spinal cord still has a great deal to tell us about how inhibition shapes neural activity at a circuit level.

Inhibition in the spinal cord serves two major functions. First, it regulates the reception and processing of sensory information via presynaptic pathways that directly gate sensory afferent transmission [4–10], and by classic postsynaptic inputs to other dorsal horn neurons that are interposed in nociceptive and mechanoreceptive sensory transmission pathways [9–11]. Second, inhibition plays a critical role in patterning and coordinating the motor activity needed for reflex movements, locomotion and postural control [12–16]. Many inhibitory interneurons synapse directly with motor neurons to control their excitability [12]. They also function indirectly through their actions on other interneurons, either to directly reduce excitability, or increase excitability via disinhibition [12,13]. In this review I will briefly summarize recent efforts to probe the development and functioning of inhibitory circuits in the spinal cord, drawing comparisons with studies in the forebrain, where appropriate. Classic electrophysiological techniques are now being coupled with molecular genetics and optogenetics to manipulate and probe discrete cohorts of inhibitory neurons. The impetus for employing these genetic approaches has come from studies aimed at molecularly parsing inhibitory neurons in the spinal cord according to their developmental provenance. To date, five cardinal classes of inhibitory neuron have been identified in the developing mammalian spinal cord (Figure 1; refs 14–16). These are the V2b, V1, V0_D, dI6 and dI4/dIL_A interneuron classes, the latter of which is composed of early born dI4 cells and late born dIL_A cells. Dorsally-derived dI4/dIL_A neurons are an extremely diverse population of inhibitory neurons [17–22]. They give rise to most of the inhibitory cells in the intermediate and dorsal spinal cord, including presynaptic “GABApre” interneurons and dorsal glycinergic inhibitory neurons. dI6 and V0_D interneurons are commissural neurons that project their axons rostrally and caudally, respectively [23, LG-C and MG, unpublished]. V1 and V2b IN interneurons, the two major classes of ventral inhibitory neurons, are also composed of multiple cell types, including Ia inhibitory interneurons and Renshaw cells [14].

Presynaptic inhibition

A unique feature of inhibition in the spinal cord is the prominent role that presynaptic inhibition plays in modulating sensory afferent transmission [4–10]. Presynaptic inhibition is mediated by specialized GABAergic axoaxonic synapses on proprioceptive and cutaneous sensory afferent fibers, thus gating sensory inputs by feedback inhibition onto sensory neurons [4–10,24,25]. This form of inhibition is commonly referred to as primary afferent depolarization (PAD) to describe the effect that GABA transmission has on the membrane potential of sensory fibers. The depolarization of sensory fibers by GABA is primarily mediated by GABA_A receptors on the axoaxonic sensory afferent membrane, which leads to Cl[−] efflux from the afferent terminal [25]. PAD is thought to reduce afferent transmission either via a shunting mechanism or by inactivating Na⁺ channels [4,24–26], and it has been observed a variety of sensory transmission pathways, i.e. muscle spindle (Ia, group II), Golgi tendon (Ib), cutaneous mechanoreceptor and nociceptor afferents [4–10,24].

The GABAergic neurons that mediate PAD in proprioceptive afferents are primarily located in the intermediate spinal cord, while those providing presynaptic inhibition to cutaneous afferents are located more dorsally in the dorsal horn [4,8–10,12,27]. In the intermediate spinal cord presynaptic inhibition of Ia muscle spindle afferents and Group II muscle spindles is sourced from different inhibitory neuron populations [8,12,24]. These findings reveal a high degree of specificity in the organization of the presynaptic circuits in the spinal cord. They also raise the following important questions: What are the molecular pathways that direct the formation of axoaxonic synapses as opposed to postsynaptic synapses? Are there general determinants that drive presynaptic versus postsynaptic synapse formation? Do specific subpopulations of presynaptic GABA interneurons possess unique molecular recognition systems that ensure they contact their appropriate targets and avoid others, and finally, what role, if any, does activity play in presynaptic circuit formation?

A recent paper from the labs of Julia Kaltschmidt and Tom Jessell [28**] addresses the first of these issues by identifying a signaling pathway that is important for the establishment of axoaxonic synapses on proprioceptive afferents. This analysis was founded on earlier work showing the inhibitory neurons that form axoaxonic synapses are derived from dorsal Lbx1⁺ dILA/dILA cells that transiently express the Ptf1a transcription factor [29]. Ashrafi et al. [28**] now show that the immunoglobulin protein NB2 and the contactin-associated protein, Caspr4, contribute to the formation of GABA boutons on proprioceptive afferent fibers in the ventral spinal cord. Mice lacking NB2 and/or Caspr4, show a significant reduction in the density of Gad65⁺ presynaptic GABA boutons on Ia sensory axons. NB2 and Caspr4 are expressed by proprioceptive neurons, and NrCAM, the putative ligand for the NB2/Caspr4 complex, is expressed in Ptf1a-derived “GABApre” neurons. The loss of NrCam-NB2/Caspr4 signaling does not lead to the complete loss of presynaptic GABA boutons on Ia afferents. This suggests that additional recognition molecules participate in the formation/stabilization of axoaxonic synapses. Interestingly, Caspr4 expression in dorsal root ganglia is broad, encompassing sensory neurons that do not express the proprioceptor marker parvalbumin. NB2 is also expressed more widely in cutaneous sensory neurons, thus raising the possibility of NB2/Caspr4 being employed to establish axoaxonic synapses on the central processes of cutaneous mechanosensory neurons. If NB2/Caspr4 do turn out to have a more general role, there are likely to be additional recognition pathways that generate the more selective patterns of presynaptic connectivity that have been described for cutaneous versus proprioceptive afferents and Group I versus Group II proprioceptive afferents [4,8,10,12]. Efforts to molecularly parse the dILA/dILA interneuron population, which is composed of multiple inhibitory cell types, is likely to be an important step in defining the mechanisms that impart specificity to the “wiring up” of presynaptic inhibitory circuits in the spinal cord.

Many GABAergic interneurons form a combination of presynaptic and postsynaptic synapses with postsynaptic target neurons and the sensory fibers that innervate these target neurons [30–32]. These connections are referred to as triads, where the axon terminal of the GABAergic neuron forms synaptic contacts with both the afferent terminal and postsynaptic dendrite. The anatomical organization of triads poses a number of interesting questions: Do all GABApre neurons form triads or only a subset thereof? With regard to the triad assembly, how do GABAergic neurons recognize and form contacts with particular

postsynaptic cells and their associated afferents, and what are the cellular mechanisms that direct these two processes in the same cell? In this regard, subcellular trafficking is likely to be important. Presynaptic localization of Gad65 at GABAergic boutons requires BDNF signaling from sensory afferents [29]. This and the recent discoveries that presynaptic inhibition requires electrical coupling mediated by connexin 36 [33], and Ia afferent presynaptic inhibition matures over the first postnatal week [34], raises the specter of activity playing a prominent role in the development and/or maturation of presynaptic GABAergic synapses in the spinal cord.

Inhibition and its role in shaping locomotor movements

Inhibition in the spinal cord has been studied extensively in the context of locomotion. Over the past 10 years, significant progress has been made on identifying the premotor interneurons that comprise the locomotor central pattern generator (CPG). One of the key drivers for this research has been the identification of developmentally defined populations of interneurons bearing unique molecular signatures [14–16]. Inhibition is particularly important for establishing the different patterns of motor activity that drive swimming, walking and other locomotor gaits. It may also contribute to rhythm generation under certain conditions [35,36]. In aquatic vertebrates, crossed inhibition mediated by inhibitory commissural neurons establishes the alternating left-right activity that drives the axial bending movements needed for swimming [16,37]. In quadrupedal vertebrates, inhibitory commissural neurons generate the alternating limb movements for walking and trotting [14–16]. A number of studies have highlighted the prominent role V0 inhibitory commissural neurons play in securing an alternating left-right gait. The first of these studies used the isolated spinal cord preparation to monitor locomotor activity in mice lacking V0 interneurons. Lanuza et al. [38] showed that *Dbx1* mutant mice display bouts of synchronous left-right bursting. V0_D inhibitory interneuron numbers are markedly reduced in these cords, pointing to a role for the V0_D interneurons in left-right alternation. Talpalar et al. [39**], by examining the contribution V0 INs make in more detail, found that ablating the V0 population as a whole leads to hopping in awake behaving mice. Interestingly, removing just the V0_D neurons caused a loss of alternation at slow speeds, while removing the glutamatergic V0_V neurons caused hopping at higher cadences. The mechanism that underlies the change in gait caused by the ablation of the *Evx1*⁺ V0_V population is not known. It is however, tempting to speculate that the hopping phenotype arises from the loss of a disynaptic inhibitory pathway in which V0_V neurons excite inhibitory interneurons on the contralateral side of the spinal cord.

Lanuza et al. [38] had found that strengthening glycinergic and/or GABAergic transmission reversed the left-right synchronous activity that occurs in the *Dbx1*^{lacZ} mutant cord, suggesting other inhibitory pathways can partially substitute for the loss of the V0_D neurons. The identity of these cells remains to be determined, although a recent study by Andersson et al. [40**] points to a possible role for dI6 neurons. dI6 cells are also inhibitory commissural neurons, and a substantial proportion of them express the transcription factor *Dmrt3*. Icelandic horses that are capable of an expanded repertoire of gaits, possess a mutation that truncates the *Dmrt3* protein. Moreover, spinal cords from *Dmrt3* mutant mice display a disorganized pattern of locomotor activity. The extent to which these changes in

motor activity reflect the normal function of the $Dmrt3^+$ dI6 neurons remains to be determined. The defects do, however, point to a role for $Dmrt3^+$ dI6 cells in coordinating stepping movements, including a potential function in coordinating left-right motor activity.

There are two major classes of ipsilaterally-projecting inhibitory neurons in the ventral spinal cord, V1 and V2b interneurons (Figure 1). While V1 interneurons have been studied extensively, with respect to their development, mature phenotypes and function, much less is known about the V2b interneurons. In mice, V1 interneurons are required for fast locomotor activity [41], a function that appears to be shared between aquatic and terrestrial vertebrates [42]. As noted previously, the V1 class of interneurons includes Renshaw cells and reciprocal Ia inhibitory interneurons (IaINs) [14], with the latter thought to be a major source of the inhibition that secures reciprocal flexor-extensor activity. Interestingly, the loss of V1 interneurons does not compromise flexor-extensor alternation in the isolated spinal cord [41], nor does it abrogate reciprocal IaIN inhibition in the cord [43]. It is only when neurotransmission is inactivated in both V1 and V2b interneurons that flexor-extensor alternation and IaIN-derived reciprocal inhibition is disrupted [44**]. This finding, together with the discovery that a subpopulation of V2b cells bear the signature features of IaINs [44**], argues that these two populations are the long sought after source of Sherrington's Schalt-Zellen.

Inhibition, push-pull and fine motor control

The genetic dissection of inhibitory pathways in the spinal cord, while providing a broad outline of the role that inhibition plays in shaping coarse motor movements, has so far failed to capture the more nuanced aspects of motor control where inhibition is also important. Standing, maintaining balance, delicate finger movements, grasping and holding objects, are complex motor behaviors that require a high level of fine motor control. Past studies in the cortex point to the potential contribution that mixed excitation and inhibition might make to shaping the activity of neural networks [45–48]. Neurons typically exist in a high conductance state that changes their input-output properties and gain (Figure 2a) This form of gain modulation appears to be a fundamental mechanism for modulating neural activity and circuit dynamics [45,48]. Three recent papers have highlighted the potential contribution that inhibition makes to balanced motor neuron excitability. Berg and his co-authors [49,50], in examining inhibitory and excitatory currents in motor neurons during bouts of fictive scratching, found a parallel increase in excitation and inhibition. This pattern of inputs to motor neurons seems counterintuitive given that flexion-extension movements during scratching involve the production of an alternating pattern of inhibitory and excitatory drive to motor neurons by reciprocal inhibition. What might the function of this coupled inhibition-excitation be? One possibility is that it changes the gain in motor neurons and thus their dynamic range [51], in much the same way that coupled inhibition-excitation has been proposed regulate the input-output properties of cortical neurons. Berg and colleagues [50] suggest that balanced excitation-inhibition is likely to be important for fine motor control, one example being grip movements. During precision gripping, there is increased phasic and tonic activity in pre-motor interneurons as compared to wrist flexion-extension movements [52].

In a further effort to determine the contribution that tonic or background inhibition makes to motor control Johnson et al. [53**] examined the contribution inhibition makes to push-pull in motor neurons. Push-pull is when excitation and disinhibition summate to increase excitability, while coincident inhibition and disfacilitation increase inhibitory drive (see ref. 53, Figure 2). They began by asking whether motor neurons receive tonic background inhibition and whether Ia inhibitory pathways are tonically active and might therefore contribute to push-pull. They surmised that if the Ia inhibitory inputs were simply reciprocal in nature, then abolishing them would leave just the excitatory component intact. However, if there is a tonic component to Ia inhibition, then reducing inhibition would also reduce any depolarization that arises from disinhibition. By comparing the synaptic currents produced in motor neurons with and without Ia reciprocal inhibition they were able to observe a marked reduction when inhibition was absent that is consistent with a push-pull mechanism (Figure 2b). Furthermore, when they examined the reflex forces generated in motor neurons under push-pull versus non-push-pull conditions, they detected a clear reduction in force generation, which supports the idea that push-pull modulates motor neuron gain in a task-dependent manner (Figure 2c). These findings reveal a more complex and dynamic architecture with regard to the inhibitory inputs that shape motor activity. A simple reciprocal organization of inhibitory inputs to flexor and extensor motor neurons, while accounting for the gross patterns of flexor-extensor alternation seen in the isolated spinal cord, fall short of accounting for the complex muscle synergies that are necessary for postural control and fine motor movements. In this respect, we still have a long way to go before we fully understand “the extensive and fundamental” contributions that inhibition makes to the rich repertoires of motor activity vertebrate animals display.

Future perspectives

Molecular genetic approaches, coupled with optogenetics, imaging and classical electrophysiological techniques, hold great promise with regard to dissecting inhibitory circuits in the spinal cord. However, squaring the relationship between and molecularly-defined cell types and functional cell types that are defined by their cellular properties, connectivity and function remains a big issue. Furthermore, defining different cell types by functional criteria, e.g. role in sensory transmission and/or motor control, is going to require more sophisticated behavioral tests and readouts of neuronal activity. While Renshaw cells and IaINs possess unique features that allow them to be classified with relative ease, identifying and classifying other interneuron cell types is likely to be more problematic. In this respect, the lessons learned from classifying and characterizing cortical inhibitory neurons should be valuable [54].

Efforts to elucidate the mechanisms that generate inhibitory neuron diversity may also provide important clues about cell identity and function. The developmental programs that specify neuronal identity in the spinal cord are comprised of spatial and temporal elements, perhaps best exemplified by the differentiation of V1 cell types. The V1 interneuron population displays a strict temporal program of that segregates Renshaw cell neurogenesis from the development of other V1 neuron cell types [55**,56**]. There is now growing evidence that similar temporal programs govern the differentiation of other inhibitory cells types, including those that are generated from the dI4/dIL_A, dI6 and V2b populations [15,

MG, G. Lanuza and LG-C, unpublished observations]. The degree of diversity that exists within these broader inhibitory neuron populations has not been determined. The dI4/dIL_A population appears to be comprised of multiple populations that are marked by the expression of different neuropeptides and transcription factors [47–50]. This suggests a high degree of complexity in the composition of inhibitory circuits in the spinal cord. While this complexity may seem daunting, the ability to precisely manipulate sensory stimuli and measure changes in motor activity and behavior makes the spinal cord a rich fishing ground for neuroscientists who are game enough to venture downunder.

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Highlights

1. Presynaptic inhibitory synapse formation
2. Inhibitory commissural neurons and left-right coordination
3. Inhibition and push-pull in motor neurons
4. Inhibitory neuron specification

Figure 1. Classes of inhibitory neurons in the developing spinal cord

(a) Five major classes of inhibitory interneurons are generated in mouse spinal cord. The dl4/dILA class is generally regarded as being composed of two subclasses, dl4 interneurons that develop early and late born dILA cells that bear the same molecular profile. In the adult spinal cord, dl4 derived cell types are enriched in the intermediate dorsal horn (laminae V-VI), while dILA cells generate the majority of inhibitory cell types in laminae I-IV of the dorsal horn. dl4/dILA, Vi and V2b interneurons project their axons ipsilaterally. dl6 and V0 interneurons are predominantly commissural. Asterisk indicates two excitatory V0 subtypes, V0_V and V0_C neurons.

(b) Schematic showing the known cell types that are derived from each class of interneuron and our current knowledge of their function.

Figure 2. Balanced excitation inhibition and push-pull

(a) The effect of background conductances on the input-output relationship of a hypothetical neuron. (Adapted from refs. 35–38). Increased background noise reduces gain and increases dynamic range.

(b) Schematic showing the change in the effective synaptic current in a motor neuron during a short bout of flexion and extension movements. The blue line indicates the average currents ($\Delta I(pp)$) measured in the presence of push-pull excitation-inhibition. The red line indicates currents when facilitation/dis-facilitation by push-pull is absent (ΔI). (Adapted from ref. 43).

(c) Force production by a motor unit in the presence (blue line) or absence (red line) of push-pull conductances.