Spinal interneurons providing input to the final common path during locomotion

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Abstract

As the nexus between the nervous system and the skeletomuscular system, motoneurons effect all behaviour. As such, motoneuron activity must be well-regulated so as to generate appropriately timed and graded muscular contractions. Accordingly, motoneurons receive a large number of both excitatory and inhibitory synaptic inputs from various peripheral and central sources. Many of these synaptic contacts arise from spinal interneurons, some of which belong to spinal networks responsible for the generation of locomotor activity. Although the complete definition of these networks remains elusive, it is known that the neural machinery necessary to generate the basic rhythm and pattern of locomotion is contained within the spinal cord. One approach to gaining insights into spinal locomotor networks is to describe those spinal interneurons that directly control the activity of motoneurons, so called “last-order” interneurons. In this review, we briefly survey the different populations of last-order interneurons that have been identified using anatomical, physiological, and genetic methodologies. We discuss the possible roles of these identified last-order interneurons in generating locomotor activity, and in the process, identify particular criteria that may be useful in identifying putative last-order interneurons belonging to spinal locomotor networks.

Introduction

A critical question in neuroscience is how neural circuits produce behaviour. In the early 20th century, Sherrington studied neural circuits responsible for motor reflexes and muscle contraction (Creed et al., 1932). His work spawned generations of scientists investigating the neural control of movement (Stuart, 2005). The honourees of this symposium are several generations removed from Sherrington; their contributions to our understanding of the circuits underlying rhythmic movement are legion. There is no doubt that our current understanding of breathing, walking, and chewing can be attributed in large measure to the efforts of Jack Feldman, Serge Rossignol, and James Lund.

It is almost the centenary of the publication of Thomas Graham Brown's works demonstrating that the mammalian spinal cord has the intrinsic capacity to produce locomotor activity (Brown, 1911; Brown, 1914). Our understanding of the spinal circuits responsible for this behaviour in terms of connectivity, neuronal intrinsic properties, and modulation has been steadily increasing (for review, see (Goulding, 2009; Grillner, 2006; Kiehn, 2006). Although descending and sensory inputs are critical for normal locomotion, the spinal locomotor network, or "central pattern generator" (CPG), regulates the basic rhythm (or speed) and pattern (or coordination) of walking, as well as the degree of motoneuron output (leading to strength of muscle contraction) during locomotion. One strategy in defining the neural network underlying locomotion is to use a bottom-up approach in which the network is described by peeling back the layers starting from the neurons that generate the output of the circuit – motoneurons, or "the final common path" (Creed et al., 1932). Hence, in this review we will focus on last-order spinal interneurons – those neurons that project to and synapse directly with motoneurons and thus produce the motor output of locomotion.

Many classes of spinal interneurons have been identified based upon anatomical and electrophysiological characteristics in the cat (Jankowska, 2008) and based upon genetic characteristics in the mouse (Goulding, 2009). Given the diversity of spinal interneurons and the difficulty in identifying them, it would be reasonable to ask the question: how would one know if any particular interneuron is directly involved in controlling motoneuron activity during locomotion? To answer this question, a list of criteria could be developed (cf. Brownstone and Wilson, 2008); one could then determine whether or not the criteria are fulfilled for any given neuronal population. Given that motoneurons receive alternating excitatory and inhibitory input during locomotion (Jordan, 1983; Perret, 1983), tentative criteria lists for both excitatory (Table 1) and inhibitory (Table 2) last-order interneurons can be proposed. It is expected that these lists will be modified as further data regarding locomotor networks is obtained. It should also be recognised that each subset of these last-order interneurons (i.e. excitatory and inhibitory) is likely comprised of more than one population of neurons. Hence, a single population may not fulfill all criteria (particularly related to their inputs). [In addition, it is now recognised that there are also last-order spinal modulatory inputs to motoneurons (Miles et al., 2007; Zagoraoui et al., 2009). These will be considered separately below.]

From the time of Sherrington to recent years, mammalian locomotor circuits have been explored largely in the cat, in which several populations of last order neurons have been identified. In recent years, attention has turned to the mouse spinal cord. Advances in molecular biology have generated new tools to dissect, study, and manipulate these circuits, with one aim being to identify interneurons responsible for rhythmic motor output. In particular, these advances have led to the use of fluorescent proteins as markers of gene expression (Chalfie et al., 1994; Zacharias et al., 2000) and tools to activate or silence (Zhang et al., 2007) neurons; these tools will be important in determining whether neurons meet the criteria and are involved in locomotor behaviour. Here, we will briefly review some last-order interneuronal populations in mammals that may be involved in the regulation of motoneuron activity during locomotion. It should be noted that even though many of these populations have been found to be rhythmically active during locomotion, none has been

found to be critical for the production of motor neuron output during locomotion. The interneurons we will discuss are depicted in Figure 1. Although the neurons defined in the cat seem to be distinct from those described in rodents, there has been recent progress to relate these functionally and molecularly defined populations. Note that we will not discuss the fundamental work done in invertebrates (see Marder and Bucher, 2007 for review) or lower vertebrates (see Grillner et al., 2008 for review), nor will we discuss the experimental approaches to studying these neurons in humans (see Hultborn and Nielsen, 2007).

Last-Order Inhibitory Interneurons

Renshaw cells

The first two sources of inhibition to motoneurons to be described were Renshaw cells (RCs) (Eccles et al., 1954; Renshaw, 1941, 1946) and Ia inhibitory interneurons (IaINs) (Jankowska and Roberts, 1972). Renshaw cells are responsible for “recurrent inhibition” – they receive inputs from α -motoneuron axon collaterals (Eccles et al., 1954; Lamotte d’Incamps and Ascher, 2008), and in turn monosynaptically inhibit α -motoneurons (as well as γ ’s) through glycinergic/GABAergic synapses (Geiman et al., 2002; Schneider and Fyffe, 1992); for review, see (Alvarez and Fyffe, 2007) and (Hultborn, 2006). Recurrent inhibition is topographically organised, with strongest effects in nearby motoneurons, in both homonymous and synergist motor pools (McCurdy and Hamm, 1994; Trank et al., 1999). They project to other neurons as well, including ventral spinocerebellar tract neurons (Windhorst, 1996) and IaINs (Hultborn et al., 1971) as well as other RCs (Ryall, 1970). There is no evidence of direct reticulospinal input to RCs (Engberg et al. 1968), which receive sparse monoaminergic inputs from brain stem nuclei (Carr et al., 1999). While they receive primary afferent input early in development (Mentis et al., 2006; Naka, 1964), this becomes non-functional in the adult (Mentis et al., 2006).

The precise functional role of this recurrent inhibition to motoneurons is still not clear. Several studies have raised various suggestions for their role (Alvarez and Fyffe, 2007; Windhorst, 1996), including: they may reverse the size-order recruitment of motoneurons (Friedman et al. 1981); they may change the “gain” of motor pools (Hultborn et al., 2004; Hultborn and Pierrot-Deseilligny, 1979); they may play a role in de-correlation of motoneuron firing (Maltenfort et al., 1998); and/or they may reduce the amplification of motoneuron inputs mediated by persistent inward currents (Bui et al., 2008; Hultborn et al., 2003; Kuo et al., 2003).

Although RCs are rhythmically active during locomotion (McCrea et al., 1980; Nishimaru et al., 2006; Pratt and Jordan, 1987), their role during locomotion is not clear; however, they are not involved in the generation of the rhythm (Pratt and Jordan, 1987). They are believed to receive rhythmic synaptic input from the CPG, possibly via commissural interneurons (Nishimaru et al., 2006). Elimination of RC activity by application of the cholinergic antagonist mecamylamine during locomotion leads to an increase in MN firing rate (Noga et al., 1987), suggesting that RCs may play a role in reducing MN firing during locomotion. However, there is no loss of interburst membrane hyperpolarisation of motoneurons, nor an increased burst duration in motoneurons (Noga et al., 1987). There is also no evidence of any change in the fidelity of flexor-extensor alternation following RC block. Therefore,

while they fulfill several of the criteria that we have listed as belonging to last-order interneurons involved in locomotion, and may play a role in modulating the frequency of MN spike trains, they do not appear to be involved in the termination of rhythmic motoneuron bursts.

Ia Inhibitory Interneurons

IaINs are responsible for “reciprocal inhibition.” That is, they are monosynaptically excited by spindle primary (Ia) afferents, and project to and inhibit antagonist motor pools (Eccles and Lundberg, 1958). In addition they inhibit Ia inhibitory interneurons that receive Ia afferent inputs from antagonist muscles (Hultborn et al., 1976a). Despite their nomenclature, inputs to Ia inhibitory interneurons are not restricted to Ia afferents – they are excited, for example, by flexor reflex afferents and cutaneous afferents (Hultborn et al., 1976b) and inhibited by RCs (Hultborn et al., 1971). Descending inputs to IaINs include monosynaptic connections from the ipsilateral vestibulospinal tract, and disynaptic inputs from motor cortex and the red nucleus (Hultborn et al., 1976c).

A logical role for IaINs would be that they could ensure appropriate alternation of flexor and extensor activity during locomotion. They meet the majority of the criteria laid out in Table 2 wherein they receive primary afferent input, are glycinergic, project to motoneurons and are rhythmically active during locomotion ((Feldman and Orlovsky, 1975; McCrea et al., 1980; Pratt and Jordan, 1987)). In fact, the latter study observed that Ia inhibitory interneuron activity preceded that of the associated motoneurons, suggesting that they receive an excitatory drive from the central pattern generator (these experiments were performed while the cats were paralyzed, thus the excitatory drive could not come from primary afferent inputs). Their ability to ensure proper alternation between flexor and extensors may be enhanced by their ability to reduce motoneuron excitability by inhibiting persistent inward currents (Hyngstrom et al., 2007; Kuo et al., 2003), or by “de-selecting” specific motoneurons by reducing their PICs (Heckman et al., 2008; Hyngstrom et al., 2008). Interestingly, systemic administration of strychnine, a glycine receptor antagonist, significantly reduced inter-burst inhibition in motoneurons but did not seem to extend motoneuron burst duration during fictive locomotion evoked by brainstem stimulation in the decerebrate cat (Pratt and Jordan, 1987). The relationship between flexor and extensor activity was not studied in this preparation, but in young rat and mouse in vitro spinal cord preparations, strychnine typically leads to coactivity in flexor and extensor MNs (Cowley and Schmidt, 1995; Jiang et al., 1999). Taken together, these data suggest that IaINs are involved in rhythmic inhibition of motoneurons during locomotion, but that there may be additional mechanisms (possibly GABA-mediated) involved in terminating motoneuron bursts.

Non-reciprocal inhibitory INs

In contrast to reciprocal inhibition, Laporte and Lloyd (1952) reported inhibition of synergist motoneurons when the strength of group I stimulation was increased slightly. This “non-reciprocal inhibition” was felt to be mediated largely by sensory afferents from group Ib fibres which originate in Golgi tendon organs. However, since many interneurons mediating non-reciprocal inhibition also receive input from Ia afferents, this reflex pathway is often

termed non-reciprocal group I inhibition (Jankowska et al., 1981). Non-reciprocal inhibition is mediated by glycinergic interneurons in laminae V and VI (Brink et al., 1983; Jankowska, 1992; Bannatyne et al. 2009). The organization of Ib inhibition has been shown to be divergent, with the effects of Ib afferent from one muscle being distributed to different muscles through subsets of interneurons for each target motor pool (Jankowska, 1992). Ib inhibitory interneurons have been observed to be mutually inhibitory (Brink et al., 1983). A number of descending inputs to these interneurons include excitatory input from ipsilateral corticospinal and rubrospinal neurons, and inhibitory input from reticulospinal inputs (Jankowska, 1992). In a recent study, monosynaptic excitation from the medial longitudinal fascicle (MLF) was demonstrated in ipsilaterally projecting, inhibitory interneurons receiving inputs from group I afferents, and located in intermediate laminae (Bannatyne et al. 2009). Given the mixed descending excitatory and inhibitory inputs to these neurons, their contribution, if any, to motoneuron inhibition during locomotion is not clear. However, in keeping with disynaptic group I inhibition being replaced by excitation in extensor motoneurons during fictive locomotion (Gossard et al., 1994; *vide infra*), recordings from two group I non-reciprocal inhibitory interneurons in the cat revealed that their activity was depressed during fictive locomotion (Angel et al., 2005). Whether the population of these neurons is inhibited during locomotion is not yet clear (see Wilson et al., 2010).

Other last-order inhibitory interneurons identified in the cat

Other last-order inhibitory neurons have been identified, including group II interneurons (receiving input from muscle spindle secondaries) (Bannatyne et al., 2009), and commissural inhibitory INs (Arya et al., 1991; Jankowska et al., 2003). Both of these populations receive inputs from reticulospinal pathways (Jankowska et al. 2003; Bannatyne et al. 2009). The possible involvement of inhibitory group II interneurons in locomotion will be treated concurrently with a discussion of excitatory group II interneurons (*vide infra*, Section 3.2).

V1 interneurons

Understanding of the development of the mouse spinal cord has led to the ability to genetically define populations of spinal interneurons. Interneurons of the ventral spinal cord are largely derived from four progenitor domains (p0-p3), resulting in four classes of post-mitotic neurons (V0-V3) (Jessell, 2000). Three of these classes are known to include interneurons that send inhibitory inputs to motoneurons: V0, V1, and V2b.

V1 interneurons are specified by the expression of the transcription factor engrailed-1, and include RCs, IaINs, and other yet-to-be-defined inhibitory neurons (Alvarez et al., 2005; Sapir et al., 2004). Recordings of these neurons (other than RCs) in the mouse during locomotor activity have not been reported. Genetic ablation or acute silencing of V1 interneurons reduces locomotor speed (Gosgnach et al., 2006). Perhaps somewhat surprisingly, however, motoneurons still received rhythmic inter-burst inhibitory input (Gosgnach et al., 2006), suggesting that IaINs (and RCs and the other V1 INs) are not solely responsible for rhythmic inhibition of MNs. Another possible explanation would be that IaINs are not exclusively derived from the V1 population (Wang et al., 2008). This finding illustrates that the physiological and molecular definitions of spinal neurons may not be

100% concordant, and combining data from these different yet complementary paradigms will be helpful in the elucidation of spinal circuits.

V0 interneurons

The homeobox gene *Dbx1* controls the specification of a class of commissural interneurons (V0 interneurons), 70% of which are inhibitory and 30% of which are excitatory (Lanuza et al., 2004)(vide infra Section 4, Spinal Modulatory Neurons). The ventral component of this class (V0_V) is composed of commissural interneurons located in lamina VIII. Injection of GFP-expressing pseudorabies virus into hindlimb muscles allowed for mapping of synaptic inputs to motoneurons and demonstrated that 30% of commissural neurons were *Dbx1* positive. Therefore, some V0 interneurons project to and synapse directly onto contralateral motoneurons. Postsynaptic inhibition of motoneurons in response to commissural interneuron activation has been demonstrated; whether or not these were V0 interneurons is not known (Quinlan and Kiehn, 2007). V0 neurons have been shown to be rhythmically active during locomotion (Dyck and Gosgnach, 2009). Although ablation of *Dbx1* neurons leads to impairments in left-right alternation in neonatal spinal cord preparations (Lanuza et al., 2004), rhythmic activity persists. It is not known whether rhythmic inhibition of motoneurons persists, or whether they simply receive rhythmic excitation in these mutants. Of note, there is no apparent change in the flexor-extensor fidelity or motor burst duration when V0 interneurons are eliminated, suggesting that they are not critical for rhythmic inhibition of motoneurons. Thus, the role of V0 INs in regulating motoneuron output during locomotion remains unknown.

V2b interneurons

V2b interneurons are derived from the *Lhx3*-expressing V2 population, and defined by their expression of *GATA2/3* (Zhou et al., 2000). It has recently been shown that these neurons express either GABA or glycine, and project to motoneurons (Al-Mosawie et al., 2007; Joshi et al., 2009; Lundfald et al., 2007). These neurons have not been studied during locomotion.

Medial lamina V/VI GABAergic neurons

We have recently described a population of GABAergic neurons in medial laminae V/VI (Wilson et al., 2010). These neurons receive low threshold primary afferent input, and seem to have diverse projections, including to motoneurons. Using 2-photon excitation calcium imaging (Wilson et al., 2007), we demonstrated that they are rhythmically active during locomotion, leading to the suggestion that they may be involved in motoneuronal burst termination. A clearer definition of their role in locomotion awaits future studies in which, for example, these neurons are silenced.

Last Order Excitatory Interneurons

Group I excitatory interneurons

Similar to the identification of inhibitory spinal interneurons in the cat, excitatory spinal interneurons have been identified primarily based on their connectivity. The best described source of sensory-derived motoneuron excitation is the direct monosynaptic Ia afferent excitation of motoneurons originating from muscle spindles responsive to muscle stretch.

Whether Ia axons, which are rhythmically depolarised during fictive locomotion (Duenas and Rudomin, 1988; Gossard, 1996), contribute to motoneuron excitation is not known. Interestingly, spikes produced by primary afferent depolarisation may contribute to rhythmic motoneuron excitation during fictive mastication (Westberg et al., 2000).

In the anaesthetized cat, group Ib afferents mediate non-reciprocal inhibition of homonymous and synergist motoneurons (vide supra). However, during locomotion in acutely spinalized cats, stimulation of Ib afferents leads to excitation rather than inhibition of extensor motoneurons (Gossard et al., 1994). In the presence of active descending systems, this excitation is mediated by a disynaptic excitatory pathway (McCrea et al., 1995). These excitatory responses to Ib stimulation recorded in motoneurons are phase-modulated, indicating the interneurons mediating this reflex pathway are rhythmically active. Indeed, candidate interneurons have been identified (Angel et al., 2005), and are rhythmically active during locomotion. Evidence has been presented demonstrating convergence of mesencephalic locomotor region (MLR)-evoked and Ib excitatory post-synaptic potentials in motoneurons (Brownstone et al., 1992b), and group I excitatory interneurons receive reticulospinal input (Bannatyne et al., 2009). Taken together, these data point towards this population of interneurons as a potential source of rhythmic excitation of motoneurons during locomotion (see Table 1).

Mid-lumbar group II interneurons

Although other sensory afferents such as group II primary afferents from secondary endings of muscle spindles can contact motoneurons directly (Lundberg et al., 1977; Lüscher et al., 1979; Stauffer et al., 1976), their main influence on motoneuron activity is mediated via ipsilaterally projecting interneurons located in laminae VI and VII (Bannatyne et al., 2009; Edgley and Jankowska, 1987; Lundberg et al., 1987; Riddell and Hadian, 2000). These neurons described in the cat can be divided into excitatory and inhibitory populations, are located in midlumbar segments, and have descending (Cavallari et al., 1987) and/or ascending (Riddell and Hadian, 2000) projections of several segments. Most mid-lumbar group II interneurons receive monosynaptic input from descending inputs, including rubrospinal, vestibulospinal, corticospinal or reticulospinal neurons (Davies and Edgley, 1994). In addition, they have been shown to receive excitatory inputs from the MLR which are mediated via reticulospinal pathways (Edgley et al., 1988) suggesting a possible role in locomotion. In fact, some mid-lumbar group II interneurons are rhythmically active during fictive locomotion (Shefchyk et al., 1990). However, DOPA administration, which can be used to elicit locomotor activity, depresses these neurons (Edgley et al., 1988), and group II field potentials are depressed during MLR-evoked locomotion (Perreault et al., 1999), suggesting that their role in locomotion may be limited.

Excitatory interneurons mediating cutaneous inputs

Excitation from last-order interneurons related to cutaneous sensory afferents have also been described. Activation of hindlimb cutaneous afferents in the cat can generate short-latency EPSPs estimated to be disynaptic in nature (Moschovakis et al. 1991; LaBella et al., 1992). The strength of these EPSPs is modulated in different phases of MLR-evoked locomotion, suggesting that these interneurons receive phasic inputs from locomotor networks during

locomotion (Burke et al. 2001, see however Gossard et al. 1989 regarding presynaptic inhibition of cutaneous afferents during locomotion). This may translate to gating of behavioural responses to cutaneous stimulation during the step cycle. For example, contact of the paw dorsum with an object can elicit two stereotyped patterns of hindlimb muscle response depending on whether contact was made during the swing or stance phase (Quevedo et al. 2005). There is also evidence that interneurons mediating excitation from cutaneous afferents also receive inputs from muscle afferents, suggesting that some of these interneurons play a multimodal integrative role (Perrier et al. 2000). Whether these interneurons are important in mediating rhythmic motoneuron excitation during locomotion remains to be determined.

Excitatory commissural interneurons

Excitatory inputs to motoneurons from commissural interneurons have also been demonstrated in the cat. These neurons receive inputs from the reticular formation and vestibular nuclei (Jankowska et al., 2003; Krutki et al., 2003; Jankowska et al., 2009). Commissural interneurons receiving reticulospinal inputs (their excitatory or inhibitory nature was not identified) have been observed to be active during fictive locomotion in the cat (Matsuyama et al. 2004). In the mouse, last-order commissural interneurons have also been identified (Quinlan and Kiehn, 2007). These neurons may be rhythmically active during locomotion (Zhong et al., 2006). Whether commissural input is a major source of motoneuron excitation during locomotion is questionable given that motoneuron activity is robust in hemisectioned spinal cords (see Cowley et al., 2009).

V2a interneurons

V2a interneurons, which are defined by the expression of the homeobox gene *Lhx3* and further specified by the homeodomain proteins *chx10* and *sox14*, send ipsilateral glutamatergic connections to motoneurons (Al-Mosawie et al., 2007; Lundfald et al., 2007). These neurons are located in lamina VII (Al-Mosawie et al., 2007). Ablation of these cells using transgenic methods leads to deficits in right-left coordination of locomotion (Crone et al., 2008). These deficits are apparent at higher locomotor speeds, evidenced by a switch from normal running to an abnormal hopping pattern (Crone et al., 2009). Interestingly, motoneurons are still rhythmically active when these neurons are eliminated, indicating that they are not the sole source of rhythmic excitation of motoneurons during locomotion.

V3 interneurons

Another class of ventral interneurons that has been shown to send glutamatergic inputs to motoneurons is the V3 class of interneurons as defined by the expression of *Sim1* (Zhang et al., 2008). Unlike the V2a class of interneurons, V3 interneurons project primarily to contralateral motoneurons. Similar to the V2a class however, ablation of the V3 class compromises the robustness of the locomotor rhythm in the isolated mouse spinal cord (Zhang et al., 2008). It is not known whether these neurons are rhythmically active during locomotion. However, there is no obvious change in the amplitude of motoneuron output, indicating that V3 interneurons do not provide a critical source of rhythmic excitation of motoneurons during locomotion.

Spinal Modulatory Neurons

During locomotion, the post-spike after hyperpolarisation is modulated in spinal motoneurons (Brownstone et al., 1992a). We have recently demonstrated that this modulation can be produced by activity in neurons supplying the prominent cholinergic C-boutons synapsing on motoneuronal somata (Miles et al., 2007). The neurons supplying these C-boutons are the medial partition neurons (Miles et al., 2007; named by Barber et al., 1984). These neurons, identified by expression of the transcription factor Pitx2, have now been shown to derive from the V0 domain and hence are called V0_C (for cholinergic) neurons (Zagoraïou et al., 2009). V0_C neurons do not receive direct primary afferent input. They are rhythmically active during locomotion, indicating they receive input from spinal locomotor networks. Silencing the output of these neurons through elimination of the synthetic enzyme for acetylcholine (choline acetyl transferase) limits the increase in motoneuron output necessary for some locomotor tasks (Zagoraïou et al., 2009). These data indicate that the spinal cord not only provides rhythmic excitatory and inhibitory input to motoneurons, but also provides modulatory input in a motor task-specific manner.

Concluding Remarks

In this review, we have briefly outlined some last-order interneurons identified in the cat and mouse. While the adult cat preparation has been very useful for the physiological identification of interneurons and the mouse has been useful for molecular identification and manipulation, it is the combination of these approaches, physiological and molecular, that will lead to our understanding of the spinal control of movement.

It is likely that there is significant redundancy in these circuits. This is demonstrated by the genetic manipulations which eliminate broad classes of interneurons (V0, V1, V2a, V3). None of these deletions leads to the elimination of rhythmic motoneuron excitation, and it is not clear if any eliminates rhythmic inhibition. That is, either there is redundancy (perhaps physiologically similar interneurons derive from different domains, see Wang et al., 2008), and/or the input is derived from dorsal interneurons (eg. Wilson et al. 2010). On the other hand, the spinal neurons responsible for AHP modulation seem to be discretely defined both physiologically and molecularly (Zagoraïou et al., 2009).

The understanding of neural circuits in relation to behaviour is a key area of investigation in neuroscience today. The contributions of Feldman, Rossignol, and Lund form a solid foundation on which to build this understanding. Using a specific motor output (eg. breathing, walking, or chewing) as a direct “read-out” of the behaviour enables the direct correlation of neural circuit activity and behaviour. The combination of new developments in electrophysiology (eg. adult mouse spinal cord recordings; Manuel et al., 2009), developmental biology (eg. transcription factors for definition of unique neuronal populations), physics (eg. optical methods), chemistry (eg. calcium- and voltage-sensitive dyes), biochemistry (eg. genetically-encoded calcium- and voltage-sensitive proteins) as well as advances in computational neuroscience (eg. Grillner, 2006) will lead to the understanding of what once seemed to be an intractable problem: how do spinal circuits produce motor behaviour?

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Figure 1.

Diagram depicting last-order spinal interneurons and some of their known excitatory inputs from descending and muscle afferent pathways. It is likely that each population receives inputs from many more types of afferents than those depicted. Filled circles represent classes of neurons identified using molecular biological approaches. Unfilled circles represent classes of neurons identified through anatomical and electrophysiological techniques. These are not mutually exclusive: for example, Renshaw cells and Ia inhibitory interneurons belong to the V1 class of spinal interneurons (indicated by yellow text) and some excitatory interneurons expressing EphA4 belong to the V2a class of interneurons. Note that not all neurons of each class are depicted; for example, some EphA4⁺ neurons may be last-order inhibitory interneurons and some V0 interneurons are excitatory. Red designates excitatory neurons and boutons, blue designates inhibitory neurons and boutons and green designates

neuromodulation. Solid lines represent monosynaptic inputs, and dashed lines represent supraspinal descending inputs that are either mono- or oligo-synaptic.

TABLE 1

Key criteria for last-order excitatory interneurons involved in locomotion

•	Glutamatergic interneurons
•	Located in the thoracolumbar spinal cord
•	Project to and excite limb alpha-motoneurons
•	At least two distinct populations, one providing excitation primarily to extensor and the other to flexor motoneurons
•	These two populations reciprocally inhibit each other (see Jankowska et al., 1967)
•	Those projecting to extensor motoneurons may receive low threshold primary afferent input (but see (Weber et al., 2007))
•	Receive direct reticulospinal input (Noga et al., 2003; Shefchyk and Jordan, 1985)
•	Rhythmically active during locomotion (Kiehn et al., 2008)
•	Their silencing leads to a reduction in output during locomotion
•	Their selective activation may lead to stepping

TABLE 2

Key criteria for last-order inhibitory interneurons involved in locomotion

<ul style="list-style-type: none">• Glycinergic and/or GABAergic interneurons• Located in the thoracolumbar spinal cord• Project to and inhibit limb alpha-motoneurons• At least two distinct populations, one providing inhibition primarily to extensor and the other to flexor motoneurons• Likely receive input from last-order excitatory interneurons• Receive disynaptic reticulospinal input, likely mediated via last-order excitatory interneurons (Noga et al., 2003; Shefchyk and Jordan, 1985)• Rhythmically active during locomotion (Kiehn et al. 2008)• Their silencing leads to some overlap of flexor and extensor output during locomotion as well as prolongation of motoneuron bursts