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SYMPOSIUM

The Role of Genetically-Defined Interneurons in Generating the Mammalian Locomotor Rhythm

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Synopsis Locomotor behavior in mammals requires a complex pattern of muscle activation. Neural networks, known as central pattern generators (CPGs) and located entirely within the spinal cord, are responsible for generating much of the timing and pattern required for locomotor movements. Historically, identification of interneuronal components of the locomotor CPG in walking mammals has proven troublesome, primarily because of the difficulty in identifying functionally homogeneous groups of neurons in the spinal cord. Recently, a molecular approach has been used to identify populations of genetically similar interneurons based on the expression of transcription factors early in embryonic development. Preliminary work on these cell populations has shown that many comprise essential components of the locomotor CPG. Here I identify populations of genetically-defined interneurons that are candidate "first-order" cells of this neural network, potentially responsible for generating the locomotor rhythm in the mammalian spinal cord. Identification of the cell population(s) responsible for this key function will provide valuable insight into the structure and function of the locomotor CPG and could potentially lay the groundwork for the development of strategies aimed at regenerating motor pathways following injury to the spinal cord.

The locomotor central pattern generator: an overview

Central Pattern Generators (CPGs) are functional neural networks that are able to generate intrinsic patterns of rhythmic activity. They exist throughout the CNS and control such behaviors as breathing, swallowing, chewing, and walking. CPGs were first postulated to exist in the spinal cord of walking mammals and control locomotor behavior a century ago (Brown 1911). In the years since, there has been a great deal of investigation into the detailed structure and mechanism of function of mammalian locomotor CPGs and it is now accepted that these circuits can generate rhythmic, locomotor-like motor patterns in the absence of sensory input (Grillner and Zangger 1984) or descending inputs from the brain (Kudo and Yamada 1987; Cazalets et al. 1992; Clarac et al. 2004). Injury to the spinal cord often compromises one's ability to maintain normal posture, execute coordinated movements, and walk. Much of the ongoing research attempting to promote functional regeneration of walking following spinal cord injury has involved attempts to restore connections across the site of injury (Thuret et al. 2006) to the locomotor CPG, so as to take advantage of its capacity to generate rhythm. A detailed characterization of the interneurons that comprise the locomotor CPG, and a better understanding of how they are activated and interconnect as a network, has the potential to benefit the development of therapies aimed at enhancing functional recovery after spinal cord injury.

For much of the past century, studies investigating the locomotor CPG have relied on electrophysiological approaches to identify and characterize component interneurons of this neural circuit, and on

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conventional techniques of anatomical tracing to determine their connectivity. While these approaches have been effective in characterizing locomotor circuits in two nonmammalian species, i.e. lamprey (Grillner 2003) and *Xenopus* tadpoles (Roberts et al. 1998), relatively little is known about the neuronal connectivity of these circuits in mammals (Kiehn and Kjaerulff 1998; McCrea 1998; Jankowska 2001; Kiehn and Butt 2003; Clarac et al. 2004; Kiehn 2006). This is primarily due to the large number of neurons present in the mammalian spinal cord combined with the fact that cells with a similar function are intermingled with other, functionally unrelated, neurons. Although these factors have limited our ability to identify components of the mammalian locomotor CPG and unravel its specific structure, a series of experiments have demonstrated that this network is located throughout the ventromedial portion of the lower thoracic and lumbar spinal cord (Kjaerulff and Kiehn 1996; Cowley and Schmidt 1997; Kremer and Lev-Tov 1997).

A logical approach to identifying components of the locomotor CPG, as well as the manner in which they are connected, is to identify ''first-order'' spinal interneurons that receive input from command centers in the brain that are known to initiate locomotor activity. The connectivity of these cells can then be studied in order to identify downstream components. This approach becomes more attractive when it is taken into account that the descending systems that are responsible for activating the locomotor CPG in mammals have been well defined. The most prominent of these is the reticulospinal tract that originates in the reticular formation and has been found to be essential for the production of locomotor activity evoked by brainstem stimulation in many different species (Shik et al. 1966; Grillner 1975; Garcia-Rill and Skinner 1987; Grillner and Dubuc 1988). In mammals, activation of the reticulospinal tract is necessary and sufficient for the initiation of locomotion (Steeves and Jordan 1980; Shefchyk et al. 1984; Drew et al. 1986) and it has been shown that locomotor-initiating centers in the midbrain/pontine tegmentum (Jones and Yang 1985; Garcia-Rill and Skinner 1987) and cerebellum (Noga et al. 1995) converge in the reticular nucleus before descending to the spinal cord. It stands to reason, therefore, that identifying the cells in the spinal cord that are monosynaptically activated by the reticulospinal tract will be a significant step forward in our understanding of the structure and mechanism of function of the locomotor CPG. Some progress has been made in this regard. Studies have shown that reticulospinal tract cells make monosynaptic contacts onto interneurons located in the ventro-medial region (lamina VII/VIII) of the lumbar spinal cord (Bannatyne et al. 2003; Jankowska et al. 2003; Matsuyama et al. 2004; Szokol et al. 2011), and many of these cells are rhythmically active during locomotor activity (Matsuyama et al. 2004).

Despite knowledge of the presence of these neurons for almost two decades, there has been no conclusive data detailing their role during locomotion or determining whether they project to other components of the locomotor CPG. This is primarily due to a limitation of the anatomical and electrophysiological techniques used to study these cells. Only a select few lamina VII/VIII neurons receive projections from the reticulospinal tract and they are densely intermingled with cells that do not. This makes the task of identifying, tracing, and/or recording from a substantial number of them extremely difficult using a ''blind'' approach.

Incorporating molecular genetic techniques to study the locomotor CPG

Over the past decade, there has been a great deal of enthusiasm that a novel experimental approach incorporating molecular techniques with traditional anatomical and electrophysiological approaches, will enable key components of the locomotor CPG to be identified (Goulding et al. 2002; Kiehn and Kullander 2004; Goulding and Pfaff 2005; Goulding 2009; Garcia-Campmany et al. 2010). Molecular genetic experiments have shown that the developing neural tube in the embryonic mouse can be divided into eleven distinct populations of spinal neurons (dI1–dI6, V0–V3, VMN) based on the expression of transcription factors (Tanabe and Jessell 1996). These populations can first be identified at about embryonic Day 10 (E10). By E13, they begin to migrate toward their settling position which they reach before birth and where they remain postnatally (see Fig. 1). Since the transcriptional profile directs cell fate (Bang and Goulding 1996; Goulding and Lamar 2000; Goulding and Pfaff 2005), pattern of axonal projection (Betley et al. 2009) and neurotransmitter phenotype (Cheng et al. 2005; Mizuguchi et al. 2006; Pillai et al. 2007), it is possible that neurons with a similar genetic background will have similar characteristics and a similar function in locomotor activity.

A molecular strategy can thus be used to investigate the structure and function of locomotor circuits by labeling entire populations of genetically defined neurons with reporter proteins (such as GFP), thereby enabling all cells belonging to a given population

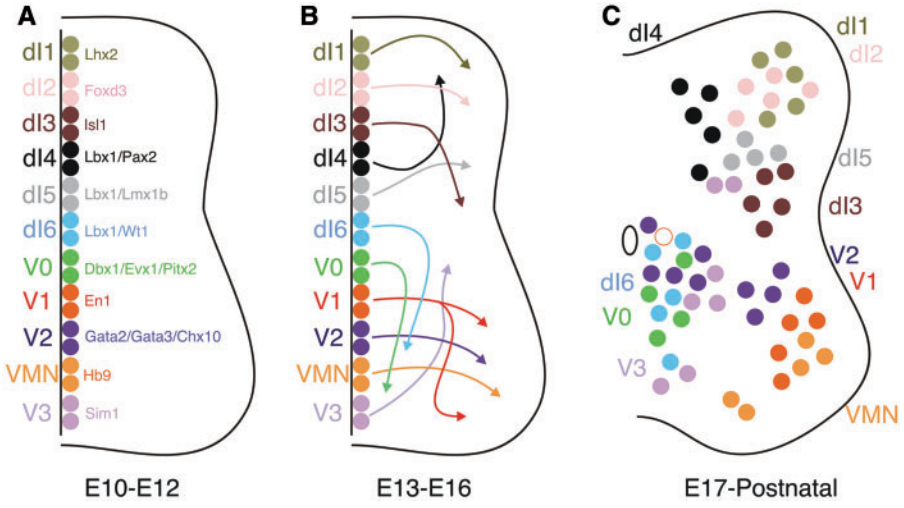

Fig. 1 (**A**) Transcription factors expressed from embryonic Days 10–12 (E10–E12) allow spinal cord neurons to be divided into several genetically distinct populations. (**B**) From E13 to E16, each population migrates toward their settling position. (**C**) Just before birth, populations reach the positions in the spinal cord where they remain throughout adulthood. Note: empty orange circle close to central canal in C represents a small population of interneurons that are derived from Hb9 progenitors (referred to as Hb9 interneurons).

to be visualized in live tissue with a fluorescent microscope. This renders anatomical and electrophysiological approaches much more efficient since they can be focused on interneurons with a specific genetic background. Alternatively, techniques can be used to selectively silence or ablate entire populations, which allows their specific function during behaviors, such as locomotion, to be elucidated. Initial evidence that certain genetically-defined interneurons are directly related to functionally-defined cell types in the adult came from the demonstration that a subpopulation of the V1 interneurons differentiate as Renshaw cells, the interneuronal cell type that mediates recurrent inhibition of motoneurons (Sapir et al. 2004). Further validation for this multidisciplinary approach has come from several studies performed in the past decade, characterizing many of the neuronal populations that originate in the ventral neural tube and defining their specific function during locomotor activity (see Table 1 for summary).

To this point, studies implementing this multidisciplinary approach have not resulted in the identification of a genetically-defined population of neurons that receive monosynaptic input from the reticular formation and is responsible for generating the locomotor rhythm. Experiments studying the mechanisms underlying initiation of locomotion have shown that the rhythm can be elicited by increasing neuronal excitability (Bracci et al. 1998; Whelan et al. 2000), or selectively activating glutamatergic neurons (Talpalar and Kiehn 2010; Hägglund et al. 2010). Furthermore, locomotor activity has been shown to persist in the absence of all fast inhibitory synaptic transmission (Bracci et al. 1996) and following midline hemisection (Kjaerulff and Kiehn 1996; Bonnot and Morin 1998). Collectively, these studies suggest that locomotor activity occurs due to the activation of a group of ipsilaterally projecting, excitatory neurons. In addition, previous work suggests that these cells should be located in lamina VII/VIII of the thoraco-lumbar spinal cord (Kjaerulff and Kiehn 1996; Cowley and Schmidt 1997) and possess intrinsic membrane properties of rhythm-generating neurons (Hochman et al. 1994; Kiehn et al. 1996; Schmidt et al. 1998; Tazerart et al. 2007; McCrea et al. 2007; Zhong et al. 2007; Tazerart et al. 2008; Brownstone and Wilson 2008; Harris-Warrick 2010).

Based solely on their location (lamina VII/VIII) in the thoraco-lumbar spinal cord, five populations of genetically defined interneurons meet the anatomical criteria of rhythm-generating cells. A significant number of the V0, V2, and V3 interneurons are located in this region, as are the dI6 cells (which originate in the dorsal neural tube and migrate ventrally during embryonic development), and a small population of interneurons that express the homeobox transcription factor Hb9. In subsequent sections, I review work that has characterized the electrophysiological and anatomical properties of each of these populations in mammals as well as their specific activity pattern and function during locomotion. Finally, I discuss whether each population is a candidate to receive input from locomotor initiating sites in the brainstem and generate the locomotor rhythm.

Table 1 Genetically-defined interneuronal populations currently thought to be involved during locomotor activity

Cell population	Subpopulations	Neurotransmitter phenotype	Axonal projection	Knockout phenotype	Primary references
dI6		Some cells inhibitory	Some cells commissural	Not determined	Goulding 2009; Rabe et al. 2010
V0	$V0_D$	Inhibitory/excitatory	Primarily commissural	Loss of left–right coordination	Pierani et al. 2001; Lanuza et al. 2004; Zagoraiou et al. 2009
	$V0_V$	Inhibitory/excitatory	Primarily commissural		
	$V0_{C/G}$	Excitatory	Ipsilateral		
V1		Inhibitory	Ipsilateral	Slow rhythm	Saueressig et al. 1999; Sapir et al. 2004; Gosgnach et al. 2006
V2	V2a	Excitatory	Ipsilateral	Left–right synchronyat high speed	Lundfald et al. 2007; Crone et al. 2008; Crone et al. 2009; Dougherty et al. 2010b; Zhong et al. 2010
	V2b	Inhibitory	Primarily ipsilateral	Not determined	Lundfald et al. 2007; Lanuza et al. 2007
V3		Excitatory	Ipsilateral and commissural	Unbalanced rhythm	Zhang et al. 2008
Hb9		Excitatory	Ipsilateral	Not determined	Hinkley et al. 2005; Wilson et al. 2005; Kwan et al. 2009

V0 interneurons

Primarily due to their settling position in the postnatal spinal cord, one of the first genetically defined populations postulated to be part of the locomotor CPG were the V0 interneurons. These cells are generated between E10 and E13 from progenitors located at the dorsal–ventral boundary of the developing neural tube that express Pax7, Dbx1, and Dbx2 (Pierani et al. 1999). Postmitotically, V0 neurons express the homeodomain proteins Evx1 and Evx2 (Burrill et al. 1997; Matise and Joyner 1997; Pierani et al. 1999). Detailed analysis of the V0 interneurons during development demonstrates that these cells settle in the ventro-medial spinal cord, and are present in all spinal segments (Pierani et al. 1999) with approximately two-thirds of the population inhibitory and one-third excitatory (Lanuza et al. 2004).

Intensive study into the development of the V0 interneuron population has resulted in the identification of multiple, molecularly distinct, subclasses of V0 cells. Initially, the population was divided into the dorsally located ($V0_D$), and ventrally located ($V0_V$) cells. Both of these subpopulations express the homeodomain protein Dbx1; however the $V0_V$ cells can be distinguished by postmitotic expression of the homeodomain protein Evx1 (Moran-Rivard et al. 2001). Both the $V0_D$ and $V0_V$ subpopulations exhibit similar migratory patterns toward lamina VII/VIII of the spinal cord and the majority extend their axons commissurally (Moran-Rivard et al. 2001; Pierani et al. 2001). Recently, a third subpopulation of V0 cells has been identified ($V0_{C/G}$), which express the transcription factor Pitx2 (Zagoraiou et al. 2009). These neurons are a subset of the $V0_V$ cells as they transiently express Evx1, and are either cholinergic ($V0_C$) or glutamatergic ($V0_G$). Interestingly, these neurons share few characteristics with other members of the V0 population as they project their axons ipsilaterally and provide excitatory input onto motoneurons (Zagoraiou et al. 2009).

The fact that there are multiple subpopulations of V0 interneurons opens up the possibility that this is a functionally diverse population, with each genetically distinct subclass having unique properties and playing a specific, perhaps complimentary, role to that of other subpopulations. During locomotor activity the function of this population as a whole was assessed using Dbx1 mutant mice, which lack all three subpopulations of V0 interneurons. Fictive locomotion was able to be elicited in these mice using the standard pharmacological cocktail (NMDA/5-HT), suggesting that these cells alone are not responsible for initiating locomotor activity. These mice did, however, exhibit frequent episodes of co-contraction between contralateral motoneurons, leading to the conclusion that the primary role for the V0 populations is to establish alternating left–right motor activity during locomotion (Lanuza et al. 2004). Selective elimination of the Evx1

transcription factor (which results in the loss of functional $V0_V$ neurons) had no effect on the fictive locomotor pattern (Lanuza et al. 2004), suggesting that either the $V0_D$ interneurons alone are responsible for coordinating left–right alternation, or that both the $V0_D$ and $V0_V$ populations have similar, additive effects.

Interestingly, a subsequent study investigating the role of the $V0_{C/G}$ interneurons during locomotor activity demonstrated that selective inactivation of this subpopulation exhibits no deficit in left–right coordination, but rather impairs a task-dependent increase in firing of motoneurons and in activation of muscles (Zagoraiou et al. 2009), a subtle phenotype which may have been overlooked in the study in which all Evx1-expressing cells or Dbx1-expressing cells had been ablated. The authors of this study suggested that $V0_{C/G}$ interneurons are responsible for providing neuromodulatory input to motoneurons (Zagoraiou et al. 2009).

Although one or more subpopulation of V0 interneurons is clearly essential for coordinating left–right alternation during locomotion, the recent characterization of the $V0_{C/G}$ interneurons suggests that the V0 population as a whole is much more functionally diverse than was initially thought. As mentioned previously, approximately one-third of V0 cells are glutamatergic. Since the majority of these excitatory cells do not belong to the $V0_G$ subpopulation (which comprise significantly <5% of the entire V0 population) (Zagoraiou et al. 2009), the specific role of these remaining glutamatergic neurons during locomotor activity is unknown. One possibility is that they are also involved in coordinating left–right alternation by disynaptically inhibiting contralateral motoneurons (Quinlan and Kiehn 2007; Dougherty and Kiehn 2010a; Kiehn et al. 2010). Based on recent studies that demonstrate inspiratory pacemaker neurons in the respiratory CPG originate from Dbx1-expressing progenitor cells (Bouvier et al. 2010; Gray et al. 2010), an alternative hypothesis is that these excitatory V0 cells are part of a locomotor–rhythm-generating network consisting of interneurons from several populations. In the absence of the excitatory V0 cells (in the Dbx1 mutant mouse), interneurons belonging to other populations are able to compensate for their loss resulting in the persistence of rhythmic locomotor activity.

V2 interneurons

Much like the V0 interneurons, the V2 population, which is derived from Lhx3 progenitors, can be divided into multiple subtypes, each having unique characteristics. V2a cells express the transcription factor Chx10 (Peng et al. 2007) while the V2b subpopulation can be identified by expression of Gata2 and Gata3 (Ericson et al. 1997; Zhou et al. 2000; Karunaratne et al. 2002; Smith et al. 2002). Cell bodies of both subpopulations are scattered in lamina VII of the postnatal spinal cord, extending from the central canal out toward the lateral edge of the gray matter (Dougherty and Kiehn 2010b). While all V2a interneurons have been shown to extend their axons ipsilaterally, a small number of V2b cells make commissural projections (Lundfald et al. 2007). Marked differences between the subpopulations begin to appear when the neurotransmitter phenotype of each is analyzed. Approximately 95% of $Chx10^+$ V2a cells are excitatory, while $Gata2/3^+$ V2b cells are almost entirely inhibitory (Lundfald et al. 2007; Lanuza et al. 2007). Since excitatory interneurons are known to be essential for the initiation and maintenance of locomotor activity in mammals (Kiehn et al. 2008), and electrophysiological studies have demonstrated that many of the $Chx10^+$ V2a cells display intrinsic membrane properties of oscillatory neurons (Dougherty and Kiehn 2010b; Zhong et al. 2010), a great deal of effort has gone into characterizing their activity and specific function during fictive locomotion. Thus far, a thorough characterization of V2b cells, and an investigation of the role that this inhibitory population plays in fictive locomotion has yet to be carried out.

Deficits in the *in vitro* fictive locomotor pattern of mice lacking V2a cells led to the suggestion that, like the V0 cells, this population is involved in coordinating left–right alternation (Crone et al. 2008). Since this subpopulation of cells project ipsilaterally, it was postulated that they make excitatory synapses onto commissural interneurons and coordinate contralateral motoneurons via disynaptic mechanism (Crone et al. 2008). This hypothesis was supported by anatomical tracing of axons from V2a interneurons onto $Evx1^+$ $V0_V$ cells (Crone et al. 2008).

Subsequent studies demonstrated that a greater percentage of V2a interneurons are rhythmically active during bouts of high-frequency fictive locomotion *in vitro* (Zhong et al. 2010), and that during a treadmill task adult mice lacking V2a interneurons are unaffected at low speeds, but lose left–right alternation and "gallop" at high speeds (Crone et al. 2009). Taken together, the *in vitro* and *in vivo* experiments have led to the conclusion that activation of V2a cells and their excitation of the commissural pathways that coordinate left–right alternation occurs primarily during walking at high

speeds (Crone et al. 2009; Zhong et al. 2010; Dougherty and Kiehn 2010b).

Despite their location in the postnatal spinal cord, their excitatory neurotransmitter phenotype, and ipsilateral pattern of projection, the fact that locomotor outputs persist in the absence of the V2a cells demonstrates that this population is not solely responsible for generating the locomotor rhythm. While recent studies have demonstrated that many of these cells are driven from the rhythm-generating core of the locomotor CPG (Dougherty and Kiehn 2010b; Zhong et al. 2010), their anatomical and electrophysiological characteristics raise the possibility that a subset of this population may comprise part of the aforementioned rhythm-generating network and work together with cells belonging to other populations to initiate locomotor activity.

V3 interneurons

The V3 interneurons are another population of primarily excitatory cells in the mammalian spinal cord (Zhang et al. 2008). These cells originate in the ventral extreme of the developing neural tube from Nkx2.2/2.9 progenitors. Postmitotically these cells express the transcription factor Sim1 (Briscoe et al. 1999; Goulding et al. 2002). V3 neurons take up positions in dorso-medial and ventro-medial regions of the spinal cord postnatally. The projection pattern of these cells is mixed, with the majority (~85%) extending axons contralaterally. An initial characterization of these cells indicated that they project to contralateral motoneurons and lack the required intrinsic membrane properties (Zhang et al. 2008).

To identify the specific role of the V3 neurons during locomotor activity a combination of *in vitro* and *in vivo* approaches was used. During *in vitro* fictive locomotion in a transgenic mouse line in which the V3 cells were selectively silenced, there was a marked irregularity of the locomotor rhythm, in which step-cycle period and burst duration varied from step to step, and asymmetry was seen between ventral roots on the left and right sides of the spinal cord (Zhang et al. 2008). This phenotype was nicely recapitulated in the *in vivo* mouse during an overground locomotor task when the V3 cells were silenced. It was concluded from these experiments that V3 interneurons are not involved in the generation of rhythm, but rather balance the rhythmic motor outputs generated by the independent oscillators located on either side of the spinal cord (Zhang et al. 2008). One caveat with this study, that does not preclude the V3 cells from a role in rhythm generation, is that the only V3 neurons that were studied were those located in the ventral-most regions of the spinal cord. These cells make up a small proportion of the entire V3 population and primarily make monosynaptic connections to motoneurons. It is highly unlikely that last order interneurons would contribute to rhythm generation; thus, it is not surprising that these cells lack intrinsic oscillatory properties. A definitive description of the role of these neurons in generation of rhythm awaits a full characterization of the more dorsal populations of V3 cells.

dI6 interneurons

In addition to the interneuronal populations that originate in the ventral aspect of the developing neural tube (i.e. V0–V3) the dI6 cells, which originate from Lbx1-expressing progenitors immediately dorsal to the V0 interneurons, migrate ventromedially during embryogenesis, and take up positions in laminae VII/VIII of the postnatal spinal cord (Gross et al. 2002). Based on their settling position alone, these cells are candidates to be involved in generating the locomotor rhythm. Until recent work demonstrated that dI6 cells express the transcription factor Wt1 (Goulding 2009), the lack of a unique molecular marker of this population has hindered their characterization; however, preliminary studies investigating these cells have suggested that they are a mixed population of ipsilaterally and commissurally projecting interneurons and are thought to be a component of the locomotor CPG (Goulding 2009; Rabe et al. 2010).

Interestingly, these cells share many similarities with the V0 interneurons. In addition to having a similar pattern of migration, both the dI6 and the $V0_D$ cells develop from Pax7 and Dbx2 progenitors ($V0_D$ cells can be differentiated by the expression of the transcription factor Dbx1). Furthermore, in the Dbx1 mutant mouse, many V0 neurons acquire characteristics of dI6 cells soon after their generation (Lanuza et al. 2004). This has led to the suggestion that the dI6 population plays a complimentary role to the V0 cells, and that they are involved in coordinating left–right alternation during locomotion. Now that a unique transcription factor has been found that identifies this population, these cells will be able to be characterized and the specific role that they play during locomotion can be assessed.

Hb9 interneurons

The homeobox gene Hb9 is a transcription factor that is expressed by embryonic motoneurons (Arber et al. 1999) and has also been shown to define a specific

class of interneurons (Hinckley et al. 2005; Wilson et al. 2005). Interneurons expressing Hb9 are located in the ventro-medial spinal cord, abutting the ventral commissure from the cervical to mid-lumbar segments (Hinckley et al. 2005; Wilson et al. 2005; Ziskind-Conhaim et al. 2010), the most rhythmogenic segments of the mammalian spinal cord (Kjaerulff and Kiehn 1996; Cowley and Schmidt 1997).

In addition to their location in lamina VII/VIII of the postnatal spinal cord, they exhibit many signature criteria of rhythm-generating cells of the locomotor CPG. Hb9 interneurons receive monosynaptic input from low threshold sensory afferents (Hinckley et al. 2010), are glutamatergic, synaptically interconnected, and project their axons ipsilaterally (Hinckley et al. 2005). Furthermore, this population has been shown to be rhythmically active during fictive locomotion (Hinckley et al. 2005; Wilson et al. 2005) and exhibit appropriate electrophysiological characteristics, including a persistent sodium current (Ziskind-Conhaim et al. 2008), prominent postinhibitory rebound, and the ability to burst rhythmically when synaptically isolated and exposed to pharmacological agents that evoke fictive locomotion (Wilson et al. 2005).

Despite this evidence supporting their role in generating rhythm, a number of findings have cast serious doubt on the assertion that these cells are solely responsible. First, the sheer number of $Hb9^{+}$ interneurons (~40 per spinal segment in the mouse) seems insufficient to generate activity in a large network such as the locomotor CPG (Kwan et al. 2009). Second, it has been shown that these neurons are seldom present below the third lumbar segment; however, rhythmic activity can be generated in these caudal segments when they are isolated (Kjaerulff and Kiehn, 1996). Finally, recent studies have demonstrated that initiation of activity in Hb9 interneurons lags behind the onset of ipsilateral ventral-root bursting during fictive locomotion, and that these cells are only active during the first few locomotor cycles (Kwan et al. 2009).

Unfortunately it has not been straightforward to examine the specific role that this intriguing population of cells plays during locomotion. While the role played by other ventral interneuron populations has been studied by employing a genetic strategy to either selectively ablate or silence all neurons expressing a specific transcription factor and analyze locomotor behavior in their absence, fictive locomotion cannot be elicited in the absence of Hb9 as it is expressed in all motoneurons. These experiments have thus been put on hold until a specific marker of the Hb9-expressing interneurons is identified.

Closing comments

Almost a decade has passed since the first studies demonstrated that a molecular approach could be combined with traditional electrophysiological and anatomical techniques to identify components of the locomotor CPG. Studies that have characterized the genetically defined interneuronal populations that originate in the ventral spinal cord, and investigated their specific function, have yet to lead to the identification of a population of cells that is necessary and sufficient for the initiation of activity in the locomotor CPG. In spite of this, many neurons belonging to the genetically defined cell groups discussed in this review exhibit the electrophysiological and anatomical properties of rhythm-generating cells.

An attractive hypothesis, therefore, is that subpopulations of several genetically distinct interneuronal groups work together to generate the locomotor rhythm. In this arrangement, the absence of any one population of cells would not affect the initiation of locomotor activity, as the majority of cells comprising this rhythm-generating network would remain present and functional. This hypothesis will be able to be tested in the upcoming years via experiments which incorporate recently developed techniques (Szokol et al. 2011) to identify the genetically defined subpopulations of cells in the mammalian spinal cord that receive monosynaptic input from the reticular formation. Subsequent to this, selective silencing of these subpopulations in unison will be used to determine whether they are required for the initiation of mammalian locomotion. Identification of these neurons will be essential for understanding how locomotor circuits in mammals are organized and function.

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