available at www.sciencedirect.com

ScienceDirect

BRAIN RESEARCH REVIEWS

ELSEVIER

Review

The mammalian central pattern generator for locomotion

Pierre A. Guertin*

Neuroscience Unit, Laval University Medical Center (CHUL-CHUQ), Quebec City, Quebec, Canada
Faculty of Medicine, Department of Neuroscience and Psychiatry, Laval University, Quebec City, Quebec, Canada

ARTICLE INFO

ABSTRACT

At the beginning of the 20th century, Thomas Graham Brown conducted experiments that after a long hiatus changed views on the neural control of locomotion. His seminal work supported by subsequent evidence generated largely from the 1960s onwards showed that across species walking, flying, and swimming are controlled largely by a neuronal network that has been referred to as the central pattern generator (CPG) for locomotion. In mammals, this caudally localized spinal cord network was found to generate the basic command signals sent to muscles of the limbs for locomotor rhythm and pattern generation. This article constitutes a comprehensive review summarizing key findings on the organization and properties of this network.

© 2009 Elsevier B.V. All rights reserved.

Contents

* Laval University Medical Center (CHUL-CHUQ), Neuroscience Unit, RC-9800, 2705 Laurier Blvd, Quebec City, Quebec, Canada G1V 4G2.
Fax: +1 418 654 2753.
E-mail address: pierre.Guertin@crchul.ulaval.ca.

1.   Introduction

It is well known by now that the spinal cord is a structure of the central nervous system (CNS) that produces in large part simple reflexes (e.g., monosynaptic excitatory, reciprocal inhibitory, withdrawal and crossed-extension reflexes). The spinal cord also contributes substantially to the control of complex motor functions such as locomotion. The control of locomotion has been mainly studied in animal models although inferential evidence of the existence of a central pattern generator (CPG) for locomotion has been provided for the human (recently reviewed in Hultborn and Nielsen, 2007; Minassian et al., 2007). CPGs have been extensively studied in non-mammalian and invertebrate species (see, e.g., Hugues and Wiersma, 1960; Stein, 1971; Delcomyn, 1977; Robertson and Pearson, 1985; Clarac and Pearlstein, 2007). In fact, studies on sea slugs, leeches, cockroaches, stick insects and crustacean locomotor (swimmeret) and motor (e.g., somatogastric system) pattern-generating networks have played a pivotal role in understanding the cellular and network bases of rhythmic motor and locomotor patterns in both invertebrate and vertebrate species (e.g., Hugues and Wiersma, 1960; Getting, 1977; Kristan and Weeks, 1983; Hopper and DiCaprio, 2004; Buschges et al., 2008). The concept of the CPG itself as a neuronal network that is capable of generating an organized pattern of rhythmic motor activity independently of sensory inputs was first described in invertebrates (Bullock, 1961; Wilson and Wyman, 1965).

The mammalian CPG associated specifically with the control of the hindlimbs constitutes the main subject of the present review. It has been the subject of previous reviews that focused on different aspects of its function such as the role of reflexes (Grillner, 1975), inputs from other systems (e.g., Shik and Orlovsky, 1976), intrinsic spinal rhythms (e.g., Delcomyn, 1980), and seminal observations and divergent opinions of pioneers (Clarac, 2008; Stuart and Hultborn, 2008). The reader is referred also to other articles, and references therein, for reviews on other rhythmic motor networks (e.g., respiration, mastication or scratching) for which findings have often been made in parallel with those on locomotor networks (e.g., Syed et al., 1990; Smith et al., 1991; Nakamura and Katakura, 1995; Arshavsky et al., 1997; Alford et al., 2003; Stein, 2005; Guertin and Steuer, 2009).

2.   Early evidence of its existence

It had already been reported by the late 19th century that spinal cord-transected animals could display locomotor-like movements (e.g., Flourens, 1824; Freusberg, 1874; for even earlier historical reports and thoughts, see Clarac 2008). Spinal cord-transected dogs were shown in 1874 to generate short episodes of locomotor activity when dropping one of the limbs from a flexed position (Freusberg, 1874). Comparable observations filmed and analyzed by Philippson (1905) led him to conclude that the spinal cord controls locomotion using both central and reflex mechanisms. Sherrington's (1910) extensive work on spinal cord-transected cats and dogs provided evidence suggesting that the basic motor pattern for walking is the result of reflex actions from proprioceptors onto spinal centers. After a brief period of spinal shock following a transection of the spinal cord, the hindlimbs were capable of executing movements called 'reflex stepping' which strikingly resemble those of the natural step. In order to produce stepping movements in decerebrate, acute spinal preparations, the animals were lifted from the ground with the spine vertical and the hindlimbs pendent which under their own weight sufficed to elicit stepping that could be stopped by passively flexing one limb at the hip joint. Because stepping could still be obtained after cutting all hindlimb cutaneous nerves, Sherrington believed that the locomotor rhythm was supported by cyclic input from proprioceptors of the hip flexor muscles and other reflexes (i.e., extensor thrust and 'umkehr'). Sherrington already knew that reflex stepping is not solely the result of peripheral input mediated via the flexion and crossed-extension reflex pathways since passive immobilization of one hindlimb during vigorous stepping did not prevent stepping in the contralateral limb. Since stepping could also be evoked by continuous stimulation of the skin (e.g., pinching the perineum or ear's pinna) or the spinal cord (i.e., faradization of the cut surface), Sherrington suggested the existence of specialized neurons in the spinal cord that can transform the tonic peripheral input into basic central stepping motor commands for stepping. However, it is essentially one of his junior collaborators, Thomas Graham Brown, who described independently (see next section) the potential existence of a spinal neuronal network for locomotion (see Stuart and Hultborn, 2008, for a thorough review of exchanges between Sherrington and Graham Brown and of Graham Brown's original contribution).

3.   Conceptual models of organization

3.1.   Half-center model

Sherrington's term "half-center," which he used temporarily to explain the spinal pathway for reciprocal inhibition, was used later by Graham Brown in his model of spinal locomotor control based on his experiments showing that the basic pattern for stepping was generated entirely in the spinal cord in the absence of peripheral afferent input in spinal cord-transected cats, rabbits and guinea-pigs (Graham Brown, 1911, 1914). The animals, under general anaesthesia, were lying on

one side when stepping movements in the hindlimbs were spontaneously evoked ('narcosis progression') after a transection of the cord at the lower thoracic level. Since the level of anaesthetic used was shown to abolish proprio- and exteroceptive reflexes but not locomotor activity, Graham Brown proposed a 'half-center' model made of two groups of spinal neurons reciprocally organized and mutually inhibiting each others that were capable of producing the basic rhythm and pattern for stepping. Activity in the first group of neurons (e.g., extensor half-center) would send motor commands to motoneurons (exciting extensors) and would inhibit simultaneously the reciprocal group of neurons (flexor half-center) preventing the excitation of antagonists (silencing flexors) (Fig. 1). After a period of 'depression' (e.g., fatigue, adaptation, post-inhibitory rebound) of the extensor half-center, the flexor half-drive would predominate for a new phase of activity. Despite these findings, the general opinion of scientists between 1920 and 1960 remained that basic locomotor activity largely depends upon sensory input from the peripheral nervous system (Delcomyn, 1980; Stuart and Hultborn, 2008). However, it is Elzbieta Jankowska and Anders Lundberg who have provided in the 1960s using intracellular recording techniques the first direct evidence supporting the existence of Graham Brown's model (Lundberg, 1965; Jankowska et al., 1967a,b). They identified intracellularly interneurons located in the lumbar segments of the cord (specifically in the lamina VII) that are active following flexion reflex afferent (FRA) stimulation. Specifically, one group of neurons was found to be activated by ipsilateral FRA, a second group by contralateral FRA (coFRA), and a third group by both ipsi- and contralateral stimulation. After injection of L-DOPA and nialamide in spinal cord-transected cats, FRA stimulation evoked a high-frequency burst followed by a long-lasting self-sustained series of discharges. Some neurons did not even show any short latency effects during the stimulus train. These interneurons were found to be monosynaptically excited by ventrolateral funiculus stimulation which contains descending fibers from the reticular formation. One of the most important features was the reciprocal organization between these groups of interneurons since coFRA stimulation abolished the long-latency discharges evoked by ipsilateral FRA and vice versa. Finally, it was proposed that Ia interneurons could participate in the production of the locomotor pattern by receiving strong excitatory input from FRA interneurons given their corresponding rhythmic activity in L-DOPA-treated cats. However, in the 1970s, several neuroscientists began to provide evidence suggesting that, as it was, this half-center organization could not fully explain the complex patterns of muscle activation found during terrestrial locomotion (e.g., in quadrupeds) (Grillner, 1975, see also section below on the unit burst generator model).

3.2. Miller and Scott model

Sharing similarities with the half-center model, the Miller and Scott hypothesis (1977) proposed that Renshaw cells rather than fatigue are responsible for the alternation between flexion and extension. Increasing activity in one pool of motoneurons (e.g., extensors) would be gradually inhibited by a corresponding increase of recurrent inhibition. Architecturally, this model takes into account known neuronal connections — it is constituted of a closed chain of neurons to which flexor and extensor motoneurons are connected in different parts. Renshaw cells and Ia inhibitory interneurons which are part of this chain of neurons are mainly responsible for reciprocal activation of the flexor and extensor motoneurons. Simultaneously, Renshaw cells would remove reciprocal inhibition of antagonists (recurrent facilitation) via their spindle Ia monosynaptic inhibitory input onto Ia interneurons allowing the flexor excitatory drive to take over for a new phase of activity (i.e., flexion). Interestingly, by varying the tonic input to the alpha motoneurons and Ia inhibitory interneurons, coactivation of the flexors and extensors may be achieved. Although, the reciprocity between flexors and extensors is nicely explained by this model, the origin of the rhythmicity itself is rather unclear. Other criticisms came from results showing that Renshaw cell activity may be inhibited during locomotor activity (Orlovskii et al., 1966; Bergmans et al., 1969) although discrepancies were reported during fictive locomotion (McCrea et al., 1980) whereas both classes of neurons were reported not to be essential for the production of a basic locomotor pattern in motoneurons (Pratt and Jordan, 1987). Other evidence against the Miller and Scott model was provided by Noga and colleagues (1987) who showed that the basic locomotor pattern and Ia interneuron activity remain after i.v. injection of the nicotinic antagonist mecamylamine (MEC), which greatly reduces Renshaw cell activation.

Half-center model

Fig. 1 – Schematic representation of the classical and simplest half-center model. Blue and black/white circles are interneurons and motoneurons, respectively. Excitatory and inhibitory connections are represented by lines ending with (V) or (O), respectively. This model is largely inspired from observations made in spinal cord-transected cats.

3.3. Ring model

The highly conceptual 'ring' model (Székely et al., 1969; Gurfinkel and Shik, 1973) was one of the models subsequently proposed to explain the existence of complex locomotor
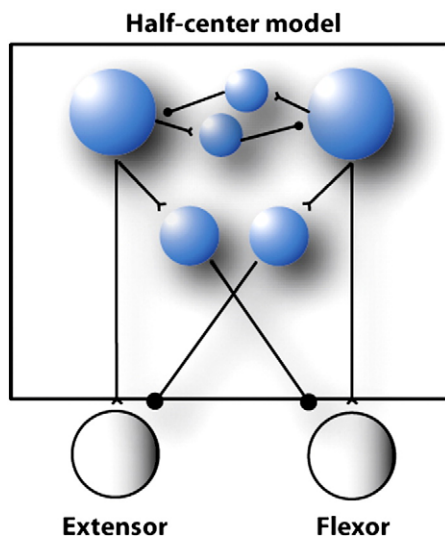
patterns (e.g., taking into account differences between backward or forward walking, synchronization and phase coupling between activity of the ring and the cyclic afferent input, etc.). It is made of a closed chain of at least five groups of neurons (e.g., 2 pure extensors, 2 pure flexors and 1 bifunctional) that project to the motoneurons either directly or through specific interneurons. The sequence of these projections determines the order of activation of various muscles during the step cycle. At rest, a number of ring neurons are tonically inhibited by a certain group of spinal neurons. Activation of the monoaminergic descending system would result in the inhibition of the inhibitory neurons with consequent disinhibition of the ring neurons. Activity within the ring is based on a cyclically propagated inhibitory drive that would travel at different speeds from one group to the other depending on the excitability (e.g., modulated by afferent input) of the path ('ring') interconnecting them. The activity within the ring starts when the excitability level is raised (through disinhibition), so the neurons would discharge when not inhibited. A slow-propagated drive would activate neurons of a group for a longer period of time (e.g., pure extensor during the stance phase) whereas a fast-propagated one would activate a group of neurons for a brief moment in motoneurons to bifunctional muscles (Shik and Orlovsky, 1976). However, this highly conceptual model has generally failed to convince most scientists in this field (i.e., no more than ten related articles may be found on PubMed!).

3.4. Flexor burst generator model

During those same years, other models such as the flexor burst generator were also proposed. Pearson and Duysens (1976) introduced this model for insects and cats. It consists of a rhythmic excitatory drive from the flexor burst generator to populations of flexor motoneurons. The burst generator would inhibit, via an inhibitory interneuron, the activity of extensors otherwise activated during the flexor silence by a tonic excitatory input. This asymmetrical model was abandoned later on in favour of a more symmetrical bipartite organization (i.e., in which both extensor and flexor portions are equally driven; see Pearson, 1995). This change in views is likely related to the subsequent description of a powerful feedback system associated with ankle extensor group I afferents that can reset rhythms and strongly excite most pools of hindlimb extensor motoneurons during locomotion (Guertin et al., 1995; Whelan et al., 1995). This said, recent data mainly from Brownstone's group provided evidence suggesting that an asymmetrical CPG organization may be re-considered. They proposed that a rhythm-generating layer, composed of a kernel of heterogeneous and electrotonically coupled neurons, would project directly to the flexor half-center of the pattern formation layer (Brownstone and Wilson, 2008).

3.5. Unit burst generator model

In the 1970s and early 1980s, the unit burst generator model contributed to the demonstration of a symmetrically organized generator in the spinal cord that can produce the basic pattern of motor commands for walking even in absence of peripheral input. It was proposed essentially to explain that

locomotion is not only a strictly alternating pattern of flexor and extensor activity (requiring all motoneurons to belong to one of these two groups) as proposed by the half-center model (initially by Graham Brown and subsequently by Lundberg and colleagues). Patterns of locomotor activity are often complex and may include some motoneuron pools that display activity during both the flexion and extension phases of the step cycle or that display differences in the onset and offset of activity in individual flexor and extensor pools. The persistence of such complex activity patterns following bilateral deafferentation of the hindlimbs in decerebrate cats, led Grillner and Zangger (1974, 1979) to conclude that the locomotor CPG does not simply generate an alternating activation of flexors and extensors but a more delicate pattern that will sequentially start and terminate the activity in the appropriate muscles at the correct instance. Their idea was further developed in a proposal for a CPG architecture in which separate "modules" or unit burst generators would control subsets of motoneurons (see Grillner, 1981). First, Székely et al. (1969) showed that the locomotor pattern (in the forelimbs) in freely moving newts was similar before and after a bilateral section of the dorsal roots. This was also shown in decerebrate cats (Grillner and Zangger, 1974) which has then led to the suggestion that a CPG could exist for each joint of each limb (Edgerton et al., 1976; Grillner, 1981; see Fig. 2). Activity from these 'units' would be tightly coupled during 'normal' walking but individually controlled by supraspinal input to produce different types of motor patterns. This model emerged after analyzing more complex patterns of locomotor activity such as backward walking, climbing, etc. For instance, it was occasionally observed during fictive locomotion that one hindlimb motor
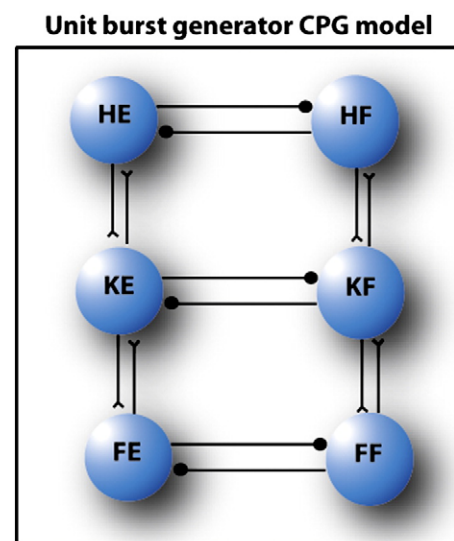

Fig. 2 – Unit burst generator CPG model. Blue circles are interneurons controlling hip (H), knee (K) and foot (F) extensors (E) and flexors (F). Excitatory and inhibitory connections are represented by lines ending with (V) or (O), respectively. This model originated mainly from observations made in spinal cord-transected cats.

nerve can display tonic activity while the others display a normal rhythmic pattern. Also, the activity of pluriarticular muscle nerves such as *semitendinosus* is sometimes in phase with extensors, or flexors, or both, which some authors found difficult to explain with a half-center type of model (Edgerton et al., 1976). Along this idea, Bizzi's group provided experimental and analytical results suggesting instead that sensory-dependent linear combinations of a small number of muscle synergies may generate diverse motor and locomotor patterns (Tresch et al., 1999; Bizzi et al., 2008). Some of the cellular components of Grillner's CPG model was identified in the 1980s using a simpler non-mammalian vertebrate nervous system preparation — the *in vitro* isolated lamprey preparation (see Fig. 3). However, despite the attractiveness of this proposal, the unit burst generator model has not generally explained the existence of other complex patterns of motoneuronal activity such as those found during spontaneous deletions (see section below).

3.6.   *Two-level half-center models*

Thus far, most models had failed to entirely explain the many patterns that can occur in the generally alternating activity of flexors and extensors during locomotion (see McCrea and Rybak, 2008). In particular, unpredictable changes called 'dele-

Lamprey's CPG model
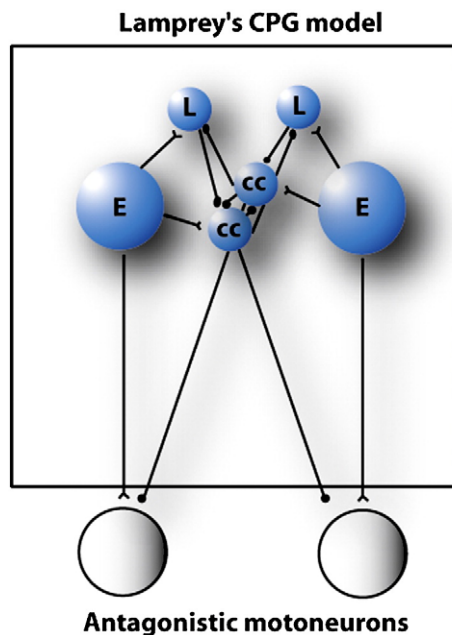
Antagonistic motoneurons

Fig. 3 – Schematic representation of the basic cellular components underlying the CPG for swimming in lampreys. Blue and black/white circles are interneurons and motoneurons, respectively. Excitatory and inhibitory connections are represented by lines ending with (V) or (O), respectively. Cc and L interneurons are inhibitory whereas E interneurons are excitatory. This model was largely based from electrophysiological data obtained using the *in vitro* isolated lamprey spinal cord preparation. Note that for clarity reasons, sensory inputs were omitted. Adapted from Grillner et al. (1988).

tions' which refer to periods of silenced activity in some populations of motoneurons (e.g., extensors such as the *soleus*) accompanied of sustained or rhythmic activity in antagonist motoneurons (e.g., flexors such as the *tibialis anterior*) while post-deletion rhythm is generally maintained. This is essentially why attempts to explain these other types of changes have first been proposed based on bipartite CPG levels. Indeed, a detailed analysis of nerve and muscle activity during spontaneous walking in non-paralyzed cats or during fictive locomotor activity in paralyzed decorticated cats let Perret and Cabelguen (1980) to initially propose a bipartite or two-level (half-center-like) model that explains the complex biphasic activity in so-called bifunctional motoneurons (e.g., *semitendinosus*). They proposed that not only one half-center but both half-centers (extensor and flexor ones) would send motor commands to bifunctional motoneurons. A variety of motoneuron patterns could be produced by modulating the half-centers output 'en route' to these motoneurons. They also suggested that a rhythm generator would be functionally separated from a pattern generator since the rhythm and the amplitude of the locomotor drive potentials appear to be two distinct characteristics that can be independently and spontaneously changing. This paved the way to other studies from Kriellaars (1992) and Kriellaars et al. (1994) who proposed a functional separation of pattern and amplitude modules (as proposed earlier also by Perret and Cabelguen, 1980). They showed that locomotor drive potentials monitored simultaneously (by dual intracellular recordings *in vivo*) in pairs of motoneurons generally covary in amplitude in homonymous motoneurons while antagonist motoneurons inversely covary. The complex locomotor pattern in bifunctional motoneurons receiving input from both half-centers would be sculpted by controlling the amplitude of the flexor and extensor locomotor drive 'en route' to these motoneurons. A similar separation of CPG function (rhythm vs. pattern and amplitude) was suggested in other studies to explain how sensory stimulation can also alter locomotor cycle timing without altering the level of motoneuron activity (Kriellaars et al., 1994; Guertin et al., 1995; Perreault et al., 1995; Guertin, 1996) which has served in the 1990s as basis to the elaboration of the most recently proposed multi-level models (2+ and 3 levels, see Rybak et al., 2009). Additional evidence from non-resetting deletions reported by McCrea and colleagues during fictive locomotion and scratch in the decerebrate cat strongly supported also this two-level CPG organization (Lafreniere-Roula and McCrea, 2005). Finally, mathematical models recently developed by McCrea and Rybak (2008) have constituted additional supports for the existence of multi-level CPG (half-center-like) organizations that can explain spontaneous deletions and other complex patterns of activity during locomotion. All in all, none of the above models have been refuted.

4.   Cellular constituents

4.1.   *Activity-dependent labeling and selective lesions experiments*

Because the mammalian CPG was still largely considered as a 'black box' at the end of the 1980s, researchers have begun exploring, using activity-dependent labeling, the localization

of spinal cord neurons active during locomotor activity. Activity-dependent methods using [¹⁴C]2-deoxyglucose or c-fos labeling revealed intermediate zone-labeled neurons in the lumbar area of the spinal cord (rabbits and cats) (Viala et al., 1988; Dai et al., 2005). Using *in vitro* isolated spinal cord preparations, Kjaerulff et al. (1994) used sulforhodamine-101, an activity-dependent marker/dye, to identify CPG neuron candidates in the rat. Following sustained spinal CPG-mediated locomotor activity induced by bath-applied N-methyl-D-aspartate (NMDA) and serotonin (5-HT), they found mainly labeled cells in L1–L6 located bilaterally near the central canal and in the medial intermediate zone (Kjaerulff et al., 1994). A comparable approach used by Cina and Hochman (2000) suggested that a relatively small number of neurons may compose the CPG. Using sulforhodamine and fictive locomotor activity induced by bath-applied 5-HT, they showed a restricted number of labeled cells (presumably CPG neurons) mainly in the spinal segments L1–L5. The labeled cells correspond to less than 0.1% of all cells constituting these segments according to Cina and Hochman (2000) (Figs. 4A and
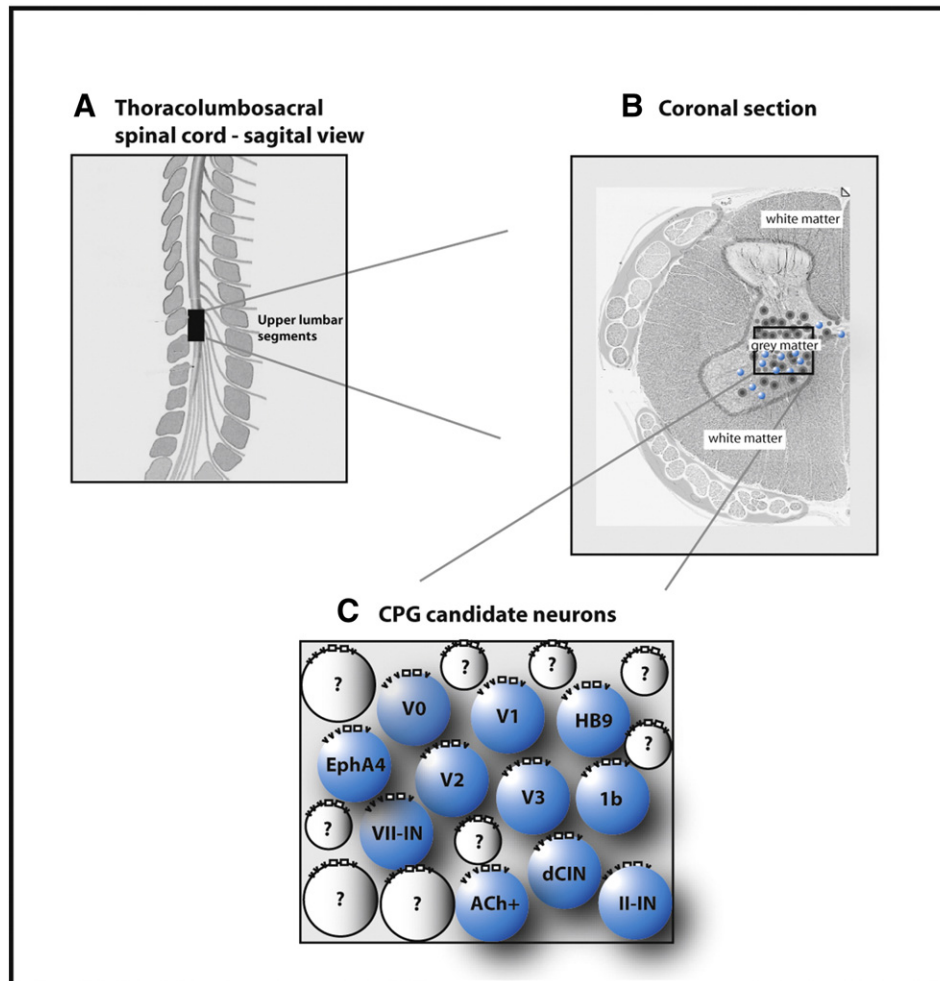

Fig. 4 – Schematic representation of the CPG including most current findings in rodents. (A) Although its conceptual organization remains unclear (e.g., unit burst generator, half-center, etc.), compelling evidence suggests its localization as a network in the thoracolumbosacral spinal cord with key elements in the upper lumbar cord segments (e.g., mice, Nishimaru et al., 2000; humans, Dimitrijevic et al., 1998). (B) Numerous locomotor activity-labeled (e.g., using c-fos or sulforhodamine) lumbar cord neurons have been found specifically in the gray matter intermediate zone, ventral horn and central canal areas (e.g., Kjaerulff et al., 1994; Cina and Hochman, 2000) as well as most of the other CPG-candidate neurons identified electrophysiologically or genetically (see below for references). (C) Although the CPG largely remains a black box, a number of CPG candidate neurons have been identified mainly genetically (see main text for details), such as the populations of HB9 (Wilson et al., 2005), EphA4 (Kullander et al., 2003), V0 (Lanuza et al., 2004), V1 (Gosgnach et al., 2006), V2a/b (Lundfald et al., 2007), V3 (Zhang et al., 2008), excitatory Ib (Guertin et al., 1995; Angel et al., 2005), lamina VII-IN (Jankowska et al., 1967a,b), ChAT-positive/c-fos-labeled ascending (Huang et al., 2000), rhythmic interneurons activated by group II afferent (Edgley and Jankowska, 1987) and descending commissural (dCIN) interneurons (reviewed in Butt et al., 2002). A number of receptors [V] and channels [□] have been associated with locomotor-like activity generation including the NA/DA (Jankowska et al., 1967a,b), 5-HT1A (Landry et al., 2006), 5-HT2A, 5-HT7 (Landry et al., 2006), D1 (Lapointe et al., 2009), NMDA (Guertin, 2004) and CaV1.3 (in vitro evidence, Guertin and Hounsgaard, 1998b).

B). These findings are supported also by other studies that showed, using electrical stimulation or selective lesions, key CPG elements in upper lumbar cord segments (paraplegic patients, Dimitrijevic et al., 1998; isolated spinal cord from mice, Nishimaru et al., 2000, see also Kiehn, 2006; Christie and Whelan, 2005, for further details regarding a rostrocaudal distribution of the CPG). Discrepancies may exist in other species such as in cats where key CPG elements are apparently more caudal (e.g., in mid-lumbar segments in cats, Langlet et al., 2005).

4.2. Genetic and molecular assessment

In recent years, advances in genetics and transgenic murine models have largely contributed to the identification of new CPG neuron candidates. Several populations of interneurons involved in locomotor activity were indeed characterized genetically (e.g., V0–V3). One of them is the population of V0 interneurons that is associated with left-right alternation since mice without V0 interneurons (lacking the transcriptional factor Dbx1) displayed bilateral synchrony rather than bilateral alternation during locomotion (Lanuza et al., 2004). Another population referred to as the V1 inhibitory interneurons (expressing the transcription factor Engrailed 1) is associated with high locomotor frequencies since slow rhythms were found in En1-DTA mice (Gosgnach et al., 2006). Other genetically identified populations include the Chx10-expressing cells (V2a glutamatergic and V2b gabaergic interneurons, Lundfald et al., 2007) located in the intermediate zone of the gray matter in the lumbar spinal cord. These neurons were associated with frequency, amplitude and bilateral coordination since all of these parameters were affected in Chx10-DTA mice (lacking V2a interneurons, Crone et al., 2008). V3 interneurons (Sim-1 expressing cells) constitute another recently identified population shown to participate in the production of a robust and balanced rhythm during locomotion. Indeed, rhythmic activity was found to be partially disrupted in mice lacking V3 interneurons (Zhang et al., 2008) (Fig. 4C). Genetically engineered animals were also utilized to show that hopping instead of normal walking is displayed in mice lacking the ephA4 receptor-expressing lumbar interneurons (Kullander et al., 2003; Butt et al., 2005). Finally, another population of CPG neuron candidate called HB9 neurons was reported to play a role in excitation during locomotion. Although, no corresponding knockout model has been tested, compelling evidence suggests that HB9 excitatory interneurons are part of an asymmetrically organized rhythm-generating network (Wilson et al., 2005; Brownstone and Wilson, 2008).

Taken altogether, results from these exciting new studies in mice suggest that these genetically characterized interneurons (V0–V3, ephA4, HB9) may constitute different cellular components of the CPG. For instance, V0 interneurons form many cell types including commissural neurons which could establish inhibitory reciprocal connections between the two sides of the spinal cord. In turn, V1 interneurons may be associated with inhibitory interneurons such as the Ia inhibitory and Renshaw cells whereas HB9s may be excitatory interneurons constituting at least part of the rhythm generating layer. However, additional studies

need to be conducted in order to fully characterize these promising new CPG neuron candidates (Brownstone and Wilson, 2008).

4.3. Electrophysiological techniques

These techniques have been instrumental to characterize the rhythms and patterns of discharge of individual neurons as well as their sources of input during locomotor activity generally in anesthetized and curarized animals or in *in vitro* isolated spinal cord preparations. Although motoneurons have been most studied electrophysiologically during locomotor activity, they are not generally considered as part of the CPG *per se* (for recent reviews of motoneuronal properties during CPG activity, see Brownstone, 2006; Grob and Guertin, 2007). This said, a few populations of CPG interneuron candidates have been identified using electrophysiological techniques mainly in cat preparations (see below).

4.3.1. Results from in vivo animal models
As mentioned earlier (Section 2.1), Jankowska and Lundberg recorded intracellularly from lamina VII interneurons in the lumbar spinal cord that may constitute the first identified cellular component of the mammalian locomotor network (Jankowska et al., 1967a,b). These interneurons were found to generate long-lasting discharges (centrally rather than reflexively generated) in L-DOPA and nialamide-treated spinal cord-transected cats. Following preliminary observations made by Guertin and colleagues (1995) and Angel, Jankowska and McCrea (2005) identified a second class of CPG interneuron candidate in cats. These interneurons located in L7 are excitatory, active mainly during the extension phase (silent and non-responsive in absence of locomotion or during flexion), and responsive to group I afferent stimulation from extensor nerves. In the absence of peripheral nerve stimulation, most of them remain active during the extension phase of MLR-induced fictive locomotion in decerebrate paralyzed cats (Angel et al., 2005). Compelling evidence suggests that these interneurons contribute also to mediate the powerful group Ia and Ib excitation of extensor motoneurons triggered by muscle spindles and Golgi tendon organs (from extensor muscles), respectively, during locomotion (Gossard et al., 1994; Guertin et al., 1995; Angel et al., 1996). Another population of interneurons was also identified in the intermediate zone of the lumbar spinal cord. It receives strong excitation from group II afferents and weak excitation from group I afferents (Edgley and Jankowska, 1987). Half of these interneurons are active during flexion and the other half are tonically inhibited throughout locomotion (Shefchyk et al., 1990). Finally, a population of cholinergic CPG interneuron candidate was also found in cats. They are located mainly in the intermediate zone of the lumbar spinal cord, ChAT-positive, activity-dependent-labeled with c-fos during locomotion, in-phase with ipsilateral extensor activity and projecting contralaterally (Huang et al., 2000).

4.3.2. Results from in vitro isolated spinal cord preparations
Using *in vitro* preparations from neonatal rodents, a heterogeneous population of descending commissural interneurons (dCINs) was identified in the ventromedial area of the lumbar

spinal cord (L2 and L3) (Hoover and Durkovic, 1992; Puskar and Antal, 1997; Stokke et al., 2002; Nissen et al., 2005). These neurons fire in-phase with the ipsilateral or contralateral L2 activity during fictive locomotion induced by NMDA/5-HT in rats. They were found also to project caudally several segments away (L4–L5) where most hindlimb motoneurons are localized. Using electrophysiological techniques in *in vitro* isolated preparations (slices or whole cords), it has been also possible to investigate many of the currents and channels involved in spinal rhythm generation. An important role for persistent sodium currents in rhythm generation was recently shown in neonatal rodents (Zhong et al., 2007; Tazerart et al., 2008) whereas calcium, I_h, I_{CAN}, various potassium currents and many other types of currents were found to contribute to spinal locomotor rhythm generation (reviewed in Harris-Warrick, 2002).

4.4. Pharmacological in vivo approaches

In recent years, an increase in commercially available blood brain barrier permeable selective ligands has been advantageously used to pharmacologically 'dissect' *in vivo* the contribution of specific receptors and channels to locomotor rhythm generation. Using *in vivo* adult spinal cord-transected models and quantitative approaches to assess drug-induced hindlimb movements, insightful data on CPG-related channels and receptor have recently been reported. Clear CPG-activating effects induced by specific drugs have also been found in various *in vitro* preparations from invertebrate and vertebrate species, although it is beyond the scope of this review to report on all of them (e.g., rats, Sigvardt et al., 1985; Cazalets et al., 1990; Cowley and Schmidt, 1994a, 1994b; Kiehn and Kjaerulff, 1996; mice, Nishimaru et al., 2000; Whelan et al., 2000; turtles, Guertin and Hounsgaard, 1998a).

As mentioned earlier, the noradrenergic system and specifically L-DOPA was associated with CPG activation in acutely spinal cord-transected cats and rabbits (e.g., Jankowska et al., 1967a,b; Grillner and Zangger, 1974; Pearson and Rossignol, 1991; Viala and Buser, 1969). Clonidine, an alpha-2 adrenergic receptor agonist, administered during sensory stimulation (e.g., tail pinching) was also reported to largely enhance the effects of training and sensory stimulation on locomotor rhythmogenesis in spinal cord-transected cats (Forssberg and Grillner, 1973; Barbeau and Rossignol, 1991; Chau et al., 1998a,b). However, it has been difficult to determine site-specific actions (e.g., on motoneurons, CPG neurons or primary afferents) from results in many of these earlier studies that were not designed to specifically assess drug-induced CPG activation *per se* (i.e., given that experimenter-associated manipulations such as tail pinching, regular training or weight support assistance with harnesses that can affect CPG activation were typically used).

4.4.1. In vivo data from non-assisted, non-stimulated and untrained paraplegic animals
More recently, experiments conducted in our laboratory using a simple and reliable semi-quantitative assay (ACOS, Guertin, 2005) and a mouse model (a complete low-thoracic transection) with no assistance (e.g., no training, no tail stimulation,

and no weight support assistance to avoid unspecific non-drug induced effects) have contributed to identify a subset of transmembranal receptors involved in pharmacologically-elicited, CPG-mediated locomotor-like movements. For instance, L-DOPA, serotonin (5-HT) or dopamine (DA) receptor ligands such as 8-OH-DPAT, buspirone (5-HT1A/7 agonists), quipazine (5-HT2A/2C agonist) and SKF-81297 (D1-like agonist) were found to trigger significant locomotor-like movements (i.e., rhythmic bilaterally alternating flexions and extensions involving one or several hindlimb joints) whereas agonists such as TFMPP (5-HT1B), m-CPP (5-HT2B/2C), SR57227A (5-HT3) or clonidine (adrenergic alpha-2 agonist) induced mainly non-locomotor movements (i.e., non-bilaterally alternating movements, twitches, cramps, etc.) in spinal cord-transected mice (Guertin, 2004; Landry and Guertin, 2004; Landry et al., 2006; Lapointe et al. 2008, 2009).

Although data using these pharmacological approaches in *in vitro* preparations or *in vivo* non-assisted spinal cord-transected animals strongly suggest that specific receptor subtypes may be associated with CPG activation, they do not demonstrate that CPG neurons contain the corresponding neurotransmitters. While suggesting that CPG neurons possess some of the identified receptor subtypes (targeted by the selective ligands and further confirmed with selective antagonists and knockout animal models, see section below), it can not be excluded based on these data that some of the identified receptor subtypes may also be localized presynaptically (e.g., on presynaptic terminals of afferent fibers or non-CPG neurons that project to CPG neurons).

4.4.2. Target specificity confirmed with selective antagonists and knock-out animals
Using selective antagonists and genetically-manipulated animals (e.g., 5-HT7KO mice), it has been established that NMDA, 5-HT1, 5-HT7, 5-HT2A and D1 receptors were specifically involved in mediating *in vivo* such CPG-activating locomotor-like effects (e.g., Landry et al., 2006; Ung et al., 2008). For instance, endogenous glutamate release and NMDA receptor activation were reported as critically important for quipazine-induced effects since a complete loss of induced movement was found in NMDA antagonist (MK-801)-treated animals (Guertin, 2004). Regarding DA receptors, administration of D2, D3 or D4 agonists was found not to generate significant hindlimb locomotor movements whereas D1/D5 agonists such as SKF-81297 potently elicit locomotor-like movements that are prevented in selective D1-like (D1/D5) antagonist-pretreated spinal cord-transected mice but no in D5–/– paraplegic mice suggesting a specific contribution of the D1 subtype to CPG activation (Lapointe et al., 2009). All and all, such pharmacological approaches used *in vivo* in untrained and non-assisted spinal cord-transected animals have contributed to identify a subset of CPG-activating compounds (and corresponding receptors confirmed with selective antagonists and knockout animal models). However, none of these molecules were found to generate large amplitude weight bearing stepping movements *per se* in untrained, non-assisted and non-sensory stimulated spinal cord-transected animals suggesting that only partial CPG activating effects can be induced by these ligands administered separately (Guertin, 2009).

4.4.3.   Synergistic effects of drug combinations: evidence suggesting that several receptor subtypes need to be simultaneously activated for full CPG activation

Simultaneously activating several populations of receptors was found, using similar pharmacological approaches and animal models, to apparently induce greater locomotor effects. Partial CPG-activating effects (i.e., associated with crawling rather than full weight bearing stepping) induced by some ligands, as mentioned above, were found indeed to turn into full CPG-activating effects (i.e., weight bearing stepping in non-assisted, untrained and non-stimulated paraplegic animals) by simultaneously administrating some of these drugs (e.g., 8-OH-DPAT, quipazine, L-DOPA, and SKF-81293; Guertin, 2008, 2009). This said, the extent to which the cellular network activated pharmacologically *in vivo* corresponds to already identified CPG neuron candidates (electrophysiologically or genetically) remains unclear. However, dual immunohisto-chemical experiments recently showed locomotor activity-labeled (c-fos) 5-HT1A-, 5-HT2A- or 5-HT7-positive neurons in the cat lumbar cord (specifically in laminae VII-VIII; Noga et al., unpublished data) suggesting that some of the locomotor activity-related receptors identified recently in *in vivo* models (Sections 3.3.2 and 3.3.3) may indeed be located on CPG neurons.

5.   Conclusion

Although evidence of its existence goes back to Graham Brown's early experiments conducted a century ago, much remain to be learnt about this fascinating and complex neuronal network that is the CPG. Indeed, the CPG is increasingly being dissected using novel tools, techniques and approaches but is still, in most parts, a 'black box' for which many of the components are incompletely character-ized. No clear consensus has been reached regarding its organization as a network (e.g., two-level half-center-like or unit burst generator-like, etc.) and it remains unclear whether some of the recently discovered cellular properties found genetically, molecularly, electrophysiologically, pharmacolog-ically or behaviourally belong to the same CPG neuron candidates across studies, experimental conditions, animal models or species. There are still active discussions as to whether or not Renshaw cells, Ia interneurons or motoneurons are part of the CPG (i.e., do they significantly contribute to rhythm or pattern generation) and we do not know for sure if 'pacemaker-like' properties are critical for rhythmogenesis within the network. Finally, it is unclear whether the organi-zation and cellular properties are well-conserved phylogenet-ically and if we will ever be able to undisputedly demonstrate its existence in humans (i.e., given that it can be studied only by inference). Although many questions remain and only 0.1% of all upper lumbar cord cells are believed to constitute the CPG, tremendous progress has been made recently suggesting that its identity and entire organization will eventually be unra-veled. Although not addressed in this review, it goes without saying that in normal life (unlike in reduced models) CPG activities are likely to be several times more complex than what has been depicted in this article. Indeed, the CPG is also known to receive additional commands and signals from other structures of the CNS (e.g., cortex, basal ganglia cerebellum, mesencephalic region, and brainstem structures) which, altogether, may constitute a global command center controlling holistically goal-directed gait and locomotion (for recent reviews, see Grillner et al. 2008; Jordan et al., 2008; Drew et al., 2008). Advances in this field of research are likely to contribute to understanding further the mechan-isms underlying locomotor deficits after spinal cord injury and help the development of rehabilitation strategies to improve reflex or sensorimotor integration and CPG function during walking (Stein and Mushahwar, 2005; Lapointe et al., 2007; Petruska et al., 2007; Guertin, 2008; Thompson et al., 2009). Promising research avenues that may contribute to future advances include, for instance, the use of imaging approaches (e.g., calcium concentration-related) to study the spatiotemporal aspects of locomotor activity in spinal neurons (O'Donovan et al., 2005; Wilson et al., 2007).

REFERENCES

Alford, S., Schwartz, E., Viana di Prisco, G., 2003. The pharmacology of vertebrate spinal central pattern generators. Neuroscientist 9 (3), 217–228.

Angel, M.J., Guertin, P., Jimenez, I., McCrea, D.A., 1996. Group I extensor afferents evoke disynaptic EPSPs in cat hindlimb extensor motorneurones during fictive locomotion. J. Physiol. 494 (Pt 3), 851–861.

Angel, M.J., Jankowska, E, McCrea, D.A., 2005. Candidate interneurones mediating group I disynaptic EPSPs in extensor motoneurones during fictive locomotion in the cat. J. Physiol. 563 (Pt 2), 597–610.

Arshavsky, Y.I., Deliagina, T.G., Orlovsky, G.N., 1997. Pattern generation. Curr. Opin. Neurobiol. 7 (6), 781–789.

Barbeau, H., Rossignol, S., 1991. Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. Brain Res. 546 (2), 250–260.

Bergmans, J., Burke, R., Lundberg, A., 1969. Inhibition of transmission in the recurrent inhibitory pathway to motoneurones. Brain Res. 13 (3), 600–602.

Bizzi, E., Cheung, V.C., d'Avella, A., Saltiel, P., Tresch, M., 2008. Combining modules for movement. Brain Res. Rev. 57, 25–33.

Brownstone, R.B., 2006. Beginning at the end: repetitive firing properties in the final common pathway. Prog. Neurobiol. 18, 156–172.

Brownstone, R.B., Wilson, J.M., 2008. Strategies for delineating spinal locomotor-rhythm generating networks and the possible role of Hb9 interneurones in rhythmogenesis. Brain Res. Rev. 57, 64–76.

Bullock, T.H., 1961. The origin of patterned nervous discharge. Behaviour 17, 48–59.

Buschges, A., Akay, T., Gabriel, J.P., Schmidt, J., 2008. Organizing network action for locomotion: insights from studying insect walking. Brain Res. Rev. 57, 162–171.

Butt, S.J., Harris-Warrick, R.M., Kiehn, O., 2002. Firing properties of identified interneuron populations in the mammalian hindlimb central pattern generator. J. Neurosci. 22 (22), 9961–9971.

Butt, S.J., Lundfald, L., Kiehn, O., 2005. EphA4 defines a class of excitatory locomotor-related interneurons. Proc. Natl. Acad. Sci. USA 102 (39), 14098–14103.

Cazalets, J.R., Grillner, P, Menard, I, Cremieux, J., Clarac, F., 1990. Two types of motor rhythm induced by NMDA and amines in

an in vitro spinal cord preparation of neonatal rat. Neurosci. Lett. 111 (1-2), 116–121.

Chau, C., Barbeau, H., Rossignol, S., 1998a. Effects of intrathecal alpha1- and alpha2-noradrenergic agonists and norepinephrine on locomotion in chronic spinal cats. J. Neurophysiol. 79 (6), 2941–2963.

Chau, C., Barbeau, H., Rossignol, S., 1998b. Early locomotor training with clonidine in spinal cats. J. Neurophysiol. 79 (1), 392–409.

Christie, K.J., Whelan, P.J., 2005. Monoaminergic establishment of rostrocaudal gradients of rhythmicity in the neonatal mouse spinal cord. J. Neurophysiol. 94 (2), 1554–1564.

Cina, C., Hochman, S., 2000. Diffuse distribution of sulforhodamine-labeled neurons during serotonin-evoked locomotion in the neonatal rat thoracolumbar spinal cord. J. Comp. Neurol. 423 (4), 590–602.

Clarac, F., 2008. Some historical reflections on the neural control of locomotion. Brain Res. Rev. 57, 13–21.

Clarac, F, Pearlstein, E., 2007. Invertebrate preparations and their contribution to neurobiology in the second half of the 20th C. Brain Res. Rev. 54, 113–161.

Cowley, K.C., Schmidt, B.J., 1994a. A comparison of motor patterns induced by N-methyl-D-aspartate, acetylcholine and serotonin in the in vitro neonatal rat spinal cord. Neurosci. Lett. 171 (1-2), 147–150.

Cowley, K.C., Schmidt, B.J., 1994b. Some limitations of ventral root recordings for monitoring locomotion in the in vitro neonatal rat spinal cord preparation. Neurosci. Lett. 171 (1-2), 142–146.

Crone, S.A., Quinlan, K.A., Zagoraiou, L., Droho, S., Restrepo, C.E., Lundfald, L., Endo, T., Setlak, J., Jessell, T.M., Kiehn, O., Sharma, K., 2008. Genetic ablation of V2a ipsilateral interneurons disrupts left-right locomotor coordination in mammalian spinal cord. Neuron 60 (1), 70–83.

Dai, X., Noga, B.R., Douglas, J.R., Jordan, L.M., 2005. Localization of spinal neurons activated during locomotion using the c-fos immunohistochemical method. J. Neurophysiol. 93 (6), 3442–3452.

Delcomyn, F., 1977. Co-ordination of invertebrate locomotion. In: Alexander, RM, Gold Spink, G (Eds.), Mechanics and Energetics of Locomotion. Chapman and Hall, London, pp. 82–114.

Delcomyn, F., 1980. Neural basis of rhythmic behavior in animals. Science 210, 492–498.

Dimitrijevic, M.R., Gerasimenko, Y., Pinter, M.M., 1998. Evidence for a spinal central pattern generator in humans. Ann. N.Y. Acad. Sci. 860, 360–376.

Drew, T., Andujar, J.E., Lajoie, K., Yakovenko, S., 2008. Cortical mechanisms involved in visuomotor coordination during precision walking. Brain Res. Rev. 57, 199–211.

Edgerton, V.R., Grillner, S., Sjostrom, A., Zangger, P., 1976. Central generation of locomotion in vertebrates. Neural control of locomotion, 18. Plenum Press, New-York, pp. 439–464.

Edgley, S.A., Jankowska, E., 1987. An interneuronal relay for group I and II muscle afferents in the midlumbar segments of the cat spinal cord. J. Physiol. 389, 647–674.

Flourens, M.-J.-P., 1824. Recherches expérimentales sur les propriétés et les Fonctions du Système Nerveux, dans les Animaux Vertébrés, Experimental Studies on the Properties and Functions of the Nervous System in Vertebrate Animals. Chez Crevot, Paris.

Forssberg, H., Grillner, S., 1973. The locomotion of the acute spinal cat injected with clonidine i.v. Brain Res. 50 (1), 184–186.

Freusberg, A., 1874. Reflexbewegungen beim Hunde. Pflügers Arch. 9, 358–391.

Getting, P.A., 1977. Neuronal organization of escape swimming in Tritonia. J. Comp. Physiol. 121, 325–342.

Gosgnach, S., Lanuza, G.M., Butt, S.J., Saueressig, H., Zhang, Y., Velasquez, T., Riethmacher, D., Callaway, E.M., Kiehn, O., Goulding, M., 2006. V1 spinal neurons regulate the speed of vertebrate locomotor outputs. Nature 440 (7081), 215–219.

Gossard, J.P., Brownstone, R.M., Barajon, I., Hultborn, H., 1994. Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. Exp. Brain Res. 98 (2), 213–228.

Graham Brown, T., 1911. The intrinsic factors in the act of progresson in the mammal. Proc. R. Soc. Lond. 84, 309–319.

Graham Brown, T., 1914. On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. J. Physiol. 48, 18–46.

Grillner, S., 1975. Locomotion in vertebrates: central mechanisms and reflex interaction. Physiol. Rev. 55, 247–304.

Grillner, S., 1981. Control of locomotion in bipeds, tetrapods, and fish. In: Brookhart, Mountcastle (Eds.), Handbook of Physiology. The nervous system II. Am. Physiol. Sco., Bethesda, pp. 1179–1236.

Grillner, S., Zangger, P., 1974. Locomotor movements generated by the deafferented spinal cord. Acta Physiol. Scand. 91, 38A–39A.

Grillner, S., Zangger, P., 1979. On the central generation of locomotion in the low spinal cat. Exp. Brain Res. 34 (2), 241–261.

Grillner, S., Buchanan, J.T., Lansner, A., 1988. Simulation of the segmental burst generating network for locomotion in lamprey. Neurosci. Lett. 89, 31–35.

Grillner, S., Wallen, P., Saitoh, K., Kozlov, A., Robertson, B., 2008. Neural bases of goal-directed locomotion in vertebrates — an overview. Brain Res. Rev. 57, 2–12.

Grob, M., Guertin, P.A., 2007. Role of Ca2+ in the pacemaker-like property of spinal motoneurons. Med. Sci. 23 (1), 64–66.

Guertin, P., 1996. Central mediation of grouop 1 muscle afferent evoked adaptation of the locomotor step cycle in decerebrate cats. Ph.D. thesis, University of Manitoba.

Guertin, P., Angel, M.J., Perreault, M.C., McCrea, D.A., 1995. Ankle extensor group I afferents excite extensors throughout the hindlimb during fictive locomotion in the cat. J. Physiol. 487 (Pt 1), 197–209.

Guertin, P.A., 2004. Role of NMDA receptor activation in serotonin agonist-induced air-stepping in paraplegic mice. Spinal Cord 42 (3), 185–190.

Guertin, P.A., 2005. Semiquantitative assessment of hindlimb movement recovery without intervention in adult paraplegic mice. Spinal Cord 43 (3), 162–166.

Guertin, P.A., 2008. A technological platform to optimize combinatorial treatment design and discovery for chronic spinal cord injury. J. Neurosci. Res. 86 (14), 3039–3051.

Guertin, P.A., 2009. Recovery of locomotor function with combinatory drug treatments designed to synergistically activate specific neuronal networks. Curr. Med. Chem. 16 (11), 1366–1371.

Guertin, P.A., Hounsgaard, J., 1998a. Chemical and electrical stimulation induce rhythmic motor activity in an in vitro preparation of the spinal cord from adult turtles. Neurosci. Lett. 245 (1), 5–8.

Guertin, P.A., Hounsgaard, J., 1998b. NMDA-Induced intrinsic voltage oscillations depend on L-type calcium channels in spinal motoneurons of adult turtles. J. Neurophysiol. 80 (6), 3380–3382.

Guertin, P.A., Steuer, I., 2009. Key central pattern generators of the spinal cord. J. Neurosci. Res. 87 (11), 2399–2405.

Gurfinkel, V.S., Shik, M.L., 1973. The control of posture and locomotion. In: Gydikov, F., Tankov, F., Kosarov, F. (Eds.), Motor control. Plenum Press, New York, pp. 217–234.

Harris-Warrick, R.M., 2002. Voltage-sensitive ion channels in rhythmic motor systems. Curr. Opin. Neurobiol. 12, 646–651.

Hoover, J.E., Durkovic, R.G., 1992. Re Retrograde labeling of lumbosacral interneurons following injections of red and green

fluorescent microspheres into hindlimb motor nuclei of the cat. Somatosens. Mot. Res. 9 (3), 211–226.

Hopper, S.L., DiCaprio, R.A., 2004. Crustacean motor pattern generator networks. Invertebrate neural networks. Neuro-Signals 13, 50–69.

Huang, A., Noga, B.R., Carr, P.A., Fedirchuk, B., Jordan, L.M., 2000. Spinal cholinergic neurons activated during locomotion: localization and electrophysiological characterization. J. Neurophysiol. 83 (6), 3537–3547.

Hugues, G.M., Wiersma, C.A.G., 1960. The coordination of swimmeret movements in the crayfish, *Procambarus clarkii* (Girard). J. Exptl. Biol. 39, 657–670.

Hultborn, H., Nielsen, J., 2007. Spinal control of locomotion — from cat to man. Acta Physiol. 189, 111–121.

Jankowska, E., Jukes, M.G., Lund, S., Lundberg, A., 1967a. The effect of DOPA on the spinal cord. 6. Half-centre organization of interneurones transmitting effects from the flexor reflex afferents. Acta Physiol. Scand. 70 (3), 389–402.

Jankowska, E., Jukes, M.G., Lund, S., Lundberg, A., 1967b. The effect of DOPA on the spinal cord. 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors. Acta Physiol. Scand. 70 (3), 369–388.

Jordan, L.M., Liu, J., Hedlund, P.B., Akay, T., Pearson, K.G., 2008. Descending command systems for the initiation of locomotion in mammals. Brain Res. Rev. 57, 183–191.

Kiehn, O., 2006. Locomotor circuits in the mammalian spinal cord. Annu. Rev. Neurosci. 29, 279–306 Review.

Kiehn, O., Kjaerulff, O., 1996. Spatiotemporal characteristics of 5-HT and dopamine-induced rhythmic hindlimb activity in the in vitro neonatal rat. J. Neurophysiol. 75 (4), 1472–1482.

Kjaerulff, O., Barajon, I., Kiehn, O., 1994. Sulphorhodamine-labelled cells in the neonatal rat spinal cord following chemically induced locomotor activity in vitro. J. Physiol. 478 (Pt 2), 265–273.

Kriellaars, D., 1992. Generation and peripheral control of locomotor rhythm. University of Manitoba, Ph D thesis.

Kriellaars, D.J., Brownstone, R.M., Noga, B.R., Jordan, L.M., 1994. Mechanical entrainment of fictive locomotion in the decerebrate cat. J. Neurophysiol. 71 (6), 2074–2086.

Kristan, W.B., Weeks, J.C., 1983. Neurons controlling the initiation, generation and modulation of leech swimming. In: Roberts, A, Roberts, B (Eds.), Neural origin of rhythmic movements. Cambridge University Press, Cambridge.

Kullander, K., Butt, S.J., Lebret, J.M., Lundfald, L., Restrepo, C.E., Rydstrom, A., Klein, R., Kiehn, O., 2003. Role of EphA4 and EphrinB3 in local neuronal circuits that control walking. Science 299 (5614), 1889–1892.

Lafreniere-Roula, M., McCrea, D.A., 2005. Deletions of rhythmic motoneuron activity during fictive locomotion and scratch provide clues to the organization of the mammalian central pattern generator. J. Neurophysiol. 94 (2), 1120–1132.

Landry, E.S., Guertin, P.A., 2004. Differential effects of 5-HT1 and 5-HT2 receptor agonists on hindlimb movements in paraplegic mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 28 (6), 1053–1060.

Landry, E.S., Lapointe, N.P., Rouillard, C., Levesque, D., Hedlund, P.B., Guertin, P.A., 2006. Contribution of spinal 5-HT1A and 5-HT7 receptors to locomotor-like movement induced by 8-OH-DPAT in spinal cord-transected mice. Eur. J. Neurosci. 24 (2), 535–546.

Langlet, C., Leblond, H., Rossignol, S., 2005. Mid-lumbar segments are needed for the expression of locomotion in chronic spinal cats. J. Neurophysiol. 93 (5), 2474–2488.

Lanuza, G.M., Gosgnach, S., Pierani, A., Jessell, T.M., Goulding, M., 2004. Genetic identification of spinal interneurons that coordinate left-right locomotor activity necessary for walking movements. Neuron 42 (3), 375–386.

Lapointe, N.P., Ung, R.V., Guertin, P.A., 2007. Plasticity in sublesionally located neurons following spinal cord injury. J. Neurophysiol. 98, 2497–2500.

Lapointe, N.P., Ung, R.V., Rouleau, P., Guertin, P.A., 2008. Effects of spinal alpha(2)-adrenoceptor and I(1)-imidazoline receptor activation on hindlimb movement induction in spinal cord-injured mice. J. Pharmacol. Exp. Ther. 325 (3), 994–1006.

Lapointe, N.P., Rouleau, P., Ung, R.V., Guertin, P.A., 2009. Specific role of D1 receptors in spinal network activation and rhythmic movement induction in vertebrates. J. Physiol. 587, 1499–1511.

Lundberg, A., 1965. Interactions entre voies reflexes spinales (interaction between the spinal reflex patheays). Actual Neurophysiol. (Paris) 16, 121–137.

Lundfald, L., Restrepo, C.E., Butt, S.J., Peng, C.Y., Droho, S., Endo, T., Zeilhofer, H.U., Sharma, K., Kiehn, O., 2007. Phenotype of V2-derived interneurons and their relationship to the axon guidance molecule EphA4 in the developing mouse spinal cord. Eur. J. Neurosci. 26 (11), 2989–3002.

McCrea, D.A., Rybak, I.A., 2008. Organization of mammalian locomotor rhythm and pattern generation. Brain Res. Rev. 57 (1), 134–146.

McCrea, D.A., Pratt, C.A., Jordan, L.M., 1980. Renshaw cell activity and recurrent effects on motoneurons during fictive locomotion. J. Neurophysiol. 44 (3), 475–488.

Miller, S., Scott, P.D., 1977. The spinal locomotor generator. Exp. Brain Res. 30, 387–403.

Minassian, K., Persy, I., Rattay, F., Pinter, M.M., Kern, H., Dimitrijevic, M.R., 2007. Human lumbar cord circuitries can be activated by extrinsic tonic input to generate locomotor-like activity. Hum. Mov. Sci. 26, 275–295.

Nakamura, Y., Katakura, N., 1995. Generation of masticatory rhythm in the brainstem. Neurosci. Res. 23 (1), 1–19.

Nishimaru, H., Takizawa, H., Kudo, N., 2000. 5-Hydroxytryptamine-induced locomotor rhythm in the neonatal mouse spinal cord in vitro. Neurosci. Lett. 280 (3), 187–190.

Nissen, U.V., Mochida, H., Glover, J.C., 2005. Development of projection-specific interneurons and projection neurons in the embryonic mouse and rat spinal cord. J. Comp. Neurol. 483, 30–47.

Noga, B.R., Shefchyk, S.J., Jamal, J., Jordan, L.M., 1987. The role of Renshaw cells in locomotion: antagonism of their excitation from motor axon collaterals with intravenous mecamylamine. Exp. Brain Res. 66 (1), 99–105.

O'Donovan, M.J., Bonnot, A., Wenner, P., Mentiz, G.Z., 2005. Calcium imaging of network function in the developing spinal cord. Cell Calcium 37, 443–450.

Orlovskii, G.N., Severin, S.V., Shik, M.L., 1966. Locomotion induced by stimulation of the mesencephalon. Dokl. Akad. Nauk. SSSR. 169 (5), 1223–1226.

Pearson, K.G., 1995. Proprioceptive regulation of locomotion. Curr. Opin. Neurobiol. 5 (6), 786–791.

Pearson, K.G., Duysens, J., 1976. Function of segmental re exes in the control of stepping in cockroaches and cats. In: Herman, R.E., Grillner, S., Stuart, D., Stein, P. (Eds.), Neural Control in Locomotion. Plenum Press, New York.

Pearson, K.G., Rossignol, S., 1991. Fictive motor patterns in chronic spinal cats. J. Neurophysiol. 66 (6), 1874–1887.

Perreault, M.C., Angel, M.J., Guertin, P., McCrea, D.A., 1995. Effects of stimulation of hindlimb flexor group II afferents during fictive locomotion in the cat. J. Physiol. 15;487 (Pt 1), 211–220.

Perret, C., Cabelguen, J.M., 1980. Main characteristics of the hindlimb locomotor cycle in the decorticate cat with special reference to bifunctional muscles. Brain Res 187 (2), 333–352.

Petruska, J.C., Ichiyama, R.M., Jindrich, D.L., Crown, E.D., Tansey, K.E., Roy, R.R., Edgerton, V.R., Mendell, L.M., 2007. Changes in motoneuron properties and synaptic inputs related to step training after spinal cord transection in rats. J. Neurosci. 27, 4460–4471.

Philippson, M., 1905. L'autonomie et la centralisation dans le système nerveux des animaux [Autonomy and centralization in the animal nervous system]. Trav. Lab. Physiol. Inst. Solvay (Bruxelles) 7, 1–208.

Pratt, C.A., Jordan, L.M., 1987. Ia inhibitory interneurons and Renshaw cells as contributors to the spinal mechanisms of fictive locomotion. J. Neurophysiol. 57 (1), 56–71.

Puskar, Z., Antal, M., 1997. Localization of last-order premotor interneurons in the lumbar spinal cord of rats. J. Comp. Neurol. 389, 377–389.

Robertson, R.M., Pearson, K.G., 1985. Neural circuits in the flight system of the locust. J. Neurophysiol. 53 (1), 110–128.

Shefchyk, S., McCrea, D., Kriellaars, D., Fortier, P., Jordan, L., 1990. Activity of interneurons within the L4 spinal segment of the cat during brainstem-evoked fictive locomotion. Exp. Brain Res. 80 (2), 290–295.

Sherrington, C.S., 1910. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. J. Physiol. 40 (1-2), 28–121.

Shik, M.L., Orlovsky, G.N., 1976. Neurophysiology of locomotor automatism. Physiol. Rev. 56 (3), 465–501.

Sigvardt, K.A., Grillner, S., Wallen, P., Van Dongen, P.A., 1985. Activation of NMDA receptors elicits fictive locomotion and bistable membrane properties in the lamprey spinal cord. Brain Res. 336 (2), 390–395.

Smith, J.C., Ellenberger, H.H., Ballanyi, K., Richter, D.W., Feldman, J.L., 1991. Pre-Boetzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science 254, 726–729.

Stein, P.S., 1971. Intersegmental coordination of swimmeret motoneuron activity in crayfish. J. Neurophysiol. 34 (2), 310–318.

Stein, P.S., 2005. Neuronal control of turtle hindlimb motor rhythms. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 191 (3), 213–229.

Stein, R.B., Mushahwar, V., 2005. Reanimating limbs after injury or disease. Trends Neurosci. 28, 518–524.

Stokke, M.F., Nissen, U.V., Glover, J.C., Kiehn, O., 2002. Projection patterns of commissural interneurons in the lumbar spinal cord of the neonatal rats. J. Comp. Neurol. 446, 349–359.

Stuart, D.G., Hultborn, H., 2008. Thomas Graham Brown (1882–1965), Anders Lundberg (1920–), and the neural control of stepping. Brain Res. Rev. 59, 74–95.

Syed, N.I., Bulloch, A.G., Lukowiak, K., 1990. In vitro reconstruction of the respiratory central pattern generator of the mullusk Lymnaea. Science 250 (4978), 282–285.

Székely, G., Czéh, G., Voros, G., 1969. The activity pattern of limb muscles in freely moving normal and deafferented newts. Exp. Brain Res. 9 (1), 53–72.

Tazerart, S., Viemari, J.C., Darbon, P., Vinay, L., Brocard, F., 2008. Contribution of persistent sodium current to locomotor pattern generation in neonatal rats. J. Neurophysiol. 98, 613–628.

Thompson, A.K., Estabrooks, K.L., Chong, S., Stein, R.B., 2009. Spinal reflexes in ankle flexor and extensor muscles after chronic central nervous system lesions and functional electrical stimulation. Neurorehabil. Neural Repair. 23, 133–142.

Tresch, M.C., Saltiel, P., Bizzi, E., 1999. The construction of movement by the spinal cord. Nat. Neurosci. 2, 162–167.

Ung, R.V., Landry, E.S., Rouleau, P., Lapointe, N.P., Rouillard, C., Guertin, P.A., 2008. Role of spinal 5-HT2 receptor subtypes in quipzine-induced hindlimb movements after a low-thoracic spinal cord transection. Eur. J. Neurosci. 28, 2231–2242.

Viala, D., Buser, P., 1969. The effects of DOPA and 5-HTP on rhythmic efferent discharges in hind limb nerves in the rabbit. Brain Res. 12 (2), 437–443.

Viala, D., Buisseret-Delmas, C., Portal, J.J., 1988. An attempt to localize the lumbar locomotor generator in the rabbit using 2-deoxy-[14C]glucose autoradiography. Neurosci. Lett. 86 (2), 139–143.

Whelan, P., Bonnot, A., O'Donovan, M.J., 2000. Properties of rhythmic activity generated by the isolated spinal cord of the neonatal mouse. J. Neurophysiol. 84 (6), 2821–2833.

Whelan, P.J., Hiebert, G.W., Pearson, K.G., 1995. Stimulation of the group I extensor afferents prolongs the stance phase in walking cats. Exp. Brain Res. 103 (1), 20–30.

Wilson, D.M., Wyman, R.J., 1965. Motor output patterns during random and rhythmic stimulation of locust thoracic ganglia. Biophys. 5, 121–143.

Wilson, J.M., Hartley, R., Maxwell, D.J., Todd, A.J., Lieberam, I., Kaltschmidt, J.A., Yoshida, Y., Jessell, T.M., Brownstone, R.M., 2005. Conditional rhythmicity of ventral spinal interneurons defined by expression of the Hb9 homeodomain protein. J. Neurosci. 25 (24), 5710–5719.

Wilson, J.M., Cowan, A.I., Brownstone, R.M., 2007. Heterogeneous electronic coupling and synchronization of rhythmic bursting activity in mouse HB9 interneurons. J. Neurophysiol. 98, 2370–2381.

Zhang, Y., Narayan, S., Geiman, E., Lanuza, G.M., Velasquez, T., Shanks, B., Akay, T., Dyck, J., Pearons, K., Gosgnach, S., Fan, C.M., Goulding, M., 2008. V3 spinal neurons establish a robust and balanced locomotor rhythm during walking. Neuron 60 (1), 84–96.

Zhong, G., Masino, M.A., Harris-Warrick, R.M., 2007. Persistent sodium currents participate in fictive locomotion generation in neonatal mouse spinal cord. J. Neurosci. 27, 4507–4518.