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Abbreviations

5HT	5-hydroxytryptamine
BG	Basal ganglia
BSN	Brainstem nuclei
Cer	Cerebellum
Chx10	Ceh-10 homeodomain-containing homolog
CN	Commissural Neuron
CPG	Central Pattern Generator
Dbx1	Developing brain homeobox 1
DSCT	Dorsal spinocerebellar tract
eCN	excitatory commissural neuron
eIN	excitatory interneuron
En1	Engrailed 1
Evx1	Even-skipped homeobox
FP	Floor Plate
GATA	GATA binding protein
Hb9	Homeobox 9
Hyp	Hypothalamus
I _A	Transient potassium current
iCN	Inhibitory commissural neuron
I _h	Hyperpolarization activated inward current
IN	Interneuron
iIN	inhibitory interneuron
I _T	Transient calcium current
L2; L5	lumbar roots 2 and 5
L-DOPA	L-3,4-dihydroxyphenylalanine
MCtx	Motor Cortex

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MLR	Mesencephalic Locomotor Region
MN	Motor Neuron
NMDA	<i>N</i> -Methyl-D-aspartic acid
pPCTX	posterioparietal cortex
rIa	reciprocally connected inhibitory Ia-interneurons
RbS	Rubrospinal
RC	Renshaw Cell
RF	Reticular Formation
RS	Reticulospinal
Sim 1	Single-minded homolog 1
VCtx	Visual Cortex
VL	ventrolateral, thalamus
VLF	Ventrolateral Funiculus
VS	Vestibulospinal
VSCT	Ventral Spinocerebellar tract

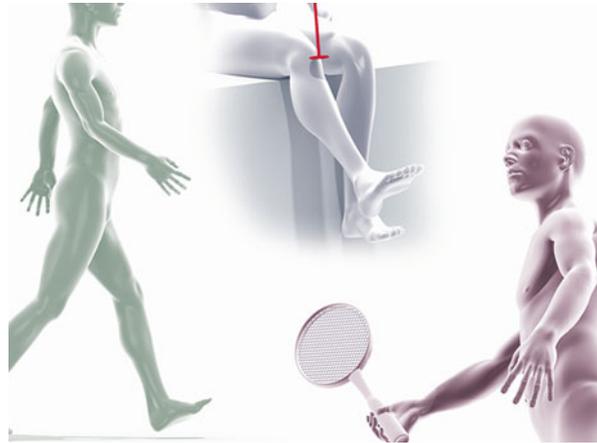
Brief History

Locomotion is the motor act that allows animals or humans to move through the environment. Locomotion is expressed in many forms and includes such diverse motor acts as swimming, flying, walking, running, and hopping. The motor act is essential for survival and allows individuals to find food, escape danger, mate, and migrate to suitable environments.

Locomotor movements are different from other motor acts that the nervous system produces, like the knee reflex that is stereotypically repeated in response to sensory stimulation, or skilled movements, like playing tennis or the piano that are learned and involve a complex coordination of muscles often in non-repeated manners (Fig. 38.1). Locomotion is a recurrent motor activity that involves sequential activity in limb and body muscles in a precise rhythm and pattern. Although there is some adaptation and maturation of locomotion, it is an innate behavior that in vertebrates, including humans, is laid down in the nervous system before birth but often not executed before the appendages and postural activity is matured.

The first precise description of locomotion came with the use of photographic techniques developed in the 1880s by Étienne-Jules Marey and Eadweard James Muybridge that allowed viewers to capture snapshots of the different moments of locomotor movements in animals and humans. These aggregated sequences of pictures showed the exact timing of the movements in detail that had not been seen before. A breakthrough in understanding the neural substrate for generating mammalian locomotion came from experiments carried out by the English neurophysiologist Thomas Graham Brown in the beginning of the twentieth century. He showed that flexor-extensor hind limb movements could be evoked in cats that had their spinal cords transected at the thoracic level and had sensory inputs

Fig. 38.1 *Locomotion is an innate rhythmic motor act different from the simple reflex that is stereotypically repeated in response to sensory stimulation or skilled movements, like playing tennis, that are learned and involve a complex coordination of muscles often in non-repeated manners*



removed. Graham Brown concluded that spinal cord neural networks can – when appropriately activated – organize rhythmic movements into alternating flexor and extensor activity in the absence of sensory influence. These findings clearly showed that the spinal cord itself contains a neural network that can generate rhythmic movements without sensory information. These ideas challenged the prevailing idea at that time, namely, that locomotion was a result of repeated reflex responses. Despite the clear evidence, Graham Brown's experiments were forgotten for almost 50 years until they were brought to light again by Swedish neurophysiologists in Gothenburg whose experiments demonstrated that rhythmic activity can be evoked in spinalized cats that are given L-DOPA, a precursor of noradrenalin. This neurochemical activation was sufficient to activate the dormant neural networks and produce rhythmic activity without the need for sensory inputs. There is now overwhelming evidence from studies of many different vertebrates, including man, that the precise phasing and timing of locomotion is, for the most part, generated by circuits in the spinal cord. These networks are called central pattern generators or CPGs. Neurons in the CPG receive an input from the brain, from which they are able to produce the rhythm and pattern of activity that is conveyed to motor neurons and then to the muscles. Almost simultaneous with the rediscovery of the CPG in the cat in the 1960s, a Russian group found that electrical stimulation of a circumscribed area in the mesencephalon initiated locomotion in decerebrated cats. This region was named the mesencephalic locomotor region and has since been found in all vertebrates. Neurons in the mesencephalic locomotor region do not project directly to the spinal cord but mediate their effects through cells in the reticular formation in the lower brainstem. Neurons in the mesencephalic locomotor region itself are under the control of other brain structures that select the behavior. Although the CPG may produce a rhythm and pattern without sensory inputs, much research has shown that when actual locomotion is performed, sensory inputs from receptors in muscle and skin are active so that the locomotion can be adapted to the environment.

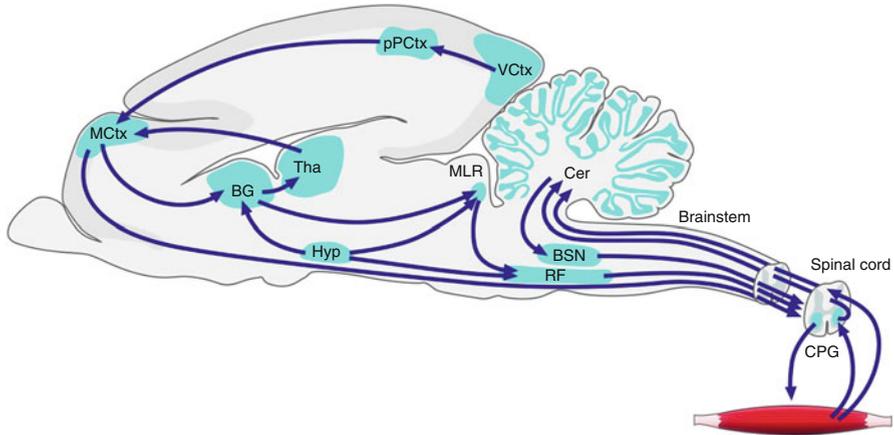


Fig. 38.2 Organization of the neural structures controlling locomotion in vertebrates. The selection of locomotor behavior is performed by the basal ganglia (BG) and enabled by circuits in the lateral and medial hypothalamus (Hyp). Output neurons of the basal ganglia project to the thalamus which then projects to the motor cortex (Mctx) and to the mesencephalic locomotor region (MLR). The initiation of locomotion is mediated by the neurons in the MLR that project to neurons in the medial reticular formation (RF) in the lower brainstem. Neurons in the medial reticular formation project to the locomotor CPG in the spinal cord that executes locomotion. Descending fibers from the vestibular and rubrospinal spinal pathways (brainstem nuclei, BSN) are maintaining equilibrium and modulating the ongoing locomotor CPG activity. The cerebellum (Cer) coordinates motor behavior by mediating movement generated feedback and internal feedback as well as by modulating the activity in the descending reticulospinal, rubrospinal, and vestibulospinal pathways. Proprioceptive sensory feedback modulates the activity of the locomotor CPG and is sent to the cerebellum. The cerebellum also receives a signal that monitors the internal activity of the CPG. Cortical activity (Mctx) provides visuomotor (VCtx) correction of locomotion via the posterior parietal cortex (pPctx)

These signals both influence the CPG activity in the spinal cord and are sent to supraspinal areas including cerebellum. Recent research has also shown that while cortical input is not needed to perform normal overground locomotion, cortical visual integration is essential for skilled locomotion, like avoiding an obstacle. It has also become clear that the spinal locomotor network is subject to neuromodulation that can cause slow and long-lasting changes in the network function.

The cat was the prevailing model for studying locomotion in vertebrates for many years. Throughout the 1980s, a number of other vertebrate models have been developed including the lamprey, tadpole, zebrafish, and neonatal rodents. These models have given new insights to the overall function of vertebrate locomotor networks. The advent of molecular genetics has also opened possibilities to study locomotor networks in ways that were not possible before.

All in all locomotion is controlled by a number of different regulatory neuronal components (Fig. 38.2):

1. Neuronal systems in the brain that select the behavior
2. Neuronal systems in the midbrain and lower brainstem that initiate the behavior

3. Neuronal networks in the spinal cord that generate the behavior
4. Sensory signals that adapt and fine-tune the activity of the neuronal networks in the spinal cord to the environment
5. Sensory signals that are sent to supraspinal structures, including the cerebellum, and are used to correct and adapt the locomotor behavior
6. Neuronal systems in the cortex that perform visual adjustment of locomotor movements
7. Neuromodulatory systems that modulate the locomotor activity by causing long-lasting changes in network activity

Finally, locomotion requires tight integration with postural activity to be performed well.

Basal Ganglia Select Locomotion

The first step in locomotion is to select the behavior. Experiments in a number of animal models have shown that the dominating selection system is the basal ganglia that select the appropriate motor patterns in a particular behavioral context, for instance, the exploratory locomotion, that allows us to migrate through the environment. The striatum receives input from the cortex as well as other brain structures, including thalamus. Striatum neurons project to the external segment of the globus pallidus, substantia nigra pars compacta, and the output nuclei of the basal ganglia: the internal segment of globus pallidus and substantia nigra pars reticulata. Neurons in the output nuclei of the basal ganglia in turn project to neurons in the mesencephalic locomotor region. The neurons in the striatum and the output nuclei of the basal ganglia are inhibitory GABAergic neurons, and those of the output nuclei of the basal ganglia have a high tonic activity at rest. When explorative locomotion is selected, the high resting activity in these neurons decreases which in turn relieves the tonic inhibition of neurons in the mesencephalic locomotor region. When released from this inhibition, mesencephalic locomotor region neurons can become active and initiate locomotion.

Two other brain regions may be involved in enabling locomotor behavior: the lateral and medial hypothalamus. Stimulation in the lateral hypothalamus may enable locomotion in a behavioral context where an animal is searching for food. This system may bypass the mesencephalic locomotor region and project directly to locomotor initiating regions in the brainstem reticular formation. Activity in the medial hypothalamus may enable defensive or escape locomotor behavior. The medial hypothalamus projects through nuclei in the brainstem to the mesencephalic locomotor region and possibly also directly to neurons in the brainstem reticular formation. It is possible that these hypothalamic nuclei also exert part of their effect via the basal ganglia.

Locomotion may therefore be initiated in different behavioral contexts where the initial selection is mediated through different selection systems. A common feature of the selection systems is that they converge onto neural networks in the midbrain and lower brainstem.

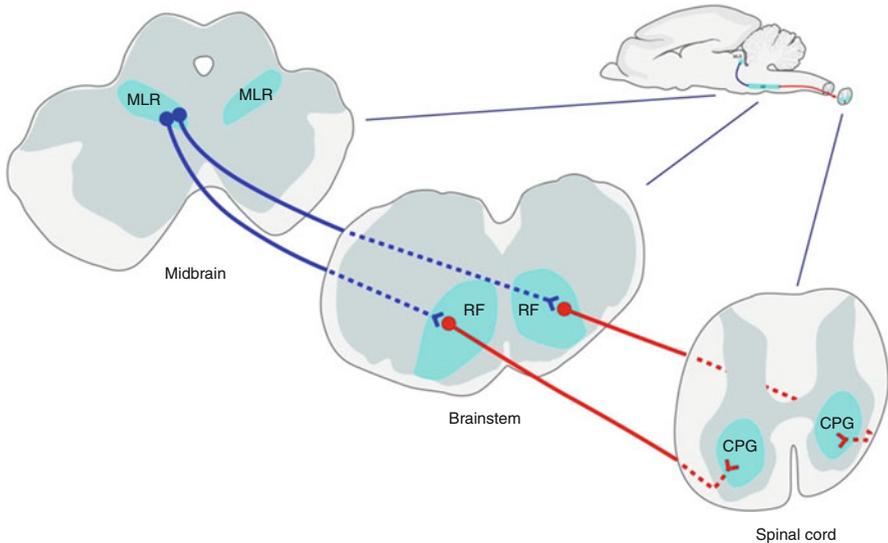


Fig. 38.3 *The mesencephalic locomotor region and the neurons in the reticular formation initiate locomotion.* Neurons in the MLR project to the medial reticular formation (RF) whose axons descend the ventrolateral funiculus (VLF) to the spinal locomotor network. Both glutamatergic and serotonergic cells located in the reticular formation project to the locomotor CPG

The Mesencephalic Locomotor Region and Reticulospinal Neurons Initiate Locomotion

Two neuronal structures in the midbrain and the lower brainstem are directly involved in initiating locomotion in vertebrates: the mesencephalic locomotor region in the midbrain and neurons in the reticular formation in the lower brainstem.

Stimulation of the mesencephalic locomotor region, just below the inferior colliculus in the midbrain, in a resting animal makes the animal stand up and start to walk, and as the frequency of stimulation increases, the animal will start to increase the speed of walking and eventually start to gallop. The mesencephalic locomotor region is found in all vertebrates (Fig. 38.3). The mesencephalic locomotor region is a hub integrating locomotor commands from higher brain structures and receives inputs from the basal ganglia and hypothalamus. The mesencephalic locomotor region serves as a control unit: increased activity of this region will gradually increase the locomotor speed.

Neurons in the mesencephalic locomotor region are excitatory and contain glutamate or acetylcholine as their neurotransmitters. Their excitatory effect is not mediated by direct projection to the spinal cord. They project to and activate neurons in the reticular formation in the lower brainstem that provide the final command signal that initiates locomotion. Experiments in lamprey have shown that the mesencephalic locomotor region also projects to cholinergic neurons in the brainstem that indirectly activate the neurons in the reticular formation.

This feed-forward pathway serves much like a volume control that will boost the excitation of the neurons in the reticular formation.

Another area that when stimulated evokes locomotion is the subthalamic (or diencephalic) locomotor region that also projects to neurons in the reticular formation. The input to the subthalamic locomotor region is not well described.

The exact identity of the neurons in the reticular formation in the lower brainstem that send the final command signal that initiates locomotion is not fully understood. Two systems seem to be involved: a glutamatergic locomotor pathway, which is found in all vertebrates, and a serotonergic locomotor pathway that is involved in initiating locomotion in mammals. The evidence for the presence of a glutamatergic locomotor pathway is that initiation of locomotion from the mesencephalic locomotor region can be blocked by interfering with glutamatergic receptors in the spinal cord and that direct and specific activation of glutamatergic neurons in the lower brainstem evokes locomotor activity. The glutamatergic locomotor pathway probably originates in large reticulospinal neurons in the lower brainstem. These neurons project directly or indirectly via a chain of propriospinal glutamatergic neurons to locomotor neurons in the spinal cord. Reticulospinal neurons also participate in regulating postural activity that is needed for the animal to perform locomotion. The evidence for the presence of a serotonergic locomotor pathway is from experiments in rats. Stimulation in the parapyramidal area in the lower brainstem that contains many serotonergic neurons evokes locomotor activity that is blocked by interfering with the activation of serotonergic receptors in the spinal cord. It therefore appears that there are, at least in mammals, parallel pathways for activating the spinal locomotor networks. It is unknown whether these pathways are activated simultaneously or in specific behavioral contexts.

Sustained locomotor activity in lamprey can also be elicited by mechanical stimulation of the skin that evokes escape locomotion. Typically, a very short sensory stimulus is transformed into a prolonged escape firing that outlasts the sensory stimulation by multiple times in duration. The reticulospinal neurons are involved in this mechanically evoked response. It has been shown both in lamprey and tadpole that these cells receive direct input from sensory afferents activated by the escape stimulus. In the lamprey, it has been shown that trigeminal primary afferents from the head region project to the reticulospinal neurons and release of glutamate from these primary afferents prolongs firing in the reticulospinal neurons. This prolonged firing is carried by a sustained depolarization in the reticulospinal neurons and a sustained rise in the intracellular calcium concentration. This increase in the calcium concentration triggers a calcium-activated nonselective cationic conductance called I_{CAN} . It is the activation of this sustained non-inactivating current that carries the prolonged firing in the reticulospinal neurons. In the tadpole, the sustained firing is less dependent on intrinsic membrane properties but seems to be generated by a strong excitatory coupling between the excitatory reticulospinal neurons.

The initiation system is therefore organized in parallel pathways. The behavioral contexts in which these parallel initiation systems may be recruited are not known.

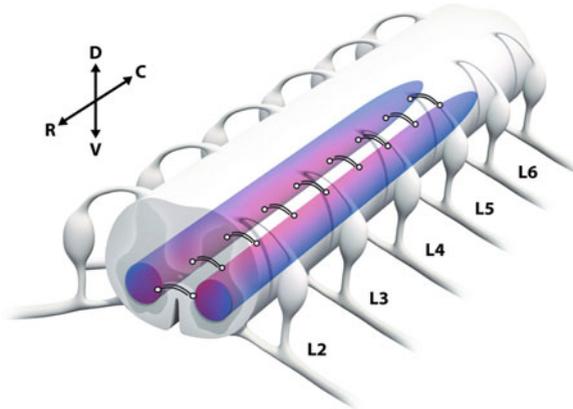


Fig. 38.4 Schematic of the localization of the locomotor hind limb CPG in the rodent spinal cord. The CPG is localized in the ventromedial part of the spinal cord and is a distributed network that extends along the lumbar spinal cord (lumbar segments, L1–L6) on either side. The color code indicates that the most rostral segments are more excitable (red) than the more caudal segments (blue). The two sides of the cord are connected by commissural neurons that have axons crossing in the midline in the ventral commissure (Adapted from Kjaerulff and Kiehn 1996)

Spinal Networks Are Complex Networks that Generate the Rhythm and Pattern of Locomotor Behavior

Spinal locomotor networks are capable of generating a rhythmic, patterned output, which can be seen as muscle activation when activated by descending input from the lower brainstem. The timing and phasing of the complex muscle activity can be generated without sensory input, and the spinal locomotor network is therefore often referred to as the central pattern generator or CPG. All of the components of the CPG are found within the spinal cord, with rostral and caudal but interconnected CPG networks controlling the forelimbs and the hind limbs in four-legged animals and CPG networks distributed along the spinal cord in legless animals. The CPG network is localized in the ventral spinal cord, and for the hind limb CPG network, it has a rostro-caudal excitability gradient with the highest excitability in the most rostral segments in the lumbar spinal cord, thereby making them the leading segments (Fig. 38.4).

Much of the early work on CPGs was carried out in the spinalized cat where it was shown that monoamine precursors like L-DOPA could evoke fictive locomotion. Now, this preparation is complemented with the use of many other spinal or in vitro preparations including the lamprey, tadpole, zebrafish, turtle, mouse, and rat. In in vitro preparations, such as the isolated spinal cord-brainstem preparation, the nervous system is isolated from the body, kept alive in artificial cerebrospinal fluid, and electrodes are attached to motor nerves so that fictive locomotion (locomotion without movements) can be recorded extracellularly. Fictive locomotion can either be evoked by electrical stimulation of the mesencephalic locomotor

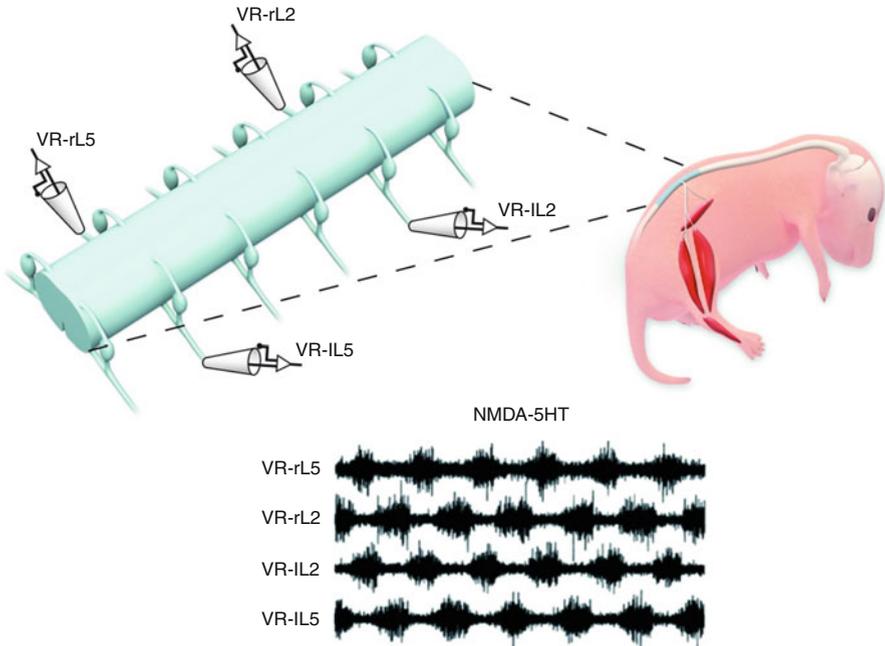


Fig. 38.5 The isolated lumbar spinal cord from newborn rodents can produce a locomotor-like pattern in vitro. Motor activity is recorded by glass electrodes placed on the ventral roots, and locomotor-like activity is induced by application of serotonin (5HT) and the glutamate agonist, NMDA. Locomotor-like activity is represented by alternation between the flexor-related bursts in the L2 ventral roots and the extensor-related bursts in the L5 ventral roots (e.g., rL2–rL5) together with segmental left-right alternation (e.g., rL2–IL2)

region or the lower brainstem or be induced by neuroactive substances, like glutamate, NMDA, serotonin, or dopamine, applied directly to the isolated spinal cord (Fig. 38.5). Lamprey, tadpole, and zebrafish have the advantage of a more limited number of neurons and less complex motor behaviors. Turtle, mouse, rat, and cat have the added complexities associated with the coordination of limbs. Zebrafish and mice have the additional advantage that they are amenable to genetic techniques that allow interactive network studies. The next sections will provide a description of the basic organization for swimming CPGs and walking CPGs.

Swimming CPG. The network structure for the swimming CPG is known in great detail from studies in the adult lamprey and the embryonic tadpole.

The lamprey is an eel-like vertebrate (cylostome), which locomotes by a traveling wave of muscle contractions down one side of the body that is out of phase with the wave of muscle contractions on the other side of the body. A lamprey has about 100 spinal segments, and within each segment, there is a neural network that generates the rhythm and alternation between the left and the right side of the body. The rhythm generator of the lamprey is made up of excitatory glutamatergic

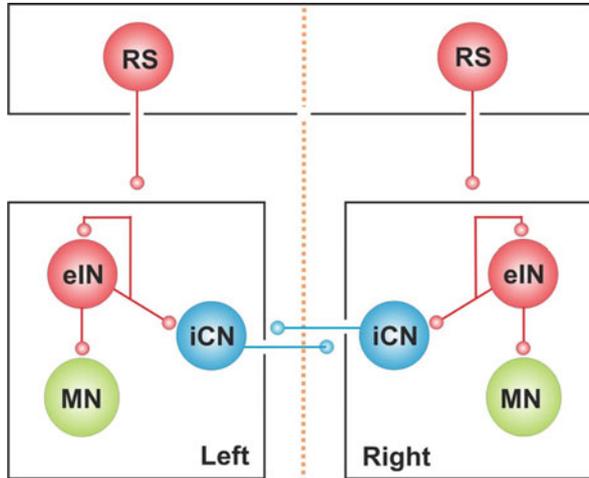


Fig. 38.6 Canonical view of the swimming CPG in lamprey and tadpole. The network is made up of ipsilaterally projecting excitatory neurons (*eIN*) that make an excitatory network and also excite ipsilateral motor neurons (*MN*) and inhibitory commissural neurons (*iCN*). These inhibitory commissural neurons project to excitatory neurons, inhibitory commissural neurons, and motor neurons on the other side of the cord. When one side of the cord is active, the other side is actively inhibited. The CPG neurons receive excitatory external drive from excitatory reticulospinal (*RS*) neurons located in the lower brainstem (Adapted from Grillner 2006)

neurons with axons projecting on the same side of the cord. These excitatory neurons excite other excitatory neurons, inhibitory commissural neurons with axons crossing in the midline, and motor neurons (Fig. 38.6). The excitatory neurons are excited by the descending command signal from reticulospinal neurons that also excites other CPG neurons and motor neurons. The feed-forward excitation between excitatory neurons and interaction with their intrinsic membrane properties generates the rhythm. The signal to motor neurons drives muscle activity on the same side. The inhibitory commissural neurons project to excitatory neurons, inhibitory commissural neurons, and motor neurons on the other side. As long as inhibitory commissural neurons are active on one side of the cord, the other side will be suppressed. The rhythmic inhibitory signal to motor neurons from inhibitory commissural neurons alternates with the rhythmic excitatory signals from excitatory neurons. Calcium entry into excitatory neurons activates calcium-activated potassium channels which eventually hyperpolarize the cells and terminate their bursting. This releases the inhibition on the other side of the cord, allowing the excitatory neurons to take over, leading to an inhibition of the side of the cord that was previously active. The inhibitory commissural neurons are required to generate left-right alternation but are not required for rhythmicity on each side of the cord.

These segmental networks are connected along the cord to secure that successive body segments are activated in an undulatory wave that produces swimming. In the lamprey, there are descending excitatory and inhibitory neurons which

coordinate activity between segments. The axons of descending inhibitory neurons are longer than descending excitatory neurons resulting in a rostral-caudal gradient in synaptic input that contributes to the traveling wave of muscle activation on each side of the body.

The tadpole swimming is an escape swimming away from predators. Many of the principles for the tadpole swimming CPG appear to be similar to those found in the lamprey. First, the neurons responsible for rhythm generation are glutamatergic excitatory neurons with axons projecting on the same side of the cord. These excitatory neurons have a descending axon and are located in the spinal cord and hindbrain. Second, the alternation of the left and right sides of the body is coordinated by inhibitory commissural neurons. Lastly, there is a traveling wave of muscle activation that propels the animal forward.

Walking CPG. Limbed walking also consists of rhythm and pattern. Glutamatergic neurons with axons projecting on the same side of the cord are also thought to generate the rhythm in mammals. The specific identity of these rhythm-generating neurons remains elusive. However, ablation experiments have shown that one ventral hemisegment of lumbar spinal cord is sufficient for rhythmicity and pharmacological experiments have shown that a rhythm can persist when inhibitory synaptic inputs are blocked. Moreover, the rhythm is blocked by pharmacological inhibition of glutamatergic receptors. Therefore, the rhythm-generating neurons must be ipsilateral, excitatory, and local interneurons. These neurons drive other neurons in the network into rhythmicity and provide rhythmic excitation of motor neurons either directly or indirectly (Fig. 38.7a). It is likely that flexors and extensors are driven by separate populations of rhythm-generating neurons (Fig. 38.7a).

The pattern in mammals is more complex than in lamprey and tadpole because muscles in a limb with joints must be coordinated in a precise temporal pattern. There are two main aspects to the coordination of limbs: flexor-extensor alternation and left-right alternation.

Flexor and extensor activity must be coordinated on each side in order to control the limbs. The CPG components responsible for flexor-extensor alternation are organized in flexor and extensor modules which seem to be reciprocally connected via inhibitory interneurons. When these inhibitory connections are blocked, flexor and extensor muscles can only be activated in synchrony. The flexor-extensor alternation is retained in a hemicord, showing that inhibitory neurons with ipsilateral projections are essential for this role. The nature of these inhibitory network components is only partly known. There is evidence that reciprocally connected inhibitory Ia-interneurons (rIa) (Fig. 38.7a) are essential for coordinating flexor-extensor activity. Ia-interneurons are activated from proprioceptive group Ia-afferents originating in muscle spindles. From cat experiments, it is known that Ia-interneurons are organized in flexor- and extensor-related groups that are reciprocally connected around a joint. Flexor-related Ia-interneurons inhibit extensor motor neurons while extensor-related Ia-interneurons inhibit flexor motor neurons (Fig. 38.7). Since Ia-interneurons are rhythmically active during locomotion, this reciprocal network may provide a neural substrate for flexor-extensor alternation (Fig. 38.7a) although it is likely that the other inhibitory neurons

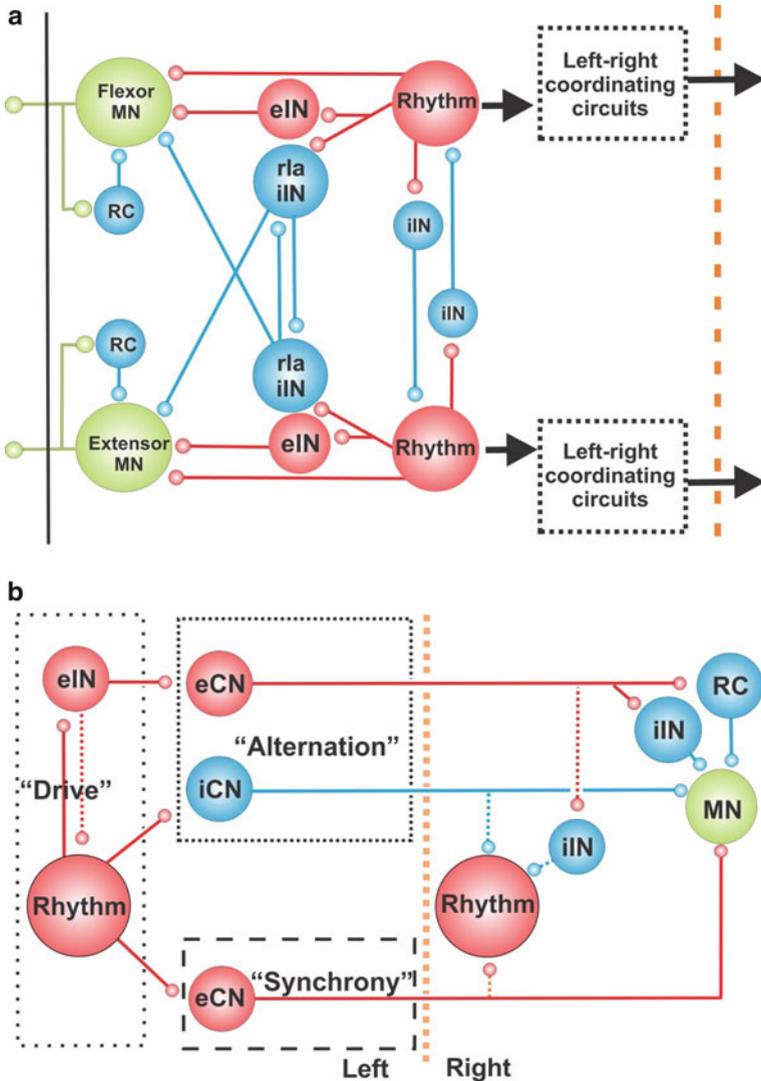


Fig. 38.7 (a) Organizational diagram of the segmental rodent CPG. The output neurons of the network are flexor and extensor motor neurons (MN, green) that are driven into rhythmicity by alternating excitation and inhibition. The locomotor CPG is divided into three major components: the rhythm-generating networks, the networks responsible for flexor-extensor alternation, and a networks securing left-right alternation. Excitatory rhythm-generating neurons (red) provide direct or indirect excitation (eIN) of flexor and extensor motor neurons. Electrophysiological studies suggest that the neurons responsible for flexor-extensor alternation are at least partly composed of reciprocally connected inhibitory Ia-interneurons (rla) that are driven by rhythm-generating neurons. Other inhibitory neurons may also participate (iIN). The rhythm-generating neurons controlling flexor and extensor motor neurons may also be reciprocally connected by inhibitory neurons (iIN; blue). The rhythm-generating neurons and possibly also other excitatory

are also involved in this important network function. The Ia-interneurons are driven by the rhythm-generating network (Fig. 38.7a).

Motor neurons also receive rhythmic inhibition from Renshaw cells (RC) that are activated by motor neuron collaterals (Fig. 38.7a). Renshaw cells generally fire late in the phase of its related motor neuron and may participate in terminating the motor neuron firing.

Left-right alternation, like in the lamprey and tadpole, is dependent on crossed inhibition. Experiments in cat and rodents have revealed details of this system. There are two ways in which this inhibition can be accomplished – directly by inhibitory commissural neurons acting on motor neurons and indirectly by excitatory commissural neurons which act on premotor inhibitory neurons (Fig. 38.7b). This dual inhibitory system may regulate alternation at different speeds of locomotion. Separate excitatory commissural neurons are responsible for coordinating left-right activity into synchrony as seen in hopping (Fig. 38.7b). The dual inhibitory left-right alternating pathways may be driven directly by the rhythm-generating neurons or indirectly by other excitatory neurons in the spinal cord (Fig. 38.7b). The left-right synchronous pathways seem to be driven directly by the rhythm-generating neurons (Fig. 38.7b).

The segmental flexor-extensor coordinating CPG with left-right circuit units may be repeated along the cord to produce a rhythm that coordinates activity in all joints in a limb.

The identity of the CPG neurons that receive the locomotor command to initiate locomotion is not known in detail. It is known, however, that commissural neurons, as well as motor neurons, receive inputs from neurons in the reticular formation, which suggests that many neurons in the mammalian spinal cord will receive a descending drive during locomotion.

By comparing the known network organization of the swimming and walking CPGs, it is clear that there are both similarities and differences. In both CPGs, excitatory neurons are responsible for rhythm generation and inhibitory commissural neurons are involved in left-right alternation. However, the left-right coordinating circuits appear more complex in the walking CPG than the swimming CPG, and ipsilateral inhibitory networks are involved in rhythmic inhibition and flexor-



Fig. 38.7 (continued) neurons (see Fig. 38.7b) drive the networks responsible for left-right alternation. Renshaw cells (RC) are inhibiting motor neurons. One neuron in the diagram represents a group of neurons. Inhibitory neurons are *blue*, excitatory neurons *red*, and motor neurons *green*. The *dotted line* indicates the midline (Adapted from Kiehn 2011). **(b)** *Organization of left-right coordinating CPG circuits in limbed animals.* The core of the left-right coordinating system in rodents is composed of commissural neurons (CNs). Left-right alternation is secured by a dual inhibitory system composed of inhibitory CNs (*iCN*) acting directly on contralateral motor neurons (*MNs*) or excitatory CNs (*eCN*) connecting indirectly to contralateral motor neurons via local inhibitory neurons (*iIN*), possibly including Renshaw cells (*RCs*). Left-right synchrony is obtained via a single excitatory system (*eCN*) acting directly on motor neurons. In order to obtain left-right coordination during locomotion, these cross-connections should also be connected to the rhythm-generating core on the other side of the cord. The left-right alternating system is driven by rhythm-generating neurons and other excitatory neurons (*eIN*) (Adapted from Kiehn 2011)

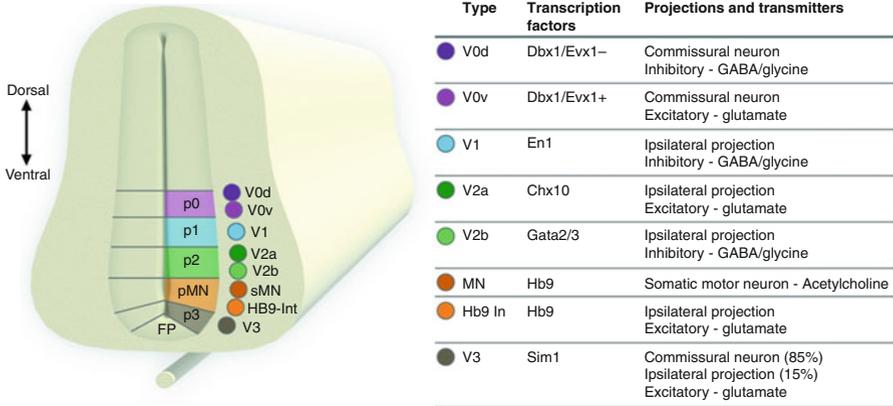


Fig. 38.8 *Molecular code determines the identity of ventral spinal neurons.* Morphogens secreted from the floor plate and roof plate set up concentration gradients in the ventricular zone to specify progenitor domains p0–p3 and pMN, characterized by their differential expression of transcription factors. When the progenitor cells mature, they migrate laterally and are called V0–V3, Hb9, and motor neurons. Table depicting the main transcription factors in the five cardinal classes of ventrally located neurons (V0–V3, Hb9) and motor neurons, the projection pattern, and the transmitter phenotype of these neurons. *Dbx1* developing brain homeobox 1, *Evx1* even-skipped homeobox, *En1* engrailed 1, *Chx10* Ceh-10 homeodomain-containing homolog, *GATA* binding protein, *Sim 1* Single-minded homolog 1, *Hb9* homeobox 9, *FP* floor plate, *Int* interneuron

extensor coordination. These differences in organization may be imposed by the increased complexity in having to control appendages during walking.

A Molecular Code May Determine the Layout of the Spinal CPG and Provide Insights into Its Function

Early in development, morphogens secreted from the floor and roof plate set up concentration gradients in the ventricular zone that lead to the differentiation of five ventral and six dorsal progenitor domains characterized by their differential expression of transcription factors. As development progresses, these progenitor cells mature and migrate laterally to their final position in the spinal cord. In the ventral spinal cord, where the CPG is localized, neurons have been divided into six groups, V0–V3 neurons, Hb9 interneurons, and motor neurons (Fig. 38.8). The V0 population, identified by the transcription factors *Dbx1* and *Evx1*, is made up of both excitatory (ventral, V0_v) and inhibitory (dorsal, V0_d) commissural neurons (Fig. 38.8). The V1 population, expressing *Engrailed 1*, is exclusively inhibitory neurons with axons on the same side of the cord and contains well-described interneurons, including Renshaw cells and inhibitory Ia-interneurons, in addition to ones to which a function has yet to be attributed (Fig. 38.8). V2 neurons, marked by *Lhx3* expression, are all ipsilaterally projecting neurons and have been further

subdivided into two populations (Fig. 38.8). The V2a neurons (Chx10 expressing) are excitatory and the V2b neurons (Gata2/3 expressing) are inhibitory. V3 neurons, expressing Sim1, are excitatory and predominantly commissural neurons (Fig. 38.8). A fifth group of neurons are excitatory and express the transcription factor Hb9, also found in motor neurons. Hb9 neurons are ipsilaterally projecting. Certainly, it is expected that a more elaborate transcription factor code will be defined in the future to account for the further subdivisions within each of these main groups.

The transcription factor code is found in mouse, chick, and zebrafish and is presumably conserved phylogenetically. It has been taken advantage of both to mark cells belonging to a certain group with a marker protein, such as green fluorescent protein, and to determine the functional consequences of knocking out or ablating a particular population. When a fluorescent protein is linked to a transcription factor, cells belonging to that group can be visually identified in isolated spinal cord preparations and spinal cord slices, thereby allowing one to reliably identify cells over and over again. In addition to the transmitter content and general projection pattern described above, this can be used to determine the cellular properties, connectivity, and activity of that cell type during different motor outputs by various electrophysiological recording techniques.

Studies combining molecular genetics and electrophysiology in the mouse, in which cells expressing a certain transcription factor are knocked out, ablated, or inactivated and the effects on locomotor activity are monitored, have shown that at least parts of all of the main populations of ventral horn neurons play a role in coordinated locomotor activity. These studies have provided valuable insights into the overall network structure in rodents.

Left-right alternation is disrupted when V0 or V2a cell populations are knocked out or ablated. Knockout of Dbx1 or ablation of these neurons, which eliminates both excitatory and inhibitory V0 neurons, causes an uncoupling in the left-right alternation or increased synchrony. The rhythm and flexor-extensor alternation remains normal. The Dbx1 neurons may, therefore, make up the commissural components of the dual left-right alternating system (Fig. 38.7b).

When V2a neurons are genetically ablated, left-right alternation is also disrupted, with no change in the flexor-extensor alternation. As the V2a neurons are ipsilaterally projecting, excitatory neurons, the effect on left-right alternation must be indirect suggesting that the V2a neurons are located between the rhythm-generating core and the dual left-right alternating system (red neuron, Fig. 38.7b). In addition to the deficits in left-right coordination, these mutants also show an increase in the variation in motor burst amplitude and burst duration. This suggests that the V2a neurons provide input to motor neurons and feedback to the rhythm-generating core, respectively (Fig. 38.7b).

Ablating or silencing of V1 neurons decreases the frequency of locomotion. However, both left-right and flexor-extensor alternations remain normal. In these V1 ablated animals, there are still some Ia-interneurons present which may uphold the flexor-extensor alternation. The source of these remaining Ia-interneurons is possibly the V2b neurons. The mechanism for the decrease in the frequency of locomotion is not understood but possibly related to the fact that inhibition is

important for terminating neuronal bursting. In the absence of this inhibition, bursting will be prolonged and the frequency of locomotion will decrease.

When the excitatory V3 neurons are removed, there is an imbalance between motor activity on the left and right sides of the cord. Since the V3 neurons are predominantly commissural, these findings may suggest that they are active in the dual left-right alternation pathways (Fig. 38.7b). V3 commissural neurons also project directly to contralateral motor neurons, which may give them a role in coordinating left-right synchrony (Fig. 38.7b).

The excitatory Hb9-positive interneurons constitute a very small group of cells located close to the central canal in the rostral lumbar spinal cord. These cells are vigorously rhythmically active. They have intrinsic properties that promote bursting and have therefore been proposed to be part of the rhythm-generating core in the rodent locomotor CPG. Since Hb9 is also found in motor neurons, it has not been possible to test this hypothesis directly by selective ablation of Hb9-positive interneurons.

By using molecular genetic studies applied to these cardinal groups of ventrally located neurons, it has thus been possible to gain functional insights to the organization of the locomotor CPG in rodents. Similar experiments have also started in the zebrafish.

Cellular Properties Contribute to CPG Activity

It is not only the synaptic connectivity that determines the function of a CPG. Neurons are equipped with a variety of ionic potassium, sodium, and calcium conductances that determine their response to synaptic inputs. Some of these ionic conductances may actively promote rhythmicity and change patterning of cell firing and activation. From studies of CPG circuits in many different experimental models, it has been shown that a number of membrane properties play a role for CPG function. These are pacemaker or plateau properties that amplify and promote bursting, ionic conductances that initiate phase-transitions, and ionic conductances that affect the rate of firing.

Bursting and Plateau Properties Amplify Cellular Responses

In some simple motor CPG circuits like those in the stomatogastric ganglia that control rhythmic movements in the gut of Crustacea, pacemaker properties producing sustained oscillations are essential for maintaining the rhythm. These pacemaker properties are most often conditional which means that they are only seen when neuromodulatory substances are present.

Conditional bursting properties have also been described in spinal cord neurons and motor neurons in lamprey, rodents, and amphibians. The bursting is elicited by activation of glutamate receptors by NMDA used to initiate the locomotion. The membrane potential oscillations arise from a negative slope region in the current-voltage relationship brought about by a voltage-dependent block of the

NMDA-receptor channel by Mg^{2+} that is released by depolarization. In amphibians, the NMDA-mediated voltage oscillations require the presence of serotonin suggesting that serotonin in these species is needed for the nonlinearity in the current–voltage relationship.

These properties are found in spinal neurons of different types. In lamprey, it has been shown that NMDA-induced oscillations play a role in generating slow swimming. It is uncertain whether conditional NMDA-mediated bursting plays an essential role for the rhythm generation itself in mammals. Nevertheless, it is certain that the nonlinearity in membrane properties which NMDA may impose will work as an amplifier of rhythmic activity. Thus, the NMDA-induced bursting properties will facilitate excitatory information flow in the circuit.

Another current that may facilitate neuronal activity is the persistent sodium current that is a slowly inactivating sodium current activated in the subthreshold range for the transient sodium current underlying the action potential. The current is native to many spinal neurons. Selective blockage of the persistent sodium current may severely affect rhythm generation possibly because the spike mechanism is impaired in the absence of the persistent sodium current.

Plateau properties are also a postsynaptic amplification mechanism. Plateau properties are voltage-dependent properties that are conditional. When present, a short barrage of synaptic input can cause the neuron's membrane potential to jump to a depolarized state, where it will continue to fire action potentials for a prolonged period of time in the absence of synaptic bombardment. The depolarized plateau can be terminated either spontaneously because of a time-dependent change in the balance of ionic conductances or by brief inhibitory synaptic inputs. Plateau properties thus enhance brief synaptic inputs, and in rhythmic systems, they may allow activity to be switched on as a result of a brief excitatory input. In vertebrate motor neurons, their expression is controlled by neuromodulatory monoamines, like serotonin and noradrenalin, and therefore, they are under regulatory control from neurons located in the brainstem. Typically, such neuromodulators induce plateau potentials by enhancing calcium currents and/or decreasing outward currents such as calcium-activated potassium currents or other potassium currents.

Phase Transition Is Regulated by Ionic Conductances

Since reciprocal inhibition between neurons appears to be a common design in locomotor CPGs, ionic conductances that are essential for phase transition may affect patterning and rhythmicity. There are three conductances that may affect phase transitions: a transient low threshold calcium current, a hyperpolarization-activated inward current, and a transient potassium current.

The transient low threshold calcium current, I_T , has properties which are important for phase transitions. This current has an activation threshold around or slightly depolarized to the resting membrane potential. It inactivates rapidly upon depolarization so at a depolarized level, I_T is inactivated after a short period of time.

The inactivation is removed by hyperpolarization and, therefore, inhibitory synaptic input will remove the inactivation. Upon release of the hyperpolarization, I_T will cause a short-lasting rebound excitation before inactivating again. In the lamprey spinal cord, metabotropic GABA_B receptor activation depresses a low-threshold calcium current, similar to I_T , in interneurons involved in producing the swimming motor pattern. The suppression leads to a longer hyperpolarized phase and therefore to a slower alternation between antagonistic muscles, a possible mechanism for the slowing of swimming seen following GABA_B receptor activation. I_T is also found in excitatory neurons, including the Hb9-positive cells, in the rodent spinal cord.

The hyperpolarization-activated inward current, I_h , may help neurons to escape from inhibition. The conductance underlying the current is activated by hyperpolarization, such as occurs during synaptic inhibition. The current is inward and will depolarize the cell and counteract the hyperpolarization that activates the conductance. Finally, the kinetics of activation and deactivation are slow. So when the membrane potential is hyperpolarized, the current activates slowly, causing the membrane potential to slowly “sag” back toward the resting membrane potential, and when the hyperpolarization is released, it slowly deactivates, so I_h contributes to the formation of a depolarizing overshoot. This behavior has two important consequences for the integrative properties of the cell. First, the depolarizing sag limits the effect of sustained inhibitory inputs and helps the cell escape from inhibition. Second, the depolarizing overshoot can trigger a burst of action potentials. In rodent motor neurons, I_h is enhanced by serotonin which will help them from escape inhibition.

The third current which may regulate the rebound from inhibition is the transient potassium current, I_A . This outward potassium current is usually inactivated at resting membrane potential. Hyperpolarization removes the resting inactivation of I_A , and a subsequent depolarization will cause a transient activation of the conductance. Activation of I_A will counteract the rebound activation – brought about, for example, by I_h or I_T – and delay its onset.

Thus, I_T , I_h , and I_A may be important for phase transitions in the locomotor CPG. These currents are found in variable amounts in many spinal neurons and may act at a subthreshold level to regulate phase transitions in the network.

Regulation of Spiking by Intrinsic Membrane Properties Affects the Function of CPG Neurons

The rate at which a neuron fires is not purely a function of how much excitatory synaptic drive it receives. The firing rate of a neuron is also determined by intrinsic membrane properties.

A number of different ionic conductances play a role in determining the firing rate of a cell. Activation and inactivation kinetics of sodium channels is one factor. For example, decreased threshold of action potential generation during locomotion has been described both in cat and rodent neurons as a factor which will help neurons to fire stronger during the depolarizing phase. Other important

conductances are sodium- and calcium-activated potassium currents. The effect of sodium- and calcium-activated potassium currents is often seen as a slow after-hyperpolarization following an action potential or a train of action potentials. Activation of these conductances therefore causes spike train adaptation and post-activation inhibition which contribute to burst termination.

Activation of Muscle Afferents and Skin Afferents Modulates Ongoing Locomotor Activity

Even though the activity of the CPG can produce the precise timing and phasing of the muscle activity that is needed to locomote without sensory information, its activity is normally modulated by sensory signals. There are two types of sensory inputs that modulate CPG activity. The first is sensory information that is generated by the active movement of the limb. The second is sensory input generated when the moving limb meets an obstacle in the surrounding.

The sensory information that is transmitted from the moving limb is generated in proprioceptive receptors in the muscles and joints. This proprioception includes stretch sensitivity from muscle spindles and force sensitivity from muscle Golgi tendon organs and is important for facilitating the stance to swing phase transition. It is primarily proprioception from the ankle joint extensor muscles and the hip joint flexor muscles that is important. Activity in force-sensitive Ib-afferents in ankle extensor muscles inhibits the flexor burst generation while activity in length-sensitive Ia-afferents in hip flexor muscles enhances the flexor burst activity during locomotion (Fig. 38.9a–c). This means that proprioceptive signals from these ankle extensor muscles and hip flexor muscles work in synergy to facilitate stance to swing phase transitions. Thus, in the late stance phase when the limb is unloaded, the inhibitory signal from Golgi tendon organs in extensor muscles decreases while at the same time, the activity in muscle afferents around the joints will increase because the hip is stretched. Together, these signals will facilitate the transition from stance to swing phase by promoting the activity in flexor motor neurons. Interestingly, while the afferents from Golgi tendon organs at rest have an inhibitory influence on their own motor neurons, during locomotion, this effect reversed in sign to excitation. Such a context-dependent reflex reversal has also been seen in other species.

The transition from swing to stance phase may also be regulated by sensory afferents, but the nature of these sensory signals is not as well characterized as those promoting the stance to swing phase transition.

The second important effect of proprioceptive inputs during ongoing locomotion is to enhance the amplitude of the locomotor burst. These activities take place during the actual locomotor activity and provide boosting of the muscle amplitude during locomotion.

The sensory signals that are activated when the moving limb meets an obstacle in the surrounding environment are mainly cutaneous and can be thought of as cutaneously evoked reflexes that are adapted to the ongoing locomotion. One of

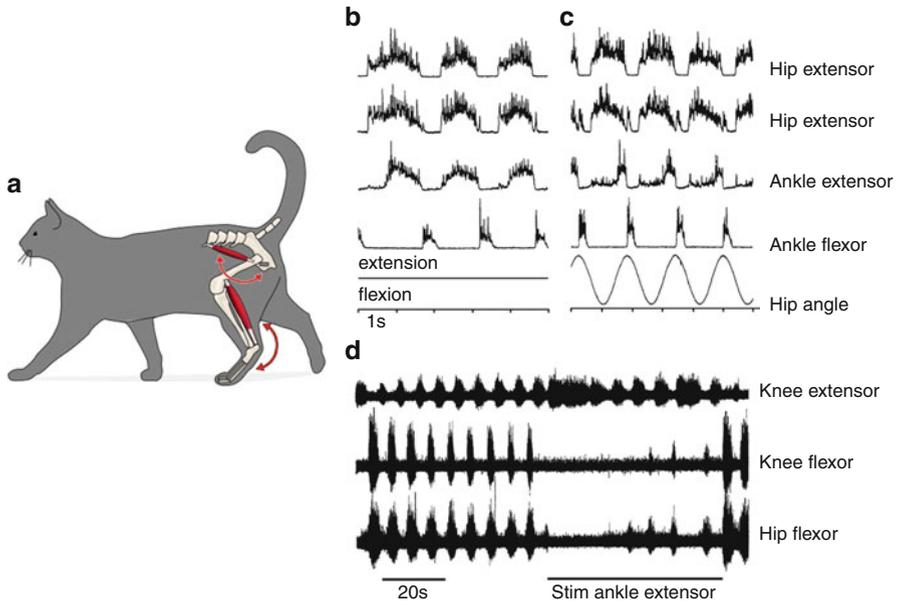


Fig. 38.9 Sensory information from the hip and the ankle contribute to transition from stance to swing (a). (b, c) Entrainment of the locomotor rhythm by passively moving the hip joint in an immobilized decerebrated cat. The stance to swing phase is initiated when the hip is fully extended at a point where there is a maximal input from Ia-afferents in the hip flexor muscles (Adapted from Kriellaars et al. 1994). (d) Motor nerve recordings from a knee extensor, a knee flexor, and a hip flexor showing that stimulation of proprioceptive fibers in an ankle extensor inhibits the flexor activity and causes an increased extensor activity. The stimulation of the ankle extensor proprioceptive afferents corresponds to a persistent loading of the ankle extensor, which will prevent the flexor activity from being initiated. Therefore, when the ankle extensor starts to be unloaded at the end of the stance phase, swing can be initiated (Adapted from Conway et al. 1987)

these reflexes that has been well characterized is the so-called stumbling correction reflex where the sensory input is gated depending on the locomotor cycle. So, for example, if an animal meets an obstacle when the limb is in the swing phase, this reflex will enhance the swing phase so that the foot clears over the obstacle. However, if a corresponding sensory stimulus is provided to the dorsum of the foot during the stance phase, there will be no correction of the stepping because this will provide an inappropriate balance for the animal.

Cerebellar Motor Circuits Integrate Internal Locomotor Commands with External Movements and Coordinate Locomotion

In addition to selection and initiation of locomotion, motor circuits in the brain are engaged in adapting the ongoing CPG activity and maintain the upright position and

balance needed to locomote properly. The cerebellum plays a central role in coordinating this interaction and damage to the cerebellum leads to a disorganized gait with variable step length and foot placement accompanied by balance problems such as increased postural sway and abnormal responses to perturbations.

The medial cerebellum (vermis) and the intermediate zone of the cerebellar hemisphere, collectively called the spinocerebellum (Fig. 38.10a), receive inputs from two groups of excitatory spinal neurons that are rhythmically active during locomotion: the dorsal spinocerebellar tract (DSCT) neurons and the ventral spinocerebellar tract (VSCT) neurons (Fig. 38.10a).

The DSCT neurons are located in the deep dorsal horn in the so-called Clarke's column, and their input is from proprioceptive afferents, mainly stretch-sensitive Ia-afferents originating in muscle spindles. Their rhythmic activity is entirely dependent on the rhythmic drive in Ia-afferents generated by the moving limb (Fig. 38.10b, c). Thus, during fictive locomotion or after deafferentation, the DSCT neurons do not exhibit rhythmic activity. The DSCT neurons can therefore be thought of as providing the spinocerebellum with information about the actual movement of the limb. In addition to the input from proprioceptive afferents, the DSCT cells also receive a descending excitation from corticospinal tract neurons. Recent studies have shown that the DSCT cells can be marked by a *glia-derived neurotrophic factor* gene, *GDNF*, and be visualized directly in the rodent spinal cord.

The VSCT neurons are located in the ventral spinal cord. Their rhythmic activity is not dependent on sensory information from the moving limb, but they are rhythmically modulated by the locomotor CPG directly (Fig. 38.10d, e). The VSCT neurons, therefore, provide the spinocerebellum with information about the degree and phase of rhythmicity generated by the locomotor CPG. Since the VSCT neurons send a readout or a copy of what is going to be the efferent motor output to spinocerebellum, this signal is often called an efference copy. The exact cells in the CPG that activate the VSCT neurons are not known.

Both the DSCT and VSCT pathways enter the cerebellum via mossy fibers and activate excitatory granule cells, which in turn activate the Purkinje cells (Fig. 38.10a). The Purkinje cells in spinocerebellum project to the deep cerebellar fastigial and the interpositus nuclei. The projection targets of the fastigial nucleus are the vestibulospinal and reticulospinal neurons in the brainstem while the interpositus nucleus projects to rubrospinal neurons and to ventral thalamus. Together, these neural circuits compose what is called a spinocerebellar loop that can modulate the ongoing CPG activity in relation to changes in the environment. A mismatch between the intended movement and the executed movement may be detected in spinocerebellum by comparing the signal from the VSCT neurons (efference copy) and DSCT neurons (limb-generated sensory signal) and be corrected by sending signals via the brainstem descending motor pathways to the spinal cord.

Recordings from Purkinje cells, deep cerebellar nuclei neurons, and neurons in the three brainstem descending motor pathways show that all of these neurons are rhythmically active as long as the locomotor CPG is rhythmically active. The CPG

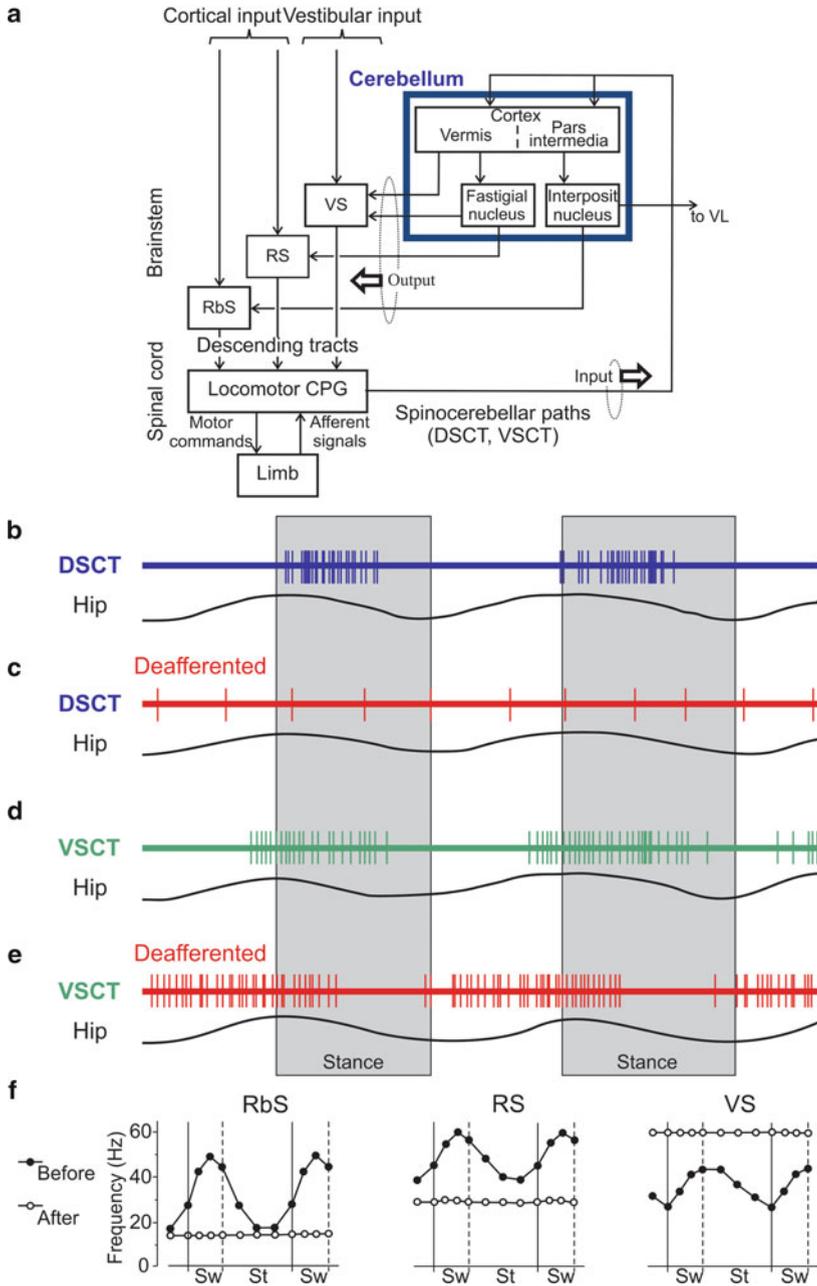


Fig. 38.10 Cerebellar circuits involved in the control of locomotion. (a) Spinocerebellar circuits composed of spinocerebellum, motor-related brainstem nuclei, the locomotor CPG, afferent sensory input from the limb, and spinocerebellar pathways are important for coordination of locomotion. VS, RS, and RbS vestibulospinal, reticulospinal, and rubrospinal neurons,

therefore imposes a rhythm mediated via spinocerebellar pathways on these supraspinal structures and not the other way around. The functional role of this rhythmicity seems to be that the cerebellum has a gating function that makes descending motor commands dependent on the locomotor phase. Thus, vestibulospinal neurons are rhythmically active in the early stance phase while rubrospinal and reticulospinal neurons are mainly active in the swing phase (Fig. 38.10f). These descending pathways therefore transmit signals efficiently during their peak of activity. External inputs to rubrospinal and reticulospinal neurons come from cortex while vestibulospinal neurons receive inputs from the balance organs. These signals will also be gated.

Neuronal Circuits in Cortex Allow Visual Adjustment of Locomotor Movements

The motor cortex is not essential for normal overground locomotion in cats and dogs. Such animals can walk and run remarkably well on flat ground when the motor cortex or the pyramidal tracts are destroyed. However, when the locomotor situation becomes more demanding as when, for example, a cat is tiptoeing on a fence or clearing the foot from an obstacle, the motor cortex is needed to perform appropriate changes in the basic locomotor pattern. This type of “skilled locomotion” requires visuomotor coordination that is imparted on the ongoing locomotor movement.

Even though the motor cortex is not needed for normal overground locomotion, the corticospinal tract neurons that project to the spinal cord are rhythmically active during locomotion. When recording their activity, corticospinal tract neurons are active in both the flexor and extensor locomotor phases and therefore apparently seem to drive neurons in the spinal cord (Fig. 38.11a, b). However, this rhythmic activity is not generated in the cortex but by positive feedback from the CPG in the spinal cord, most likely through cerebellum. The excitability of corticospinal tract neurons is therefore rhythmically modulated so that some are more excitable in the flexor phase and others are more excitable in the extensor phase, all of which is controlled by the activity of neurons in the spinal locomotor network. When skilled locomotion starts, the corticospinal tract neurons increase their activity but maintain a phasic activity. For example, corticospinal tract neurons that are active in the



Fig. 38.10 (continued) VL ventrolateral thalamus, *DSCT* dorsal spinocerebellar tract, *VSCT* ventral spinocerebellar tract (Adapted from Orlovsky et al. 1998). (b, c) The activity of the dorsal spinocerebellar tract neurons signals the movement of the limb. These cells are rhythmically active when the limb moves (b) but fall silent when the dorsal roots are cut (deafferented) (c) (Adapted from Orlovsky et al. 1998) (d, e) The activity of the ventral spinocerebellar tract neurons sends an “efference copy” of the activity in the central pattern generator (d) which is unaffected by cutting the dorsal roots (e) (Adapted from Orlovsky et al. 1998). (f) Rhythmicity in reticulospinal (*RS*), rubrospinal (*RbS*), and vestibulospinal (*VS*) descending neurons in the brainstem depends on intact cerebellar circuits. Activity of the descending neurons is shown before (*closed circles*) and after (*open circles*) removing the cerebellum (Adapted from Orlovsky et al. 1998)

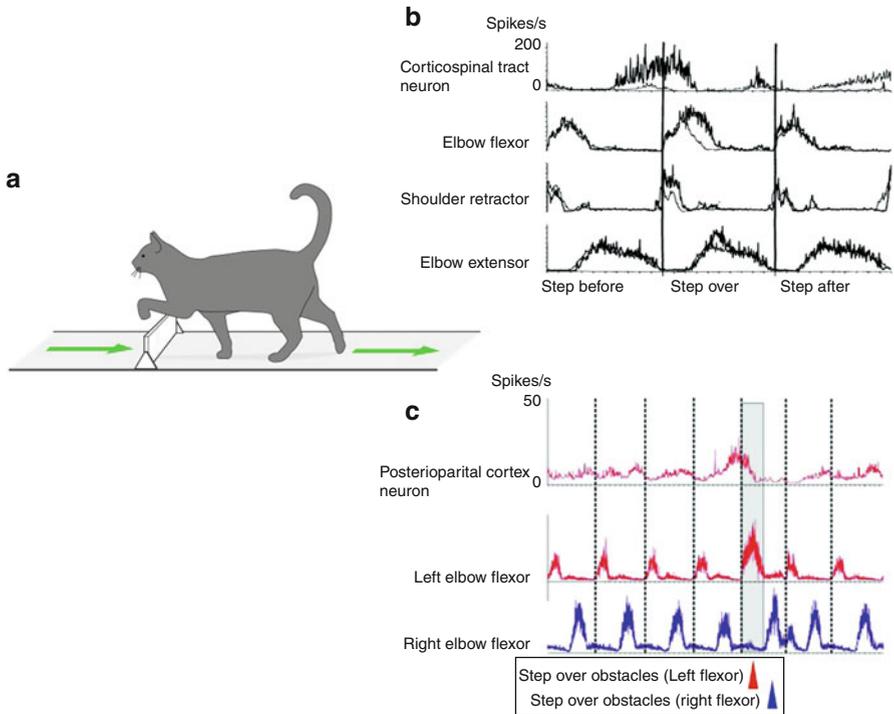


Fig. 38.11 *Neurons in motor cortices are active during skilled locomotor movements (a). (b)* During overground locomotion in a normal cat, there is rhythmic activity in corticospinal tract neurons in primary motor cortex. The intensity of this rhythmic activity increases during tasks that require skilled locomotion, like lifting the limb over an obstacle. The increased activity leads to phase-dependent enhancement of the motor output (Adapted from Drew 1993). *(c)* Neurons in the posterioparietal cortex increase their firing frequency in advance of the EMG activity recorded in the limb as an indication that these neurons are involved in planning rather than executing the changes in skilled locomotor movements (Adapted from Drew et al. 2008)

flexor phase will increase their activity when the animal lifts its foot over an obstacle. The activity is tightly correlated with the size of the object suggesting that the activity of the corticospinal tract neurons during skilled movements provides the activity needed to make the correction which is smoothly incorporated in the ongoing locomotion so that the basic pattern is not disturbed. Recordings from neurons in the posterioparietal cortex show that these neurons are active before the corrections, suggesting that the planning of the corrections is initiated by visual inputs that reach the posterioparietal cortex and via premotor cortex reach the motor cortex (Fig. 38.11a, c). The cerebellum is presumably also involved in this planning. Thus, the lateral cerebellar hemisphere receives input from most motor cortical areas and projects back via the thalamus to motor cortical areas. Damage of the lateral cerebellar hemispheres affects the possibility for skilled locomotion.

Neuromodulatory Systems Cause Long-Lasting Changes in Synaptic Strength and Ionic Conductances of the Locomotor CPG

The function of the operation of the locomotor CPG depends upon two things: the synaptic interconnections between neurons in the network and the intrinsic membrane properties of the component neurons. These circuit characteristics are not fixed but may be dynamically changed to reshape the motor output by neuromodulators.

There are two sources of neuromodulation for the locomotor CPG: extrinsic neuromodulation that originates from other parts of the nervous system and intrinsic neuromodulation that originates from within the locomotor CPG itself.

The most pronounced extrinsic neuromodulation comes from neuromodulatory neurons located in the brainstem. These neurons are located in the lower brainstem and include serotonergic raphe neurons, dopaminergic neurons in the hypothalamus, and noradrenergic neurons in locus coeruleus. These cells are relatively few in number, but they project fibers in a divergent fashion to the spinal cord and may affect many neurons at the same time. For example, individual noradrenergic neurons innervate both cervical and lumbar parts of the spinal cord, acting on both motor neurons and premotor neurons in those areas. The main effect of these neuromodulatory inputs is that they can change the frequency of locomotion and burst amplitude of the ongoing locomotor activity. In mammals, serotonergic cells may act both to initiate the rhythm (see above) and to modulate the ongoing rhythm, possibly through different descending serotonergic pathways.

Intrinsic neuromodulation provides a local control of the locomotor CPG. The presence of neuromodulation intrinsic to the CPG may be important for monitoring the activity of the CPG and thus causing use-dependent alterations in the behavior. Such use-dependent changes have been described both for the walking and swimming CPGs. In tadpoles, it has been shown that ATP released from spinal neurons during swimming episodes increases the excitability of the swimming CPG by reducing voltage-activated potassium currents in spinal neurons. After it is released, ATP is metabolized into adenosine. Adenosine causes a reduction in voltage-activated calcium currents and thereby lowers the excitability of the rhythmic network. Thus, the ATP and adenosine dynamically regulate the swimming CPG activity in opposite directions. At the onset of swimming, the relative concentration of ATP will be high. That causes the network excitability to start off high. As swimming progresses, the adenosine concentration increases and the tadpole will stop swimming due to the buildup of ADP. Thus, accumulation of a neuromodulator produced by a motor network can play a role in self-termination of a motor behavior.

A state-dependent amplification of network output is seen in the lamprey spinal cord where gaseous nitric oxide (NO) is released from active neurons and enhances ongoing locomotor activity by decreasing inhibition and increasing excitation in the network. This effect of NO seems to be obtained by a synergistic effect mediated by endocannabinoids that are also released from neurons in an activity-dependent manner. The release of endocannabinoids from spinal neurons leads to long-term enhancement of motor output due to changes in intrinsic membrane currents and

synaptic transmission. The endocannabinoid response is a type of memory function in the CPG that makes it produce a maintained output.

In the rodent spinal cord, a small group of cholinergic neurons marked by the transcription factor, Pitx2 (Pituitary homeobox), are found close to the central canal. These cholinergic Pitx2 neurons give rise to the large cholinergic C-boutons that surround motor neurons, and they have widespread projections to motor neurons that act in synergies (e.g., extensors around a joint). They are rhythmically active during locomotion. Genetic inactivation of their output leads to a reduction in extensor amplitude during swimming but not during walking in mice, suggesting that cholinergic Pitx2 neurons are recruited in a task-dependent way to amplify and coordinate the activity in synergist motor neurons during rhythmic movements.

Outlook

Locomotion is a complex motor act that in vertebrates is generated by CPG networks in the spinal cord. These networks are at the core of generating locomotion. CPG activity is selected and initiated from supraspinal neural circuits and is modulated by sensory inputs. The rhythmic activity of the CPG itself provides the cerebellum with rhythmic signals that are used to gate, coordinate, and correct motor signals from the brain and the brainstem so that they are appropriate to the locomotor phase. When active, the CPG network provides modulatory signals that intrinsically regulate its function. Understanding the function of the CPG networks is therefore key to understanding how locomotor movements are accomplished.

The work in lamprey and tadpole has given a detailed outline of the basic CPG networks controlling swimming. The organization of spinal locomotor networks in mammals also starts to be revealed, not least because of the possibility to combine genetic and electrophysiological studies in the developing rodent spinal cord. With these methods, it has been possible to allocate populations of neurons to designated network functions. However, essential elements of the network organization still need to be determined. This includes the organization of the rhythm-generating network, which has so far escaped characterization, underscoring the need to define new molecular markers for glutamatergic neurons in the CPG. As molecular genetics cannot be performed with ease in the cat, a major challenge in the future will be to converge the CPG analysis in the developing rodent with that in the adult feline spinal cord, which for many years has been the prevailing model for studies of locomotion. The use of zebrafish, where molecular genetics can be applied, will make a direct comparison of common principles as well as differences in network organization in limbed and non-limbed animals more straightforward.

The precise organization and cellular origin of the initiating systems in the lower brainstem and the behavioral context in which these systems are activated remain to be determined in mammals, including the CPG neurons that the descending locomotor command targets. Such knowledge will be of utmost importance in selecting appropriate repair and regeneration strategies to improve motor defects after spinal cord injury, where the descending motor command is lost but the CPG is probably intact.

The details of the circuits for selection of locomotion and the higher order control of these circuits as well as the circuits for skilled locomotion also remain to be fully understood. The successful approach to this network analysis might be found in using several different animal models of various complexities.

Although multifaceted in nature, it appears within reach to provide a comprehensive and mechanistic description of the locomotor circuits and their physiology in vertebrates within the foreseeable future.

Glossary

Central Pattern Generator (CPG) Neuronal networks responsible for the phasing and timing of rhythmic activity.

Fictive Locomotion Locomotion without movements; the motor pattern recorded from motor nerves.

Locomotion Motor behavior allowing for movement through the environment; includes walking, swimming, flying, running, and hopping.

Mesencephalic Locomotor Region (MLR) Midbrain region where locomotor commands from higher structures are integrated and initiating locomotion.

Neuromodulation Alteration of ongoing CPG activity by monoamines or other neurochemicals released over groups of neurons to indicate state, use-dependence, or drive; neurons releasing neuromodulators can be extrinsic or intrinsic to the locomotor CPG.

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