CURRENT PRINCIPLES OF MOTOR CONTROL, WITH SPECIAL REFERENCE TO VERTEBRATE LOCOMOTION

GRAPHICAL ABSTRACT

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CLINICAL HIGHLIGHTS
This review summarizes the logics of the neural control of motion extending from the basal ganglia mechanisms responsible for selection of behavior and cortex for precision walking to the cellular and molecular design of the central pattern generator networks in the brain stem-spinal cord.
CURRENT PRINCIPLES OF MOTOR CONTROL, WITH SPECIAL REFERENCE TO VERTEBRATE LOCOMOTION

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Grillner S, El Manira A. Current Principles of Motor Control, with Special Reference to Vertebrate Locomotion. Physiol Rev 100: 271–320, 2020. First published September 12, 2019; doi:10.1152/physrev.00015.2019.—The vertebrate control of locomotion involves all levels of the nervous system from cortex to the spinal cord. Here, we aim to cover all main aspects of this complex behavior, from the operation of the microcircuits in the spinal cord to the systems and behavioral levels and extend from mammalian locomotion to the basic undulatory movements of lamprey and fish. The cellular basis of propulsion represents the core of the control system, and it involves the spinal central pattern generator networks (CPGs) controlling the timing of different muscles, the sensory compensation for perturbations, and the brain stem command systems controlling the level of activity of the CPGs and the speed of locomotion. The forebrain and in particular the basal ganglia are involved in determining which motor programs should be recruited at a given point of time and can both initiate and stop locomotor activity. The propulsive control system needs to be integrated with the postural control system to maintain body orientation. Moreover, the locomotor movements need to be steered so that the subject approaches the goal of the locomotor episode, or avoids colliding with elements in the environment or simply escapes at high speed. These different aspects will all be covered in the review.

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I. INTRODUCTION

“To move things is all that mankind can do, and for this the sole executant is a muscle, whether it be whispering a syllable or felling a forest.” This quote from Charles S. Sherrington couches the fact that most of the processing in the nervous system is dedicated to producing movement. The final expression of most cognitive and emotional processes includes motor actions. Our broad motor repertoire enables us to walk, reach out for different objects, maintain balance as we are standing, breathe, chew, swallow, and direct the eyes to different salient objects. Specific neural circuits are dedicated to serve each of these functions, often referred to as central pattern generator circuits (CPGs) or motor programs. They form together an innate motor infrastructure that accounts for the basic motor repertoire of a given species and are to a large degree conserved from lamprey to primates. While the different CPGs are preformed, they are also adaptable. Some are ready and fully functional at birth, whereas others mature during the first few years after birth. The final processing within the central nervous system underlying all motor actions is, however, the result of a delicate interaction between intrinsic computations within these different circuits and sensory information.

This review summarizes the logics of the neural control of motion extending from the basal ganglia mechanisms responsible for selection of behavior and cortex for precision walking to the cellular and molecular design of the central pattern generator networks in the brain stem-spinal cord.
In real life, the different circuits are combined in a dynamic fashion to endow the organism with a panoply of motor behaviors. For instance, a tennis player will run and reach out to hit the ball, while at the same time tracking its trajectory with the eyes and making appropriate predictions. Practically all parts of the nervous system are involved in such a task. To initiate a movement in a behavioral context, the brain needs to determine the overall goal of the movement, then select which circuits to activate, and finally execute the movements with precise timing, speed, and coordination.

To unravel the principles governing this process, we need to gain insight into the organization and function of the individual circuits and also the mechanisms that determine when a motor program should be called into action. Therefore, this review is intended to provide an account of the state of our knowledge of the neural circuits that control motor behavior in general, and in particular the neural bases of locomotion. The focus is on how the combined activity of circuits in the forebrain, brain stem, and spinal cord contribute to generating a flexible and adaptable motor behavior (FIGURE 1).

In all vertebrates, locomotion is one of the most basic and yet most complex motor acts. At the same time, it is the motor system in which most knowledge is available through experimental models ranging from humans to cyclostomes (368). It involves not only the actual process of propulsion, but also the adaptation of the movements to the overall goal of the animal, and to the details of the surrounding terrain depending on visuomotor processing supplemental by information from other senses. In addition, there is the role of sensory feedback in adjusting the activity to the inevitable perturbations occurring during the movements. CPG circuits in the spinal cord are essential for the propulsive aspect of locomotor movements as they produce the complex motor pattern corresponding to swimming, flying, or walking (201). In addition, they act as a processing interface to integrate sensory feedback and instructive signals from the brain to optimize the execution of locomotor movements (393).
From the analytical point, studies of the neural control of action are at an advantage over open-ended sensory or cognitive systems, because the neural activity is linked to a defined purposeful motor act. The analyses of the organization and function of locomotor circuits have traditionally relied mostly on electrophysiological and anatomical approaches to define sets of interneurons essential for generating the motor pattern and map their connectivity. In this regard, accessible vertebrate preparations such as the lamprey and Xenopus tadpole have unraveled many of the fundamental principles of organization at the spinal level with ipsilateral excitatory drive combined with reciprocal inhibition (70, 387) that represents the backbone of the spinal locomotor circuits in all vertebrates. These studies are now complemented with the introduction of molecular and genetic tools defining molecular markers of classes of interneurons in zebrafish (145, 334) and mouse (2, 194, 217, 271, 286, 312). This allows for perturbation of their function through a variety of methods including the optogenetic approach that has spurred the further analysis of the locomotor circuits.

This review will consider the control of motion with a focus on the integrated system for locomotion, which encompasses not only the brain stem-spinal cord networks generating propulsion and steering but also the forebrain mechanisms underlying selection of behavior and the integrated control system for body orientation (posture). We will include general findings in vertebrates extending from lamprey to humans. Although clearly of critical importance, we will only marginally include biomechanical aspects (94, 160, 163, 202, 213, 354, 451) and only very briefly the area of spinal cord injury.

II. THE BUILDING BLOCKS OF THE LOCOMOTOR SYSTEM: OVERVIEW

The diagram in Figure 2 summarizes the overall organization of the control system for motion (22). The choice of action and decision making represents perhaps the most complex level, and here the basal ganglia play a central role and depend on input from cortex, thalamus, and the dopamine system. The basal ganglia control the different command centers in the brain stem, which in turn activate different command lines to specific CPGs in the spinal cord or brain stem responsible for the execution of a particular motor program. The cerebellum plays an important role for the fine tuning of movements and motor learning.

The specific scheme relating to the control of locomotion is shown in Figure 3 (220, 224, 390). Goal-directed locomotion is produced by different components, extending from the forebrain to the spinal cord. We will first give a brief account of the role of the different building blocks of the locomotor system to show how they contribute. Subsequently, we will make a more detailed account for each of these components.

The networks coordinating the stereotypic locomotor pattern are located in the spinal cord in all vertebrates extending from the swimming movements of fish and salamanders to the walking movements of tetrapods, and bipeds, like birds and humans. These networks, usually referred to as CPGs, contain sufficient information to activate the different muscles in the appropriate sequence to coordinate the movements during each locomotor cycle (Figure 3). If the CPG has an appropriate excitability, spinal animals will generate walking movements on for instance a treadmill belt, or a spinal fish will produce swimming movements. In practically all vertebrates, the sensory feedback onto the CPGs also plays an important role for adapting the activity to external events (138, 139, 174, 226, 229, 230, 453). The details of the locomotor pattern will in most cases be influenced also by conditions in the periphery, like perturbations.

B. Initiation of Locomotion from Locomotor Command Areas

The activity in the spinal networks is controlled from locomotor command areas in the mesencephalon (MLR) and
the animal needs to steer the movements along a path or towards a particular goal that is of interest, such as a place to feed. The visuomotor coordination is important in this context, and much information is conveyed directly to the optic tectum (superior colliculus in mammals) that can elicit both orienting movements towards an object and evasive movements that can make an animal avoid bumping in to different obstacles (281). These effects are mediated to the spinal cord and become integrated with the locomotor propulsive movements. Visuomotor coordination is also mediated via the corticospinal system in mammals, which enables them to modify the step cycle and provides very precise placement of the feet as when walking in the forest (40, 128, 186, 315).

D. Intersegmental and Interlimb Coordination in Vertebrate Locomotion

Swimming vertebrates such as lamprey, fish, salamanders, and tadpoles swim with body undulation transmitted along the body, which are generated by the trunk muscles being activated in succession from head to tail with a constant phase lag (see Ref. 201).

The interlimb coordination among mammals and other tetrapods can be reduced to two modes at each limb girdle, either strict alternation as in walk or trot, or more or less simultaneous activation of the limbs at the same girdle (homologous), like in a bound or a gallop. In the latter, there is often a slight bias with one limb leading (as summarized in Ref. 208). With regard to the coordination between fore- and hindlimbs, in walking the limbs are activated in a rotary way with the support phases in succession. In trot, the limbs on the same side of the body alternate (diagonal coupling), whereas they are simultaneous in pace (homolateral, as for camels and certain horses). In a gallop or bound, the trunk also contributes effectively to prolong the stride.

Birds of course use their forelimbs for flying, in a strict synchronous mode. The legs when used for walking alternate in practically all species, although some species preferentially use simultaneous activation as in a jumping gait.

E. The Control of Body Orientation/Posture during Locomotion

All vertebrates control the body orientation during locomotion; a fish swims with the dorsal side up, a tetrapod walks similarly with the back upward and horizontal, and a biped walks with the trunk vertical. This is a fundamental property of the locomotor control system, and it needs to be in operation for meaningful locomotion to occur, and the transition from walking to standing needs to be smooth. Sherrington formulated this elegantly as “posture follows movement like a shadow.”

C. The Goal-Directed Aspect of the Locomotor System and Precision Walking

The circuits described above are sufficient for generating the propulsion itself, but for locomotion to become purposeful,
In fish, the vestibular apparatus plays a critical role for correcting the head-body position, with effects mediated by the vestibulospinal and reticulospinal systems. In land-living vertebrates, the vestibular system remains important, but sensory information from the limbs is also critical due to the increased effects of gravity (115).

The combined action of each of these different components is required for successful goal-directed locomotion to occur whether in fish or mammal.

III. LOCOMOTION IN HUMANS AND TETRAPODS: DEVELOPMENTAL AND EVOLUTIONARY CONSIDERATIONS

The newborn infant has a limited motor repertoire; it can cry for help, be fed, and breathe. During the first year of life, there is a progressive maturation of the motor system, and after a year or so the child can stand up by itself and maintain balance and finally walk a few steps before falling (FIGURE 4). This requires the engagement of many different parts of the nervous system from the forebrain to the spinal cord.

The situation is radically different in certain other mammals like the deer, in which the young ones are able to coordinate locomotor movements and balance within minutes after birth. Although not perfect, the fawns are sufficiently calibrated to follow their mothers around and even run (204). This radical difference to humans seemed very surprising, and in many quarters it was argued that the bipedal human locomotion was fundamentally different from that of other mammals. However, it has actually been shown that the detailed motor pattern of all mammals and birds is very similar (90, 126), including four different phases of the step cycle (support, lift up, swing, and touch down). Since the common evolutionary origin of birds and mammals are from reptiles (FIGURE 5A), it implies that the motor pattern originates from reptiles or possibly amphibians (211).

An unexpected answer to the question of why human children are so slow in developing walking came with the study of Garwicz et al. (184) that showed a linear correlation between the time it takes for an animal to be able to walk from the instant of conception (not birth!) and the weight of the brain. There is a linear relation between these two factors from the small brain of the mouse to the big brains of humans and elephants (FIGURE 5B). The elk, as most likely would the deer, falls on the regression line connecting mice with humans, as do all mammals explored. It thus means that it is only when the brain has been assembled to a certain level that it can coordinate the locomotor movements, and that it takes longer time to “put together” a brain with billions of neurons rather than the small mouse brain. The important implication is that there is no reason to believe that the neural circuits responsible for human locomotion are in any basic way different from those of other mammals; it is a matter of maturation of the nervous system rather than learning. Thus, if human mothers would have carried their young ones for 2 yr instead of 9 mo, it is likely that they would have walked directly after birth, like the young of the deer. There will of course be particular adaptations in each species dependent on the anatomy of for instance the legs, but the basic features are the same.

A. The Postnatal Development: from Reflex Stepping to the Adult Motor Pattern

The human infant displays during the first few months of life what is often called reflex stepping. The child can be held with the legs in contact with a treadmill belt, and if the belt moves, the child will perform what looks like stepping

![FIGURE 4. Progressive maturation of the motor development of the infant and young child. At 2 months after birth, a child can lift its head and at 4 months sit with support. Subsequently, it is able to stand with support, crawl, stand without support, and finally walk. The approximate time at which a child is able to perform those different motor tasks is indicated above each figure. The variability in the maturation process is substantial. [Modified from Grillner (204), copyright 2003 Elsevier.]]
movements. At birth, the full four-phase locomotor pattern (support, lift up, swing, and touch down) has not yet been developed, and the stepping movements consist simply of an alternation between flexors and extensors (168, 303). Only at around the age of 1 yr, when the permanent walking movements start to develop, do the four-phase movements evolve (126).

Similarly, in rodents at birth, the motor pattern is limited to alternation between flexors and extensors, when the pups are mainly crawling. The adult four-phase motor pattern evolves during the first few weeks as the pup becomes able to support itself while walking (63, 126, 460). This gradual maturation of the locomotor pattern raises a methodological problem for the mouse neonatal in vitro spinal cord preparation. It has for technical reasons only been possible to maintain the spinal cords in good conditions during the first neonatal week at a point when the four-phase locomotor pattern has not yet evolved. These studies, although very important, are thus performed on a network that is not yet fully developed and will not be able to capture the full four-phase motor pattern. This also applies to the zebrafish and tadpole, in which the frequently used larval stage undergoes significant changes until the adult stage (9).

Despite this limitation, the rodent neonatal in vitro spinal cord and the larval and adult zebrafish have become very useful and important experimental models in the study of the control of motion. The possibility to use genetic techniques for deletion, inactivation, or activation of identified interneuron classes, targeting the expression of fluorescent reporters or viral techniques, has revolutionized the experimental toolbox for cracking the code of complex circuits. This applies both to circuit analyses in in vitro or in vivo experiments, in acute experiments, or just to test the impact on locomotion and other aspects of motor behavior. This has the advantage that it can be tested mostly on fully matured mice, but effects on the network can only be indirectly assessed.

B. Evolutionary Perspective on the Vertebrate/Chordate Motor System

Throughout the vertebrate phylum, from lamprey to mammals, the essential “bauplan” of forebrain, midbrain, brain stem, and spinal cord is evolutionarily conserved (224, 438). It is therefore important to consider the early evolution leading up to vertebrates and the lamprey.
What is the origin of the vertebrate central nervous system (CNS)? The ascidian protochordate *Ciona intestinalis* has in its tadpole stage a brain vesicle, a motor ganglion (brain stem), and spinal cord made out of a very limited number of 80–100 neurons (357, 480). It swims with undulatory locomotor movements driven from the motor ganglion but generated by the spinal cord. The spinal cord itself, without the supraspinal structures, can generate swimming movements if glutamate is added to the perfusate, and the alternating activity is due to commissural glycnergic neurons, similar to that of other vertebrates (99, 221, 286, 386). The *Ciona* thus has a spinal CPG that may be the first to have evolved some 650 million years ago (357). Recent findings show that *Ciona* has a neural crest and placodes, representing a hallmark of the developing vertebrate nervous system (264). After the larval stage, *Ciona* becomes sessile and loses the spinal cord!

The cephalochordate *amphioxus* (lancelets) has been of central interest for comparative anatomists for more than a century, because it has a well-developed spinal cord with segmental dorsal roots and the segmental muscles are innervated through segmental synapses at the spinal cord margin (167, 355). There is a brain vesicle in the adult lancelet and a midbrain/brain stem. The lancelet is several centimeters long and swims with undulatory movements of the trunk. If the spinal cord is isolated from the brain stem, it can still generate locomotor movements. The molecular phylogenetic studies position cephalochordates as the basal group within chordates (FIGURE 6; Refs. 261, 382).

The oldest group of now living vertebrates is cyclostomes (lamprey and hagfish). They have no paired fins and swim with undulatory movements of the body (see below). One major step from amphioxus to cyclostomes is that the forebrain, midbrain, and brain stem now have evolved and in great detail are similar to that of mammals, although with fewer neurons (361, 389, 434, 438) and with critical forebrain transcription factors. The system of hox genes is important for the rostrocaudal organization of the nervous system of vertebrates (271). It has now been identified in cyclostomes (371, 436). The trunk motoneurons are located in the medial motor columns (MMC). The cyclostome lineage diverged some 560 million years ago from that leading up to mammals (FIGURE 6).

Next in line are the elasmobranchs (sharks and rays), which is the first vertebrate group to have developed paired appendages. They swim with undulatory locomotor movements of either trunk or the extended pectoral fins. The trunk muscles are controlled from the MMC while the fins are controlled from the well-characterized lateral motor column (LMC), as shown by Jung et al. (275). They recently showed that the paired pelvic fins can generate alternating walking-like movements, when the ray moves along the bottom of the sea, and also that not only the LMC is organized as in the tetrapod limb, but also the transcription factors of the interneurons are present (see also Refs. 91, 105, 203). The inference is that the basic neuronal machinery for the control of the tetrapod limb had been developed.

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**FIGURE 6.** Vertebrate evolution from amphioxus to mammals with the time indicated when the different groups became separate from the line of evolution leading up to mammals. The paired appendages emerged in elasmobranchs together with the lateral motor column (LMC). Below are indicated the LMC, important for locomotion with paired appendages, and the medial motor column (MMC) critical for undulatory locomotion. [Modified from Grillner (203).]
some 420 million years ago when the elasmobranchs diverged from the mammalian lineage (see FIGURE 6).

The elasmobranchs thus represent the first group of vertebrates with paired appendages controlled by the LMC. Next follows teleost fish like zebrafish, and the next stage is after the development of actual fore- and hindlimbs as in frogs and salamanders and then reptiles, birds, and mammals (FIGURE 6). They will be considered in the text when appropriate.

IV. SELECTION OF BEHAVIOR: ROLE OF THE FOREBRAIN

The forebrain is required for the recruitment of different movements adapted to the needs of the animal. A variety of motor patterns can still be elicited, such as locomotion or swallowing if the forebrain is incapacitated, as in the classic decerebrate cat preparation (409), but these movements are now automatized and unrelated to the behavioral demands of the animal. The main structures of importance for the control of goal-oriented movements in the forebrain are the cortex, basal ganglia, including the dopamine system, and thalamus.

A. Role of Motor Cortex for Precision Walking and Steering: Motor Capacity of Mammals Without Cortex

The motor cortex in mammals sends direct projections to the spinal cord, which are important for, e.g., precision walking, like placing the feet on the rungs of a ladder or accurately in a complex terrain (186, 284, 308, 309, 315). Recordings of cells in the motor cortex during locomotion show that they are strongly modulated during precision walking when the limbs must be accurately placed (FIGURE 7, A AND B), or the trajectory of the limb changed to overcome an obstacle (FIGURE 7C) (40, 128, 130). Different sets of cells in motor cortex are concerned with corrections of the limb-trajectory in different directions (128). The cells in motor cortex that are modulated during precision walking are also modulated in reaching tasks (FIGURE 7C) (130). These corrections depend on vision, and the parietal lobe serves as an intermediary structure.

For other aspects of locomotion, motor cortex is not needed. Actually, in mammals including cats and rodents, the motor cortex can be removed (see Ref. 284) without significant effects. Even the entire cortex can be removed...
without any visible effects on most aspects of basic motor behavior (49, 406, 468). Without all parts of cortex, a cat can still walk around, explore the environment, search for food, and survive for years in a laboratory environment. Under these conditions, the basal ganglia remain intact and can apparently still handle the selection of behavior, that is, to initiate movements appropriate to the external or internal demands of the animal. This suggests that also under normal conditions the basal ganglia play a key role in the selection of basic aspects of behavior.

B. Role of Basal Ganglia and Related Structures

A major set of evolutionary conserved forebrain nuclei below cortex is the basal ganglia (FIGURE 8), which are comprised of striatum (with input from cortex, thalamus, and the dopamine system), globus pallidus externa, the subthalamic nucleus, and the output nuclei. Dysfunction of the basal ganglia leads to major motor deficits, including Parkinson’s disease, Huntington’s disease, and involuntary movements sometimes occurring as a side effect of medication (437).

The basal ganglia play a major role in determining when a given pattern of behavior should be recruited. The direct projections from the output nuclei of the basal ganglia [globus pallidus interna (GPi) and substantia nigra, pars reticulata (SNr)] are of critical importance in this context. They consist of GABAergic neurons that are spontaneously active at a high level under resting conditions (253). They provide continuous inhibition of the different command centers for locomotion in mesencephalon (MLR) and diencephalon (DLR) and a number of other motor centers, controlling for example chewing, swallowing, postural tone, and respiration. Only when the tonic inhibition has been removed through input from striatum will a given motor center be able to generate its specific pattern of behavior due to the disinhibition.

The neurons in GPi and SNr (see FIGURE 8) are “designed” to be tonically active and need no excitatory drive to maintain their inhibitory activity (434, 437). Thus, under resting conditions, all the brain stem motor centers are kept under tonic inhibition from GPi/SNr, and only when these neurons are inhibited from striatum, the motor centers are disinhibited and allowed to come into operation. This mechanism of releasing a motor program by removal of inhibition, disinhibition, has been demonstrated for MLR, DLR (lamprey, rodent, cat), and for eye and orienting movements and other motor centers (179–182, 214, 253–258, 337, 390, 440–442).

1. The basal ganglia “direct pathway” from striatum to GPI/SNr initiates locomotion through action on MLR

The dopamine D1 receptor (D1R), expressing GABAergic projection neurons of striatum, directly inhibits GPi/SNr. They have opposite membrane properties to those of SNr/GPi and are instead difficult to activate, because they express potassium channels (inward rectifiers, Kir), which make them stabilize at a quite hyperpolarized level under resting conditions. The excitatory input to these striatal cells originates from cortex and thalamus and needs to be sufficiently strong to depolarize the cells to overcome the Kir depression. The DIR projection neurons are excited by dopamine through a combined action on several ion channels including a reduction of the Kir (437).

Roseberry et al. (390) have in an elegant study shown that an optogenetic activation of direct pathway D1R striatal neurons, via the SNr, leads to an activation of glutamatergic neurons in MLR and also to the initiation of locomotion. Conversely, an inhibition of MLR neurons leads to a cessation/reduction of ongoing spontaneous locomotor activity, indicating that MLR has a pivotal role in spontaneously generated locomotion (see Figures 3 and 8). An activation of cholinergic MLR neurons enhances ongoing locomotion, but does not by itself induce locomotion.
The activation of the striatal D1R neurons depends in turn on input from cortex, thalamus, and the dopamine system. Cortex, in particular the sensorimotor aspects, provides 55% of the glutamatergic input to striatum, and different thalamic nuclei provide the remainder (5, 125, 421). The cortical input is mostly from two types of layer 5 pyramidal neurons: 1) those that project to the brain stem-spinal cord (PT-type) and give off collaterals to the striatum as they pass through this structure and 2) the intralaminar palliothalamic (IT) neurons that originate from the cortex at the ipsilateral or contralateral side and terminate in striatum. In addition, the dopaminergic input (see below) from the substantia nigra, pars compacta (SNc), and the ventral tegmental area (VTA) is of critical importance for the operation of the basal ganglia, as is the 5-hydroxytryptamine (5-HT) input from the raphe nuclei and the histamine control.

The areas within both the ventral (nucleus accumbens) and dorsal striatum appear to be involved in the control of locomotion. An activation of D1R striatal projection neurons with local administration of dopamine in the ventral striatum can elicit locomotor activity, which is blocked by inactivating MLR, showing that the effects from the ventral striatum are channeled via MLR (66, 67, 272, 344, 475).

In the decorticate state, the direct projections from thalamus to striatum provide the only glutamatergic input, which apparently is sufficient for a well-adapted motor behavior (see above and Ref. 49). The relative importance of the thalamic versus the cortical input to striatum for initiation of movement in the behaving intact animal remains as yet unclear.

2. The basal ganglia “indirect pathway” reduces locomotor activity

Another set of GABAergic striatal projection neurons express dopamine D2 receptors (FIGURE 8; D2R), and in this case dopamine will cause a net inhibition by having essentially opposite effects to those of D1R on the different target molecules (437) (FIGURES 3 AND 8). The D2R-expressing neurons have different downstream targets, initially the inhibitory globus pallidus externa (GPe) projecting to the glutamatergic subthalamic nucleus (STN) that will excite GPi/SNr. The net effect of activating striatal D2R projection neurons will be an enhanced activity in STN and thereby also in GPi/SNr, which will further inhibit the motor centers that are targeted (103). Roseberry et al. (390) show that an activation D2R projection neurons and thereby the indirect pathway can lead to a reduction of MLR activity and locomotion.

Deep brain stimulation (high frequency) of STN can alleviate the symptoms of Parkinson’s disease. This would seem counterintuitive because a further activation of STN should be expected to further inhibit brain stem centers. It remains unclear what is the actual explanation for this effect of the STN stimulation that has proven effective in thousands of patients.

3. The role of the dopamine system: salient stimuli, reward, and direct projections to MLR

The dopaminergic input to the dorsal striatum originates from SNc (FIGURE 8), whereas that to the ventral striatum (e.g., nucleus accumbens) comes from the adjacent VTA. The tonic dopamine levels set the responsiveness of striatum: too little dopamine and it will be difficult to initiate movements (Parkinson’s disease) and too much can result in unintended movements (hyperkinesias).

Salient visual stimuli evoke a burst of activity in SNc mediated by direct projections from tectum/superior colliculus (374, 375, 461). Thought-provoking new experiments from Rui Costa's laboratory (106) have recently shown that before each bout of locomotion in freely moving mice, there is a burst of activity in dopamine neurons, likely due to salient stimuli in the environment. If the dopamine neurons are optogenetically inhibited, the mice will initiate movement episodes less often. It would thus seem that any salient stimulus that initiates dopamine bursts will promote a bout of locomotion to or from the stimulus.

Another role of dopamine neurons, particularly in the VTA, is related to the signaling of reward (reward prediction error) which requires a value-based decision. A motor pattern that is rewarded (by external rewards or perception of success) leads to reinforcement of this motor pattern and thus motor learning (30).

Many SNc dopamine neurons in addition to the projections to striatum also extend branches to other motor centers, including MLR (398) and tectum (374, 461), in vertebrates extending from lamprey to mammals. The SNc projections to MLR express not only dopamine but also glutamate and can when activated elicit locomotor activity. The combined effects will be to promote locomotion via both D1 and glutamate receptors in MLR. SNc neurons are activated by a variety of salient stimuli (374, 382), including direct projections from glomeruli in the medial olfactory bulb (200).

4. Basal ganglia in evolution: a conserved structure

It has recently been shown that the basal ganglia appeared early in evolution, and even the oldest now living group of vertebrates, the lamprey, has the same intricate organization as that of mammals (156, 224, 431–434). The dopamine system, the striatum with the D1R and D2R projection neurons, the peptidergic cotransmitters, the GPi/SNr, GPe, and the STN are all in place with the same membrane properties and connectivity. This means that this basic circuitry for the control of behavior evolved very early in vertebrate evolution, before the lineage leading to mammals became separate from that of lampreys, ~560 million years ago (296).
V. THE LOCOMOTOR COMMAND SYSTEMS IN MIDBRAIN (MLR) AND DIENCEPHALON (DLR)

The initiation and change of speed of locomotion leads to a simultaneous change in metabolic demands. Therefore, respiratory and cardiovascular changes need to occur in parallel. Actually, when locomotion is initiated from the command centers in mesencephalon (MLR) and diencephalon (DLR; Figure 9), there is a simultaneous activation of the respiratory centers to match the metabolic needs required during locomotion, and also an enhanced cardiac rate is produced (124). There is thus a built-in predictive control of the metabolic demands provided from the locomotor centers that will be fine-tuned later through feedback from respiratory and cardiovascular sensors during ongoing locomotion. This applies to both mammals and lamprey. Dubuc and co-workers (183) have shown that MLR activates monosynaptically respiratory CPG neurons. This respiratory control is thus proactive and initiated before any metabolic demands could have been manifested and is thus part of an overarching control system.

In addition to DLR and MLR that serve as the primary systems for initiating and maintaining locomotor drive, there is also a number of subsidiary systems (Figure 9) that also contribute, such as the modulatory 5-HT and noradrenergic systems, the adaptive cerebellar contribution via the vestibulo-, reticulo-, and rubrospinal systems (see below), the corticospinal system for precision walking (see above), and finally “stop cells” that terminate locomotor episodes.

A. DLR

The identification of different locomotor command areas represented an important step in our understanding of the control system for propulsion. Waller (467) and Grossman (238) showed in lightly anesthetized cats that a stimulation of an area near the subthalamic nucleus could elicit walking movements lasting for many minutes. Orlovsky (367) then showed in the thalamic preparation (devoid of forebrain and without anesthesia) that stimulation of the posterior hypothalamus near the subthalamic nucleus could elicit well-controlled locomotion from walk to trot and gallop. Lesions of MLR did not affect the ability to elicit locomotion, from this region. The exact location was, however, not further identified. Later studies (283, 370) have confirmed the importance of this general area referred to as zona incerta for eliciting locomotion, with both electrical stimulation and local pharmacological activation (rat). This area is now called the DLR and contains neurons known to project to the brain stem reticulospinal neurons that activate the spinal CPGs. Some neurons from this general area also project caudally towards the MLR, and it is possible that there is a parallel control exerted via MLR (see below and Ref. 344). DLR was incorrectly referred to as the subthalamic locomotor region in the first studies due to its proximity to the STN, but it actually has no direct relation to this structure. A question often raised is if DLR and MLR are used under different behavioral conditions, such as for foraging or escape. It has been suggested that DLR may be engaged in locomotion triggered primarily by emotional stimuli.

Similarly, in lamprey, the DLR (147) is located in the corresponding area, in the ventral thalamus, which contains neurons that project directly to the lower brain stem and provide monosynaptic glutamatergic excitation to the reticulospinal neurons that in turn activate the spinal CPG. The organization is most likely similar throughout vertebrate phylogeny from lamprey to mammals.
B. MLR

An important discovery was the demonstration by Shik et al. (412, 413) that stimulation in a location at the mesopontine border could induce locomotion in the decerebrate cat (MLR). This was achieved with stimulation at constant frequencies ranging between 20 and 30 Hz. With low stimulation strength, walking movements were initiated, and as the stimulation strength was increased, the cat shifted over to trot and then gallop. When the stimulation strength is increased, more neurons in MLR will be recruited. A non-patterned MLR command thus leads to locomotion, which involves hundreds of different muscles activated in an appropriate sequence, the latter coordinated by the spinal CPG circuits controlling the limbs and trunk. This was an important finding also from an experimental point of view, because it then became possible to study a complex motor behavior like locomotion in a decerebrate animal without any anesthesia. Stimulation of MLR in intact animals elicits locomotor movements that can be very intense and have an escape character. They avoid obstacles but otherwise without a goal-directed aspect.

MLR is present in vertebrates extending from lamprey to primates (81, 132, 331, 358, 397, 413, 418, 419) and is thus a conserved command structure controlling locomotion in tetrapods as well as swimmers. MLR (see Figure 9) at the mesopontine area is located close to both the cuneiform nucleus and the pedunculopontine nucleus (PPN). For many years it had remained uncertain whether one or the other or both represented the effective MLR, since the resolution of the techniques with electrical stimulation was not sufficient.

This has now been resolved by Caggiano et al. (82) and Josset et al. (274), using optogenetic techniques expressing channel rhodopsin in either the cuneiform nucleus or the glutamatergic and cholinergic component of PPN in mouse. The cuneiform nucleus is suggested to relate to escape behavior and tends to elicit fast locomotion, whereas PPN activates primarily slower forms of locomotion and could relate to, e.g., foraging and exploratory behavior. The cholinergic component of PPN did not by itself induce locomotor activity, but would facilitate spontaneous ongoing locomotor activity (390). In addition, it should be noted that PPN is subdivided into two populations of neurons, one that projects towards the brain stem level and another to the dopamine system, thalamus, and the basal ganglia (326, 375).

VI. DOWNSTREAM PROJECTIONS FROM THE LOCOMOTOR COMMAND CENTERS TO THE SPINAL CORD CPGs

A. Lamprey and Lower Vertebrates

Stimulation of DLR and MLR in lamprey leads to an activation of reticulospinal neurons in the middle (MRRN) and posterior (PPRN) rhombencephalic nuclei, which convey excitation to the spinal cord locomotor circuits (61, 147, 362). A unilateral activation of MLR provides symmetric locomotor movements, through a balanced bilateral excitation of the reticulospinal neurons on both sides in both lamprey and salamander (395). The monosynaptic excitatory postsynaptic potentials (EPSPs) evoked in reticulospinal neurons are mediated by both cholinergic (nicotinic) and glutamatergic [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA)] synaptic transmission (61). In addition, a plateau-like depolarization can be elicited in the reticulospinal neurons via the calcium-dependent ICAN current, related to the Ca^2+ entry through NMDA receptors (122), which will promote a prolonged locomotor drive. The reticulospinal neurons represent the final common path for activation of the locomotor system at the spinal level (362) and act via both NMDA and AMPA receptors, which have a synergistic effect on the locomotor network.

Whereas MRRN cells are activated efficiently during slow or moderate speeds, the neurons in the posterior rhombencephalic reticulospinal nucleus (PRRN) remain subthreshold, receiving only smaller monosynaptic EPSPs from MLR. Only at higher speeds are PRRN neurons recruited and contribute to the locomotor drive (60). Both MRRN and PRRN neurons activate premotor interneurons of the CPG and motoneurons (362).

When the lamprey MLR is stimulated, three subpopulations of reticulospinal neurons in MRRN become activated (276). Throughout the locomotor episode, “maintain cells” are active that drive the locomotor activity. Start cells display only an initial burst, and stop cells have a marked burst at the end of the locomotor episode. The role of the latter is to contribute to the termination of a swimming episode (see also below).

B. Mammals, the Interface Between the Locomotor Command Regions and the Spinal Cord: Role of the Reticulospinal, 5-HT, and Noradrenergic Systems

In mammals the effects from MLR and DLR are both mediated primarily by the glutamatergic reticulospinal system that provides relatively fast mediation to the spinal cord CPGs, demonstrated in lamprey, rodent, and cat. In addition, both the 5-HT and noradrenergic systems are involved in the control of the spinal locomotor circuitry (Figure 9). Below we will review each system (80, 360).

1. Glutamatergic reticulospinal neurons drive the spinal CPGs

In rodents and cats, MLR projects to reticulospinal neurons in the general area of nucleus reticularis magnus, which in
turn project to the spinal cord. Locomotor activity can be induced by stimulation in this region and also by local administration of cholinergic agonists (358). The functional role of the many different reticular subregions with reticulospinal neurons had remained unclear, since electrical stimulation experiments have not provided clear-cut results due to activation of mixed cellular populations and fibers of passage.

With the use of a combination of viral tracing, optogenetic, and behavioral analyses, the analytical situation has radically changed through the work of in particular Silvia Arber’s laboratory. Subpopulations of reticulospinal neurons have been linked to specific functions such as grasping and selective activation of flexors and extensors (21, 158, 379). Capelli et al. (87) now show in mice that glutamatergic neurons in the lateral paragigantocellular nucleus (LPGi), a small region in the caudal brain stem, can induce well-controlled locomotion over a wide range of speeds. LPGi receives input from MLR (FIGURES 9 AND 10). Intermingled with the glutamatergic neurons in LPGi are glycinergic projection neurons that instead contribute to the termination of a locomotor episode (see below and FIGURE 10). The reticulospinal activation of the spinal CPG networks interneurons depends on an activation of both NMDA and AMPA receptors and also metabotropic glutamate receptors.

In addition, locomotor activity can also be induced by stimulation of other structures, acting via reticulospinal neurons. Thus an activation of sensory input via the trigeminal nerve at the bulbar level can induce locomotor activity (348) and similarly stimulation within the fastigial nucleus (346). Finally, stimulation in the caudal tectum near the midline can also result in locomotor activity. In all cases, the action is most likely mediated via reticulospinal neurons.

2. Locomotor-related modulation of descending pathways resulting from feedback from the spinal cord

Once locomotion has been initiated, the spinal CPGs send efference copy information back to the brain stem via spinocerebellar and spinoreticular pathways in both lamprey and mammals. This provides phasic feedback mediated by cerebellum to the major fast-conducting vestibulo-, rubro-, and reticulospinal (from the caudal pontine and medullary reticular nucleus) pathways (see sect. XV). These pathways can respond to rapid corrections of the locomotor movements and posture (215, 216, 219, 363–365). Medullary reticulospinal neurons are phasically active during fictive locomotion and correlated to different muscle groups in fore- or hindlimbs, respectively (376). Moreover, cutaneous stimulation during ongoing locomotion enhances their discharge (129). In mammals, the effects are mediated mainly through cerebellum (26–28), whereas in the lamprey there are projections directly to fast-conducting reticulospinal neurons at the brain stem level (69, 131, 282, 459) (see sect. XV). In general, efference copy information plays an important role for the coordination of different motor systems, like eye and locomotor movements (304, 435).

The phasic activity will most likely provide a net reinforcing drive to the spinal premotor interneurons and motoneurons, but as important provide a phase-dependent gating of, e.g., steering signals that are channeled through these pathways (294).

3. Modulator action via the noradrenergic system contributes to spinal locomotor control

In the 1960s it was shown in the Lundberg laboratory (10, 11, 269, 270) that injection of l-DOPA in the acute spinal cat led to a release of long-lasting reciprocally organized reflex discharges in flexors and extensors combined with a suppression of short-latency reflexes. The effect of l-DOPA was caused by a release of noradrenaline from the terminals of the noradrenergic fibers, which originate in locus coeruleus near MLR. Some years later it was shown (227) that a subthreshold stimulation of MLR led to a release of similar late reflex discharges as after l-DOPA, combined with a suppression of the short-latency reflexes from skin and muscle afferents. Thus the MLR produced the same effects as the noradrenergic system.

Furthermore, in acute spinal cats (lower thoracic level) injected with either DOPA or noradrenergic agonists (clonidine), well-coordinated walking movements could be released (170, 205). These experiments taken together show that a release of noradrenaline at the spinal level can activate the locomotor circuits. It was later shown that noradrenaline is indeed released by stimulation of MLR (249). These effects are conveyed via the coeruleospinal pathway via α2- and β2-receptors (360). Although the noradrenergic
system thus can contribute, locomotion can still be elicited after it has been inactivated (429). The noradrenergic effects have been demonstrated in rodents and cats, and it is unknown if it also contributes in other classes of vertebrates. In the lamprey, the noradrenergic system is not developed to a significant degree, and it is not present in the spinal cord.

4. Modulator action via the 5-HT system contributes to spinal locomotor control

In mammals there are two descending 5-HT systems, one involved in pain transmission targeting the dorsal horn and another affecting the ventral horn including both interneurons and motoneurons (104) and considered important for the control of locomotion. The effects of 5-HT precursors have similar, but not identical, effects to that of DOPA, suggesting a 5-HT involvement in the control of locomotor circuits (10, 11). In rodents and cats, it has been shown that 5-HT neurons in the raphe magnus nucleus are activated, when locomotor activity is turned on from for instance MLR or spontaneously (80), and that 5-HT is released in the spinal cord (360). These effects are mediated by both 5-HT$_{1A}$ and 5-HT$_7$ receptor subtypes. In the rodent spinal cord in vitro preparation, 5-HT is usually coadministered with glutamate agonists to achieve a regular locomotor rhythm (194, 290, 488), which also applies to practically all vertebrate in vitro preparations. A detailed analysis of how the 5-HT affects the spinal locomotor CPG and how it changes over time has been described in the developing frog embryo can be found in References 100, 469.

The lamprey, as many other species, has a rich supply of intrinsic 5-HT neurons in the spinal cord as in many other species. These neurons are turned on when locomotion is initiated. 5-HT acts via both 5-HT$_{1A}$ and 5-HT$_3$ receptors (98, 408, 463). The former reduces the calcium entry during the action potential and thereby the post-spike afterhyperpolarization is dependent on $K_{Ca}$ channels and contributes to the spike frequency adaptation of both interneurons and motoneurons. This contributes to the enhanced intensity and duration of the bursts during locomotion. The effect of 5-HT is thus to modify neuronal properties and synaptic transmission in a way that promotes the stability of the locomotor activity. 5-HT also produces presynaptic inhibition of glutamatergic synaptic transmission (72, 150, 408). In the lamprey, the locomotor pattern deteriorates if 5-HT antagonists are administered (486), and conversely, it is improved by administering 5-HT (245) or potentiating its release.

C. Conclusion

The locomotor command thus includes the reticulospinal system for rapid control of the CPG activity. This system is glutamatergic and acts via ionotropic AMPA and NMDA receptors (362), as well as the slower $G$ protein-mediated metabotropic glutamate receptors (290). The modulatory 5-HT and noradrenergic pathways are both unmyelinated and therefore have low conduction velocities. Their role appears to be to fine-tune the properties of the spinal interneurons to be optimal for a stable operation of the spinal CPG through the mediation of $G$ protein-coupled receptors.

VII. TRANSITION FROM STANDING TO LOCOMOTION AND BACK

A. “Posture Follows Movements like a Shadow”

Mammals and other tetrapods can assume many different body positions from lying down to rest, to standing up observing, and also make the smooth transition from standing to locomotion, or as elegantly formulated by Sherrington (409) “posture follows movements like a shadow.” The knowledge of the neural mechanisms used to elicit standing, a very basic motor act, has remained insufficient. However, two regions in the dorsal and ventral tegmentum (347, 440) influence muscle tone. The latter increases the extensor muscle tone, the animal then extends the limbs and stands up and may subsequently start walking. Stimulation in the dorsal tegmentum instead decreases the muscle tone and makes the animal lie down in a natural way. This occurs in both intact and reduced decerebrate preparations (cat). These latter effects are mediated by activation of reticulospinal neurons in the gigantocellular reticular nucleus that inhibit both fore- and hindlimb motoneurons via segmental inhibitory premotor interneurons (441).

A prerequisite for initiation of walking is that the animal needs to first stand up. Therefore, the regulation of muscle tone with activity in extensor muscles in the limb and trunk needs to be integrated with the first steps, in which the extensor tone is replaced with flexor bursts in successive limbs to generate the alternating stepping movements. The reverse will happen in the transition from locomotion to standing. Clearly, the systems for regulation of muscle tone and for locomotion need to be coordinated and interacting. One may even ask if the system for muscle tone would represent a partial activation (extensor part) of the command system for locomotion and the extensor-related excitatory premotor interneurons.

B. Stop Cells Control the Termination of Locomotor Episodes

If one were to just terminate the locomotor drive from the brain stem suddenly, a collapse of muscle tone would occur. This does not occur, and instead, there is a smooth transition from locomotion to standing. Recent findings now
show that we have special brain stem command systems to initiate this transition.

Actually, a mouse slows down gracefully to stop and sit down when a subgroup of reticulospinal neurons (expressing Chx10) is activated optogenetically during locomotion (FIGURE 10) (54). This appears to be a separate subcomponent of the locomotor behavior that had not been identified previously, and it is mediated directly to the premotor networks of the spinal cord. There appears, however, to be yet another control system for controlling this transition. Capelli et al. (87) report that the LPGi that mediates the locomotor drive signals activated from MLR also contain intermingled glycinergic inhibitory projection neurons to the spinal cord. When the latter are activated optogenetically during locomotion, they elicit a smooth transition from locomotion to standing. Whether the two intermingled LPGi populations interact synaptically is as yet unknown.

Chronic spinal cats adapt to the speed of the treadmill, and when it is slowed down to finally stop, the rhythmic activity also stops and is replaced by continuous activity in the different limb extensors as in standing. This means that already at the spinal level there is a mechanism to handle the transition from locomotion to posture. The brain stem stop cells may possibly exert their action via the interneuronal mechanism responsible at the spinal level.

In the lamprey, a subpopulation of reticulospinal neurons in the caudal part of MRRN are activated with a burst of activity when a locomotor episode stops; therefore, they are called stop cells (196, 276). Activation of these cells leads to earlier termination of a locomotor episode, whether locomotion is elicited from MLR or by sensory stimuli. Conversely, if the activation of the stop cells is blocked, a locomotor episode is gradually terminated rather than abruptly. This area contains fast conducting reticulospinal glycinergic neurons intermingled with glutamatergic neurons. Whether these effects are mediated by the glycinergic cells from LPGi as in the mice data (87) or glutamatergic neurons similar to those of Bouvier et al. (54) is as yet unknown.

C. Control of Direction of Locomotion, Forward-Backward

Stimulation of MLR generates forward locomotion with significant propulsive thrust and the possibility to change from walk to trot and gallop. Animals from mammals to lamprey can also move backward. In the cat and other mammals, this entails a change of the phase relations between different muscle groups so that the hip moves backward while the lower limb is in the support phase (73). It is not yet known which system provides the commands for this change in coordination, but it has recently been shown that in decerebrate cats during epidural stimulation of the lumbosacral spinal cord backward locomotion can be initiated by stimulation over a limited area (caudal L5 to L7), while in the remainder forward locomotor movements were elicited (341). Also spinal animals walking on the treadmill can, if the direction of the belt is reversed, display backward locomotion.

If the lamprey is caught in a corner it will swim backward (267) with a reversed phase coupling from tail to head. The movement amplitude is larger, and it does not maintain body orientation (dorsal side up). A limited number of reticulospinal neurons are active exclusively during backward locomotion, and it would seem likely that they provide the command signal (481). The isolated lamprey spinal cord that normally is set for a rostrocaudal phase lag is able to produce a constant phase lag. The pattern can be reversed if extra excitation is added to the caudal spinal cord (right). The segments of the caudal part then have higher excitability and generate a somewhat higher burst rate and will then entrain the more rostral segmental networks. The rostral segments have a lower inherent burst rate and are therefore entrained but with a certain lag.

FIGURE 11. The lamprey swims by producing a mechanical wave that is transmitted along the body. As illustrated during forward locomotion, there is a lag between consecutive segments in the spinal cord. This lag is always a certain proportion of the cycle duration (a constant phase lag). It can be reversed into a wave that is propagated from tail to head, as during backward locomotion. In the isolated spinal cord (bottom panel), a rostrocaudal phase lag is also produced, and therefore the ability to generate a constant phase lag is inherent to the spinal cord. The pattern can be reversed if extra excitation is added to the caudal spinal cord (right). The segments of the caudal part then have higher excitability and generate a somewhat higher burst rate and will then entrain the more rostral segmental networks. The rostral segments have a lower inherent burst rate and are therefore entrained but with a certain lag.
VIII. THE SPINAL COORDINATION OF LOCOMOTION IN VERTEBRATES

A. Spinal Tetrapods Can Generate Well-Coordinated Locomotor Movements

In amphibians (198, 199) as well as birds and reptiles (417, 445), efficient locomotor movements can be generated after a spinal lesion leaving the spinal cords without contact with the rest of the central nervous system, and even forceful wing movements have been reported in spinal birds (summarized in Ref. 208).

After a spinal lesion in the lower thoracic level in a young mammal, it may display locomotor movements more or less directly after the lesion. The detailed motor pattern with regard to movement and electromyogram (EMG) corresponds to that of intact animals, and the animal is able to adapt to the treadmill speed and can actually change over from the alternating pattern of walk to the more or less simultaneous hindlimb movements as in gallop at higher treadmill speeds (171, 172, 207). Clearly, the central locomotor network is activated, but the coordination is adapted to external events through sensory input (see sect. XII).

Some of these animals could even walk over ground supported by their hindlimbs, although with a tendency to fall to one side or the other now and then.

Cats, rats, and mice spinalized as adults are as a rule paralyzed for some period, but if trained regularly on a treadmill, they will recover well-coordinated locomotor movements over time that can be maintained over years (6, 310, 445), efficient locomotor movements can be generated after a spinal lesion leaving the spinal cords without contact with the rest of the central nervous system, and even forceful wing movements have been reported in spinal birds (summarized in Ref. 208).

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Some of these animals could even walk over ground supported by their hindlimbs, although with a tendency to fall to one side or the other now and then.

The chronic spinal cat is the most well-studied species with regard to recovery of locomotor function, but the same results apply also to spinal rats and mice (6). It is very important to realize this fact, since many studies of therapy for spinal cord injury have failed to take into account the ability of the spinal cord to generate locomotor movements after training. The effects reported have in many cases not been due to the particular intervention made, often claimed to be due to regeneration, but rather to the spinal cord’s inherent ability to generate locomotor movements.

B. Plasticity of the Spinal CPG: Sequential Hemisections

The Rossignol laboratory designed an elegant way of showing that the spinal CPG itself is subject to plasticity (38, 157, 176, 193, 325). They hemisected the spinal cord at lower thoracic level, and bilateral locomotor activity rapidly recovered. Some weeks later they transected the other half of the spinal cord, and the newly hemisected side started to walk within the first day. This would take several weeks after a complete transection. To further test whether this depended on changes within the CPG or on other factors like sensory afferents activated by the movement, they immobilized a preparation subjected to the same procedure and induced locomotor activity (176). The same enhanced activity occurred on the newly transected side. The conclusion is that plastic changes of the CPG network itself had occurred.

C. Spinal Coordination of Undulatory Locomotion

Cyclostomes, fish, *Xenopus* tadpoles, and salamanders swim with undulatory locomotor movement from head to tail, generated by segmental activity generated sequentially with an intersegmental phase coupling ([FIGURE 11](#)). After a high spinal transection, locomotor movements can occur spontaneously (dogfish and eel; Refs. 45, 212, 430) or be induced by sensory input (e.g., lamprey, fish, *Xenopus* tadpole or salamander; Refs. 212, 277, 278, 396, 465). Moreover, the isolated spinal cord of these different species can be induced by pharmacological activation or stimulation of descending axons to generate the motor pattern underlying locomotor activity and thus without any phasic sensory input (223, 277, 278, 465).

The spinal cord itself can thus generate coordinated locomotor-like movements even when deprived of any supraspi-
nal control, as shown in experiments on eel and dogfish from far back in the 19th century (42, 45, 430). In these species, the excitability of the spinal cord circuitry is so high that the spinal cord generates spontaneous locomotor movements even directly after a transection. The observation that locomotor-like movements occur was the first step in the analysis, but EMG recordings were required to establish that the motor pattern is actually conserved (212). In the case of the dogfish it could be shown that the detailed EMG pattern with the undulatory wave was generated by a motor pattern with a constant phase delay in the rostrocaudal direction (from head to tail). This intersegmental phase lag remained a fixed proportion of the cycle duration, even as the frequency of swimming changed with increasing speed of locomotion. At low speed, mostly the red muscle fibers were active, but as the frequency of swimming increased, white muscle fibers were progressively recruited. Moreover, this motor pattern is also produced when the dogfish has been immobilized through curare and is thus of central origin (223). The spinal cord is thus set up to generate this rostrocaudal phase coupling at rest, but it can easily be reversed to generate backward swimming with a caudorostral propagation of the wave by, e.g., sensory stimuli applied to the rostral segments.

Similarly, after a spinal transection in the lamprey, locomotor movements can be induced in a swim-mill, and also in the isolated spinal cord (465). In the latter condition, the descending reticulospinal drive can be replaced by superfusion of the spinal cord with excitatory amino acids (222), electrical stimulation of the cut axons in the spinal cord (86), or providing additional sensory input from for instance the tailfin (64). The motor pattern is virtually identical to that of the intact swimming fish/lamprey.

**D. Spinal Cord Injury: Brief Comment**

The fact that the basic sensorimotor machinery for locomotion resides in the spinal cord (see above) has implication for spinal cord injury. If the lesion of the descending pathways controlling the locomotor circuitry is complete, there are clearly no possibilities for the subject to volitionally control the locomotor circuits, and thereby locomotion. In most cases the lesions are, however, not complete, although the subjects are unable to walk. The question then arises if one can facilitate the descending control by somehow elevating the excitability or sensitivity of the spinal circuits and thereby allow the reduced supraspinal control to get into operation.

The fact that complete spinal mammals could be made to walk on the treadmill and that brief daily training episodes lead to significant improvement of the quality and strength of the locomotor movements (37, 171, 172, 320) opened up the possibility that training of spinal cord injury pa-
tients on the treadmill could lead to an improved operation of the locomotor circuits. This led to a series of studies on human patients (39, 242–244, 470–472), and for some patients this has markedly improved their ability to walk for short distances and also the ability to stand. Since locomotor activity can be induced by agonists of for instance monoamines in cats and other mammals (35, 37, 170), the ability to promote locomotion pharmacologically has been investigated. To deliver drugs orally tends to have side effects, so intrathecal administration has been considered (36), with the idea to elevate the excitability in the spinal circuits locally so that it should be easier for the remaining axons from the brain stem to initiate locomotor activity. If the noradrenergic agonist clonidine is instead injected intrathecally in patients with spinal cord injury, it will promote locomotor activity in a proportion of the patients (385). Clonidine elicits locomotor activity also in spinal cats directly after a spinal cord transection (170).

Sensory stimulation has experimentally been shown to elevate the excitability of the CPG and induce activity (236). Intrathecal electric stimulation of afferents and particularly the dorsal columns or peripheral stimulation have been shown to promote locomotor activity or facilitate other movements (19, 88, 89, 241, 243, 330, 404, 405, 462). Currently, this approach seems to be the most promising way to elevate the excitability in the spinal cord networks of spinal cord injury patients to a degree so that the remaining brain stem fibers from LGPi can initiate the locomotor activity, particularly if combined with locomotor training. We will not comment here on the ultimate goal to promote regeneration across the spinal cord lesion with the aim for individual axons to synapse on the appropriate neuronal targets.

**IX. CENTRAL PATTERN GENERATION VERSUS SENSORY SCULPTING OF A SIMPLE RHYTHM: MAMMALIAN LOCOMOTION**

**A. Historical Account**

An issue raised towards the end of the 19th century was whether the locomotor movements were generated by central networks or entirely dependent on sensory information. There were early strong proponents of both views, and by the last part of the 20th century it became clear that there is a CPG that provides a detailed motor pattern combined with an important sensory overlay (FIGURE 12A). An initial hypothesis put forward to explain the origin of spinal stepping was the chain-reflex hypothesis whereby reflex mechanisms were considered as the underpinning of alternating activity between antagonistic muscles (197, 409, 410). If this were true, the alternating pattern of flexors and extensor muscles controlling each joint should be abolished in the...
absence of sensory feedback. Indeed, Graham Brown (65) used spinal cats in which the dorsal roots were transected and practically all nerves to the hindlimb muscles were sectioned (or a surgical removal of entire muscles) and only a flexor and an extensor muscle were left intact. When pinching the cut spinal cord with a pair of forceps, rhythmic alternating activity between a single pair of flexor and extensor muscles could be elicited under these conditions, thus in the absence of all sensory feedback. Von Holst (262, 263) showed later that movements could persist in different fish and other vertebrates after dorsal root transection.

The findings of Brown (65) from the cat led to the formulation of the half-center hypothesis, which implies that there are two half-centers, one controlling flexor muscles and the other extensor muscles with reciprocal inhibition between the two, and that the reciprocal connection somehow, through an undefined mechanism (fatigue), should lead to alternating activity. However, this pioneering work and the resulting half-center hypothesis did not provide information on the neuronal underpinning or on whether the central programs alone were able to produce the complex pattern of activity of the different muscles underlying stepping.

A further complication is that the pattern of muscle activation during the step cycle of the hindlimbs in both mammals and in birds consists of four phases rather than two distinct flexor and extensor phases (90, 126, 153). The reciprocally activated main flexor and extensor muscles during swing and stance represent the two main phases of the step cycle. In addition, there is the lift up phase at the end of the stance phase, engaging double joint muscles such as semitendinosus, and the touch down at the termination of the swing phase, when the foot makes contact with ground, during which there is a transient activity in several muscles, including the short dorsiflexors of the foot (151–153, 391). The EMG activity of muscles active during lift up and touch down occurs partially in between the activity of the main flexors and extensors, respectively.

This complex pattern of activation of the different flexor and extensor muscles could not be captured by the original half-center hypothesis in which all flexors would be active simultaneously in alternation with all extensors. To accommodate the half-center hypothesis with the complex pattern of muscle activity during locomotion, it was suggested that proprioceptive afferent feedback would be responsible for sculpting a simple flexor-extensor alternating activity into a more complex locomotor pattern (321). The central element of the adapted half-center hypothesis is that there would be a simple central program producing flexors and extensors alternation and that the differential activation pattern of different muscle pools underlying stepping is the consequence of proprioceptive reflexes that give the gait its required characteristics (321). This hybrid half-center hypothesis was supported by the fact that administration of l-DOPA in spinal cats induced long-latency and long-lasting flexor bursts and contralateral extensor bursts (269, 270) thought to represent Brown’s half-centers (65). Furthermore, stimulation of high-threshold muscle and cutaneous afferents was shown to induce short bouts of alternating flexor and extensor activity (205, 269, 270). However,
this, at the time, reasonable view of how locomotor activity could be generated was contradicted by experiments.

B. The Complex Motor Pattern Retained After Deafferentation

Whether this hypothesis would hold was tested by determining if removal of sensory feedback could switch the complex pattern of muscle activity into a simple alternating program between all extensors and flexors (234, 235). After both deafferentation (section of all dorsal roots; FIGURE 12B) and in curarized animals (no movement-related sensory feedback), the complex pattern of activation of different motoneuron pools persisted (134, 234, 235). These data provided a firm proof that proprioceptive reflexes are not a prerequisite for the generation of the complex locomotor program. Thus central neural networks (locomotor CPGs) alone can generate the delicate timing pattern that will sequentially start and terminate the activity of individual muscles at the correct phase of the step cycle (210). However, the motor pattern can at times become more variable in the absence of sensory feedback.

The conclusion that CPGs are sufficient to produce the complex pattern of muscle activity should not be mistaken to mean that sensory feedback does not play a role during locomotion. On the contrary, sensory information is essential for adapting the ongoing activity by changing the intensity and duration of muscle activity in different phases of the locomotor cycle (see sect. XII and Refs. 138, 225, 329, 400).

C. Alternating Activity in Swimming: Is There a Half-Center?

Whereas the limb motor control is complex and cannot be generated by a half-center, true alternation occurs for instance between the left and right side in a segment during swimming. The half-center hypothesis provided a conceptual framework for the analysis of the organization of the locomotor CPGs, but there were important constraints regarding the basic architecture of these networks. Namely, each half-center by itself can only produce tonic activity, and the rhythmic and alternating pattern requires reciprocal inhibitory connections between the two half-centers. Are these criteria fulfilled? A blockade of reciprocal inhibitory synaptic transmission between the left and right side did not switch the rhythmic alternating pattern recorded in ventral roots on opposite sides into tonic activity (84–86, 99); instead, rhythmic activity continues on both sides, and it can also be generated when the spinal cord has been subdivided into a left and right half. Moreover, an inhibitory blockade can generate simultaneous bursting on the left and right (99). In both lamprey and the Xenopus tadpole, rhythmic activity can be generated in a completely isolated hemi-segment of the spinal cord, showing that each hemi-segment contains the necessary elements to generate recurring burst activity (84, 86, 278). The half-center hypothesis in its classical form must thus be rejected. Another matter is that two burst-generating circuits can be coordinated through reciprocal inhibition.

D. Distribution of the Ability to Generate Rhythmic Activity Along the Spinal Cord in Mammals

Both in the cat and rodent spinal cord, rhythmic activity can be produced if different parts of the lumbosacral spinal cord are separated from each other. There is, however, a tendency that the segments controlling the proximal muscles (hip) provide a more stable rhythmic activity (84, 86, 93, 236, 240, 278, 393). In mice it was shown with optogenetic techniques activating glutamatergic interneurons that any select part of the lumbosacral spinal cord could elicit rhythmic bursting, whether unilateral or bilateral (240). This clearly shows that this ability is distributed within the entire spinal cord. Similarly, the 100-segment-long lamprey spinal cord can be subdivided into pieces down to a segment and still be able to elicit rhythmic bursting (86).

E. Concepts Regarding the Overall Organization of the Limb Control in Mammals

The two hindlimbs can generate their intrinsic motor coordination independently, because they can alternate as in trot or walk or be almost simultaneous as in gallop or bound. Each limb can thus generate its own motor pattern. The previous section shows in addition that in each part of the cord there is the ability to generate rhythmic burst activity, and thus that the capacity to generate rhythmic activity is distributed. It would thus suggest that the spinal cord is composed of a set of circuits, each of which can generate burst activity that are coordinated through inhibitory or excitatory interaction. If such unit burst generators (202, 210) controlled groups of close synergists motor nuclei at hip, knee, and ankle, the interaction between them could account for the overall pattern of coordination (FIGURE 13), allowing for alternation between some groups and the additional four phase motor pattern. This organization would allow for the flexibility of the motor pattern, which is required as we can walk not only forwards, backwards, and sideways but even on our knees. The network of each limb needs thus to be very flexible, if there should not be a separate CPG for each pattern of coordination (FIGURE 13). A flexible network design of this type has also been demonstrated in the stick insect (77, 78).

An alternative concept favored by some research groups is that the spinal cord would contain only one rhythm...
generating circuit (see below) that would set the rhythm for one limb, and then a set of downstream interneurons would activate interneurons that in turn would activate a certain set of motoneurons and inhibit others (see Ref. 411). Modifications of the motor pattern would have to redistribute the downstream control to the different groups of motoneurons. Rybak and colleagues (32, 33, 110) have extended the model to include the spinal coordination of the different gaits (walk, trot, and gallop) between forelimbs and hindlimbs, and also the supraspinal drive from the different brain stem command centers.

X. THE INTRINSIC FUNCTION OF CPGs: SPINAL INTERNEURONS CONTROLLING LOCOMOTION

A detailed locomotor activity is generated by spinal circuits that are endowed with the ability to transform non-patterned commands from locomotor centers in the brain into well-coordinated rhythmicity. The pattern of activity generated by these circuits ensures a precise coordination of muscles controlling movements on the two sides of the body and around different joints. They also ensure the control of the speed and strength of locomotor movements. Much of the insights into neuronal and circuit processes by which the spinal networks control the different aspects of locomotor movements has been gained using accessible model systems, initially Xenopus tadpoles and lamprey and more recently zebrafish. In parallel, key molecular and genetic principles specifying the neuronal types and their assembly during development have been elucidated in the mouse together with the role of some molecularly defined interneurons.

A. CPG for Rhythm and Coordination?

In its most stereotyped form, locomotion consists of a single repeating sequence of alternating movements. The question is how these two basic aspects of locomotion, namely, the rhythmicity and coordination, are produced. Results in lamprey and Xenopus in which the detailed cellular and synaptic properties of the locomotor circuits have been characterized indicate that these two aspects are intimately linked. The locomotor rhythm and pattern are an emerging property of the circuit construction and connectivity between identified component neurons. In this sense, the networks consist of interconnected ipsilateral glutamatergic interneurons and commissural inhibitory interneurons. The ipsilateral glutamatergic interneurons form a recurrent circuit that drives the locomotor rhythm and provides direct excitation to motoneurons. The alternation of activity between the two sides of the body is mediated by inhibitory commissural interneurons. The combined interaction be-
between on-cycle excitation and mid-cycle inhibition sets the rhythm and pattern of locomotor movements.

The impenetrability of rodent and cat spinal cord to detailed construction of circuit connectivity in a similar detail as in *Xenopus* and lamprey led to the formulation of circuit organization based on changes in the activity recorded extracellularly and in some cases from single neurons combined with their responses to sensory inputs. Rather than having an inclusive circuit controlling the rhythmicity and coordination, it has been suggested that there are two separate circuits hierarchically organized. One generates the rhythm and the other controls the coordination (pattern).

This dual organization of the locomotor CPG initially proposed for the cat spinal cord and embraced in mouse rests on the assumption that rhythm-generating interneurons (circuit) are several synapses upstream of motoneurons, while those responsible for pattern formation make direct synaptic contacts with motoneurons. This organization has been suggested as an alternative to explain the modulation of afferent inputs (76, 287, 295, 301), change in motor burst amplitude, and omission of motor burst referred to as deletions. However, most available experimental results do not support this dual organization, and alternative CPG organization such as the unit burst generators combined with intrinsic plasticity of synaptic transmission can account for all these experimental observations. Indeed, the rhythm and pattern formation are intimately linked and there are no simple perturbations that affected the coordination without affecting the rhythm. Furthermore, in model systems (lamprey, tadpole, zebrafish) accessible for precise reconstruction of the locomotor CPG connectivity it has been demonstrated that excitatory interneurons generating the rhythm also make monosynaptic connections with motoneurons. And recent evidence in zebrafish and mouse show that motoneurons themselves can profoundly influence the function of the locomotor CPG making them an integral part of the circuit generating locomotion. This is not to say that the roles of the different excitatory and inhibitory interneurons are interchangeable, rather they are controlling different moments of the locomotor cycle as they are active sequentially to ensure burst initiation, termination and left-right coordination.

### B. Identification of the Interneurons Generating Locomotion

In all vertebrates, the different classes of neurons develop according to a conserved plan controlled by the concentration gradient of morphogens from the floor and roof plates (58, 95, 154, 155, 317, 450, 477). The combination of the two gradients results in the differentiation in 11 progenitor domains, 6 in the dorsal part of the spinal cord (dI1-dI6) and 5 in the ventral part (one giving rise to the motoneurons and four domains, termed V0-V3) (FIGURE 14A). These cardinal neuronal classes express specific transcription factors and account for the different types of CPG excitatory and inhibitory interneurons involved in the generation of locomotion and the sensory processing from afferent feedbacks to the locomotor CPG (58, 155, 187, 194, 195, 237, 251, 271, 288, 307, 311, 316, 318, 402). It is becoming clear that the transcriptional code in the developing spinal cord is conserved across the vertebrate lineage.

**FIGURE 14.** Schematic representation of the precursor groups in the developing vertebrate spinal cord and the lamprey and mouse central pattern generator (CPG). **A:** the spinal cord is subdivided into six dorsal and five ventral progenitor domains (pd) giving rise to different interneuron populations and motoneurons. To the right are indicated the different classes of neurons referred to in the text in the context of locomotion. The color code is blue for inhibitory and red for excitatory interneurons and green for motoneurons. **B:** the lamprey CPG with the same color code. **C:** the mouse CPG.
Local excitatory interneurons in the spinal cord are the initial point for the transformation of commands from the brain into a patterned locomotor rhythm. These neurons are glutamatergic and are confined to each half of the spinal cord. They were initially identified in *Xenopus* tadpoles and lamprey; they have ipsilateral axonal projections and use glutamate as the main transmitter to activate different interneurons and motoneurons via NMDA and AMPA receptors. These interneurons share many similarities with V2a interneurons in zebrafish and mouse that were initially defined by the expression of the transcription factor Chx10 (102, 144, 271, 288, 489) as well as interneurons expressing the transcription factor Shox2 studied in mouse and also present in zebrafish (127) (FIGURE 14) (see also sect. X, C and D).

1. Lamprey and Xenopus, where it all began

The lamprey and *Xenopus* are the two model systems that have so far provided the most comprehensible characterization of the organization of the spinal locomotor CPG (68, 70, 72, 107–109, 201, 209, 314, 388, 415, 416) (FIGURE 14B). Locomotion in these animals consists of a caudally directed series of left-right muscle contractions underlying a forward propulsion. The swimming movements are generated by segmentally repeated CPGs, each capable of generating the segment’s coordinated rhythmic motor pattern (84, 86, 423). Because of the lack of molecular markers, these original studies used pain-taking blind dual recordings to map the functional connectivity of the locomotor network and provided a framework of the basic organization of the locomotor network and how it generates the propagating wave of swimming movements, its left-right alternation, as well as rostrocaudal coordination.

2. Rhythm generation

A key question that these two classical model systems addressed is whether the rhythmicity depends on the whole network connectivity or if it is produced by local excitatory circuits independent of reciprocal inhibition. It was shown that even a single hemi-segment is able to produce rhythmic activity due to the local recurrent excitatory connections between interneurons generating the rhythm (86, 201, 209, 314, 388). These interneurons are confined to one side of the spinal cord and form an excitatory circuit mutually activating each other; in addition, they also drive the activity of motoneurons and commissural interneurons ensuring the left-right pattern. Studies in lamprey and *Xenopus* established that the basic rhythm is mediated by local excitatory interneurons (70, 72, 108, 109). In addition, these studies not only identified the excitatory interneurons driving locomotion, but also provided their detailed connectivity with other components of the locomotor CPG and hence a circuit framework of how locomotion is generated in vertebrates (70). However, the molecular identity of these excitatory interneurons has not been determined, although they bear similarities with molecularly identified interneurons in mouse and zebrafish (see below).

Locomotor activity is turned on by brain stem reticulospinal neurons (RS in FIGURE 15), and they activate populations of excitatory interneurons (E in FIGURE 15) in the spinal cord through NMDA and AMPA receptors. This makes the excitatory E-neurons fire, but how come they turn into burst mode? The explanation is due to a contribution of membrane properties. When E-neurons are activated through voltage-dependent NMDA channels, which have longer lasting EPSCs than AMPA receptors, Ca\(^{2+}\) ions start to accumulate inside the E-neurons, and the action potentials will activate Ca\(^{2+}\) channels, further adding to the Ca\(^{2+}\) load. The Ca\(^{2+}\) will in turn activate calcium-dependent K\(^{+}\) channels, which will cause a hyperpolarization helping to terminate the burst (34, 149, 232, 293). At high levels of activity the sodium level increases inside the cell, leading to an activation of sodium-activated K\(^{+}\) channels, which further contributes to the hyperpolarization (464). In conclusion, the burst activity of segmental E-neurons is due to the supraspinal drive, their connectivity, and intrinsic membrane properties.

3. Coordination of swimming at the segmental level

The first evidence for the role of reciprocal inhibition in coordinating the left-right alternation comes from experiments in lamprey spinal cord. Pharmacological blockade of glycinergic receptors switched the left-right alternation into synchronous rhythmic bursting in ventral roots on both sides of the spinal cord (99, 231). In addition to the demonstration of the necessity of inhibition for alternation, these experiments also revealed the existence of commissural interneurons ensuring the synchronicity of the rhythmic activity. The inhibitory commissural interneurons were identified using dual recordings both in lamprey and *Xenopus* (68, 422). These inhibitory I-neurons (FIGURE 15) receive direct excitation from rhythm-generating interneurons and mediate inhibition of motoneuron and other interneurons on the contralateral half of the spinal cord.

4. Undulatory locomotion through intersegmental coordination

As noted above (sect. VIII and FIGURE 11), undulatory locomotion is characterized by constant intersegmental phase coupling along the spinal cord in cyclostomes fish and salamanders. This is a property of the spinal cord itself, since the isolated cord can generate this pattern. As studied in the
lamprey, both the E- and I-interneurons are not strictly segmental, rather E-neurons extend around 7 segments caudally and a few segments rostrally, and the crossed I-neurons can extend their axons over 20 segments. There is thus a concatenated circuit along the spinal cord rather than a strict segmental organization.

To analyze if the available data regarding E- and I-neurons, their connectivity, and specific membrane properties could account for the intersegmental coordination, large-scale simulations were performed with an approximately correct number of model cells in the spinal network and also reticulospinal neurons (218, 293). With 10,000 spinal simulated neurons (100 segments) and a detailed compartmental modeling for each neuron, the network could generate a rostrocaudal phase lag along the simulated spinal cord (FIGURE 16, A–C). Noteworthy is that a phase lag can be created also by a hemicord intersegmental chain composed by only E-neurons (292).

Moreover, by changing the excitability in a few most rostral segments, the phase lag along the entire spinal cord could be changed. If the excitability was enhanced, the phase lag increased gradually. Conversely, if the excitability of these same rostral segments were progressively reduced, the phase lag was reduced and could be reversed as during backward locomotion. It is thus sufficient to tinker with the excitability of a small part of the network to change the
performance of the entire network, which could be regulated by different brain stem systems.

5. Fine-tuning of the locomotor network through metabotropic receptors

The locomotor network operates dynamically through ionotropic glutamatergic receptors (NMDA, AMPA) and glycinergic receptors. This core network is, however, embedded in a number of modulatory systems operating at a slower time scale. The metabotropic, G protein-coupled receptors operate primarily through phosphorylation of a variety of targets such as ion channels. They have longer latency for activation and are thus not optimal for a phasic cycle-to-cycle operation but instead set the background level of activity in different target molecules. **FIGURE 17** summarizes the effects of different modulators known to be endogenously released during locomotion (lamprey). 5-HT is paracrinically released from 5-HT neurons onto the dendrites of network interneurons, and it is a modulator that uniformly promotes a stable locomotor activity in vertebrates. 5-HT acts on several levels including the afterhyperpolarization (sAHP; via Ca\(^{2+}\) channels) and spike-frequency adaptation, presynaptic inhibition, and postinhibitory rebound (47, 352, 408, 463). Metabotropic glutamate receptors are of several types that activate either pre- or
postsynaptic sites in the locomotor network. mGluR$_1$ potentiates the NMDA receptor transmission resulting in a burst frequency increase acting partially through endocannabinoids (285). GABA$_B$ receptor activation in contrast lowers the burst frequency through their effects on the sAHP, postinhibitory rebound, and presynaptic inhibition of network interneurons. Dopamine, tachykinins, endocannabinoids, and nitric oxide (145, 298, 300, 373, 424, 447, 449) also contribute as indicated in FIGURE 17. There is thus an intricate balance between the different modulator systems that serves to promote a stable operation of the locomotor network throughout its operation from a slow swimming to fast escape.

C. Zebrafish: Modular Circuit Organization of the CPG

The accessibility of zebrafish from larval stages to adult allows for a detailed analysis of how circuits are organized and reconfigured during development to control swimming at different speeds and in different behavioral context. At larval stages the recruitment of motoneurons and interneurons obeys a topographic organization and is dictated by the difference in input resistance of these neurons (333). During maturation towards adult stages, the mechanisms governing the recruitment of motoneurons and interneurons instead mature, become more sophisticated, and rely on a combined computation of synaptic input and biophysical properties, which makes up a higher level of integration than the input resistance rule. The zebrafish larvae swim at frequencies above 20 Hz using mostly fast muscles, while adults swim at much lower frequencies (<1 Hz and never exceed 21 Hz) (8, 299). The changes in the mechanisms of recruitment of neurons and the decrease in swimming speed are associated with the transformation of muscle composition, which switches from mostly a single fast layer in larvae to a three-layer organization with slow, intermediate, and fast muscles (8, 178). Three pools of motoneurons innervating selectively each of the three muscle layers occupy specific positions in the spinal cord and display distinguishable intrinsic properties (8, 178). Zebrafish, like other organisms, undergo changes during development in the organization in the neuromuscular apparatus to accommodate an increase in their behavioral repertoire and social behaviors (473).

1. Rhythm generation

In zebrafish, the rhythm generation is mediated by V2a interneurons defined by the expression of the transcription factor Chx10 (288). These interneurons are necessary and sufficient for the generation of locomotion (144, 319). Acute and local two-photon ablation of the spinal V2a interneurons showed that they are necessary for the generation of rhythmic locomotor movements. All features associated with the generation of the locomotor rhythm were dramatically affected; the transformation of command signals into a swimming pattern was impaired, and the swimming across all the frequency range was dramatically reduced. Even the ablation of a proportion of these interneurons suppressed the generation of swimming activity induced by stimulation of descending commands from the brain. Very high stimulation intensities (>6 times those used in control animals) were required to produce a very brief swimming bout with much lower burst frequency. In this case, the whole frequency span shifted towards lower ranges. In addition, selective ablation of V2a interneurons involved in fast swimming suppressed the highest swimming frequencies, without affecting the slower ones (144). These findings were further corroborated using genetic silencing of synaptic transmission from V2a interneurons which resulted in impairment of the swimming activity at all frequencies (476). Conversely, channelrhodopsin was selectively expressed in V2a interneurons, and optogenetic activation of these interneurons produced a well-coordinated
locomotor rhythm confirming that they are sufficient for the generation of locomotion. Optogenetic activation of V2a interneurons mostly elicited locomotor activity of slow/intermediate frequency because the interneurons involved in fast locomotion have a high activation threshold and could not be recruited optogenetically (319). The frequency and the duration of the swimming activity were correlated with the duration and the intensity of the optogenetic stimulation, which was mediated by increased activity in V2a interneurons.

2. Modular organization

Aside from generation and coordination of locomotor movements, the spinal CPG needs to accommodate the smooth transition between different locomotor speeds to adapt to environmental requirements. The spinal CPG needs to be endowed with an optimal construction to enable transformation of the excitatory drive from the brain into a gradual recruitment of motor units to produce appropriate speed of movements. An understanding of the circuit computation for speed control requires a true mapping of the function circuits. The pertinent question is how the locomotor CPG is organized to convey the excitation to the different motoneurons to enable their sequential recruitment to control the speed of locomotion. Until recently, the conceptual consensus has been that the locomotor network even in its most modular form, such as the unit burst generator, consists of a single network, and the increase in the speed is merely a result of an overall increase in the excitability of the whole CPG network. In adult zebrafish, the rhythm-generating V2a interneuron population is functionally heterogeneous and is instead composed of three subtypes defined by their order of activation as a function of the swimming speed (slow, intermediate, or fast V2a interneurons) (9, 31). This organization mirrors that described in motoneurons innervating slow, intermediate, and fast muscle fibers (8, 178). A detailed connectivity analysis between V2a interneurons and motoneurons in adult zebrafish has revealed that V2a interneuron and motoneuron connectivity is precisely structured to form three speed modules (FIGURE 18). The strength and probability of connections within modules are much higher within modules compared with between modules (9, 426). The three speed modules are engaged in a sequence from slow to intermediate and then to fast to increase the swimming speed. This organization with three speed modules permits a more flexible control of the speed of swimming. Each module can be recruited either separately as during slow swimming, or fast swimming following escape, or it can be combined sequentially with other modules to increase the locomotor speed. Thus the circuit-level analysis in adult zebrafish has shown a more sophisticated circuit construction that accommodates a more precise control of the speed of locomotion. It also shows that the locomotor CPG does not consist of the single network, but it is modularly organized in a manner that reflects the organization of the motor apparatus. It is likely that a similar organization will also be found in mammals.

3. Neuronal diversity and task-specific circuit architecture

Another key question that was addressed using dual recordings in adult zebrafish is whether the three circuit modules represent reiterations of a canonical circuit obeying the same connectivity rules and driven by V2a interneurons with homogeneous properties. The results show that each circuit module is selectively tailored to ensure the primacy with other modules to increase the locomotor speed. Thus the circuit-level analysis in adult zebrafish has shown a more precise control of the speed of locomotion. It also shows that the locomotor CPG does not consist of the single network, but it is modularly organized in a manner that reflects the organization of the motor apparatus. It is likely that a similar organization will also be found in mammals.

FIGURE 18. The modular zebrafish central pattern generator (CPG). As described in the text, the excitatory V2a interneurons are further subdivided into three modules, each controlling fast (blue), intermediate (green), and slow (red) motoneurons and muscle fibers. Each module consists of excitatory interneurons, some of which also have gap junctions (as indicated by the resistor in the drawing). The gap junctions play a new role in providing feedback between motoneurons and interneurons, thereby amplifying the chemical synaptic transmission when depolarized (see text). To the left is indicated that at slow speeds the slow (red) module is recruited, and with increasing speed the intermediate (green) and the fast (blue), respectively.
tral and caudal directions. This non-bursting type makes synapses on the somata of fast motoneurons where its produces weaker excitatory drive without any NMDA-dependent short-term plasticity. These results have provided novel insights into how the interplay between the V2a interneuron diversity, and their properties, which are tuned according to the modular identity and the locomotor speed they control. The slow speed module generating less vigorous locomotor movements involves mainly bursting-type V2a interneurons that are sufficient to drive firing in slow motoneurons. This module is endowed with an amplification in the form of NMDA-dependent short-term potentiation that scales down the number of V2a interneurons required to recruit slow motoneurons. On the other hand, the fast module encoding more vigorous movements relies on the convergence of a large number of non-bursting-type V2a interneurons to drive the fast motoneurons to fire. This module would require more synchronized synaptic inputs from many non-bursting V2a interneurons. This fast module is reserved only for salient stimuli such as escaping a predatory attack. This is a clear example of how the interneuron functional diversity endows the locomotor CPG with module-specific organization that enables a more flexible computation that meets the behavioral needs.

4. Interneurons involved in coordination

In zebrafish, a detailed morphological and physiological characterization of V0 interneurons is beginning to reveal a large degree of diversity. The distinction between V0v and V0d does not account for the full diversity of these commissural interneurons. At larval stages, the V0v interneurons could be differentiated into three subclasses based on their temporal order of development and their axonal projections. The ascending ones arise first, followed by the bifurcating, and lastly, the descending V0v interneurons (402). While there are many similarities between zebrafish and mice in terms of the V0 interneuron developmental mechanisms, there are also some differences. Ablation of V0v interneurons in immature mice produced changes in the locomotor pattern that are consistent with a role in maintaining left-right coordination at relatively fast speeds. In contrast, in larval zebrafish, a subtype of V0v interneurons MCoDs have been shown to be active only during slow swimming (164, 333, 335). In adult zebrafish, there is an additional layer of diversity of V0v interneurons. There is a larger degree of functional diversity of their pattern of recruitment during swimming with some being active at slow speed, while others become recruited only at intermediate or fast speeds (48). A large proportion of V0v interneurons are of the fast category, suggesting that the V0v interneurons in adult zebrafish are predominantly involved in coordinating swimming at faster speeds. It seems that V0v interneurons undergo developmental changes in the zebrafish. The prevalence of fast V0v interneurons and the absence of interneurons with morphology resembling the MCoDs described in larval zebrafish are evidence for such development changes. In the larvae, the MCoDs are responsible for driving slow-frequency locomotion (335), whereas in the adult zebrafish this task appears to be carried out largely by slow V2a interneurons (31).

5. Motoneurons are bona fide members of the CPG: evidence from zebrafish

The motoneurons represent the final pathway from the spinal CPG that allows the execution and the scaling of the speed and vigor of locomotor movements by coordinating the sequential activation of muscles around many joints. A well-established route through which motoneuron influence interneurons in the spinal cord is via their collateral activation of Renshaw cells (305, 306, 340, 356). However, this circuit has been mainly implicated in limiting motoneuron excitation without any direct influence on the locomotor CPG (359). One of the long-held concepts that has been traditionally accepted in vertebrates is that motoneurons serve largely as passive recipients of motor programs elaborated by CPG interneurons, and they themselves do not have any influence on the generation of locomotion. Recent evidence in zebrafish and mouse has invalidated this prevailing view. It is becoming clear that motoneurons play a more prominent role within the locomotor CPG, and therefore they should be considered as bone fide components of the locomotor CPG.

In zebrafish, the rhythm-generating V2a interneurons make mixed electrical and chemical synapses with motoneurons. While the chemical synapses with their short-term plasticity scales the excitatory drive necessary for the sequential recruitment of motoneurons to control the vigor and speed of movements (9, 426), the electrical synapses enable a retrograde influence from motoneurons back to the V2a interneurons (425) (FIGURE 18). These electrical synapses allow a propagation of any change in the membrane potential of motoneurons in an analog fashion that enables them to influence the firing and synaptic transmission from the V2a interneurons. In V2a-motoneuron pairs connected with mixed synapses, a tonic depolarization is transferred from the motoneuron dendrites to the V2a interneuron presynaptic terminals to locally enhance transmitter release. This depolarization is also able to propagate backward to the soma of V2a interneurons to change their firing. Conversely, tonic hyperpolarization of the motoneuron decreased the firing frequency of the V2a interneurons and their synaptic strength. Indeed, during locomotion, the membrane potential of motoneurons is continuously fluctuating due to in-phase excitation and mid-cycle inhibition. The gradual depolarization of the membrane potential of motoneurons during the in-phase excitation is propagated online retrogradely to the excitatory (V2a) CPG interneurons to enhance their transmitter release, decrease their firing threshold, and increase their firing frequency. In contrast, as the membrane potential of motoneurons begins repolarizing during mid-cycle inhibition, the synaptic drive
and firing of the excitatory CPG interneurons become depressed. These results thus reveal a novel route of influence of motoneurons onto the locomotor CPG via gap junctions.

In newborn rodent, antidiromic stimulation of motoneurons via ventral roots can induce rhythmic locomotor-like activity (340) and entrain the spontaneous rhythmic activity induced (51). In neonate mouse, optogenetic manipulation of the activity of motoneurons during ongoing drug-induced locomotor activity also altered the frequency, phase, and stability of the rhythm (159). Similar to zebrafish, when motoneurons were inhibited or activated optogenetically, there was a strong influence on the frequency of the locomotor rhythm. In mouse, these effects occur independent of gap junctions and seem to be mediated via activation of iGluRs. These results suggest that newborn rodent motoneurons are also endowed with ability to influence the locomotor CPG (159).

Previous evidence has highlighted the potential of motoneurons to provide a positive feedback to other motoneurons and the locomotor CPG (44, 323). For example, it has been shown in lamprey that retrograde signaling via endocannabinoids endows motoneurons with the capacity to influence the strength of synaptic input they receive from CPG interneurons and hence influence the locomotor activity (146, 285, 298, 427, 448). Several converging lines of evidence are now providing support for a strong direct role of motoneurons in the elaboration of the locomotor rhythm; motoneurons should be considered integral members of the locomotor CPG as has already been shown in invertebrates.

D. Mouse Circuit Organization: Towards Understanding Circuits for Limbed Locomotion

The accessibility of lamprey, _Xenopus_, and zebrafish afforded a true characterization of the circuit connectivity using dual recordings from the constituent neurons. These model systems use mostly undulatory locomotion. The analysis of circuit controlling limb locomotion requires access to mouse models, which is amenable to many of the new tools but in which the detailed circuit connectivity is far from being easy.

1. Rhythm generation

Like in lamprey, _Xenopus_, and zebrafish, also in the mouse the generation of locomotion relies on excitatory interneurons. The core CPG for hindlimbs is distributed in the lumbar segments with more prominent rhythmogenic abilities in the rostral two segments compared with more caudal ones (93, 290), and the excitatory interneurons use the vesicular glutamate transporter 2 (vGluT2), although rhythmic coordinated activity can still be induced pharmacologically after deletion of this transporter (444, 466). Furthermore, optogenetic activation of neurons expressing vGluT2 at the level of the spinal cord or close to specific motor pools produces rhythmic activity (240). The availability of molecular markers for different types of interneurons in the mouse has enabled genetic ablation/silencing of specific populations of excitatory interneuron to probe their contribution to the generation of the locomotor rhythm. While this approach has been applied to many molecularly identified interneuron populations, it has not yet yielded conclusive results linking a specific excitatory interneuron subtype to the generation of the locomotor rhythm in the mouse (102, 127, 444, 466, 489). For example, elimination of V2a interneurons (actually all neurons expressing Chx10 in the CNS) in mice early during development impaired the generation of locomotor-like activity by stimulation of descending inputs from the brain and by stimulation of dorsal root. However, it did not prevent the generation of pharmacologically induced rhythmicity in vitro, but it mostly altered the left-right coordination at intermediate to high frequencies (102, 489). In mice, like in zebrafish (426), V2a interneurons have been suggested to consist of two types based on the expression level of the transcription factor Chx10, which shows postnatal rostro-caudal expression differences (247). Type I V2a maintains Chx10 expression and is present at both lumbar and cervical level, while type II V2a interneurons have a low Chx10 expression and are preferentially restricted to cervical segments.

The uncertain results about the role of V2a interneurons led to a shift to other interneuron subtypes such as those expressing Shox2 (127) (FIGURE 14A). The expression of this transcription factor largely overlaps with that of Chx10, which is expressed in V2a interneurons. But a small proportion of interneurons seem to express Shox2 exclusively. These interneurons are electrically coupled to each other (239). Perturbation of synaptic transmission in all Shox2-expressing interneurons appears to affect the upper frequency range of the locomotor rhythm, while when this perturbation was limited to Shox2-expressing V2a interneurons, little effect was seen on the rhythm frequency. It was suggested, by deduction, that Shox2-non-V2a interneurons may contribute to the generation of the locomotor rhythm. However, the apparent effect seen when synaptic transmission of all Shox2 versus only Shox2-V2a interneurons could also be because more interneurons were affected.

Another class, however, of ipsilateral excitatory interneurons that could contribute to the generation of locomotion in the mouse are those expressing the transcription factor Hb9. Anatomical analysis suggests that they are premotor interneurons receiving inputs from sensory afferents and having axonal projections terminating directly onto motoneurons (83, 259, 297, 474, 490). These interneurons also display pacemaker properties mediated by activation of persistent sodium currents (61, 62, 446, 490) that enable...
them to generate rhythmicity in the absence of fast excitatory and inhibitory synaptic transmission (12, 327). While these Hb9 interneurons were rhythmically active during drug-induced locomotor-like activity in vitro, the onset of their activity occurred after that of motoneurons, which prompted uncertainty whether these interneurons could be driving locomotor activity (297). However, selective deletion of vGlut2 from Hb9 interneurons, which is supposed to block synaptic transmission from these interneurons, decreased the frequency of drug-induced locomotor-like rhythm compared with control animals (83).

Thus, in mice, it is as yet not clear how the locomotor rhythm is generated and which interneurons are involved. The strategy that has been used is the genetic ablation or blockade of synaptic transmission of several excitatory interneurons on drug-induced locomotor-like rhythm in neonate spinal cord in vitro. For example, the little impact of ablation of V2a interneurons on drug-induced locomotion versus the profound impairment of descending and sensory input-induced locomotor rhythm was considered to imply that this class of interneurons is not essential for rhythm generation. However, the genetic ablation or synaptic silencing of a whole interneuron population at an early stage of the locomotor circuit development could activate compensatory mechanisms to overcome the decrease in intrinsic excitability in the spinal cord. The role of these different interneurons, including V2a interneurons, needs to be assessed using acute interference with their activity in the spinal cord to reassess their role in the generation and coordination of locomotor movements in mice. Such studies will allow the discrepancy in the effect of stimulation of sensory and descending inputs (natural route for induction of locomotion) versus drug-induced locomotor-like activity in neonate in vitro to be addressed.

2. Flexor-extensor coordination

The coordination around single joints and across multiple joints requires mechanisms that precisely control the activity of flexor and extensor motoneurons. Ia interneurons, first described within the Ia afferent reflex circuit, ensure reciprocal inhibition between flexor-extensor motoneurons. These interneurons are rhythmically active during locomotion and scratching (116, 162, 185, 381) The two molecularly defined interneurons providing ipsilateral inhibition are of the V1 and V2b classes (7, 401, 485), which give rise to several types of ipsilateral inhibitory interneurons identified by their physiological effects. These include Ia, Ib, and Renshaw interneurons. The V1 interneurons have been described in zebrafish and Xenopus and play a role in sensory integration (252, 313). In the mouse, selective silencing of V1 interneuron population drastically reduced the speed of locomotor-like rhythm in vitro by prolonging the duration of locomotor bursts (188), suggesting that these interneurons contribute to the termination of locomotor bursts. Anatomical analysis combined with sensory stimulation has revealed that Renshaw cells and Ia interneurons represent a small fraction of V1 interneurons. The functional identity and role of a large proportion of V1 interneuron population are yet to be characterized. Recent studies have suggested a very large diversity of V1 interneurons based on combinatorial expression of a large number of transcription factors (46, 177, 439). The different subtypes of V1 interneurons could be incorporated within different subcircuits to control the coordination of movements of the different joints, hence enabling a modular construction. Another population that also give rise to Ia interneurons is the V2b class derived from same progenitor domain as V2a. It has been shown in zebrafish that the two classes of V2 interneurons are generated by a final asymmetrical division (289). Silencing of synaptic transmission from V1 and V2b interneurons disrupted both the flexor-extensor coordination, as well as the Ia reciprocal inhibition (59, 485). These studies are thus beginning to uncover the interneuron subtypes required for ipsilateral coordination.

3. Left-right coordination

Limbed animals also need to coordinate movements across the left-right axis, around single joints, and across multiple joints. V0 interneurons represent a major class of commissural interneurons, which is evolutionarily conserved from zebrafish to mice (194). It is characterized by the expression of the transcription factor Dbx1, which comprises two subtypes early during development: one excitatory located ventrally (V0v) and the other inhibitory and located dorsal (V0d) (307, 345, 378). In mice, genetic deletion of the transcription factor Dbx1 or DTA-induced ablation of Dbx1-expressing V0 interneurons switched the left-right alternation into synchronization, hence recapitulating the effect of blocking glycineergic inhibition with strychnine (307, 443). As the V0 consists of two distinct subclasses, it was important to probe the effect of ablation of each of them individually. The two V0 interneuron subclasses underlie a differential left-right coordination as a function of speed. The inhibitory V0d interneurons are responsible for coordination at slow but not at high speeds, while the excitatory V0v interneurons appear to be predominantly involved at higher but not at lower speeds. Other mechanisms could also be implicated in the observed behavioral effects of ablation of the two types of V0 interneurons such as their differential recruitment or a selective modulation of their synaptic transmission (see Ref. 79) (FIGURE 14C).

A profound effect on gait control has been seen in horses with a mutation in a gene belonging to the DMRT family of transcription factors (DMRT3). In mice, DMRT3 is expressed in a subset of dI6 interneurons. Knockout of the dmrt3 gene produced marked changes in the frequency and coordination of drug-induced activity in vitro (14, 187, 458). These studies in horse and mice have revealed conserved functions of the DRMT3 gene that might be carried out by dI6 interneurons to control the flexibility of coordi-
nation and gait during locomotion. These interneurons are active during drug-induced locomotion in neonate mouse (377) and seem to contact motoneuron on the contralateral side (14).

V3 interneurons represent an additional class of commissural excitatory interneurons that are broadly distributed along the dorsal-ventral and rostral-caudal axes in the postnatal spinal cord (50, 52, 487). It has been shown that V3 interneurons, in addition to their commissural role, also provide ipsilateral excitation to each other and to motoneurons on the same side (96). Acute silencing of interneurons in the lumbar region of freely moving adult mice produced uneven gaits, suggesting that they play a role in limb coordination (487).

4. Coordination along the body

During locomotion, there is a need to coordinate movement of many body parts while ensuring stabilization of the entire body. The smooth execution of the quadrupedal locomotion depends on limb coordination aligned with postural adjustments. Long-range projections must exist between cervical and lumbar circuits to coordinate their activity in behaving animals. These interactions are mediated by long projecting neurons belonging to diverse genetic populations (394). Specifically, V0v interneurons provide the excitatory commissural long projections, whereas V2a interneurons provide the ipsilateral cervicolumbar projections. Ablation of these interneurons at the cervical level predominantly affected the left-right hindlimb, but not forelimb interlimb coordination at higher locomotor speeds. These results point out the importance of long projecting neurons in ensuring interlimb coordination where cervicolumbar projection neurons may therefore also be involved in regulating gait transitions or changes in limb coordination when animals have to adjust quadrupedal stepping.

XI. THE SPINAL COORDINATION OF UNDULATORY LOCOMOTION: SENSORY MODULATION OF THE SPINAL CPGs

A. Sensory Interaction with the Spinal CPG

In most species the excitability is decreased after the spinal cord is transected, and some form of pharmacological or electrical stimulus of the excitatory input to the spinal networks is required to elicit locomotion, as in lamprey or zebrafish (9, 465). In the case of the lamprey, it is sufficient to put the spinal animal in a water current (swim-mill), and the peripheral stimulus will then initiate locomotor movements that become reinforced by the actual sensory feedback from the movement itself, again with the appropriate motor pattern.

The feedback signals are from mechanoreceptors (233) that sense the lateral movements of the body and project to the CPG interneurons and motoneurons. Actually, if locomotor activity is initiated in the lamprey or the dogfish, which are immobilized through curare, a superimposition of locomotor-like movements will entrain the locomotor rhythm (221, 229, 230). Thus, if the resting frequency of the network is at a given level, the superimposition of a movement (FIGURE 19A) at a somewhat higher frequency will entrain the central network and conversely if a lower movement rate is applied. However, at the higher than rest rate, the entrained burst will occur later in the cycle than with lower than rest rate entrainment both in experiments and simulations (453) (FIGURE 19A). This emphasizes the close interaction between sensory feedback and central network; normally they operate as one unit. Under natural conditions,
however, the central network initiates the activity, whereas the outcome of the movement with different perturbations, like currents in the water, will modify the movements and then the feedback becomes of critical importance (143, 324).

In lamprey and dogfish, the stretch receptors that sense the locomotor movements are located at the lateral margin of the spinal cord (228, 229) and most likely also in other vertebrates that move with lateral undulations like reptiles (407). The stretch receptors are of two kinds (FIGURE 19B): one inhibitory that projects to the contralateral CPG and motoneurons, and one with ipsilateral axons that provides excitation to the ipsilateral locomotor network itself and enhances the burst frequency (123).

The dorsal fin motoneurons (414) are activated during each swim cycle, but are out of phase (reciprocal) with the myotomal motoneurons on the same side (339) to be able to stabilize the fin position during the locomotor movements. The dorsal fins provide lateral stability during locomotion and have a separate feedback system originating from cutaneous afferents near the base of the dorsal fins. The fin motoneurons sense the strain in the skin as the fin bends, when the body is moved laterally during one phase of the swim cycle. The cutaneous afferents elicit monosynaptic excitation to fin motoneurons, and thus provide something akin to a stretch reflex, acting to restore the position of the fin (148). They receive excitation from contralateral commissural excitatory interneurons (338). They are thus synergistically activated from the contralateral side, and part and parcel of the same locomotor program.

XII. SENSORY CONTROL OF THE TETRAPOD LOCOMOTOR CYCLE AND INTERACTION WITH THE CPG

For tetrapods, the step cycle is divided into four phases: the support phase, lift off, the flexion phase, and touch down. The transition from stance to swing is obviously important (FIGURE 20, A AND B) and similarly the accurate placement of the foot on the ground (FIGURE 20C), which needs to move backward already when it touches the ground. With regard to the sensory contribution to the step cycle control, the most complete information is from the cat and recently also the rodent nervous system. Signals related to hip movements inform about the overall position of the limb in the step cycle and when lift off should occur (226), while the load on the limb during the support phase prevents a too early transition (FIGURE 20B) (138). Although much less information is available in other groups including rodents, birds, reptiles, and amphibians, all indications are that the same type of solutions is used within the tetrapod and biped groups (90, 126). There are also examples from the invertebrate world like for example walking insects, like the cockroach, locust, and stick insect, being dependent on both load and position feedback from the most proximal limb segment (78, 372). This area has been extensively reviewed by Rossignol, Dubuc, and Gossard (393).

A. Role of Sensory Signals Reporting Hip Position

The hip movement during the step cycle signals dynamically in which part of the step cycle the limb is at a given moment. This is clearly important information, and it is not surprising that signals from afferents influenced by hip position help regulate the step cycle at the spinal cord level (226), but they are also transmitted to the cerebellum via the dorsal spinocerebellar tract (456) together with information regarding the length of the limb from hip to toe.

1. Support phase and transition to swing: lift off

The hip position regulates the transition from stance to swing (FIGURE 20, A AND B) that is the termination of extensor activity and onset on flexor activity. In particular, knee flexors lifting the lower limb above the ground (16, 17, 226) are activated first. If the stance phase progresses slower
than expected so that the limb does not reach the posterior angle at which it normally would switch from stance to swing, the transition will be delayed, and conversely, if the movement proceeds faster than expected, the transition will occur earlier. Receptors in muscles operating at the hip joint (iliopsoas and sartorius) will contribute and possibly also sensory input from ligaments at the hip (15, 302).

2. Swing phase and the transition to support: touch down

One critical phase of the step cycle is when the limb makes contact with the ground, and it is critical that the extensor activity and backward movement have started well before the limb touches ground (153). If the forward movement of the limb (hip) during the swing phase (FIGURE 20C) is hampered, the period of flexor activity will be prolonged and the subsequent period of extensor activation delayed. Conversely, if the forward movement of the limb is faster than control, the period of flexor activity will instead be shortened (15–17, 206, 336).

B. Role of the Load on the Limb for the Extensor/Flexor Transition

Receptors sensing the load on the limb during the support phase (FIGURE 21, A AND B) will act to maintain the extensor activity, and conversely, a decreased load will tend to initiate a transition to the swing phase (136, 138, 139). During mid-stance, the limb carries the maximal load, but towards the end of the stance phase, the load will decrease and this will facilitate the transition to swing. Clearly, a premature flexion occurring in the middle of the support phase would be disastrous and needs to be prevented. Contributing to this response may be afferents originating from the Golgi tendon organ (force transducers) that signal both the muscle force exerted, and perhaps also receptors on the footpads, which integrate the total load on a limb.

Stimulation of Golgi tendon organ afferents (1b) from knee and ankle extensors facilitates the ongoing extension phase during locomotor activity, while the stimulation during the flexion phase rapidly terminates flexion and activates extensor activity. The synaptic response is thus modulated during the step cycle (101, 189, 332). During locomotion, the extensor 1b input thus provides excitation to the extensors, while during resting conditions it would instead provide autogenetic inhibition (137, 393).

C. Position-Dependent Reflex Reversal of Effects from Contralateral Afferents Gated by Hip Position

The position of the hip can also gate reflex activation elicited by sensory input from the contralateral side when the locomotor network is active at a subthreshold level. Stimulation of a peripheral nerve on one side will elicit a discharge in contralateral muscles; if the limb is flexed, extensor activity will be elicited, and conversely flexor activity if the limb is extended (FIGURE 21) (225). The hip position will thus determine (gate) whether flexors or extensors will become activated by an identical contralateral sensory signal. This type of gating can be described as a position-dependent reflex reversal and can most likely play a role also for determining the effects of signals of supraspinal origin (201). These long-lasting reflex discharges are thought to reflect activation of different parts of the locomotor CPG (225), and the limb position thus provides a gating of the effects distributed to antagonist muscle groups (137, 393).

D. Contribution of Muscle Spindles and Cutaneous Input: Selective Eliminations

In mice, Akay et al. (1) investigated the effect of a selective elimination of muscle spindle afferents (1a/II), while retaining Golgi tendon organs, joint, and cutaneous afferents. These animals retained a complex motor pattern, with one difference: ankle flexor activity was somewhat prolonged. This resulted in an enhanced swing phase trajectory and effects on the touch-down of the limb with ground. The support phase was not affected. Later work by Mayer and Akay (329), with selective deletions from individual muscle groups, showed that the ability to increase the activity in extensor muscles was dependent on muscle spindle input, and in particular from the ankle extensors.

![FIGURE 21. Position-dependent gating of sensory stimuli. If the locomotor central pattern generator is kept at a subthreshold level, the position of the contralateral limb (flexed or extended) will determine if an ipsilateral stimulus gives rise to activation of flexors or extensors.](Image)
During swimming in mice (1), the coordination between the different flexors of the limb changes markedly, as compared with walking. This change does not occur when the muscle spindle input has been eliminated. It indicates that muscle spindles contribute to the changes induced in the swimming motor pattern due to radically changed mechanical conditions, when the limb meets very different resistance by the water. The final locomotor movements are thus the result of a continuous adaptation of the CPG activity by sensory feedback.

The input from cutaneous receptors on the footpads integrates the overall load on the limb. It has nevertheless been assumed that they do not contribute importantly (410). However, recent findings show that even a partial denervation of the foot pads has profound effects on the ability to place the feet appropriately during precision walking, for instance, placing the feet accurately on the rungs of a ladder or in complex terrain (55–57, 393). In spinal animals, a cutaneous denervation of the foot has a negative effect on the foot placement during locomotion.

E. Phase-Dependent Reflex Reversals Help Correct for Obstacles Impeding the Limb Movements During Locomotion

If an obstacle impedes the movement of the foot during the swing phase, the necessary correction will be an enhanced flexion to overcome the obstacle (FIGURE 22, left), but the same stimulus occurring during the support phase should not give rise to flexion, if so the supporting limb would collapse in both cats and rodents (169, 173, 174, 329). Touching the hairy skin on the dorsum of the foot will indeed elicit an enhanced flexion during the swing phase, but instead an enhanced extensor activity when applied during the support phase (FIGURE 22, right). We thus have a phase-dependent reflex reversal! Light touch activating just a few hairs will actually be sufficient to either flex or extend the leg, depending on the phase of locomotion. This phase-dependent gating of the two alternative pathways is due to effects from the spinal CPG gating the transmission at the interneuronal level (FIGURE 22, middle) (18). In this case, we thus have the CPG adapting the reflex response in a phase-dependent manner. The reflex circuitry utilized corresponds to the spinal component of the placing reaction (175).

Which interneurons are responsible for phasically gating the sensory transmission? One likely component is the dl3 interneurons that are activated by low-threshold cutaneous afferents and project to both motoneurons and the CPG (74, 75). Moreover, they are rhythmically modulated by the CPG and would thus seem well suited to be part of this circuit. The inhibitory RORβ interneurons (from embryonic dl4/dl5 progenitors) modulate the motor output during walking by gating sensory afferent transmission, which, however, may preferentially be involved with gating of proprioceptive information (291).

F. CPG-Induced Presynaptic Gating of Sensory Afferents and Premotor Interneuron’s Terminals

Both cutaneous and muscle spindle axons receive phasic presynaptic input during locomotion with one pronounced depolarization during the flexion phase followed by a pronounced repolarization at the end of flexion followed often by a weaker depolarization during the extension phase (133, 190–192). The GABA-induced presynaptic depolarization leads to a presynaptic shunting and reduced synaptic transmission from the terminals of the axons to their intraspinal targets in the spinal cord. The particular subset of GABAergic interneurons in the dorsal horn mediating the presynaptic action have been identified (43), and the elimination of them genetically leads to deficient movements particularly during forelimb reaching (166). These results emphasize the importance of regulating the efficiency of sensory input in different behavioral situations and in different parts of the movement cycle. Sensory presynaptic locomotor-related gating is present in vertebrates extending from lamprey to mammals and in invertebrates (92), and
thus must be considered a general feature of the locomotor control system.

Also in the lamprey, the cutaneous sensory input is subject to phasic presynaptic inhibition during ipsilateral locomotor bursts (148) mediated by GABA/neuropeptide Y interneurons (97). Intra-axonal recordings from the presynaptic terminals of excitatory and inhibitory targeting motoneurons showed that they are also depolarized in phase with the ipsilateral CPG-driven motor bursts. These effects are mediated by GABAergic interneurons operating through both GABA<sub>A</sub> and GABA<sub>B</sub> receptors on the presynaptic terminals (3, 4). Presynaptic locomotor-related gating at the interneuronal level has only been established in the lamprey, but it would not seem unlikely that this would not occur in other vertebrates since there are no examples from recordings of interaxonal terminals in other species.

### G. Rapid Corrections During the Support Phase: Muscle Stiffness Followed by Sensory Control

When a mammal like a horse is running at high speed in for instance deep grass, it needs to instantaneously handle sudden changes in the support phase. Under many conditions, the sensory feedback system from, e.g., muscle spindles would not be sufficiently fast, considering the short duration of the support phase at top speed and the inevitable delays involved. This includes the conduction delays from peripheral sensory receptors to and within the spinal cord and back to the muscle, and furthermore, the neuromechanical delay until sufficient muscle force has been developed. Fortunately, the first line of compensation lies in the muscle stiffness of the already contracting muscles. The length-tension curve of each extensor muscle provides increased force as the muscle length is extended, as will inevitably happen during part the stance phase and of course even more so during an unexpected extra load (213). Actually, the whole limb acts as a spring when a cat, rabbit, kangaroo, or horse bounces along at high speed (160, 163, 354).

At low speeds of locomotion, there may be ample time for sensory feedback from muscle spindles, tendon organs, and cutaneous receptors, but still the muscle stiffness will be first in line to protect against unexpected perturbations. The primary muscle spindle afferents will clearly be excited by muscle lengthening and provide monosynaptic excitation to the motoneurons of the muscle being stretched (1, 140, 321), which will add to the contraction, and similarly the input from Golgi tendon organ will contribute excitation during locomotion (101, 189, 332) in contrast to the inhibitory effects exerted during resting conditions.

### XIII. VESTIBULAR AND SPINAL MECHANISMS CONTROLLING BODY ORIENTATION DURING LOCOMOTION

#### A. Vestibular Stabilization During Swimming

Let us first consider aquatic locomotion in which there is much less of a problem with gravity than for terrestrial locomotion. In fish and lamprey, the vestibular apparatus is the main instrument for correction of body orientation during swimming (dorsal side up). The Deliagina-Orlovski laboratory has in a series of studies elucidated these mechanisms. Essentially, the vestibular apparatus on the left and right side signal the orientation of the head during swimming, and via relay interneurons they impinge on contralateral reticulospinal neurons (FIGURE 23A). A tilt to the left will increase the activity of the reticulospinal neurons on the right side, and vice versa (FIGURE 23B). The reticulospinal neurons concerned will act on spinal motoneurons to counteract the rotation that has occurred (483, 484). The reticulospinal neurons have some level of resting activity, and the head oscillates horizontally somewhat during each swim cycle, and therefore the activity of the reticulospinal neurons will oscillate in anti-phase on the two sides. The position will be stabilized at an equilibrium point at which the activity of the reticulospinal neurons on the left and right sides become symmetric (see FIGURE 23B). If, however, the lamprey is turned towards one side for instance by a water current, let us say 90 degrees in FIGURE 23, the reticulospinal activity will be markedly enhanced on one side, and reduced on the other side. This will lead to a rapid compensation of the body orientation through reticulospinal neurons.

The lamprey has three brain stem reticular nuclei that receive the vestibular input. One is maximally activated at 45 degrees tilt, another at 90 degrees, and the third at 180 degrees that is when the lamprey is upside down (117, 118, 120, 121). The connectivity of different reticular nuclei is such that they target different combinations of either dorsal or ventral segmental myotomal motoneurons, although there is also variability within each nucleus (483, 484). If the vestibular apparatus on one side is damaged, the input to the brain stem will be asymmetric, and the lamprey will consequently swim with a continuous rotation of the body.

During forward swimming, there is thus a very precise and accurate control of body orientation. In contrast, if backward swimming is induced in the behaving animal (266), the animal rotates without lateral stabilization, and the small reticulospinal population activated only during backward swimming is not responsive to lateral tilt. In contrast, the remaining reticulospinal population responds mostly to lateral tilt (481).

Under some conditions, the fish or lamprey may like to stabilize a position in which the body is tilted to one side.
during swimming. One case is the “dorsal light response,” which encompasses a rotation of the dorsal black side of the body towards the direction of the sunlight to reduce the possibility of being detected by predators, like birds flying over the water. This reaction is elicited by an asymmetric distribution of the light into the two eyes, which makes the lamprey rotate the body to get a more symmetric illumination of the two eyes (115, 273). The rotation is achieved by light activation of the reticulospinal neurons particularly on one side, which in turn results in an enhancement of the activity of one side over that of only exclusively the vestibular input, leading to a new equilibrium point tilted to one side (482) (see \textbf{FIGURE 23}). In this way the body position can be stabilized at any angle.

\section*{B. Stabilization of Swimming with Different Pitch Angles (Downward or Upward)}

The same type of reasoning applies to a situation in which the lamprey stabilizes the pitch (dorsoventral) angle (119). The vestibular compensation will then stabilize that position. In this case, the reticulospinal neurons can be subdivided in two populations: those that respond to an upward deviation of the course of swimming, and those that respond to a downward deviation with an enhanced activity. During swimming in the horizontal plane, the stabilized equilibrium point will be when the activity of both groups is symmetric. If an additional excitatory drive would occur to for instance the upward responsive groups of reticulospinal neurons, this would mean that they become more responsive than their downward sensitive reticulospinal neurons, and a new equilibrium point would be generated. The net result in this case would be that the lamprey would stabilize a downward swimming direction. The reverse would be true if the additional excitatory input were directed to downward sensitive reticulospinal neurons. This is a mechanism that allows for setting the pitch angle in a graded way and in addition to stabilizing this particular angle.

\section*{C. The Control of Body Orientation in Tetrapods During Standing and Locomotion}

Also in mammals, the vestibular control is important in that it detects movements of the head, which can be used for rapid compensations, and an asymmetric activation of the vestibular apparatus leads to changes in posture during standing and also during locomotion (114, 115, 118, 121, 135, 353). These effects are mediated primarily by the vestibulospinal projections from Deiters nucleus, which convey monosynaptic excitation to extensor motoneurons (216, 350).

The corrections after perturbations (e.g., tilt) of the fore- or hindlimb girdles during standing are to a large extent mediated by postural limb reflexes (PLR), and not severely disturbed in labyrinthectomized mammals (265). The PLR reflexes are relatively weak in spinal animals, but well-developed in decerebrate and intact animals. It seems likely that the PLR reflexes are actually facilitated from supraspinal sources. A limited set of muscle synergies can account for the postural compensations in different directions (452). The input sensing the perturbation acts not only on the spinal level, but is also conveyed to the brain stem level. Actually neurons in motor cortex, the rubro- and reticular nuclei are modulated by postural challenges (41, 428). The
importance of motor cortex in this context is unclear, since after lesions of this structure postural control is not detectably affected.

During locomotion, a lateral perturbation is compensated by abducting or adducting the respective limbs during the next swing phase so that they are placed, e.g., in a more lateral position on one side in the next support phase. This will clearly stabilize the next support phase. There are, however, only limited effects on the speed and the overall limb trajectory (279, 351). During vestibular input (tilt), vestibulospinal neurons are markedly affected, but the identical tilt stimulus applied during locomotion will cause a smaller response, thus the signal is gated when locomotion is turned on (369).

XIV. STEERING OF LOCOMOTOR MOVEMENTS

As discussed in section IVA, visuomotor coordination in terms of placing the feet accurately while walking in a complex terrain or avoiding obstacles identified visually involves the parietal lobe and subsets of corticospinal neurons in mammals (128).

Another form of everyday visuomotor coordination is when walking along the pavement of a busy street. We then continuously steer our movements to avoid bumping into fellow pedestrians or orient towards the goal of an interesting item. The steering movements depend to a large extent on the midbrain tectum (superior colliculus), which receives visual input arranged in a retinotopic map of the surrounding space (273, 349), which is formed by the afferents from the eye. On the output side there are two sets of efferents: one producing evasive and the other orienting movements in vertebrates extending from lamprey to mammals (113, 248, 280, 281, 294, 399). The tectal output neurons project to different sets of reticulospinal neurons that convey the different steering signals to motoneurons on opposite sides of the body and also controlling the dorsoventral axis (281, 483, 484).

Stimulation of tectum in the dogfish provides a clear integrated steering coordination with a body curvature superimposed on the locomotor movements, combined with a prominent change of angle of the pectoral and anal fins promoting the turn; thus a specific turning motor program is used to elicit the steering movement (228). In contrast to teleost fish, the large pectoral fins of the dogfish do not take part in the propulsive movements, but can steer the forward movements by changing the angle of attack in three dimensions (sideways, up, or down dependent on stimulus location). Teleost fish use the fins when exploring at low speeds or position themselves in relation to food objects, but at higher speeds of swimming they fold the fins close to the body and rely on changes of the body curvature to steer, as do salamanders. Larval zebrafish orient towards small food objects, but respond with avoidance or escape when exposed to larger objects (165).

In mammals, stimulation of tectum (superior colliculus) elicits a “tectal response” (260), which includes inducing an enhanced curvature of the body and a rotation of the limbs to induce turning. As in the dogfish, there is thus a superimposed separate motor program for turning. In rodents, the superior colliculus is more important than the visual cortex for orienting movements (343), and stimulation of the superior colliculus in fish as well as mammals will be dependent on stimulus location eliciting an avoidance or orienting movement (113, 250). The asymmetry of the limb movements, on the concave and convex sides of the body, respectively, during turning can be handled by the spinal reflex machinery of the two limbs (171, 172).

XV. ROLE OF CEREBELLUM: FINE TUNING OF THE LOCOMOTOR MOVEMENTS

Although the basic features of locomotion can be coordinated by the spinal cord itself, the overall coordination between the limbs is less precise. Cerebellum receives detailed dynamic information from the spinal cord about ongoing and planned movements, and it can through descending signals target both motoneurons directly and the CPG (see FIGURE 24) and provide corrections.

**FIGURE 24.** Interaction between cerebellum and the locomotor central pattern generator (CPG). Cerebellum receives input from the moving limb through the dorsal spinocerebellar tract (DSCT), efferent CPG commands via the ventral spinocerebellar tract (VSCT), and error information through the spinoolivary tract. Cerebellum provides compensatory adaptations through the vestibulo-, rubro-, and reticulospinal pathways.
A. The Dorsal Spinocerebellar Tract Transmits Critical Information from the Moving Limb During Locomotion

Arshavsky and co-workers (27, 28) reported that the dorsal spinocerebellar tract (DSCT) conveys sensory information reporting about the progress of the hindlimb movements during each step cycle. It has been known for a long time that DSCT neurons receive a variety of information from proprioceptors (1a, 1b) and cutaneous afferents (322). Moreover, Poppele and co-workers (53, 380) have shown that DSCT transmits an integrated signal resulting from sensory input from different muscles and joints (see FIGURE 24). They investigated the message transmitted by applying passive locomotor-like movements to the hindlimbs while recording from DSCT neurons, and discovered that the primary information transmitted was 1) the length of the limb (from hip to foot), and 2) the progress of the limb movements throughout the step cycle, corresponding to the dynamic hip angle. These two measures are obviously critical for being able to control the limb within different parts of the step cycle. The cerebellum is apparently continuously updated regarding these important parameters. Recently, Fedirchuk et al. (161) have shown that in a curarized preparation locomotor activity driven from the CPG can also be represented in two-thirds of the DSCT cells, so the situation is not as “clean” as originally conceived, i.e., that DSCT only transmitted sensory information.

B. Efference Copy Information from the Locomotor CPG Transmitted via the Ventral Spinocerebellar and the Spino-Reticulo-Cerebellar Pathways

Whereas DSCT reports primarily sensory events, Arshavsky et al. (26) could show that the ventral spinocerebellar tract (VSCT) conveys dynamic information about the CPG and primarily about the flexion phase of the movements. Thus a form of efference copy of the commands issued to the flexor motoneurons is forwarded to cerebellum. The corresponding efference copy of the CPG commands to extensor motoneurons is conveyed via the spino-reticulo-cerebellar pathway (SRCP) to the cerebellum (28). It is obviously important information to the cerebellum regarding the upcoming movements.

C. Sensory Information and CPG Activity Is Transmitted to Purkinje Cells in the Anterior Cerebellar Lobe

In the immobilized animal, activity in the spinal CPGs is transmitted via VSCT and SRCP, which reaches the Purkinje cells in the anterior cerebellar lobe via the granular cells (23–25, 142, 457). The Purkinje cells become strongly modulated in different phases of the step cycle, and in turn modulate the activity of the different cerebellar nuclei and downstream motor structures (20, 29).

D. The Cerebellar Output Is Rhythmically Modulated and Provides Phasic Drive to the Fast Descending Pathways

The input from the spinocerebellar pathways modulates subsets of Purkinje cells so that they become phasically modulated in each step cycle. The Purkinje cells in turn drive, either directly or via the different cerebellar nuclei, the fast-conducting vestibulospinal neurons that activate extensors in the extensor phase of the limb movement, while the activity of reticulo- and rubrospinal neurons primarily is flexor-related (364–366). This step-cycle-related modulation of the descending pathways is due to cerebellum, because it disappears if cerebellum is removed. Cerebellum will thus through the descending pathways be able to modulate or fine tune the overall activity at the spinal level. Interfering with the locomotor movements affects the pattern of activity in the descending pathways. If the cerebellar anterior lobe is inactivated through cooling, the locomotor movements become modified and a marked hyperflexion may occur (454, 455). This means that cerebellum continuously monitors the locomotor activity and is able to modify or correct ongoing locomotor activity. Due to the inherent time delays, it is likely that the ongoing step is not fully corrected but rather the subsequent, at least during fast movements.

E. Cerebellar Learning and Locomotor Coordination

The cerebellum is in general considered to adapt movements over longer periods through a form of learning. This is an error-driven process mediated through the climbing fibers, through which movements can be modified, whether eye or locomotor movements (112, 268, 342, 478). One way to test locomotor adaptations both in humans and animals has been to have them walk on a split-belt treadmill, where the left and the right pairs of limbs can be made to walk with different speeds (383, 384, 479). This results in an initial adaptation in which the limb on the fast belt adapts by shortening the support phase, while the leg on the slow belt instead prolongs the support phase. This initial adaptation occurs via spinal mechanisms (171, 172), but a subsequent more complex adaptation concerning both temporal and spatial adaptations of the four limbs develops over several trials and depends on cerebellum (111). Recordings of climbing fibers in the paravermal cortex during the initial split belt induction have shown that there is an enhanced climbing fiber activity, particularly in the touch down phase (E1) of the limb movement, representing a
critical phase of the limb trajectory in each step cycle (478). Lesions of the paravermal cortex in the anterior lobe that projects to the interpositus nucleus lead to an inability to adapt, whereas inactivation of the fastigial or lateral nucleus has no effect. The interpositus nucleus projects to the midbrain, from which the rubrospinal pathway originates, a nucleus known to be modulated in each step-cycle in the cat (364). Sudden perturbations occurring during locomotion lead to a marked increase in climbing fiber activity (13), presumably providing an error signal (FIGURE 24).

F. In Conclusion

The cerebellum is tightly involved in the adaptation of the locomotor movement to short- and long-term perturbations signaled via climbing fibers, serving as the error detectors. The mossy fiber input provides information about the actual ongoing movements in each step cycle, as well as efference copy information regarding the flexor and extensors commands provided by the locomotor CPG.

XVI. CONCLUDING REMARKS

We have tried to show over the preceding pages how the knowledge about the motor system has evolved, and in particular the many interacting systems that control locomotion.

It has become clear that the basic neural organization of the oldest now living vertebrates (lamprey) has very much in common with the mammalian nervous system. This applies to the detailed organization of the forebrain including the basal ganglia, the dopamine system, the midbrain, brain stem, and spinal cord, although the number of cells in each location is much lower. This means that the basic organization of the vertebrate motor system had evolved very early in vertebrate evolution when the lamprey line of evolution was isolated from that leading to mammals (some 560 million years ago). The forebrain circuits are critical for selection of behavior and the goal-directed aspects of motion.

There are CPGs in the spinal cord and brain stem-midbrain that control different patterns of behavior in all vertebrates. Green fluorescent protein expression in subsets of CPG interneurons combined with optogenetic techniques and viral technology has markedly facilitated the analyses of the intrinsic function of the CPGs and the importance of identifying the relevant interneurons with their membrane properties and synaptic connectivity. Likewise, the specific roles of subsets of different descending pathways each controlling different aspects of motor behavior have been unraveled. The overarching interaction between the postural and locomotor system is critical as is the steering control.

From our perspective, there has been an amazing development of this entire field. In a review by one of us (S. Grillner) for Physiological Reviews already in 1975, the major building blocks of the locomotor system had become established in terms of the MLR, spinal networks, and sensory control. However, the cellular and synaptic bases of the CPGs were in the dark, and even glutamate’s role as a transmitter was unknown! The role of the cortex and the basal ganglia in terms of locomotion was not appreciated nor were the descending pathways. Most experiments were performed on the cat, and experiments on mice, zebrafish, tadpole, and lamprey with their technical advantages had not yet started in the context of locomotion. They now contribute to new fundamental insights regarding the operation of this complex control system.

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