DOI 10.1007/s11055-016-0299-5
Neuroscience and Behavioral Physiology, Vol. 46, No. 6, July, 2016

Central Pattern Generators: Mechanisms of Operation and Their Role in Controlling Automatic Movements

Y. I. Arshavsky,[1] T. G. Deliagina,[2] and G. N. Orlovsky[2]

UDC 591.1,612

Translated from Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova, Vol. 65, No. 2, p. 156–187, March–April, 2016.

Central pattern generators consist of sets of interconnected neurons able to generate a basic motor output pattern underlying automatic movements (respiration, locomotion, chewing, swallowing, etc.) without any afferent signals from the executive motor apparatus. They are divided into constitutive pattern generators, which are active throughout life (the respiratory generator), and conditional pattern generators, which control episodic movements (locomotion, chewing, swallowing, etc.). As the motor output of a pattern generator is defined by its internal organization, the activity of conditional pattern generators is initiated by a simple command arriving from the higher centers. The structural-functional organization of the locomotor pattern generators in the marine mollusk *Clione*, the lamprey, the frog embryo, and laboratory mammals (cats, mice, and rats) are described, along with pattern generators controlling respiratory and swallowing movements in mammals and pattern generators for discharges of the electric organs of gymnotiform fish. Generation of the rhythmic motor output is shown in all cases to be based on the endogenous (pacemaker) activity of specific groups of interneurons and interneural interactions. These two interacting mechanisms supplement each other, ensuring the reliable operation of pattern generators. The question of whether experience gained from studies of central pattern generators can be used for understanding the mechanisms of more complex brain functions, including cognitive functions, is discussed.

Keywords: central pattern generator, constitutive and conditional pattern generators, command area, pacemaker interneuron, interneural interactions, locomotion, respiration, swallowing, electric organ.

Introduction

This review was inspired largely by publication of the Round Table "Central Pattern Generators" by Balaban et al. [Balaban et al., 2013]. Unable to take part personally in the Round Table discussions, we would like to use the present article to respond to the discussion by presenting our views of the mechanisms of operation of central pattern generators and their roles in controlling movements. We will work mainly on the basis of data obtained from studies of vertebrates. An exception was made for the marine pteropod mollusk *Clione*, which we have used as a study object for many years.

The motor repertoire of animals includes a series of quite stereotypical "automatic" movements, such as respiration, various types of locomotion (walking, swimming, flying, etc.), chewing, swallowing, and others. The question of the roles of reflex and central mechanisms in controlling this type of movement arose with the classical studies of Sherrington and Brown, who addressed the mechanism generating hindlimb stepping movements in spinal cats. Sherrington's initial view was that stepping consisted of a complex chain reflex in which each subsequent phase of the step was initiated by afferent signals arriving from proprioceptors activated during the preceding phase [Sherrington, 1910]. This purely reflex interpretation was shaken up by Brown, who had worked in Sherrington's laboratory and showed that stepping movements could be evoked in spinal cats with dorsal root transection [Brown, 1911]. Assessing Brown's contribution, Sherrington wrote: "Indeed, from the observations of Professor Brown, an intrinsic automatic activity of spinal centres seems the essential nervous mechanism responsible for inconscient stepping, a central activity comparable with that of the respiratory centre in the bulb,

[1] University of California, San Diego, USA; e-mail: yarshavs@ucsd.edu.
[2] Karolinska Institute, Stockholm, Sweden.

and like the latter, highly regulated by reflex action" [Sherrington, 1924].

With the development of electrophysiological methods, the phenomenon described by Brown was confirmed in many studies on the isolated central nervous system (CNS) of invertebrates and lower vertebrates and on mammals immobilized curare-like substances. In these conditions, the neural centers retain the ability to generate their motor output, which underlies most, if not all, automated movements. The concept of central pattern generators (CPG) was built on these data [Arshavsky et al., 1997, 1998; Grillner, 2003, 2006; Marder and Calabrese, 1996; Orlovsky et al., 1999; Selverston, 2010]. The term central pattern generator was formulated in the framework of motor control physiology. Attempts to use it in other areas of neurophysiology are, in our view, rather artificial. Such attempts make little contribution to our understanding of other, especially cognitive, brain functions, merely diluting the sense of the term.

CPGs are defined as sets of interconnected neurons able to generate the basic motor output pattern underlying the movement concerned without any afferent signals from the executive motor apparatus. The motor output itself, not accompanied by any actual movement, is termed a "fictive movement" (fictive locomotion, fictive scratching, fictive chewing, etc.). This definition of a central pattern generator is entirely relative, as what lies behind the words "set of interconnected neurons" does not emerge from it. The number of generators which have been studied at the level of single identified neurons is very limited. In invertebrates, these are the cardiac rhythm generators in crustacea [Cooke, 2002] and leeches [Kristan et al., 2005], the pyloric and gastric generators located in the stomatogastric ganglion of crustacea that control rhythmic contractions of the stomach [Marder and Calabrese, 1996; Selverston, 2005, 2010], the locomotor generators in the gastropod mollusks *Clione* [Arshavsky et al., 1998, 1993] and *Tritonia* [Getting et al., 1989] and leeches [Kristan et al., 2005], and digestive rhythm generators in several gastropod mollusk species [Arshavsky et al., 1988; Elliot and Susswein, 2002; Murphy, 2001]. In vertebrates, locomotor rhythm generators in lampreys [Grillner, 2003, 2006; Grillner et al., 1995] and clawed frog *Xenopus laevis* [Arshavsky et al., 1993; Li, 2011; Roberts et al., 2008] have also been studied at cellular level. A well-studied group of neurons located in the medulla oblongata of so-called weakly electric fish can also be added to this list [Heiligenberg, 1991]. These neurons control the continuous discharges of the electric organ used mainly for electrolocation. As the electric organ is a muscle fiber derivative and is innervated by motoneurons, the set of cells controlling its discharges can be regarded as a central pattern generator. The models of the structural-functional organization of all these CPGs are based on quite complete knowledge of their neurons and interneural connections.

Unfortunately, this cannot be said for most other CPG studied to date, especially in mammals. Despite major advances in this area, knowledge of the neurons forming central pattern generators for automatic movements and interneural connections is clearly inadequate. The models proposed to date do not therefore show the actual organization of CPGs but rather attempt to answer the question of the properties required by a CPG to explain the whole set of observed physiological facts.

The existence of CPGs does not deny the importance of reflex mechanisms for automatic movements. It may be that movements in some invertebrates are controlled mainly by CPGs with afferent signals playing a minor role. However, the situation in vertebrates is different. An example is locomotion of terrestrial mammals [Hultborn et al., 2007; Orlovsky et al., 1999; Pearson, 2008; Rossignol et al., 2006]. Here we will mention three items: 1) the level of motoneuron (MN) excitation and, therefore, the strength of muscle contractions during natural walking or running in cats are determined by signals arriving both from the locomotor CPG and from muscle and tendon receptors; 2) afferent signals coming from primary muscle spindle receptors are to a significant extent responsible for determining the reciprocal activity of antagonist muscles; 3) the transfer from the stance phase to the swing phase of the stepping limb and, thus, the duration of the whole cycle of the step depends mainly on signals arriving from receptors located in the hip joint and from receptors sending signals relating to loadings on the extensor muscles at the end of the stance phase of the step. Besides, the important role of the locomotor CPG is that it modulates the thresholds of reflex reactions at different phases of the cycle. This interaction between reflex and central mechanisms provides for reliable control of locomotion at the spinal level.

To conclude the Introduction, we want to make the following general comment. When addressing such vitally important CNS functions as the control of automatic movements, the alternative "either-or" approach (either central mechanisms, or reflexes) is usually rather unproductive. As a rule, the regulation of complex vitally important functions is based on several mutually supplementary mechanisms, which ensures the reliable operation of the control system. A more productive approach to understanding CNS functions will therefore be the synthetic "both-and" approach. We still face this issue when addressing the various aspects of the operation of CPGs.

General Characteristics of Central Pattern Generators

CPGs in vertebrates constitute the lowest level of motor control. This is especially apparent in mammals, with their clearly hierarchical CNS organization. CPG in these animals are located either in the spine (for example, locomotor CPG) or medulla oblongata (CPGs controlling respiratory, sucking, and swallowing movements).

The structural-functional organization of CPGs is genetically determined. CPG are formed during embryogenesis and have no need for training to start their func-

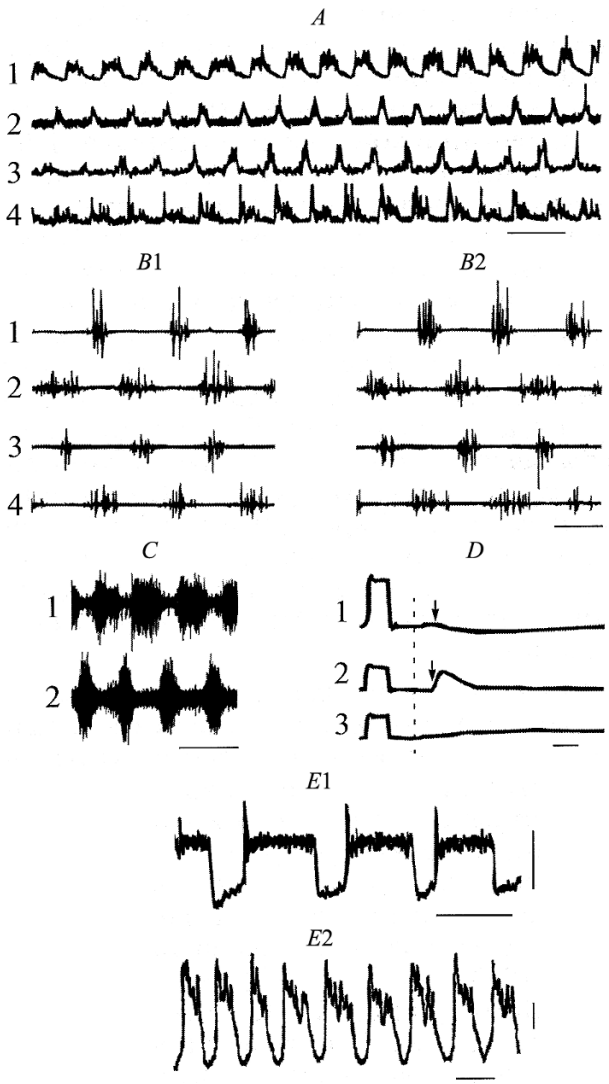

Fig. 1. A mammalian locomotor CPG: initiation of activity and mechanism of operation. *A*) Fictive locomotion in an immobilized cat evoked by stimulation of the mesencephalic region. *1–4*) Electroneurograms (ENG) recorded from the left sartorius nerve (*1*), the left gastrocnemius nerve (*2*), the right semitendinosus nerve (*3*), and the right tibialis anterior nerve (*4*). The scale bar shows 1 sec. Modified from [Kudo and Nishimaru, 1998]. *B*) Locomotor activity evoked by application of NMDA (20 μM) solution before (*B1*) and after (*B2*) transection of the posterior roots of the spinal cord; spinal cord preparation from a neonatal rat connected to the hindlimbs. *1–4*) Electromyograms (EMG) recorded from the left (*1*) and right (*3*) tibialis anterior muscles and the left (*2*) and right (*4*) gastrocnemius muscles. The scale bar shows 1 sec. Modified from [Douglas et al., 1993]. *C*) Fictive locomotion evoked by application of solution containing a mixture of serotonin (15 mM) and dopamine (75 mM); isolated spinal cord from a neonatal mouse. *1* and *2*) ENG recorded from the left (*1*) and right (*2*) anterior roots. The scale bar shows 10 sec. Modified from [Whelan, 2010]. *D*) Relationship between reflex reaction and phase of the locomotor cycle. Mean intracellularly recorded response of a gastrocnemius muscle motoneuron to stimulation of the plantar nerve with a strength of 1.6 times threshold; the moment of stimulation is marked by the dotted line. *1*) At rest; *2*) extensor phase; *3*) flexor phase; arrows show the onset of postsynaptic potentials. The square-wave impulses at left are 2-mV calibration markers; the scale bar on the time axis shows 2 msec. Modified from [McCrea, 1998]. *E*) Pacemaker activity of spinal interneurons. Intracellular recording of an unidentified interneuron (*E1*) and an Hb9 interneuron (*E2*) in transverse sections of spinal cord sections from a rat and a mouse, respectively. Fictive locomotion was evoked by application of NMDA (20 μM) (*E1*) or a mixture of serotonin (20 mM), dopamine (50 mM), and NMDA (20 μM) (*E2*) supplemented with tetrodotoxin (1 μM). Calibration voltages are 40 and 10 mV and time markers are 10 and 5 sec, respectively. Modified from [Hochman et al., 1994] and [Wilson et al., 2005].

tioning. This is well known from everyday experience. Children, like other mammals, perform respiratory, sucking, and swallowing movements from the moment of birth. This also applies to the locomotor generator. One example is provided by precocial birds, whose chicks start to walk immediately after hatching from the egg. Another example is provided by ungulates, whose offspring are able to walk within hours, if not minutes, after birth. If this were not so, these animals, living on open spaces, would soon be taken by predators.

The fact that locomotor CPGs are formed during embryogenesis has been confirmed in electrophysiological experiments. A series of studies of the swimming CPG was performed on *Xenopus* embryos (Fig. 6). Locomotor CPG activity has also been recorded in a spinal cord preparation isolated from neonatal rodents (Fig. 1, *B*, *C*). Therefore, the inability of neonatal rodents that mature after birth, being protected in the burrow, to perform locomotion can probably be explained by muscle weakness rather than by the lack of "training" of the control system.

CPGs are often divided into constitutive and conditional generators. The former include CPG whose activity persists throughout the organism's life. These are the respiratory generators, the cardiac generator in crustacea, and the generator of rhythmic discharges of the electric organ in fish. By contrast, conditional CPGs control movements such as locomotion, chewing, swallowing, etc., which occur episodically in relation to various behaviors. The question of the mechanism by which these CPGs are activated will be addressed in the following section.

Initiation of Central Pattern Generator Activity

As the motor output of CPGs is determined by their internal organization (i.e., the properties of the CPG-forming neurons and interneural connections; see below), CPG activity can be switched on by a simple command coming from above-lying centers. This has been demonstrated in studies of the neural mechanisms controlling locomotion in cats [Shik et al., 1966; Orlovsky et al., 1999; Shik and Orlovsky, 1976]. An area was found in the midbrain (the "mesencephalic locomotor area") which, when stimulated electrically at frequencies of 20–60 Hz, induced coordinated locomotion on a treadmill band in cats decerebrated at the premammillary level [Shik et al., 1966]. By analogy with command neurons in invertebrates [Wiersma and Ikeda, 1964], areas of this type, evoking coordinated motor

behavior, can also be termed command areas. Changes in the strength of the stimulation applied to the mesencephalic area can be used to control both the speed of locomotion (from slow walking to running) and the animal's gait (from diagonal locomotion to a trot and then to a gallop). Stimulation of the mesencephalic area evokes locomotion not only in decerebrate, but also in intact cats [Sirota and Shik, 1973]. This effect occurs independently of the animals' initial state (at rest, during sleep, or while eating) and continues during the whole period of the stimulation.

Speaking of CPGs, we should emphasize that stimulation of the mesencephalic area also induces locomotor activity in the absence of afferent signals from receptors in the executive motor apparatus. For example, stepping movements could be evoked in animals with transection of the dorsal roots or in immobilized animals [Douglas et al., 1993; McCrea, 1998; McCrea and Rybak, 2008; Orlovsky et al., 1999; Sholomenko et al., 1991] (Fig. 1, *A*). This means that signals arriving from the command area convert the CPG from the passive state to the active, rhythm-generating state (the question of the mechanism of the transfer of locomotor and other conditional CPG from the passive to the active state will be discussed below).

Cats can go backwards as well as forwards. Recent studies on decerebrate cats showed that stimulation of the mesencephalic area induces locomotion only in the forward direction, while direct stimulation of the spinal cord can induce locomotion in both the forward and backward directions depending on the direction of treadmill band movement [Musienko et al., 2012]. This result suggests that apart from the mesencephalic area, there is a command area evoking locomotion in the reverse direction. We note that in some animals, this type of locomotion constitutes a significant proportion of locomotor behavior. For example, during defensive behavior, a porcupine will quickly run backwards to plunge its needles into its enemy.

The mesencephalic locomotor area has also been found in other mammals [Skinner and Garcia-Rill, 1984] and in members of various vertebrate classes – Cyclostomata [Sirota et al., 2000], fish [Kashin et al., 1974], amphibia [Cabelguen et al., 2003], reptiles [Kazennikov et al., 1980, and birds [Sholomenko et al., 1991].

The mesencephalic locomotor area is not the only command area initiating locomotion in mammals. Another area which when stimulated evokes locomotion in cats was found in the hypothalamus, the "diencephalic locomotor area" [Orlovsky 1969]. The effect of hypothalamic stimulation persisted after removal of the mesencephalic area. Thus, these two command areas are able to act independently of each other. A diencephalic command area has also been found in lampreys [El Manira et al., 1997].

The question of why there exist two command areas initiating locomotor CPG activity remains unclear. One suggestion is that they are used in different forms of behavior [Jordan, 1998]. However, another suggestion is also possible. The existence of two or perhaps a larger number of command areas may be a manifestation of the "redundant" organization of the CNS. This redundancy ensures the reliable control of physiological functions.

Both locomotor areas send projections to the medial reticular system, which gives rise to the reticulospinal tract [Jordan et al., 2008; Orlovsky et al., 1999]. This tract directly carries out the command function, i.e., it converts the locomotor CPG from the passive to the rhythm-generating state. The reticulospinal tract is heterogeneous, at least pharmacologically. Different fractions of the reticulospinal tract act on spinal targets using different transmitters such as glutamate, serotonin, adrenaline, and dopamine. Experiments on animals immobilized with curare-like substances and preparations of isolated CNS or spinal cord have shown that fictive locomotion can be evoked by application of one of these transmitters or their agonists simulating the effect of the descending command signals (Fig. 1, *B*, *C*). Among transmitter agonists, the most commonly used are glutamate agonists: NMDA (N-methyl-D-aspartate), NMA (N-methyl-DL-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and kainate. The functional significance of the heterogeneity of the descending command pathway and of the multiplicity of transmitters employed is unclear. The most obvious suggestion is that different fractions of the command tract are used to produce different types of modulation of the activities of locomotor CPG and other spinal mechanisms, adapting ongoing locomotion to the environmental conditions. However, experimental support for this suggestion is absent, at least at present.

Activation of locomotor CPG occurs simultaneously with modification of proprioceptive spinal reflexes. For example, at rest, signals from the Golgi tendon receptors of the extensor muscles, transmitted by group Ib afferents, evoke disynaptic inhibition of their motoneurons. In real and fictive locomotion, Ib afferents evoke not inhibition, but disynaptic excitation of extensor motoneurons during the extensor (stance), but not during the flexor phase of the cycle (Fig. 1, *D*). Signals from tendon receptors maintain the activity of the extensor muscles during the stance phase of the step. This change in the response has been suggested to result from inhibition of inhibitory interneurons and disinhibition of excitatory interneurons in the spinal cord. In addition to effects at the level of the simplest disynaptic reflexes, signals from Golgi receptors of extensor muscle exert significant influences on the operation of the locomotor CPG itself [Hultborn and Nielson, 2007; Orlovsky et al., 1999; Pearson, 2008; Rossignol et al., 2006].

It is interesting to note that modification of proprioceptive reflexes during transition from rest to locomotion has also been seen in insects. Experiments on stick insects have shown that, at rest, limb flexion at the femorotibial joint activating the sensory chordotonal organ led to excitation of extensor motoneurons. This reflex promotes stability to ex-

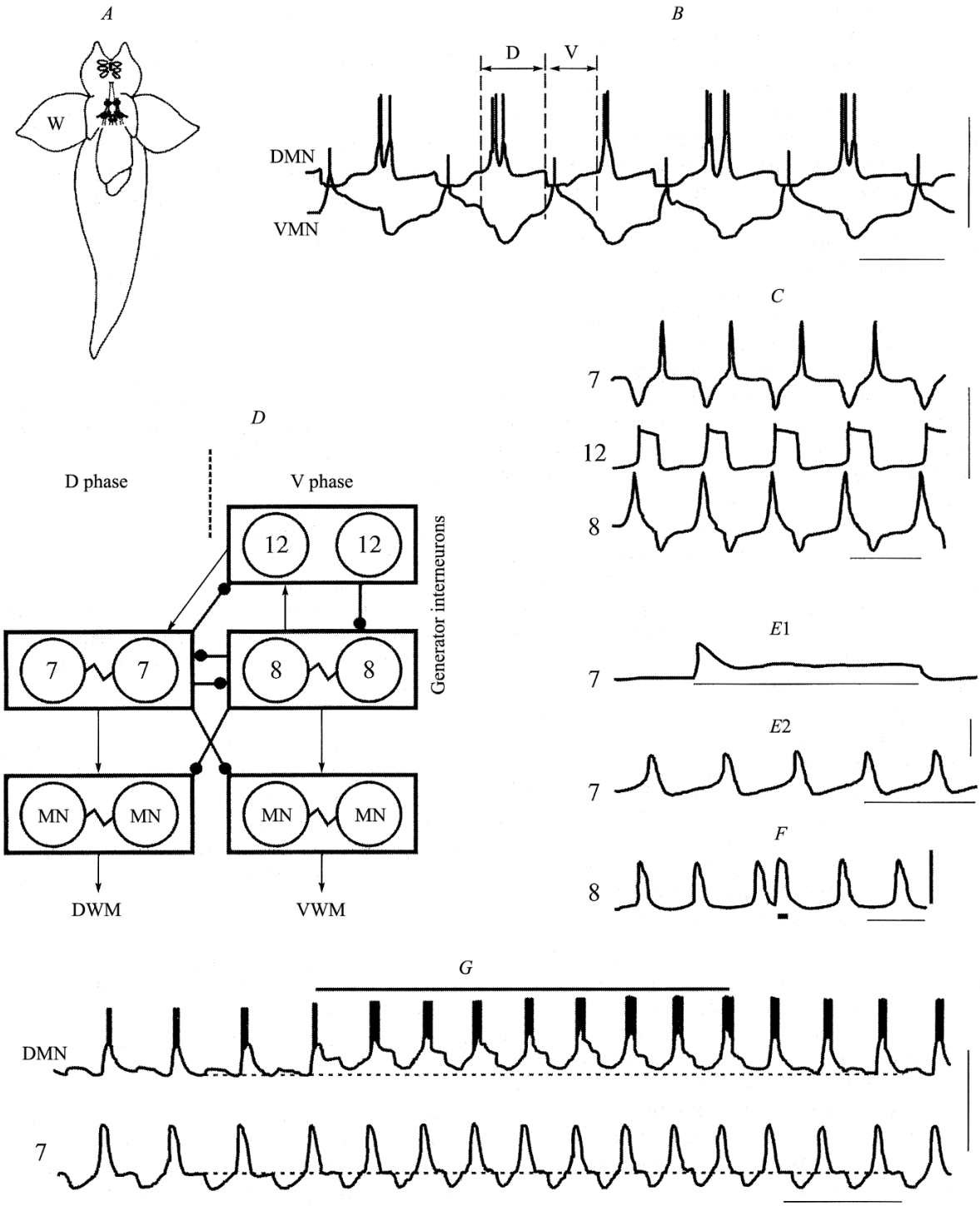

Fig. 2. Locomotor CPG in the mollusk *Clione*. *A*) Diagram showing the mollusk. W – wing. *B*, *C*) Fictive swimming; isolated CNS preparation. *B*) Intracellular recording of motoneurons in the pedal ganglion active during the dorsal (DMN) and ventral (VMN) phases of the cycle. The calibration voltage shows 50 mV; the time scale bar shows 0.5 sec [Arshavsky et al., 1985]. *C*) Simultaneous recording of interneurons of groups 7, 8, and 12 (see text). The calibration voltage shows 20 mV; the time scale bar shows 1 sec [Panchin et al., 1995]. *D*) Diagram of interneural connections in the locomotor CPG in *Clione*; DWM and VWM are the dorsal and ventral wing muscles, respectively. *E*, *F*) Intracellular recording of isolated types 7 and 8 interneurons. *E*) Application of serotonin (0.5 μM) evoked rhythmic activity in an interneuron (*E*2) while depolarization with direct current (black line) evoked a single action potential (*E*1). The calibration voltage shows 20 mV; the time scale bar shows 1 sec (Panchin et al., 1996]. Electrical excitation of an interneuron evoked by a pulse of depolarizing current (marked by short black line). The calibration voltage shows 50 mV; the time scale bar shows 2 sec [Arshavsky et al., 1986]. *G*) Effect of stimulation of a serotoninergic command neuron in the cerebral ganglion (the stimulation period is shown by the black line) on the activity of a pedal motoneuron and interneuron (intracellular recording). Isolated CNS preparation; the dotted line shows the baseline membrane potential. The voltage calibration shows 100 mV; the time scale bar shows 1 sec [Panchin et al., 1996].

ternal perturbations (*a resistance reflex to imposed movements*). However, during locomotion, activation of the chordotonal organ leads to excitation of flexor motoneurons, which helps maintain their activity in the swing phase of the cycle [Bässler, 1993]. As noted above, data of this type show that at least in vertebrates and higher invertebrates, the central and reflex mechanisms interact with each other, ensuring reliable control of movements.

Not only locomotor, but also other conditional CPGs are activated by higher centers by means of simple commands. For example, two closely located, albeit not identical, areas have been identified in the frontal lobe of the cerebral cortex in several mammal species. Stimulation of these areas evokes rhythmic chewing or swallowing movements, as well as fictive chewing or swallowing, respectively [Morguette et al., 2012; Sumi, 1969]. Command signals are transmitted to the medulla oblongata via glutamatergic pathways. Accordingly, the command effect can be reproduced by injecting glutamate or its agonists into the corresponding areas of the medulla oblongata (Fig. 4, *A–C*).

Mechanisms of Operation of Central Pattern Generators

Although CPG control not only rhythmic, but also single movements (for example, standard defense reactions [Arshavsky et al., 1994] or swallowing movements which, depending on the nature of the food, can be either single or rhythmic), the mechanism of activity has been studied mainly for CPG controlling rhythmic movements. An understanding of the neuronal mechanisms of CPG operation presupposes answering to two basic questions:

1. What is the mechanism of the origin of rhythmic activity in the frequency range determining mechanical characteristics of executive motor apparatuses?

2. What is the mechanism forming the motor output (number of phases in the cycle, transfers from one phase of the cycle to the next, distribution of activity to motoneuron pools at different phases of the cycle)?

Before the 1980s, the question of the origin of rhythmic activity was usually formulated as follows. The rhythmic activity typical of a given CPG arises either as a result of complex excitatory and inhibitory interactions between neurons which themselves are able to generate only continuous spike trains or are the result of the endogenous (pacemaker) activity of specific neurons within the CPG. The atmosphere in this field started to change in the 1980s. Detailed investigation of a number of CPGs in invertebrates and vertebrates showed that this strictly alternative approach to the problem – either interneural interaction or pacemaker – is incorrect. These two mechanisms are not mutually exclusive, but supplement each other. Before describing some of the central pattern generators in vertebrates, we will provide a brief illustration of this point using the locomotor CPG of the pteropod mollusk *Clione* as an example.

The locomotor CPG of the mollusk *Clione limacina*. The marine mollusk *Clione* (Fig. 2, *A*) swims by rhythmic movements with its pair of wings at a frequency of 1–5 Hz [Arshavsky et al., 1982; Arshavsky et al., 1985, 1998, 1993]. Each locomotor cycle consists of two phases – dorsal and ventral flexion of the wings. The motoneurons active in the dorsal and ventral phases of the cycle (Fig. 2, *B*) are located in the pedal ganglia. The basis of the CPG consists of two antagonistic groups of interneurons – 7 and 8 – that are active in the dorsal and ventral phases of the cycle, respectively (Fig. 2, *C*, *D*). These two groups of interneurons inhibit each other, such that they operate in antiphase. Interneurons 7 and 8 generated long (~100 msec) tetrodotoxin-resistant action potentials – one per cycle (Fig. 2, *C*). These action potentials, as well as the inhibitory postsynaptic potential elicited by them, determine the durations of the phases of the locomotor cycle. It is interesting to note that in young mollusks, which for mechanical reasons wave their wings at a higher frequency than adult individuals, the duration of the action potentials generated by interneurons is ~40 msec.

Interneurons in each group are electrically connected with each other. These connections support the synchronous excitation of interneurons of both ganglia in the corresponding phase of the cycle. Interneurons of groups 7 and 8 excite motoneurons innervating the dorsal and ventral wing muscles, correspondingly, and also inhibit the antagonistic motoneurons. The synchronous activation of synergistic motoneurons is determined not only by their common input, but also by their electrical connections (interneural connections are diagrammed in Fig. 2, *D*).

Naturally, the locomotor CPG in *Clione* is a conditional CPG. Its activity is initiated by cerebropedal command neurons. The two groups of command neurons use serotonin as transmitter. Experimental stimulation of these neurons evokes locomotor activity in resting preparations or increases the frequency of ongoing activity (Fig. 2, *G*). One of the functions of serotoninergic command neurons is the defensive swimming acceleration reaction ("avoidance reaction") evoked by stimulation of tactile receptors in the tail. In addition, one neuron was identified in each cerebral ganglion which initiates the activity of different motor systems, including the locomotor CPG, which are involved in hunting behavior in *Clione*. The presumptive transmitter used by hunting command neurons is acetylcholine as fictive hunting behavior can be induced in vitro by a cholinesterase inhibitor.

Interneurons of both groups are "conditional" (also termed "latent") pacemakers. After isolation from ganglia, they continue, under the influence of serotonin, to generate periodic action potentials with a frequency close to the frequency of the locomotor rhythm (Fig. 2, *E*2, *F*). Each neuron discharge is preceded by a slow rise in membrane potential (the pacemaker prepotential). Additional excitation of isolated interneurons leads to a shift in the phase of the rhythmic activity just as occurs in the heart during a sinus extrasystole (Fig. 2, *F*). It is important to note that depolarization of isolated interneurons, in contrast to the action of

serotonin, induces single discharges, but not rhythmic activity (Fig. 2, *E*1). Correspondingly, the increase in the frequency of the locomotor rhythm evoked by stimulation of serotoninergic command neurons is not accompanied by depolarization of interneurons (Fig. 2, *G*). In this respect, interneurons differ from motoneurons, in which stimulation of command neurons evokes depolarization leading to increases in their discharges in the corresponding phase of the cycle (Fig. 2, *G*). These results suggest that the effect of serotonin on interneurons is mediated not by ionotropic but by metabotropic receptors. This produces a cascade of reactions altering the properties of the membranes of pacemaker cells, converting them to the rhythm-generating state.

Although the main role in generating the locomotor rhythm is played by the pacemaker properties of interneurons, interneural connections also make their contribution to this process. One of these "network mechanisms" is the mutual inhibition of antagonistic interneurons, which not only supports their excitation in antiphase, but also, due to post-inhibitory rebound, ensures a reliable transfer from one phase of the cycle to the next. Rebound is based on the inward current I_h (the hyperpolarization-activated inward current). Another "network mechanism" is associated with the existence of group 12 interneurons (Fig. 2, *C*). These are non-spiking neurons, which generate prolonged plateau-like potentials. Type 12 interneurons are included in CPG operation only during intense swimming, when the probability of rhythm disruptions increases. As shown in Fig. 2, *D*, these cells receive excitatory inputs from type 8 interneurons and inhibitory inputs from type 7 interneurons. Depolarized, type 12 interneurons have a recurrent inhibitory influence on type 8 interneurons and an excitatory influence on type 7 interneurons. Thus, in intense swimming, type 12 interneurons drive the end of the ventral and initiation of the next dorsal phase of the cycle.

These results lead to the following conclusions regarding the mechanism of CPG operation:

1) The basis of CPGs is formed by interneurons that control motoneuron activity.

2) Rhythmic activity of CPGs is based on the endogenous properties of pacemaker interneurons.

3) The pattern of motor output is formed as a result of interneural interactions leading to synchronous excitation of synergistic neurons and alternative excitation of neurons active in different phases of the cycle. This does not mean that the generation of rhythmic activity and the formation of the motor output are independent of each other, as pacemaker interneurons are included in the pattern-forming network.

4) Although pacemakers play the main role in generating the rhythm, interneural interactions also contribute to maintaining rhythmic activity and ensuring reliable transfer from one phase of the cycle to the next.

As will be shown below, these conclusions, based on studies of the control of swimming movements in such an animal as the primitive pteropod mollusk *Clione* are relevant to understanding of the mechanisms of CPG operation in vertebrates. Before moving on to vertebrates, we will make two comments regarding the universality of these conclusions. The usually cited example incompatible with the first conclusion are the pyloric and gastric CPGs controlling rhythmic contractions of the stomach in crustacea that are mainly formed by motoneurons [Marder and Calabrese, 1996; Selveston, 2005, 2010]. However, neurons in the somatogastric ganglion in crustacea can only formally be termed motoneurons. These cells have a very complex organization, consisting of three main parts – body, huge dendritic tree, and axon – which perform different functions. The bodies of these neurons have practically no active ion channels and, therefore, perform a purely trophic function. Most of the active ion channels are located on the dendrites, which play the main role in generating the pyloric and gastric rhythms. Furthermore, interneural interactions are mediated mainly by dendro-dendritic synapses. Therefore, dendrites perform the functions of the interneurons that are formally absent from the stomatogastric ganglion. As regards the axon, this transmits normal spikes to muscles and is, therefore, the only part of the cell functioning as a motoneuron.

Another objection is related to the second conclusion. The disproving example here is the locomotor CPG of the mollusk *Tritonia*, which generates rhythmic activity as a result of the interaction of several groups of cerebral neurons, which themselves are not pacemakers [Getting, 1989]. However, in reality, *Tritonia*, unlike *Clione*, does not generate a stable rhythm. "Swimming" in *Tritonia* arises in response to contact with a starfish and consists of several rhythmic ventrodorsal flexions of the trunk. As a result, the mollusk is lifted off the ground and the water flow carries it away from the starfish. The generation of several cycles probably does not require any pacemaker and can be based solely on interneural interactions.

CPG controlling electric organ discharges in gymnotiform fish. South American fish of the order *Gymnotiformes* use electric organ discharges, with frequencies ranging in different species from 50 to several hundred Hz, for social communication and electrolocation. The CPG inducing electric organ discharges is a constitutive generator, as these discharges continue throughout the whole life. The CPG is located in the medulla oblongata [Heiligenberg, 1991]. The main role in the operation of these CPG is played by short-axon interneurons. In isolated CNS preparations these generate continuous spike discharges at a frequency corresponding to the discharge frequency of the electric organ. These interneurons have been suggested to be the constitutive endogenous pacemaker, as each spike is preceded by a slow pacemaker prepotential. In contrast, the pacemaker prepotential is not seen in the projection neurons sending axons to the spinal cord, where they activate electromotor neurons.

A characteristic feature of this generator is that it is a monophasic CPG. Each cycle of its rhythm includes just one

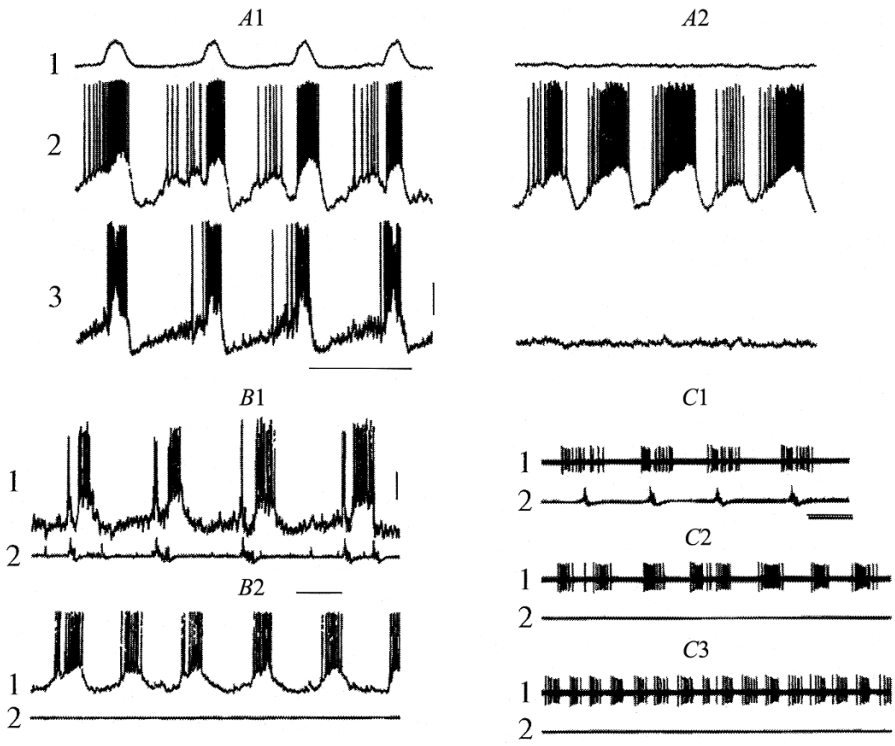

Fig. 3. Mammalian respiratory CPG. *A*) Patch clamp recording of two inspiratory interneurons (2 and 3) and integral extracellular recording of inspiratory interneurons before (*A*1) and after (*A*2) pharmacological blockade of excitatory and inhibitory synapses (see text). Transverse medulla oblongata slice from neonatal mouse. The calibration current is 1 nA and the time scale bar is 4 sec [Peña et al., 2004]. *B*) Intracellular recording of an inspiratory interneuron (*1*) before (*B*1) and after (*B*2) immersion of preparation consisting of brainstem and spinal cord from a neonatal rat into solution containing a low Ca^{2+} concentration (0.2 mM) and a high Mg^{2+} content (5.0 mM); *2*) recording from 4th anterior cervical root. The calibration voltage shows 10 mV; the time scale bar shows 5 sec [Onimaru et al., 1995]. *C*) Extracellular recording of an inspiratory interneuron (*1*) before (*C*1) and after (*C*2 and *C*3) use of solution containing low Ca^{2+} and high Mg^{2+}, same preparation. *C*3) Effect of decrease in solution pH from 7.4 to 7.1. The time scale bar shows 5 sec (Onimaru et al., 1989].

active phase. The interneural interactions within the CPG are organized in an extremely simple manner. Pacemaker interneurons are electrically connected to each other, and this determines their synchronous activity. After uncoupling of electrical connections by increasing the intracellular Ca^{2+} concentration, pacemakers discharge independently of each other. Pacemaker interneurons excite projections neurons via electrical and chemical synapses. There are no inhibitory interactions between neurons in the CPG controlling the operation of the electrical organ. It should be noted that other monophasic CPG – the CPG controlling cardiac functioning in crustacea [Cooke, 2002] and the locomotor CPG of medusas [Satterlie, 2002] – also lack inhibitory interactions between neurons.

CPG controlling respiration in mammals. Another example of a constitutive generator is provided by the respiratory CPG in vertebrates. Recently, significant progress has been achieved in understanding the mechanism of respiratory rhythm generation in mammals [Feldman et al., 2013; Ramirez et al., 2011; Rybak et al., 2014; Smith et al., 2000]. We will restrict ourselves to a description of quiet respiration when the respiratory center operates in the regime of a monophasic CPG generating only the inspiratory phase of the cycle, whereas the expiratory phase is passive.

The respiratory CPG is located in the ventrolateral area of the medulla oblongata. Most data on the operation of the respiratory CPG have been obtained in rodents. Isolated nervous system preparations from neonatal animals, consisting of the medulla oblongata and spinal cord or transverse slices (0.2–0.6 mm) of medulla oblongata have been used. Despite the strong reduction in the neural network forming the respiratory CPG, medulla oblongata slices continue to generate a stable respiratory rhythm. A group of interneurons was identified which is active during the preinspiratory and inspiratory phases of the cycle. These interneurons form both bidirectional excitatory connections with each other via glutamate synapses and electrical connections. These connections determine simultaneous excitation of interneurons in the inspiratory phase of the cycle. Inspiratory interneurons send signals to diaphragmatic motoneurons, motoneurons of the XII nerve, and descending neurons running to motoneurons controlling the external intercostal

muscles. Furthermore, they excite one more group of interneurons, active at the boundary of the inspiratory and expiratory phases of the cycle. These are inhibitory cells using γ-aminobutyric acid (GABA) as transmitter. These neurons elicit recurrent inhibition of excitatory interneurons and, therefore, facilitate termination of the inspiratory phase. Blockade of $GABA_A$ receptors with bicuculline increases the duration of the inspiratory phase of the cycle.

Experiments with functional uncoupling of interneural synaptic connections showed that some of the inspiratory interneurons are endogenous pacemakers. Synaptic transmission was blocked by using various experimental approaches. One of these was application of physiological saline containing both specific antagonists of excitatory receptors – CPP (3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid) and CNQX (6-cyano-7-nitroquinoxaline- 2,3-dione) – which block of excitation in NMDA-sensitive and NMDA-insensitive glutamate synapses, and antagonists of inhibitory receptors – bicuculline and strychnine (Fig. 3, *A*). This solution suppresses interneural interactions in the respiratory CPG, as evidenced by disappearance of overall respiratory activity recorded with extracellular electrodes from the ventrolateral areas of medulla oblongata slices (Fig. 3, *A*2, upper trace). Some inspiratory interneurons completely lost their rhythmic activity (Fig. 3, *A*2, lower cell), while other interneurons continued to generate rhythmic discharges at a frequency close to the respiratory frequency (Fig. 3, *A*2, upper cell). One can see that the discharges of the pacemaker neuron are preceded by the slowly growing depolarization. The somewhat higher frequency of the endogenous activity of the pacemaker interneuron than the initial respiratory rhythm can be explained by the absence of recurrent inhibition from interneurons active at the boundary between the inspiratory and expiratory phases of the cycle.

The existence of endogenous pacemakers in the respiratory CPG was also demonstrated by using another method of uncoupling interneural interactions – immersion of preparations in solutions with decreased Ca^{2+} ion concentrations and increased Mg^{2+} ion concentrations (Fig. 3, *B*, *C*). After blockade of synaptic transmission (evidenced by the disappearance of the respiratory rhythm recorded from the ventral root C4), some inspiratory interneurons continued to produce rhythmic activity (Fig. 3, *B*2 and *C*2). The frequency of endogenous activity increased in response to a decrease in the solution pH simulating an increased CO_2 concentration (Fig. 3, *C*3). The same result – continuation of rhythmic activity in some inspiratory interneurons and an increase in their discharge frequency in response to decreased solution pH – was obtained in experiments with blockade of synaptic transmission by Cd^{2+} ions [Koizumi et al., 2010].

The ionic mechanisms underlying the pacemaker activity of inspiratory neurons have received insufficient study. It has been suggested that the so-called persistent sodium current (I_{NaP}) plays an important role in rhythm generation. In contrast to the fast sodium current underlying generation of action potentials, I_{NaP} has a lower threshold of activation and is inactivated more slowly. Sustained depolarization induced by the persistent sodium current produces a prolonged spike discharge by the neuron. When interneural interactions are blocked, termination of discharges results from inactivation of I_{NaP} and/or development of the slowly activating potassium current (I_{KS}). Then, after completion of inactivation of I_{NaP} and inactivation of I_{KS}, reactivation of I_{NaP} occurs, which leads to the end of the expiratory phase and the beginning of a new cycle. The speed of I_{NaP} activation and inactivation depends on the neuron membrane potential and, probably, other factors, such as the CO_2 and O_2 concentrations and pH. There is one more current involved in generating the rhythmic activity of respiratory pacemaker neurons – the calcium-dependent nonspecific cation current (I_{CAN}). Experimental results have been obtained suggesting that the I_{NaP} and I_{CAN} currents are used in two different neuron populations [Peña, 2004]. However, there are no data showing whether these two populations have different functional roles or they simply duplicate each other.

Thus, the results described in this section lead to the conclusion that generation of the respiratory rhythm is based on the endogenous activity of inspiratory pacemaker interneurons. Reliable operation of the respiratory CPG is ensured by the fact that pacemaker interneurons excite each other, maintaining a high level of activity during the inspiratory phase of the cycle. The end of the inspiratory phase is determined not only by the final duration of pacemaker discharges, but also by recurrent inhibition provided by the group of cells active at the end of the inspiratory phase. During intensive respiration, when both phases of the cycle are active, an additional respiratory CPG in a different location starts to function [Feldman et al., 2013]. However, both the mechanisms of operation of this CPG and its interaction with the respiratory CPG located in the ventrolateral area of the medulla oblongata have received insufficient study. For this reason it is not addressed in the present review.

CPG controlling swallowing movements in mammals. Another quite well studied CPG located in the medulla oblongata is the CPG controlling swallowing movements in mammals [Jean, 2001]. Swallowing is an extremely complex stereotypical act involving more than 25 pairs of muscles in the mouth, pharynx, larynx, and esophagus. Swallowing movements may be single or rhythmic. The latter are particularly typical of neonates taking mother's milk. In experiments, rhythmic swallowing – real or fictive – can be elicited by stimulation of the internal branch of the superior laryngeal nerve or the command area located in the frontal lobe of the cerebral cortex (see above).

The swallowing CPG consists of two parts, located in the dorsal and ventral areas of the medulla oblongata. The dorsal part is located in the solitary tract nucleus and adjacent reticular formation. This part plays the main role in generating the swallowing rhythm. This area receives signals from afferent fibers running in the superior laryngeal

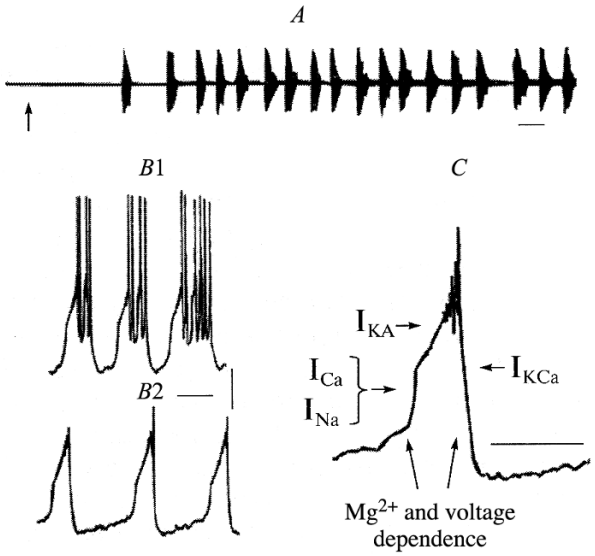

Fig. 4. The mammalian swallowing CPG. *A*) Rhythmic swallowing evoked by microinjection of NMDA (50 pM) into the solitary tract nucleus of a rat (the arrow shows the beginning of injection) recorded from the superior suprahyoid muscle [Jean, 2001]. *B*) Intracellular recording of a neuron in the solitary tract nucleus in a transverse slice of the medulla oblongata of a rat before (*B*1) and after (*B*2) exposure to tetrodotoxin (2 μM), fictive swallowing evoked by application of NMDA solution (60 μM). The calibration voltage shows 25 mV. Modified from [Tell and Jean, 1993]. *C*) Ion currents underlying the pacemaker activity of generator neurons initiated by activation of NMDA receptors [Tell and Jean, 1993]. The time scale bar shows 1 sec.

nerve and from the cerebral command areas, i.e. signals transforming the CPG from the passive to the active state. Both the cerebromedullary command pathways and the afferent inputs use glutamate as transmitter. Injection of glutamate or its agonists into the dorsal part of the CPG also elicits rhythmic swallowing (Fig. 4, *A*). In contrast, injection of glutamate receptor antagonists – CNQX and APV (DL-2-amino-5-phosphonovalerate) – blocks the ability of the superior laryngeal nerve to evoke reflex swallowing.

The swallowing CPGs located in the right and left halves of the medulla oblongata can work independently of each other. After longitudinal sectioning of the medulla oblongata, stimulation of one of the laryngeal nerves induces unilateral swallowing movements. The mechanism of the concordant operation of the right and left CPG remains unclear. As afferent fibers in the superior laryngeal nerve terminate only ipsilaterally, it was suggested that their synchronous activity is due to bilateral connections between the two dorsal parts of the CPG [Jean, 2001].

The most important data on the mechanism generating the swallowing rhythm were obtained in experiments on rat medulla oblongata slices through the solitary tract nucleus [Tell, 1993]. The results of these experiments showed that generation of the swallowing rhythm is based on the pacemaker activity of the neurons in the dorsal part of the CPG. As shown in Fig. 4, *B*1, immersion of the brain slice in solution containing NMDA evoked generation of the swallowing rhythm recorded from solitary tract nucleus neurons. After addition of tetrodotoxin to the solution to block spike activity and, therefore, interneural interactions, the neuron continued to generate rhythmic oscillations in the membrane potential (Fig. 4, *B*2).

Figure 4, *C* shows the main ion currents underlying the pacemaker activity of neurons in the swallowing CPG evoked by activation of NMDA receptors. NMDA receptors include an ion channel, which at rest is blocked by Mg^{2+} ions. Channel blockage by Mg^{2+} ions is voltage-dependent. Activation of NMDA receptors, leading to a minor and slow depolarization of neurons, unblocks these channels. This leads to rapid depolarization due to inward Na^+ and Ca^{2+} currents (the I_{Na} and I_{Ca} currents in Fig. 4, *C*). These inward currents are opposed by the outward potassium current (I_{KA}). This current leads to a delay of spike discharges in pacemaker neurons. It is interesting that the strength of the I_{KA} current differs in different neurons. This suggests that, along with the organization of interneural connections, this mechanisms determines the sequential recruitment of neurons responsible for different phases of the swallowing cycle. Influx of Ca^{2+} ions into cells activates the Ca^{2+}-dependent potassium current (I_{KCa}), which leads to a drop in the membrane potential to the initial hyperpolarized level and, thus, to blockade of NMDA channels by Mg^{2+} ions. The intracellular Ca^{2+} concentration then decreases and Ca^{2+}-dependent potassium permeability is inactivated allowing the next cycle to start.

Signals from the dorsal part of the swallowing CPG reach the ventral part of the CPG, which is adjacent to the nucleus ambiguus. This part performs a distributive function. From here, signals are sent to motoneurons located in the nuclei of the V, VII, IX, X, and XII cranial nerves and to segments C1–C3 of the spinal cord. In contrast to the mechanism generating rhythmic activity, the details of interneural interactions in both the dorsal and ventral parts of the CPG have received insufficient study. The question of the mechanism of formation of the motor output underlying swallowing will not, therefore, be discussed.

Although the pattern of muscle activity during swallowing is determined mainly by central signals, reflex mechanisms also participate in the formation of the motor output. Both acute and chronic experiments have shown that the amplitude and duration of muscle contractions to some extent depend on the size and consistency of the portion of food swallowed. The effects of signals from peripheral receptors are largely realized at the level of the swallowing CPG [Jean, 2001].

The locomotor CPG of the lamprey. The mechanism generating the locomotor rhythm in vertebrates has been studied in most detail in the cyclostomata (lampreys), amphibia (embryos of *Xenopus laevis*) and mammals (cats, mice, rats). We will start with the lamprey. Lampreys swim by making undulating movements of the trunk (Fig. 5, *A*),

which result from alternating contractions of antagonistic lateral muscles in each segment. Undulation frequency in lampreys about 15 cm long ranges from 1 to 10 Hz. At lower frequencies, only superficial red muscle contracts, while the deeper white muscle is involved at higher frequencies.

As already noted, swimming in lampreys is initiated and maintained by mesencephalic and diencephalic command areas, signals from which reach the spinal cord via glutamatergic reticulospinal fibers [Buchanan, 2001; Grillner, 2003, 2006; Grillner et al., 1995; Orlovsky et al., 1999]. The locomotor CPG is spread along the spinal cord. In experiments on isolated spinal cord, the CPG could be activated by application of glutamate on its agonists (Fig. 5, *B*). The effect can be obtained on preparations consisting of 1.5–2 segments isolated from different parts of the spinal cord. Figure 5, *C* diagrams the organization of the segmental CPG, which consists of two symmetrical neural networks which inhibit each other ("two hemicenters" according to Brown's terminology [Brown, 1911]). The central role in generating rhythmic activity is played by glutamatergic excitatory interneurons (E cells in Fig. 5, *C*), which respond to command signals by transferring to the rhythm-generating state. These interneurons excite each other increasing the effectiveness of command signals arriving via glutamatergic reticulospinal fibers. In addition, glutamatergic interneurons excite all other spinal neurons – motoneurons, inhibitory interneurons (I cells in Fig. 5, *C*), which send their axons to the opposite half of the brain, lateral inhibitory interneurons found only in the rostral part of the spinal cord (L cells in Fig. 5, *C*), as well as other interneurons not shown in the diagram.

Commissural inhibitory interneurons determine the reciprocal activity of the two hemicenters of the segmental CPG (Fig. 5, *C*). These are not necessary for generating rhythmic activity. This was demonstrated in experiments with longitudinal sectioning of the spinal cord or with pharmacological blockade of inhibitory effects. After longitudinal sectioning and removal of one of the halves of the spinal cord, the remaining half retained the ability to generate rhythmic activity in response to application of glutamate agonists. Similarly, the ability to generate the rhythm persisted after blockade of inhibition by strychnine. Elimination of the effects of inhibitory interneurons led, as expected, to an increase in the frequency of the rhythmic activity. Commissural interneurons, in turn, receive inhibitory inputs from L cells. As L cells have low excitability, they are activated at the end of the phase of the locomotor cycle. Therefore, L cells do not influence on the commissural interneurons at the beginning of the phase of the cycle, but inhibit these cells at the end of the phase. This leads to disinhibition of the contralateral hemicenter and, thus, promotes the onset of the opposite phase of the locomotor cycle.

Excitatory interneurons are conditional pacemakers. This is apparent in Fig. 5, *D*, which shows intracellular recording of a presumptive excitatory interneuron during fictive swimming induced by application of NMDA. After addition of tetrodotoxin, leading to complete suppression of spike activity (as evidenced by the disappearance of spikes not only in the interneuron itself, but also in the ventral root of the spinal cord), the neuron continued to generate rhythmic oscillations in the membrane potential at a frequency close to the frequency of the initial locomotor rhythm (Fig. 5, *D*2). It should be noted that other spinal neurons, including motoneurons, also displayed endogenous rhythmic activity in response to glutamate or its agonists. It can be suggested that

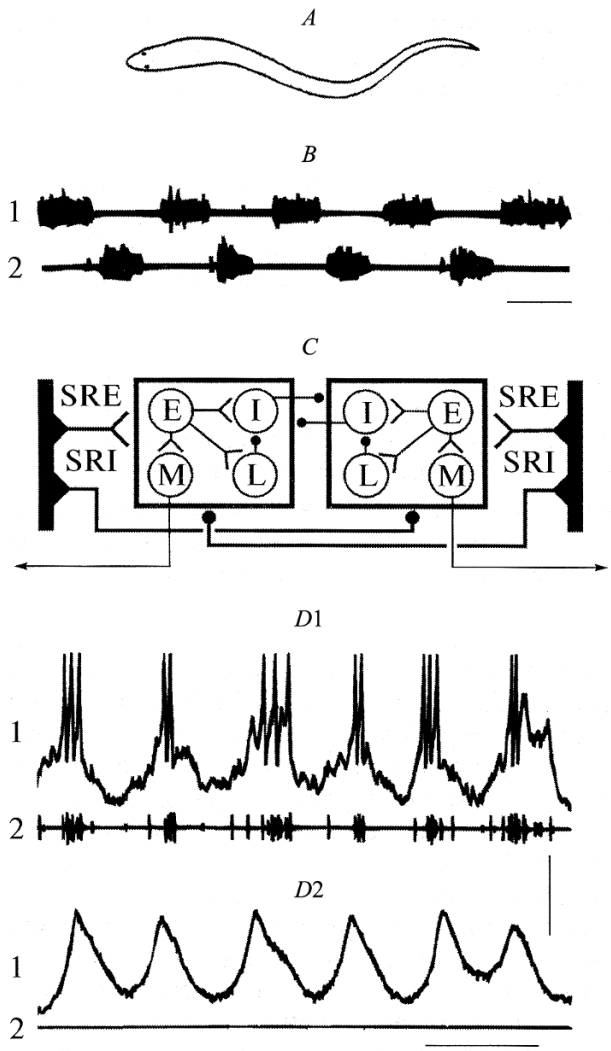

Fig. 5. The locomotor CPG of the lamprey. *A*) Diagram showing swimming in lampreys. *B*) Fictive locomotion evoked by application of NMA solution (100 μM); isolated spinal cord preparation. *1* and *2*) ENG recorded from the right (*1*) and left (*2*) anterior roots [Brodin et al., 1985]. *C*) Diagram of interneural connections in the locomotor CPG including connections formed by stretch receptors in the spinal cord. E – excitatory interneuron; I – commissural inhibitory interneuron; L – inhibitory interneuron; SRE and SRI – excitatory and inhibitory stretch receptors [Grillner et al., 1995]. *D*) Intracellular recording of a presumptive excitatory interneuron (*1*) before (*D*1) and after (*D*2) exposure to tetrodotoxin (3 μM). Isolated spinal cord preparation, fictive swimming evoked by application of NMDA (150 μM); 2) ENG from anterior root. The calibration voltage shows 10 mV [Wallén and Grillner, 1987]. The time scale bar shows 1 sec.

this ability to produce intrinsic rhythmic activity allows them to follow signals from excitatory interneurons more reliably.

The ionic currents underlying neuron pacemaker activity arising in response to activation of NMDA receptors are analogous to the currents described for pacemaker neurons in the swallowing CPG (Fig. 4, *C*). Interaction of NMDA with receptors induces slow neuron depolarization leading to unblocking of the receptor channel. This results in inward Na^+ and Ca^{2+} currents leading to rapid depolarization, which reaches the level of the equilibrium potential. With increases in the intracellular Ca^{2+} concentration, Ca^{2+}-dependent potassium channels open, activating an outward K^+ current which leads to slow repolarization of the neuron. When the membrane potential reaches a critical level, voltage-dependent potassium channels open. This leads to rapid hyperpolarization of the neuron to the level of the potassium equilibrium potential. The intracellular Ca^{2+} content, which maintains high permeability for K^+ ions, then decreases, and NMDA again induces slow depolarization of the neuron; this leads to the onset of the new cycle. Suppression of Ca^{2+}-dependent potassium permeability with apamin leads to an increase in the duration of the plateau-like period of depolarization and, thus, to a decrease in the frequency of NMDA-evoked rhythmic activity [Grillner, 2003, 2006; Grillner et al., 1995].

Activation of AMPA receptors in spinal cord preparations elicits the generation of a higher-frequency locomotor rhythm than activation of NMDA receptors does. The resulting ion currents are independent of Mg^{2+} ions.

During real swimming in lampreys, one more type of neurons – the edge cell – participates in organization of the motor output. The bodies of these neurons are located among the axons of the lateral tracts, while the terminals of their dendrite branch at the lateral surface of the spinal cord. As marginal cells are located within the spinal cord, they must formally be regarded as interneurons [Buchanan, 2001]. Meanwhile, edge cells operate as stretch receptors responding to changes in the curvature of the spinal cord. Thus, functionally these cells are analogous to the peripheral joint and tendon stretch receptors in mammals.[1] There are two types of receptor cell – excitatory glutamatergic cells, whose axons terminate in the ipsilateral half of the spinal cord, and inhibitory glycinergic cells, which send their axons to the contralateral half of the cord (SRE and SRI in Fig. 5, *C*). Excitatory receptors are, thus, synergists of generator interneurons (E cells in Fig. 5, *C*), while inhibitory receptors are synergists of commissural interneurons (I cells in Fig. 5, *C*). It is interesting to note that the locomotor generator imposes presynaptic control of the effectiveness of afferent signals coming from receptor cells depending on the phase of the cycle. This control is mediated via GABAergic interneurons that are located around the central aqueduct of the spinal cord and receive signals from generator interneurons. As a result, the locomotor generator blocks all incidental afferent inputs except for those coming in the "correct" phase of the cycle.

It should be emphasized that even during quiet, undisturbed real swimming in lampreys, switching from one phase of the cycle to another occurs not only due to the internal activity of the locomotor CPG, as in fictive swimming, but mainly due to afferent signals coming from edge cells. This is another example showing that the central and reflex mechanisms supplement each other ensuring a high level of reliability of locomotor control at the spinal level.

We will now move on from describing the segmental generator to intersegmental interactions. The spinal cord in lampreys consists of about 100 segments. During swimming, there is sequential propagation of a wave of activity along the spinal cord in the rostrocaudal direction that produces a directed forward driving force (Fig. 5, *A*). The absolute duration of the intersegmental delay is not constant, but depends on the frequency of undulations. At any swimming speed, the phasic intersegmental delay is approximately 1% of the duration of the cycle. This means that the body of the lamprey always looks like one sinusoidal wave (Fig. 5, *A*). Intersegmental interactions occur due to horizontal connections formed by the excitatory interneurons in the CPG. Collaterals of these interneurons run across several segments in both the rostral and especially the caudal directions. The intersegmental delay is determined by a rostrocaudal gradient of reticulospinal influences, which controls the intrinsic frequencies of segmental CPGs. Activation of each sequential segment, therefore, depends not only on the intrinsic characteristics of its CPG, but also on the glutamatergic excitatory inputs from one or several preceding segments.

In natural conditions, lampreys can swim not only forward, but also backward. In experiments on isolated spinal cords, changes in the direction of propagation of periodic waves from the rostrocaudal to the caudorostral could be obtained by applying higher concentrations of glutamate agonists to the caudal segments. This suggests that in natural conditions, backward swimming is also due to creation of an opposite gradient of reticulospinal influences.

When lampreys swim forward without backward turning, reticulospinal neurons exert identical influences on both halves of the spinal cord, leading to symmetrical activation of the hemicenters of the segmental CPGs. Lamprey's lateral turns occur due to asymmetrical reticulospinal influences on the hemicenters of the segmental CPGs. This leads to increases in the frequency and duration of discharges of excitatory neurons in the corresponding hemicenters, resulting in flexion of the lamprey to the side receiving the more intense reticulospinal influences. Asymmetry in hemicenter activity is enhanced by the fact that commissural interneurons produce stronger inhibition of cells on the opposite side.

[1] This is one of many examples illustrating the relative conventionality of our terms. In the same way as it is believed that the retina does not relate to the peripheral nervous system, but rather is a part of the CNS located at the periphery, edge cells can be regarded as part of the peripheral nervous system located within the spinal cord.

Another mechanism is involved in the case of dorsoventral turns. This involves a special group of reticulospinal neurons. The excitatory influences of these neurons address not the segmental CPG as a whole, but exclusively motoneurons innervating the dorsal or ventral musculature, depending on the direction of the turn. The effect of reticulospinal neurons is realized only during activity of motoneurons controlled by the locomotor CPG. Thus, during swimming, supraspinal centers can change the direction of a lamprey's movement both via asymmetry in the activity of segmental CPG and via changes in the activity of specific groups of motoneurons [Deliagina et al., 2002].

The locomotor CPG of the clawed frog (*Xenopus laevis*) embryo. *Xenopus* embryos extracted from eggs not long before natural hatching are about 5 mm long. They are usually in a resting state, attached to the substrate by mucus produced by the so-called cement gland. Swimming occurs when a shadow falls on the tadpole or in response to touching, and can continue for tens of seconds. As in lampreys, swimming occurs as a result of undulating body movements at a frequency of 10–25 Hz (Fig. 6, *A*). Undulations arise as a result of alternating contractions of the right and left lateral segmental muscles. Waves of muscle contractions propagate only in the rostrocaudal direction.

Most data on the mechanism of operation of the locomotor CPG have been obtained in studies of fictive swimming of tubocurarine-immobilized *Xenopus* embryos [Arshavsky et al., 1993; Li, 2011; Li et al., 2010; Roberts et al., 2008; Soffe and Roberts, 1982]. The locomotor CPG in the tadpole is located not only in the spinal cord, but also in the caudal part of the brainstem. One method producing fictive swimming initiation was by stimulating the skin in the posterior part of the body (Fig. 6, *B*, *C*). Since this effect is mediated by glutamatergic mechanisms, another method of initiating fictive swimming was by direct application of glutamate or its agonists to the nervous system.

The organization of the CPG in the tadpole is similar to that in the lamprey. It also consists of two antagonistic hemicenters located in the right and left halves of the spinal cord. Each hemicenter includes three main groups of neurons – excitatory interneurons, commissural inhibitory interneurons, and motoneurons. Segmental excitatory interneurons activate each other, as well as inhibitory interneurons and motoneurons. As in the lamprey, excitatory interneurons use glutamate as transmitter. Glycinergic inhibitory interneurons sending their axons to the opposite half of the brain determine the reciprocal activity of the antagonistic hemicenters (Fig. 6, *B*). The specific feature of motoneurons is that neighboring cells are electrically connected to each other. In addition to the common input from excitatory interneurons, this provides another mechanism promoting synchronization of the discharges of segmental motoneurons in the corresponding phases of the cycle.

As in many other cases, glutamatergic fibers form two types of synapse on spinal neurons, terminating either on AMPA/kainate or on NMDA receptors. Activation of AMPA/kainate receptors leads to the appearance of short excitatory postsynaptic potentials, while activation of NMDA receptors induces longer-lasting postsynaptic potentials. Summation of postsynaptic potentials due to activation of NMDA receptors leads to stable depolarization of spinal neurons during fictive swimming evoked by stimulation of tactile receptors (Fig. 6, *C*).

For a long time, attempts to detect endogenous rhythmic activity initiated by glutamate agonists in spinal neurons of *Xenopus* embryos were unproductive. This led to the conclusion that tadpoles lack pacemaker neurons, and that generation of their locomotor rhythm is based exclusively on interneural interactions – each sequential phase of the cycle arises as a result of rebound from the inhibition occurring in the previous phase (see also the section "The locomotor CPG of the mollusk *Clione limacina*"). Experiments using intracellular injections of short pulses of hyperpolarizing current showed that post-hyperpolarization rebound arises only at a particular level of neuronal depolarization. Therefore, it was suggested that the role of the command reticulospinal pathways is restricted by creating the stable depolarization of spinal neurons necessary for realizing the phenomenon of post-inhibitory rebound occurs. This concept of the origin of rhythmic activity was supported by results obtained from analysis of a computer model of the neural network simulating the tadpole locomotor CPR.

However, data have been obtained in recent years which have significantly altered our views of the mechanisms generating rhythmic activity in tadpole embryos [Li et al., 2010]. These studies showed that in tadpoles, in contrast to lampreys, excitatory pacemaker interneurons are not distributed along the spinal cord, but are located only in the caudal part of the brainstem. One feature of these interneurons is that they are connected to each other not only via glutamatergic synapses, but also electrically. The fact that excitatory interneurons in the hindbrain are pacemakers has been demonstrated in several series of experiments. For example, local application of glutamate or NMDA from a micropipette applied to the caudal part of the brainstem (diagram, Fig. 6, *D*1) evoked the rhythmic activity of interneurons at frequencies of 7–27 Hz occurring on the background of depolarization after addition of strychnine and gabazine to the solution, which block glycinergic and GABAergic receptors, respectively, and Cd^{2+} ions, which block other ionotropic receptors. In preparations treated with tetrodotoxin, microperfusion of the caudal part of the brainstem with NMDA solution also evoked rhythmic oscillations of the membrane potential in excitatory interneurons; the oscillation frequency was about 10 Hz. Oscillations of the membrane potential persisted after pharmacological uncoupling of the electrical connections between interneurons. Tetrodotoxin-resistant oscillations of membrane potential disappeared after preliminary perfusion of preparations with solution lacking Mg^{2+} ions (see

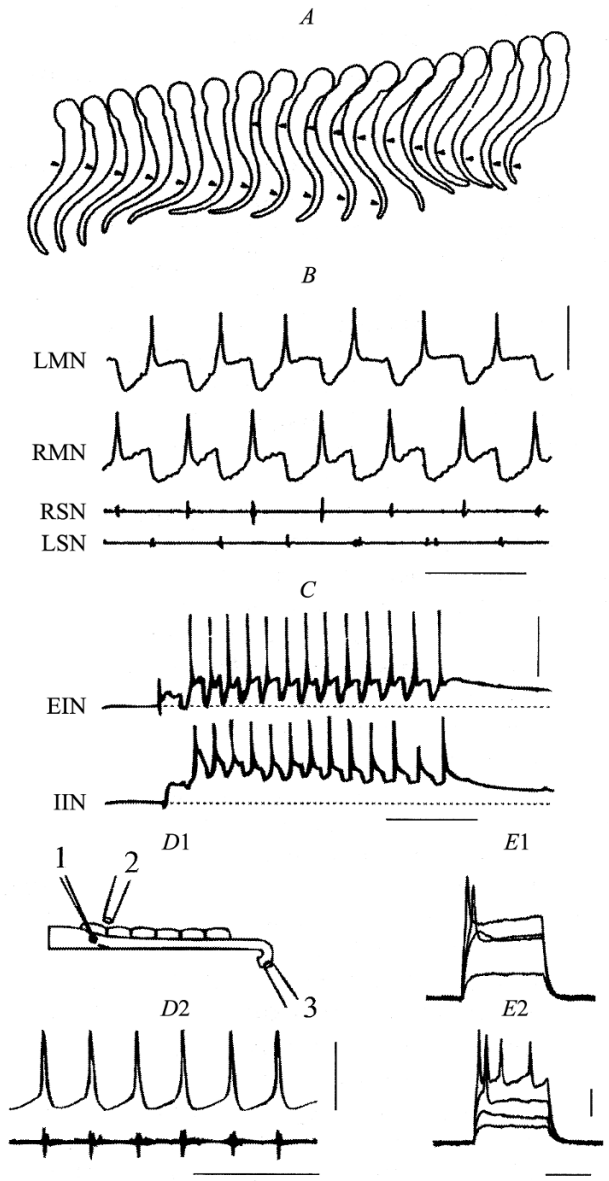

Fig. 6. The locomotor CPG in the *Xenopus* embryo. *A*) Diagram showing a swimming tadpole; the arrows show the propagation of the wave of undulation [Kahn et al., 1982]. *B*, *C*) Fictive swimming in an immobilized tadpole evoked by stimulation of the skin. *B*) Intracellular recording of the left (LMN) and right (RMN) motoneurons; RSN and LSN: ENG recorded from the right and left spinal nerves. *C*) Intracellular recording of excitatory (EIN) and inhibitory (IIN) interneurons; the dotted line shows the baseline membrane potential. The calibration voltage is 40 mV; the time scale bars show 100 and 200 mV, respectively [Arshavsky et al., 1993] and [Dale and Roberts, 1985]. *D*) A pacemaker interneuron from the caudal part of the brainstem. *D*1) Diagram showing the experiment on a longitudinally transected preparation. *1*) microelectrode; *2*) micropipette for local application of solutions; *3*) extracellular electrode. *D*2) Intracellular recording of an excitatory interneuron, fictive swimming evoked by local application of NMDA (100 μM); strychnine (1 μM) and gabazine (20 μM) were added to the solution to block possible inhibitory connections. The lower trace shows the ENG recorded in the caudal part of the preparation. The calibration voltage shows 50 mV; the time scale bar shows 200 msec [Li et al., 2010]. *E*) Responses of neurons to intracellular injection of a depolarizing current impulse in a frog embryo (*E*1) and a tadpole the day after hatching from the egg (*E*2). The voltage calibration shows 20 mV; the time scale bar shows 50 msec [Sillar et al., 1992].

the description of the mechanism of action of NMDA in the sections "CPG controlling swallowing movements in mammals" and "The motor CPG of the lamprey"). Tetrodotoxin-resistant oscillations in potential were either absent in motoneurons and other interneurons located in the brainstem or had much lower frequency. Finally, a series of experiments was performed on preparations consisting of the longitudinal halves of the caudal part of the brainstem and spinal cord (as illustrated in Fig. 6, *D*1). Although these preparations lacked reciprocal interactions between antagonistic hemicenters, local perfusion of the brainstem with NMDA solution evoked rhythmic activity in excitatory interneurons (Fig. 6, *D*2).

These observations leave no doubt that the main source of the locomotor rhythm in the *Xenopus* embryos is the activity of pacemaker interneurons located in the caudal part of the brainstem. Strictly speaking, only this area of the brain can be called the locomotor CPG in the narrow sense of the term. The spinal cord reproduces the incoming rhythm. This is clearly shown in Fig. 6, *D*2. Local application of NMDA evoked rhythmic neuron activity not only in the brainstem, but also in the spinal cord. In contrast to the lamprey, the absolute magnitude of the intersegmental delay in the *Xenopus* embryo is independent of the frequency of fictive swimming. This is additional evidence that in the *Xenopus* embryos the generator is not located in the spinal cord. The generator is located in the brain, from where rhythmic activity propagates along the spinal cord at a constant speed.

However, two points should be emphasized. First, the spinal cord does not simply reproduce the incoming rhythmic activity, but also, participates in organizing the motor output due to appropriate interneural connections at the segmental level. Second, evidence of the existence of pacemaker interneurons does not mean that the rebound phenomenon does not play any role in generating rhythmic activity. As described in the section on locomotion in the mollusk *Clione*, these two mechanisms interact with each other supporting the reliable operation of the locomotor CPG in swimming in natural conditions.

As already noted, the frequency of undulations in tadpole embryos of length about 5 mm extracted from eggs can reach 25 Hz. Such a high frequency results from the fact that both the motoneurons and the interneurons involved in controlling locomotion never generate series of spikes, but only one spike per cycle. Experiments with intracellular injections of pulses of current also demonstrated that artificial depolarization of neurons generated only single spikes (Fig. 6, *E*1). The inability of neurons to generate rhythmic discharges is due to long-term postspike hyperpolarization arising as a result of increased permeability to K^+ ions. For purely mechanical reasons, this high frequency of undulations cannot be maintained in growing tadpoles. Experiments on tadpoles of length about 8 mm (the day after natural hatching) showed that their neurons are already able to generate

rhythmic discharges both during fictive swimming and in response to artificial depolarization (Fig. 6, *E*2) because of decreased postspike hyperpolarization. This change in neuron membrane properties during tadpole development is due to the modulatory influences of serotoninergic brainstem-spinal tracts.

The locomotor CPG of mammals. The question of the command pathways eliciting locomotion in mammals was addressed above. Here we will focus on describing the CPG itself. It should be noted that when we refer to the locomotor CPG in mammals, we are generally thinking in terms of a generator involved in controlling the locomotor movements of the hindlimbs. For technical reasons, the neuronal mechanisms controlling forelimb movements have received incomparably less study.

The spinal mechanisms controlling hindlimb movements are located mainly in the lumbar enlargement of the spinal cord (segments L3–S1 in cats and T12–L6 in rodents). Chronic experiments on cats showed that they retain the ability to perform hindlimb stepping movements after transection of the spinal cord rostral to the L3 and even the L4 segment, but lose this ability after more caudal transection [Afel't et al., 1973]. In acute experiments, hindlimb stepping movements were obtained after transection of the spinal cord rostral to the L5 segment by injection of the dopamine precursor, L-DOPA [Grillner and Zanger, 1979]. The need for the rostral spinal segments for generating the locomotor rhythm has also been demonstrated in experiments on rodents [Kiehn and Kjaerulf, 1998]. All these data lead to the conclusion that the CPG generating the locomotor rhythm is located in the rostral area of the lumbar enlargement – segments L3 and L4 and, to a lesser extent, L5 in cats and segments L1–L2 in rodents. During locomotion, this area ensures the rhythmic activity of all other neurons in the lumbar enlargement, including the activity of motoneurons located caudal to the generator area. We should note that the CPG generating the rhythmic activity underlying hindlimb scratching movements in cats was found in the same rostral segments of the lumbar enlargement as the locomotor CPG [Deliagina et al., 1983].

The locomotor CPG is located in the ventrolateral area of the gray matter of the spinal cord [Orlovsky and Feldman, 1972; Kiehn and Kjaerulf, 1998]. Recent studies using both physiological and embryological and molecular-genetic methods have made significant progress in understanding the functional organization of the locomotor CPG in rodents [Gosgnach, 2011; Goulding, 2009; Whelan, 2010]. At least five classes of interneuron involved in forming the locomotor CPG have been identified in the ventral part of the spinal cord (V0, V1, V2, V3, and Hb9). During embryonic development, these neurons derive from various precursor cells and express different transcription factors.

V0 cells include a subclass of inhibitory glycinergic interneurons ($V0_D$), which send their axons to the other side of the spinal cord. These cells are homologous to commissural inhibitory interneurons in lampreys and *Xenopus* embryos. They play a significant, if not decisive, role in the control of alternate hindlimb movements during diagonal locomotion. This was demonstrated in experiments on genetically modified mice. Precursor cells of the V0 interneurons express transcription factor Dbx1, which is needed for formation of commissural connections. During fictive locomotion in Dbx1 knockout ($Dbx1^{-/-}$), spinal mice, antiphase activation of the right and left hemicenters of the locomotor CPG typical of normal animals (Fig. 1, *B*, *C*) was impaired. In contrast, these mice showed a tendency to synchronous activity of the two hemicenters.

Interneurons of subclass $V0_V$ and class V3 are excitatory glutamatergic neurons whose axons mainly project to the opposite side. This suggests that these interneurons are responsible for a tendency to synphasic activity of the two hemicenters during fictive locomotion in Dbx1 knockout mice. These two groups of interneurons, or at least one of them, are homologous to those interneurons in cats that are active at the boundary between the flexor (swing) and extensor (stance) phases of the limb movement [Orlovsky and Fel'dman, 1972]. Contralaterally projecting excitatory interneurons active at the boundary between the two phases of the locomotor cycle have to facilitate more reliable alternation of the activity of the two hemicenters during diagonal locomotion.

Cells of classes V1, V2 (including subclasses V2a and V2b), and Hb9 form projections to the ipsilateral half of the spinal cord. In particular, they send axons to the caudal segments of the lumbar enlargement, where most of the motoneurons innervating the hindlimb muscles are located. V2a and Hb9 interneurons have been identified as excitatory and V1 and V2b interneurons as inhibitory. Recent studies have shown that V1 and V2b interneurons are involved in regulating the reciprocal activity of the extensor and flexor motoneurons [Zhang et al., 2014]. However, final formation of the motor output (a sequence of switching on and off motoneurons of various muscles, reciprocal inhibition of antagonistic motoneurons, etc.) even during fictive, not to mention real, locomotion occurs in the caudal segments where motoneurons and associated interneurons (such as Renshaw cells, Ia interneurons, and others) are located.

The question of the source and mechanism of the locomotor rhythm in mammals remains incompletely understood. The first result showing that rhythmic CPG activity in mammals may be based on the pacemaker activity of a particular group (or groups) of neurons was published as long ago as 1994 [Hochman et al., 1994]. Interneurons were recorded in transverse sections of the spinal cord in neonatal rats, in which application of solution containing both NMDA and tetrodotoxin evoked slow oscillations in the membrane potential with a frequency close to the frequency of locomotion (Fig. 1, *E*1). This result was subsequently confirmed by other investigators [Brocard et al., 2010; Brownstone and Wilson, 2008]. According to many authors

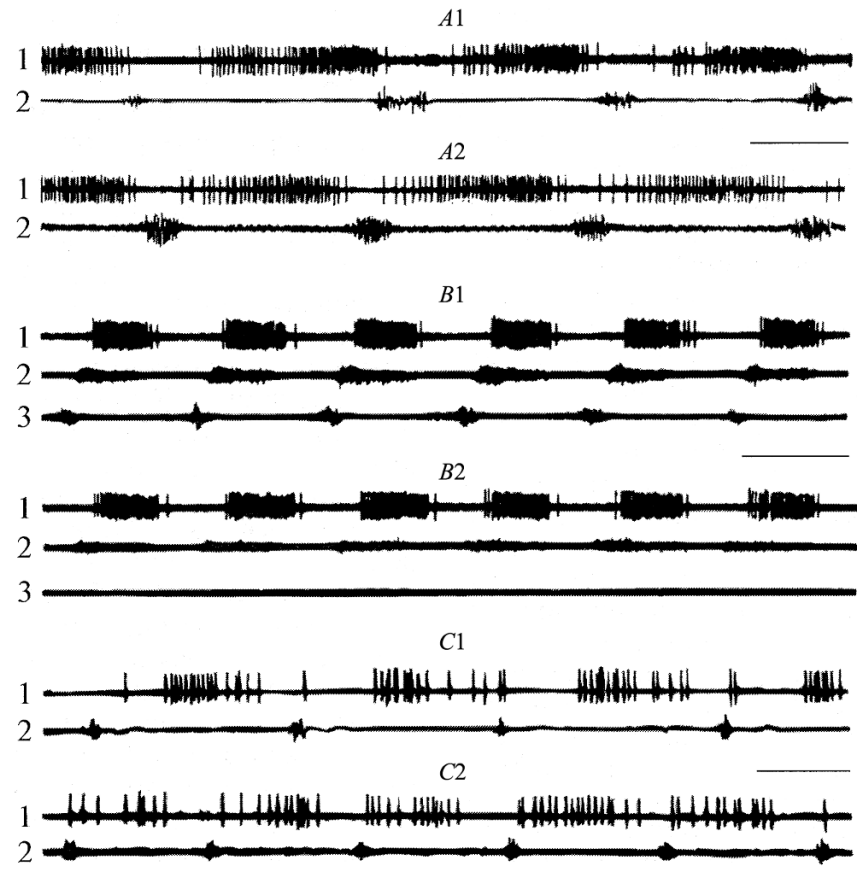

Fig. 7. Signals reporting the operation of the scratching CPG reaching the brain. *A*) A ventral spinocerebellar tract neuron (*1*) recorded during real and fictive scratching before (*A*1) and after (*A*2) immobilization of the cat; *2*) gastrocnemius muscle EMG (*A*1) and gastrocnemius nerve ENG (*A*2) [Arshavsky et al., 1978]. *B*) Activity of a ventral spinocerebellar tract neuron (*1*) during fictive scratching before (*B*1) and after (*B*2) cooling of the spinal cord at the level of segment L5; *2* and *3*) ENG from the sartorius (*2*) and gastrocnemius (*1*) nerves [Arshavsky et al., 1984]. *C*) Activity of a rubrospinal tract neuron before (*C*1) and (*C*2) immobilization of the animal; *2*) EMG of the gastrocnemius and ENG of the gastrocnemius nerve, respectively [Arshavsky et al., 1978]. The time scale bar shows 200 msec.

[Brocard et al., 2010; Brownstone and Wilson, 2008], conditional pacemakers generating the locomotor rhythm in mammals are class Hb9 interneurons, so named because they express the corresponding transcription factor. These are glutamatergic excitatory neurons which, as already noted, send their axons to the caudal segments of the lumbar enlargement. The synergistic Hb9 interneurons form both chemical and electrical connections with each other that determine their synchronous activity. Experiments on transverse spinal cord sections from the rostral part of the lumbar enlargement showed that Hb9 interneurons respond to conditioning factors applied in combination with tetrodotoxin by generating rhythmic oscillations of the membrane potential (Fig. 1, *E*2). Tetrodotoxin-independent oscillations in the membrane potential persisted after uncoupling of the electrical connections between cells. V1a interneurons are another possible source of the locomotor rhythm. These are also glutamatergic excitatory neurons sending projections to the caudal segments of the lumbar enlargement. However, there are no data on the pacemaker activity of V2a interneurons in the literature.

As described above, the command reticulospinal pathway converting the locomotor CPG from the passive to the rhythm-generating state is pharmacologically heterogeneous. Its various fractions use different transmitters to act on spinal neurons. Correspondently, it was shown that the rhythmic activity of pacemaker neurons is based on different ionic mechanisms. At least two mechanisms are discussed in the literature. One of these is linked with activation of NMDA receptors (Fig. 4, *C*). Another mechanism is associated with activation of the persistent sodium current (I_{NaP}). This mechanism has also been discussed above in relation to description of pacemaker neurons in the respiratory CPG. It is possible that both mechanisms are used in the same neurons. For example, according to one group of authors, the endogenous rhythmic activity of Hb9 interneurons is linked with activation of NMDA receptors [Masino et al., 2012], while data from other authors indicate that this

activity is associated with activation of I_{NaP} [Ziskind-Conhaim et al., 2008]. The differences in these results may arise because the authors used different conditioning factors to produce fictive locomotion.

It should be noted that realization of connections between the neurons forming locomotor CPG depends on the animal's gait. As mentioned above, strengthening of the command signal in cats leads to substitution of diagonal locomotion by trotting. One can suggest that this change in gait involves disconnection of the mutual inhibitory connections between the right and left hemicenters of the locomotor CPG, which are performed by the $V0_D$ interneurons. In contrast, mutual excitatory connections between the right and left hemicenters, which are mediated by the $V0_V$ and V3 interneurons, are strengthened. Other examples can also be given. As mentioned above, humans and many animals are able to walk backwards. Different interactions between spinal neurons occur during forward and backward locomotion because these two gaits require different sequences and durations of the contractions of the same limb muscles. One more example is walking down a slope. In this case, the force of gravity is directed not only vertically in relation to the animal's body as in walking on a horizontal surface, but also has a horizontal component, the magnitude of which increases with increases in the steepness of the slope. Therefore, during the stance phase of step when walking on a sloping surface, the extensor and flexor muscles operate not as antagonists, but rather as synergists to prevent the animal from sliding down.

The organization of the connections in different types of locomotion, as well as the frequency of limb movements is controlled by several mechanisms. One of these has been noted above. This consists of signals descending from the supraspinal command centers to the locomotor CPG. The other mechanism consists of afferent signals coming from receptors in the executive motor apparatus. Finally, it is possible that the higher motor centers in some cases control locomotion not only by using a CPG, but also by directly addressing α- and γ-motoneurons and their associated interneuronsm, bypassingthe CPG. We will return to this mechanism in the Conclusion.

To conclude this section, we will touch on one more point illustrating the flexibility of the activity and interconnections of spinal neurons. The lumbar segment of the spinal cord controls not only locomotor, but also scratching movements that take place in many mammals, birds, and reptiles. Rhythmic scratching movements are performed by one of the hindlimbs. These movements differ from locomotor movements in having a higher frequency and shorter duration of the extensor phase of the cycle compared to the flexor phase (Fig. 7). In experiments on cats, real or fictive scratching could be induced by tactile stimulation of the pinna or electrical stimulation of the superior cervical segments of the spinal cord [Arshavsky et al., 1984; Orlovsky et al., 1999]. As mentioned, the neural network generating the scratching rhythm is located in the same rostral segments of the lumbar enlargement as the locomotor CPG [Deliagina et al., 2002]. In experiments on immobilized cats, a gradual spontaneous transition from fictive scratching with its typical ratio of extensor to flexor phases to slower fictive locomotion with a different ratio of these phases was sometimes observed [Berkinblit et al., 1973]. This fact suggests that locomotion and scratching are controlled not by different, but by the same CPG operating in different regimes depending on the incoming command signal. If this suggestion is correct, we have another example demonstrating the wide range over which a locomotor CPG can operate. Command signals evoking scratching movements arriving via propriospinal pathways from the cervical segment of the spinal cord transfer pacemaker neurons to the active state with the frequency of the generated rhythm typical of scratching. These command signals simultaneously block the connection of the hemicenter controlling the movements of the limb with the contralateral hemicenter and with the CPGs controlling forelimb movements, and also establish the appropriate temporary relationships between the activities of V1 and V2n interneurons, which are involved in controlling extensor and flexor motoneurons.

Spinal generators send signals about their operation to the brain. It is widely accepted that during motor activity, the brain corrects the operation of the spinal mechanisms using signals arriving both from distant receptors and from receptors in the executive motor apparatus. However, studies of the activity of neurons in the spinocerebellar tracts during locomotion and scratching showed that the brain also receives signals about the operation of the central generators [Arshavsky et al, 1984; Orlovsky et al., 1999].

During locomotion and scratching, rhythmic signals arrive in the cerebellar cortex via three pathways – the dorsal and ventral spinocerebellar tracts (DSCT and VSCT) and the spinoreticulocerebellar pathway.[2] Fibers of the DSCT and VSCT terminate directly in the cerebellum, while fibers of the spinoreticulocerebellar pathway run from the spinal cord to the lateral reticular nucleus of the medulla oblongata, whose neurons send their axons to the cerebellum. Experiments on decerebrate cats running on a treadmill belt (see above) showed that the DSCT transmits signals about the operation of the executive motor apparatus. In contrast to cats with intact dorsal spinal roots, rhythmic activity of DSCT neurons related to limb movement was absent during locomotion of cats with transected dorsal roots. Similarly, rhythmic activity of DSCT neurons was absent during the fictive, but not during the real scratch reflex. A different result was obtained in studies of the VSCT. Rhythmic, limb movement-related activity of VSCT neurons was present during locomotion in cats with both intact and transected

[2] There is also the spinoolivocerebellar pathway, but it does not transmit signals relating to rhythmic movements [Arshavsky et al., 1984].

dorsal roots. The correlation between the rhythmic activity of VSCT neurons and the operation of the CPG was shown particularly clearly in experiments with the scratch reflex. Figure 7, *A* shows an example of the activity of a VSCT neuron recorded initially during real and then during fictive scratching. This Figure shows that immobilization of the animal and, therefore, elimination of the afferent signals from receptors in the moving limb practically exerted no effect on neuron activity. The activity of VSCT neurons during fictive scratching did not alter after inactivation of the caudal part of the lumbar enlargement by cooling of the spinal cord at the level of the L5 segment (Fig. 7, *B*). These results lead to the conclusion that the VSCT does not transmit signals on the operation of the executive motor apparatus, but on the activity of the spinal CPG. This same conclusion was made in relation to the spinoreticulocerebellar pathway as analogous results were obtained for neurons of the lateral reticular nucleus. Comparison of the activity of neurons in the rostral area of the lumbar enlargement with the activity of neurons in the VSCT and lateral reticular nucleus during scratching showed that these spinocerebellar pathways transmit signals relating to the operation of all groups of interneurons forming the CPG. It is important to note that the existence of pathways transmitting signals to higher centers not only about activity of the motor apparatus, but also about activity of the CPG has also been found in the lamprey [Vinay and Grillner, 1992] and in invertebrates [Davis and Kovacs, 1981].

From the cerebellum, rhythmic signals are transmitted to neurons giving rise to the four major descending tracts through which the brain controls the operation of the spinal mechanisms – the pyramidal, rubrospinal, reticulospinal, and vestibulospinal tracts. Here we will describe only the activity of the brainstem-spinal tracts, as the experiments were performed on decerebrate animals. Signals coming from the cerebellum constitute the only source of rhythmic activity in the descending pathways. In the decerebellated cats, in contrast to cats with an intact cerebellum, there is no rhythmic activity in the brainstem-spinal tracts during locomotion or scratching.

Rhythmic activity in the descending pathways is formed mainly if not exclusively on the basis of signals about the operation of the spinal CPG. This was demonstrated in studies of the scratch reflex. Neuron activity in the brainstem-spinal tracts was essentially the same during real and fictive scratching. One example is shown in Fig. 7, *C*, which shows a rubrospinal neuron recorded initially during real and then during fictive scratching. The rhythmic discharges generated by this neuron did not change when the cat was immobilized.

Information on the activity of the spinal CPG can be essential for the adequate control of spinal mechanisms by the brain. We will consider a simple example. Imagine that a running animal has to jump over an obstacle. To do this, the brain has to send an additional excitation to extensor motoneurons in a strictly defined phase of the cycle when the corresponding hindlimb (or both hindlimbs in the case of galloping) is on the substrate. Signals relating to the spinal CPG provide information on the phase of the locomotor cycle.

Conclusions

Summarizing the results described in this review, we will touch on two points: what are the further prospects for studies of CPGs and what can the results obtained in studies of CPGs contribute to our understanding of the common principles of CNS operation? Further studies of the CPGs underlying various automatic movements in vertebrates and invertebrates will undoubtedly throw light on many unknown details of the organization of particular CPGs. However, we do not think that these results can exert any great influence on current understanding of the general principles of CPG organization and the mechanisms of their operation. As the authors of this review were involved mainly in studying the locomotor CPG, we will discuss two possible research directions in this field that seem to be the most promising. One is the role of the CPG in performing locomotor movements controlled by the higher motor centers. We will illustrate this with the following example. One of the studies reported by Orlovsky described a cat walking along a fence consisting of stakes of different heights [Orlovsky, 1991]. In this situation, each step was performed under visual control and consisted of a voluntary movement (as far as this anthropomorphic term can be applied to animals). The cat did not look directly beneath its feet, but rather a few steps ahead.[3] It seems that motor centers of the brain preprogram each step and then send the relevant commands to the spinal cord. The following question arises: Are these commands addressed directly to α- and γ-motoneurons, bypassing the locomotor CPG, or does the locomotor CPG take part in controlling this type of "voluntary" locomotion? The answer to this question seems to have a significant importance for understanding the mechanisms controlling locomotion in humans.

Another perspective direction in this field has an applied value – attempts to use advances obtained in studies of the mammalian locomotor CPG in clinical practice for the rehabilitation of patients with spinal cord injuries. Studies of this kind are currently being conducted in a number of laboratories [Moshonkina et al., 2012; Rossignol and Frigon, 2011].

It is more interesting to consider what we have learned from studies of CPGs for understanding the general principles of CNS functioning. Throughout this review we have tried to emphasize two points. One is the redundancy of CPG organization. Various aspects of the operation of each of the CPG considered here are generally based on at least two, if not on several, mechanisms supplementing each other to ensure high reliability of CPG functioning. One can

[3] A pianist told us that when he plays from music, he also does not look at the next note to be played, but at several notes ahead.

suggest that this redundancy is typical of all central formations performing various functions. For example, this type of redundancy ensures the reliable sensory perception of the surrounding world and the permanent storage of memory of individual experience necessary for adequate behavior of an organism.

Another point relates to the role of individual neurons and neural interactions in generating rhythmic activity. As mentioned, the question of the origin of rhythmic activity was originally discussed in a strictly alternative way: rhythmic activity arises either as a result of interaction between excitatory and inhibitory neurons, which themselves generate continuous spike discharges or are silent, or results from the pacemaker activity of a specific group of neurons in the CPG. The dominant, if not the only, was the former, purely connectionist point of view, which remained popular for a long time. Many analytical and computer models have been described, which reproduced stable rhythm generation as a result of interactions between neuron-like elements – from very simple models in which each sequential cycle arises as a result of rebound from inhibition arising during the preceding phase of the cycle [Roberts et al., 2008; Satterlie, 1985] to more complex models generating periodic spike discharges [Friesen and Stent, 1978; Psujek et al., 2006; Rybak et al., 2006].

The facts presented in this review show that the rhythm generation in all sufficiently well studied CPGs is based on pacemaker activity. Interneural interactions also make a certain contribution to maintaining and stabilizing rhythmic activity. Furthermore, neural interactions play the decisive role in forming the motor output of a CPG. Thus, CPG operation is based on interactions between interconnected cellular (pacemaker) and network mechanisms.

A further example of a function based on the interaction between cellular and network mechanisms is the circadian rhythm generated by the hypothalamic suprachiasmatic nucleus. It is evident that generation of a rhythm with a period close to 24 h is a more complex task than generating a rhythm with periods typical of automatic movements measured in seconds and tens of seconds. It seems that this is a reason why generation of the circadian rhythm is based solely on complex molecular processes occurring within individual suprachiasmatic neurons [Reppert and Weaver, 2011]. Neural interactions do not participate in rhythm generation, but ensure the synchronous activity of autonomously operating neurons.

Surprisingly, the purely connectionist concept continues to prevail in studies of the much more complex cognitive functions of the brain. It is suggested that cognitive functions are performed exclusively at the neural network level as a result of complex interneural interactions. We can illustrate this view by citing the widely used textbook on neurophysiology [Kandel et al., 2000]: "The complexity of human behavior depends less on the specialization of individual nerve cells and more on the fact that a great many of these cells form precise anatomical circuits" (p. 19). Neurons themselves are regarded as primitive elements whose function is limited to generating electrical potentials and transmitting signals to other cells. This point of view was clearly expressed in a recent article on neurolinguistics [Ullman et al., 2001]: "Modern connectionist theory has offered a computational framework for the single-system view. It has been argued that the learning, representation and processing of grammatical rules and lexical items take place over many interconnected, simple processing units" (p. 719). Below we will consider two examples suggesting that the operation of centers involved in performing cognitive functions is also based on the interaction of cellular and network mechanisms.

The first example came from neurolinguistics. The unique intellectual abilities of humans include the ability communicate using language. Language – oral, written, and sign in deaf people – is the ability to to produce a practically infinite number of meaningful messages by using a finite number of lexical elements and a set of grammatical rules. Unlike humans, communication between animals includes only a limited set of standard signals [Lorentz, 1978]. Even apes are unable to use their rich set of gestures and facial expressions to create even simple sentences (see [Arshavsky et al., 2011] for details). As the connectionist concept does not assume any intrinsic differences between human and animal neurons, the only explanation for humans' linguistic abilities is the large size of the brain consisting of an enormous number of cells (just the cerebral cortex in humans has about $2 \cdot 10^{10}$ neurons and 10^{14} synapses) forming neural networks which are much more complex than those in animals. The complexity of the structural organization of the "language areas" of the cerebral cortex, i.e., Broca's and Wernicke's areas, is particularly emphasized [Rilling, 2014]. However, this explanation is refuted by results from studies of the linguistic abilities of microcephalic individuals.

Microcephalics are individuals with the occipital-frontal head circumference three standard deviations below the mean [Ross and Frias, 1977]. Brain weight in people with microcephaly (430–600 g) is ~2.5-times less than normal (1350 g). Microcephaly has various etiologies. One type of inherited microcephaly is due to abnormalities in the late stages of precursor cell division during formation of the cerebral cortex. This type of microcephaly is accompanied by relatively moderate intellectual deficit and the ability to communicate with languages at least at the level of a five-year-old child [Woods et al., 2005]. Moreover, microcephalics demonstrating practically normal intellect and language ability have been described [Evans, 1991; Hennekam et al., 1992; Ramirez et al., 1983; Rizzo and Pavone, 1995; Rossi et al., 1987]. Some of these individuals have graduated from high school and worked as a postman [Widler, 1911], bank employee [Rizzo and Pavone, 1995], a secretary [Rossi et al., 1987], a kindergarten teacher [Evans, 1991], and even

a doctor [Ramirez et al., 1983]. We believe that these facts suggest (if we remain within the framework of scientific knowledge and do not invoke Cartesian dualism) that human neurons are qualitatively different from neurons in animals and that linguistic, as well as other cognitive functions, are to a great extent performed at the intracellular level. With regard to intercellular interaction, this determines the coordinated operation of the cells performing elementary cognitive functions.

The other example is related to sensory perception – the kinds of mechanisms determining the different sensory sensations arising in response to stimulation of various receptors. In view of the absence of experimental approaches, this problem is traditionally ignored by neurophysiologists [Smit, 1973; Kandel et al., 2000] and was the prerogative of philosophy, where it is known as the *Qualia* problem. We have formulated this problem in neurophysiological terms. Why are signals coming from retinal receptors via the lateral geniculate body to the occipital cortex perceived as visual images, while signals coming from cochlear receptors via the medial geniculate body to the temporal cortex are perceived as auditory images? This different quality of sensation cannot be explained by learning, as precocial birds and ungulate mammals can see with their eyes and visual centers and hear with their ears and auditory centers from birth. It is difficult to explain the mechanism underlying the different qualities of sensations within the framework of the purely connectionist concept. Spikes reach the visual and auditory areas through thalamocortical projections formed by fibers arising from the lateral and medial geniculate bodies, respectively. These tracts have similar organization and form synaptic connections with the same types of cortical neurons. The visual and auditory areas of the cortex have a similar laminar structure. They consist of identical types of neurons that generate identical potentials and use the same transmitter for signal transmission. What is different? It can be suggested that the differences lie in some unknown intrinsic properties of neurons that make them "visual" or "auditory." In other words, it is suggested that the process of brain development includes not only morphological differentiation of neurons, including formation of specific interneural connections, but also their functional differentiation, with the effect that excitation of some neurons is transformed into visual images and excitation of others produces auditory images. This suggestion is supported by recent data showing that neurons from different sensory areas express not only identical genes, but also specific genes [Leamey et al., 2008; Mühlfriedel et al., 2007; Stansberg et al., 2011].

These two examples suggest that we can expect a paradigm shift in the physiology of higher nervous activity from the purely connectionist concept towards a cellar approach as has occurred in studies of the mechanism of operation of central pattern generators controlling automatic movements.

REFERENCES

Afel't, Z., Veber, N. V., and Maksimova, N. V., *Reflex Activity in the Chronically Isolated Cat Spinal Cord*, Nauka, Moscow (1973).

Arshavsky, Yu. I., "The role of neural networks and individual neurons in brain function," *Sens. Sist.*, **25**, No. 1, 3–16 (2011).

Arshavsky, Yu. I., Gel'fand, I. M., and Orlovsky, G. N., *The Cerebellum and the Control of Rhythmic Movements*, Nauka, Moscow (1984).

Arshavsky, Yu. I., Orlovsky, G. N., Pavlova, G. A., and Panchin, Yu. V., "Generation of the locomotor rhythm by the pedal ganglia in *Clione limacina*," *Neirofiziologiya*, **14**, No. 1, 102–104 (1982).

Arshavsky, Y. I., Beloozerova, I. N., Orlovsky, G. N., et al., "Control of locomotion in marine mollusc *Clione limacina*. I. Efferent activity during actual and fictitious swimming," *Exp. Brain Res.*, **58**, No. 2, 255–262 (1985).

Arshavsky, Y. I., Deliagina, T. G., and Orlovsky, G. N., "Pattern generation," *Curr. Opin. Neurobiol.*, **7**, No. 6, 781–789 (1997).

Arshavsky, Y. I., Deliagina, T. G., Okshtein, I. L., et al., "Defense reaction in the pond snail *Planorbis corneus*. II. Central pattern generator," *J. Neurophysiol.*, **71**, No. 3, 891–897 (1994).

Arshavsky, Y. I., Deliagina, T. G., Orlovsky, G. N., et al., "Control of locomotion in marine mollusc *Clione limacina*. VI. Activity of isolated neurons of pedal ganglia," *Exp. Brain Res.*, **63**, No. 1, 106–112 (1986).

Arshavsky, Y. I., Deliagina, T. G., Orlovsky, G. N., et al., "Analysis of the central pattern generator for swimming in the mollusk *Clione*," *Ann. N. Y. Acad. Sci.*, **860**, 51–69 (1998).

Arshavsky, Y. I., Deliagina, T. G., Orlovsky, G. N., Panchin, Y. V., "Control of feeding movements in the freshwater snail *Planorbis corneus*. III. Organization of the feeding rhythm generator," *Exp. Brain Res.*, **70**, No. 2, 332–341 (1988).

Arshavsky, Y. I., Gelfand, I. M. Orlovsky, G. N., and Pavlova, G. A., "Messages conveyed by spinocerebellar pathways during scratching in the cat. 2. Activity of neurones of the ventral spinocerebellar tract," *Brain Res.*, **151**, No. 2, 493–506 (1978).

Arshavsky, Y. I., Orlovsky, G. N., Panchin, Y. V., et al., "Neuronal control of swimming locomotion: analysis of the pteropod mollusc *Clione* and embryos of the amphibian *Xenopus*," *Trends Neurosci.*, **16**, No. 6, 227–233 (1993).

Arshavsky, Y. I., Orlovsky, G. N., Pavlova, G. A., and Perret, C., "Messages conveyed by descending tracts during scratching in the cat. 2. Activity of rubro-spinal neurones," *Brain Res.*, **159**, No. 1, 111–123 (1978).

Balaban, P. M., Vorontsov, D. D., D'yakonova, V. E., et al., "Central pattern generators," *Zh. Vyssh. Nerv. Deyat.*, **63**, No. 5, 520–541 (2013).

Bässler, U., "The femur-tibia control system of stick insects – a model system for the study of the neural basis of joint control," *Brain Res. Rev.*, **18**, No. 2, 207–226 (1993).

Berkinblit, M. B., Deliagina, T. G., Feldman, A. G., and Orlovsky, G. N., "Generation of scratching. II. Nonregular regimes of generation," *J. Neurophysiol.*, **41**, No. 4, 1058–1069 (1978).

Brocard, F., Tazerart, S., and Vinay, L., "Do pacemakers drive the central pattern generator for locomotion in mammals?" *Neuroscientist*, **16**, No. 2, 139–155 (2010).

Brodin, L., Grillner, S, and Rovainen, C. M., "N-Methyl-D-aspartate (NMDA), kainate and quisqualate receptors and the generation of fictive locomotion in the lamprey spinal cord," *Brain Res.*, **325**, No. 1–2, 302–306 (1985).

Brown, T. G., "The intrinsic factors in the act of progression in the mammal," *Proc. Roy. Soc. London B*, **84**, No. 572, 308–319 (1911).

Brownstone, R. M. and Wilson, J. M., "Strategies for delineating spinal locomotor rhythm-generating networks and the possible role of Hb9 interneurones in rhythmogenesis," *Brain Res. Rev.*, **57**, No. 1, 64–76 (2008).

Buchanan, J. T., "Contributions of identifiable neurons and neuron classes to lamprey vertebrate neurobiology," *Prog. Neurobiol.*, **63**, No. 4, 441–466 (2001).

Cabelguen, J. M., Bourcier-Lucas, C., and Dubuc, R., "Bimodal locomotion elicited by electrical stimulation of the midbrain in the salamander *Notophthalmus viridescens*," *J. Neurosci.*, **23**, No. 6, 2434–2439 (2003).

Cooke, I. M., "Reliable, responsive pacemaking and pattern generation with minimal cell numbers: the crustacean cardiac ganglion," *Biol. Bull.*, **202**, No. 2, 108–136 (2002).

Dale, N. and Roberts, A., "Dual-component amino-acid-mediated synaptic potentials: excitatory drive for swimming in *Xenopus* embryos," *J. Physiol.*, **363**, No. 1, 35–59 (1985).

David, W. J. and Kovac, M. P., "The command neuron and the organization of movement," *Trends Neurosci.*, **4**, No. 3, 73–76 (1981).

Deliagina, T. G., Orlovsky, G. N., and Pavlova, G. A., "The capacity for generation of rhythmic oscillations is distributed in the lumbosacral spinal cord of the cat," *Exp. Brain Res.*, **53**, No. 1, 81–90 (1983).

Deliagina, T. G., Zelenin, P. V., and Orlovsky, G. N., "Encoding and decoding of reticulospinal commands," *Brain Res. Rev.*, **40**, No. 1–3, 166–177 (2002).

Douglas, J. R., Noga, B. R., Dai, X., and Jordan, L. M., "The effects of intrathecal administration of excitatory amino acid agonists and antagonists on the initiation of locomotion in the adult cat," *J. Neurosci.*, **13**, No. 3, 990–1000 (1993).

El Manira, A., Pombal, M. A., and Grillner, S., "Diencephalic projection to reticulospinal neurons involved in the initiation of locomotion in adult lampreys *Lampetra fluviatilis*," *J. Comp. Neurol.*, **389**, No. 4, 603–616 (1997).

Elliott, C. J. and Susswein, A. J., "Comparative neuroethology of feeding control in mollusks," *J. Exp. Biol.*, **205**, No. 7, 877–896 (2002).

Evans, D. G., "Dominantly inherited microcephaly, hypotelorism and normal intelligence," *Clin. Genet.*, **39**, No. 2, 178–180 (1991).

Feldman, J. L., Del Negro, C. A., and Gray, P. A., "Understanding the rhythm of breathing: so near, yet so far," *Annu. Rev. Physiol.*, **75**, 423–452 (2013).

Friesen, W. O. and Stent, G. S., "Neural circuits for generating rhythmic movements," *Annu. Rev. Biophys. Bioeng.*, **7**, 37–61 (1978).

Getting, P. A., "Emerging principles governing the operation of neural networks," *Ann. Rev. Neurosci.*, **12**, 185–204 (1989).

Gosgnach, S., "The role of genetically-defined interneurons in generating the mammalian locomotor rhythm," *Integr. Comp. Biol*, **51**, No. 6, 903–912 (2011).

Goulding, M., "Circuits controlling vertebrate locomotion: moving in a new direction," *Nat. Rev. Neurosci.*, **10**, No. 7, 507–518 (2009).

Grillner, S. and Zangger, P., "On the central generation of locomotion in the low spinal cat," *Exp. Brain Res.*, **34**, No. 2, 241–261 (1979).

Grillner, S., "Biological pattern generation: the cellular and computational logic of networks in motion," *Neuron*, **52**, No. 5, 751–766 (2006).

Grillner, S., "The motor infrastructure: from ion channels to neuronal networks," *Nat. Rev. Neurosci.*, **4**, No. 7, 573–586 (2003).

Grillner, S., Deliagina, T., Ekeberg, O., et al., "Neural networks that co-ordinate locomotion and body orientation in lamprey," *Trends Neurosci.*, **18**, No. 6, 270–279 (1995).

Heiligenberg, W., *Neural Nets in Electric Fish*, MIT Press, Cambridge (1991).

Hennekam, R. C., van Rhijn, A., and Hennekam, F. A., "Dominantly inherited microcephaly, short stature and normal intelligence," *Clin. Genet.*, **41**, No. 3, 248–251 (1992).

Hochman, S., Jordan, L. M., and MacDonald, J. F., "N-methyl-D-aspartate receptor-mediated voltage oscillations in neurons surrounding the central canal in slices of rat spinal cord," *J. Neurophysiol.*, **72**, No. 2, 565–577 (1994).

Hultborn, H. and Nielsen, J. B., "Spinal control of locomotion – from cat to man," *Acta Physiol. (Oxford)*, **189**, No. 2, 111–121 (2007).

Jean, A., "Brain stem control of swallowing: neuronal network and cellular mechanisms," *Physiol. Rev.*, **81**, No. 2, 929–969 (2001).

Jordan, L. M., "Initiation of locomotion in mammals," *Ann. N. Y. Acad. Sci.*, **860**, 83–93 (1998).

Jordan, L. M., Liu, J., Hedlund, P. B., et al., "Descending command systems for the initiation of locomotion in mammals," *Brain Res. Rev.*, **57**, No. 1, 183–191 (2008).

Kahn, J. A., Roberts, A, and Kashin, S. M., "The neuromuscular basis of swimming movements in embryos of the amphibian *Xenopus laevis*," *J. Exp. Biol.*, **99**, 175–184 (1982).

Kandel, E. E., Schwartz, J. H., and Jessel, T. M. (eds.), *Principles of Neural Science*, McGraw-Hill, New York (2000), 4th ed.

Kashin, S. M., Feldman, A. G., and Orlovsky, G. N., "Locomotion of fish evoked by electrical stimulation of the brain," *Brain Res.*, **82**, No. 1, 41–47 (1974).

Kazennikov, O. V., Selionov, V. A., Shik, M. L., and Yakovleva, G. V., "The rhomboencephalic 'locomotor area' in turtles," *Neirofiziologiya*, **12**, No. 4, 382–390 (1980).

Kiehn, O. and Kjaerulff, O., "Distribution of central pattern generators for rhythmic motor outputs in the spinal cord of limbed vertebrates," *Ann. N. Y. Acad. Sci.*, **860**, 110–129 (1998).

Koizumi, H., Smerin, S. E., Yamanishi, T., et al., "TASK channels contribute to the K^+-dominated leak current regulating respiratory rhythm generation in vitro," *J. Neurosci.*, **30**, No. 12, 4273–4284 (2010).

Kristan, W. B., Calabrese, R. L., and Friesen, W. O., "Neuronal control of leech behavior," *Prog. Neurobiol.*, **76**, No. 5, 279–327 (2005).

Kudo, N. and Nishimaru, H., "Reorganization of locomotor activity during development in the prenatal rat," *Ann. N. Y. Acad. Sci.*, **860**, 306–317 (1998).

Leamey, C. A., Glendining, K. A., Kreiman, G., et al., "Differential gene expression between sensory neocortical areas: potential roles for Ten_m3 and Bcl6 in patterning visual and somatosensory pathways," *Cereb. Cortex*, **18**, No. 1, 53–66 (2008).

Li, W. C., "Generation of locomotion rhythms without inhibition in vertebrates: the search for pacemaker neurons," *Integr. Comp. Biol.*, **51**, No. 6, 879–889 (2011).

Li, W. C., Roberts, A., and Soffer, S. R., "Specific brainstem neurons switch each other into pacemaker mode to drive movement by activating NMDA receptors," *J. Neurosci.*, **30**, No. 49, 16609–16620 (2010).

Lorentz, K. Z., *King Solomon's Ring* [Russian translation], Znanie, Moscow (1978).

Marder, E. and Calabrese, R. L., "Principles of rhythmic motor pattern generation," *Physiol. Rev.*, **76**, No. 3, 687–717 (1996).

Masino, M. A., Abbinanti, M. D., Eian, J., and Harris-Warrick, R. M., "TTX-resistant NMDA receptor-mediated membrane potential oscillations in neonatal mouse Hb9 interneurons," *PLoS One*, **7**, No. 10, e47940 (2012).

McCrea, D. A. and Rybak, I. A., "Organization of mammalian locomotor rhythm and pattern generation," *Brain Res. Rev.*, **57**, No. 1, 134–146 (2008).

McCrea, D. A., "Neuronal basis of afferent-evoked enhancement of locomotor activity," *Ann. N. Y. Acad. Sci.*, **860**, 216–225 (1998).

Mühlfriedel, S., Kirsch, F., Gruss, P., et al., "Novel genes differentially expressed in cortical regions during late neurogenesis," *Eur. J. Neurosci.*, **26**, No. 1, 33–50 (2007).

Morquette, P. Lavoie, R., Fhima, M. D., et al., "Generation of the masticatory central pattern and its modulation by sensory feedback," *Prog. Neurobiol.*, **96**, No. 3, 340–355 (2012).

Moshonkina, T. R., Makarovskii, A. N., Bogacheva, I. N., et al., "Effects of electrical stimulation of the spinal cord in patients with vertebrospinal pathology," *Byull. Eksperim. Biol. Med.*, **153**, No. 1, 21–26 (2012).

Murphy, A. D., "The neuronal basis of feeding in the snail, *Helisoma*, with comparisons to selected gastropods," *Prog. Neurobiol.*, **63**, No. 4, 383–408 (2001).

Musienko, P. E., Zelenin, P. V., Lyalka, V. F., et al., "Spinal and supraspinal control of the direction of stepping during locomotion," *J. Neurosci.*, **32**, No. 48, 17,442–17,453 (2012).

Onimaru, H, Arata, A., and Homma, I., "Firing properties of respiratory rhythm generating neurons in the absence of synaptic transmission in rat medulla in vitro," *Exp. Brain Res.*, **76**, No. 3, 53–536 (1989).

Onimaru, H., Arata, A., and Homma, I., "Intrinsic burst generation of preinspiratory neurons in the medulla of brainstem-spinal cord preparations isolated from newborn rats," *Exp. Brain Res.*, **106**, No. 1, 57–68 (1995).

Orlovsky, G. N. and Fel'dman, A. G., "Classification of neurons in the lumbospinal segment of the spinal cord depending on their discharges during evoked locomotion," *Neirofiziologiya*, **4**, No. 3, 410–417 (1972).

Orlovsky, G. N., "Spontaneous and evoked locomotion in thalamic cats," *Biofizika*, **14**, No. 5, 1095–1102 (1969).

Orlovsky, G. N., "Cerebellum and locomotion," in: *Neurobiological Basis of Human Locomotion*, Shimamura, M., Grillner, S., and Edgerton, V. R. (eds.), Japan Scientific Societies Press, Tokyo (1991), pp. 187–199.

Orlovsky, G. N., Deliagina, T. G., and Grillner, S., *Neuronal Control of Locomotion*, Oxford University Press (1999).

Panchin, Y. V., Arshavsky, Y. I., Deliagina, T. G., et al., "Control of locomotion in the marine mollusk *Clione limacina*. XI. Effects of serotonin," *Exp. Brain Res.*, **109**, No. 2, 361–365 (1996).

Panchin, Y. V., Sadreev, R. I., and Arshavsky, Y. I., "Control of locomotion in the marine mollusk *Clione limacina*. X. Effects of acetylcholine antagonists," *Exp. Brain Res.*, **106**, No. 1, 135–144 (1995).

Pearson, K. G., "Role of sensory feedback in the control of stance duration in walking cats," *Brain Res. Rev.*, **57**, No. 1, 222–227 (2008).

Peña, F., Parkis, M. A., Tryba, A. K., and Ramirez, J. M., "Differential contribution of pacemaker properties to the generation of respiratory rhythms during normoxia and hypoxia," *Neuron*, **43**, No. 1, 105–117 (2004).

Psujek, S. Ames, J., and Beer, R. D., "Connection and coordination: the interplay between architecture and dynamics in evolved model pattern generators," *Neural Comput.*, **18**, No. 3, 729–747 (2006).

Ramirez, J. M., Koch, H., Garcia, A. J., et al., "The role of spiking and bursting pacemakers in the neuronal control of breathing," *J. Biol. Phys.*, **37**, No. 3, 241–261 (2011).

Ramírez, M. L., Rivas, F., and Cantú, J. M., "Silent microcephaly: a distinct autosomal dominant trait," *Clin. Genet.*, **23**, No. 2, 281–286 (1983).

Reppert, S. M. and Weaver, D. R., "Molecular analysis of mammalian circadian rhythms," *Annu. Rev. Physiol.*, **63**, 647–676 (2001).

Rilling, J. K., "Comparative primate neurobiology and the evolution of brain language systems," *Curr. Opin. Neurobiol.*, **28**, No. 1, 10–14 (2014).

Rizzo, R. and Pavone, L., "Autosomal-recessive microcephaly in two siblings, one with normal IQ and both with protruding mandible, small ears, and curved nose," *Am. J. Med. Genet.*, **59**, No. 4, 421–425 (1995).

Roberts, A., Li. W. C., Soffe, S. R., and Wolf, E., "Origin of excitatory drive to a spinal locomotor network," *Brain Res. Rev.*, **57**, No. 1, 22–28 (2008).

Ross. J. J. and Frias, J. L., "Microcephaly," in: *Congenital Malformations of the Brain and Skull*, Vinken, P. J. and Bruyn, G. W. (eds.), Elsevier, Amsterdam (1977), Part 1, pp. 507–524.

Rossi, L. N., Candini, G., Scarlatti, G., et al., "Autosomal dominant microcephaly without mental retardation," *Am. J. Dis. Child.*, **141**, No. 6, 655–659 (1987).

Rossignol, S and Frigon, A., "Recovery of locomotion after spinal cord injury: some facts and mechanisms," *Annu. Rev. Neurosci.*, **34**, 413–440 (2011).

Rossignol, S, Dubuc, R., and Goddard, J. P., "Dynamic sensorimotor interactions in locomotion," *Physiol. Rev.*, **86**, No. 1, 89–154 (2006).

Rybak, I. A, Molkov, Y. I., Jasinski, P. E., et al., "Rhythmic bursting in the pre-Bötzinger complex: mechanisms and models," *Prog. Brain Res.*, **209**, 1–23 (2014).

Rybak, I. A,. Shevtsova, N. A., Lafreniere-Roula, M., and McCrea, D. A., "Modelling spinal circuitry involved in locomotor pattern generation: insights from deletions during fictive locomotion," *J. Physiol.*, **577**, No. 2, 617–639 (2006).

Satterlie, R. A., "Neuronal control of swimming in jellyfish: A comparative story," *Canad. J. Zool.*, **80**, No. 12, 1654–1679 (2002).

Satterlie, R. A., "Reciprocal inhibition and postinhibitory rebound produce reverberation in a locomotor pattern generator," *Science*, **229**, No. 4711, 402–404 (1985).

Selverston, A. I., "A neural infrastructure for rhythmic motor patterns," *Cell. Mol. Neurobiol.*, **25**, No. 2, 223–244 (2005).

Selverston, A. I., "Invertebrate central pattern generator circuits," *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **365**, No. 1551, 2329–2345 (2010).

Sherrington, C. S., "Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing," *J. Physiol.*, **40**, No. 1, 28–121 (1910).

Sherrington, C. S., "Problems of muscular receptivity," *Nature*, **113**, 929–932 (1924).

Shik, M. L. and Orlovsky, G. M., "Neurophysiology of locomotor automatism," *Physiol. Rev.*, **56**, No. 3, 465–501 (1976).

Shik, M. L., Severin, F. V., and Orlovsky, G. N., "Control of walking and running by electrical stimulation of the midbrain," *Biofizika*, **2**, No. 5, 659–666 (1966).

Sholomenko, G. N., Funk, G. D., and Steeves, J. D., "Locomotor activities in the decerebrate bird without phasic afferent input," *Neuroscience*, **40**, No. 1, 257–266 (1991).

Sillar, K. T., Simmers, A. J., and Wedderburn, J. F., "The post-embryonic development of cell properties and synaptic drive underlying locomotor rhythm generation in *Xenopus* larvae," *Proc. Biol. Sci.*, **249**, No. 1324, 65–70 (1992).

Sirota, M. G. and Shik, M. L., "Locomotion in cats on stimulation of the midbrain," *Ros. Fiziol. Zh.*, **59**, No. 9, 1314–1321 (1973).

Sirota, M. G., Di Prisco, G. V., and Dubuc, R., "Stimulation of the mesencephalic locomotor region elicits controlled swimming in semi-intact lampreys," *Eur. J. Neurosci.*, **12**, No. 24, 4081–4092 (2000).

Skinner, R. D. and E. Garcia-Rill, "The mesencephalic locomotor region (MLR) in the rat," *Brain Res.*, **323**, No. 2, 385–389 (1984).

Smit, K., *Biology of Sensory Systems*, BINOM, Moscow (2005).

Smith, J. C., Butera, R. J., Koshiya, M., et al., "Respiratory rhythm generation in neonatal and adult mammals: the hybrid pacemaker-network model," *Respir. Physiol.*, **122**, No. 2, 131–147 (2000).

Soffe, S. R. and Roberts, A., "Activity of myotomal motoneurons during fictive swimming in frog embryos," *J. Neurophysiol.*, **48**, No. 6, 1274–1278 (1982).

Stansberg, C., Ersland, K. M., van der Valk, P., and Steen, V. M., "Gene expression in the rat brain: high similarity but unique differences between frontomedial-, temporal- and occipital cortex," *BMC Neurosci.*, **12**, 15, (2011).

Sumi, T., "Some properties of cortically-evoked swallowing and chewing in rabbits," *Brain Res.*, **15**, No. 1, 107–120, (1969).

Tell, F. and Jean A., "Ionic basis for endogenous rhythmic patterns induced by activation of N-methyl-D-aspartate receptors in neurons of the rat nucleus tractus solitarii," *J. Neurophysiol.*, **70**, No. 6, 2379–2390 (1993).

Ullman, M. T., "A neurocognitive perspective on language: the declarative/procedural model," *Nat. Rev. Neurosci.*, **2**, No. 8, 717–726 (2001).

Vinay, L and Grillner, S., "Spino-bulbar neurons convey information to the brainstem about different phases of the locomotor cycle in the lamprey," *Brain Res.*, **582**, No. 1, 134–138 (1992).

Wallén, P. and Grillner, S., "N-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey," *J. Neurosci.*, **7**, No. 9, 2745–2755 (1987).

Whelan, P. J., "Shining light into the black box of spinal locomotor networks," *Philos. Trans. R. Soc. Biol. Sci.*, **365**, No. 1551, 2383–2395 (2010).

Wiersma, C. A. and Ikeda, A., "Interneurons commanding swimmeret movements in the krayfish, *Procambarus clarki* (Girard)," *Comp. Biochem. Physiol.*, **12**, No. 5, 509–525 (1964).

Wilder, B. G., "Exhibition of, and preliminary note upon, a brain of about one-half the average size from a white man of ordinary weight and intelligence," *J. Nervous Ment. Dis.*, **30**, No. 1, 95–97 (1911).

Wilson, J. M., Hartley, R., Maxwell, D. J., et al., "Conditional rhythmicity of ventral spinal interneurons defined by expression of the Hb9 homeodomain protein," *J. Neurosci.*, **25**, No. 24, 5710–5719 (2005).

Woods, C. G., Bond, J., and Enard, W., "Autosomal recessive primary microcephaly (MCPH)," *Am. J. Hum. Genet.*, **76**, No. 6, 717–728 (2005).

Zhang, J., Lanuza, G. M., Britz, O., et al., "V1 and v2b interneurons secure the alternating flexor-extensor motor activity mice require for limbed locomotion," *Neuron*, **82**, No. 1, 138–150 (2014).

Ziskind-Conhaim, L, Wu. L, and Wiesner, E. P., "Persistent sodium current contributes to induced voltage oscillations in locomotor-related hb9 interneurons in the mouse spinal cord," *J. Neurophysiol.*, **100**, No. 4, 2254–2264 (2008).