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**ANALYSIS OF DIFFERENT FORMS OF
LOCOMOTOR BEHAVIOR IN LAMPREY**

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ABSTRACT

The ability to locomote, that is move actively in space, is characteristic of the animal kingdom. Many species are capable of different forms of locomotion. They include forward and backward locomotion, fast and slow progression, etc. A general goal of this study was, by comparing three different forms of locomotion observed in the lamprey (a lower vertebrate), to estimate the similarities and distinctions of their control mechanisms.

The main and well studied form of locomotion in the lamprey is fast forward swimming (FFS) based on periodical waves of lateral body flexion propagating from head to tail. The lamprey is also capable of two other forms of undulatory locomotion, slow forward swimming (SFS) and slow backward swimming (SBS). In the present study, it was shown that continuous tactile stimulation of a large area in the middle part of the body evokes SFS. By contrast, continuous tactile stimulation of a large area in the head or gill region causes SBS. Lesions of afferent inputs showed that sensory information critical for elicitation of SFS is transmitted through the dorsal roots in the middle part of the spinal cord. By contrast, sensory signals that induce SBS are transmitted not only through the dorsal roots in the gill region of the spinal cord but also through afferents of the lateral line nerves and trigeminal nerves.

In the present study, the kinematical and electromyographic patterns of SFS and SBS were characterized and then compared with those of FFS. During SBS, the waves of lateral body undulations propagated from tail to head. The EMG bursts on the two sides alternated, and the EMG in more caudal segments led in phase the EMG in more rostral segments. During SFS, the waves of lateral body undulations propagated from head to tail. The EMG bursts on the two sides alternated, and the EMG in more rostral segments led in phase the EMG in more caudal segments. The most striking feature of SFS was non-uniformity of the shape and speed of locomotor waves propagating along the body: in the vicinity of the site of stimulation, the waves slowed down, and the body curvature increased several-fold due to the enhanced muscle activity.

It was found that SFS and SBS have some important features in common. In both forms of slow swimming, the cycle duration was similar, and it was much longer than the cycle duration of FFS. Besides that, both SFS and SBS had a lower speed of progression and larger amplitude of head and tail excursions as compared to FFS. Another characteristic feature of both modes of slow swimming was the lack of stabilization of the body orientation in space, which differed from the very efficient stabilization of orientation during FFS. Taken together, these characteristic features of SFS and SBS suggest that these two modes of locomotion are not effective for long distance migrations. Instead, SFS and SBS can be used for escape and withdrawal behavior.

Persistence of SFS and SBS after different lesions of the spinal cord suggests that the ascending and descending pathways, necessary for initiation of SBS and SFS, are dispersed over the cross-section of the spinal cord. A complete transection of the spinal cord did not abolish the generation of SBS rostral to the lesion, suggesting that SBS can be initiated from different levels of the spinal cord.

Role of the spinal cord and the brainstem in generation of different forms of undulatory locomotion is discussed.

Key words: motor coordination, undulatory locomotion, postural corrections, spinal cord damage, lamprey.

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- II. **Islam S. S.**, Zelenin P. V. Uneven propagation of locomotor waves in the lamprey. *The Journal of Neurophysiology* (submitted).

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INTRODUCTION

General characteristics of locomotor behavior and locomotor control system in vertebrates

Different forms of locomotion

Locomotion is the self-powered, patterned motion of limbs or other anatomical parts by which an individual moves itself from place to place. Locomotion is one of the basic motor behaviors in practically all animals, including man (Orlovsky et al. 1999). Animals can display different forms of locomotion. The rhythmic movements of limbs in the legged animals propel them through space (cat, monkey, human, etc.). Swimming by means of undulatory movements of the trunk is the main form of locomotion in aquatic vertebrate species (lamprey, fish, frog tadpole, etc.). In case of airborne animals (birds, bats, etc.), rhythmic flapping of their wings in order to fly in the air is another form of locomotion.

An animal can switch between completely different modes of locomotion, like the bipedal and quadrupedal locomotion of monkey (Nakajima et al. 2004) and in lizards (Irschick and Jayne 1999); walking and swimming of salamander (Frolich and Biewener, 1992); side winding and concertina crawling in snakes (Jayne 1988a,b). Also, a usual forward locomotion pattern can be modified to produce backward progression (Buford et al. 1990; Thorstensson 1986; Vilensky and Cook 2000). Legged animals can also use a variety of gaits differing in the interlimb coordination – walking, running, trotting, pacing, galloping (Engberg and Lundberg 1969). Usually, a change of the gait correlates with a change of the speed of locomotion. For example, at slow and moderate speeds, terrestrial quadrupeds use walk and tort, with strict alternation of the two limbs of one pair and different phase shifts between the two pairs. At the highest speeds, animals are galloping, with in-phase coordination in each pair of limbs.

General organization of locomotor control system

A choice of a given form of locomotion is a function of higher levels of CNS. They operate on the basis of sensory information about external and internal state of the animal.

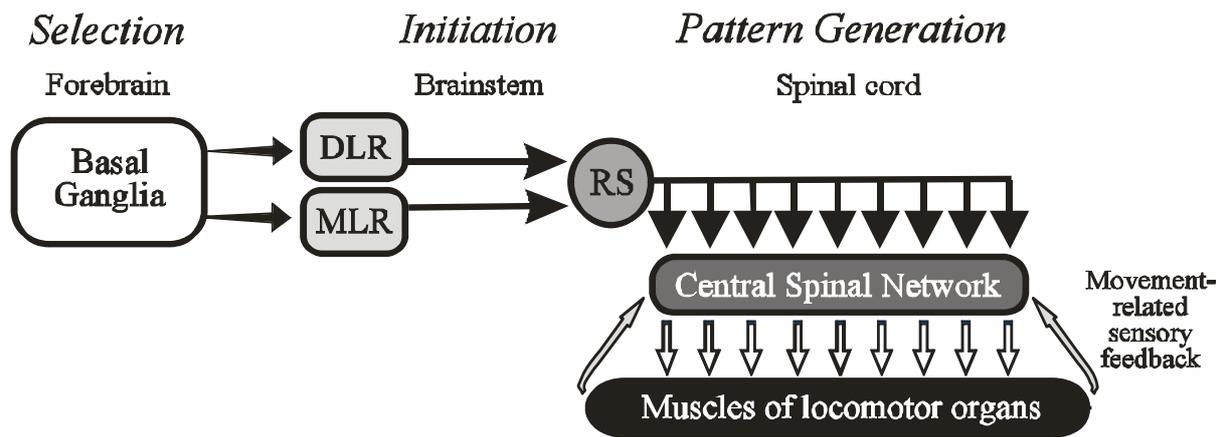


Figure 1. Basic components of locomotor control system in vertebrates. The basic locomotor pattern is generated by the spinal locomotor network in close interaction with sensory feedback. The spinal locomotor network is activated by locomotor regions of the brainstem (diencephalic locomotor region, DLR; metencephalic locomotor region, MLR) through reticulospinal system (RS). The decision to locomote is made by a system responsible for behavioural choice, which includes the basal ganglia.

Basic locomotor mechanisms reside in the spinal cord. The higher levels of CNS send commands to the spinal cord through descending pathways, and activate and modulate the spinal locomotor networks. This functional organization is characteristic for all vertebrates (Orlovsky et al. 1999) (Fig. 1).

Animals propel themselves through space during swimming, walking or flying by rhythmic oscillatory movements of their locomotor organs (body, wings, limbs, etc.). Evidence collected from many studies indicate that locomotor movements are patterned centrally by spinal network (Fig. 1, Grillner, 1981; Orlovsky et al. 1999). This is a locomotor central pattern generator (CPG) – a specialized neuronal circuit that can generate basic rhythmical motor pattern in the absence of sensory movement-related feedback. Sensory inputs can, however, exert strong effects on the motor output (Grillner and Wallen, 2002). In addition, the CPG can exert gating of sensory inputs to adapt their effects to the ongoing phase of a movement (Anderson et al., 1978). Sensory feedback can influence the magnitude and the timing of motor activity (Fig. 2). In vertebrates, the locomotor CPGs are distributed along the spinal cord. Spinal networks integrate sensory feedback about body position and the environment, together with supra-spinal motor commands, to produce a precise and goal-directed locomotion (Orlovsky et al. 1999).

The spinal locomotor networks are activated and modulated by supraspinal structures,

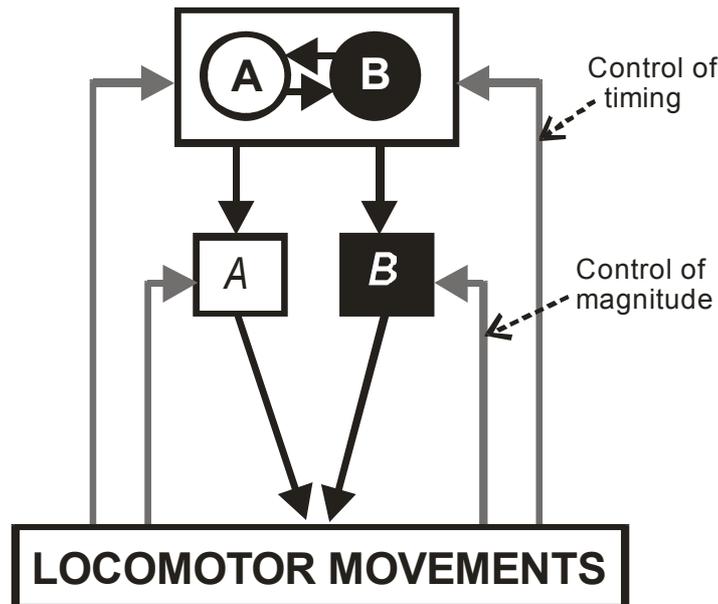


Figure 2. Contribution of sensory movement-related feedback to generation of locomotor pattern. The rhythm generating network of the locomotor CPG (depicted in a square box with two neurons, A and B) drives sets of antagonistic motor neuron pools and muscles (combined in square boxes and marked with letters in italics). Sensory movement-related feedback from limb receptors can contribute either to the control of magnitude of motor output or to the control of timing of motor activity. *Modified from Büschges 2005.*

which is necessary for initiation and adaptive control of the goal-directed locomotion (Fig. 1, Grillner 1997; Mori et al. 2001, Orlovsky et al. 1999). There are several areas in the brainstem and cerebellum which, when stimulated, elicit locomotion in the intact animal. One of these areas is the ‘mesencephalic locomotor region’ (MLR), first described in cats (Shik et al. 1966). It controls the intensity of locomotion in a nearly linear fashion; such as progressively higher stimulation intensities lead to a higher frequency rhythm. There are several lines of evidence showing that stimulation of the MLR activates the CPG through a monosynaptic activation of reticulospinal (RS) neurons located in the pons and medulla. A different area in the brainstem eliciting locomotion when stimulated is the diencephalic (or subthalamic) locomotor region, DLR (Orlovsky 1969; Waller 1940). The DLR also projects to the medial reticular formation (Baev *et al.* 1988), and a large number of RS neurons receive excitatory input from the DLR (Orlovsky 1970). It was found that the fastigial nucleus of the cerebellum projects to a wide area in the reticular formation (Walberg et al. 1962). Stimulation of this nucleus also elicits locomotion in the cat, and activates a large proportion of RS neurons (Mori et al., 1992).

A fundamental question of neuroscience is how different forms of behaviour are selected and initiated in the CNS. Experimental evidence indicates that the basal ganglia are important for the initiation of locomotion. In vertebrates, the basal ganglia project to the DLR and MLR. Activation of ventral striatum (nucleus accumbens) in rodents, elicited by local injection of dopamine or glutamate agonists, leads to the initiation of locomotion channelled through the MLR. Conversely, lesions of the dopamine input to the striatum lead to characteristic deficits in locomotor initiation. It was shown that basal ganglia can contribute to the initiation of locomotion through disinhibition of MLR (Takakusaki et al. 2003).

The lamprey – a simple animal model for analysis of locomotor behaviour

Advantages of lamprey as an animal model

The lamprey (lower vertebrate, cyclostome) originates from a group of animals that diverged from the main evolutionary line of vertebrates around 450 million years ago, when ordinary fish had not yet appeared. The anatomical structure of the lamprey brainstem, spinal cord, sensory organs, and motor apparatus is in many respects similar to that in higher vertebrates (Rovainen 1979). This aquatic animal displays a number of motor behaviours, including forward and backward locomotion, crawling, turning in different planes, postural reflexes, etc. The lamprey presents good opportunities for analytical studies of the neural networks controlling different motor functions. This is because, first, the lamprey has orders of magnitude fewer nerve cells of each type than higher vertebrates. Second, an *in vitro* preparation of the brainstem and spinal cord has been developed which can remain active for several days. Third, the motor patterns underlying different types of motor behaviours (locomotion, turning, postural reflexes, etc.) can be elicited in this isolated nervous system. These features of the lamprey allow analysis of motor functions at the behavioural, network and cellular levels. The lamprey has been used extensively as an experimentally amenable animal model for studying the basic principles of the locomotor control (Grillner et al. 1995; Grillner 1997, 2003).

Locomotor behaviour in lamprey

The lamprey has two main behavioural states, quiescent and active (Rovainen 1979). In the quiescent state, the animal is attached to a substratum by its sucker mouth, and the trunk musculature is almost completely relaxed. In the active state, the animal is detached from the substratum and locomotes.

There are several forms of locomotion in lampreys: forward and backward crawling, fast forward swimming, slow forward swimming and slow backward swimming. Two forms of non-undulatory locomotion, forward and backward crawling, are exhibited by lampreys in natural habitats for moving about or out of tight places. Some aspects of crawling (kinematical and neuronal) have been analysed (Archambault et al. 2001; Zelenin 2005).

The main form of undulatory locomotion, most effective for long-distance migrations, is a fast forward swimming (FFS). It was analysed in considerable details at the behavioural (kinematical), network and cellular levels (Cangiano and Grillner 2003, 2005; Grillner 1997; Grillner and Wallen 2002; Grillner et al. 1981, Wallen and Williams 1984; Williams et al. 1989).

Two other forms of undulatory locomotion strikingly differ from FFS; these are slow backward swimming (SBS) and slow forward swimming (SFS). They can be considered as withdrawal and escape behaviour. Usually, the lamprey exhibits short episodes of SBS when encountering obstacles (McClellan 1998). The lamprey exhibits SFS during attempts to escape when it is caught in the middle part of the body. SBS and SFS in intact lampreys have not been investigated systematically because of difficulties in their elicitation. In the present study, a regular way to evoke long episodes of SBS and SFS by tactile stimulation of large areas in the anterior and middle part of the body, respectively, was found. *The general goal of the present study was, by comparing SBS, SFS and FFS, to reveal the possible similarities and distinctions in the control mechanisms for these three forms of locomotion.*

Kinematical characteristics and EMG pattern of fast forward swimming and crawling

All characteristics of fast forward swimming (FFS) in the lamprey are very similar to the general scheme of undulatory locomotion in fish (Grillner and Kashin, 1976). The lamprey swims by producing lateral undulating movements of the body, usually within a frequency range of 1-8 Hz. The alternating contractions of the myotomes along the left and right side of the body takes place with a phase lag, from rostral to caudal. As a result, waves

of lateral flexions propagate from the anterior part towards the tail. These undulatory waves push the animal forward through the water. The higher the speed of propagating locomotor waves, the faster the lamprey swims. The mechanical wave of body undulations is caused by a corresponding wave of muscle contraction. The body musculature in the lamprey has a segmental organisation. Altogether, there are about 100 muscular segments (myotomes); each myotome is innervated by the left and right ventral roots of the corresponding segment of the spinal cord. Each myotome consists of a superficial layer of red (slow) muscle fibres and a deeper layer of white (fast) fibres. Muscle fibres of a few successive segments are mechanically linked together to form a chain that produces a body flexion when the fibres contract (Rovainen 1979). Each myotome is subdivided into dorsal and ventral parts that are innervated by two separate pools of motoneurons. During rectilinear swimming, both the dorsal and ventral parts of a myotome contract simultaneously and to an equal extent, which results in pure laterally directed body flexion (Grillner and Kashin 1976; Wallen et al. 1985; Williams et al. 1998).

By comparing the EMG pattern with the mechanical pattern (Wallén and Williams 1984; Williams et al. 1989) the following important characteristics of fast forward swimming were revealed: (i) In any myotome, the burst of activity lasts for about 40% of the locomotor cycle, and up to 40% of the myotomes on one side of the body are contracting simultaneously; (ii) The excitation of the ipsilateral parts of myotomes is accompanied by the relaxation of their contralateral parts; (iii) The wave of excitation of myotomes (“electrical wave”) propagates in the caudal direction slightly faster than the mechanical wave; (iv) As in a number of investigated species of fish, in the lamprey the speed of propagation of electrical and mechanical wave and the frequency of undulations change approximately in direct proportion to each other (Grillner and Kashin 1976; Williams et al 1989). Thus the wavelength of the mechanical or electrical wave remains constant and does not depend on the locomotor frequency. In the lamprey, the mechanical wave is about 0.8 of the body length, and the electrical wave is about one full body length during FFS. In other words, the phase lag between oscillations in two different points along the body axis is constant and does not depend on the frequency of oscillations. For the electrical wave, the phase lag per segment at any frequency is around 1% of the locomotor cycle. By contrast, the time lag changes in inverse proportion to the frequency.

During crawling, the lamprey adapts its body shape to the configuration of external constraints. In contrast to FFS, crawling is a non-undulatory locomotion: forward or backward progression is caused by propagation of a solitary wave. The muscle activity

responsible for propulsion is confined to the area around the body bend. Muscles on the inner (concave) side are activated when approaching the turn, and inactivated on the top of the arc. Muscles on the outer (convex) side are co-active with their antagonists, but also active in the area of straightening of the body bend. This pattern of motor activity propagates along the body (Archambault et al. 2001). Thus, in contrast to the reciprocal activity of muscles in any point along the body during FFS, the motor pattern used for crawling is characterized by (i) the muscle activity located near the curved part of the body, and (ii) co-activation of the right and left muscles. The spinal mechanisms responsible for generation of this pattern of muscle activity have not yet been investigated.

Neuronal control of fast forward swimming

Neuronal mechanisms responsible for the control of fast forward swimming (FFS) has been extensively studied and their organization and function have been understood to a considerable extent (Fig. 3, for review see Deliagina et al. 2002; Grillner 2003; Grillner et al. 1981, 1995, 2000; Sirota et al. 2000).

The motor pattern of FFS is generated by the spinal CPG (Fig. 3) (Wallén and Williams 1984). Motor output in each segment consists of alternating bursts of activity of the left and right motoneuron pools. The onset of activity in each segment is delayed in relation to its rostral neighbour segment. This basic pattern can be generated by the spinal cord deprived of sensory feedback. A small piece of the spinal cord, at any location along the body, can generate rhythmic oscillations (Cohen and Wallen 1980), and the spinal CPG for FFS can thus be viewed as a chain of unitary oscillators (segmental CPGs). Each segmental CPG consists of two symmetrical parts – the left and right half-centres, which are active in alternation and inhibit each other through crossed inhibition from glycinergic interneurons (Grillner et al. 1995).

The main components of each half-center are excitatory interneurons, which provide a phasic excitatory drive to motoneurons during locomotor cycle (Buchanan and Grillner 1987) and also to glycinergic interneurons with crossed axons that inhibit all neurons of the contralateral half-center (Buchanan 1982, Grillner 1985). The circuitry of each half-center is able to generate rhythmical bursts by itself (Cangiano and Grillner 2003); the crossed

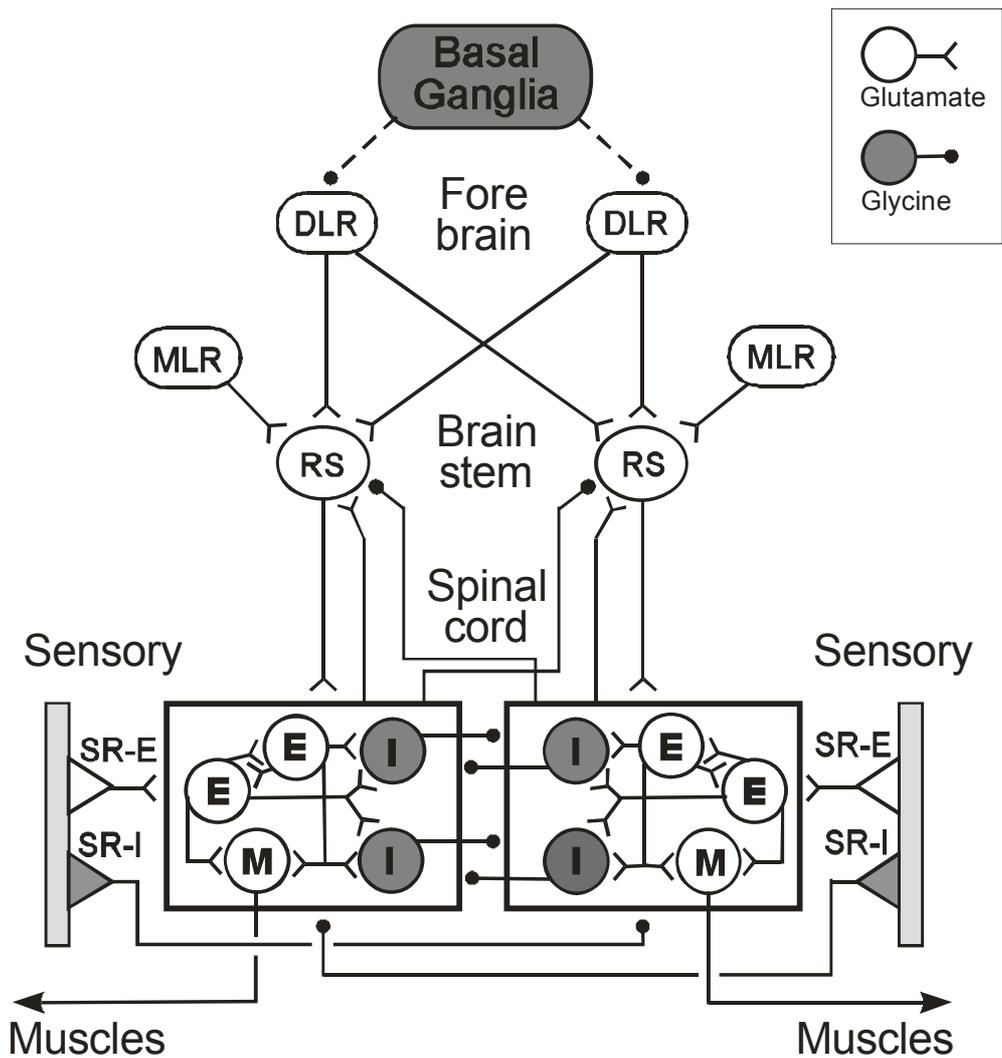


Figure 3. Neuronal organization of locomotor control system in lamprey. The basic locomotor pattern is generated by the spinal network. Segmental CPG consists of the left and right half-centres (in square boxes). Each half-centre contains excitatory glutamatergic interneurons (E) (which affect all ipsilateral neurons) and inhibitory glycinergic interneurons (I) (which cross the midline and inhibit all neurons of the contralateral half-centre). Excitatory intraspinal stretch receptor neurons (SR-E) project ipsilaterally, and inhibitory ones (SR-I) project contralaterally. Spinal locomotor network is activated by reticulospinal (RS) neurons. Locomotion can be initiated and controlled from diencephalic (DLR) and mesencephalic (MLR) locomotor regions, which in their turn are presumably controlled by basal ganglia. *Modified from Grillner 2003.*

inhibitory connections not only ensure alternation but also down-regulate the endogenous unilateral frequency (Cangiano and Grillner 2003; Grillner and Wallen 1980). Ipsilateral glycinergic inhibition is not required for rhythm generation and even a single hemisegment is sufficient to produce rhythmic bursting (Cangiano and Grillner 2003).

Both cellular and network properties contribute to the generation of rhythmicity in this system. First, some of the interneurons are conditional bursters with NMDA-dependent pacemaker properties (Wallén and Grillner 1987). Second, the interneurons can be excited upon rebound after termination of the inhibitory input from the contralateral half-centre (Grillner and Wallen 1980).

The segmental CPGs affect each other through propriospinal connections and form a chain of oscillators along the spinal cord. Two different hypotheses have been formulated to explain rostro-caudal propagation of locomotor waves. According to the “network reconfiguration” hypothesis (Kopell and Ermentrout 1986, 1988), all the unitary oscillators in the spinal cord are equivalent to each other, and the direction of propagation of the waves is determined by the asymmetry of connections of each oscillator with its rostral and caudal neighbours. The alternative “trailing oscillators” hypothesis (Cohen 1987; Rand et al. 1988; Matsushima and Grillner 1990, 1992) suggests that the rostral oscillator, with the shortest intrinsic period, plays the leading role in the chain, and determines the frequency of oscillations in all spinal segments, as well as the rostro-caudal direction of locomotor wave propagation. Signals from intraspinal mechanoreceptors give rise to the sensory feedback, which may affect the locomotor pattern and adapt it to a variety of environmental conditions (Viana Di Prisco et al. 1990).

Activation of the spinal locomotor network is produced by an excitatory drive from a population of reticulospinal (RS) glutamatergic neurons (Brodin et al. 1988; Grillner et al. 1981, 1995, 2000). This drive increases the excitability of the oscillator interneurons and motor neurons, and switches the neurons that are conditional bursters from the resting state into the oscillatory state.

In the lamprey, locomotion can be initiated and controlled from the diencephalic (DLR) and mesencephalic (MLR) locomotor regions, both of which project to RS neurons (Brocard and Dubuc 2003; El Manira et al. 1997; Le Ray et al. 2003; Sirota et al. 2000). These two structures are presumably involved in the control of goal-directed locomotion in different behavioural contexts. Recent studies (Ménard et al., 2007) demonstrated that MLR receives GABA-ergic inputs from the medial pallium (which might be related to the amygdala or pallidal structures of higher vertebrates, and thought to be involved in the selection of motor programs), from the thalamus and the hypothalamus, which also could take part in the motivational aspect of motor behavior in lampreys. It also has been shown that MLR is maintained under tonic GABAergic inhibition that, once discontinued, releases locomotor command (Ménard et al., 2007).

A switch of the lamprey from the quiescent state to the active (locomotor) state can take place spontaneously but can also be caused by different sensory stimuli (illumination of eyes or illumination of tail dermal photoreceptors, tactile stimuli, water vibration, vestibular stimuli) provided that the stimuli are strong and/or long enough (Deliagina et al. 2002). Initiation of locomotion is always preceded by a sharp, bilateral activation of RS neurons (Deliagina et al. 2000). This bilateral activation occurs irrespective of the modality and laterality of the applied sensory stimulus, and swimming continues as long as a high level of RS activity is present. One of the possible sites of the transformation of the unilateral sensory signal into the bilateral RS activity is the MLR, which has bilateral projections to RS neurons and evokes symmetrical locomotion. In the lamprey, a high activity of RS neurons and swimming can last for many seconds after termination of the stimulus, presumably due to the NMDA-mediated plateau properties of some RS neurons (Viana Di Prisco et al. 1997).

Recently the activity of individual RS neurons during FFS and crawling was characterized (Zelenin 2005). It was shown that these forms of locomotion in lampreys can be caused by considerably overlapping groups of RS neurons. Based on correlation of the RS activity with the direction of crawling, two groups of RS neurons were revealed. One group is presumably responsible for initiation of forward crawling and the other one – for initiation of backward crawling (Zelenin 2005).

Modification of the pattern of fast forward swimming by sensory signals

Sensory signals of different modalities (vestibular, visual and tactile) can have unspecific and specific effects on FFS (Deliagina et al 2002). Sufficiently strong and/or long stimuli evoke unspecific response: initiation or acceleration of FFS. Weaker stimuli evoke specific responses, that is, modification of the FFS pattern with the aim to adapt it to the environmental conditions.

During FFS, the lamprey actively maintains the horizontal, dorsal side-up body position by stabilizing its orientation in the sagittal (pitch) and transversal (roll) planes. It also stabilizes the direction of swimming in the horizontal (yaw) plane. Deviation from the desirable body orientation in any of these three planes (caused by an external force) leads to activation of a specific group of vestibular afferents (Deliagina et al. 1992b) which, through the neurons of the octavomotor (vestibular) nuclei (Northcutt 1979a) activate a specific population of RS neurons (Deliagina et al. 1992a; Deliagina and Fagerstedt 2000;

Karayannodou et al. 2007; Pavlova and Deliagina 2002;). The activated population of RS neurons causes a corrective motor response through modification of the basic locomotor pattern of FFS generated by the spinal CPG (Zelenin et al. 2007). In the pitch and yaw planes, the corrections occur due to the body bending in the corresponding plane. This is due to predominant activation of both dorsal or both ventral myotomes, or due to predominant activation of the dorsal and ventral myotomes on one side, respectively) (McClellan and Hagevik, 1997; Ullén et al., 1995a; Zelenin et al. 2007). In the roll plane, the corrections occur due to a change of the direction of locomotor body undulations, from lateral to oblique (through predominant activation of the ipsilateral ventral and contralateral dorsal parts of myotomes) (Zelenin et al. 2003; Zelenin et al. 2007).

Unilateral eye illumination applied during FFS causes two types of responses: *the dorsal light response* and *the negative phototaxis*. The mechanism determining which of these responses will be evoked remains to be revealed.

The dorsal light response is a change of body orientation in the roll plane caused by asymmetrical visual input. When the lamprey swims into a zone with asymmetrical illumination, it tilts around the longitudinal axis towards the light (Ullén et al. 1995b). Functionally, it can be viewed as a behavioural phenomenon aimed at maintaining an orientation where the illumination of the left and right eye is symmetrical (Bischof, 1974; von Holst 1935). Together with the camouflage pigmentation of the animal's skin, the dorsal light response presumably serves to prevent detection by predators: by turning the dark dorsal side towards the light, exposure of the white belly upwards and dark back downwards is avoided (Guthrie and Muntz 1986). The putative pathway underlying the dorsal light response is the following: afferents activated by ipsilateral eye illumination affect excitatory commissural pretectal neurons in the contralateral pretectum, which in turn activate a specific population of ipsilateral RS neurons involved in the control of body orientation in the roll plane (Deliagina and Fagerstedt 2000; Deliagina et al. 1993; Ullén et al. 1997; Zompa and Dubuc 1996).

The neuronal mechanism underlying the dorsal light response was studied in considerable detail (Deliagina and Fagerstedt 2000; Deliagina et al. 1993). It was shown that unilateral illumination of eyes modifies operation of the roll control system responsible for stabilization of the body orientation in the transversal plane. Body stabilization in roll plane is based on the interaction of two antagonistic postural reflexes mediated by two populations of RS neurons. The animal stabilizes the orientation at which these reflexes are equal to each other. This is an equilibrium point of the system, which (in the case of symmetrical eye

illumination) corresponds to the dorsal side-up orientation. Unilateral eye illumination shifts the equilibrium point of the system by changing the gain in one of the reflex chains through activation of the ipsilateral population of RS neurons. Activation of this population leads to predominant activation of ipsilateral ventral and contralateral dorsal parts of myotomes and therefore to a change of the direction of locomotor body undulations, from lateral to oblique (Zelenin et al. 2003; Zelenin et al. 2007). This leads to rotation of the animal around the longitudinal body axis towards the source of light (Zelenin et al. 2003).

Negative phototaxis is a horizontal turn of the swimming animal away from the source of light. This behavioural reaction is clearly an effective way of avoiding illuminated areas, where the risk of detection by predators is higher. The putative pathway underlying the negative phototaxis was revealed in the lesion study (Ullén et al. 1997). It includes (i) afferents activated by ipsilateral eye illumination, which activate (ii) excitatory neurons in the contralateral pretectum, which activate (iii) a specific group of contralateral RS neurons, which cause the turn away from the light. The neuronal mechanism underlying the negative phototaxis has not yet been investigated in detail.

Brief mechanical stimulation of the head in the swimming lamprey causes a lateral turn away from the stimulus (McClellan 1984; McClellan and Hadevik 1997). The correlates of this behaviour (“fictive” lateral turn) can be evoked in the *in vitro* preparation (Fagerstedt and Ullén 2001; Fagerstedt et al. 2001). It was shown that this reaction is mediated by trigeminal afferents. The pathway mediating trigeminal influences on RS neurons was characterized only partly. Trigeminal afferents terminate ipsilaterally in the descending trigeminal nucleus, which extends through the whole rhombencephalic alar plate (Koyama et al. 1987; Northcutt 1979b). Cells within this nucleus project to the middle and posterior rhombencephalic reticular nuclei (Viana Di Prisco et al. 2005). The role of these cells for the transmission of excitatory and inhibitory influences to RS neurons is not clear.

In the intact lamprey, lateral turns are associated with asymmetry in the bilateral mass RS activity, the activity on the ipsilateral (to a turn) side being higher than that on the opposite side (Deliagina et al. 2000). Intracellular recording from individual RS neurons during “fictive” lateral turn has revealed the population of neurons activated during the ipsilateral turn and inhibited during contralateral turn (Fagerstedt et al. 2001). This population can be responsible for modifications of FFS pattern, which underlie the lateral turn. It is possible that the same population of RS neurons is responsible for initiation of the lateral turn in different behaviours, since modifications of FFS pattern during lateral turns in different behaviours are similar. During lateral turns in the swimming lamprey (McClellan 1984; Ullén

et al. 1993), a mechanical wave with a larger amplitude propagates along the body, so that the orientation of the body in the yaw plane changes, initially in the rostral part, then progressively in more caudal parts (McClellan 1984, McClellan and Hagevik 1997; Ullén et al. 1993). The pattern of muscle activity during turning is characterized by an increase in EMG burst amplitude and duration on the side towards which the animal is turning.

Specific aims of the present study

1. To investigate kinematical and EMG patterns of two forms of swimming, slow backward and slow forward ones, and to compare these patterns with the pattern of fast forward swimming. (*Papers I and II*)
2. To describe the receptive fields for elicitation of slow backward and slow forward swimming, as well as to reveal groups of afferents transmitting signals from the receptive field to the CNS. (*Papers I and II*)
3. To elucidate the effect of different lesions in the spinal cord on the initiation of slow backward and slow forward swimming. (*Papers I and II*)
4. To assess if the lamprey stabilizes its spatial orientation during slow backward and slow forward swimming. (*Papers I and II*)

METHODS

All experiments in this study were performed on adult river lampreys (*Lampetra fluviatilis*) *in vivo* (Papers I-II). Animals were kept in an aerated freshwater aquarium at 5-7°C, with a 12h:12h light/dark cycle. All experiments were approved by the local ethical committee (Norra Djurförsöksetiska Nämnden).

Surgical procedures

Surgery was performed under MS-222 (Sandoz) anaesthesia (100 mg/l). To analyse the EMG pattern of SBS, SFS and FFS, chronic implantation of up to four bipolar EMG electrodes in the dorsolateral left and right muscles in the mid-body area, at two rostro-caudal levels was performed.

To reveal groups of afferents causing SBS and SFS, denervation of their receptive fields was performed. For this purpose, the roots of the spinal cord at the level of receptive field, as well as the trigeminal nerve, the posterior and anterior branches of lateral line nerves were transected bilaterally. The trigeminal nerve and the anterior branch of the lateral line nerve were cut at the level of entering the brainstem, the posterior branch of the lateral line nerve was cut at the level of the 1st gill. *Post mortem* investigation showed that, in all cases, transection was complete.

To elucidate the effect of different lesions in the spinal cord on SBS and SFS, the following damages were performed: (i) a complete transection of the spinal cord at different rostro-caudal levels; (ii) a lateral hemisection of the spinal cord in the rostral or middle segments; (iii) a bilateral transection of the lateral columns at the level of the 2nd gill; (iv) a transection of the medial part of the spinal cord at the level of the 2nd gill.

Histological control of spinal lesions

The extent of lesions was determined histologically *post mortem*. A part of the spinal cord containing the lesion was fixed for 12 h in 3% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Then it was placed in 30% sucrose in

0.1 M phosphate buffer until sank, and then sectioned on a cryostat microtome at 40 μm . Sections were stained with cresyl violet.

Experimental procedure

Slow swimming was evoked by tactile stimulation. For this purpose, an elastic ring (0.5-3.5 cm in length) or, for initiation of SBS, an elastic “cap” (a finger of a surgical glove, ~5 cm in length) was put over the receptive field (head and/or rostral part of the body - for SBS; middle part of the body - for SFS). When released in water, the lamprey usually exhibited slow swimming until the ring or cap slid off. The FFS was initiated either spontaneously or by a strong pinch of the tail.

In most experiments, swimming was studied in a shallow aquarium (80 X 80 cm, 10 cm depth). Movements of the animal were recorded from above by a video camera (25 frames/s), positioned at the distance of 2 m from the aquarium. Video and EMG recordings were performed simultaneously and synchronized by pulses recorded simultaneously by both systems.

For testing the capacity for spatial orientation during slow swimming, the lamprey with the stimulating cap or ring was released into a deeper aquarium (110 X 35 cm, 37 cm depth) and video recorded in the free water, i.e., before the animal contacted the walls or the bottom. The side view and the top view (by means of a mirror) were recorded simultaneously.

Processing of data

Signals from implanted EMG electrodes were amplified by conventional AC amplifiers, rectified, smoothed (time constant, 50 ms), and then stored on a PC-computer.

Characteristics of the swim motor pattern were defined as in the preceding studies (see e.g. Matsushima and Grillner, 1992; Williams *et al.*, 1989). Briefly, the positions of the points of maximal concave and convex curvature were determined in video images. The distance between two such neighbouring points was taken as a half of the locomotor wavelength; this value was multiplied by 2 and divided by the body length to obtain the wavelength expressed in body lengths. The speed of locomotor waves was calculated by

tracking a maximum curvature point or a zero curvature point along the body; the speed was expressed in body length per second. Tracking positions of one body point (the head tip or the tail tip) provided the trajectory of the point; it allowed calculating the speed along trajectory. Averaging of the trajectory across the cycle duration provided an estimate of the whole body (center of mass) progression, and allowed calculating the speed of progression.

Onset and termination of an EMG burst were measured, and the midburst point was then calculated. The cycle duration was defined as the time interval between the midpoints of two successive bursts. The burst proportion was defined as the ratio of burst duration and cycle duration. The phase difference between two EMGs was calculated as the ratio of the time interval between the midpoints of the corresponding bursts divided by the cycle duration. The phase lag values were further divided by the number of segments between the two EMG electrodes to obtain the phase lag per segment value. All values are presented as mean \pm SD.

RESULTS AND DISCUSSION

Comparison of kinematical and EMG characteristics underlying slow backward, slow forward and fast forward swimming

A comparative analysis of kinematics and motor patterns for three forms of locomotion in the lamprey (SFS, SBS and FFS) was done in *Papers I and II*. It was shown that SBS and SFS are based on the same principle as FFS, i.e., the waves of periodic lateral body flexion propagate along the body. During SFS and FFS, they propagate in the rostral-caudal direction. By contrast, during SBS, the direction of wave propagation is opposite (caudo-rostral).

It was found that main kinematical characteristics of SBS and SFS are similar but strongly differ from those of FFS (Fig. 4A,B,D,E): the amplitude of lateral undulations was much larger, their frequency was lower, speed of the undulatory wave along the body was slower. Because of large head undulations during SBS (Fig. 4D) and tail undulations during SFS (Fig. 4E), the swim trajectory became very curvilinear, and the speed of whole body progression (Fig. 4B) constituted only a small proportion of the speed along trajectory (Fig. 4C). Thus SFS and SBS are not effective for long distance migrations. Instead, they are used for escape and withdrawal behaviour, respectively.

It was found (*Paper I*) that during both SBS and FFS the length of the mechanical waves travelling along the body was approximately equal to the body length. Also, the phase shift per segment was similar at different swim frequencies.

The most striking feature of SFS was a non-uniformity of the shape and speed of locomotor waves propagating along the body: close to the site of stimulation, the waves slow down, and the body curvature increases several-fold due to an enhanced muscle activity (*Paper II*). This can be interpreted as an attempt to use the stimulating (holding) object as a support for pulling the body out of the grip, using the rostral part of the body as a lever. If the attempt with flexion to one side fails, the next SFS wave with flexion to another side is generated. If the pulling is effective and the body slides forward in relation to the stimulus, it means that the stimulus slides backwards in relation to the body. It was shown that the site of the body, where the characteristics of locomotor pattern are modified, follows the stimulus. Thus it seems likely that the local tactile stimulation causes a local decrease of the wave

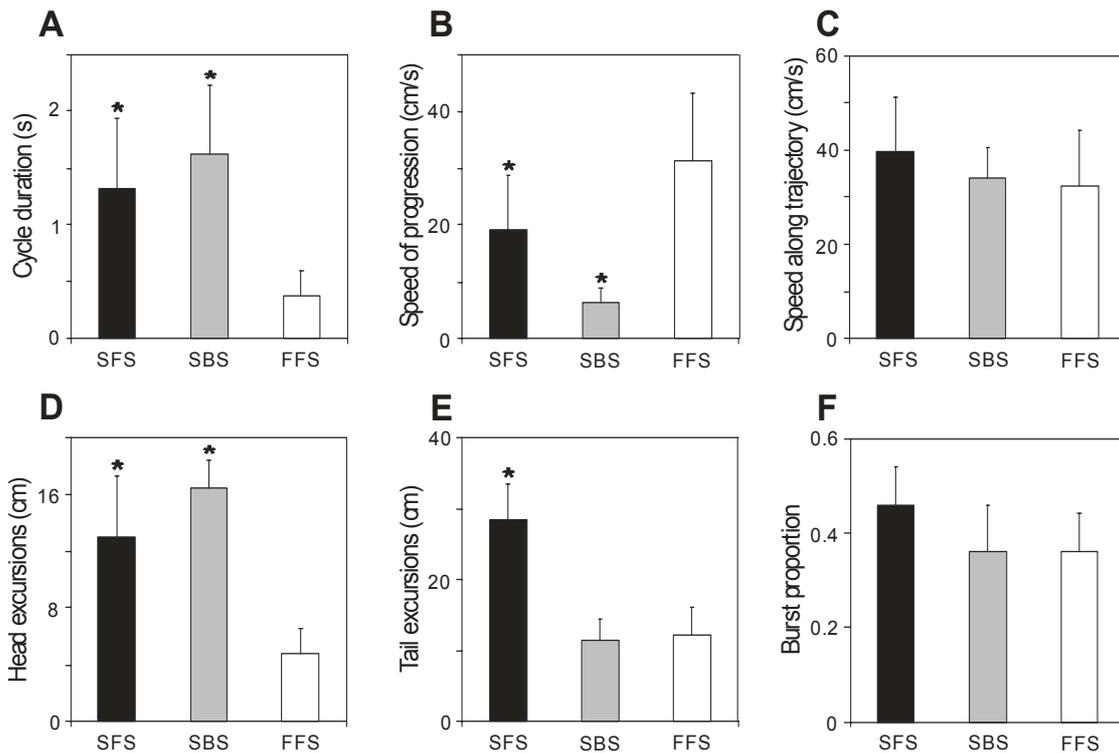


Figure 4. Comparison of main characteristics of kinematical and EMG patterns for SFS, SBS and FFS. *A:* swim cycle duration. *B:* speed of progression. *C:* speed of head movement along trajectory. *D:* lateral head excursions. *E:* lateral tail excursions. *F:* proportion of the EMG burst in the swim cycle. In each graph, mean values \pm SD are indicated for SFS (black bar), SBS (grey bar) and FFS (unfilled bar). Means for SBS and SFS, statistically different from those for FFS are indicated by asterisks (t-test, $p < 0.05$).

speed and an increase of muscle activity, resulting in strong body flexion. These local modifications may be done through 5-HT system. This system in the spinal cord is known to cause intense efferent bursts during fictive locomotion, with much longer burst duration and with a reduced wavelength (Harris-Warrick and Cohen 1985; Wallén et al. 1989). On the other hand, 5-HT fibers in the dorsal roots of the lamprey spinal cord have been revealed immunohistochemically (Harris-Warrick et al. 1985; Van Dongen et al. 1985).

Relationships between the muscle activity and the body flexion during three modes of locomotion were determined (*Papers I and II*). The EMG burst proportion in the swim cycle during SFS, SBS and FFS were similar (Fig. 4F). During SFS and SBS, as well as during FFS, the EMG burst on the two sides alternated. During FFS and SFS, the EMG in more rostral points led in phase the EMG in more caudal points, whereas during SBS the opposite relationship was observed.

Active muscles initially caused a progressive decrease of the contralateral body

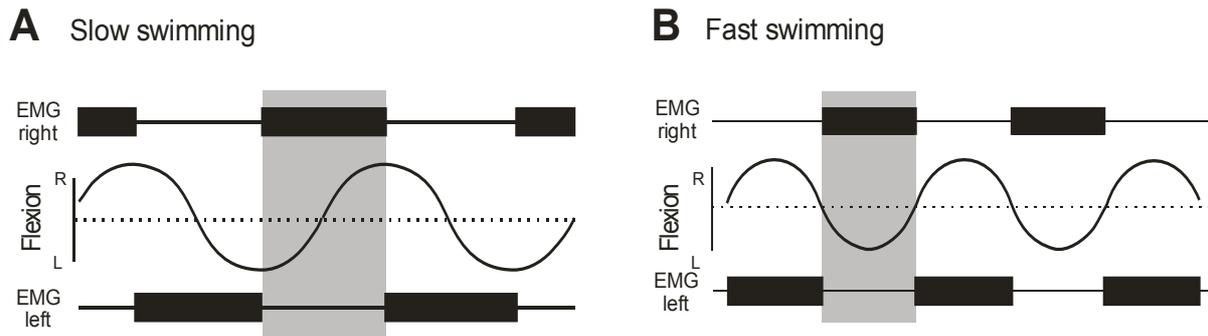


Figure 5. Schematic representation of the correlation between body kinematics and muscular activities during slow (A) and fast (B) swimming.

flexion and then they caused an increasing ipsilateral body flexion both in SBS and SFS (Fig. 5A), while in FFS the active muscle initially decelerated the contralateral flexion and then caused a decrease of the contralateral body flexion followed by the ipsilateral flexion (Fig. 5B) (Wallén and Williams, 1984). This difference in timing of body flexion and EMG bursts for slow and fast swimming may be caused by higher velocities of body bending necessary for the faster FFS rhythm.

Receptive fields and afferents causing slow forward and slow backward swimming

In the present study, a regular way to evoke long episodes of SBS and SFS by tactile stimulation of large areas in the anterior and middle part of the body, respectively, was found (*Papers I and II*). By positioning the stimulus (an elastic ring, 0.5-3.5 cm in length) on different rostro-caudal parts of the body, the receptive fields causing different forms of swimming could be revealed (*Paper II*). It was found that the receptive field for the SBS occupies the anterior part of the body (area from 0 to 20% of body length, Fig. 6A), the receptive field for the SFS – middle part of the body (area from 20 to 65% of the body length, Fig. 7A). Sometimes, but very rarely, the FFS was elicited from the SFS receptive field. Locations of the receptive fields well corresponded to the behavioral (functional) role of SBS and SFS, which can be considered as withdrawal and escape behaviour, respectively. The lamprey exhibits SBS if something threatening is encountered during forward progression, or if the rostral part of the body (the head and gill region) got into a tight split. When the animal

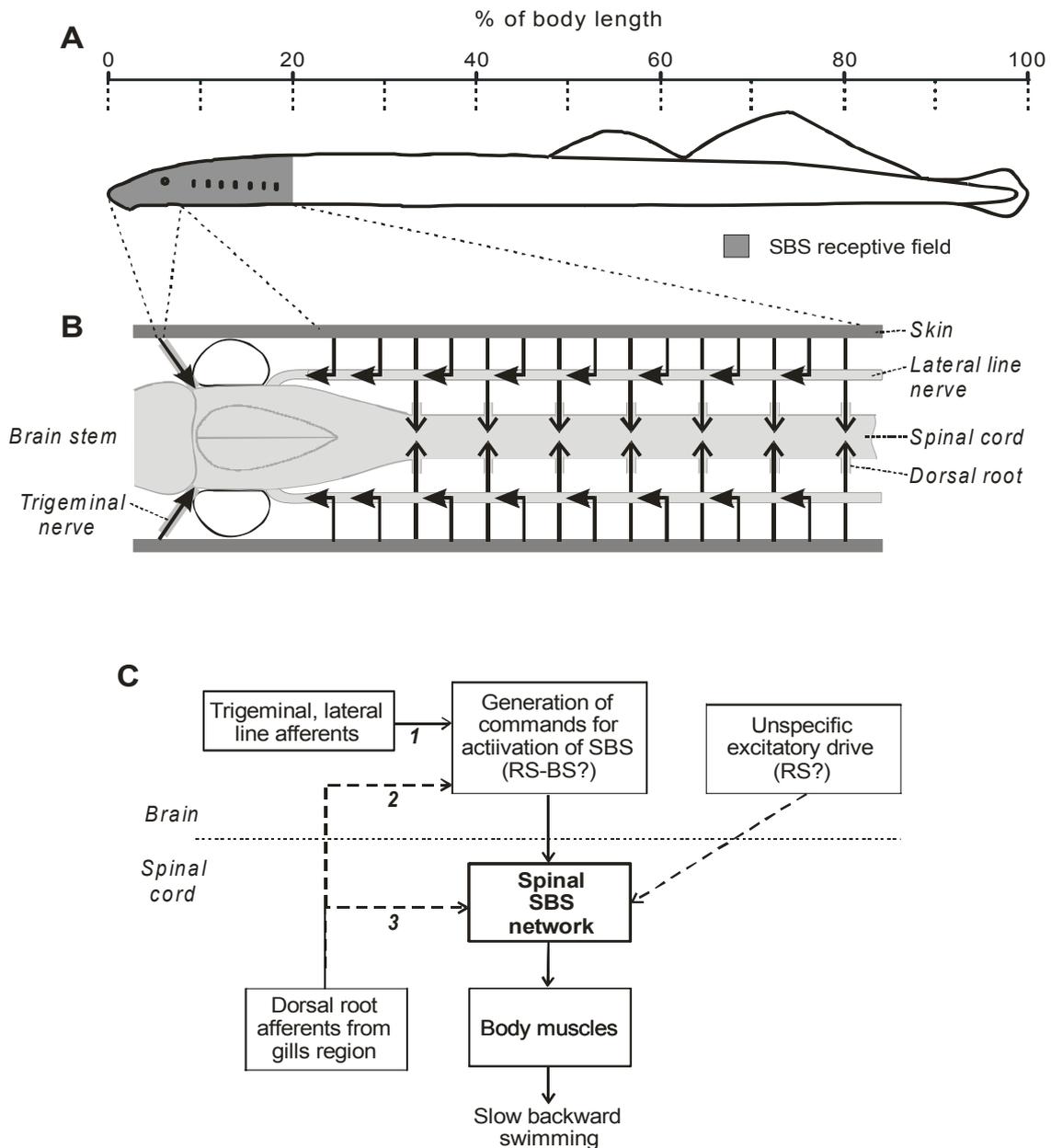


Figure 6. Organization of control system for SBS. *A*: receptive field. *B*: schematic representation of the afferents (shown by black arrows) transmitting signals from different areas of the receptive field to CNS. Trigeminal afferents transmit signals from the head area. Dorsal roots afferents and posterior branch of lateral line nerve transmit signals from the gill region of the receptive field. *C*: proposed scheme for initiation of SBS. Activation of trigeminal and lateral line nerve afferents (1) (transmitting sensory signals from the head and gill area of the receptive field) leads to formation of supraspinal commands for SBS, transmitted presumably by a specific population of RS neurons (RS-BS). This command activates the spinal network responsible for generation of SBS pattern. Afferents of dorsal roots (transmitting sensory signals from the gill area of the receptive field) may activate the spinal network either through brainstem (2) or directly (3). The level of excitability of the spinal SBS network is maintained by unspecific supraspinal tonic drive transmitted by another population of RS neurons. Solid arrows indicate connections suggested by experimental data. Interrupted arrows indicate suspected connections.

is held in the middle part of the body (between the gill region and the caudal dorsal fin), the SBS may be ineffective, and a different motor pattern, SFS, is used.

The afferents transmitting signals from the receptive fields to the CNS and responsible for initiation of SBS and SFS were determined (*Paper II*). For this purpose, different nerves (trigeminal nerves, posterior branch of lateral line nerves, dorsal and ventral roots) innervating the skin areas that correspond to the receptive fields were transected. It was found that tactile information necessary for induction of SBS comes via a number of routes – (i) dorsal roots in the gill region of the spinal cord, (ii) the posterior branches of the lateral line nerves (from the gill area of the receptive field), (iii) the trigeminal nerves (from the head area of the receptive field) (Fig. 6B). Tactile information necessary for induction of SFS comes via dorsal roots of the middle segments of the spinal cord (Fig. 7B).

The afferents of the posterior branch of the lateral line nerve enter the brainstem and terminate bilaterally in the medial octavolateral nucleus by crossing through the cerebellar commissure (Ronan and Northcutt 1987). Trigeminal afferents, after entering the brainstem, terminate in the sensory part of the ipsilateral trigeminal nucleus (Koyama et al 1987; Matthews and Wickelgren 1978). Cells within this nucleus project to the middle and posterior rhombencephalic reticular nuclei (Viana Di Prisco et al. 2005). The fact that trigeminal afferents as well as afferents of the poster branch of the lateral line nerves (when stimulated alone) are able to evoke SBS indicates that this form of slow swimming is evoked by specific commands coming from the brain (Fig. 6C). In lamprey, the main descending system transmitting the commands to all regions of the spinal cord is the RS system (Rovainen, 1979). One may expect that specific population of RS neurons is responsible for elicitation of SBS (RS-BS in Fig. 6C).

In lampreys, the dorsal roots afferents have their cell bodies located in either the dorsal root ganglia, or in the spinal cord (“dorsal cells”, Freud 1878). The dorsal cells are good candidates for initiation of SFS and SBS. They are sensitive to tactile stimulation (pressure and touch) (Christenson et al. 1988). Stimulation of dorsal cells evokes local muscle reflexes (Teräväinen and Rovainen 1971), which could be responsible for the enhancement of muscle activity close to the site of stimulation during SFS. Dorsal cells also relay sensory information to the reticular nuclei (Dubuc et al. 1993). It is not clear at present if the dorsal roots afferents causing SFS and SBS directly affect the spinal networks (3 in Fig. 6C and 1 in Fig 7D) and induce respective form of slow swimming, or their signals are transmitted to the brain (2 in Fig. 6C and 1 in Fig. 7C) and used for formation of specific supraspinal commands presumably transmitted by RS neurons (RS-FS in Fig. 7C).

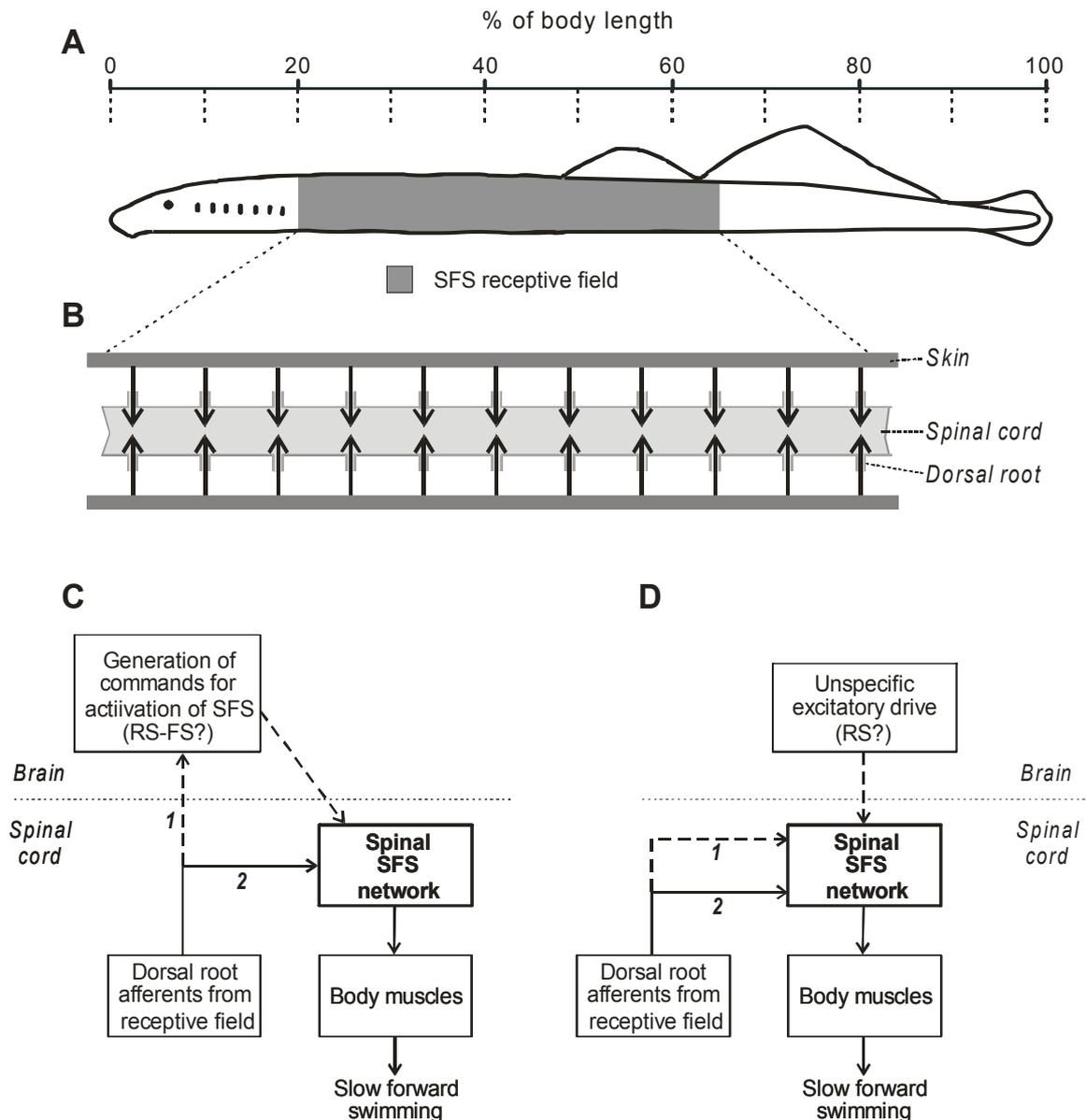


Figure 7. Organization of control system for SFS. *A*: receptive field. *B*: schematic representation of the dorsal root afferents (shown by black arrows) transmitting signals from the receptive field to CNS. *C, D*: possible schemes for initiation of SFS. Afferents of dorsal roots may activate the spinal network responsible for generation of SFS either through brainstem (*C, 1*) presumably through population of RS neurons (RS-FS), or directly (*D, 1*). The afferents are also responsible for slowing down the locomotor wave and increase of body curvature close to the site of stimulation (*C, 2*; *D, 2*). Solid arrows indicate connections suggested by experimental data. Interrupted arrows indicate suspected connections.

Effects of spinal cord lesions on slow forward and slow backward swimming

It was found that transection of the spinal cord did not affect the pattern of SBS rostrally to the lesion, suggesting that SBS can be initiated from different levels of the spinal cord (*Paper I*). After transection of the spinal cord at the level of the second gill, stimulation of the receptive fields evokes neither SFS nor SBS. However, this result may have different interpretations. Absence of swimming can be caused by abolishing of specific supraspinal commands (Figs. 6C; 7C). It can also be caused by deprivation of spinal networks of unspecific excitatory drive (Figs. 6C; 7D). As a result of such deprivation, the level of excitability of spinal networks becomes low, and they cannot be activated by signals from the receptive fields. The fact that backward swimming in the spinal dogfish can be evoked by stimulation of the rostral cutaneous innervation field (Grillner 1974) supports the latter suggestion.

It was found that partial lesions of the spinal cord (bilateral lesion of lateral funiculi, lesion of the medial part, and hemisection) at the level of the 2nd gill, did not interfere with initiation of SBS or SFS from different parts of receptive fields (*Paper II*). This means that descending pathways, responsible for the transmission of supraspinal commands for SBS, are evenly distributed across the spinal cord, and a unilateral command is sufficient to evoke SBS. If the SFS is also caused by supraspinal commands, the obtained result suggests that the descending pathways responsible for transmission of these commands, as well as the ascending pathways transmitting the signals necessary for the formation of these commands, are also dispersed across the spinal cord.

It was shown that, in contrast to a lateral hemisection performed in rostral segments of the spinal cord, a lateral hemisection in the midbody area did not affect the alternating EMG pattern rostral to the lesion; whereas caudal to the lesion, the EMG bursts persisted on the intact side, but were reduced or absent on the lesioned side (*Paper I*). The possible interpretation of this finding is that there is a difference in the intensity of supraspinal excitatory drive to motoneurons located in the rostral and caudal segments of the spinal cord. It was shown that the majority of RS neurons terminate in the rostral part of the spinal cord (Bussi eres 1994). As a result, the level of excitability of the motoneurons below the rostral hemisection remains high and they respond to the signals from the rhythm generator. By contrast, the level of excitability of the motoneurons caudal to the hemisection in the midbody level is very low, and they do not respond to signals from the rhythm generator.

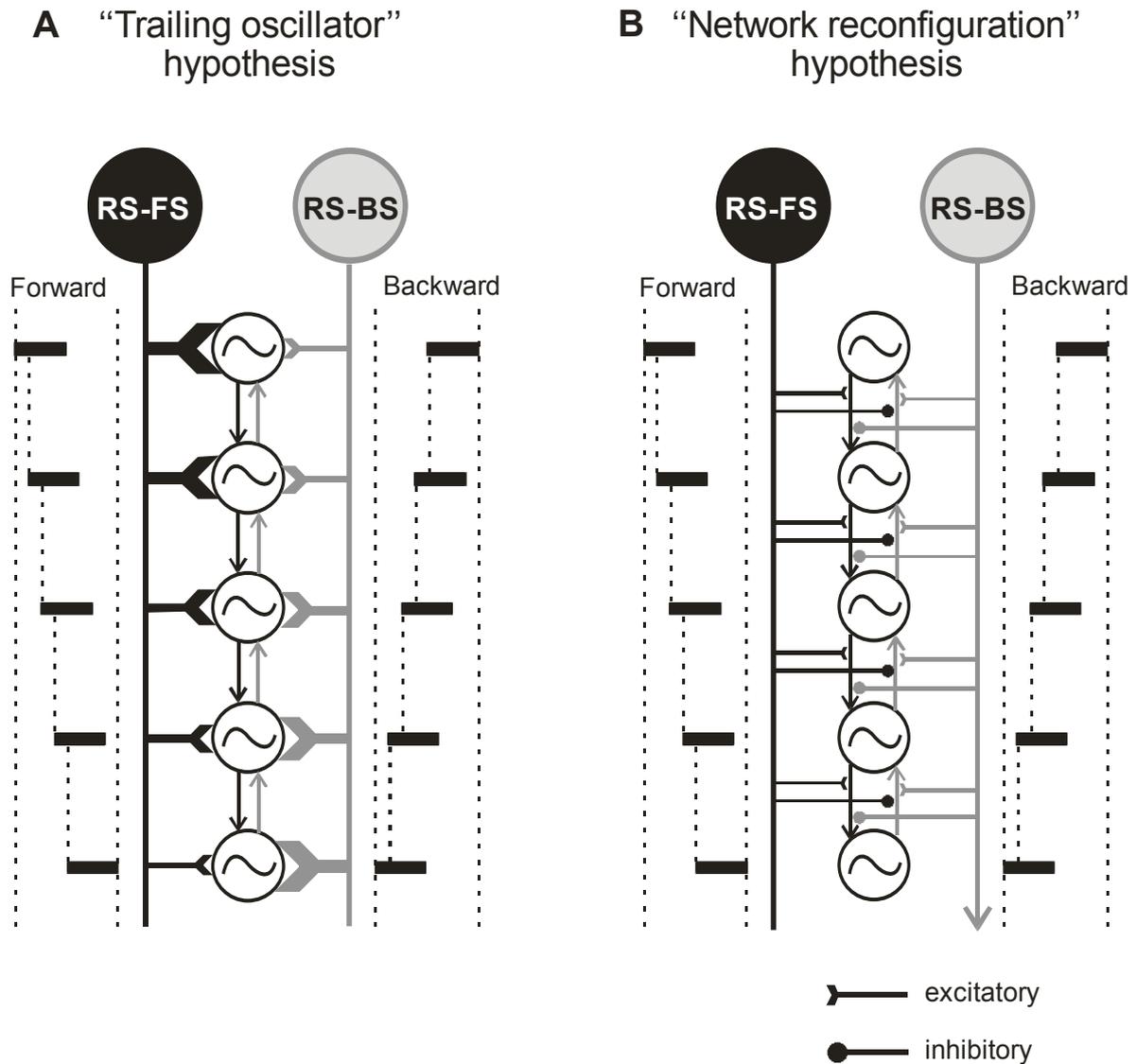


Figure 8. Two hypotheses on generation of different forms of swimming. *A:* The “trailing oscillator” hypothesis. The population of RS neurons responsible for activation of SFS (RS-FS) creates the rostro-caudal gradient of excitability in the chain of coupled oscillators. The rostral segment with higher excitability becomes the leading one and entrains the whole spinal cord, which causes SFS. The population of RS neurons responsible for activation of SBS (RS-BS) creates the caudo-rostral gradient of excitability in the chain of coupled oscillators and thus causes SBS. *B:* the “network reconfiguration” hypothesis. RS-FS and RS-BS activate the corresponding system of connections between the unitary oscillators along the whole extent of the spinal cord necessary for FS and BS, respectively.

Generation of the pattern of slow forward and slow backward swimming

Both SFS and SBS in lampreys are based on same principle as FFS, i.e., the waves of EMG activity and lateral body flexions propagate along the body either from head to tail or in

opposite direction. Two hypotheses (see INTRODUCTION, *Neuronal control of fast forward swimming*) can explain different directions of wave propagation in SFS and SBS. (i) In the framework of the “trailing oscillator” hypothesis, one can suggest that tactile stimulation of the head and neck area leads to activation of a special “SBS group” of reticulospinal neurons, (RS-BS in Fig. 8A). These neurons activate differently the unitary oscillators along the spinal cord, thus creating a gradient of their intrinsic frequency, so that any oscillator along the cord has higher intrinsic frequency than its rostral neighbour. The frequency gradient could also explain our finding that, after a complete transection of the spinal cord at any level, SBS could still be elicited rostral to the lesion. It is noteworthy that the sensory input from the rostral region could also directly influence the pattern generator in this part of the spinal cord to lower its excitability, as appear to be the case in the spinal dogfish (Grillner, 1974).

By contrast, a rosto-caudal gradient of the intrinsic frequency of unitary oscillators, which causes the backward propagating locomotor waves required by SFS, is created either by “SFS group” of reticulospinal neurons (RS-FS in Fig. 8A) or by dorsal root afferents from the receptive field, acting through the propriospinal neurons.

(ii) In the framework of the “network reconfiguration” hypothesis, one can suggest that RS-FS and RS-BS or dorsal root afferents from the receptive field activate the corresponding system of connections between the unitary oscillators along the whole extent of the spinal cord, necessary for SFS and SBS, respectively. This hypothesis also explains persistence of SBS (rostral to the lesion) after a transection of the spinal cord.

A distinctive feature of SBS and SFS is a very large amplitude of body undulations and low frequency of these undulations as compared with those of FFS. To explain these differences, each of the two hypotheses should be supplemented by a suggestion that the RS-FS and RS-BS or dorsal root afferents from the receptive field not only activate the unitary oscillators, but also modify their intrinsic properties (as compared to the FFS mode), and thus cause the oscillations with low frequency and large amplitude. The latter suggestion was supported by recent findings that two different, distinct modes of rhythmic activity could be evoked in the spinal cord by application of NMDA, one mode with a fast rhythm and the other with a slow rhythm, which are similar to the rhythms of fast and slow swimming, correspondingly (Cangiano and Grillner, 2003). Different neuro-modulators could also be responsible for the modifications of properties of the CPG network during BS; in particular, 5-HT is known to cause more intense bursts with much longer burst duration (Harris-Warrick and Cohen, 1985; Wallén et al., 1989).

Lack of spatial orientation during slow swimming

The ability to stabilize the spatial orientation during two different modes of locomotor behaviour (SBS and SFS) was tested in the present study. It was found that there is no stabilization of spatial orientation during SBS (*Paper I*) as well as during SFS (*Paper II*). This result well corresponds to the suggestion that these two forms of locomotion represent the withdrawal and escape behaviours which do not require stabilization of the body orientation in space.

During FFS, the propulsive system based on the body undulation and the postural system based on the vestibular-driven postural reflexes closely interact with each other and constitute a gross motor synergy, which allows the animal to stabilize the dorsal side up orientation (Deliagina et al. 2002; Zelenin 2005). The commands for postural corrections are transmitted by the reticulospinal system (Deliagina and Fagerstedt 2000; Deliagina et al. 1992; Karayannodou et al. 2007; Pavlova and Deliagina 2002). Gating of the vestibular postural reflexes during SBS and SFS could occur at different points of the reflex loop: (i) as a result of blocking vestibular input to RS neurons, (ii) as a result of inactivation of the group of RS neurons transmitting commands for postural corrections, (iii) through reconfiguration of the spinal network that receives postural commands. Revealing the site of gating of postural reflexes during SBS and SFS is a goal for future studies.

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