HOW DOES THE LAMPREY CENTRAL NERVOUS SYSTEM MAKE THE LAMPREY SWIM?

BY STEN GRILLNER  AND  PETER WALLÉN

Department of Physiology III, Karolinska Institutet, Lidingövägen 1, S-114 33 Stockholm, Sweden

SUMMARY

The lamprey spinal cord, in isolation or with the brainstem, can be used in vitro. The motor patterns underlying the swimming movements can be elicited by: (1) a pharmacological activation of a specific type of neuronal receptor (NMDA-receptor), that may in other systems give rise to an unstable membrane potential, (2) by stimulation of the brainstem or (3) by tactile activation of skin regions left innervated. In the latter case the initiation of 'fictive' swimming is partially caused by a release of a transmitter activating NMDA-receptors, as judged by the effect of NMDA-receptor blockers. The central pattern generator (CPG) is strongly influenced by feedback from mechanosensitive elements, which at least partially reside within the spinal cord. The edge cell in the lamprey spinal cord serves as an intraspinal mechanoreceptor.

The ability to generate a coordinated motor output is distributed, since spinal cord sections down to 1.5–2 segments can be made to generate alternating activity. Motor neurones receive an approximately synchronous alternating excitatory and inhibitory drive in each swim cycle and do not appear to be part of the CPG. Motor neurones supplying different parts of the body wall on the same side of a body segment have different morphology with ramifications around different descending axons. The input drive signal during fictive locomotion to motor neurones located close to each other but with different morphological characteristics may differ substantially with regard to the $\gamma$-relationship ($\pm 25\%$) and the shape of the oscillation. This implies that even at a segmental level motor neurones may be further subdivided, and furthermore that the ipsilateral network generating the drive signal to ipsilateral motor neurones generates a more complex and individualized output than previously assumed. Motor neurones are not part of the rhythm-generating circuit. The large identifiable interneurones are not required for rhythmic activity to occur although they may be phasically active in the swim cycle. The small segmental interneurones have not yet been completely characterized. Many are phasically active during 'fictive locomotion' and lack an apparent axon. Their phase relationships in relation to the burst patterns vary over the entire swim cycle.

INTRODUCTION

The major components of the control system for locomotion in higher vertebrates have been identified over the last fifteen years (cf. Grillner, 1981). However, a specific

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knowledge of how these function will be very difficult to attain, due to the vast complexity of the mammalian central nervous system (CNS) and to the large number of neurones involved. Rather than pursuing this difficult task on mammals, we have turned to a primitive vertebrate, the lamprey, in the hope that we will be able to deepen our understanding of the crucial mechanisms of initiation, pattern generation and feedback control. The general principles of the neural organization remain similar from cyclostomes, fish, amphibians, reptiles and birds to primates (Grillner, 1975, 1981). It is, therefore, reasonable to believe, that a knowledge of the relevant mechanisms in the lamprey will allow us to design experiments to discover whether these apply in higher vertebrates.

A most important advantage is the ability to study the nervous system while it is actively generating the motor pattern, and, at the same time, being able to apply the relevant neurobiological techniques. A variety of in vitro preparations (reduced spinal cord or brainstem-spinal cord) have been developed, which can generate a neural correlate of locomotion. Such a motor pattern obviously does not represent the real pattern of behaviour, and it is, therefore, referred to as fictive (i.e. not functional locomotion). This chapter summarizes some basic facts about the components of the lamprey CNS involved in locomotion, as well as the current knowledge on the neurobiology of lamprey locomotion – viewed as a model network.

**PROPULSION BY MEANS OF AN UNDULATING WAVE IN FISH AND CYCLOSTOMES**

**Basic movement**

As an eel or a lamprey swims, an undulatory wave is propagated towards the tail with an increasing amplitude of movement (Gray, 1933; Grillner & Kashin, 1976). This wave pushes the animal forward in the water. The speed of the wave, travelling backwards along the body, is proportional to, but somewhat larger than, the resulting forward speed of the animal due to the inevitable slip in the water (Lighthill, 1969). This mechanical wave results from alternating contractions of the muscle fibres in each half of each myotome with a rostro-caudal lag between the activation of consecutive segments. This lag is not fixed but is proportional to the cycle duration in each segment. Between each segment it is usually about 1% of the cycle duration and, thus, 75–100% in an animal with a corresponding number of segments (i.e. if the cycle duration is 0.1 s or 1 s the lag between two adjacent segments will be 0.001 s or 0.01 s, respectively). Such a lag is referred to as a constant phase-lag (Grillner, 1974; Wallén & Williams, 1984).

The mechanical lag between two segments need not be exactly the same as the lag between the electromyographical activation of the same segments, since the mechanical lag is the end result of the contracting muscle fibres acting on the mechanical system which the body constitutes. A mechanical wave may be transmitted along the body in an entirely passive way just as a snatch in one end of a rope will be transmitted towards the other end. In the dogfish the mechanical lag is significantly larger than the corresponding electromyographical lag (S. Grillner, S. Rossignol & P. Wallén in Grillner & Kashin, 1976). Thus electrical and mechanical lags should never be
Lamprey locomotion

quated without prior investigation, as was unfortunately done even in a recent study by Ayers, Carpenter, Currie & Kinch (1983).

The lamprey has no paired fins which can act in steering and propulsion. The dorsal fin presumably has a stabilizing effect against rolling and enlarges the lateral surface. In locomotion, dorsal fin motor neurones can be activated in antiphase to the myotomal muscle fibres on the same side (Buchanan & Cohen, 1982).

Changes in the intersegmental phase lag – reversed phase coupling

A lamprey or an eel may also reverse the phase coupling to swim backwards from a corner (McClellan & Grillner, 1983). In fact, even fish with a more ‘cod like’ body, like dogfish, can reverse the coordination to achieve a reversed phase lag when the caudal segments lead the rostral ones. This coordination can be produced within the spinal cord even when it has been transected into smaller lengths (Grillner, 1974). In some instances, a fish will activate all segments simultaneously, to bend the body in a C-shaped form. Under these conditions there is no lag.

Steering coordination during locomotion

Since lamprey and the related hagfish have no paired fins, they steer by superimposing bending movements on the travelling wave. If the body is curved to the left, while the undulatory wave is passed along the body, the lamprey will turn to the left, or if it is curved upwards (i.e. a contraction of the dorsal parts of the myotomes), it will

Fig. 1. Schematic representation of the brainstem of the dogfish (Squalus acanthias). Electrical stimulation of indicated regions gives rise to locomotor movements and, with mesencephalic stimulation, superimposed turning responses (S. Grillner & P. Wallén, unpublished observations).
swim upwards. No direct investigations have been made of this type of coordination but patterns of coordination used in righting responses have been studied (Rovainen, 1979a, b, 1983).

In dogfish, stimulation of different areas of the mesencephalon will give rise to swimming combined with 'steering signals'. For example, stimulation in the left mesencephalon gives rise to a turning response to the left with a left body curvature and appropriate coordination of pectoral and anal fins (Fig. 1). Midline stimulation gives rise to upward or downward swimming (S. Grillner & P. Wallén, unpublished data).

**MYOTOMAL ORGANIZATION**

*General organization of the myotome and its innervation*

Each myotome is separated, by a myoseptum, from neighbouring rostral and caudal myotomes. The muscle fibres run longitudinally from the rostral to the caudal myoseptum and will, when activated, shorten the myotome. The organization of the muscle fibres differs from that in fish in that smaller segmental subunits are found with a few fast muscle fibres (with action potentials) surrounded by a layer of thinner, slow parietal fibres which are rich in oxidative enzymes and lack propagated action potentials. Each such segmental subunit is flattened and extends from near the midline laterally towards the skin (Teräväinen, 1971). The fast muscle fibres in each subunit are electrically coupled and only the most medial of them appear to be directly innervated, while the more lateral ones are indirectly activated by action potentials in medial fibres. The ventral roots leave the spinal cord in the middle part of the myotome between the myosepta, and make synapses on the central part of the medial fast muscle fibres. In contrast, the motor neurones supplying the slow parietal muscle fibres innervate the individual muscle fibres and their axons ramify between the individual subunits (Teräväinen, 1971; Teräväinen & Rovainen, 1971; Tretjakoff, 1927). The motor neurones supplying fast muscle fibres are similar to those innervating the slow fibres, except that they appear to be somewhat larger and to have a lower input resistance (Teräväinen & Rovainen, 1971).

The dorsal roots exit at the level of a myoseptum and the afferent fibres course along a septum to reach the surface of the skin (Tretjakoff, 1927). The afferents are of two categories, one with cell bodies in the dorsal root ganglia (Freud, 1878) and the other with dorsal cell bodies in the spinal cord (Freud, 1877; Rovainen, 1967; see below).

*The control of the dorsal and ventral parts of the myotome*

As everyone knows who has eaten fish, its myotomes are divided into a dorsal muscle mass, referred to as the epaxial part (above the level of the vertebral column), and a ventral hypaxial part (cf. Bone, 1966). The central part in each subdivision contains fast 'white' fibres and the outer part is surrounded by a layer of slow 'red' muscle fibres. Along the lateral margin between the hypaxial and epaxial parts extends a band of red muscle fibres of varying thickness (cf. mackerel), which are the prime movers during swimming at cruising speed (cf. Grillner, 1974). The geometric arrangement of the epaxial and hypaxial parts of each myotome is very complex and,
S. Grillner and P. Wallén

Fig. 2. Transverse section of the lamprey spinal cord. Sectors (solid and interrupted lines) indicate the locations of axons of different neurones. Abbreviations: gi, giant interneurone axons; drs, different reticulospinal axons; m, motor neurones; cci, CC-interneurones; li, lateral interneurones; M, Mauthner cell axon; lIi, axon of the isthmic Müller cell lIi; bM, axons of bulbar Müller cells; mM, axons of mesencephalic Müller cells; cc, central canal. (Based on information given in Baumgarten, 1972; van Dongen, Hökfelt & Grillner, 1984 and in preparation; Rovainen, 1979a,b; P. Wallén, S. Grillner, J. L. Feldman & S. Bergelt, in preparation.) Scale bar, 100 μm.
Lamprey locomotion

They form cornet-like structures in which the individual myotomes project into each other.

The lamprey myotomes have no anatomical subdivision into epaxial and hypaxial compartments, and a myotome forms a shallow 'V' pointing forwards. Nevertheless, there is a selective control of different parts of a myotome from the spinal cord. Individual motor neurones supply only restricted parts of the myotome and those supplying the most dorsal and ventral parts have a different morphology (see below, Wallén, Grillner, Feldman & Bergelt, 1984b).

The central nervous system

The lamprey CNS is unmyelinated. The brainstem and spinal cord can be maintained in vitro for several days, since it is thin and flattened, about 200–300 μm thick and 1 mm wide (Fig. 2). The spinal cord is normally oxygenated from the cerebrospinal fluid, since it has no vascular supply of its own, and this is of obvious advantage in the in vitro preparation. It extends along the length of the animal (10–100 cm depending on age and species), and contains around 100 segments. The ventral and dorsal roots exit separately and never unite. Except for the roots there is no clear segmentation within the spinal cord (Rovainen, 1979a,b; Wallén et al. 1984b). However, there are regional differences in cell types between the rostral and the caudal part (see below). The number of cells in one hemisegment is said to be around 500, of which 40–100 are motor neurones, 15–20 segmental 5-hydroxytryptamine (5-HT) neurones, 7–9 dorsal sensory cells and 20 edge cells (cf. Rovainen, 1979a, 1983; van Dongen et al. 1984).

The spinal cord has a thin grey matter extended in the lateral-medial direction, and surrounded by axonal bundles, and a dorsal column which contains the ascending axons of sensory neurones. The dendrites of intraspinal neurones extend, as a rule, into the 'white' matter, and descending axons form en passant synapses with others, often of a mixed chemical and electrical type (see Rovainen, 1979a). The general organization is similar to that of other primitive vertebrates and the number of descending axons is around 200. Below we summarize briefly some of the neuronal systems, which have been defined and, particularly, those which are important in locomotion.

Descending brainstem control

The lamprey brainstem has several large Müller cells with large ipsilateral descending axons. Three Müller cells originate from the mesencephalon (M1–M3) and descend in the ventromedial spinal cord; another two (I1 and I2) descend from the isthmus region (i.e. the transition between mesencephalon and rhombencephalon). The I2 cell differs from other Müller cells and has, at least in Petromyzon, input synapses on the axon itself within the spinal cord. From the middle of the fourth ventricle another set of large Müller cells (bulbar cells, B1–B5) send their axons in a somewhat more lateral position in the ventral tract (Rovainen, 1974b, 1983). The Müller cells receive a variety of inputs from different sensory systems (Wickelgren, 1977). M1–M3 and I1 are activated by one set of vestibular afferents and the bulbar
cells by another (Rovainen, 1979a,b), and it is likely that they contribute to righting responses in different directions. Some Müller fibres may excite motor neurones monosynaptically (Rovainen, 1974b), and recent data indicate that some medial Müller fibres preferentially influence motor neurones to the ventral myotome and that bulbar Müller cells control dorsal myotome motor neurones (P. Wallén, S. Grillner, J. L. Feldman & S. Bergelt, in preparation). Different Müller cells may activate different subtypes of inhibitory premotor interneurones (Rovainen, 1974b; Buchanan, 1982). It would seem probable that one should regard Müller cells as specific rapidly conducting pathways, which can be used to elicit rapid modifications of the motor pattern under a variety of conditions.

In addition to the Müller cells there are, as in many other vertebrates, two Mauthner cells located in the floor of the fourth ventricle. They have crossed axons descending in the lateral tract, which may activate motor neurones. The role of Mauthner neurones in escape responses is not as clear as in other vertebrates (Rovainen, 1978).

A number of smaller reticulospinal fibres originate from three separate areas in the rostral (i-type), middle (b-type) and caudal (v-type) rhombencephalon and descend ipsilaterally throughout the larger part of the spinal cord often in a dorsolateral position.

Vestibulospinal fibres from the nucleus octavomotorius descend ipsilaterally to the most rostral part of the spinal cord, and affect motor neurones in this area and one large type of propriospinal inhibitory interneurone with a long descending axon (lateral interneurone). In addition they activate ipsilateral reticulospinal fibres from the most caudal area (v-type; Rovainen, 1979a,b), which are also excitatory to motor neurones. A crossed vestibular projection from the same nucleus activates descending reticulospinal fibres.

*Ascending spinal and spinobulbar neurones*

*Dorsal cells and dorsal columns*

Dorsal cells are located medially in the spinal cord and have one afferent process in the dorsal root, and another which projects into the dorsal column towards the brainstem, as in higher vertebrates. Most cells have a caudal descending axonal branch as well (Rovainen, 1967; Martin & Wickelgren, 1971; Martin & Bowsher, 1977). Dorsal cells also influence segmental neurones (Rovainen, 1974b), but it is not yet known whether this is through *en passant* synapses or by the small processes that can be seen from the cell body and the axonal branches (T. A. Boman & J. Christensson, unpublished observations). Dorsal cells convey three different modalities, touch, pressure and nociception (Martin & Wickelgren, 1971). The role of dorsal ganglion cells remains to be explored.

*Giant interneurones and other spinobulbar neurones*

In the caudal half of the spinal cord there are 15–20 cells with very large cell bodies (Fig. 3). They have large bilateral dendrites which extend over the entire cross section of the spinal cord and an axon that ascends in the contralateral dorsolateral funiculus, in some cases to the brainstem (see Rovainen, 1979a). The giant interneurones are activated by cutaneous stimuli: (1) monosynaptically, (2) polysynaptically via
Lamprey locomotion

Fig. 3. Diagrams of the lamprey spinal cord, indicating the locations and axonal projection patterns of some identified cell types. Numbers indicate the number of cells of a particular type in the spinal cord. Abbreviations, see Fig. 2. (See text for further details.)

unidentified interneurones, and (3) from more caudally located giant interneurones via synapses, which may release aspartate (Rovainen, 1974a; Homma, 1981). The giant interneurones probably constitute a rapidly conducting spinobulbar pathway conveying information related to peripheral events in the caudal part of the body or the fins. During ‘fictive’ locomotion, giant interneurones appear to show either no, or very little phasic modulation, which suggests that they do not convey efference copy information.

Other types of neurones, with uncrossed and crossed ascending axons have been found in the spinal cord but they have not been described in any detail (Rovainen, Johnson, Roach & Mankovsky, 1973; S. Grillner & K. Sigvardt, unpublished observation).

Edge cells – intraspinal mechanoreceptors

Laterally in the ‘white matter’, cells with varying form are located. They have characteristic lateral nest-like ramifications along the lateral margin in the rostro-caudal plane (see Fig. 4). Ultrastructurally they resemble the crayfish stretch receptor neurone. Edge cells are stretch sensitive and serve as intraspinal mechanoreceptors (Grillner, McClellan & Sigvardt, 1982a; Grillner et al. 1982b; Grillner, Williams & Lagerbäck, 1984), but they also have synaptic inputs from the I2 Müller axon and from some giant interneurones (Rovainen, 1974b), and probably from other sources as well. Their axons ascend for several segments (around 5–10) on the ipsi- or the contralateral side, and it can be inferred that they project to the interneurones of the spinal central pattern generator for locomotion (Grillner, McClellan & Perret, 1981a; Grillner et al. 1981b, see below). Intraspinal mechanoreceptors can sense the form
of the body in animals like the lamprey, in which the spinal cord is bent each time the body is bent as during swimming or turning.

**Long descending propriospinal premotor interneurones**

*Lateral interneurones*

These are located in the lateral part of the grey matter in the rostral part of the spinal cord (Fig. 3) and their axons inhibit, monosynaptically, some ipsilateral motor neurones and probably several other neuronal types. They relay effects from bulbar Müller cells, vestibulospinal and some reticulospinal fibres from the middle and caudal part of the rhombencephalon (b and v-types; Rovainen, 1974a,b).

*CC-interneurones (crossed-caudal axon)*

These are located along the spinal cord and have a long crossed descending axon (>20 segments). Some have a smaller ascending branch (Fig. 3). They have a general resemblance to motor neurones and an extensive ipsilateral dendritic tree (Buchanan,
Lamprey locomotion

They have been subdivided into three types; two are inhibitory premotor interneurones, one of which is excited from B1 and the other from B1–B4 Müller axons. The third type has an excitatory effect on contralateral motor neurones.

Segmental interneurones

5-HT interneurones

Below the central canal the cell bodies of 5-HT interneurones are located (15–20 segment; Baumgarten, 1972; van Dongen et al. 1984; P. A. M. van Dongen, T. Hökfelt & S. Grillner, in preparation). Their neurites extend in a ventral direction and form a dense ventromedial plexus in which medial motor neurone and lateral interneurone dendrites ramify (P. A. M. van Dongen, T. Hökfelt & S. Grillner, in preparation). They also send axonal branches into the larger part of the white matter. Some 5-HT interneurones also appear to contain a tachykinin peptide (substance P family, van Dongen et al. 1984; P. A. M. von Dongen, T. Hokfelt & S. Grillner, in preparation). The cells are multipolar but their input has not yet been defined. Judging from these histochemical results 5-HT neurones may exert direct effects on motor neurones and one type of premotor interneurone, the lateral interneurone.

Interneurones without an apparent axon

A number of small interneurones have a thin (10–20 µm) elongated cell body and only a few medial and lateral dendrites (Tretjakoff, 1909; Sigvardt & Grillner, 1981 and in preparation). Lucifer Yellow-filled cells of this type, which are regarded as well filled, do not have an axon-like process. In the light microscope all appear as dendrite-like processes. This is in striking contrast to other spinal neurones, which have clearly distinguishable axons. Despite the fact that they appear to be local interneurones, most of them could be shown to generate an action potential.

Motor neurones

The cell bodies of motor neurones are located in the intermediate part of the grey matter (see Fig. 7). They have dendrites that extend into the white matter, often to the surface of the spinal cord (Tretjakoff, 1909; Teräväinen & Rovainen, 1971). The dorsal columns are spared only on the ipsilateral side. Motor neurones supplying the most dorsal, or the most ventral aspect of the myotome may have dendrites which cross the midline ventral to the central canal and extend to the contralateral side (Wallén et al. 1984b). Medial dendrites ramify in the ventromedial plexus of 5-HT neurites (van Dongen et al. 1984; P. A. M. van Dongen, T. Hökfelt & S. Grillner, in preparation) and around the large reticulospinal Müller axons, some of which excite motor neurones monosynaptically (Rovainen, 1974b). Motor neurones supplying the most ventral aspect of the myotome have dendrites that ramify preferentially near the midline and surround the medial mesencephalic Müller axons. Motor neurones which supply the most dorsal aspect of the myotome have either no, or few dendrites in the most medial region of the spinal cord, but ramify near the more lateral bulbospinal Müller axons. Dorsal myotome motor neurones have more extensive ramifications in the rostro-caudal direction than ventral myotome motor neurones (Wallén et al. 1984b). The motor neurones supplying the dorsal fin have not yet been described in detail.
(Rovainen & Birnberger, 1971), but they appear to resemble dorsal myotome motoneurones.

Fig. 5
Lamprey locomotion

REAL AND FICTIVE SWIMMING

The motor pattern used by an intact lamprey when swimming at different velocities in a swim-mill has been compared with that of the same animal after a high spinal transection, and then with recordings of the motor pattern from ventral roots of its isolated spinal cord-notochord (fictive locomotion, see below). In all three preparations a phase lag of similar value occurred at different velocities of swimming (real or fictive), the burst duration remained a certain proportion of the cycle duration. The frequency of swimming overlapped (Fig. 5); in the intact lamprey it varied between 7.6–0.8 Hz, in the spinal preparation between 4.1–0.4 Hz, and in the in vitro preparation between 2.0 (1.4)–0.2 Hz (Wallén & Williams, 1982, 1984; McClellan & Grillner, 1983, and cf. Grillner et al. 1981). It is to be noted that the reinforcing effect of the movement-related feedback is missing in the in vitro preparation. These findings suggest that the same spinal circuitry is used in all three (Wallén & Williams, 1982, 1984). Ayers et al. (1983) recently challenged this interpretation by claiming that the frequency ranges of the intact and spinal preparations did not overlap, which is a fallacy. They equated mechanical and electromyographical phase lags, which cannot be done (see above). Moreover, they compared the movement pattern of an intact lamprey moving through water with that of a spinal lamprey, fixed so that it did not move forwards. Clearly that will change the lateral resistance for the movement. Their finding that burrowing as well as swimming has a similar phase lag may simply imply that they represent the same type of behaviour adapted in a slightly different way (see below). What has so far not been compared between fictive and real swimming is the amplitude of the ventral root bursts (i.e. the degree of muscle activation).

THE NEURAL GENERATION OF FICTIVE LOCOMOTION

Initiation

The brainstem-spinal cord, in vitro preparation

Stimulation of the trigeminal nerve or the skin on the head, if left innervated, can give rise to a coordinated swimming motor pattern in the spinal cord (McClellan, 1984) as recorded in the ventral roots. These effects are elicited by long descending fibres which presumably activate the local networks for locomotion along the length of the spinal cord (McClellan, 1984; McClellan & Grillner, 1982). Micro-stimulation in different brainstem regions shows that a restricted parasagittal region is effective...
in evoking locomotion. The anatomical counterpart to this physiologically identified region is not yet established (McClellan & Grillner, 1984).

During pharmacologically-elicited locomotion (see below) stimulation of Müller cells (I₁, B₁–B₄, Buchanan & Cohen, 1982) can modify the rate of fictive locomotion, which implies that they have a direct or indirect input to the pattern-generating circuitry. This finding can presumably not be taken to indicate that locomotion is normally elicited via Müller cells. It appears more likely that they serve to modulate the locomotor activity in addition to their other effects, which perhaps all relate to steering functions.

The isolated spinal cord and tailfin-spinal cord, in vitro preparation and role of NMDA and kainate receptors

The isolated spinal cord can be dissected from the animal with ventral root stumps and then pinned down in a Sylgard dish, or left on the notochord. The motor pattern can be recorded from the ventral roots and from neurones. In an ordinary physiological solution, these preparations are almost always silent and the motor neurones have a flat membrane potential. Very occasionally they may exhibit a slow rhythmic activity

![Graph of NMA and D-glutamate effects on fictive locomotion](image)

Fig. 6. Induction of fictive locomotion by bath application of amino acids. The effects of different concentrations of NMA and D-glutamate were compared by recording the efferent activity in two adjacent ventral roots (B–F). At concentrations of 0.05 and 0.10 mmol l⁻¹, NMA is effective (B,D) but only at 0.25 mmol l⁻¹ does D-glutamate have an effect (C,E,F). Time calibration in (F) applies to all records. The graph illustrates that the cycle duration is always longer with D-glutamate (upper curve) than with NMA (lower curve) at the same concentration. In this case the shortest cycles were about 0.5 s, i.e. a burst rate of 2 Hz. NMA itself is more potent in Mg²⁺-free solution (A). (From Grillner et al. 1981b.)
Lamprey locomotion

Ordinated as for swimming (Grillner et al. 1981a,b; Wallén & Williams, 1984). These spontaneously active preparations are, as far as one can see, in a good condition. In most preparations excitatory amino acids must be applied to the bath to elicit a motor pattern (Poon, 1980; Cohen & Wallén, 1980). Amino acids can act on three well characterized type of receptors, i.e. quisqualate, kainate and N-methyl-D-aspartate (NMDA) receptors (Watkins, 1981). An activation of the latter two can lead to a well coordinated motor pattern. Kainate is effective in a narrow concentration range (5–15 µmol l⁻¹, Brodin, Grillner & McClellan, 1984, and in preparation), and above this range it gives rise to tonic activity, i.e. the effect first ascribed to kainate (Poon, 1980). In contrast N-methyl aspartate (NMA) is effective (Fig. 6) at a wide concentration range (20–2000 µmol l⁻¹) leading to a gradually increased burst rate (Grillner et al. 1981b; L. Brodin & S. Grillner, unpublished data). Thus NMDA and kainate exert an effect directly on the central pattern generator (CPG) interneurones, or on neurones with input to the CPG.

The fact that NMA and kainate as well as the naturally occurring aspartate and L-glutamate can give rise to fictive locomotion does of course only mean that cells in a certain strategic position have receptors for these amino acids. To test if these receptors are actually used under physiological initiation of locomotion, we have made use of an in vitro preparation in which the tailfin and the caudalmost dorsal roots were left intact (McClellan & Grillner, 1983). A tactile or electric stimulus to the skin can activate the motor pattern. If 2-aminophosphono-valerate, a specific NMDA receptor blocker, is added to the solution, the activation of the motor pattern is depressed but not abolished. PDA, a combined kainate and NMDA receptor blocker, has a more potent effect. It thus appears that the initiation of locomotion is dependent on a continuous release of transmitters, perhaps corresponding to aspartate and L-glutamate, which may activate both NMDA receptors and kainate receptors (Brodin et al. 1984).

NMDA-receptors and membrane properties

NMDA-receptors have recently been reported to act via a new type of voltage-dependent Na-channel, which is not blocked by tetrodotoxin (TTX) (Flatman, Schwindt, Crill & Stafstrom, 1983; MacDonald, Porietis & Wojtowic, 1982). When the ordinary TTX-sensitive channels have been blocked, NMDA application changes the I-V relation of the cell from a curvilinear to an S-shaped form. In other words, at a given current the membrane potential can take on two different values (e.g. −70 and −55 mV). Thus NMDA induces a negative slope conductance in cells with an ordinary I-V relationship, (i.e. NMDA induces an unstable membrane potential). A negative slope conductance is characteristic of cells with pacemaker properties and presumably also plateau potentials (see Boisson & Gola, 1976; Ducreux, 1976; Ducreux & Chalazonitis, 1976; Gola, 1976).

Although it may be a coincidence that NMDA exerts this type of effect in other systems, it would seem likely that the effect of the transmitter that activates NMDA receptors is exerted not only by conventional EPSPs but also by changing the membrane properties of critical cells. In fact, preliminary results from intracellular recordings of 40 neurones in the lamprey spinal cord showed that nine of them exhibited a slow oscillating membrane potential (around 10–15 mV) after application of TTX (Sigvardt & Grillner, 1981 and in preparation; Grillner et al. 1983).
The frequency regulation of vertebrate neurones is controlled by the after-hyperpolarization. In the lamprey the after-hyperpolarization may have two components, an early part which is blocked by tetra ethyl ammonium chloride (TEA) and a late one which disappears in low-Ca-solution containing Ca-channel blockers (Hill, Arhem & Grillner, 1984 and in preparation). This can be interpreted as indicating that two separate potassium channels are involved: an early voltage-dependent one, and a late one that is dependent on the entry of calcium. In recent experiments on other preparations the latter type of channel was influenced by noradrenalin, which modified the firing properties of the cells (Madison & Nicoll, 1982).

**Spinal network – general aspects**

If the spinal cord is divided into small parts, then each can generate ‘fictive swimming’ under the influence of NMA. Pieces containing as few as 1-5 segments may still generate rhythmic, but less regular, activity. From this it follows that local circuits in one segment can generate rhythmic activity and that long propriospinal neurones are not required.

Sometimes the spinal cord circuitry is viewed as represented by one oscillator in each half of each segment. Whether this is so cannot be said with certainty. The motor neurones and other neurones are lined up in one continuous column without apparent segmentation. Neighbouring motor neurones which exit through different roots may have a virtually identical input signal during fictive locomotion, whereas neighbouring motor neurones in the same segment which supply different parts of a myotome may have quite a large difference in input signal (Fig. 7, Wallén et al. 1984b). This implies that even within one segment there may be a subdivision of the circuitry, perhaps in some form of modular arrangement.

**Motor neurones and interneurones**

Motor neurones function in the lamprey as in most other vertebrates as an output from the spinal cord and do not take part in the pattern-generating network. Trains of antidromic stimuli applied to all motor neurones in one pair of ventral roots during fictive locomotion fail to affect the motor pattern in adjacent ventral roots, which would be expected if they played a part in the coordinating process (Wallén & Lansner, 1983, 1984).

Motor neurones receive periods of excitation alternating with chloride-dependent IPSPs in each swim cycle (Russell & Wallén, 1980, 1983). In each cycle there is thus activity in excitatory premotor interneurones alternating with inhibitory premotor interneurones. At present the only known well-defined types of premotor interneurones are the long inhibitory propriospinal interneurones. The lateral interneurones are active in phase with local motor neurones, but project caudally on the ipsilateral side and would thus be inadequate. The crossed descending CC-interneurones which inhibit contralateral motor neurones are phase advanced by 25 % or so (Buchanan & Cohen, 1982). Their effect cannot, therefore, be segmental or serve as the main source for phasic inhibition.

In all likelihood local small interneurones are important in generating the rhythm. Different bipolar interneurones without apparent axons spike phasically during different parts of the cycle, so that they are neither exclusively in phase with motor
neurones nor in antiphase (Sigvardt & Grillner, 1981; Grillner et al. 1982b, 1983). Their exact pattern of connectivity is still not defined.

The local midline 5-HT interneurones are presumably inactive during fictive locomotion since 5-HT blockers do not affect the motor pattern (Grillner et al. 1981b, see also below). Dorsal cells, giant interneurones or edge cells all on the sensory side of the spinal circuitry show little or no phasic activity during fictive locomotion and are thus presumably not of prime importance in generating the rhythm.

**Nonsynaptic mechanisms — extracellular potassium levels**

In some nervous systems large extracellular fields appear to synchronize the discharge of individual neurones (Jefferys & Haas, 1982; Taylor & Dudek, 1982). In the lamprey grey matter the extracellular phasic activity linked to the locomotor burst appears negligible, presumably since the extracellular fields of the individual neurones cancel each other. This factor can, therefore, probably be discarded as being of main importance in the generation of locomotion.
Another factor, discussed over the years (e.g. Jankowska, Jukes, Lund & Lundberg, 1967), is whether a possible accumulation of extracellular potassium at the end of the neuronal bursts could give rise to changes in threshold of certain neurones or even terminate a burst in others. The potential significance of this factor became apparent when Kriz, Sykova, Ujec & Vyklicky (1974) showed, in the cat, that a short synchronous volley in a hindlimb nerve could change the extracellular potassium from 3 to 9 mmol l\(^{-1}\) in the dorsal horn. During fictive locomotion phasic changes with each ventral root burst occur within the grey matter (Fig. 8), but they are small, of the order of 0.2 mmol l\(^{-1}\). When locomotion is turned on, the general level of extracellular potassium may increase by 0.2 mmol l\(^{-1}\). This may cause minor changes in membrane potential of around 1 mV, but the net effect is presumably not of major importance (Wallén, Grafe & Grillner, 1984a).

**Glycine, GABA and 5-HT during fictive locomotion**

If the blockers of glycine, \(\gamma\)-amino butyric acid (GABA) or 5-HT are added to the bath during fictive locomotion, the pattern is influenced only by the first. The glycine-receptor blocker, strychnine, causes an acceleration of the rhythmic activity. Hence during fictive locomotion neurones are active, and release a transmitter (glycine?) activating these receptors. They are located either within, or at a stage prior to, the pattern-generating network. With the same reasoning, neurones releasing GABA or 5-HT appear not to be important in this context. On the other hand, the application of GABA or 5-HT to the bath will slow down the rhythmic activity, and 5-HT will in addition increase the burst amplitude (Grillner & Wallén, 1980; Grillner et al. 1981b and unpublished data; Harris-Warrick & Cohen, 1983). Whether this means...
at GABA and 5-HT neurones would exert such an effect under some conditions is not yet known.

**INTERACTION BETWEEN THE CENTRAL PATTERN GENERATING NETWORK AND MOVEMENT RELATED FEEDBACK**

A central spinal network and a powerful movement related feedback system has been documented in all vertebrates in which the organization of locomotion has been investigated. The feedback system and the central organization together constitute the control system for locomotion (e.g. Grillner, 1981; Andersson, Forssberg, Grillner & Wallén, 1981; Grillner & Wallén, 1982; Grillner et al. 1981a; Pearson & Duysens, 1976). The feedback system is powerful enough to slow down or speed up the activity in the central network so that the movement progresses in an effective way even if unexpected external events affect the movements.

In animals which bend their body as they move, the spinal cord will also be bent by each movement. The flattened spinal cord of the lamprey contains mechanoreceptors located bilaterally along the lateral margin. They will sense lateral movements such as those occurring during locomotion (Grillner et al. 1982a,b, 1984, see above). These receptors are presumably part of the feedback system controlling the locomotor CPGs. During fictive locomotion in a spinal cord-notochord preparation, in which dorsal and ventral roots have been cut, a bending of the notochord and thus the spinal cord will affect the burst pattern (Fig. 9, Grillner et al. 1981a). In fact, if the imposed sinusoidal movements are faster or slower than the resting burst rate during fictive locomotion, the motor pattern will become entrained in both cases (Fig. 9). Whether mechanoreceptors in the skin also take part is not known.

A feedback system of this sort would be very useful as the lamprey swims in unpredictable water currents (Grillner et al. 1981a,b; cf. Grillner & Wallén, 1982). A slightly different condition occurs when the lamprey attempts to accelerate during swimming. The central drive will then act to increase the frequency, but the end result will depend on the outcome of the movements and the feedback signals. In all likelihood, the combination of central and feedback mechanisms is crucial to optimize the amplitude of the movement.

Another case is when the animal swims and meets with an increased physical resistance such as a belt of seaweed or a layer of sand. The speed of the forward movement will then slow down. For the sake of simplicity it is assumed that it becomes zero, as if the animal swims into an unyielding surface. The mechanical situation is as follows. (1) The 'water resistance' for lateral body movements will decrease, since the slower the body moves through the water the lower the lateral resistance will be. Thus, all else being equal, the amplitude of the lateral movements should increase. (2) The resistance to the backward push of the travelling wave on the body will increase drastically. During ordinary swimming the backward speed of the travelling wave is only somewhat larger than the forward speed of the body through the water. In the present case, however, the forward speed will, instead, be zero, and the body wave will then have to move the water backwards (i.e. the load will be very much larger). The feedback system, discussed above, should slow down the movements to adapt the central drive to the mechanical situation. Burrowing, which is like swimming into a sandbank,
is characterized by the changes we have just discussed (i.e. an increased lateral movement amplitude and a lowered frequency). In other words, we predict that the feedback system discussed here is also used to adapt the swimming motor pattern to suit other activities, such as burrowing.

**CONCLUDING REMARKS**

The question posed in the title (How does the lamprey CNS make the lamprey swim?) has not been fully answered. Nevertheless, many of the neuronal elements...
Lamprey locomotion

In the generation of locomotion have been identified and their neuropharmacology and histochemistry investigated. Several aspects of the spinal control system underlying the swimming motor pattern have also been described. This knowledge is a prerequisite for an understanding of some crucial mechanisms in initiation of pattern generation and feedback control. However, the lamprey locomotor system (like all other vertebrate systems underlying a pattern of behaviour) is not yet fully understood. Nevertheless, more pertinent knowledge about the neural generation of locomotion may now be available from lamprey than from the long-studied cat. This new knowledge has provided not only new facts but fresh insights into the general mechanisms of motor coordination.

REFERENCES


Lamprey locomotion

I. Introduction

II. Molecular and Cellular Mechanisms

A. Motor Neurons

1. Identification of Motoneurons

2. Characteristics of Motoneurons

B. Synaptic Inputs

1. Excitatory Synapses

2. Inhibitory Synapses

C. Neurotransmitters

1. Amino Acids

2. Peptides

III. Neural Networks

A. Segmental Networks

1. Intersegmental Connections

2. Segmental Coordination

B. Central Networks

1. Reticulospinal System

2. Vestibulospinal System

IV. Behavioral Aspects

A. Swimming Patterns

B. Locomotion in Different Conditions

V. Conclusion

References