ELSEVIER

Brain Research Reviews 26 (1998) 184–197

BRAIN RESEARCH REVIEWS

Intrinsic function of a neuronal network — a vertebrate central pattern generator [1]

Sten Grillner [a,*], Örjan Ekeberg [b], Abdeljabbar El Manira [a], Anders Lansner [b], David Parker [a], Jesper Tegnér [a,b], Peter Wallén [a]

[a] *Nobel institute for Neurophysiology, Department of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden*
[b] *Department of Numerical Analysis and Computing Science, Royal Institute of Technology, S-100 44 Stockholm, Sweden*

Abstract

The cellular bases of vertebrate locomotor behaviour is reviewed using the lamprey as a model system. Forebrain and brainstem cell populations initiate locomotor activity via reticulospinal fibers activating a spinal network comprised of glutamatergic and glycinergic interneurons. The role of different subtypes of Ca^{2+} channels, Ca^{2+} dependent K^+ channels and voltage dependent NMDA channels at the neuronal and network level is in focus as well as the effects of different metabotropic, aminergic and peptidergic modulators that target these ion channels. This is one of the few vertebrate networks that is understood at a cellular level.

Keywords: Locomotion; Ion channels; Networks; Glycine; Glutamate; Tachykinin; Modellin

Contents

1. Introduction 185
2. Forebrain and brainstem control of locomotion 186
3. Brainstem–spinal cord network underlying locomotion 187
4. Role of low voltage-activated calcium channels 189
5. Calcium imaging in lamprey neurons 190
6. Role of sensory feedback 190
7. Mathematical modelling of the locomotor network 191
8. Modulation of the central pattern generator network – presynaptic effects 192
9. Synaptic plasticity induced by tachykinins in the locomotor network 195
10. Concluding remarks 195
Acknowledgements 195
References 195

* Corresponding author: Tel.: +46 (8) 728 6900; fax: +46 (8) 34 95 44; E-mail: Sten.Grillner@neuro.ki.se
[1] Published on the World Wide Web on 8 April 1998.

1. Introduction

One major challenge of current neuroscience is to bridge the huge gap between the studies at molecular–cellular level and the behavioural level, in the process of gaining further insight into nervous system function. Actually, even with a 'complete molecular description' of every single cell present in the entire central nervous system (CNS), one would still know nothing about the function of the CNS, because the essence of the CNS is its intricate and specific neural organization. The nervous system is modular, with discrete networks sub-serving various functions such as generating different patterns of motor behaviour, being feature detectors in sensory systems, critical modules in memory formation, or being involved in the expression of emotions. In order to account for the intrinsic function of these networks, it is necessary to define the dynamic synaptic interactions between the different nerve cells that form the network, the blend of ion channels expressed in the component nerve cells, as well as the different transmitters/modulators and their receptors. This is easy to state, but more difficult to achieve.

During the last few decades, innumerable new facts about the molecular and cellular components of the CNS have accumulated in mammals, but there has been no corresponding gain in insight concerning the intrinsic function underlying the network and systems level. In all areas of biology, simple experimental model systems have played a crucial role. In neuroscience, the squid giant axon has provided important information, and invertebrate integrative models like the stomatogastric system or the gill withdrawal reflex [52,36,29,5] have been very influential. Due to the vast complexity of the mammalian CNS, to understand a particular aspect of vertebrate behaviour at the cellular level, it has become necessary to use simple, experimentally amenable vertebrate model systems with relatively few neurons. Moreover, the particular neural function of interest should be conserved in other vertebrates, to allow a possible generalization of the findings. The vertebrate CNS has, with the exception of the cerebral cortex, most basic and characteristic features in common from cyclostomes to man. These include the gross neuronal organization with the brainstem — forebrain, the different nuclei, connectivity and transmitter systems, etc. [48,3].

The neural network underlying locomotion is recruited as a component of most goal-directed patterns of vertebrate behaviour (Fig. 1). In all classes of vertebrates the overall locomotor control system is designed in a similar way [23,25], which is not surprising when viewed in an

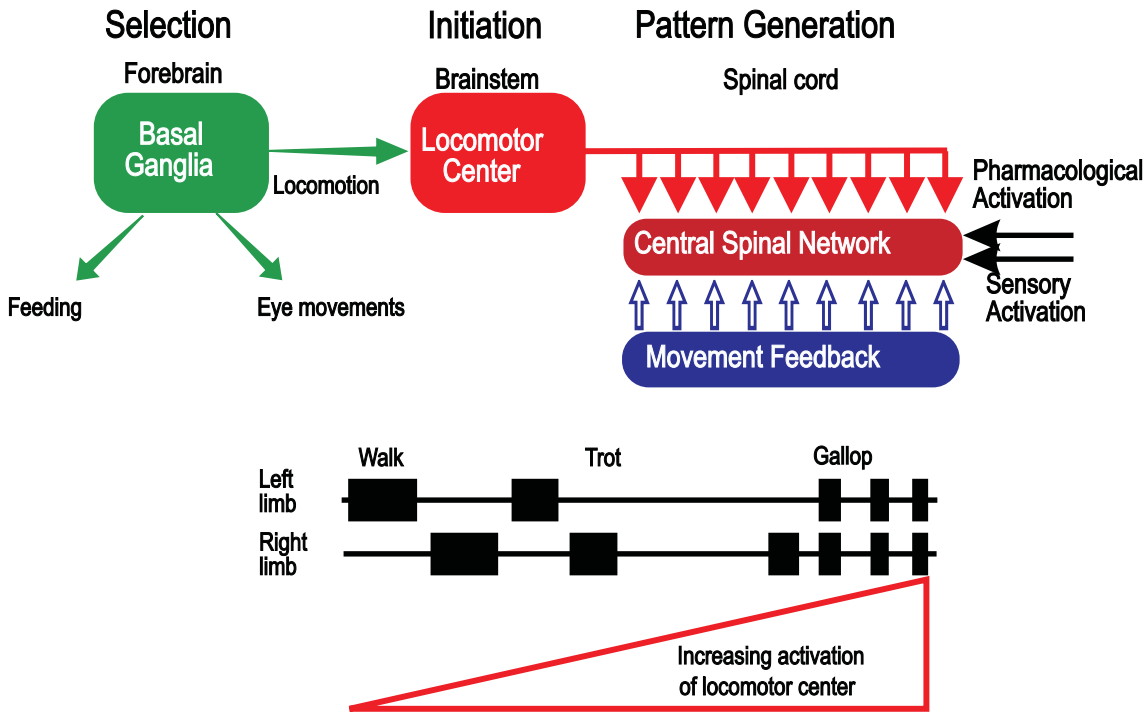

Fig. 1. General control scheme for vertebrate locomotion. The basal ganglia exert a tonic inhibitory influence on different motor centres. Once a pattern of motor behaviour is selected the inhibition is released, allowing in this case the locomotor centre in the brainstem to be activated. Locomotion is then initiated by an increased activity in reticulospinal neurons which activate the central spinal network, which in turn produces the locomotor pattern in close interaction with sensory feedback. With increased activation of the locomotor centre the speed of locomotion will also increase. In quadrupeds this also leads to a shift in interlimb coordination, from walk to trot and then to gallop. Experimentally, locomotion can also be elicited pharmacologically by administration of excitatory amino acid agonists and by sensory input.

evolutionary perspective. Thus, the same meso-pontine and diencephalic centres initiate locomotor activity in lampreys as in primates, via an activation of lower brainstem reticulospinal neurons. These in turn activate the spinal networks of nerve cells (CPG) which generate the motor pattern, be it swimming or walking. Sensory feedback acting on the CPG is an integral part of the control system and helps adapt the motor pattern to external events. The larger building blocks in the control system have thus been defined in mammals [53,32], but the intrinsic mode of operation on the cellular level is now being revealed using simple model systems from frog embryo [47,55] and lamprey [24,6].

In this chapter we will review the cellular basis of the neural control system for locomotion in the lamprey CNS. The lamprey is a lower vertebrate that separated from the main vertebrate line 450 million years ago. Its nervous system contains comparatively few neurons. The brainstem spinal cord can be isolated and maintained in vitro for one or several days. Moreover, different brainstem areas that evoke locomotion can be stimulated and the motor activity underlying locomotion can then be recorded in the ventral roots or intracellularly in the different components of the neural control system (Fig. 2). These two conditions have allowed a comparatively detailed analysis on the cellular level. Below we will discuss the forebrain control of locomotion, followed by a review of the network including a detailed account for the contribution of different types of ion channels, and the importance of presynaptic modulation and synaptic plasticity involving peptidergic modulation of glutamatergic pathways.

2. Forebrain and brainstem control of locomotion

Fig. 3 shows a block diagram demonstrating that locomotion can be initiated from a diencephalic (ventral thalamus–zona incerta) and mesopontine area, both of which project to glutamatergic reticulospinal neurons, which in turn activate the spinal network underlying locomotion [38,19,65]. These different structures are presumably involved in the control of goal-directed locomotion in different behavioural contexts [25]. The diencephalic locomotor area in turn receives fiber projections from the olfactory bulb and the opthalmic nerve — two sensory inputs which are known to activate goal-directed locomotor behaviour [34,61].

The lamprey basal ganglia has a neural organization that histochemically appears closely related to that of mammals and primates [45,46]. The striatum contains neu-

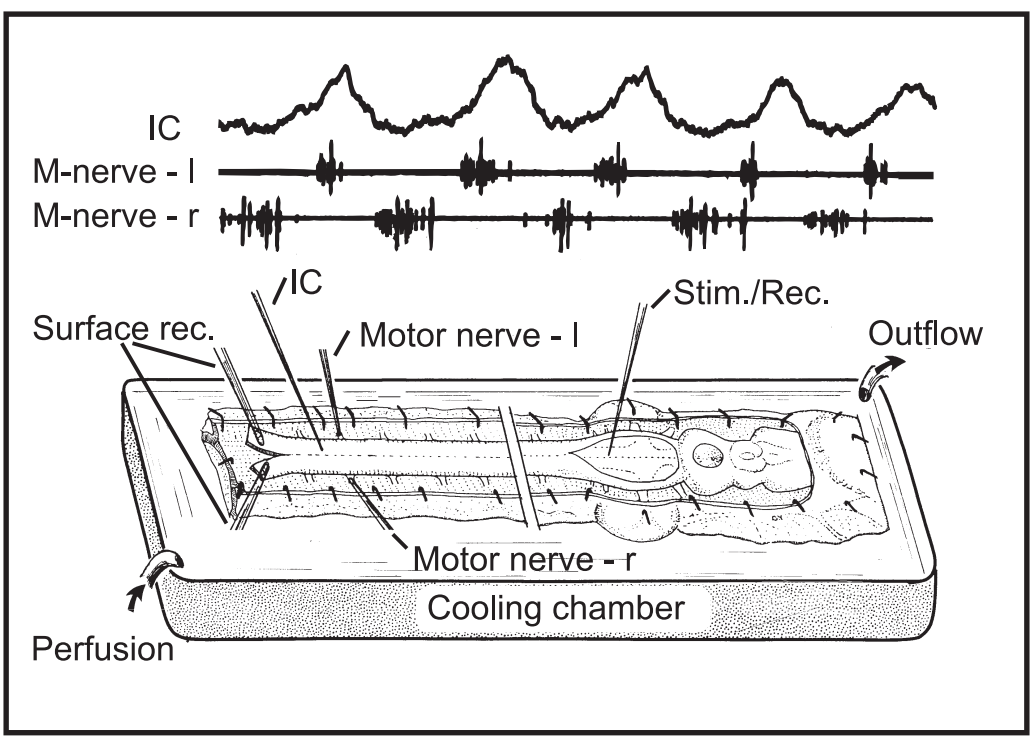

Fig. 2. In vitro preparation of the lamprey CNS. The brainstem–spinal cord of the lamprey can be maintained alive for several days in isolation, and the motor pattern underlying locomotion can be produced by stimulating the brainstem locomotor centres. The motor activity can be recorded in the ventral roots (motor nerves) that normally activate the musculature on the left (l) and right (r) sides. The activity in single or pairs of cells can be recorded intracellularly with microelectrodes (IC). An intracellular record (IC) of a network neuron with subthreshold membrane potential oscillations is shown above together with the alternating motor activity in the ventral roots on the left and right sides. The experimental chamber is kept cold (4–7°C), and it is continuously perfused with physiological solution.

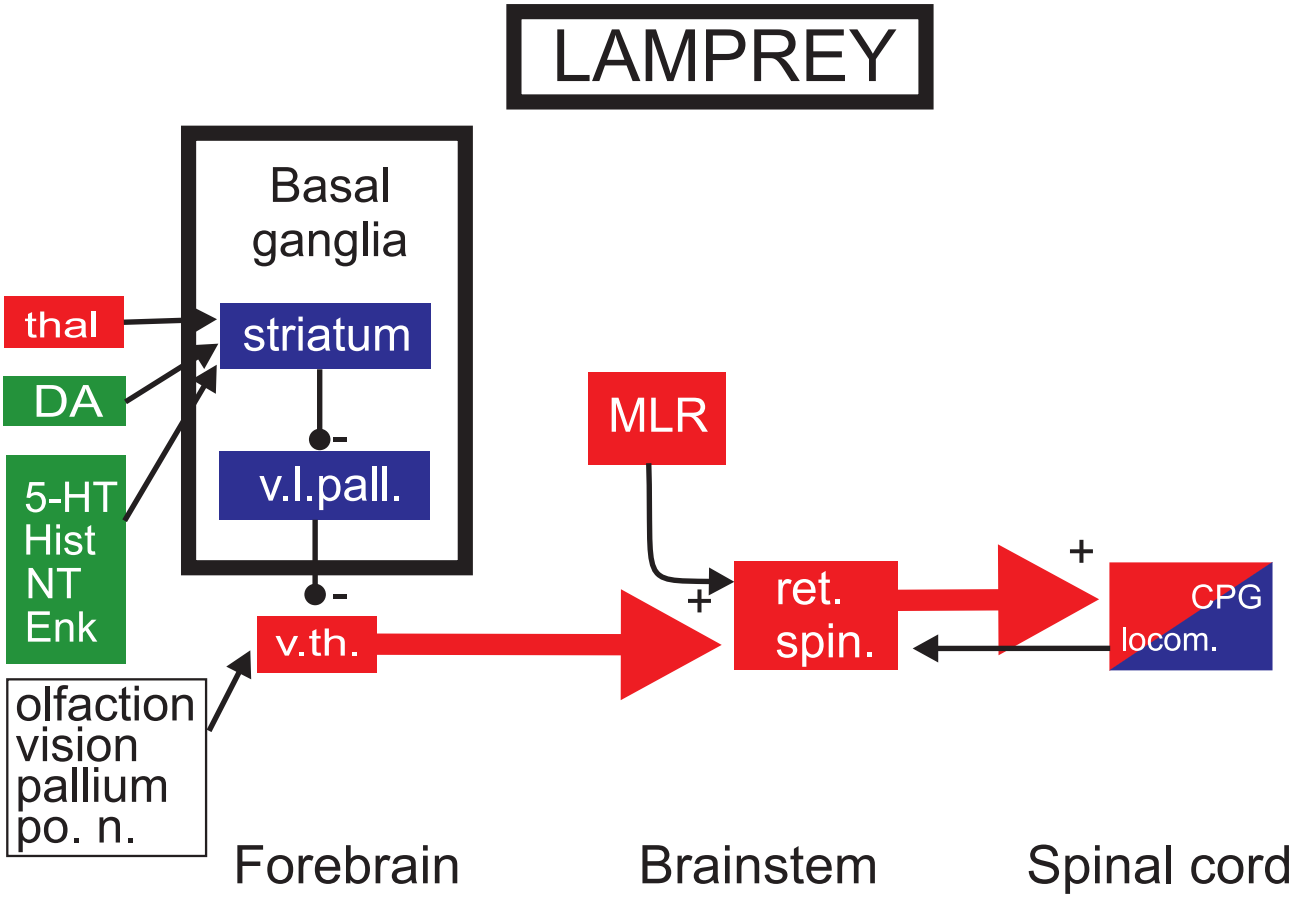

Fig. 3. Forebrain and brainstem structures important for initiation of locomotion in lamprey. The striatum of the basal ganglia receives dopaminergic, serotonergic, histaminergic and peptidergic inputs, as well as input from thalamus and telencephalon. GABAergic striatal neurons project to ventrolateral pallium, which in turn sends GABAergic projections to ventral thalamus. This nucleus also receives olfactory and visual input, and projects to the brainstem where reticulospinal neurons are excited. In addition to this diencephalic locomotor control, the brainstem MLR area also may initiate locomotion by exciting reticulospinal neurons. The brainstem reticulospinal neurons will then in turn activate the spinal locomotor network.

rons with extensive dendritic trees with spines distributed in the neuropil. Striatal neurons consist of GABAergic neurons and cells which contain substance P and AChesterase. The inputs to striatum include a dense dopaminergic, 5-HT-ergic and histaminergic inputs, as well as peptidergic (enkephalin, neurotensin) inputs and presumed glutamatergic input from thalamus and telencephalon. GABAergic projections from the striatum extend to the ventrolateral pallium, which contains GABAergic neurons that in turn project to the ventral thalamus (Fig. 3). The latter nucleus gives rise to the diencephalic locomotor control. Stimulation of the ventral thalamus elicits locomotor activity which can be recorded in the ventral roots of the isolated brainstem–spinal cord. The basal ganglia may thus exert a gate control of goal-directed locomotion elicited by olfactory and visual stimuli. Since olfactory and visual stimuli are both potent activators of locomotion [25], it is likely that these effects are exerted over the ventral thalamus. This would mean that goal-directed locomotion can be elicited via an oligosynaptic pathway which involves only two relays (ventral thalamus, reticulospinal nuclei) from sensory structures to the spinal cord.

3. Brainstem–spinal cord network underlying locomotion

Fig. 4 shows the cellular components of the brainstem–spinal cord network underlying locomotion. It consists of glutamatergic excitatory and glycinergic inhibitory neurons (see [24]). These provide dynamic interactions on the millisecond time scale. In addition there are a number of G-protein mediated modulatory effects exerted by monaminergic and peptidergic systems.

The brainstem reticulospinal neurons (RS) are responsible for initiating and maintaining the excitatory drive to the locomotor pattern generators. They exert their effects through both NMDA and AMPA kainate receptors onto all neurons in the spinal network [39]. The main components of the spinal network that are responsible for the actual

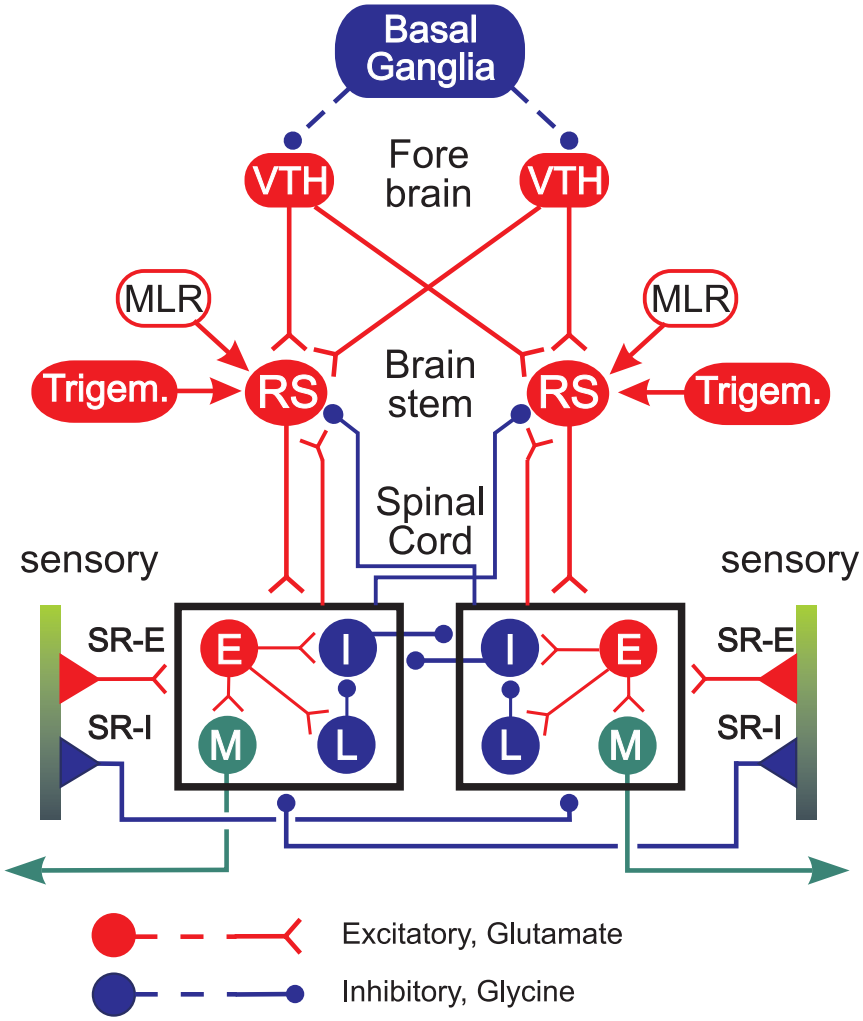

Fig. 4. Lamprey locomotor network. A schematic representation of the forebrain, brainstem and spinal components of the neural network that generate locomotor burst activity. All neuron symbols denote populations of neurons rather than single cells. The reticulospinal (RS) glutamatergic neurons excite all classes of spinal interneurons and interneurons. The excitatory interneurons (E) excite all types of spinal neurons, that is, the inhibitory glycinergic interneurons (I) that cross the midline and inhibits all classes of neurons on the contralateral side, the lateral interneurons (L), which inhibits I interneurons and motoneurons (M). The stretch receptor neurons are of an excitatory type (SR-E) that excites neurons on the ipsilateral side and an inhibitory type (SR-I) that inhibits all neurons on the contralateral side. RS neurons receive excitatory synaptic inputs from cutaneous afferents (Trigem.), the mesencephalic locomotor region (MLR) and from the ventral thalamus (VTh), which in turn receives inputs from the basal ganglia.

pattern generation are the ipsilateral excitatory interneurons, which provide a phasic excitatory drive to motor neurons during each locomotor cycle [6], and glycinergic interneurons with crossed axons that inhibit all contralateral network neurons, including motor neurons, at the segmental level [8,24]. The inhibitory interneurons provide phasic inhibition during contralateral activity. In addition there are in the rostral part of the spinal cord other types of inhibitory interneurons called lateral interneurons which receive phasic depolarization and hyperpolarization during locomotor activity but do not appear to play any major role in pattern generation [22]. The motor neurons on the left and right side show alternating activity. All motor neurons undergo one half cycle with excitatory drive and another half cycle with inhibitory drive [49,70]. During slow activity many motor neurons generate sub-threshold oscillations while at higher burst rates the motor neurons are recruited progressively.

Dynamic interactions among network interneurons are responsible for the pattern generation. The more tonic excitatory drive the interneurons are provided with (from the brainstem or by administering glutamate agonists in the bath) the faster the network will oscillate (range 0.2–10 Hz). The reciprocal inhibition is of prime importance for generating the alternating pattern (see [24]). During activity on one side, both excitatory and inhibitory interneurons will be active. The net result will be that ipsilateral motor neurons are excited, while contralateral interneurons and motor neurons become inhibited. This inhibition is strong enough to overcome the excitatory drive from the brainstem. One crucial factor in a network like this is the control of the locomotor burst termination (Fig. 5). In the

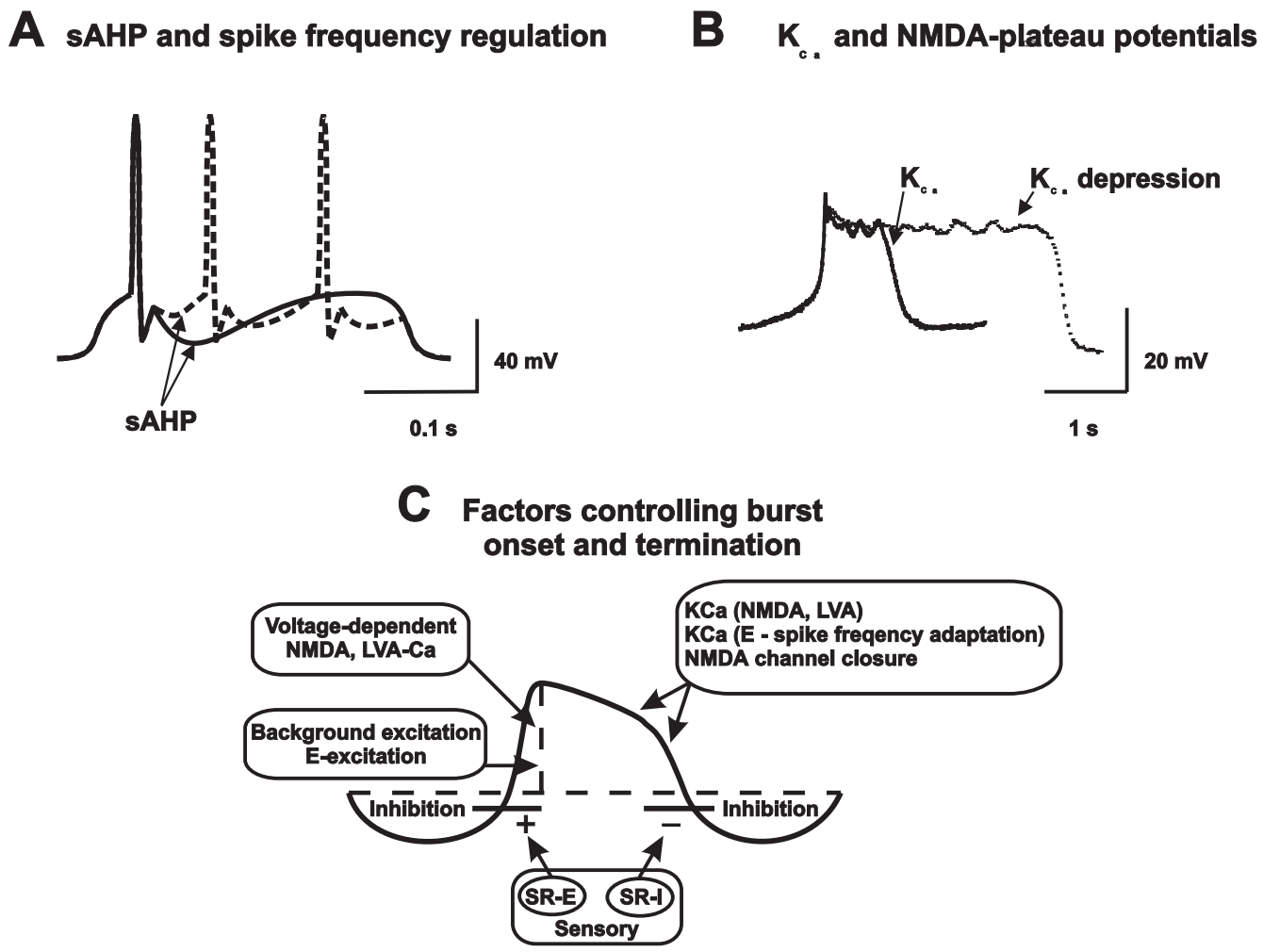

Fig. 5. Spike frequency regulation, NMDA-plateau potentials and control of burst termination. A: The amplitude of the slow afterhyperpolarization (sAHP) will determine whether one or several action potentials will occur during the phase of synaptic excitation in locomotor cycle. A large and long-lasting sAHP will make locomotor bursts shorter. B: Ca^{2+}-dependent K^+ channels (K_{Ca}) not only cause the sAHP but will also promote the termination of NMDA-receptor induced plateau potentials. The control plateau (solid trace) is markedly prolonged in the presence of the K_{Ca}-channel blocker apamin (dotted trace). C: Several different factors contribute to the initiation of the depolarizing phase, its maintenance, and its termination. In addition to conventional synaptic excitation, voltage-dependent NMDA receptors and low-voltage activated Ca^{2+} channels (LVA-Ca) are activated. Ca^{2+} will enter the cell through these channels, cause activation of K_{Ca}, and thereby a progressive hyperpolarization leading to closure of the NMDA channels. The initiation of the depolarizing phase is facilitated by activation of ipsilateral excitatory stretch receptor neurons (SR-E), while the termination of the depolarized phase is partially a result of activation of contralateral inhibitory stretch receptor neurons (SR-I). Abbreviation: E, excitatory interneuron.

isolated spinal cord, the membrane properties of the inhibitory interneurons play an important role. In particular, these include the calcium dependent potassium channels (K_{Ca}) which are of two types, activated by (1) the calcium entry through high voltage activated calcium channels of N and P/Q type (Fig. 5A) [20,57,71], and (2) a separate set of K_{Ca} channels that are activated by calcium entry through NMDA channels (Fig. 5B [57]). Frequency adaptation in the interneurons through summation of the afterhyperpolarization due to K_{Ca} is one major factor for controlling burst termination. Plateau-like depolarizations elicited by activation of NMDA channels may also contribute, as well as low voltage activated calcium channels (Fig. 5C) [24,69,58].

The network can thus operate at different burst rates. To prove that the K_{Ca} channels have a critical role, experiments with channel-specific toxins have been carried out. Apamin blocks the K_{Ca} channels activated by the calcium entry through N and P/Q channels occurring during the action potential. If apamin is administered during continuous burst activity, burst duration will increase (cf. Fig. 5), but burst frequency decrease, and at lower frequencies the burst pattern will break down altogether [20,59]. Endogenous modulators like 5-HT, that also act on K_{Ca} channels, cause more intense bursts with much longer burst durations [30,68,73].

4. Role of low voltage-activated calcium channels

Low voltage-activated (LVA) calcium channels are also present in network neurons [37,18]. These are activated

when the cells are depolarized from a comparatively hyperpolarized level, and open below the threshold for the action potential. The LVA Ca^{2+} channels can thus boost the membrane depolarization enabling it to reach the threshold for an action potential. The calcium entry through LVA Ca^{2+} channels thus provides a post-inhibitory rebound. This can contribute to the stability of network activity [58], since during rhythmic burst activity, a blockade of LVA Ca^{2+} channels in modelling experiments may cause a change from a strict reciprocal pattern to more or less irregular activity. Thus, both LVA Ca^{2+} channels and voltage- dependent NMDA channels may contribute to burst stability.

5. Calcium imaging in lamprey neurons

In calcium imaging experiments in the spinal cord of the lamprey [4], it was shown that in both soma and dendrites of the network, there is entry of Ca^{2+} ions during activation (Fig. 6). During an action potential, Ca^{2+} entry occurs in both soma and dendrites (Fig. 6A), and during synaptic activation (sub-threshold EPSPs) there are local increases of calcium in the dendrites at the synaptic region (Fig. 6B). The Ca^{2+} entry during the glutamatergic EPSPs was due to both NMDA channels (50%) and to LVA Ca^{2+} channels, and possibly AMPA channels (Fig. 6B). During locomotor activity there is a phasic Ca^{2+} entry occurring in the dendrites in neurons, even in the absence of action potentials. This synaptically driven Ca^{2+} entry peaks during the ipsilateral burst and reaches a minimum during the trough (Fig. 6C). To what extent these changes in Ca^{2+} levels during the locomotor cycle affect the activation of K_{Ca} channels is thus far unknown.

6. Role of sensory feedback

The segmental network can thus operate in its physiological frequency range with a surprisingly simple drive signal, i.e. changing the level of excitatory drive in the bath, that is level of excitatory amino acid [13,27]. Under normal conditions, however, the undulatory movements of the body provide phasic sensory feedback from stretch

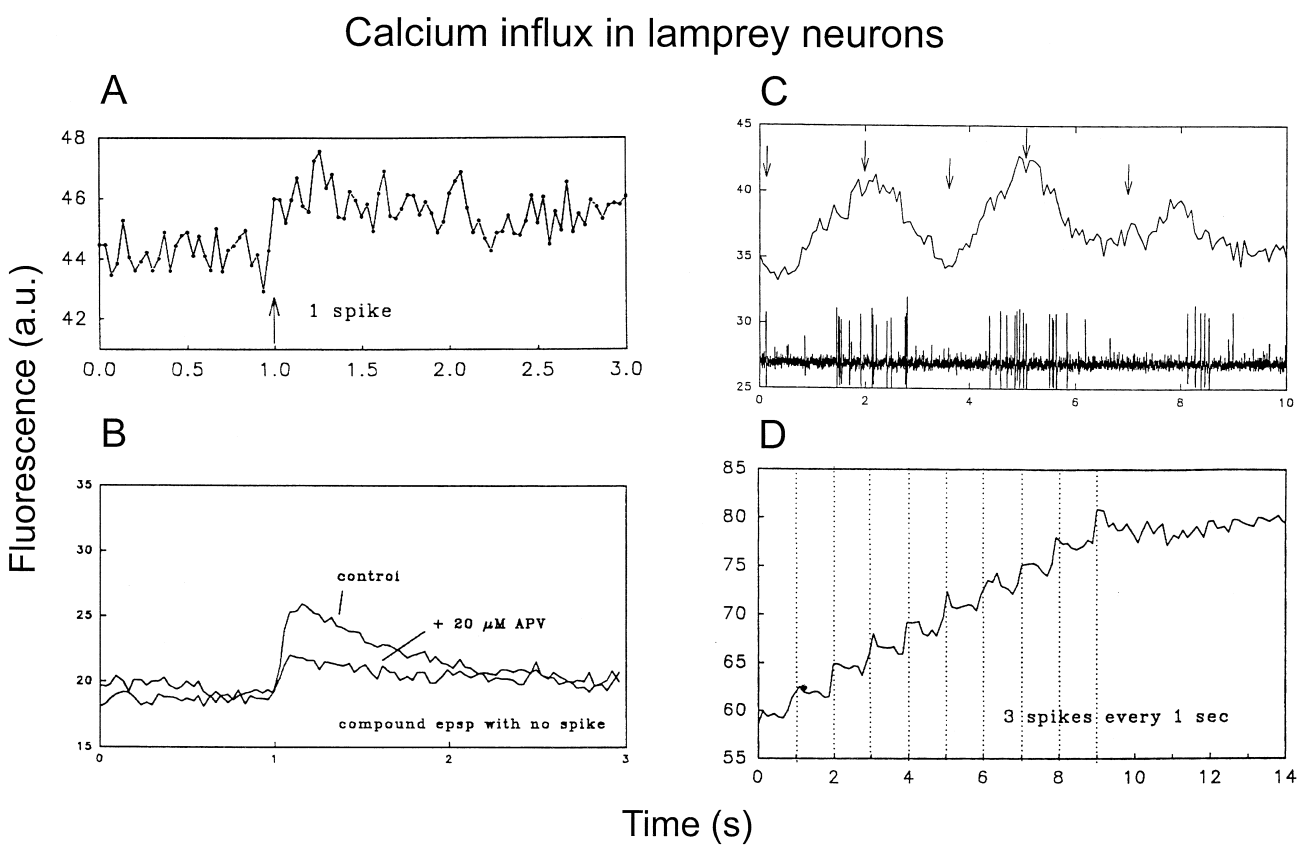

Fig. 6. Calcium dynamics in lamprey neurons. A: Fluorescence increase (Fluo-3; arbitrary units) in a portion of a proximal dendrite of a motoneuron, in response to a single action potential, evoked by intracellular stimulation. B: Calcium transients recorded in a small portion of a distal dendrite of a motoneuron, elicited by synaptic activation (resulting in subthreshold EPSPs) via stimulation of reticulospinal axons. The NMDA-receptor blocker 2-APV did not completely block the calcium transient. C: Phasic fluctuations of the calcium level in a motoneuron dendrite during ongoing fictive locomotion. Lower trace is the corresponding ventral root recording. The peak of the calcium trace occurs in phase with the burst, corresponding to the depolarizing phase of the locomotor cycle. D: A train of 3 action potentials was evoked by intracellular stimulation every 1 s. Calcium increases in a stepwise fashion with each spike train. (Modified from [4]).

receptors located at the lateral margin of the spinal cord (edge cells; [28,26]). The stretch receptor neurons are of two kinds, one that provides excitation to ipsilateral interneurons and motor neurons, and a second that provides inhibition contralaterally [66] (See Fig. 4). The sensory input is powerful enough to entrain the central network activity. Sensory feedback thus provides an overlay that can adapt the motor activity to different types of perturbations that may occur during locomotion under natural conditions, for instance by changing water currents. It is easy to realize that the connectivity discussed above can produce entrainment of the central network. Essentially if the muscles on one side contract, the curvature of the other half of the segment will be affected and the stretch receptors on this side activated. This will result in an inhibition of the ongoing activity on the contracting side and ipsilateral excitation. This coupling will thus make the activity of the central pattern generator become entrained by peripheral movements within a certain range above or below the rest rate of the spinal network.

7. Mathematical modelling of the locomotor network

Even with a fair knowledge of the properties of each type of neuron, the types of synaptic interaction, and the neuronal activity that occurs, it is very difficult to understand intuitively how the network operates (Fig. 4). This is due to the fact that an immense number of events occur in parallel in each cell, as well as in the interaction between a number of cells. In order to test rigorously if different plausible interpretations could apply, mathematical modelling is a very useful tool. We have therefore developed biophysical models of each type of neuron in the network [17]. These neurons are simulated with five different compartments, each of which may have Na^+, K^+, Ca^{2+}, K_{Ca} conductances, as well as synaptic conductances for EPSPs and IPSPs and voltage dependent NMDA receptors (Fig. 7A). These neurons are thus assigned a certain input resistance and respond to simulated current injection in a way similar to their natural counterparts. Populations of excitatory and inhibitory interneurons with properties vary-

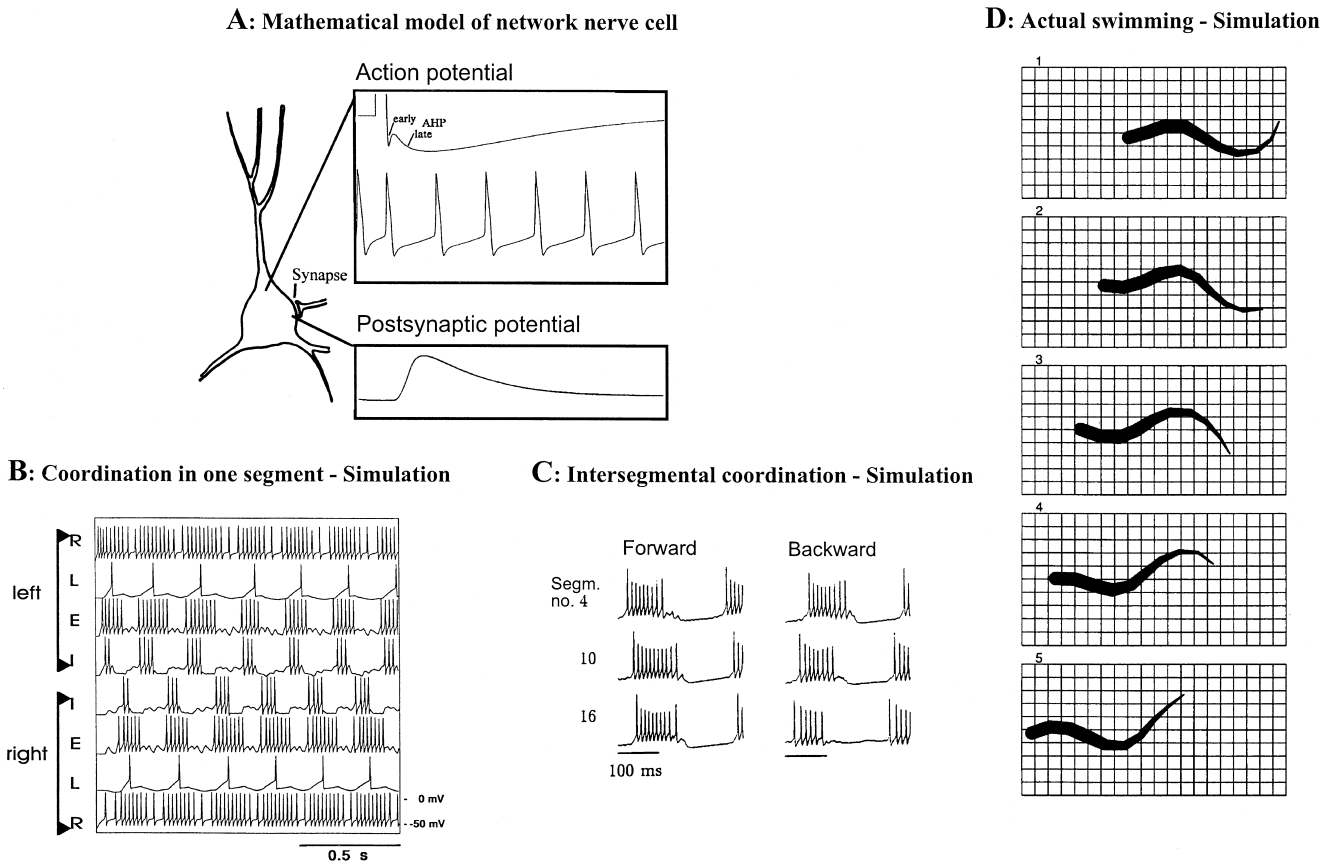

Fig. 7. Modelling of the lamprey locomotor network-simulations at neuronal, network and behavioral levels. A: Neurons of the network were simulated in a realistic fashion, with the different voltage-dependent (Na^+, K^+, Ca^{2+}), Ca^{2+}-dependent K^+ channels, and ligand-gated channels (AMPA/kainate, NMDA, glycine). Action potentials with early and late afterhyperpolarization (AHP), and spike frequency adaptation, can be simulated, together with postsynaptic potentials occurring in different compartments. B: Simulation of the segmental network using a pool of excitatory (E) and inhibitory (I) interneurons and lateral (L) interneurons. The activity is driven by excitatory reticulospinal neurons (R). Activity on the left and right sides alternates. C: Pattern of intersegmental coordination, produced by a simulated network of 60 segments. This circuitry will produce a rostro-caudal phase lag along the simulated spinal cord, and this lag can be reversed if the excitability is increased in the caudal end, which results in backward locomotion. D: Simulation of actual swimming movements using a neuro-mechanical model. Frames show steady-state swimming at 4 Hz, resulting from tonic excitation of the network, with the model lamprey moving forward at a speed of 0.73 m/s. Time interval between frames is 50 ms. See text for further details. (Modified from [24]).

ing as in the natural population of cells were then connected as in the network described above [60,31]. Such a network will respond to excitatory drive with burst activity with the appropriate phasing of the different cell types (Fig. 7B). The simulated network can be made to cover the physiological range of burst rates between 0.2 and 10 Hz. This means that the simulated network can mimic the segmental central pattern generator, and consequently that sufficient biological information is available to account for the behaviour. Obviously that does not mean that all relevant factors have been identified.

If, in addition, segmental networks along the cord are connected in series, a rostrocaudal pattern of activation with a constant phase lag can be achieved, similar to that occurring in the swimming lamprey (Fig. 7C). This means that the intersegmental coordination can also be mimicked. Subsequently, this network was used to control a segmental viscoelastic muscle model such that motor activity in the rostral segments control rostral myotomes, which are coupled in series with caudal myotomes, which in turn are controlled from caudal network oscillators. By also simulating the viscous properties of water, it is possible to simulate actual swimming movements (Fig. 7D). The lamprey movements are controlled by the neural network which activates the different segments along the body in the appropriate sequence. This results in a mechanical undulatory wave that is propagated from head to tail and which pushes the lamprey model through the simulated water [67,16,24]. The model was initially made in 2D but later developed into a 3D model that can 'swim' through the simulated water and turn left, right, upwards, downwards and even produce screw-like movements. This is achieved by dividing the myotomes into dorsal and ventral compartments (cf. [70]) on each side, which in turn is controlled by a dorsal and ventral compartment of the ipsilateral spinal network. The actual motor pattern of this model lamprey is similar to its biological counterpart, not only with regard to the propulsive movements but also with regard to the movement pattern used during turning. At the network level turning is produced by providing extra excitation via reticulospinal neurons to different combinations of the spinal compartments. For example, providing extra excitation to the dorsal and ventral compartments of the network on one side will produce turning toward this side. Correspondingly, an excitatory bias of the dorsal compartments on the left and right side will produce an upward turning movement.

8. Modulation of the central pattern generator network — presynaptic effects

The prime targets for different monoamine modulators (dopamine, 5-HT), metabotropic GABA and glutamate receptors, and peptides (somatostatin, NPY, tachykinins) are the different types of Ca^{2+} channels, K_{Ca} channels, and K^+ channels in the network neurons. Each of the target ion channels may affect either the somadendritic membrane properties of the interneurons, or synaptic transmission. This will in turn affect the firing properties of the cells and the strength of synaptic transmission. The properties of the cells then determine the activity pattern of the network. Dopamine and $GABA_B$ receptors act on N and P/Q Ca^{2+} channels [37,51,18,9], and reduce Ca^{2+} entry occurring during the action potential, thereby reducing the activation of K_{Ca} channels. This will result in a smaller post-spike afterhyperpolarization and thereby higher frequency of firing, and at the network level, longer bursts. At the presynaptic level, dopamine and $GABA_B$ receptors reduce Ca^{2+} entry and thereby depress synaptic transmission [12,2].

At the interneuronal level in the pattern generator network, a phasic GABAergic modulation of synaptic transmission occurs from both excitatory and inhibitory interneurons to motor neurons, which results in a gating of the synaptic efficacy in phase with the locomotor burst activity (Fig. 8A–C) [1]. A similar $GABA_B$ modulation occurs at the sensory side and there is also a phasic modulation of afferent neuron synaptic transmission [12,21]. Sensory dorsal cells provide glutamatergic excitation to target interneurons. Small bi-polar interneurons which contain both GABA and NPY immunoreactivity [42,56] form close appositions onto the axons of the dorsal cells (Fig. 8E). Both GABA (via $GABA_B$ receptors) and co-stored NPY cause a depression of synaptic transmission by a presynaptic action. The action of GABA is shorter-lasting than that of NPY. NPY is stored in dense core vesicles in the terminals and GABA immunoreactivity occurs over clusters of small vesicles. It would therefore seem likely that GABA is released during low levels of activity in the bi-polar interneurons, whereas NPY would be released from the dense core vesicles only when the level of cytoplasmic calcium is elevated, as will occur during high activity levels. If so, the presynaptic GABAergic action would be potentiated by co-released NPY only during high levels of activity. The dorsal root monosynaptic sensory transmission is also modulated by 5-HT (Fig. 8D) [21] which provides presynaptic inhibition, and by tachykinins which instead generate presynaptic facilitation [41]. Both these modulatory inputs to originate from sensory afferents with smaller diameters than dorsal cells which mediate touch and pressure (Fig. 8E).

Synaptic transmission from the large reticulospinal axons is the target for a presynaptic modulation by metabotropic group II and III glutamate receptors (mGluRs), which are co-localized on the same reticulospinal axon (Fig. 9A) [35]. This glutamatergic presynaptic inhibition of a glutamatergic synapse will thus presumably serve to provide autoinhibition, perhaps during overflow of glutamate during high levels of activity. In addition to the presynaptic Group II and II mGluR, there are also postsynaptic Group I MgluRs that increase the ex-

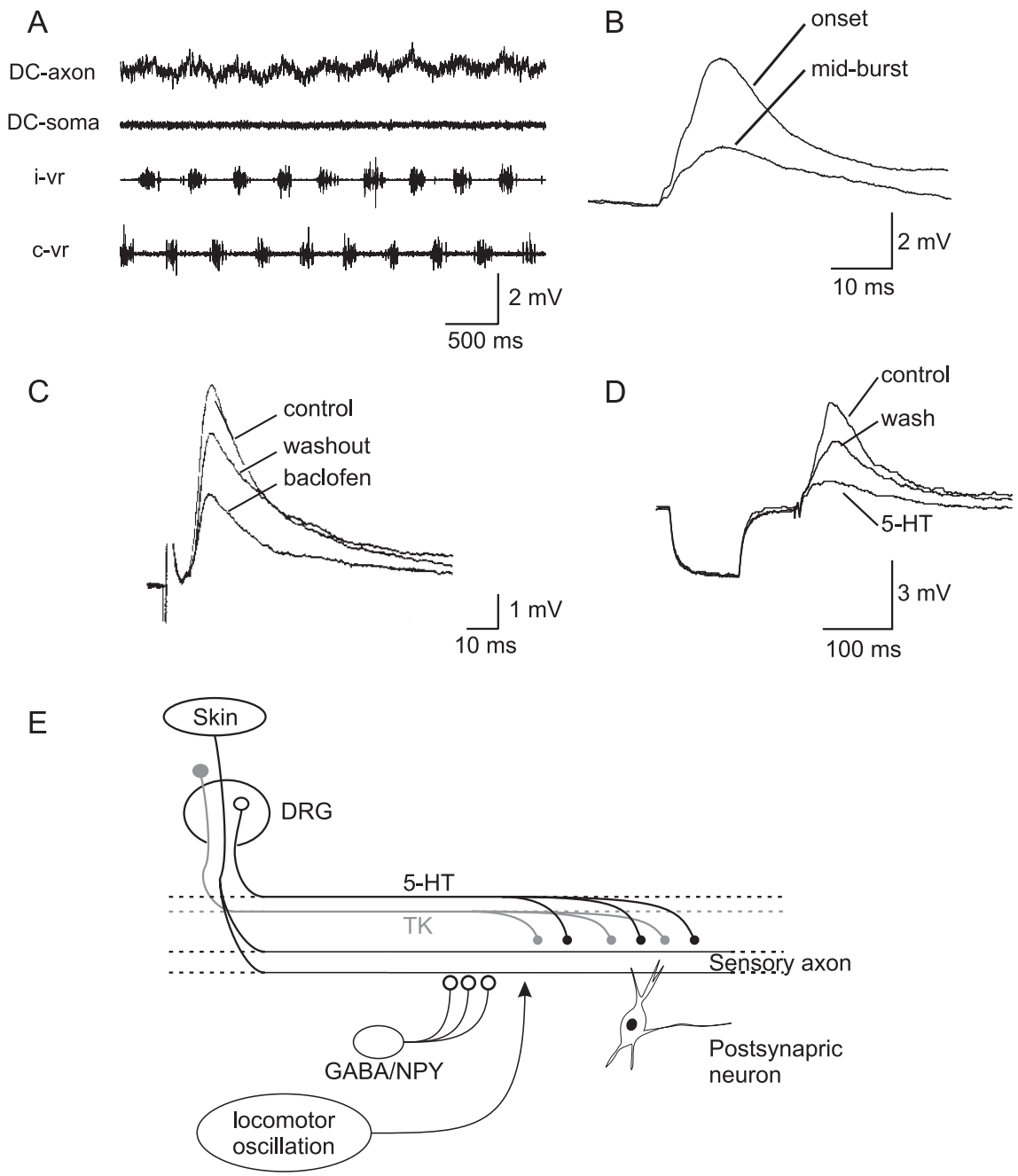

Fig. 8. Phasic membrane potential depolarization and modulation of sensory transmission. A: Dual intracellular recordings are made from a dorsal cell soma (DC-soma) and its axon (DC-axon) during fictive locomotion. The DC-axon recording shows phasic membrane depolarizations with the peak amplitude at the midpoint of the ipsilateral ventral root (i-vr) burst, whereas the membrane potential of DC-soma shows no phasic modulation. B: Monosynaptic compound EPSPs elicited in a giant interneuron by stimulation of dorsal column stimulation, which activates axons of dorsal cells as well as other sensory neurons. The EPSP amplitude is smaller when the stimulation is delivered at the midburst, as compared with the burst onset. C: The $GABA_B$ receptor agonist baclofen depresses a sensory-evoked monosynaptic EPSP. D: 5-HT reduces the amplitude of monosynaptic EPSP elicited in a giant interneuron by dorsal column stimulation. E: Presynaptic modulation of sensory transmission. Dorsal root ganglion (DRG) contains small cells with 5-HT immunoreactivity and tachykinin fibers with their cell body in the peripheral dorsal root. 5-HT causes presynaptic inhibition of sensory dorsal cell synaptic transmission. GABA and NPY, which are co-localized in bipolar interneurons, also mediate presynaptic inhibition of sensory transmission. The locomotor oscillator also provides presynaptic modulation of excitatory glutamatergic transmission to postsynaptic neurons.

citability of network neurons and increase the frequency of the locomotor rhythm (Fig. 9B). mGluRs can be activated by their specific agonists, and the presynaptic receptors require somewhat higher glutamate concentration than the postsynaptic metabotropic glutamate receptors belonging to the type I category.

Just below the central canal in the midline of the spinal cord, there is a group of cells that are immunoreactive to

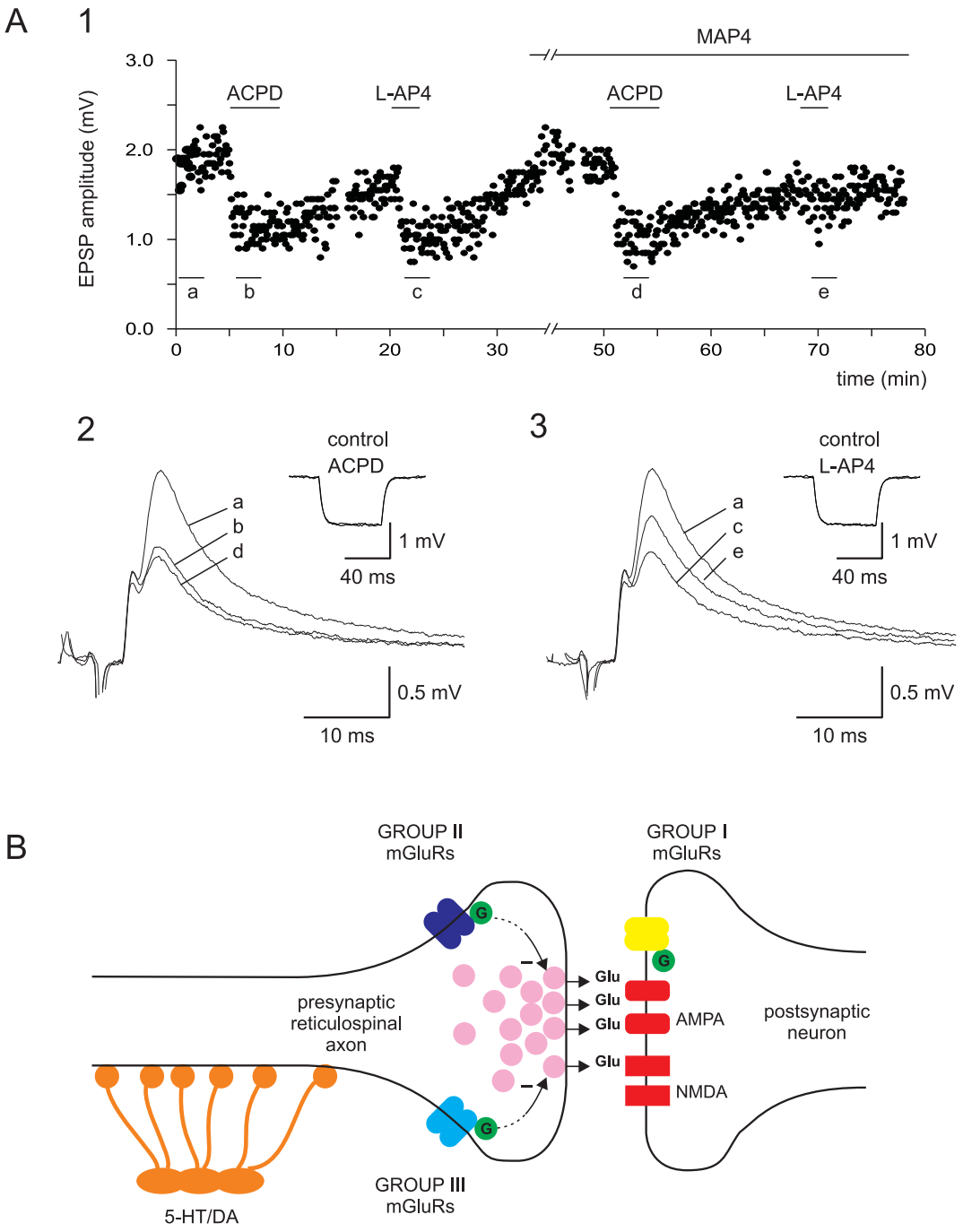

Fig. 9. Metabotropic glutamate receptor-mediated presynaptic inhibition of reticulospinal synaptic transmission. A1: The effects of the mGluR agonists (1S,3R)-ACPD and L-AP4 on the amplitude of the reticulospinal evoked EPSP were tested before and during application of the group III mGluRs antagonist MAP4. A2: The decrease in the monosynaptic EPSP amplitude by (1S,3R)-ACPD was not antagonized by MAP4. A3: The L-AP4-induced decrease of the monosynaptic EPSP was antagonized by MAP4. Neither (1S,3R)-ACPD nor L-AP4 affected the electrical component of the EPSP or the input resistance of the postsynaptic target neuron. B: Two types of mGluRs are co-localized on single reticulospinal axons and mediate presynaptic inhibition. These receptors are pharmacologically similar to group II and III mGluRs. Group I mGluRs are present at the postsynaptic soma-dendritic level where they increase the excitability of neurons and thereby increase the frequency of the locomotor rhythm. Reticulospinal axons are also subject to presynaptic inhibition by 5-HT and dopamine (DA). Both 5-HT and DA are co-localized in neurons located ventral to the central canal and which make close appositions with reticulospinal axons. Modified from [35].

5-HT, dopamine and tachykinins. They form a dense bilateral plexus at the ventromedial surface of the spinal cord. In this plexus the network interneurons and motor neurons distribute their ventromedial dendrites. The varicosities in this plexus do not form point to point synapses, and thus rely on paracrinic release of the transmitters [11]. The plexus extends throughout the spinal cord and is very dense. The concentration of transmitter/modulator in the

ventromedial part of the spinal cord presumably will be substantial. Co-release of 5-HT and dopamine will target K_{Ca} and calcium channels, respectively, on network interneurons, and thereby exert complementary actions (see above). Furthermore, both dopamine and 5-HT elicit presynaptic inhibition on synaptic transmission from reticulospinal axons [7,72] (Fig. 9B). The fact that the neurons of the 5-HT/dopamine plexus are distributed along the spinal cord and are activated by sensory as well as descending fibers means that the activity in this plexus can be regulated segmentally [50]. This in turn suggests that the level of presynaptic inhibition along the reticulospinal axons can be regulated locally. In fact, the segment can presumably control how efficient synaptic transmission from descending supraspinal fibers will be. Consequently the level of descending drive may be varied through segmental presynaptic inhibition in different parts of the spinal cord.

9. Synaptic plasticity induced by tachykinins in the locomotor network

Tachykinins are also distributed in the ventromedial plexus as well as in the dorsal horn and in sensory afferents [62–64,41,43]. If tachykinins are applied to the bath during ongoing fictive locomotion, they cause a concentration-dependent increase in the locomotor rate. At higher, but still physiological, concentrations (1 μM) a short-lasting (10 min) application of tachykinins may cause a doubling of the locomotor burst rate, and this increased burst rate will remain elevated over 24 h [44]. The initial increase in burst rate is due to a protein kinase-C mediated increase in NMDA mediated synaptic transmission lasting over a period of one to two hours. This leads to a potentiation of synaptic transmission in glutamatergic synapses, which involves activation of post-synaptic NMDA receptors. The subsequent period (2–24 h) requires protein synthesis. Synthesis blockers like anisomysin applied prior to/or within 1 h of the short-lasting tachykinin application will entirely block the second phase of facilitation of the burst rate, while the first NMDA dependent component remains unchanged.

10. Concluding remarks

In this brief review we have mainly dealt with the neural control of the propulsive locomotor synergy and in particular reviewed the cellular bases of this behaviour. In addition to the standard configuration of the brainstem–spinal cord network, we have reviewed many of the different modulatory mechanisms directed at specific ion channels or receptors which in turn produce specific modifications of the activity pattern of single cells, which determine the specific motor pattern produced at the network level. This network resembles in many aspects the locomotor network of the developing frog embryo/larva, particularly at the later stages when a burst pattern has evolved [54]. In mammals (cat, neonatal rat) comparatively little information is available at the cellular level, although the general neural organization, as well as its neuropharmalogical data indicate that the basic mechanisms are similar [10,33].

We have limited this chapter only to the propulsive synergy and have not included the other motor component which is critical for behaviourally successful locomotion, that is maintaining an appropriate postural control during ongoing movements. In the lamprey model, we now have a detailed understanding also of the visuovestibular control, which is critical for maintaining an appropriate body orientation during swimming in turbulent water [40,14,15].

Acknowledgements

This work was supported by the Medical Research Council (proj. no. 3026), Marianne and Marcus Wallenberg foundation, the Swedish Natural Science Research Council (proj. no. B-AA/BU03531), the Swedish National Board for Industrial and Technical Development, NUTEK, (proj. no. 8425-5-03075) and the Swedish Society for Medical Research.

References

[1] S. Alford, J. Christenson, S. Grillner, Presynaptic $GABA_A$ and $GABA_B$ receptor-mediated phasic modulation in axons of spinal motor interneurons, Eur. J. Neurosci. 3 (1991) 107–117.
[2] S. Alford, S. Grillner, The involvement of $GABA_B$ receptors and coupled G-proteins in spinal GABAergic presynaptic inhibition, J. Neurosci. 12 (1991) 3718–3728.
[3] C. Ariens Kapper, G. Huber, E. Crosby, The Comparative Anatomy of the Nervous System of Vertebrates, including Man, The Macmillan Company, Vol. 1, 1936, p. 2.
[4] B.J. Bacskai, P. Wallén, V. Lev-Ram, S. Grillner, R.Y. Tsien, Activity-related calcium dynamics in lamprey motoneurons as revealed by video-rate confocal microscopy, Neuron 14 (1995) 19–28.
[5] J. Bao, E. Kandel, R. Hawkins, Involvement of pre- and postsynaptic mechanisms in posttetanic potentiation at aplysia synapses, Science (Washington) 275 (1997) 969–973.
[6] J. Buchanan, S. Grillner, Newly identified 'glutamate interneurons' and their role in locomotion in the lamprey spinal cord, Science (Washington) 236 (1987) 312–314.
[7] J. Buchanan, S. Grillner, 5-Hydroxytryptamine depresses reticulospinal excitatory postsynaptic potentials in motoneurons of the lamprey, Neurosci. Lett. 112 (1991) 71–74.
[8] J.T. Buchanan, Identification of interneurons with contralateral, caudal axons in the lamprey spinal cord: Synaptic interactions and morphology, J. Neurophysiol. 47 (1982) 961–975.
[9] N. Bussiéres, A. El Manira, $GABA_B$ receptor activation in lamprey sensory neurons modulates N- and P/Q-type calcium channels, Soc. Neurosci. Abstr. 23 (1997) 1182.
[10] J. Cazalets, M. Borde, F. Clarac, Localization and organization of the central pattern generator for hindlimb locomotion in newborn rat, J. Neurosci. 15 (1995) 4943–4951.
[11] J. Christenson, J. Franck, S. Grillner, Increase in endogenous 5-hy-

droxytryptamine levels modulates the central network underlying locomotion in the lamprey spinal cord, Neurosci. Lett. 100 (1989) 188–192.
[12] J. Christenson, S. Grillner, Primary afferents evoke excitatory amino acid receptor mediated EPSPs, that are modulated by presynaptic ($GABA_B$) receptors in lamprey, J. Neurophysiol. 66 (1991) 2141–2149.
[13] A.H. Cohen, P. Wallén, The neuronal correlate of locomotion in fish. 'Fictive swimming' induced in an in vitro preparation of the lamprey spinal cord, Exp. Brain Res. 41 (1980) 11–18.
[14] T. Deliagina, G. Orlovsky, S. Grillner, P. Wallén, Vestibular control of swimming in lamprey: 2. Characteristics of spatial sensitivity of reticulospinal neurons, Exp. Brain Res. 90 (1992) 489–498.
[15] T. Deliagina, G. Orlovsky, S. Grillner, P. Wallén, Vestibular control of swimming in lamprey: 3. Activity of vestibular afferents. Convergence of vestibular inputs on reticulospinal neurons, Exp. Brain Res. 90 (1992) 499–507.
[16] Ö. Ekeberg, A. Lansner, S. Grillner, The neural control of fish swimming studied through numerical simulations, Adaptive Behavior 3 (1995) 363–384.
[17] Ö. Ekeberg, P. Wallén, A. Lansner, H. Tråvén, L. Brodin, S. Grillner, A computer based model for realistic simulations of neural networks. I: The single neuron and synaptic interaction, Biol. Cybern. 65 (1991) 81–90.
[18] A. El Manira, N. Bussiéres, Calcium channel subtypes in lamprey sensory and motor neurons, J. Neurophysiol. 78 (1997) 1334–1340.
[19] A. El Manira, M. Pombal, S. Grillner, Diencephalic projection to reticulospinal neurons involved in the initiation of locomotion in adult lampreys *lampetra fluviatilis*, J. Comp. Neurol., (1997) in press.
[20] A. El Manira, J. Tegnér, S. Grillner, Calcium-dependent potassium channels play a critical role for burst termination in the locomotor network in lamprey, J. Neurophysiol. 72 (1994) 1852–1861.
[21] A. El Manira, W. Zhang, E. Svensson, N. Bussiéres, 5-HT inhibits calcium current and synaptic transmission from sensory neurons in lamprey, J. Neurosci. 17 (1997) 1786–1794.
[22] P. Fagerstedt, P. Wallén, S. Grillner, Activity of interneurons during fictive swimming in the lamprey, IBRO Congress Neurosci. Abstr. 1 (1995) 346.
[23] S. Grillner, Neurobiological bases of rhythmic motor act in vertebrates, Science (Washington) 228 (1985) 143–149.
[24] S. Grillner, T. Deliagina, Ö. Ekeberg, A. El Manira, R. Hill, A. Lansner, G. Orlovsky, P. Wallén, Neural networks that coordinate locomotion and body orientation in lamprey, Trends Neurosci. 18 (1995) 270–279.
[25] S. Grillner, A. Georgopoulos, L. Jordan, Selection and initiation of motor behaviour, in: P. Stein, D. Stuart, A. Selverston, S., Grillner (Eds.), Neurons, Networks, and Motor Behaviour, MIT Press, Cambridge, MA, 1997, Ch. 1, pp. 3–19.
[26] S. Grillner, A.D. McClellan, C. Perret, Entrainment of the spinal pattern generators for swimming by mechanosensitive elements in the lamprey spinal cord in vitro, Brain Res. 217 (1981) 380–386.
[27] S. Grillner, A.D. McClellan, K. Sigvardt, P. Wallén, M. Wilén, Activation of NMDA receptors elicits 'fictive locomotion' in lamprey spinal cord in vitro, Acta Physiol. Scand. 113 (1981) 549–551.
[28] S. Grillner, T. Williams, P. Lagerback, The edge cell, a possible intraspinal mechanoreceptor, Science (Washington) 223 (1984) 500–503.
[29] R.M. Harris-Warrick, Pattern generation, Curr. Opin. Neurobiol. 6 (1993) 982–988.
[30] R.M. Harris-Warrick, A.H. Cohen, Serotonin modulates the central pattern generator for locomotion in the isolated lamprey spinal cord, J. Exp. Biol. 116 (1985) 27–46.
[31] J. Hellgren, S. Grillner, A. Lansner, Computer simulation of the segmental neural network generating locomotion in lamprey by using populations of network interneurons, Biol. Cybern. 68 (1992) 1–13.
[32] L. Jordan, R. Brownstone, B. Noga, Control of functional systems in the brainstem and spinal cord, Curr. Opin. Neurobiol. 2 (1992) 794–801.
[33] O. Kiehn, J. Hounsgaard, K. Sillar, Basic building blocks of vertebrate spinal central pattern generators, in: P. Stein, D. Stuart, A. Selverston, S. Grillner (Eds.), Neurons, Networks, and Motor Behaviour, MIT Press, Cambridge, MA, 1997, Ch. 4, pp. 47–59.
[34] H. Kleerekoper, Role of olfaction in the orientation of *petromyzon marinus*. i. response to a single amine in prey's body odor, Physiol. Zool. 36 (1963) 347–360.
[35] P. Krieger, A. El Manira, S. Grillner, Activation of pharmacologically distinct metabotropic glutamate receptors depresses reticulospinal-evoked monosynaptic EPSPs in the lamprey spinal cord, J. Neurophysiol. 76 (1996) 3834–3841.
[36] E. Marder, R.L. Calabrese, Principles of rhythmic motor pattern generation, Physiol. Rev. 76 (1996) 687–717.
[37] T. Matsushima, J. Tegnér, R. Hill, S. Grillner, $GABA_B$ receptor activation causes a depression of low- and high-voltage-activated (Ca^{2+}) currents, postinhibitory rebound, and postspike afterhyperpolarization in lamprey neurons, J. Neurophysiol. 70 (1993) 2606–2619.
[38] A.D. McClellan, S. Grillner, Activation of 'fictive swimming' by electrical micro-stimulation of brainstem locomotor regions in an in vitro preparation of the lamprey central nervous system, Brain Res. 300 (1984) 357–361.
[39] Y. Ohta, S. Grillner, Monosynaptic excitatory amino acid transmission from the posterior rhombencephalic reticular nucleus to spinal neurons involved in the control of locomotion in lamprey, J. Neurophysiol. 62 (1989) 1079–1089.
[40] G. Orlovsky, T. Deliagina, P. Wallén, Vestibular control of swimming in lamprey: 1. Responses of reticulospinal neurons to roll and pitch, Exp. Brain Res. 90 (1992) 479–488.
[41] D. Parker, S. Grillner, Tachykinin-mediated modulation of sensory neurons, interneurons, and synaptic transmission in the lamprey spinal cord, J. Neurophysiol. 76 (1996) 4031–4039.
[42] D. Parker, C. Söderberg, E. Zotova, O. Shupliakov, U. Langel, T. Bartfai, D. Larhammar, L. Brodin, S. Grillner, Co-localized neuropeptide Y and GABA have complementary actions in modulating spinal sensory inputs, under revision, Europ. J. Neurosci. (1997).
[43] D. Parker, E. Svensson, S. Grillner, Substance P modulates sensory action potentials in the lamprey via a protein kinase c-mediated reduction of a 4-aminopyridine-sensitive potassium conductance, Eur. J. Neurosci. 9 (1997) 2064–2076.
[44] D. Parker, W. Zhang, S. Grillner, Tachykinin-mediated potentiation of NMDA responses results in protein synthesis-dependent long-lasting modulation of the lamprey locomotor network, Abstract for Göttingen, N. Elsner, R. Wehrer (Eds.), Georg Thiesne, Stuttgart, 1998.
[45] M. Pombal, A. El Manira, S. Grillner, Afferents of the lamprey striatum with special reference to the dopaminergic system: A combined tracing and immunohistochemical study, J. Comp. Neurol. 386 (1997) 71–91.
[46] M. Pombal, A. El Manira, S. Grillner, Organization of the lamprey striatum — transmitters and projections, Brain Res. 766 (1997) 249–254.
[47] A. Roberts, M. Tunstall, E. Wolf, Properties of networks controlling locomotion and significance of voltage dependency of NMDA channels: stimulation study of rhythm generation sustained by positive feedback, J. Neurophysiol. 73 (1995) 485–495.
[48] C.M. Rovainen, Neurobiology of lampreys, Phys. Rev. 59 (1979) 1007–1077.
[49] D.F. Russell, P. Wallén, On the control of myotomal motoneurones during 'fictive swimming' in the lamprey spinal cord in vitro, Acta Physiol. Scand. 117 (1983) 161–170.
[50] J. Schotland, O. Shupliakov, S. Grillner, L. Brodin, Synaptic and nonsynaptic monoaminergic neuron systems in the lamprey spinal cord, J. Comp. Neurol. 372 (1996) 229–244.

[51] J. Schotland, O. Shupliakov, M. Wikström, L. Brodin, M. Srinivasan, Z.B. You, M. Herrera-Marschitz, W. Zhang, T. Hökfelt, S. Grillner, Control of lamprey locomotor neurons by colocalized monoamine transmitters, Nature (London) 374 (1995) 266–268.
[52] A. Selverston, Modeling of neural circuits: What have we learned, Annu. Rev. Neurosci. 16 (1993) 531–546.
[53] M. Shik, G. Orlovsky, Neurophysiology of locomotor automatism, Physiol. Rev. 56 (1976) 465–501.
[54] K. Sillar, O. Kiehn, N. Kudo, Chemical modulation of vertebrate motor circuits, in: P. Stein, D. Stuart, A. Selverston, S. Grillner (Eds.), Neurons, Networks, and Motor Behaviour, MIT Press, Cambridge, MA, 1997, Ch. 17, pp. 183–193.
[55] K. Sillar, A. Simmers, Presynaptic inhibition of primary afferent transmitter release by 5-hydroxytryptamine at a mechanosensory synapse in the vertebrate spinal cord, J. Neurosci. 14 (1994) 2636–2647.
[56] C. Söderberg, Molecular evolution of the neuropeptide Y family of peptides, PhD thesis, Department of Medical Pharmacology, Uppsala University, 1996.
[57] J. Tegnér, Modulation of Cellular Mechanisms in a Spinal Locomotor Network — An Experimental and Computational study in Lamprey, PhD thesis, Nobelinstitute for Neurophysiology, Karolinska Institute, 1997.
[58] J. Tegnér, J. Hellgren-Kotaleski, A. Lansner, S. Grillner, Low voltage activated calcium channels in the lamprey locomotor network-simulation and experiment, J. Neurophysiol. 77 (1997) 1795–1812.
[59] J. Tegnér, A. Lansner, S. Grillner, Modulation of burst frequency by calcium-dependent potassium channels in the lamprey locomotor system-dependence of the activity level, J. Comp. Neurosci., (1998) in press.
[60] H. Tråvén, L. Brodin, A. Lansner, Ö. Ekeberg, P. Wallén, S. Grillner, Computer simulations of NMDA and non-NMDA receptor-mediated synaptic drive–sensory and supraspinal modulation of neurons and small networks, J. Neurophysiol. 70 (1993) 695–709.
[61] F. Ullén, T. Deliagina, G. Orlovsky, S. Grillner, Visual pathways for postural control and negative phototaxis in lamprey, J. Neurophysiol. 78 (1997) 960–976.
[62] P. Van Dongen, T. Hökfelt, S. Grillner, A. Verhofstad, H. Steinbusch, A. Cuello, L. Terenius, Immunohistochemical demonstration of some putative neurotransmitters in the lamprey spinal cord and spinal ganglia: 5-hydroxytryptamine-, tachykinin-, and neuropeptide-y-immunoreactive neurons and fibers, J. Comp. Neurol. 234 (1985) 501–522.
[63] P. Van Dongen, E. Theodorsson-Norheim, E. Brodin, T. Hökfelt, S. Grillner, A. Peters, A. Cuello, W. Forssmann, M. Reinecke, E. Singer, L. Lazarus, Immunohistochemical and chromatographic studies of peptides with tachykinin-like immunoreactivity in the central nervous system of the lamprey, Peptides 7 (1986) 297–313.
[64] P. Van Dongen, E. Theodorsson-Norheim, E. Brodin, T. Hökfelt, S. Grillner, A. Peters, A. Cuello, W. Forssmann, M. Reinecke, E. Singer, L. Lazarus, Immunohistochemical demonstration of some putative neurotransmitters in the lamprey spinal cord and spinal ganglia: 5-hydroxytryptamine-, tachykinin-, and neuropeptide-y-immunoreactive neurons and fibers, J. Comp. Neurol. 234 (1986) 501–522.
[65] G. Viana Di Prisco, E. Pearlstein, R. Robitaille, R. Dubuc, Role of sensory-evoked NMDA plateau potentials in the initiation of locomotion, Science (Washington) 278 (1997) 1122–1125.
[66] G. Viana Di Prisco, P. Wallén, S. Grillner, Synaptic effects of intraspinal stretch receptor neurons mediating movement-related feedback during locomotion, Brain Res. 530 (1990) 161–166.
[67] T. Wadden, J. Hellgren-Kotaleski, A. Lansner, S. Grillner, Intersegmental co-ordination in the lamprey — simulations using a continuous network model, Biol. Cybern. 76 (1997) 1–9.
[68] P. Wallén, J. Buchanan, S. Grillner, R. Hill, J. Christenson, T. Hökfelt, Effects of 5-hydroxytryptamine on the afterhyperpolarization, spike frequency regulation, and oscillatory membrane properties in lamprey spinal cord neurons, J. Neurophysiol. 61 (1989) 759–768.
[69] P. Wallén, S. Grillner, *N*-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey, J. Neurosci. 7 (1987) 2745–2755.
[70] P. Wallén, S. Grillner, J.L. Feldman, S. Bergfelt, Dorsal and ventral myotome motorneurons and their input during fictive locomotion in lamprey, J. Neurosci. 11 (1985) 654–661.
[71] M. Wikström, A. El Manira, S. Grillner, N-type Ca^{2+} channels activates apamin-sensitive Ca^{2+} dependent K^{+} channels that generate the afterhyperpolarization in lamprey neurons, Int. Cong. Phys. 33 (1997) P012.15.
[72] M. Wikström, A. El Manira, W. Zhang, R. Hill, S. Grillner, Dopamine and 5-HT modulation of synaptic transmission in the lamprey spinal cord, Soc. Neurosci. Abstr., (1995).
[73] W. Zhang, M.A. Pombal, A. El Manira, S. Grillner, Rostrocaudal distribution of 5-HT innervation in the lamprey spinal cord and differential effects of 5-HT on fictive locomotion, J. Comp. Neurol. 374 (1996) 278–290.