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ARTICLE

L. Prime · Y. Pichon

Role of ligand-gated ion channels in the swimming behaviour of *Xenopus* tadpoles: experimental data and modelling experiments

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Abstract The swimming behaviour of lower vertebrates has been used as a model to study the function of simple neuronal circuits. Good examples are the lamprey and the *Xenopus* tadpole. In these two cases, glutamate-activated NMDA receptors are involved, and the relative importance of the NMDA and non-NMDA receptors as well as the involvement of other ion channels has been studied using a combination of electrophysiological recordings and modelling experiments, but little attention had been paid to their evolution during development. In the present experiments, which have been performed on *Xenopus* embryos from stages 31 to 42, we have probed the relative importance of the two categories of receptors using selective blockers [respectively DL-2-amino-5-phosphonovaleric acid (APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)]. The sensitivity of the swimming behaviour to APV was found to increase during development and that to CNQX to decrease. Furthermore, it has been observed that the spike activity recorded from the ventral roots is more complex in late embryonic stages that in early embryos. These modifications are associated with changes of the neuronal circuit, some of which correspond to a lengthening of the axon and an increased complexity of the dendritic tree of the motoneurons. We have incorporated these modifications in a simplified model of the central pattern generator built with Neuron software. The results indicate that at least part of the observed changes can be associated with changes in the length of the dendrites and axons.

Keywords Electrophysiology · Ion channels · Modelling · Swimming · *Xenopus* tadpole

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L. Prime · Y. Pichon (✉)
Equipe Canaux et Récepteurs Membranaires,
UMR CNRS 6026, Université de Rennes 1, Bâtiment 13,
Campus de Beaulieu, Avenue du Général Leclerc,
35042 Cedex Rennes, France
E-mail: yves.pichon@univ-rennes1.fr
Tel.: +33-2-23235061
Fax: +33-2-23235052

Introduction

The locomotor activity of several invertebrate species, as well as that of lower vertebrates, has revealed interesting properties of simple neuronal circuits, the best examples being fictive swimming behaviour of the lamprey (Grillner 1985; Buchanan and Grillner 1987; Wallen and Grillner 1987; Grillner 1997; Grillner 2003) and of the *Xenopus* tadpole (Clarke and Roberts 1984; Roberts 1990; Roberts and Tunstall 1990). In both cases, detailed electrophysiological and pharmacological experiments have enabled the dissection of the neuronal circuits which underlie this simple behaviour and thereby facilitated their realistic modelling. In the *Xenopus* tadpole, this neuronal circuit is made of a limited number of spinal cord neurons (Clarke and Roberts 1984): Rohon–Beard sensory neurons, two sets of secondary sensory neurons, three sets of interneurons and one set of motoneurons which controls the contraction of the homolateral segmental myotomes. The pattern of activity during "fictive swimming" (i.e. swimming in immobilized or paralysed animals) is characterized by an alternating activity of the motoneurons located on each side of the body (Roberts and Kahn 1982; Soffe and Roberts 1982a, 1982b). These motoneurons are excited by several excitatory inputs, the most important being mediated by glutamate and associated with cells located on the same side of the spinal cord (Soffe and Roberts 1982b; Dale and Roberts 1984, 1985). They are inhibited by glycinergic inputs from contralateral inhibitory interneurons (Dale 1985; Soffe 1987). This basic pattern has been more recently shown to be modulated by several neuro-

transmitters, including ACh (Perrin and Roberts 1995), GABA (Reith and Sillar 1999), 5-HT (Sillar et al. 1992; McDearmid et al., 1997), noradrenaline (McDearmid et al. 1997) and adenosine (Fisher et al. 2001) . Furthermore, it has been shown that the circuit changed during postembryonic development. Thus, the duration of the ventral root discharge at 23 °C has been shown to increase from 7 ms at stage 37/38 to 20 ms at stage 42 in 27 h (Sillar et al. 1991) and, according to Reith and Sillar (1999), endogenous activation of GABA(A) receptors plays an increasingly important role during the first day of postembryonic development.

Since it has been shown, on the one hand, that both NMDA and non-NMDA receptors provide excitatory drives for swimming (Dale and Roberts 1984 and 1985) and, on the other hand, that functional AMPA receptors appear before NMDA receptors in differentiating spinal neurons (Gleason and Spitzer 1998), it seemed important to follow the evolution of the relative importance of these two systems in the swimming behaviour. Furthermore, it appeared essential to find out if there is a correlation between this behaviour and the known changes in the morphology of the motoneurons observed between the early embryonic stage and the larval stage by van Mier et al. (1985).

In the present experiments, we show that the duration of the swimming episodes, which is hardly sensitive to the addition of the NMDA channel blocker DL-2-amino-5-phosphonovaleric acid (APV) at concentrations up to 150 μM at early stages, becomes increasingly sensitive at larval stages. Conversely, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), which blocks the non-NMDA receptors, inhibits swimming in early stages but has little effect at larval stages. Another change in the properties of the swimming behaviour is the replacement of the single spike pattern of activity at stage 37/38 by bursts of discharges at stage 42, after only one day of development (Sillar et al. 1991). These results, as well as the apparent lack of effect of direct inhibition of the presumed motoneurons impaled in situ on the firing frequency, can be accounted for by a realistic model of the central pattern generator. This suggests that part of the observed changes can be associated with changes in the length of the dendrites and axons.

Methods

Animals were obtained from the local breeding facilities. Their developmental stages were defined using morphological criteria of Nieuwkoop and Faber's (1956) table as follows: early stages (28/30), embryonic stages (32/36), postembryonic (37/39) and larval stages (40/43). They were released from their egg membranes, anaesthetized using tricaine (MS-222, Sigma) and transferred into a recording chamber containing the physiological saline in which curare (10^{-4} M) or α-bungarotoxin (10^{-6} M) (both from Sigma) had been added to immobilize the animal. The saline contained 105 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ at pH 7.2–7.4, to which were added different concentrations of the NMDA-receptor blocker APV (from Sigma) or the non-NMDA-receptor blocker CNQX (from RBI).

The embryos were spinalized in front of the first post-otic segment and the skin removed over a length of 5–6 myotomes. Extracellular recordings were made as described by Kahn and Roberts (1982) by placing a suction micro-electrode (about 50 μm in diameter) on the outer surface of the myotomal muscle over an intermyotomal cleft and a reference electrode in the bath. The signals were amplified, visualized on an oscilloscope and stored on tape for off-line analysis. Intracellular recordings were performed using fine tipped microelectrodes filled with 3 M potassium acetate (impedance ranging from 150 to 200 MΩ) in neurons located superficially in the ventral quarter of the spinal cord and are presumed motoneurons (Dale and Roberts 1984). Fictive swimming was induced by a short (20–40 ms) current pulse (10 μA) applied to the skin.

Modelling was performed using version 5.2 of the Neuron software (Hines and Carnevale 2001) running on a Windows 98 (Microsoft) operated microcomputer. This software is based on the notion of continuous cable "sections" connected to each other to form arborizations analogous to those found in real neurons. Biophysical properties of the membrane (ion channels, pumps) and of the cytoplasm (buffers, second messengers) are described by differential equations, kinetic diagrams and sets of simultaneous equations. These model descriptions are compiled so that membrane voltage and gating states can be computed efficiently using an implicit integration method optimized for complex geometrical structures such as neurons (Hines and Carnevale 2000).

Basically, the model was made of a set of six identical neurons consisting of one inexcitable dendrite connected, via a weakly excitable cell body, to an elongated excitable axon (Fig. 1A)[1]. Apart from the cell body, which was made of a single compartment, the two other parts of the neuron were built as a series of segments, the number and the length of which could be modified to accommodate neuronal growth. Three synapses were located on each neuron: two excitatory synapses (Ex1 and Ex2) on the dendrite and one inhibitory synapse on the cell body (Inh) (Fig. 1B). One more "stimulation" excitatory synapse (Stim) (corresponding to the skin sensory neurons) was placed on the dendrites of the excitatory interneurons to start the swimming episodes. The synaptic weights and synaptic delays could be adjusted between each simulation, as were the other parameters of the synapses.

Two different models were used. In the simplified model (model 1, Fig. 1B), only two neurons (E1 and E2), which are at the same time excitatory and inhibitory, are used. They possess two autoexcitatory synapses (+), one fast and one slow, and one inhibitory synapse (−). The second model (model 2, Fig. 1C), which is more realistic, comprises six neurons: two excitatory interneurons (E1 and E2), two inhibitory interneurons (I1 and I2) and two motoneurons (M1 and M2), which are in synaptic contact with the segmental muscles through neuromuscular junctions (nmj1 and nmj2). The neurons are connected through excitatory (+) and inhibitory (−) synapses. To simplify the computation, Ex1 and Ex2 were merged, in this second model, into one single excitatory synapse which could be adjusted to mimic fast or slow excitatory synaptic potentials (EPSPs).

The main parameters used in the simulations were as follows: membrane capacitance, 1 $\mu F/cm^2$; cytoplasmic axial resistance, 80 Ω cm; E_{leak}, −54.3 mV; E_{Na}, 50 mV; E_K, −77 mV; g_{Namax}, 0.120 S/ cm^2; g_{Kmax}, 0.036 S/cm^2; g_{leak}, 0.0003 S/cm^2. The Hodgkin and Huxley (1952) scheme was used for the voltage-gated conductances. Neuron files can be obtained from Y.P. (yves.pichon@univ-rennes1.fr).

[1]This simple model has been preferred to a more elaborate model for two reasons: (1) our knowledge of the morphology of the *Xenopus* spinal neurons is limited; (2) a recent comparison of the predictive values of two-compartment models of rat cerebellar Purkinje cells, a detailed compartment model (built with Neuron) and a two-compartment model, indicate that the latter has a reasonably good predictive value (Roth and Häusser 2001); see also Rall and Agmon-Snir (1989)

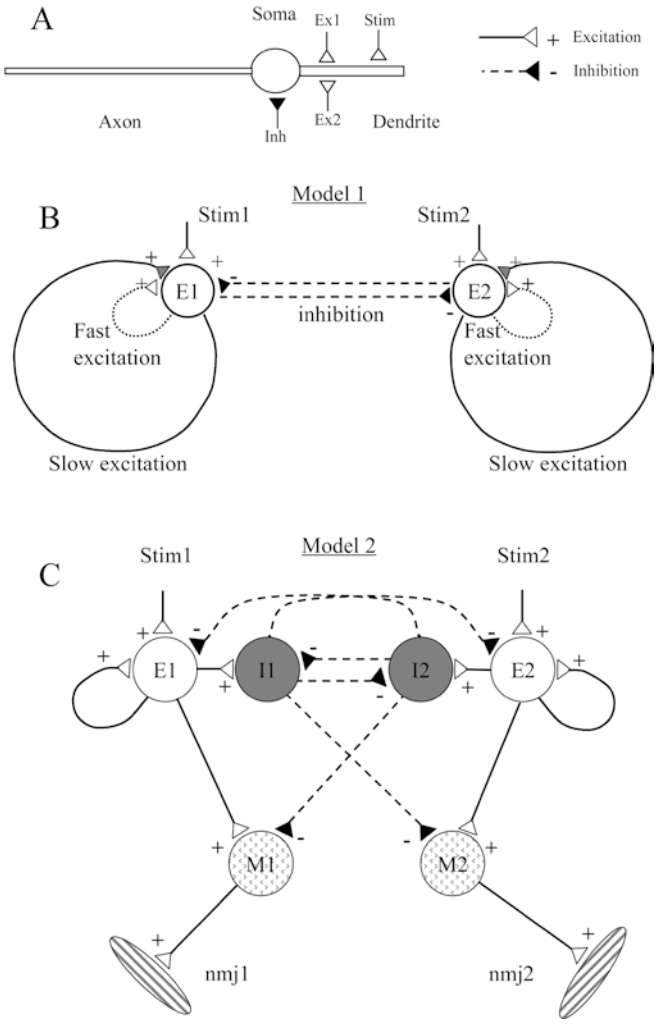

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Fig. 1 Schematic representation of the model neuron (**A**) and of the two neuronal circuits (**B** and **C**) used in the computation. **A** The neuron consisted of a cell body (soma) connected at one end to an inexcitable dendrite (dendrite) and at the other end to an excitable axon (axon). The soma was made of a single compartment (20×20 μm), whereas both dendrite and axon were made of several segments with a minimum of three for the dendrite of early embryos. The diameter of the dendrite was set to 2 μm and that of the axon to 1 μm. Their length was changed to reflect neuronal growth from 80 μm to 1000 μm. The neuron could be stimulated with an exponential signal (Stim) on the dendrite (at a distance of 30% or 50% of the dendritic length from the cell body) and could be excited by two double exponential synaptic inputs (Ex1 and Ex2) also located on the dendrite but near the soma (at a distance of 20% of the dendritic length from the cell body). An inhibitory synaptic input (Inh) was applied directly to the cell body. Not to scale. **B** Simplified circuit used to analyse the effects of dendritic and axonal growth on electrical activity of the interneurons (E1 and E2). Each neuron is stimulated independently (Stim1 and Stim2), inhibited by the other neuron (Inhibition, *dashed line* and *black triangles*) and is autoexcited by two synaptic inputs differing by their time course: a fast EPSP with a rising time constant of 0.1 ms and a falling time constant of 15 ms (*dotted line* and *open triangles*) and a slow EPSP with a rising time constant of 5 ms and a falling time constant of 75 ms (*continuous line* and *filled grey triangles*). **C** Simplified circuit of a six-neuron network used to monitor the activity of the motoneurons M1 and M2 (*dotted circles*) in the presence and absence of inhibition by the inhibitory interneurons I1 and I2 (*dark circles*). The excitation of the neurons (including autoexcitation of the interneurons E1 and E2) is modelled by one single EPSP with a rising time constant of 0.5 ms and a falling time constant of 10 ms. Nmj1 and nmj2 are neuromuscular junctions

Results

The normal "fictive swimming" behaviour of *Xenopus* tadpoles: experiments and model

Experiments

Under our experimental conditions, electrical stimulation of the skin induces "fictive swimming" behaviour characterized by regular bursts of spikes (Fig. 2A). The mean duration of a swimming episode was more or less constant for any given animal. Thus, the SEM for any given individual almost never exceeded 25% and could be less than 10% (mean 13.8 ± 1.5 s for 24 animals and 85 swimming episodes). The mean durations (± SEM) were not significantly different between the three selected stages and were, respectively, 32.2 ± 16.7 s for early stages, 30.2 ± 12.5 s for postembryonic stages and 27.2 ± 11.2 s for the larval stages in curare and 42.7 ± 9.3 s for early stages, 50.7 ± 7.0 s for postembryonic stages and 33.46 ± 3.93 s for the larval stages in α-bungarotoxin.

Model

This activity was modelled using the six-neurons model of Fig. 1C. The alternate activity of the motoneurons as well as the firing frequency could be properly mimicked as shown in Fig. 2B for the terminal part of the axons of motoneurons M1 (left) and M2 (right).

Glutamate receptor channels: evolution of the role of NMDA and non-NMDA receptors in the swimming behaviour during development: experiments and model

Experiments: effects of receptor-channel blockers

The involvement of the two categories of glutamate receptors in the swimming behaviour has been investigated using two selective blockers: APV for the NMDA receptors and CNQX for non-NMDA receptors.

APV

Three concentrations of APV (50, 100 and 150 μM) were tested. Their effects on the duration of the fictive swimming episodes are summarized in Fig. 3A for animals immobilized with curare. These effects were negligible for the early stages whereas the NMDA-channel blocker reduced significantly the duration of the swimming episodes at all concentrations for the postembryonic and

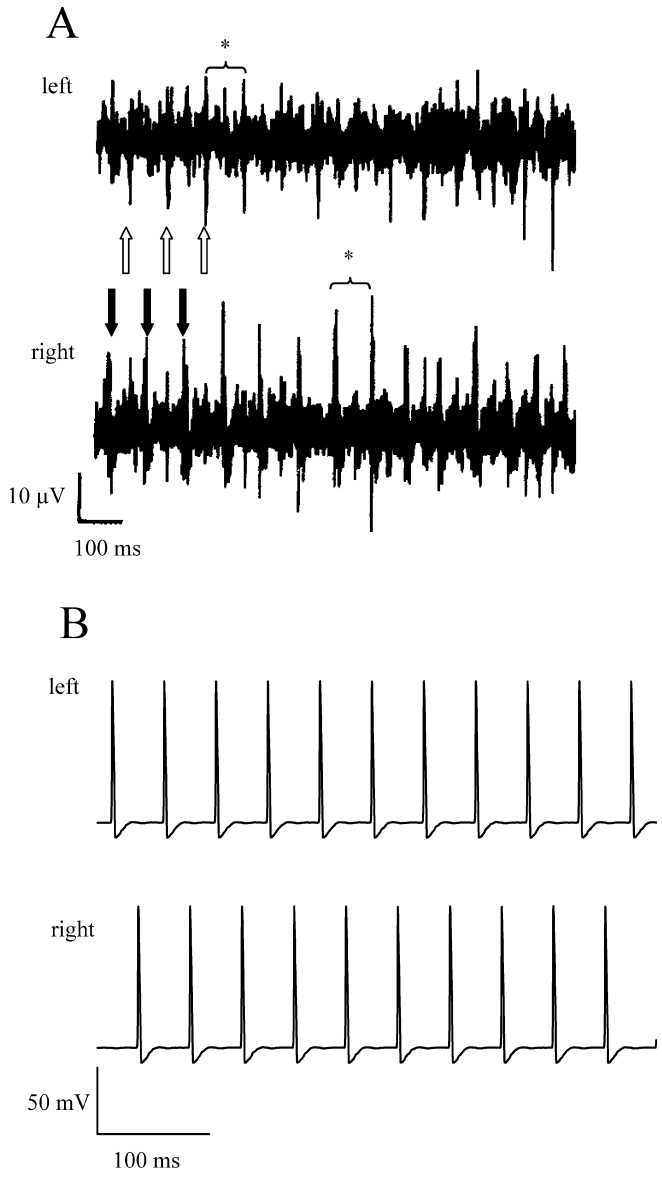

Fig. 2 Examples of extracellular recordings of the fictive swimming activity from the ventral roots of a 36/37 stage *Xenopus* tadpole (**A**) and computer simulations of the electrical activity of the motor axons (**B**). **A** The fictive swimming activity is characterized by the alternate firing of motoneurons from the two sides, which would correspond during normal swimming to alternate contractions of the body wall. They are seen here as short biphasic bursts on top of a background noise (*arrows*). In this and the following figures, typical swimming cycles are indicated by a *bracketed asterisk*. **B** Computer simulation with the six-neurons model of the electrical activity of the distal part of the axons of motoneurons M1 and M2. A regular alternate activity is seen in the two axons. The dendritic length was set to 300 µm and the axonal length to 1000 µm. The dendrites were made of 7 segments, the axons of 33 segments

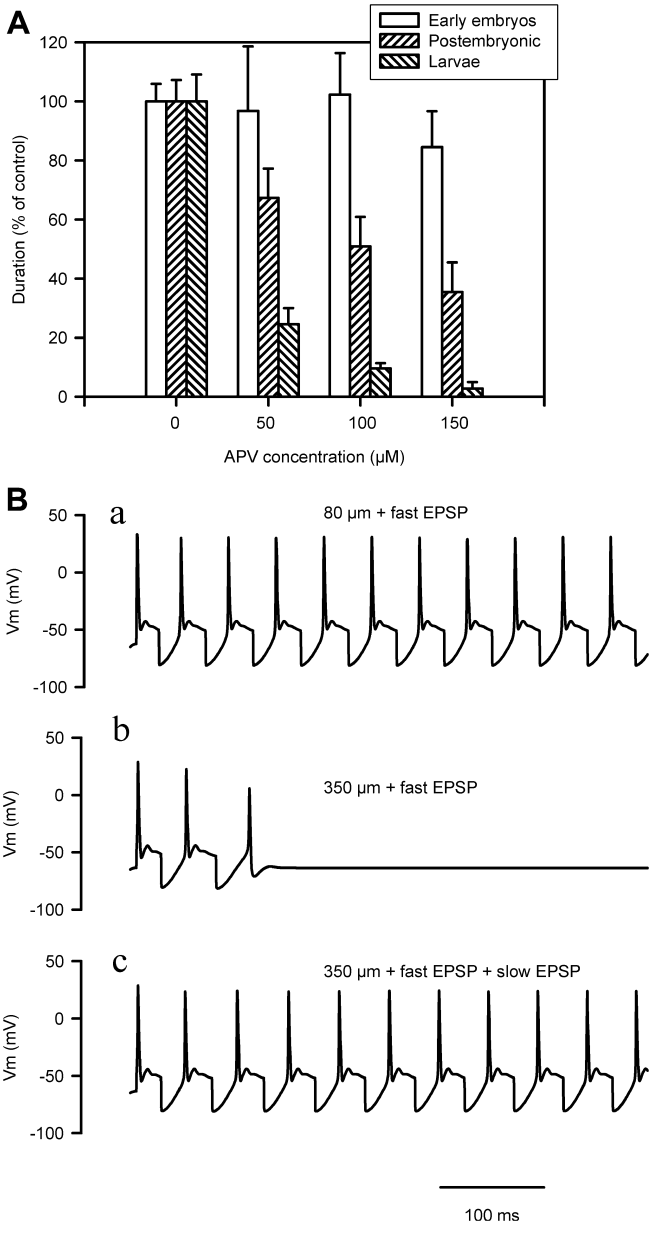

Fig. 3 Effects of growth on fictive swimming. Importance of NMDA receptor-channels: experimental data (**A**) and computer simulations (**B**). **A** Effects of increasing concentrations of APV on the duration of fictive swimming associated with an electrical stimulation of the skin. The NMDA channel blocker reduced significantly the duration for postembryonic tadpoles and larvae but had no significant effect on early embryos. *Bars*: SEM. Animals were immobilized with curare. **B** Computer simulations with the simplified two-neurons model indicate that fast EPSPs alone (which correspond to non-NMDA receptors) are sufficient to account for repetitive electrical activity when the lengths of the dendrite and axon are shorter than 350 µm (**a**). When the axonal and dendritic lengths reach this value, the oscillations stop (**b**). Repetitive firing can be restored by adding a slow EPSP component (which corresponds to NMDA receptors) (**c**). In this and in the following figures, the electrical activity of only one neuron will be represented for the sake of clarity

the larval stages. The percentage of reduction for the three concentrations were respectively 67.3 ± 10, 51 ± 10 and 35.5 ± 10 for postembryonic tadpoles, and 24.6 ± 5.5, 9.6 ± 1.8 and 2.7 ± 2.3 for larvae. Similar results were obtained when the tadpoles were immobilized with α-bungarotoxin.

APV (100 and 150 µM) was also found to reduce significantly the firing frequency at the beginning of the swimming episodes for the postembryonic and larval

stages. For animals immobilized with curare, the frequency dropped from 21.9 ± 3 to 16.8 ± 0.9 and 16.9 ± 0.4 Hz for postembryonic tadpoles and from 20.6 ± 1.2 to 12.6 ± 3.2 and 12.7 ± 4.5 Hz for larvae.

These results confirm that NMDA receptors are needed at postembryonic and larval stages, but strongly suggest that they are not required for proper fictive swimming activity in early embryos.

CNQX

Bath application of 5 μM CNQX inhibited fictive swimming completely in early stage tadpoles. A similar inhibition was found for postembryonic stages embryos immobilized with curare, whereas only 30% of larval stage embryos were affected by the non-NMDA receptor blocker. The inhibitory effects of CNQX were not reversible, thus prohibiting a reliable study of the effects of this blocker on the duration of the swimming episodes and on the firing frequency during fictive swimming.

This result confirms the previous experiments with APV and suggests that non-NMDA receptors play a prominent role in the "fictive swimming" behaviour of early embryos and that these receptors are also important at postembryonic stages and less important at larval stages.

Model: relative importance of fast and slow EPSP in fictive swimming during dendritic and axonal growth

To evaluate the relative importance of the two categories of receptors, we have studied the stability of the rhythmical activity while increasing the length of both dendrites and axons. Two categories of EPSPs were considered: fast EPSP representing the non-NMDA receptors and slow EPSPs representing the (APV-sensitive) NMDA receptors (see Dale and Roberts 1985). Fast EPSPs had an exponential rising phase of 0.1 ms and an exponential falling phase of 15 ms; slow EPSPs had an exponential rising phase of 5 ms and an exponential falling phase of 75 ms. These values correspond to those found in the literature (see Dale and Roberts 1985; Roberts and Tunstall 1990; Gauck and Jaeger 2003). The simple two-neurons (Fig. 1B) model was sufficient for this computation.

For neuritic lengths between 80 μm and 300 μm, fast EPSPs were sufficient to account for a stable repetitive activity (Fig. 3Ba). When the axonal length was increased to 350 μm, the burst was terminated on both sides (Fig. 3Bb) The addition of a very small slow component (synaptic weight of 0.0005 as compared to 0.05 for the fast EPSP) is sufficient to restore the activity (Fig. 3Bc). These results suggest that, for short axons and dendrites, the non-NMDA receptors are sufficient to account for fictive swimming and they fit well with Gleason and Spitzer's (1998) observation that non-NMDA receptors appear earlier than NMDA receptors in these neurons.

Bursting activity and development: experiments and model

Experiments: bursting activity in Xenopus tadpoles

As first mentioned by Sillar et al. (1991), during the first 24 h of post-embryonic development, the ventral root discharge increases from a single compound spike to a discrete burst of activity. This phenomenon is illustrated in Fig. 4A: the mean duration of the ventral root discharge at room temperature changed from about 5 ms for stage 32 to about 20 ms for stage 41. It has been proposed that this change could arise from changes in the properties of the neurons themselves, since the proportion of neurons firing repetitively (under patch-clamp conditions) was higher in post-embryonic than in early tadpoles (Prime 1999). It could also arise from changes in the properties of the network.

Model: effects of changes in the proportion of fast and slow EPSP on fictive swimming during dendritic and axonal growth

Using the same model as above (model 1), we have found that when the dendritic and axonal lengths are sufficiently long (more than about 300 μm), repetitive firing could be obtained by reducing the weight of the fast EPSPs. Thus, as illustrated in Fig. 4B, the single spike activity obtained in "a" with the combination of a strong fast EPSP (weight: 0.05) and a small slow EPSP (weight: 0.0005) can be transformed into a bursting activity by removing the fast EPSP component (Fig. 4Bb). It is tempting to consider that the synaptic weight of the fast (non-NMDA) EPSPs does indeed decrease during development and therefore facilitates the (experimentally observed) bursting activity. Using the same model, we have investigated the possibility that the mixture of fast and slow EPSP could also be used to model the activity at earlier stages. As illustrated in Fig. 4Bc, the same parameters as those used in Fig. 4Ba (fast EPSP with a weight of 0.05 and slow EPSP with a weight of 0.0005) fail to induce repetitive firing, demonstrating the importance of the geometry of the network in its activity.

Glycine receptors and electrical activity of the motoneurons: experiments and model

Experiments: intracellularly recorded activity in motoneurons

So far, we have used extracellular electrodes to monitor the activity of the motoneurons. Intracellular microelectrodes have been used to record the activity of single motoneurons. Most reports on this preparation (see Introduction) indicate that the electrical activity in the motoneurons during fictive swimming is made up of three components: a sustained depolarization due to the

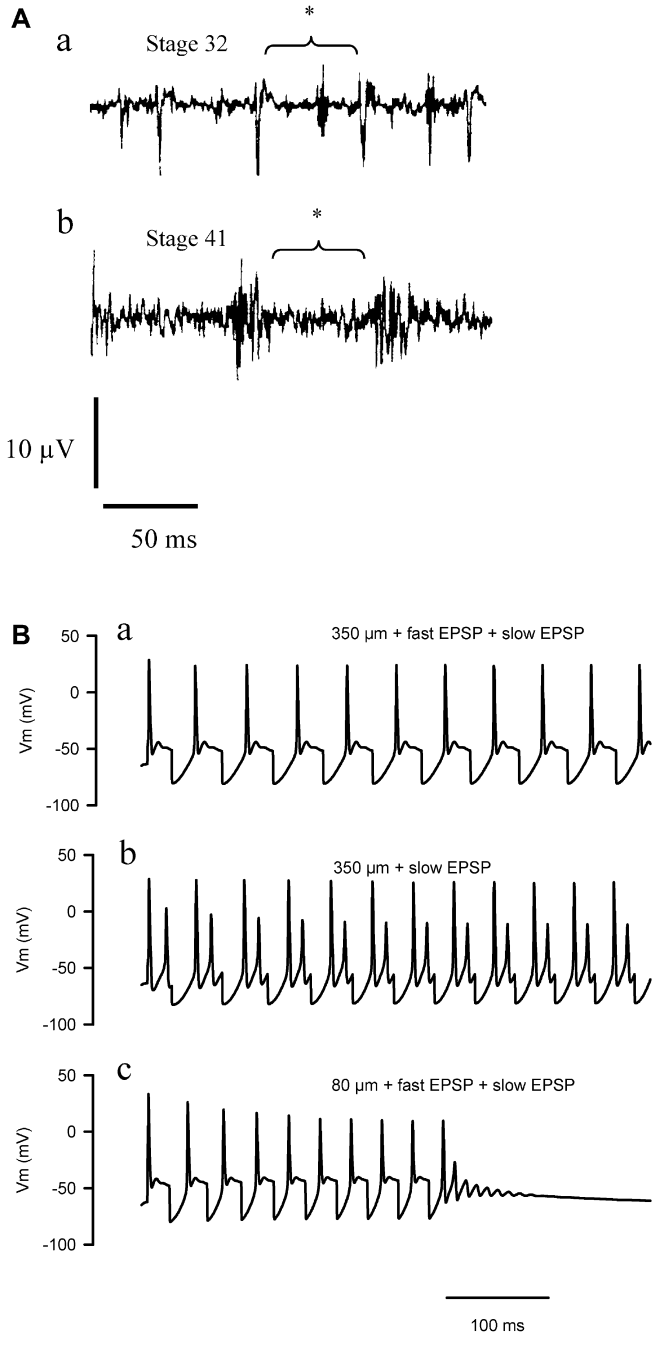

Fig. 4 Effects of growth on fictive swimming: bursting activity experimental data (**A**) and computer simulations (**B**). **A** Examples of extracellularly recorded fictive swimming activity for early embryos (stage 32, **a**) and larval (stage 41, **b**) tadpoles. Whereas the frequency of the periodic electrical discharges remained basically the same (*bracketed asterisks*), single "spikes" seen at stage 32 were replaced at stage 41 by bursts of activity made of multiple individual "spikes". **B** Computer simulations with the simplified two-neurons model. The first tracing (**a**) is the same as tracing **c** of Fig. 2 and represents the activity of the E1 neuron with the combination of fast and slow EPSPs for axonal and dendritic lengths of 350 µm. When the fast (non-NMDA) component is removed (**b**), a second spike is produced. The combination of fast and slow EPSPs, which is effective for axonal and dendritic lengths of 350 µm, fails to induce a long-lasting activity for the shorter length of 80 µm (**c**)

NMDA receptors, a single action potential and a prominent inhibitory synaptic potential (IPSP) associated with the operation of the inhibitory glycine-operated synapse. In our experiments, on several occasions we have recorded an activity which did not fit with that description. Two examples are given Fig. 5A. In Fig. 5Aa, there was a good coincidence between the intracellularly recorded activity (bottom trace) and the activity recorded extracellularly from the homolateral ventral roots (top trace). This activity, which most probably originates from a motoneuron, was maintained during several seconds despite the absence of IPSPs. The two other traces in Fig. 5A (b and c) are from another cell, trace "b" at the beginning of the "fictive swimming", trace "c" after 1 min. The size of the activity was smaller than in "a" (see the scale), most probably because of the damage caused by the microelectrode. Here again, regular depolarizations were recorded and the frequency was maintained throughout the recording. Interestingly, the activity started in the absence of IPSPs (b), then IPSPs started appearing occasionally as downward deflections from time to time and became more and more present. They were present between most depolarizations after 1 min (c). Surprisingly, the firing frequency remained unchanged throughout the entire swimming episode, suggesting that these IPSPs did not play any role in the repetitive activity.

Model: is the electrical activity dependent upon inhibition of the motoneurons?

To investigate the role of inhibition in the stability of the firing behaviour of the motoneurons, we have used the six-neurons model (model 2 of Fig. 1), as in Fig. 2. As illustrated in Fig. 5Ba, regular spike activity can be obtained in the absence of inhibitory inputs to the motoneuron if the inhibitory connections between the interneurons are maintained. As shown in Fig. 5Bb and c, the frequency of the activity is not sensitive to the presence of IPSPs in the motoneuron.

Discussion

The experiments described in the present paper indicate that "fictive swimming" of *Xenopus* tadpoles relies upon the existence of two sets of excitatory inputs, corresponding to two glutamate-activated receptors: fast excitatory EPSPs and slow excitatory EPSPs. The presence of these two sets of postsynaptic potentials has been demonstrated by Dale and Roberts (1985), who showed that motoneurons from stage 37/38 *Xenopus* embryos exhibited postsynaptic potentials which had two components, one mediated by kainate/quisqualate receptors and the other by NMDA receptors. In their experiments, the mean rise time of the fast EPSP was 3 ms and the mean fall time 50 ms, with a half fall time of about 9 ms. The mean rise time of the slow EPSP was 21 ms and the

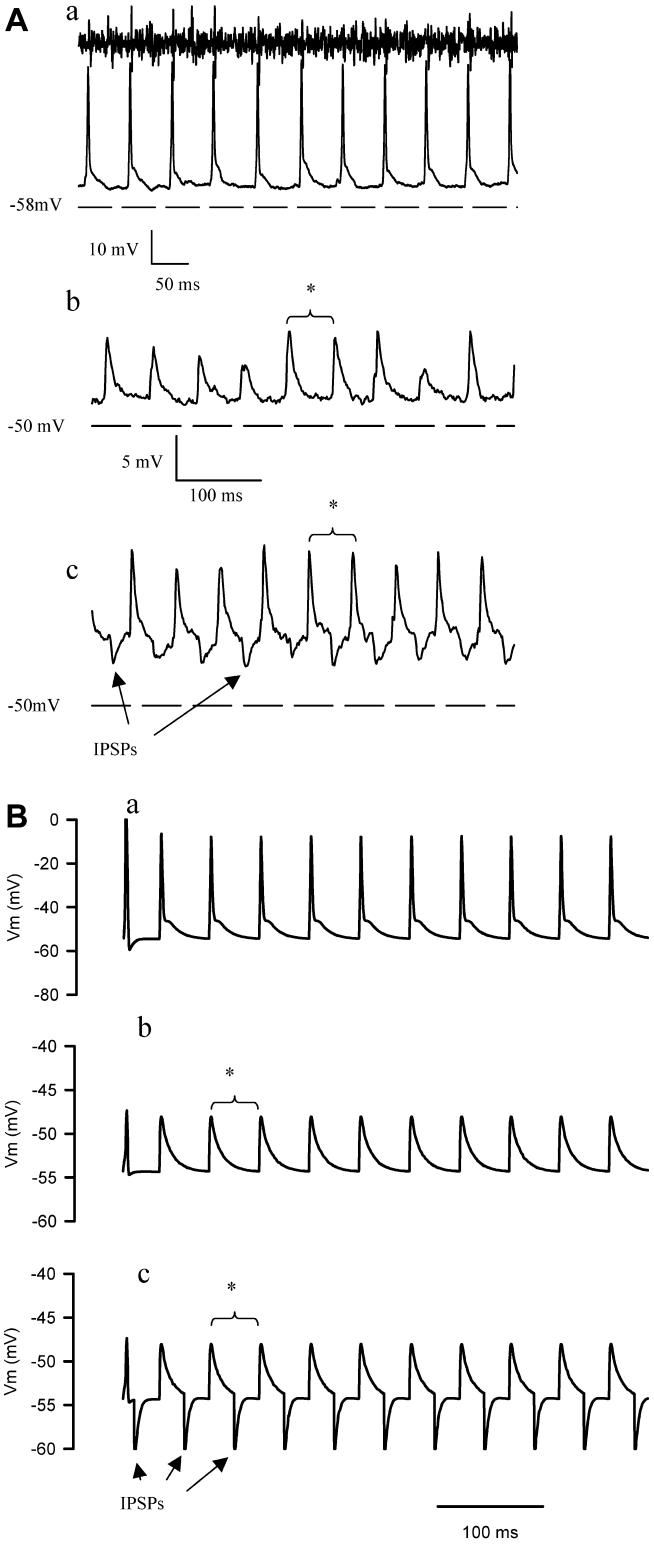

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Fig. 5 Fictive swimming and inhibition: experimental data (**A**) and computer simulations (**B**). **A** Examples of extracellularly and intracellularly recorded activity during fictive swimming. In **a**, the microelectrode was impaled into a motoneuron with a resting potential of −58 mV (*dashed line*). The intracellularly recorded spikes (*lower trace*) were monophasic and exhibited a one-to-one correspondence with the extracellularly recorded activity of the corresponding ventral roots (*upper trace*). No IPSP could be observed. In **b** and **c**, the electrical activity was recorded intracellularly from another (presumed) motoneuron which was slightly depolarized (resting potential of −50 mV, *dashed line*). Recording **b** was taken a few seconds after the beginning of the swimming episode and recording **c** after more than 1 min. The frequency of the transient depolarizations remained the same in the absence (**b**) or the presence (**c**) of transient hyperpolarizations (IPSPs). **B** Computer simulations of the electrical activity of the motoneuron M2 with the simplified six-neurons model. The inhibitory connection between interneuron I1 and M2 is suppressed in **a** and **b** and maintained in **c**. The leak conductance of the motoneuron (gl) is increased to account for the low resting potential values (0.005 in **a**, 0.02 in **b** instead of 0.0001). EPSP rising time constant: 0.5 ms, EPSP falling time constant: 10 ms. In all cases, the circuit is found to oscillate at the right frequency and to mimic the "in situ" recordings

mean falling time 200 ms, with a half fall time of 72 ms. In most cases the EPSPs were of mixed type, with a mean rise time of 4.7 ms, a mean falling time of 200 ms and a half fall time of 65 ms. The slow EPSP was selectively abolished by 50 μM APV. These results are in agreement with Dale and Robert's (1984) suggestion that the background tonic excitation responsible for the swimming behaviour is mediated by a release of an endogenous excitatory amino-acid neurotransmitter acting on NMDA and non-NMDA receptors. Based on pharmacological experiments, these two categories of receptors were also found in cultured embryonic *Xenopus* spinal neurons (Sands and Barish 1989). Indeed, Roberts and Tunstall (1990), who used a simplified three-compartment model of the neurons, and Dale (1995b), who also used a two-neuron model with elaborate voltage-dependent ion conductances, have shown that a robust rhythmic alternating activity could be obtained based on long-duration NMDA-type EPSPs and short-duration IPSPs. Tabak and Moore (1998) reached a similar conclusion using a refined version of this model and showed that the voltage dependency of the NMDA-receptor channels increases the efficiency of the post-inhibitory rebound, an observation also reported by Roberts et al. (1995) who used a realistic 24-neuron model. Tabak and Moore (1998) also found that the voltage dependency of the NMDA receptor channels allowed neurons to fire more than one action potential per cycle. Our own model also supports the basic features of Roberts and Tunstall's model and indicates that the combination of the excitatory and inhibitory inputs originating from a set of two excitatory interneurons and two inhibitory interneurons is adequate to trigger the basic firing properties of the motoneurons, as illustrated in Fig. 2B.

Our observation that APV has no effect on the duration of the swimming episodes for early embryos is of importance since it strongly suggests that NMDA receptors are not involved at that stage. As mentioned in the Introduction, this lack of effect of APV is not surprising, since Gleason and Spitzer (1998) have shown that functional AMPA receptors appear soon after neurite initiation and before NMDA neurons in differ-

entiating spinal neurons. Our modelling experiments suggest that this is possible if the lengths of the axon and dendrite are shorter than 350 μm (Fig. 3Ba). Under these conditions, the depolarization induced by the fast EPSP at the site of initiation of the action potential is large enough to trigger a spike. For longer lengths, the attenuation of the EPSP increases and the threshold cannot be reached for axon and dendrite lengths exceeding 350 μm (Fig. 3Bb). Under these conditions, the addition of a further depolarization corresponding to the slow (NMDA) EPSP is needed (Fig. 3Bc).

The fact that multiple action potentials are present for each cycle in postembryonic tadpoles and larvae can be explained in simple terms. The slow (NMDA) EPSPs add together to give a plateau depolarization, as shown experimentally by Dale and Roberts (1985). If the interval between the first spike and the IPSP is sufficiently long, a second spike is produced if the leak resistance of the motoneuron is sufficiently high and the balance between the sodium and the potassium conductances appropriate, as shown Fig. 4Bb. This does not happen if the sodium conductance is inactivated by the addition of the fast EPSP, as illustrated in Figs. 3Bc and 4Ba. This suggests that the bursting activity reflects the increase of the NMDA component of excitation and the decrease of the fast non-NMDA component and fits in well with our observation that CNQX, the non-NMDA receptor blocker, has comparatively little effect on larval stage tadpoles. As mentioned earlier, it seems likely that a change in biophysical properties of the motoneurons themselves, namely their ability to respond to DC current injections by several action potentials, can also account for the bursting activity: older neurons are more likely than young neurons to respond by several action potentials (Prime 1999). However, patch-clamp experiments have revealed that the motoneurons from early embryos can also fire multiple action potentials, a feature which was not observed with intracellular microelectrodes. It is reasonable to assume, in that respect, that microelectrodes inevitably damage these (small) neurons and therefore induce a significant shunting effect (see Dale 1995a, 1995b) and reduce their excitability.

Our observation that repetitive activity of the motoneurons can occur in the absence of IPSPs is more surprising since the post-inhibitory rebound has been consistently found to be a basic and necessary part of the locomotor rhythm production. In their 1986 review article, Roberts et al. (1986) reported that compound IPSPs of 5–20 mV in amplitude starting at mid-cycle were seen in all intracellular motoneuron recordings (Roberts and Kahn 1982; Soffe and Roberts 1982a; Soffe et al. 1984; Dale 1985; Dale and Roberts 1985). Our experiments show (1) that this is not always true and (2) that normal alternate ''fictive swimming'' activity can be obtained in the absence of direct inhibition of the motoneurons. A simple interpretation of this discrepancy between our experiments and the more ''classical'' observations of Roberts and co-workers could be that, under our experimental conditions, the motoneurons are damaged, and therefore do not exhibit their normal behaviour. This interpretation is hardly tenable since this absence of IPSPs was not always linked to reduced resting potentials, which would indicate damaged cells (the resting potential of cell 1 in Fig. 5Aa is −58 mV). Furthermore, as shown in Fig. 5Ab and c, the same cell can exhibit activity without and with IPSPs with no change in the frequency of that activity. One possibility remains that, at least for cell 1, the neuron is not a motoneuron but an inhibitory interneuron. In that case, however, one has to admit that this interneuron is located on the other side of the spinal cord since its action potentials are synchronous with those recorded extracellularly (upper trace in Fig. 5Aa), which is very unlikely. Whichever interpretation is correct, the model indicates that there is no need for direct inhibition of the motoneurons for normal rhythmical activity to occur, provided the alternate activity is maintained in the interneurons which send excitatory inputs to the motoneurons (Fig. 5Ba and b).

In conclusion, our experiments illustrate how a simple neuronal circuit which underlies simple locomotor activity is driven by a set of ion channels: voltage-dependent ion channels (Na^+, K^+, Ca^{2+}) which control excitability and conduction and ligand-gated ion channels, NMDA and non-NMDA receptors and glycine receptors, which are directly involved in the production of the rhythmical activity. Some of these parameters change during the embryonic development of the animal but remain sufficiently stable to ensure a proper functioning of the system during the entire embryonic period. Interestingly, when the swimming behaviour changes with the animal becoming a larva, the pattern of activity changes together with some properties of the neurons, such as the nature of the potassium conductance which, from being purely voltage dependent, becomes more calcium dependent (blocked by iberiotoxin) from stage 41 onwards (Sun and Dale 1998). At the same time, the spinal neurons develop TTX-resistant oscillatory properties (see Prime et al. 1999; Pichon et al. 2003) which become prominent at larval stages and would control fast swimming in these larvae (Reith and Sillar 1999).

References

Buchanan JT, Grillner S (1987) Newly identified 'glutamate interneurons' and their role in locomotion in the lamprey spinal cord. Science 236:312–314

Clarke JDW, Roberts A (1984) Interneurons in the *Xenopus* embryo spinla cord: sensory excitation and activity during swimming. J Physiol (London) 354:345–363

Dale N (1985) Reciprocal inhibitory interneurons in the *Xenopus* embryo spinal cord. J Physiol (London) 363:61–70

Dale N (1995a) Kinetic characterization of the voltage-gated currents possessed by *Xenopus* embryo spinal neurons. J Physiol (London) 489:473–488

Dale N (1995b) Experimentally derived model for the locomotor pattern generator in the *Xenopus* embryo. J Physiol (London) 489:489–510

Dale N, Roberts A (1984) Excitatory amino acid receptors in *Xenopus* embryo spinal cord and their role in activation of swimming. J Physiol (London) 348:527–543

Dale N, Roberts A (1985) Dual-component amino-acid-mediated synaptic potentials excitatory drive for swimming in *Xenopus* embryos. J Physiol (London) 363:35–59

Fisher H, Merrywest SD, Sillar KT (2001) Adrenoreceptor-mediated modulation of the spinal locomotor pattern during swimming in *Xenopus laevis* tadpoles. Eur J Neurosci 13:977–986

Gauck V, Jaeger D (2003) The contribution of NMDA and AMPA conductances to the control of spiking in neurons of the deep cerebellar nuclei. J Neurosci 23:8109–8118

Gleason EL, Spitzer NC (1998) AMPA and NMDA receptors expressed by differentiating spinal neurons. J Neurophysiol 79:2986–2998

Grillner S (1985) Neurobiological bases of rhythmic motor acts in vertebrates. Science 228:143–149

Grillner S (1997) Ion channels and locomotion. Science 278:1087–1088

Grillner S (2003) The motor infrastructure: from ion channels to neuronal networks. Nat Rev Neurosci 4:573–586

Hines ML, Carnevale NT (2000) Expanding NEURON's repertoire of mechanisms with NMODL. Neural Comput 12:839–851

Hines ML, Carnevale NT (2001) Neuron: a tool for neuroscientists. Neuroscientist 7:123–135

Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (London) 117:125–134

Kahn JA, Roberts A (1982) The central nervous origin of the swimming motor pattern of embryos of *Xenopus laevis*. J Exp Biol 99:185–196

McDearmid JR, Scrymgeour-Wedderburn JF, Sillar KT (1997) Aminergic modulation of glycine release in a spinal network controlling swimming in *Xenopus laevis*. J Physiol (London) 503:111–117

Nieuwkoop PD, Faber J (1956) Normal tables of *Xenopus laevis* (Daudin). Elsevier, Amsterdam

Perrin R, Roberts A (1995) Cholinergic and electrical synapses between synergistic spinal motoneurons in the *Xenopus laevis* embryo. J Physiol (London) 485:135–144

Pichon Y, Prime L, Benquet P, Tiaho F (2003) Some aspects of the physiological role of ion channels in the nervous system. Eur Biophys J (in press, this issue)

Prime L (1999) "Rôle des récepteurs NMDA dans le réseau neuronal de la locomotion de l'embryon de *Xénope*". Thèse, Université Rennes 1, France, pp 1–141

Prime L, Pichon Y, Moore LE (1999) *N*-Methyl-D-aspartate-induced oscillations in whole-cell clamped neurons from the isolated spinal cord of *Xenopus laevis* embryos. J Neurophysiol 2:1069–1073

Rall W, Agmon-Snir H (1989) Cable theory for dendritic neurons. In: Koch C, Segev I (eds) Methods in neuronal modelling: from ions to networks. MIT Press, Cambridge, Mass., pp 9–62

Reith CA, Sillar KT (1999) Development and role of GABA(A) receptor-mediated synaptic potentials during swimming in postembryonic *Xenopus laevis* tadpoles. J Neurophyiol 82:3175–3187

Roberts (1990) How does a nervous system produce behaviour? A case study in neurobiology. Sci Prog (Oxford) 74:31–51

Roberts A, Kahn JA (1982) Intracellular recordings from spinal neurons during "swimming" in paralysed amphibian embryos. Phil Trans R Soc London Ser B 296:213–228

Roberts A, Tunstall MJ (1990) Mutual re-excitation with post-inhibitory rebound: a simulation study in the mechanism for locomotor rhythm generation in the spinal cord of *Xenopus* embryos. Eur J Neurosci 2:11–23

Roberts A, Soffe SR, Dale N (1986) Spinal interneurons and swimming in frog embryos. In: Grillner S, Stein PSG, Stuart D, Forstberg H, Herman RM (eds) Neurobiology of vertebrate locomotion. Macmillan, London, pp 279–306

Roberts A, Tunstall MJ, Wolf E (1995) Properties of networks controlling locomotion and significance of voltage-dependency of NMDA channels: simulation study of rhythm generation sustained by positive feedback. J Neurophysiol 73:485–495

Roth A, Häusser M (2001) Compartment models of rat cerebellar Purkinje cells based on simultaneous somatic and dendritic patch-clamp recordings. J Physiol (London) 535:445–472

Sands SB, Barish ME (1989) A quantitative description of excitatory amino acid neurotransmitter responses on cultured embryonic *Xenopus* spinal neurons. Brain Res 502:375–386

Sillar KT, Wedderburn JFS, Simmers AJ (1991) The development of swimming rhythmicity in post-embryonic *Xenopus laevis*. Proc R Soc London Ser B 246:147–153

Sillar KT, Wedderburn JFS, Simmers AJ (1992) Modulation of swimming rhythmicity by 5-hydroxytryptamine during post-embryonic development in *Xenopus laevis*. Proc R Soc London Ser B 250:107–114

Soffe SR (1987) Ionic and pharmacological properties of reciprocal inhibition in *Xenopus* embryo motoneurons. J Physiol (London) 382:463–473

Soffe SR, Roberts A (1982a) Activity of myotomal motoneuron during fictive swimming in frog embryos. J Neurophysiol 48:1274–1278

Soffe SR, Roberts A (1982b) Tonic and phasic synaptic input of spinal cord motoneurons during fictive locomotion in frog embryos. J Neurophysiol 48:1279–1288

Soffe SR, Clarke JDW, Roberts A (1984) Activity of commissural interneurons in the spinal cord of *Xenopus* embryos. J. Neurophysiol 51:1257–1267

Sun Q, Dale N (1998) Developmental changes in expression of ion currents accompany maturation of locomotor pattern in frog tadpoles. J Physiol (London) 507:257–264

Tabak J, Moore LE (1998) Simulation and parameter estimation study of a simple neuronal model of rhythm generation: role of NMDA and non-NMDA receptors. J Comput Neurosci 5:209–235

van Mier P, van Rheden R, ten Donkelaar HJ (1985) The development of the dendritic organisation of primary and secondary motoneurons in the spinal cord of *Xenopus laevis*. An HRP study. Anat Embryol 172:311–324

Wallen P, Grillner S (1987) *N*-Methyl-D-aspartate receptor-induced inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. J Neurosci 7:2745–2755