Roles of Ionic Currents in Lamprey CPG Neurons: A Modeling Study

Mikael Huss,1,2 Anders Lansner,1 Peter Wallén,2 Abdeljabbar El Manira,2 Sten Grillner,2 and Jeanette H. Kotaleski1,2

1School of Computer Science and Communication, Royal Institute of Technology, Stockholm, Sweden; and 2Department of Neuroscience, Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm, Sweden

Submitted 17 May 2006; accepted in final form 2 February 2007

Huss M, Lansner A, Wallén P, El Manira A, Grillner S, Kotaleski JH. Roles of ionic currents in lamprey CPG neurons: a modeling study. J Neurophysiol 97: 2696–2711, 2007. First published February 7, 2007; doi:10.1152/jn.00528.2006. The spinal network underlying locomotion in the lamprey consists of a core network of glutamatergic and glycineric interneurons, previously studied experimentally and through mathematical modeling. We present a new and more detailed computational model of lamprey locomotor network neurons, based primarily on detailed electrophysiological measurements and incorporating new experimental findings. The model uses a Hodgkin–Huxley-like formalism and consists of 86 membrane compartments containing 12 types of ion currents. One of the goals was to introduce a fast, transient potassium current (Kt) and two sodium-dependent potassium currents, one faster (KNaF) and one slower (KNaS), in the model. Not only has the model lent support to the interpretation of experimental results but it has also provided predictions for further experimental analysis of single-network neurons. For example, Kt was shown to be one critical factor for controlling action potential duration. In addition, the model has proved helpful in investigating the possible influence of the slow afterhyperpolarization on repetitive firing during ongoing activation. In particular, the balance between the simulated slow sodium-dependent and calcium-dependent potassium currents has been explored, as well as the possible involvement of dendritic conductances.

INTRODUCTION

The lamprey CNS has widely served as a model system of the neural basis of vertebrate locomotion. There are fewer neurons in the lamprey than in higher vertebrates and the motor activity underlying locomotion can be maintained in the isolated spinal cord for days (Grillner et al. 2000, 2003). The core of the lamprey spinal central pattern generator (CPG) consists of ipsilaterally projecting glutamatergic neurons and contralaterally projecting glycineric neurons (Buchanan et al. 1982, 1987; Grillner 2003). One tool for investigating the lamprey CPG, in addition to the experimental approach, has been extensive modeling at different levels of abstraction (Grillner et al. 2001). Both biophysical single-cell and network models of the lamprey spinal CPG were previously described (see, e.g., Ekeberg et al. 1991; Grillner et al. 1988; Hellgren et al. 1992; for more abstract types of models also see Buchanan 1992; McClellan and Haguevick 1997; Williams 1992). Different aspects of the local spinal network were previously simulated using Hodgkin–Huxley types of models, including its modulation by sensory feedback, activation by supraspinal structures, and coordination along the spinal cord (Brodin et al. 1991; Ekeberg et al. 1991; Hellgren et al. 1999a,b; Kozlov et al. 2001; Tegné et al. 1998, 1999; Trävén et al. 1993; Ullström et al. 1998; Wadden et al. 1997; Wallén et al. 1992). Understanding such systems at the neuronal network level is very demanding because of the complexity of the dynamic interactions within and between neurons. Therefore to examine the possible architecture of cellular and synaptic properties that lead to an experimentally shown phenomenon, computer simulations are powerful tools of analysis. Detailed models of various cell types are necessary building blocks for such network simulations. These detailed models need to have a good grounding in electrophysiology, especially ion channel kinetics. Over the last years, new experimental data have become available that require an updated mathematical description for adequate simulation studies (see, e.g., Cangiano et al. 2002; Hess and El Manira 2001, 2002; Wallén et al. 2003, 2005; Hess et al. 2007). Improved models should also make it possible to address some previously unanswerable questions and to put forth hypotheses about the inner workings of the single lamprey spinal neuron. This article presents a computational model of a lamprey spinal cord neuron based on new experimental data.

The closest analogue to our work is a computational model of the CPG neuron in the Xenopus frog embryo (Dale 1995a). Like the lamprey, Xenopus is an important model system for studying vertebrate motor pattern generation. The swimming CPGs in the two systems share a number of similarities. In both Xenopus and lamprey, the segmental CPG is thought to consist of recurrent excitatory networks coupled by reciprocal inhibition and the voltage-dependent properties of N-methyl-D-aspartate (NMDA) receptors have a key role in pattern generation (Grillner 2003; Roberts and Tunstall 1990; Roberts et al. 1995). In the Xenopus embryo, electrophysiological properties and ionic currents are well characterized (see, e.g., Dale 1995b; Kuenzi and Dale 1998). Other computational models of neurons participating in pattern-generating networks have furthermore been created for many invertebrate systems, for instance, in the stomatogastric ganglion of the lobster (Buchholz et al. 1992) and the cardiac ganglion of the leech (Nadim et al. 1995; Olsen et al. 1995).

As in the nervous systems of most animal phyla, the lamprey spinal cord contains, among others, three basic types of neurons: sensory neurons, motor neurons, and interneurons. The focus of this study was to develop a generic model of lamprey spinal CPG neurons. This model (which is summarized in Table A1 and equations in the Appendix) can be implemented
on different morphologies, depending on the type of spinal neuron one seeks to simulate. No systematic study comparing the kinetics and densities of specific ionic currents in different CPG neuron classes has been carried out, but different lamprey spinal CPG neurons show quite similar behavior in some respects (Biró et al. 2006; Buchanan 1993, 2001; Hu et al. 2002; Wallén and Grillner 1987). For example, a study characterizing various properties of identifiable lamprey spinal neurons (Buchanan 1993) found that properties that depend largely on active ionic conductances, such as action potential amplitude and width as well as the size of the slow afterhyperpolarization (sAHP), were similar between different classes of neurons, suggesting similar mechanisms for generation of the spike and sAHP. Our generic cell model will therefore be used as a basis when simulating the various cell types involved in the lamprey CPG circuits.

However, there are still many possible sources of variability both within and between classes of spinal neurons. For instance, variability in soma size and number of dendritic branches lead to a variability in a number of measured cell properties such as rheobase and input resistance (Buchanan 1993). Also, spiking frequencies and postsynaptic potential amplitudes were found to show a marked variability in motoneurons and various classes of interneurons (Parker and Bevan 2007). In view of this variability, it would be difficult or impossible to construct a model that could reproduce all published experimental results with a fixed parameter set, even for a single class of CPG neuron. However, the present model can reproduce most important cell behaviors qualitatively and sometimes quantitatively.

The role of certain ionic currents cannot be addressed experimentally in some cases, although simulations can be used to predict their roles and suggest new experiments. Results of this kind are described and discussed herein. Work on incorporating this new cell model into a hemisegmental CPG network model is in progress and will be reported elsewhere.

**METHODS**

**Model specification**

The cell model is a compartmental model implemented using the GENESIS neural simulator (Bower and Beeman 1998). Passive properties and ion channel kinetics and distributions are described in the following sections.

**Cell morphology**

Spinal neurons in the lamprey CPG are found in a large variety of shapes and sizes. For example, interneurons typically have a much smaller cell body and dendritic tree than those of motoneurons (compare, e.g., Buchanan et al. 1989, 2001; Wallén et al. 1985). Our ionic current models (see following text) can be implemented on different cell morphologies. For illustration, we use a morphology herein that gives the model neuron an input resistance and rheobase close to mean values reported for two different types of interneuron in the lamprey spinal cord (Buchanan 1993). We used the five filled interneurons from Buchanan et al. (1989) as guidance, but the model we will use to illustrate the results is not strictly based on any of these, being instead an idealized version that has typical values for soma dimensions, rheobase, and input resistance. For purposes of comparison, we constructed three other model neurons (see Effects of changes in the morphology), each one based closely on one of the filled cells with dendritic compartments representing each branch shown in the reconstructions in the original paper (Buchanan et al. 1989). The “default” model has 86 compartments, 84 of them dendritic (Fig. 1). All compartments are modeled as cylinders. The soma compartment has a length of 20 μm and a diameter of 20 μm, which gives a membrane area within the range observed for interneuron somata (Buchanan 1982; Buchanan et al. 1989). A separate compartment represents the axon initial segment; it is 50 μm long with a diameter of 5 μm. This representation of the axon is inspired by the work described in Colbert and Pan (2002) and it allowed reasonable densities of ionic conductances (see APPENDIX). There are two primary dendrites, each branching out to two secondary dendrites, which in turn branch out into two tertiary dendrites each. The three levels of dendrites correspond to proximal, medial, and distal dendrites, respectively. Each of these is in turn divided into two, four, and eight compartments, respectively [resulting in (2 × 2) + (4 × 4) + (8 × 8) = 84 dendritic compartments]. The size of the dendritic tree can be varied to mimic different types of spinal neurons. In the parameter set presented here, primary dendrites are 90 μm long with 5-μm diameter; the secondary dendrites are 150 μm long with 3-μm diameter; and the tertiary dendrites are 240 μm long with 2-μm diameter. The ratio of the dendritic surface area to the somatic is thus about 16, which is slightly less than that estimated for mammalian motoneurons (Lüscher and Clamann 1995).

We also simulated dissociated cells because some experimental data were available for such cells only, which largely lack dendrites. The dissociated cells used in the experiments were motoneurons, contralaterally and caudally projecting interneurons (CCINs), or unidentified neurons from larval or young adult lampreys. A dissociated cell is modeled in the same way as the default model interneuron, but with all dendritic branches removed; only the soma and the axon initial segment are retained.

**Passive membrane parameters**

All passive parameters are identical for each compartment. The specific membrane resistance is 1 Ωm², the axial resistance is 1 Ωm, and the specific capacitance is 0.01 F/m². The resting membrane potential was usually set to −78 mV, the mean value reported for CC, EIN, and LIN neurons according to Buchanan (1993), except when examining the effects of varying the membrane potential or simulating experiments, in which case the model’s resting potential was set to the same value as in the experiment.
Ion current kinetics

Ion current kinetics—at least for those ion currents involved in action potential generation and termination—do not seem to vary significantly across different types of lamprey locomotor neurons (Buchanan 1993, 2001). For instance, the action potentials of different types of cells have remarkably similar amplitudes and durations. The voltage threshold for action potential generation also does not vary appreciably between cell types (Buchanan 1993, 2001). In building the model, data from different preparations were used. Thus we used information obtained from experiments on motoneurons, inhibitory interneurons, excitatory interneurons, and unidentified neurons. Sometimes these were dissociated cells; for example, kinetic data on the $K_+$, $K_{Na}$, $Ca_{VA}$, and $Ca_{LVA}$ (see Table A1 for definitions of notation) currents were measured in dissociated cells from larval and young adult lampreys.

Lamprey spinal cord neurons were recently shown to contain a larger number of distinct ion channels types than previously known (see, e.g., Hess and El Manira 2001, 2002; Wallén et al. 2005; Hess et al. 2007). Importantly, three novel currents were experimentally characterized: one transient, high-voltage–activated, inactivating $A$-type potassium current (hereafter referred to as $K_+$) and two sodium-dependent potassium currents ($K_{Na}$), which will be denoted as $K_{NaF}$ (F for fast) and $K_{NaS}$ (S for slow). Furthermore, more quantitative data were obtained on the other types of currents, that is $Na^+$, $K_+$, and subtypes of $Ca^{2+}$ currents.

Kinetic parameters for the ion channel models, along with the equations used, can be found in Table A1 in the APPENDIX and are also further described in RESULTS.

Ion conductance distribution

The soma densities of the various conductances are as follows (all in $S/m^2$): $Na^+ \, 320$, $K_+ \, 40$, $K_{Na} \, 50$, $K_{NaF} \, 50$, $K_{NaS} \, 5.2$, $Ca_{N} \, 80$, $Ca_{LVA} \, 0$, $Ca_{VA} \, 4$, $K_{CaN} \, 60$.

The low-voltage–activated $Ca^{2+}$ ($Ca_{LVA}$) conductance was set to zero in the soma because experimental evidence suggests that the $Ca_{LVA}$ current is usually not expressed in dissociated cells, where most of the dendritic tree has been removed. El Manira and Bussières (1997) found that, in dissociated larval neurons, no motoneurons and only 19% of unidentified spinal neurons expressed this type of current. For adult cells, $Ca_{LVA}$ currents were consistently found in intact preparations of both motoneurons and unidentified spinal neurons (Matsushima et al. 1993). This could mean that the $Ca_{LVA}$ current is usually present in the dendritic tree but not in the soma, as we have assumed here; however, an alternative explanation is that $Ca_{LVA}$ could be differently expressed in larval and adult neurons.

In the initial segment, the densities are (in $S/m^2$): $Na^+ \, 20,000$, $K_+ \, 6,000$. The reasons for the differences between these values and the somatic $Na^+$ and $K_+$ conductance densities are given in the APPENDIX and in the DISCUSSION. For dissociated cells, the conductance densities in the soma and axon were the same as those for the intact neuron model.

The densities of the various ion conductances in the dendrites are (in $S/m^2$): $Na^+ \, 320$, $K_+ \, 40$, $K_{Na} \, 20$, $K_{NaF} \, 150$, $K_{NaS} \, 52$, $Ca_{N} \, 80$, $Ca_{LVA} \, 575$, $Ca_{VA} \, 2$, $K_{CaN} \, 600$.

NMDA, $Ca_{NMDA}$, and $Ca_{CaNMDA}$ conductances are included, in the soma and dendrites, if bath NMDA activation is simulated. For the densities, see NMDA-induced oscillations in RESULTS. For simplicity, all dendritic compartments have the same density of each ion channel. In cases where the dendritic density of an ion conductance differs from the somatic one, a modification was made to better fit the model to an experimental observation. Rationales for these modifications can be found in the APPENDIX.

RESULTS

In this section, we compare the behavior of the model neuron to that seen in real neurons under various experimental conditions and discuss different phenomena that the modeling process has revealed.

The single action potential in intact and dissociated cells

The model can address possible consequences of using dissociated cells as an experimental preparation and how results from those can be extrapolated to intact cells. Figure 2A shows two sample somatic action potentials generated by the model: one intact cell with the default parameters and one with parameters corresponding to a dissociated cell. The action potentials were induced by simulating current injection for 2 ms (the period of stimulation is indicated in the figure). The action potential is initiated earlier in the “dissociated” model cell than in the “intact” model cell because the dissociated model cell has a higher input resistance resulting from its lack

![FIG. 2. Action potentials (APs) and ion currents in the soma of model cells. A: voltage traces of APs generated by the “intact” model cell, including dendrites (thicker line) and by the model dissociated cell, which has no dendrites (thinner line). Period of stimulation is indicated by the bar marked “stim.” Note that the AP starts earlier in the dissociated cell because of the difference in input resistance. Also, in agreement with experimental results, the amplitude of the AP in the dissociated cell is larger than that in the intact cell. B: time courses and magnitudes of different ion currents in the soma of the model dissociated cell in response to a simulated AP-like waveform (shown with a bold line topmost in the picture) used in an experimental study to measure different currents (Hess et al. 2007). This waveform was used to tune current parameters in the model. C: resulting time courses and magnitudes of different ion currents in the soma of model cells after a spontaneous AP in an intact cell with dendrites (thicker line) and in a dissociated cell (thinner line).](http://jn.physiology.org/10.1152/jn.00150.2007)
of a dendritic tree. The action potential of the intact model cell, apart from being initiated later, also has a smaller amplitude. This is in accordance with experiments: in intact cells, the action potential tends to reach +20 mV or so, whereas dissociated cell action potentials can reach +60 mV (Hess and El Manira 2001). Interestingly, experiments also show that the action potential width varies strongly between dissociated and intact cells—the half-amplitude width was around 0.5 ms in dissociated cells and around 1 ms in intact cells in one study (Hess and El Manira 2001). At present, the reason for this discrepancy is unclear. The action potentials generated by our model’s default settings agree with the former value, both in simulated intact cells and simulated dissociated cells. Our model cannot account for the discrepancy with the present settings: the action potential can be made broader by changing, e.g., the Na\(^{+}\) or K\(_{s}\) dynamics (for example, by changing the activation time constant of the K\(_{s}\) current in the model, we can make the model generate action potentials with a duration around 1 ms), but then the model does not give the right behavior for dissociated cells. It is possible that the measured differences in action potential duration can (at least partly) be attributed to the different measurement techniques (patch-clamp in dissociated cells and sharp electrodes in intact cells).

An alternative explanation might be that in intact cells, the passive load imposed on the soma by the dendritic tree tends to widen the action potential. This effect can also be observed to a certain degree in our simulations when the dendritic branches are made thicker, longer, or more numerous (not shown). Finally, it is conceivable that the K\(_{s}\) kinetics, measured in neurons from larval and young adult lampreys, might be different from that found in neurons of adult lampreys. Figure 2B shows the activation of various currents in the soma during a simulated action-potential–like waveform in a dissociated cell. The simulated voltage waveform is displayed with a bold line. This voltage signal was previously used as a voltage clamp in dissociated cells and sharp electrodes in intact cells.

The slow afterhyperpolarization (sAHP) is very important for determining a neuron’s repetitive firing characteristics. Example sAHPs are magnified in insets in Fig. 3A. The main factor—at least during low-frequency stimulation of the cell—determining the amplitude and shape of the sAHP is a calcium-dependent potassium current that is activated by calcium entering through high-voltage–activated calcium channels, mainly N-type calcium channels (Ca\(_{n}\)) (Wikström and El Manira 1998), but there is also an sAHP component that is not abolished by cadmium application and is thus not sensitive to calcium (Cangiano et al. 2002). Previous experiments showed that this potassium current is instead dependent on sodium ion influx to the cell (Wallén et al. 2003, 2005). This slow sodium-dependent potassium current accounts for roughly 20–50% of the sAHP size, depending on the activity of the cell. It is not known whether this channel is activated solely by the sodium ion concentration, or by both sodium ion concentration and voltage. In the model, we couple it to a “sodium pool” with slow dynamics (decay time constant = 50 ms). This simplified...
The sAHP is much smaller in dissociated cells than in intact cells, or even absent. Because the sAHP is the main determinant of the firing frequency—and it is mediated by $K_{CaN}$ and $K_{NaS}$ channels—it is reasonable to believe that these channels could be present in the dendritic tree. To obtain a model that had typical-looking sAHPs in intact model cells and small or no sAHPs in dissociated model cells, these currents were included in the dendritic tree at tenfold the somatic density. The very high firing frequencies observed in one study on dissociated larval cells (Hess and El Manira 2001) also required a high density of $Na^+$ and $K_+$ current in the initial segment (see APPENDIX for further details).

Figure 3A shows the responses simulated for a model intact spinal neuron (left) and a real unidentified spinal adult neuron (right) for a current injection level slightly above the current threshold for repetitive firing. The sAHP region is shown magnified in an inset for both cases. Figure 3B shows the current–frequency relationship of the model neuron, both for the first interspike interval and for the steady-state interspike interval (last interval). Experimental values, estimated from Fig. 9A in Buchanan (1993), are shown in the same plot. We used data for interneurons because the instantiation of our model used here has passive characteristics resembling those of interneurons. Current–frequency curves for motoneurons can also be well reproduced by the model, given that the soma size and extent of the dendritic tree are increased in the model to match the passive characteristics of real motoneurons (not shown). The injection current is normalized to rheobase for ease of comparison. In the physiologically relevant range shown, it is apparent that the correspondence of the steady-state firing rates between the experiments and the simulations is fairly good. The first interspike interval—although fairly well reproduced by the model at low current-injection strengths—becomes too high at very high levels of injected current. This is because, in the simulation, such strong injection currents tend to lead to a doublet spike in a simulated soma of relatively small size such as the one used here. Figure 4 shows simulated and experimentally recorded spike trains when calcium channels have been blocked. The recordings were performed on an unidentified neuron and we compare the results with a simulated trace from a model spinal interneuron. In the experiment, calcium channels were blocked using cadmium. Figure 4A shows the response in an unidentified neuron and a simulated spinal neuron when calcium was applied, blocking all high-voltage–activated calcium channels. The stimulation was just strong enough to yield repetitive firing in both the experiment and the simulation. The shape of the sAHP, which is now mediated by $K_{NaS}$ only, was noticeably altered compared with the case with intact $Ca^{2+}$ channels (compare Figs. 3 and 4; the insets in each figure show magnifications of the regions of interest). Although the shape of the interspike region is similar in both cases (lacking the “dip” that is seen in the control case), the time course of the early phase of the sAHP is faster in the simulation. This difference can be explained by the fact that the experimental recording was obtained from a larger cell (rheobase $>1$ nA), and additional simulations using a cell with larger soma and more extensive dendritic tree yielded a time course more nearly similar to the one observed experimentally, with the shape retained (not shown).

Previous experiments showed that the part of the sAHP contributed by the $K_{NaS}$ current grows as the firing frequency of the cell is increased (Wallén and Grillner 2003), suggesting that this current becomes important at high firing frequencies. This effect is replicated in the model. Figure 4B illustrates how the $K_{NaS}$ part of the sAHP varies when spike trains of different frequencies are induced in the cell. The higher the imposed spike frequency, the larger the $K_{NaS}$-mediated part of the sAHP becomes. This is the result of a slower dynamics in the $K_{NaS}$ part.

**Catechol-sensitive currents: $K_t$ and $K_{NaF}$**

After having introduced the basic factors involved in action potential generation and firing frequency control in lamprey CPG neurons, we now describe in more detail how the fast $K$ currents $K_t$ and $K_{NaF}$ influence the shape of both action potentials and slow AHPs. We begin by considering the $K_t$ current, for which there is more experimental evidence. The $K_t$ current...
current, a fast-inactivating transient potassium current, is thought to be the main determining factor of action potential width in motoneurons, contralaterally and caudally projecting interneurons (CCINs) and unidentified spinal neurons (Hess and El Manira 2001). A change in the $K_t$ current’s activation time constant in our model can change the width without having a large effect on the rising phase or amplitude (results not shown). The $K_t$ current can be blocked by catechol. In *Xenopus*, two high-voltage-activated conductances ($I_{Kf}$ and $I_{KNa}$) that are blocked by catechol also affect action potential width, although they do not seem to be as important for normal locomotor pattern generation as the $K_t$ in lamprey (Kuenzi and Dale 1998).

Figure 5 shows some characteristics of our model $K_t$ current. The half-activation value is at around 0 mV and the time constant of activation is rapid compared with the time constant of inactivation. The recovery of inactivation curve suggests that most of the $K_t$ channels are available again only 2–4 ms after an action potential has occurred. These kinetic parameters were measured in dissociated cells and, when they are used in our model, they yield action potentials with a half-amplitude width of nearly 0.5 ms both in the dissociated cell model and in the intact cell model.

In addition to the sAHP-related $K_{Na}$ current, a faster sodium-dependent potassium current (here called $K_{NaF}$) was also previously described in lamprey spinal neurons (Hess and El Manira 2002; Hess et al. 2007). Like $K_t$, this current can be blocked by catechol (Hess and El Manira 2002; Hess et al. 2007). The activation and inactivation properties of this current were found to be similar to those of the $Na^+$ current (Hess and El Manira 2002; Hess et al. 2007), i.e., the $K_{NaF}$ “mirrors” the $Na^+$ current. One interpretation of this is that $K_{NaF}$ channels are activated by a localized sodium ion pool with fast turnover. Rapid influx into this pool of $Na^+$ ions during an action potential would then activate $K_{NaF}$ in proportion to the sodium current and a rapid decay of the local concentration would explain the transient character of the $K_{NaF}$ current. According to this reasoning, a fast sodium pool was used in simulating the current here, as described in the Appendix.

Applying catechol to a dissociated lamprey spinal neuron results in a wider action potential and an inability of the cell to fire repetitively in the absence of calcium influx. It was suggested (Hess and El Manira 2001) that catechol blocks the $K_t$ channels, leading to a failure of $Na^+$ channels to properly recover from inactivation. Figure 6 shows the response of a real cell (reproduced from Hess and El Manira 2001) and a model neuron to a double-step protocol where the cell is stimulated by injected current for 20 ms, after which the injected current is removed for 2 and 13 ms, respectively, before another current step is applied for 20 ms. The procedure is done in the presence and absence of catechol, resulting in a total of four experiments. We repeated these experiments (described in Hess and El Manira 2001) in the model, where we have direct access to the activation and inactivation state of the $Na^+$ current. These experiments were performed on dissociated cells, both in the real cells and in the simulation. At the time of the experimental study, the existence of the $K_{NaF}$ current, which is also blocked by catechol, was not known, so the observed effects were attributed solely to the absence of $K_t$. We used simulations with our model to see whether blocking only $K_t$ is sufficient to explain the results or whether $K_{NaF}$ has to be blocked as well. The simulations suggest that blocking only $K_t$ currents in a model dissociated cell is sufficient to reproduce the observed results because blocking both $K_t$ and $K_{NaF}$ gives a result that is practically identical to blocking only $K_t$. (Note, however, that the effects of blocking $K_{NaF}$ in addition to $K_t$ are prominent when simulating experiments an intact cell; more on this subsequently follows in connection with Fig. 7.) As can be seen, when the $K_t$ current is intact, the

![FIG. 5. Kinetics of the modeled $K_t$ current. A: curves of normalized steady-state activation and inactivation for $K_t$ as a function of voltage. Circles are experimentally observed steady-state activation values; crosses are experimentally observed steady-state inactivation values (Hess and El Manira 2001; Hess and El Manira 2007). Solid line is the steady-state activation function used in the model; the dash–dotted line is the steady-state inactivation function. B: time constant of activation used in the model. C: time constant of inactivation used in the model. D: recovery from inactivation as a function of time. Solid line represents experimentally observed values; the dotted line represents values generated by the model for voltage steps from $V_m = -120$ mV.]
cells fire action potentials in rapid succession, but when catechol is applied (corresponding to setting $K_t$ and/or $K_{NaF}$ conductances to a few percent of the original value in the model), the cells stop firing after two spikes. In our replicated version of the experiment using the model, we also plotted the magnitude of the $Na^+$ current’s inactivation state variable. It can be seen from the plot that this variable fails to return to its original value between action potentials when the $K_t$ and $K_{NaF}$ currents are absent.

The experiments just described were performed in dissociated cells in the absence of calcium. When catechol is applied in the intact spinal cord, without blocking calcium channels, the cells are still able to fire repetitively, but their AHPs lose their biphasic look with one fast AHP and one slow AHP and instead show a single AHP with a rounded shape (Hess and El Manira 2001). Because catechol can block both $K_t$ and $K_{NaF}$, this effect could be attributed to the absence of either or both of these currents. We therefore used a model neuron to examine the effects of blocking just one or both of the currents during repetitive firing (Fig. 7). In Fig. 7A, simulations with a strong applied injection current (2 nA) are shown where $K_t$ and $K_{NaF}$, or both are blocked. For reference, a control trace is shown at the top of the figure with spikes scaled to 25% size for clarity. The spike frequency in the control trace is high but comparable to the frequency in the control case of the experiment we replicate (Hess and El Manira 2001). It is apparent that blocking only $K_t$ is sufficient to obtain the rounded sAHP. Blocking one or both of the $K_t$ currents has a significant effect on the shape of the sAHP between spikes. Blocking one or both of the $K_t$ currents results in a broadened spike. AHP but a very large sAHP. Looking at the action potential shapes, blocking either $K_t$ or $K_{NaF}$ in isolation seems to increase the spike width. Figure 7B therefore shows a magnification of action potentials from Fig. 7A; these were superimposed for clarity. Apparently, in the model, blocking $K_t$ or $K_{NaF}$ leads to a broader spike, although the effect of blocking $K_t$ is stronger. When both are blocked, there is a supralinear effect that leads to a much broader spike. Double-headed arrows mark out the half-amplitude width of each action potential. The differences in spike width would be expected to lead to changes in calcium influx during the action potential and subsequent changes in $K_{Ca}$ currents activated by the calcium that has entered. Figure 7C shows $C_{Na}$ and $K_{Ca}$ current time courses and amplitudes during the action potentials in Fig. 7B. [We plot only N-type calcium channels because calcium entering through these channels is the main activator of $K_{Ca}$ channels; there is also a small effect from calcium influx through P/Q-type channels (Wikström and El Manira 1998), although we have neglected this effect in our model.] Because dendrites have a much higher density of $K_{Ca}$ channels than soma, we plotted currents from a primary dendritic compartment to see the effects more clearly. Also, because the activation of $K_{Ca}$ currents here dominated that of the other sAHP-mediating current, $K_{CaS}$, we plot only $C_{Na}$ and $K_{Ca}$ currents. As expected, the differences in spike width are reflected by changes in resulting dendritic $K_{Ca}$ currents. Comparing the current time courses with the voltage traces in Fig. 7A suggests that the rounded shape of the sAHPs mainly reflects the activity of the $K_{Ca}$ current.

**NMDA oscillations**

Because our cell model is constructed to be useful in a network simulation, it is important that, in addition to the...
voltage- and concentration-gated ion channels described earlier, the ligand-gated ion channel mechanisms are accurate as well. Earlier experimental studies of the lamprey CPG showed that a tonic activation of NMDA receptors may give rise to intrinsic voltage oscillations in intact spinal neurons. Several variations of these NMDA-induced oscillations were examined experimentally and computationally (see, e.g., Brodin et al. 1991; Grillner and Wallén 1985). Experimental studies on single cells extensively described tetrodotoxin (TTX) and TTX + tetraethylammonium (TEA)-resistant NMDA-induced oscillations (Grillner and Wallén 1985; Wallén and Grillner 1987). We simulated these types of oscillations using our model. Although NMDA-induced oscillations are robustly observed in intact adult neurons where synaptic transmission has been blocked by TTX application, we have so far not observed them in dissociated larval neurons (El Manira and Hill, unpublished observations). This could mean that the dendrites (which are largely missing from the dissociated cells) are needed to generate NMDA oscillations or it could be attributed to developmental differences between the larval and adult life stages. Additional experiments using multiphoton imaging are under way to examine the calcium transients resulting from different kinds of activation, including bath NMDA application. These experiments will likely clarify the distribution of NMDA channels over the cell. Our main purpose concerning NMDA dynamics was to make sure that the model can generate NMDA-induced oscillations with different distributions of NMDA channels. This was found to be the case: depending on how the conductances are set, the model can generate oscillations with NMDA channels on soma only, dendrites only, or both on soma and dendrites. In this section and in the following section with NMDA channels on soma only, dendrites only, or both on soma and dendrites. The densities were set differently in soma and dendrites. Soma densities were: NMDA, CaNMDA, and KCaNMDA channels were present on both soma densities were: NMDA = 5× bath NMDA concentration; CaNMDA = 1× bath NMDA concentration; KCaNMDA = 1.75 [all in S/m²; NMDA concentration in arbitrary units (au)]. Dendrite densities were: NMDA = 5× bath NMDA concentration; CaNMDA = 1× bath NMDA concentration; KCaNMDA = 0.175 [all in S/m²; NMDA concentration in arbitrary units (au)].

**TTX oscillations**

With NMDA present while action potentials are being abolished by administering TTX to block sodium channels, one sees a characteristic type of NMDA-mediated oscillation (Grillner and Wallén 1985). The depolarized plateaus in these oscillations often have a stereotyped shape, with a small Ca²⁺-dependent depolarization in the beginning and a slowly declining phase that is suddenly terminated, whereupon the potential quickly drops toward the trough potential (Fig. 8A). This interplateau state is triggered when enough calcium has entered through the open NMDA-receptor channels to terminate the depolarized plateau through the activation of hyperpolarizing K⁺ currents. The length of the interplateau phase is likely determined by the time it takes for the accumulated calcium to be removed. When a certain depolarizing potential threshold is crossed (determined by the voltage range where the magnesium block of the NMDA channel starts to be removed), a self-reinforcing loop between membrane potential and NMDA channel activation rapidly takes the neuron to a depolarized potential. TTX oscillations are usually 10–25 mV in amplitude (Wallén and Grillner 1985). See Tegné et al. (1998) for a more detailed description of the possible mechanisms behind these TTX oscillations. Figure 8A shows experimentally recorded and simulated TTX oscillations, whereas Fig. 8B shows the sizes of various currents during one of the simulated plateaus. NMDA, CaNMDA, and K⁺ currents dominate, although there is also a fairly large L component. Sometimes, variations on the theme are seen and many TTX-resistant oscillations seem to be “broken up” or to have internal

**Fig. 8.** Various types of N-methyl-D-aspartate (NMDA)-induced oscillations. A: NMDA-induced oscillations in the presence of tetrodotoxin (TTX), which blocks Na⁺ channels. Left: experimental recording. Inset: magnification of the small-amplitude oscillations at the beginning of a plateau. Right: simulation [bath NMDA concentration was 0.7 (a.u.) and the resting potential was −70 mV]. B: somatic currents activated during the simulated experiments. NMDA-induced oscillations in the presence of TTX (same as A, right). Because the NMDA current is much larger than the other currents, it has been omitted in the plot. Relative magnitude of NMDA current compared with the other currents is shown in the inset; the y-scale is 15-fold larger in this inset than in the main plot. C: NMDA-induced oscillations in the presence of TTX and tetraethylammonium (TEA), which blocks K⁺ and L channels. Left: experimental recording. Right: simulation [bath NMDA concentration was 0.65 (a.u.) and resting potential was −70 mV]. D: somatic currents activated during the simulated NMDA-induced oscillations in the presence of TTX and TEA (same as C, right).
oscillations within them (see, e.g., Grillner and Wallén 1985). These internal oscillations (examples of which are shown in an inset to Fig. 8A) are, in the model, dependent on the dynamics of the Ca\textsubscript{N}, and K\textsubscript{Ca} channels. Both the “regular” TTX-resistant oscillations and the “irregular” ones can be mimicked by the model by varying relevant parameter values (not shown). In Fig. 8B, the NMDA current has been omitted from the main figure because it is so large compared with the other currents; a smaller version of the main plot with a different y-scale (zoomed out 15 times) is shown in an inset to give an idea of the amplitude proportions of NMDA versus other currents.

**TEA + TTX-resistant oscillations**

When in addition to TTX, TEA is applied, blocking both the K\textsubscript{r} and K\textsubscript{c} channels, the oscillations dramatically change their character, becoming much shorter in duration (Grillner and Wallén 1985). As a result of insufficient hyperpolarization, the calcium channels remain open and large amounts of calcium ions flow into the cell as Ca\textsubscript{N}, Ca\textsubscript{L}, and NMDA currents depolarize the membrane potential. The cell has time to reach a much more depolarized level before the influx of calcium activates enough calcium-dependent potassium channels to pull the potential back to hyperpolarized levels again. TEA + TTX oscillations can have amplitudes >60 mV. Figure 8C shows an experimental recording (modified from Grillner and Wallén 1985) and a simulation of TEA + TTX oscillations. Figure 8D shows the degree of activation of different currents during one oscillation period from the simulation.

**Effects of changes in the morphology**

The results shown so far were obtained with the “default” model described in Methods. To find out how modifying the model neuron’s morphology affects its properties, we constructed compartmental models by closely modeling three of the filled cells shown in Buchanan et al. (1989). All simulations previously described for the default model were run using these “reference” model cells and all results were qualitatively confirmed for each cell: simulated channel blockers (Cd\textsuperscript{2+}, catechol) had the same effects as described earlier, NMDA-induced TTX and TTX + TEA oscillations were present, and so forth. Despite the qualitative robustness of the cellular properties, there were slight quantitative differences in spiking properties such as adaptation. For conciseness, we limit ourselves to showing the variations in spike trains resulting from current injections at two levels. The reconstructed cells (modified from Buchanan et al. 1989) are shown together with graphical representations of the corresponding compartmental models in Fig. 9, B–D, whereas 9A shows the default model for ease of comparison. The lengths and widths of the dendritic branches in the graphical representations correspond to those used in the compartmental models.

The geometric orientation of the branches is arbitrary from the perspective of the compartmental model, but they were plotted with orientations roughly corresponding to those of the original reconstructed neurons. Axon collaterals are indicated with a dashed line. The neuron marked “Reference cell 1” is from Fig. 3C in Buchanan et al. (1989), whereas cell 2 is from Fig. 3D and cell 3 is from Fig. 7 in the same paper. The current densities of the default model were kept constant. The resulting input resistance values for the three new model neurons were 64, 52, and 98 M\textohm; for cells 1, 2, and 3, respectively. The current injection level needed to elicit an action potential was 0.29, 0.33, and 0.19 nA, respectively. Figure 9 compares spike trains generated by the default model and the three compartmental models based on the filled neurons. Two spike trains are shown for each model cell: one generated in response to a simulated 0.5-nA current injection and one generated in response to a simulated 1.5-nA current injection. Note that the current injection levels were constant and thus not normalized to rheobase. The lower current-injection level, 0.5 nA, is roughly 1.5- to 2.5-fold the rheobase of the model cells, whereas the higher level is roughly five- to sevenfold rheobase. All three model cells fired spike trains not unlike those generated by the default model. However, there were slight differences in the firing frequency and the degree of adaptation between the model neurons. Thus at the lower injection level, clear adaptation where the last interspike interval is noticeably longer than the first one is seen for the default cell and reference cell 2 (see C3), whereas the adaptation is only slight in reference cell 1 (see B3). Reference cell 3 displays no noticeable adaptation at this current-injection level (see D3). At the higher injection level, all cells show adaptation, although it is strongest in the default cell and cell 2 (C4): the first interspike interval is very short and is followed by a longer interval, after which the interval length settles down to a steady state.

The spike trains of reference cell 2 seem most similar to the default model cell, which may partly reflect that these cells have similar rheobase (0.33 and 0.35 nA, respectively) and input resistance (52 and 49 M\textohm, respectively.) However, these factors are not sufficient to explain all differences in spiking between the neurons: for example, reference cell 2 shows faster firing than reference cell 3 at the lower injection level (compare B3 and C3) but slower firing at the higher injection level (compare B4 and C4), suggesting that differences in the dendritic tree morphology resulted in different slopes of the steady-state frequency–current (f–I) curves in these cells.

The differences in the initial adaptation between the model neurons can be partly explained as follows. Spike-frequency adaptation is a transient phenomenon and, because the current injection is delivered to the soma, the soma size will be an important factor in determining the first interspike interval. As dendritic currents become activated with a slight delay, they will successively have a larger influence on the interspike interval. In our example simulations, the cells that show the strongest initial adaptation are both cells with a relatively small soma. [Note also that the injection current (1.5 nA) is very high: almost fivefold rheobase for these neurons.] If the other two cells are injected with a sufficiently strong current, they will also respond with a similarly strong adaptation. Simulations where soma and dendrite sizes were varied while retaining the total membrane area of the cells (not shown) confirmed that the preceding explanation is essentially correct with respect to the simulated cells—that is, an increase in soma size with concomitant reduction in dendrite size to preserve total surface area leads to smaller adaptation. Moreover, simulations with passive dendrites (all active conductances set to zero) yielded the same outcome, which shows that the observed phenomenon was not an artifact arising from the implicit
changes in dendritic conductances resulting from changing the dendritic surface area.

In summary, firing properties show considerable robustness in a qualitative sense with some quantitative variability.

DISCUSSION

A new model of a lamprey locomotor network neuron has been constructed. Although previous models (e.g., Ekeberg et al. 1991) were biophysically detailed, channel kinetics were based mostly on adapting ion channel models from other organisms to fit experimental traces. The present model is to a large extent based on voltage-clamp and other types of experiments on real lamprey neurons. It incorporates several types of ionic currents not present in previous models (Kt, KNaF, KNaS), which in itself represents a significant improvement. Although previous models contained only one high-voltage–activated calcium current, the present model has two (CaN and CaL), which have both been identified in lamprey, and both have kinetic parameters consistent with experimental results (El Manira and Bussières 1997). The new model can reproduce many experimental results that were not addressable before, such as effects of applying catechol. The separation of the high-voltage–activated calcium current into CaN and CaL also led to simulated TTX-resistant oscillations with characteristics...
more consistent with those seen in experiments. In the previous models, the dendritic tree was purely passive, whereas in actual lamprey spinal neurons several types of active conductances are thought to be located there. The new model allows an examination of the distribution of such conductances across the neuron, leading for example to the hypothesis presented in this paper about $K_{CaN}$ and $K_{NaS}$ channels being present at significantly higher densities in the dendritic tree than in the soma. In the same vein, the present model reproduces experimentally observed differences between dissociated cells and intact cells. Previous models such as that proposed by Ekeberg et al. (1991) did not yield a realistic dissociated cell when the dendritic tree was removed. Because the dissociated cell is an important experimental preparation, the model needs to be able to give a good description of it.

One important use of the model is as a “check” of the soundness of our experimentally based understanding of the single lamprey CPG neuron. The model can provide a tool to study the currents underlying the neuron’s electrical activity by showing the simulated amplitudes and time courses of selected currents during various types of activity, as well as following manipulation of some of the model components (see, e.g., Figs. 2 and 6–8). In this way insights into the functional roles of recently characterized ion channels in the lamprey CPG can be achieved. For example, the importance of $K_c$ for action potential repolarization and width in this system suggested by Hess and El Manira (2001) is supported by the model. $K_c$ is classified as an “A-type” current—a fast, inactivating potassium current. In general, the kinetics of A-type currents varies depending on the cell type (Birnbaum et al. 2004). Suggested functional roles of this current depend on the activation threshold (see, e.g., Surmeier et al. 1989). The typical role of A-type currents, which are activated at more hyperpolarized levels, below the neuron’s spiking threshold, is to act as a kind of brake or damper, so that the onset of an action potential is delayed (Hille 2001). In lamprey CPG neurons, the A-type current is activated at a high membrane potential. It repolarizes the neuron after an action potential and thereby frees the Na$^+$ channels from inactivation, making the neuron ready for another spike. A repolarizing function of an A-type current was also previously found in other systems (Storm 1987; Surmeier et al. 1989). In the context of the operation of the lamprey locomotor CPG network, it was shown that the $K_c$ current affects the number of action potentials per locomotor cycle and the regularity of the locomotor rhythm (Hess and El Manira 2001).

The interplay between Na$^+$ and $K_c$ allowed very high spiking frequencies in one study of dissociated cells in which calcium had been blocked (so that $K_{Ca}$ currents are absent) (Hess and El Manira 2001). To reach the spiking frequencies found in those experiments, our model had to include Na$^+$ and $K_c$ channels in the axon initial segment at a much higher density than the somatic one. Several computational models also found it necessary to have much higher sodium channel densities in the initial segment. A good discussion of these issues can be found in Stuart et al. 1999. Because the Na$^+$ and $K_c$ currents are present on the initial segment in such high densities in the model, the initial segment component of these currents seems to be more important for action potential generation than the somatic component. If instead enough $K_c$ channels are placed on the soma to fully repolarize the action potential, the resulting somatic $K_c$ current becomes much larger than experimental data suggest. The $K_c$ current is, in general, considerably less activated in the soma of an intact cell than in a dissociated cell (see Fig. 2C). The reason is that the maximum somatic potential reached during a spike in an intact cell is only +15 to +20 mV, whereas it can be up to +60 mV in a dissociated cell (Hess and El Manira 2001). $K_c$ is a high-voltage–activated current that reaches its maximum conductance only at +40 mV or so (Hess and El Manira 2001). Because the activation kinetics is also faster at more depolarized levels, the difference in the resulting current amplitude will be considerable.

The model also throws light on firing-frequency characteristics of the lamprey CPG neuron. It predicts that differences in firing frequency and sAHP size between dissociated and intact neurons may be explained by a high density of $K_{CaN}$ and $K_{NaS}$ currents (tenfold the somatic density) in the dendritic tree. In addition, it shows that a simplified model of the $K_{NaS}$ current is sufficient to explain many of the relevant experimental results on sodium-mediated sAHPs and the relative contribution of a calcium-dependent versus a sodium-dependent component (Wallén and Grillner 2003). As described earlier slow $K_{Na}$ channels ($K_{NaS}$) in the lamprey seem to regulate the sAHP, firing frequency, and adaptation together with $K_{Ca}$. The relative importance of $K_{Na}$ increases with activation frequency. $K_{Na}$ channels were also found in other parts of the nervous system (Dryer 1994; Yuan et al. 2003). They were suggested to play a role in such phenomena as the regulation of neural excitability and firing patterns and modulation of the action potential waveform (see, e.g., Dryer 1994). As in the lamprey, a $K_{Na}$ current was also previously found to affect the sAHP amplitude in neocortical intrinsically bursting neurons (Franceschetti et al. 2003). In that study, it was also shown that $K_{Na}$ channels can give rise to rhythmic bursting, an effect seen in our model as well if the $K_{NaS}$ conductance is sufficiently increased (not shown). As in lamprey CPG neurons, a slow $K_{Na}$ current was shown to be involved in adaptation in V1 neurons (Wang et al. 2003).

The preceding results suggest various functional roles of the different currents in the lamprey CPG neurons and can form a basis for comparison with the Xenopus embryo model, a model system that was experimentally and computationally investigated in detail (see Dale and Kuenzi 1997). Interestingly, neurons in these two spinal cord CPGs use many of the same classes of ionic currents, but these currents seem to differ in their functional and/or electrophysiological characteristics between the two model systems. Here differences in developmental stages might, however, play a role. Here we will make a short comparison of what is known about the functional roles of ionic currents in Xenopus versus lamprey. In Xenopus embryo spinal neurons, Ca$^{2+}$ currents are necessary for repetitive firing (Dale and Kuenzi 1997), whereas in lamprey spinal neurons, repetitive firing persists in cadmium for both dissociated and intact cells (Hess and El Manira 2001; Wallén, personal communication). Both the Xenopus embryo and the lamprey contain currents described as A-type–like; however, they seem to differ in their functional significance because Dale and Kuenzi (1997) state that the Xenopus A-type current is probably highly inactivated during locomotor activity and was thus omitted from their mathematical model of a Xenopus neuron. Like the lamprey CPG neuron, the Xenopus embryo
CPG neurons contain potassium currents that control action potential width. In Xenopus these currents are a type of delayed rectifier ($I_{Kr}$) and a fast sodium-dependent potassium current ($I_{KNa}$), whereas experiments in the lamprey showed the $K_s$ current to be essential. Our simulations suggest that the lamprey $K_s$ and $K_{NaF}$ could be analogous to the Xenopus $I_{Kr}$ and $I_{KNa}$. However, there are notable differences between these pairs of currents in the two model systems. For instance, the lamprey $K_s$ in addition to controlling action potential width, is important for enabling repetitive firing by controlling the action potential repolarization, which is not the case for the Xenopus $I_{Kr}$ and $I_{KNa}$ (Kuenzi and Dale 1998). In Xenopus a slow potassium current, $I_{KSc}$, controls and decreases a cell’s firing frequency (Kuenzi and Dale 1998), whereas calcium- and sodium-dependent potassium ($K_{Cf}$, $K_{Na}$) currents preferentially regulate firing frequency and adaptation in the lamprey CPG, although a slower delayed rectifier is also present. Calcium-dependent potassium channels also exist in Xenopus and are involved in a rundown of activity in the CPG (Dale and Kuenzi 1997), a phenomenon occurring over much longer timescales than spike-frequency adaptation in lamprey neurons. Comparing the fast sodium-dependent currents in the two organisms, we note that the activation of Xenopus $I_{KNa}$ channels depends on both voltage and the level of $Na^+$ ions that enter primarily through leak channels and excitatory synaptic channels; $Na^+$ influx during action potentials does not give an appreciable effect except during repetitive firing (Dale and Kuenzi 1997). In contrast, the lamprey $K_{Na}$ channels (both the fast and slow types) are noticeably activated after a single action potential (Cangiano et al. 2002; Hess and El Manira 2002; Wallén et al. 2005). In summary, although many of the same current classes seem to be present in both model systems, the currents generally have different roles.

A common criticism of this type of model is that simulation results are sensitive to parameters, and that data are too variable to constrain the model. This is a valid concern, and to show that our generic model gives qualitatively similar (although quantitatively variable) output when the morphology is varied, we constructed three reference model neurons closely based on filled cells from Buchanan et al. (1989), which is the most comprehensive study of excitatory interneurons in the lamprey CPG so far. Simulations using these morphologies showed that the results herein (roles of various currents, effects of simulated channels blockers, appearance of NMDA-induced oscillations in TTX and/or TEA, etc.) still hold true in each of the model cells, although there were slight quantitative differences in the spike trains resulting from given current injection levels. The functional implications of this kind of variability will be further examined in network simulations. There are previous results suggesting that variability in properties such as rheobase and sAHP size (which controls adaptation) can lead to an enhanced robustness and increased working range of locomotor pattern generation (Hellgren et al. 1992).

Another type of variability likely to be important for pattern generation is found on the synaptic level. For example, excitatory synapses between excitatory interneurons and motoneurons or crossed-caudal (CC) interneurons can be either facilitating or depressing (Parker 2000, 2003), whereas inhibitory synapses from small inhibitory interneurons are depressing in motoneurons and facilitating in CC interneurons (Parker 2000). Different cell types can be differently modulated by neurotransmitters, for example, substance P has opposite effects on the postinhibitory rebound in inhibitory interneurons and motoneurons (Svensson 2003). This synaptic and modulatory variability will be considered in the network simulations based on this cell model.

Some of the model’s predictions suggest new experiments to confirm or falsify them. For instance, the prediction of much higher densities of $K_{Cf}$ and $K_{Na}$ currents in the dendrites suggests a closer look at $Na^+$ and $Ca^{2+}$ transients in dendrites and how they are connected to the activity of the neuron. Experiments measuring such transients using multiphoton imaging are under way. Further, although not illustrated herein, the model predicts that slightly counterintuitively, the frequency of TTX oscillations should decrease as the applied-bath NMDA level is increased. This prediction is currently being tested experimentally.

In future work, we plan to search for putative new roles for ion channels when the model is used in a network context, such as an interneuron network inside a central pattern generator module.

APPENDIX

Kinetic parameters for the ion channel equations

The equations referred to in Table A1 are listed below.

The equation used to calculate the ionic current through each channel is given in the first column in Table A1. This equation always involves a maximum conductance and a reversal potential. In most cases, it also involves one or sometimes two activation (or inactivation) variables, which vary between 0 and 1 and may be voltage or concentration dependent. In the case of the voltage-dependent (in)activation variables, the following equation holds:

$$\frac{dx}{dt} = \alpha_x(V)(1 - x) - \beta_x(V)x$$

(A1)

where $x$ is the name of the (in)activation variable (m, h for the Na$^+$ current, for example). In two cases—the Ca$^{2+}$, channel and the Ca$^{2+}$L channel—the following (completely equivalent) equation was used instead

$$\frac{dx}{dt} = \frac{x - x_m}{\tau_m(V)}$$

(A2)

Here, $x$ is again the name of the (in)activation variable (m and h for Ca$^{2+}$ and q for Ca$^{2+}$L). This formalism was used here because it was the preferred format in the original paper describing the Ca$^{2+}$ current model (Booth et al. 1997).

The equations determining the value of the (in)activation variable in the voltage-dependent case are thus expressed either in terms of $\alpha_x$ and $\beta_x$, (corresponding to opening and closing rates) or in terms of $x_m$ and $\tau_m$ (corresponding to steady-state value and time constant). These variables, in turn, are dependent on further parameters $A$, $B$, and $C$. When the equation for the (in)activation variable is expressed in terms of $x_m$ and $\tau_m$, the following equations are used to determine these:

$$y(V) = \frac{A(V - B)}{1 - \exp\left(\frac{B - V}{C}\right)}$$

(A3)
for the opening rates ($\alpha$) of the activation variables

$$y(V) = \frac{A(B - V)}{1 - \exp\left(-\frac{V - B}{C}\right)} \quad (A4)$$

for the closing rates ($\beta$) of the activation variables and opening rates for inactivation variables, and

$$y(V) = \frac{A}{1 + \exp\left(\frac{V - B}{C}\right)} \quad (A5)$$

for the closing rate of the inactivation variables. In these equations, $y(V)$ stands for either $\alpha(V)$ (opening rate) or $\beta(V)$ (closing rate).

For the NMDA and Ca$_{\text{NMDA}}$ currents, slightly different equations for $\alpha$ and $\beta$ were used, following Brodin et al. (1991)

Not all ion channels were voltage dependent. In some cases, the concentration of some ion determined the activation level of the channel.

<table>
<thead>
<tr>
<th>Current Type</th>
<th>$E_{\text{rev}}$, V</th>
<th>Variable</th>
<th>Equations</th>
<th>$A$</th>
<th>$B$</th>
<th>$C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast sodium current (Na$^+$)$_1$</td>
<td>0.050</td>
<td>$\alpha_m$</td>
<td>$A_1, A_3$</td>
<td>$0.6 \times 10^6; 2 \times 10^6$</td>
<td>$-0.043^<em>; -0.053^</em>$</td>
<td>0.001</td>
</tr>
<tr>
<td>$I_{Na} = g_{Na} \times m(V)^3 \times h(V) \times (V - E_{Na})$</td>
<td>$\beta_m$</td>
<td>$A_1, A_4$</td>
<td>$0.18 \times 10^6; 0.6 \times 10^6$</td>
<td>$-0.052^<em>; -0.062^</em>$</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>N-type calcium channel (Ca$^+$)$_N$</td>
<td>0.050</td>
<td>$\alpha_n$</td>
<td>$A_1, A_4$</td>
<td>$75 \times 10^6; 50 \times 10^6$</td>
<td>$-0.046^<em>; -0.054^</em>$</td>
<td>0.001</td>
</tr>
<tr>
<td>$I_{CaN} = g_{CaN} \times m(V)^3 \times h(V) \times (V - E_{CaN})$</td>
<td>$\beta_n$</td>
<td>$A_1, A_5$</td>
<td>$6 \times 10^6; 4 \times 10^6$</td>
<td>$-0.042^<em>; -0.050^</em>$</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

In the Ca$_N$ and Ca$_L$ cases, where the activation and (for Ca$_N$) inactivation variables were expressed in $x_a$ and $\tau(x)$, the following equations determine these values [note that $\tau(x)$ is constant]

$$\tau(x) = A \frac{1}{x_a} = \frac{1}{1 + \exp\left(-\frac{V - B}{C}\right)} \quad (A8)$$

channels. The model includes sodium- and calcium-dependent channels. To model these concentration-dependent channels, we introduced intracellular “pools” with a characteristic influx rate and time constant of removal. The ion concentration $[C]$ in the pools increases proportionally to the size of inward currents carried by the ion type in question, and decays according to the time constant of removal

$$\frac{d[C]}{dt} = A_i I_i - \frac{[C]}{B_i}$$  \hspace{1cm} (A9)$$

where $A_i$ is the pool’s influx rate of sodium, $I_i$ is the sodium or calcium current, and $B_i$ is the pool’s time constant of decay.

Both in the sodium and the calcium case, we used two different pools (“fast” and “slow”), the concentration in which determined the activation rates of the two sodium-dependent potassium channels ($K_{NaS}$ and $K_{NaF}$) and the two calcium-dependent potassium channels ($K_{CaN}$ and $K_{CaNMDA}$), respectively. Each of these pools, naturally, can have different $A$ and $B$ values.

For most of the concentration-dependent currents ($K_{NaS}$, $K_{CaN}$, and $K_{CaNMDA}$), we let the activation variable be an instantaneous function of the ion concentration. For $K_{NaF}$, however, it was found that a better fit to experimental data could be achieved if the activation variable had fast internal dynamics so that it reached its equilibrium value after a brief delay instead of instantaneously.

Thus for $K_{NaS}$, $K_{CaN}$, and $K_{CaNMDA}$, the activation is determined by an instantaneous function

$$z = \frac{[C]}{[C] + B_i}$$  \hspace{1cm} (A10)$$

Here, $[C]$ is the ion concentration in the relevant pool and $B_i$ is a parameter describing the half-activation concentration of the pool. $B_i$ need not be the same in separate pools containing the same ion type (such as in the fast and slow Ca$^{2+}$ pools).

For $K_{NaF}$, the activation is instead determined by

$$\frac{dz}{dt} = \alpha_i ([Na^+]^z)(1 - z) - \beta_i ([Na^+]^z)z$$  \hspace{1cm} (A11)$$

where $\alpha_i$ and $\beta_i$ are determined through

$$\alpha_i = A \left( \frac{[Na^+]}{[Na^+] + B} \right) \beta_i = A \left( 1 - \frac{[Na^+]}{[Na^+] + B} \right)$$  \hspace{1cm} (A12)$$

where $A$ is a delay factor that determines the time constant of the process and $B$ is the half-activation concentration in the fast Na$^{+}$ pool. These equations yield a steady-state value $z_* = [Na^+]^z([Na^+] + B)$ for $z$ (so the steady-state value is the same as the instantaneous value from Eq. A9), and this value is approached with a time constant of $1/A$.

Sources of parameter values

The Na$^+$ current model was adapted from a previous cell model but modified to better replicate results from experiments performed after the publication of that paper (Ekeberg et al. 1991). For the initial segment, the kinetics is slightly different from the somatic kinetics (refer to Table A1 for the values). These differences in kinetics were added to obtain a better fit to the time course of sodium activation observed during action potential clamp on larval lamprey neurons (Hess et al. 2007). The $K_c$ model was based on voltage-clamp data (Hess and El Manira 2001) from larval and young adult neurons. It is notable that in the initial segment, Na$^+$ and K$^+$ conductances are needed in high densities (62.5 and 120 times soma density, respectively) to satisfy a constraint derived from experiments; in the presence of a calcium blocker, the lamprey locomotor network neuron should be able to spike indefinitely in response to a sustained current pulse up to $\pm 7$ nA (Hess and El Manira 2001). This constraint cannot be satisfied by increasing the somatic Na$^+$ and $K_c$ conductances because that would violate other constraints derived from action potential clamp studies (see Fig. 2B). The $K_c$ model was fitted to voltage-clamp data from larval neurons (Hess and El Manira, unpublished data) in combination with constraints from pharmacological experiments (Hess and El Manira 2001).

Modeling of the $K_{NaF}$ current was based on results from action potential clamp experiments (Hess and El Manira 2002; Hess et al. 2007), whereas the $K_{NaS}$ model was based on experiments that used different protocols to examine this current’s influence on the sAHP (Wallén and Grillner 2003). For both the $C_{aK}$ and the $C_{aNa}$ current, we had partial data (from El Manira and Bussières 1997), but not enough data points to do a good fit in terms of selecting an activation gate formalism. In that paper, different Ca$^{2+}$ currents in larval dissociated cells were separated from each other by the use of specific channel blockers. This allowed us to infer some characteristics of $C_{aNa}$ and $C_{aCa}$ currents. In the case of $C_{aCa}$, we borrowed the m$^h$ formalism and the equation formats from a study done on turtle neurons (Booth et al. 1997). The m$^h$ gate formalism was also used to model inactivating HVA currents in Köhr and Mody (1991). This formalism allowed a good fit to those data points that we do have. In the case of $C_{aNa}$, we know from El Manira and Bussières 1997 that it is not inactivated (or very slowly inactivated). We therefore selected a formalism with just one activation gate. This allowed a good fit of the equations to those data points presented in that paper.

In contrast to the other ion channels in this paper, the parameters are given in terms of steady-state activation and activation time constant, also the preferred format in Booth et al. (1997). Data from El Manira and Bussières (1997) were also used to estimate the effective calcium reversal potential (+50 mV) used here; this value, which may seem unusually low, was estimated from a current–voltage (I–V) curve in the paper. The $C_{aNa}$ channel does not contribute to calcium influx into the fast calcium pool. We finally checked the qualitative consistency of our HVA Ca$^{2+}$ ion channel models with results presented in Hill et al. (2003), which contains additional data about the I–V relationship of the total HVA current in dissociated spinal neurons. The kinetics for the low-voltage–activated calcium channel are taken directly from a previous modeling study (Tegnér et al. 1997) with one modification: the $C_{aNa}$ parameter was multiplied by a factor of 10. This was because in the original article, the relevant phenomenon [postinhibitory rebound, a phenomenon found in motoneurons and unidentified spinal neurons, thought to be mediated by $C_{aLVa}$ channels (Matsushima et al. 1993; Tegnér et al. 1997)] that we sought to reproduce in our model was demonstrated from a holding potential of $\pm 54$ mV. In our model, the spiking threshold is around $\pm 56$ mV, so a holding potential of $\pm 54$ mV cannot be achieved. The adjustment in $C_{aNa}$ was necessary to obtain rebound spiking at a more hyperpolarized holding potential ($\pm 58$ mV). The $C_{aLVa}$ conductance was set to zero in the soma, as discussed in METHODS, but using a nonzero value for the somatic conductance does not change any results presented here qualitatively, although the model neuron gets slightly more excitable. The NMDA, $C_{aNaMDA}$, and $C_{aCaNMDA}$ channel models were modified from a previous lamprey neuron model (Brodin et al. 1991). For example, the slow pool kinetics was changed to better reproduce experimental results. Some of the current densities were modified from the soma to the dendritic tree. Importantly, the $C_{aCaN}$ and $K_{NaS}$ currents are present at tenfold higher densities in the dendritic tree, as described in the text. This was necessary to reconcile the considerable differences in sAHP size between intact and dissociated cells; information on the frequency dependency of sAHP summaton (Fig. 4B) was also taken into consideration when setting these parameter values. The $C_{aLVa}$ density, which is zero in the soma because this current is thought to be located exclusively in the dendritic tree (Hu et al. 2002), was set to a value that allows postinhibitory rebound (see above). The $K_{NaS}$ density was increased and the $C_{aNa}$ density was reduced from the somatic level to obtain more typical-looking ADPs (adependolpolariza-
ions). The K density was decreased because keeping it at the somatic level tended to destabilize TTX-resistant NMDA oscillations.

ACKNOWLEDGMENTS

We thank Dr. Russell Hill for helpful suggestions on this manuscript.

GRANTS

This project was supported by European Union Grants QLG3-CT-2001-01241 (“Microcircuits”) and IST-001917 (“Neurobotics”) and Swedish Science Council Grants VR-M 3026, VR-M 5974, VR-M 5706, and VR-NT 3861.

REFERENCES


