Role of High-Voltage Activated Potassium Currents in High-Frequency Neuronal Firing: Evidence From a Basal Metazoan

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Buckingham, Steven D. and Andrew N. Spencer. Role of high-voltage activated potassium currents in high-frequency neuronal firing: evidence from a basal metazoan. J Neurophysiol 88: 861–868, 2002; 10.1152/jn.00552.2001. Certain neurons of vertebrates are specialized for high-frequency firing. Interestingly, high-frequency firing is also seen in central neurons in basal bilateral metazoans. Recently, the role of potassium currents with rightward-shifted activation curves in producing high-frequency firing has come under scrutiny. We apply intracellular recording, patch-clamp techniques, and compartmental modeling to examine the roles of rightward-shifted potassium currents in repetitive firing and shaping of action potentials in central neurons of the flatworm, Notoplana atomata (Phylum Platyhelminthes). The kinetic properties of potassium and sodium currents were determined from patch-clamp experiments on dissociated brain cells. To predict the effects of changing the steady-state and kinetic properties of these potassium currents, these data were incorporated into a computer model of a 30-μm spherical cell with the levels of current adjusted to approximate the values recorded in voltage-clamp experiments. The model was able to support regenerative spikes at high frequencies in response to injected current. Current-clamp recordings of cultured cells and of neurons in situ showed evidence of very-high-frequency firing. Adjusting the ratio of inactivating to non-inactivating potassium currents had little effect upon the firing pattern of the cell or its ability to fire at high frequencies, whereas the presence of the non-inactivating current was necessary for repetitive firing. Computer simulations suggested that the rightward shift in voltage sensitivity confers a raised firing threshold, while rapid channel kinetics underlie high frequency firing, and the large activation range enhances the coding range of the cell.

INTRODUCTION

Potassium currents play a major role in the sculpturing of action potential shape and in the patterns of action potential discharge (Hille 1992; Huguenard and McCormick 1992). For example, rapidly inactivating potassium currents have been shown to confer slow, non-zero firing (Connor and Stevens 1971; Rush and Rinzel 1995) and even to cause rhythmic firing (Rush and Rinzel 1995), although a more recent analysis challenges the requirement for these currents for low-frequency firing (Rush and Rinzel 1995). More recent analyses have addressed the role of K⁺ channels (such as Kv3.1) with rightward-shifted voltage dependence in producing high-frequency firing (Erisir et al. 1999; Gan and Kaczmarek 1998; Gurantz et al. 2000).

We have previously shown that, unlike most vertebrate potassium currents, the potassium currents of flatworm neurons are activated at very depolarized potentials, activate and deactivate very rapidly, and have a wide range of activation (Buckingham and Spencer 2000). Theoretical consideration of these currents raises a number of problems regarding their role in firing of action potentials. Their voltage dependence of activation suggests that they are not likely to be active in the early part of the depolarization phase of an action potential but only at its peak. The rightward shift in activation, along with the rapid activation and deactivation, suggest a role in high-frequency firing. Indeed, we have observed in intracellular recordings from flatworm brain neurons a surprising uniformity in spike shape and a capability of high-frequency firing. The inactivating potassium current of neurons in Notoplana has a time constant of inactivation that suggests that it is unlikely to play a role in the shaping of an individual action potential (Buckingham and Spencer 2000) but may have an indirect effect upon firing patterns, such as through the accumulation of inactivation. It is surprising that basal metazoans such as flatworms should share such a specialization with more derived bilaterians, and so it would be of interest to know whether high-frequency firing is brought about by the same means.

This paper addresses the question of how the observed features of a flatworm potassium channel (rapid kinetics, wide range of activation, rightward-shifted voltage dependence) contributes to high-frequency firing. Because there are inadequate data to guide the construction of a realistic model of a flatworm neuron, we have adopted the approach of incorporating models of these currents into a hypothetical cell, whose purpose is not to model real flatworm neurons but to provide a framework in which the hypothesis, that the rightward shift and rapid kinetics of flatworm potassium currents are key determinants of high-frequency firing, can be tested. We have first modeled flatworm currents using a simple compartmental computer model incorporating data on potassium and sodium currents derived from patch-clamp experiments described in this and in previously published papers. We then systematically adjusted some parameters of K⁺ currents: the levels of expression of the currents, their voltage dependence of activation and inactivation, activation range, and rates of activation and inactivation to determine their effects upon action potential shape and firing pattern and the relationship between frequency of firing and the amplitude of the injected current. The predictions

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of the model were compared with the properties recorded for in situ and dissociated neurons.

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Collection and care of animals

*Notoplana atomata* (0.5–3 cm) were collected from the underside of rocks in the inter-tidal zone near Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada, and were kept in a glass tank continuously supplied with sea water (9–12°C) from the Centre’s system. Animals were not fed and were used within 3 wk of capture.

Intracellular recording from brain neurons in situ

Individuals were anesthetized by immersion in a 1:1 (vol/vol) mixture of sea water and 0.33 M MgCl₂ for ≥20 min. Anesthetized animals were pinned ventral side up onto a silicone elastomer (Sylgard)-lined dish (Falcon, 30 mm) and the ventral epidermis overlying the brain was cut and removed. The animals were then re-pinned dorsal side up, and the dorsal epidermis overlying the brain similarly was removed so as to expose the brain. More pins were used to stabilize the opened part of the animal, and spines from the cactus *Opuntia* sp. were judiciously placed around the brain to stabilize it. Excess sea water was removed by drawing it up with a piece of tissue paper, attention being paid to ensure that water was also removed from the cavity in which the brain lies. A crystal of Pronase was placed on the brain and then quickly (≤5 s) washed off with a surplus of artificial sea water (ASW, Table 1). After ~5 min the sheath surrounding the brain was seen to disintegrate. Cells on the posterior dorsal surface of the brain were impaled with two 3 M KCl-filled electrodes pulled from fiber-filled, thin-walled glass capillaries (GC150FT-75, Warner Instruments) with resistances of 15–30 MΩ. Voltages were amplified using a Dagan 8800 amplifier (Dagan, Minneapolis, MN), which was also used to inject current. Signals were displayed on a Tektronix 5223 digitizing storage oscilloscope and recorded on a VHS video cassette recorder (MTC, Japan) after pulse-code modulation (PCM2, Medical Systems, Greenvale, NY). Recorded signals were subsequently digitized using a Digidata 1200 AD converter and analyzed using pClamp 8.0 software (Axon Instruments, Foster City, CA).

Cell dissociation and culture

Anesthetized animals were washed in filtered ASW and then pinned out in a Petri dish lined with Sylgard resin. An incision was made in the dorsal epithelium, and the nerves were severed so as to leave fairly long stubs. The brain was removed and placed in a separate Petri dish (30 mm, Falcon) containing filtered ASW and gently triturated through fire-polished, silanized Pasteur pipettes or glass capillaries.

Electrophysiological recording from dissociated cells

Cells with somata having diameters of 40–100 μm, with processes extending for more than twice the cell body diameter, were selected for electrophysiological recording. Dispersed cells were allowed to attach for 1 h and subsequently washed several times in ASW (or modified ASW if required by the experiment) and transferred to the stage of a Nikon inverted microscope. Glass pipettes were manufactured from borosilicate glass using a Sutter puller and fire-polished using a Narashige micro-forge. Pipettes had resistances in the range 300 kΩ to 2 MΩ when filled with pipette-filling media (Table 1). These pipettes readily formed seals in the 1- to 10-GΩ range and the whole cell configuration could be obtained by applying negative pressure or a combination of negative pressure and a brief (10–100 ms) pulse of depolarizing current. Access resistances were in the range 2–4 MΩ. The cells were voltage-clamped using an Axopatch 1D amplifier, and currents were recorded using the pClamp 6.0 data-acquisition suite (Axon Instruments), running on a Dell Dimension P166v personal computer. Data were analyzed using a combination of pClamp 6.02 (Axon Instruments) and Sigma-Plot 4.0 (SPSS) analysis software and Gnuplot v3.7 running on Red Hat Linux v6.0. All recordings were compensated for series resistance by 50–80% using the feed-forward circuit built into the amplifier. Greater compensation could not be used without causing saturation of the feedback amplifier. Thus a voltage error remained in some of our current recordings, which could amount to ≤20 mV for a 10-nA current after 50% compensation. The estimated worst-case error is given in the figure legends and in the text where appropriate. Capacitative and leak currents were subtracted on-line using the P/N protocol provided with the data acquisition software. N inverted copies of the test waveform (the amplitude of each being 1/N of the main trial) were applied before each trial. The sum of the currents evoked during these prepulses was subtracted from the currents recorded during the main waveform.

Salines and culture media

Salines and pipette-filling solutions were prepared as in Table 1. The volume of 1 M KOH required to balance the pH was measured to determine the final concentration of K⁺. Culture medium consisted of 75 mM ASW mixed with 25 mM Leibowitz L15 culture medium (Sigma, St. Louis, MO) and 50 μg/ml gentamycin sulfate to which 475 mg NaCl was added to balance the osmotic pressure to a value approximating that of sea water (~1 Osm).

Compartmental modeling

Neuronal modeling was implemented using the NEURON (version 4.3.1, John Moore, Michael Hines and Ted Carnevale) software obtained from Yale University running under a Linux Red Hat v7.0 operating system on a Dell OptiPlex GX1 personal computer. The neuron consisted of an isopotential sphere of 30 μm diameter, with capacitance 1.0 μF/cm². E_K and E_Na were set to -77 and +50 mV, respectively. The voltage dependence of each current was modeled using a modifications of a Hodgkin-Huxley formulation for inactivat-

<table>
<thead>
<tr>
<th>NaCl</th>
<th>KCl</th>
<th>CaCl₂</th>
<th>MgCl₂</th>
<th>EGTA</th>
<th>HEPES</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>295</td>
<td>10</td>
<td>1</td>
<td>50</td>
<td>—</td>
<td>10</td>
<td>7.5 (NaOH)</td>
</tr>
<tr>
<td>395</td>
<td>—</td>
<td>10</td>
<td>60</td>
<td>—</td>
<td>10</td>
<td>7.5 (CsOH)</td>
</tr>
<tr>
<td>50</td>
<td>400</td>
<td>1</td>
<td>50</td>
<td>11</td>
<td>10</td>
<td>7.5 (KOH)</td>
</tr>
<tr>
<td>50</td>
<td>—</td>
<td>400</td>
<td>1</td>
<td>—</td>
<td>10</td>
<td>7.5 (CsOH)</td>
</tr>
</tbody>
</table>

All values are in mM except for pH, which is dimensionless. ASW, artificial sea water.
ing sodium ($I_{Na}$) and potassium ($I_{K}$) currents and a non-inactivating potassium current ($I_{KDR}$). The values for the potassium currents were derived from Buckingham and Spencer (2000); those for sodium were obtained from physiological measurements reported in this paper. A passive conductance (0.001 S/cm²), representing a leakage current was added with a reversal potential of −70 mV. The sodium and inactivating potassium conductances were proportional to $m^3 h$ and the non-inactivating potassium conductance proportional to $n^4$.

States $n$ and $h$ for the potassium conductances were given by

$$n' = \frac{n_{inf} - n}{\tau_n}$$

$$h' = \frac{h_{inf} - h}{\tau_h}$$

where

$$n_{inf} = \frac{1}{1 + e^{(V-35.9)/15}}$$

and

$$h_{inf} = \frac{1}{1 + e^{(V-67.5)/15}}$$

The values for the time constants of activation ($\tau_n$) and inactivation ($\tau_h$) were taken from (Buckingham and Spencer 2000) and were

$$\tau_n = 0.94 \text{ ms} (I_{K})$$

$$\tau_h = 26.1 \text{ ms} (I_{KDR})$$

The half-activation potential for $I_{KDR}$ and $I_{K}$ was +16.9 mV with Boltzmann slope of the activation curve of −15, the half-inactivation potential for $I_{K}$ was −35.4 mV with slope 9.5.

The corresponding states for sodium are derived from the experimental results described in the following text.

In each simulation, the threshold for firing was determined to the nearest picoamp, and the firing rate for each increment in injected current measured over 100 or 1,000 ms until irregularities in the action potentials appeared, which current amplitude was taken as the maximum current that evokes stable, repetitive discharge. All data are presented as means ± SE.

RESULTS

Inward currents recorded in cultured brain cells

We determined the characteristics of transient, inward currents in freshly dissociated neurons in Na-ASW using the pipette-filling medium Na-pip (Table 1), which was designed to isolate sodium currents. A series of voltage steps (20 ms, 10-mV steps) from a holding potential of −70 mV to a range of potentials from −80 mV to +80 mV resulted in large, transient inward currents (Fig. 1A) being recorded in some (14 of 67) cells. The maximum voltage error for these currents was estimated as −14 mV. These currents were activated at potentials more positive than about −30 mV and reversed at +50 mV (Fig. 1A). A greater proportion of cells were seen to express these currents after ≥1 day in culture compared to freshly dissociated cells (Buckingham and Spencer 2000), suggesting that changes in ion channel expression occur during the culturing period. In addition to the rapidly inactivating currents, a non-inactivating component was also seen that was more pronounced in the outward direction. This could represent either a non-inactivating component of the sodium current or ionic currents that have not been eliminated using this protocol. This component was ignored on our analysis. The reversal potential of the current recorded in Na-ASW was similar to the equilibrium potential calculated (+46.7 mV) for sodium ions using the Nernst equation, suggesting that the current is carried by sodium ions.

Kinetic and steady-state properties of the sodium current

In ASW, the development of inward current in seven separate cells in response to depolarizing voltage steps (taken to be an estimate of the time constant of activation) could be fitted by a single exponential with time constant, $\tau_n$, given by

$$\tau_n = \frac{0.1979 + 0.04773}{0.518 + 0.07348 e^{0.16(0.80/V - 1)}}$$

where $V$ is the membrane potential (mV). The rate of inactivation, $\tau_h$, taken to be the time course of the decline of the inward current, was measured for seven separate cells and was fitted to the function

$$\tau_h = 0.672 + 1.39e^{-0.1115V}$$

Steady-state activation was determined from current-voltage data such as illustrated in Fig. 1A. The linear portion of the $I-V$ curve was fitted to a linear function and extrapolated throughout the membrane potential range from −80 to +80 mV. The proportion of the maximum current activated was taken to be the ratio of the measured current to the extrapolated maximum value. This ratio, plotted against the membrane potential, could be fitted to a sigmoidal curve of the form

$$\frac{G}{G_{max}} = \frac{1}{1 + e^{-(V-V_{50})/b}}$$

where $G/G_{max}$ = ratio of conductance to maximum conductance; $V$ = membrane potential; $V_{50}$ = half-activation potential; $b$ = slope (Fig. 1B). The Boltzmann slope factor ($b$) describes the steepness of the curve, such that a large value of $b$ corresponds to a less steep curve. Consequently, we refer to a large value of $b$ as a less-steep curve. Applying this expression individually to data from individual cells gave a half-activation value of +5.07 ± 2.41 mV ($n = 6$) and slope of +4.73 ± 0.44 mV/e ($n = 5$). Steady-state inactivation was measured using a paired-pulse protocol in which a range of 100-ms test pulses from −90 to +50 mV was applied to achieve steady-state inactivation, immediately followed by a 20-ms pulse to 0 mV. Steady-state inactivation levels were taken to be the ratio of the amplitude of the current response to the second pulse at each conditioning potential to the maximum response amplitude. This ratio, plotted as a function of the conditioning potential, could be fitted to data from individual cells with a sigmoid function with half-inactivation value of −14.42 ± 2.51 mV ($n = 4$) and slope of −6.03 ± 0.59 mV/e ($n = 4$).

Neuronal modeling

Measurements of half-activation potential, half-inactivation potential and time constants derived in the preceding text were applied to our compartmental model. Some simplifying assumptions were made based upon the limits of confidence in the physiological data. The time constant of activation of the inward current was difficult to measure accurately, and we
cannot exclude the possibility that its voltage dependence is due to inadequate space clamp. We have therefore modeled the sodium current with a voltage-independent time constant (0.22 ms) approximating to its value at +20 mV (0.28 ± 0.04 ms, n = 7). Similarly, the rate of inactivation was taken to be 0.821 ms, the predicted value for +20 mV (the measured value was 0.83 ± 0.13 ms, n = 7). At a maximum current density of 0.6 S/cm², this produced currents that reached a peak of around −6.5 nA and that resembled those recorded in whole cell voltage clamp, except, of course, that the development of the current was voltage independent. A maximum current density of 0.07 S/cm² for the non-inactivating potassium current produced peak currents of 10 nA at +80 mV, values similar to those recorded in cultured cells.

When sodium and the non-inactivating potassium currents were included in the model, injection of depolarizing current at just above spiking threshold gave rise to high-frequency action potentials (Fig. 2A) with a maximum sustainable frequency of ~320 s⁻¹ (Fig. 2B) and spike half-width of 1.58 ms. The range of current densities (the difference between the maximum and minimum) that could elicit repetitive firing was 0.039 nA/pF. When the inactivating current was added, there was a delay in the onset of firing (Fig. 2C) with little effect upon the frequency of firing, although the range of current densities that could elicit firing was slightly increased to 0.047 nA/pF (Fig. 2B). Because the presence of the inactivating current had little observable effect upon the firing frequency, all subsequent analyses were restricted to the sodium and the non-inactivating current. Raising the maximum current density of the non-inactivating potassium current to 0.14 S/cm² without changing the sodium current density increased the maximum firing rate to 430 s⁻¹ and the range of currents that evoked spikes to 0.09 pA/pF (Fig. 2B). Similarly, reducing the maximum potassium current density to 0.03 S/cm⁻² reduced the maximum firing frequency to 120 s⁻¹ and the range of currents that evoked spikes to 0.001 nA/pF.

The effects of adjusting the time constants of activation and inactivation were assessed to determine their effects upon spike frequency. Doubling the time constant of activation of the potassium current as well as the time constants for sodium activation and inactivation also increased the spike half-width to 3.33 ms, reduced the maximum firing rate to 190 s⁻¹ and only slightly increased the range of currents that evoked spikes to 0.048 pA/pF. Halving the time constants of both currents results in a reduced spike half width (0.79 ms), increased maximum firing rate (630 s⁻¹), but had little effect upon the

**FIG. 1.** Inward currents in voltage-clamped, cultured *Noto*-
*plana atomata* neurons. A: a series of depolarizing voltage steps elicits a transient, inward current when recorded under ionic conditions designed to isolate sodium currents (inset). The non-inactivating currents, which are more pronounced in the outward direction, may represent a genuine non-inactivating component of the sodium current or other ion currents that were not blocked by this protocol. The current/voltage plot of the peak current amplitude against the amplitude of the depolarizing step shows that the currents reach a peak around +10 mV and reverse around +50 mV. The estimated worst-case voltage error was 14 mV. The data points in the graph represent the mean of 5–6 independent measurements from separate cells, bars indicate SE. Cells were voltage-clamped at −70 mV and then stepped from −80 mV to +90 mV in 10-mV increments for 20 ms and then returned to −70 mV. B: steady-state activation (●) and inactivation (○, •) of the inward currents. Activation was determined by fitting the linear part of the current/voltage curves such as those illustrated in A to a linear function. The normalized steady-state activation was estimated to be the ratio of the measured current amplitudes at each membrane potential to the maximum current predicted by the linear function. Inactivation was measured by applying a 100-ms conditioning pulse to a range of potentials from −90 to +20 mV immediately before a test pulse to 0 mV. Inactivation was estimated as the ratio of the amplitudes of the current responses and plotted against the amplitude of the conditioning pulse. Both activation and inactivation of 5–6 individual cells were fitted to a Boltzmann function from which mean values for slope and V₅₀ were estimated. — and - - - , the plots of activation and inactivation respectively using these mean values.
range of currents that evoked action potentials (0.0384 pA/pF; Fig. 2D).

Effect of the rightward shift in activation and inactivation and of the wide activation range

The effect of the rightward shift was assessed by introducing a leftward shift of the activation curves of the potassium channel from $V_{50} = 16.9$ mV to $V_{50} = -33$ mV and of the sodium channel from $V_{50} = 5.1$ mV to $V_{50} = -35$ mV and the inactivation of $I_{Na}$ from $V_{50} = -14.4$ mV to $V_{50} = -54$ mV. This represents a shift of 50 mV in the leftward direction of the non-inactivating current followed by rounding to the nearest whole millivolt. Such a shift required a leftward shift in the other channels, but a simple 50 mV shift in these resulted in a model that failed to support regenerative spikes. Systematically changing the $V_{50}$ of activation and inactivation of the $I_{KA}$ and $I_{Na}$ currents revealed a range of values that supported spiking, which differed in the ranges of injected current over which they supported spiking, although all sustained very high maximum firing rates (data not shown). Such a search of parameter space was anchored to $I_{KDR}$ because this is the potassium current that plays the more major role in supporting spiking. The values for $V_{50}$ of activation and inactivation for $I_{KA}$ and $I_{Na}$ were chosen so as to sustain firing over a wide range. This leftward shift resulted in spikes firing at lower threshold, with spike width 1.32 ms, and an increase in both maximum firing frequency to 350 s$^{-1}$ and increased range of currents that evoke firing (0.074 pA/pF, Fig. 3). If the slope ($b$) of the potassium currents was adjusted from $-15$ to $-9$, thus making the activation curve steeper, while preserving their rightward shift, the peak firing rate was only slightly reduced (to 280 s$^{-1}$), but the range of current inputs for which the model could code was much reduced (to 0.014 nA/pF). Making the same adjustments to both the slope and the $V_{50}$ of activation and inactivation simultaneously gave a maximum firing frequency of 480 s$^{-1}$ and the range to 0.096 pA/pF.

Current-frequency relationship in cultured neurons

To provide a physiological comparison for the modeling, the dependence of the rate of action potential firing upon the amplitude of the injected current was determined for cultured neurons. Recordings were made in the whole cell mode under current clamp. Sufficient hyperpolarizing current was injected to keep the cells below the firing threshold. Depolarizing (relative to the holding current) current pulses (100 ms), increasing by 200-pA steps, were applied at 10- to 30-s intervals,
firing rate was \(85.93 \pm 42.62 \, \text{s}^{-1}\), \((n = 6; \text{Fig. 4B, Table 2})\). One cell had a peak sustained firing rate of \(150 \, \text{s}^{-1}\).

**Frequency/current relationships of cells recorded in situ**

A small proportion (≤10%) of cells impaled in the dorsal posterior brain could be induced to spike by the injection of current. Action potentials were brief (mean half width 0.91 ± 0.02 ms, \(n = 7\)) and overshooting (Fig. 4C) with threshold around −30 mV. Pulses of depolarizing current were applied incrementally and the mean firing frequency plotted against the amplitude of the injected current. The maximum frequencies that could be driven, given the limitations on the maximum current (100 nA) that could be passed by the amplifier, were \(\sim 107 \, \text{s}^{-1}\) (Fig. 4D).

**DISCUSSION**

**Inward currents in Notoplana neurons in culture**

Here we show the presence of at least one inward current in cultured neurons of *N. atomata* that is activated at −0 mV and inactivates rapidly. The reversal potentials are compatible with this inactivating current being carried by sodium ions. Like the potassium currents in the same cells (Buckingham and Spencer 2000), the inward current is rightward shifted with respect to steady-state activation and inactivation compared with most reported sodium currents, but the slopes of activation and inactivation are not markedly different from other Na\(^+\) currents. These currents activate and inactivate at remarkably high rates, suggesting a role in high-frequency firing.

**Modeling of action potentials**

Rather than model real neurons, the present work attempts to study the effects of adjusting K\(^+\) current parameters in isolation of other ion currents that are almost certainly present in neurons by using a minimal model cell.

The findings of the present study are summarized in Table 2, which compares spike shape and discharge patterns in cultured and in situ cells with the model, as well as the effects of changing current parameters. The rapid rates of onset of the currents forced us to assume that the rate constants of activation were voltage independent, at least for the purposes of the model. While we may be confident that such an assumption adequately describes the rate of development of currents in response to depolarizations, transitions at more negative potentials could not be obtained.

**TABLE 2. Summary of features of action potential shape and firing pattern**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In Situ Cells</th>
<th>Cultured Cells</th>
<th>Control</th>
<th>Slow</th>
<th>Fast</th>
<th>V Shift</th>
<th>Slope</th>
<th>Slope + Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum frequency, (s^{-1})</td>
<td>71.22 ± 35.18</td>
<td>85.93 ± 42.62</td>
<td>320</td>
<td>190</td>
<td>630</td>
<td>350</td>
<td>280</td>
<td>480</td>
</tr>
<tr>
<td>Minimum frequency, (s^{-1})</td>
<td>≥0</td>
<td>≥0</td>
<td>80</td>
<td>60</td>
<td>70</td>
<td>120</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Coding range, pA/pF</td>
<td>*</td>
<td>16.12 ± 8.37</td>
<td>39</td>
<td>48</td>
<td>38</td>
<td>741</td>
<td>14</td>
<td>96</td>
</tr>
<tr>
<td>10-90% Decay, ms</td>
<td>0.51 ± 0.02</td>
<td>0.75 ± 4.85</td>
<td>1.27</td>
<td>2.55</td>
<td>0.9</td>
<td>1.42</td>
<td>1.33</td>
<td>1.3</td>
</tr>
<tr>
<td>Spike half-width, ms</td>
<td>0.91 ± 0.02</td>
<td>1.03 ± 0.17</td>
<td>1.58</td>
<td>3.33</td>
<td>0.79</td>
<td>1.32</td>
<td>1.38</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Values are means ± SE. Control: model in which potassium and sodium currents of *Notoplana* are incorporated. Slow: model in which the time constants of activation and inactivation are doubled. Fast: model in which the time constants of activation and inactivation are halved. V Shift: voltage dependence of sodium and potassium current activation and inactivation are leftward shifted. Slope: steepness of the potassium current activation curve increased (reduced Boltzmann slope, b). Slope + Shift: sodium and potassium currents leftward shifted and steepness of potassium current is increased. * maximum frequency could not be obtained.
tentials may be slower or faster than predicted. This may affect the predicted membrane potential changes during the interspike interval, which in turn will influence the rate of firing.

Flatworm brain neurons are highly stereotyped in their expression of voltage-activated potassium (Buckingham and Spencer 2000) and sodium currents and in their firing patterns (personal observations). This approach, by systematically varying the recorded parameters of the sodium and potassium currents, indirectly explores the issue of possible heterogeneity in the neurons. These data show that varying the expression levels of the non-inactivating potassium current results in little changes in the maximum firing frequencies and that the presence of the inactivating current simply introduces a delay in the onset of firing in response to a prolonged depolarization. This is in contrast to the findings of other models (Connor and Stevens 1971; Rush and Rinzel 1995), which indicate that inactivating potassium currents can have profound effects upon firing pattern.

Modeling the rightward shifted ion currents has tested the assumption that the reported features of flatworm potassium channels (rightward-shifted activation curves, wide range of activation, rapid activation and deactivation) are the key determinants in sustaining high firing rates at high threshold. Here we have shown that it is possible to generate action potentials of the kind seen in all Notoplana neurons from which we have recorded using parameters derived from cultured Notoplana neurons. Furthermore, these models have shown that, like cultured neurons, action potentials can be generated at high frequencies. Firing frequencies in the model cell were very much higher than those obtained in situ. Some of this difference can be attributed to the fact that maximum firing frequencies in cultured and in situ cells may not have been reached due to limitations on the current that could be injected through the recording pipette. A greater part of the difference probably lies, however, in the assumptions made in the model. The model assumes an even distribution of ion channels over a cell, whereas in the case of real neurons the ion channels are likely to be selectively expressed on different parts of the neuron, while the shapes of the neurons are clearly not spherical. A further possibility is that there are additional currents not yet described that serve to slow the rate of depolarization and hence reduce firing frequency. It is also possible that calcium currents may contribute both to the depolarization stroke of the action potential and, indirectly through calcium-dependent potassium currents, to the repolarization. Calcium currents and calcium-dependent potassium currents are known to contribute to action potentials in some flatworm neurons (Keenan and Koopowitz 1984), and we have observed small, slowly inactivating inward currents in neurons patch-clamped in sodium-free medium (data not shown).

The failure of $I_{KA}$ to reduce firing rates is surprising in view of previously reported findings (Delord 1999; Locke and Narbonne 1997; Rathouz and Trussell 1998). The model of Connor et al. (1977) was able to fire at frequencies up to $>200\ mV^{-1}$ using a modification of the Hodgkin and Huxley (1952) model. The addition of transient $I_{KA}$ currents has been shown to allow lower, non-zero frequency firing (Av-Ron 1994; Connor et al. 1977) by prolonging the inter-spike interval (Connor and Stevens 1971; Huguenard and McCormick 1992) and can even lead to the generation of action potential bursting (Av-Ron 1994). In our model, the addition of the transient potassium current little affected the firing pattern, confirming our previous prediction (Buckingham and Spencer 2000) that the inactivation of $I_{KA}$ is not significant in determining action potential frequency. The addition of $I_{KA}$ to our model did, however, introduce a delay in the onset of firing due presumably to the transient activation of $I_{KA}$ providing a temporary opposition to depolarization. The rate of inactivation and recovery from inactivation (Buckingham and Spencer 2000) is too slow for participation in spike sculpturing. We therefore suggest that $I_{KA}$ plays a role in dendritic integration, such as the spatial control of current flow, and predict that the channels underlying $I_{KA}$ will be expressed locally at points on the dendritic tree.

The search of parameter space permitted by these models has shown different effects of the steepness of the activation curve and its voltage dependence. The effects of these parameter changes are easiest to describe in reference to Fig. 3. The “left-shift, steep-slope” curve represents a potassium current that has been adjusted to resemble more typical Shaker currents [around $-10\ mV$ for Shaker B (Papazian et al. 1991; Wei et al. 1990)]; $-28\ mV$ for inactivation-removed Shaker (Papazian et al. 1995) in its voltage dependence and steepness of the activation curve. The “steep activation slope” curve would thus represent a Shaker current that has had its voltage dependence shifted to the right. Viewed in this way, these two curves indicate that simply shifting a Shaker channel to the right would result in a loss of dynamic range. Similarly, the “control” curve could be viewed as a Shaker-like current which has been both rightward shifted and given a less steep activation curve (increased Boltzmann slope factor, $b$). This suggests that the shallow activation curve (large Boltzmann slope factor, $b$) of Notoplana potassium channels (Buckingham and Spencer 2000) is a requisite compensatory mechanism to allow a rightward shift without loss of dynamic range.

Increasing the steepness (reduced $b$ in the Boltzmann equation) of $I_{KDR}$ allowed lower firing frequencies independently of alterations in the voltage dependence. Interestingly, high-frequency firing was not lost upon shifting the voltage dependence of activation to the left but depended rather upon the kinetics of the ion currents, suggesting that whereas the rightward shift may well be an adaptation to high-frequency firing, it is not its cause. In contrast to the findings of Delord (1999), we found that a leftward shift in activation did not result in lowered firing rates, but a change in the steepness of the activation curve did.

The results of changing the steepness and the voltage dependence of activation suggest that simply introducing a rightward shift in voltage dependence of a Shaker channel would result in a restricted coding range (compare the leftward shifted, steep slope curve with the one that is rightward shifted, steep slope in Fig. 3). The shallow slope of activation seen in Notoplana and in Schistosoma (Kim et al. 1995) may therefore be a compensatory mechanism necessitated by a rightward shift in voltage dependence to preserve dynamic range. Experimental confirmation of this awaits discovery of mutations that independently alter slope and voltage dependence.

The cultured neurons and the model cell both fire at high frequencies, have short spikes and show little frequency adaptation. The current range over which the in situ cells responded were $-6.3$ compared to $39\ PA/pF$ for the modeled cell. The model was able to generate spikes at higher frequencies than cells recorded in situ and the cultured cells. This may simply
reflect differences in the respective recording conditions, such as limits in the amplitude of current that can be injected through a sharp, intracellular recording electrode. Alternatively, these differences may be due to cell morphology and/or the distribution of ion channels over the cell membranes or the presence of additional unidentified ion currents. It is possible that the simplification of the kinetics of the sodium channel may be responsible for the difference because a slower onset at lower membrane potentials might be expected to produce a slower rate of depolarization.

This model of the roles of slope and activation levels of rightward-shifted potassium channels predicts that *Schistosoma* neurons expressing the SKv1.1 channel (Kim et al. 1995) fire at high frequencies with a wide coding range and that interventions that reduce the steepness of the activation curve of these flatworm potassium channels would reduce the minimum firing frequency of flatworm brain neurons whereas interventions that introduce a leftward shift would lower the threshold for spiking without loss of high-frequency firing.

It will be necessary to clone and express a wide-range of potassium channels from all the major branches of early metazoans to establish what general features of potassium channels were selected for high-frequency firing in neurons.

REFERENCES


