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Phil. Trans. R. Soc. Lond. B 2000 **355**, 1191-1194
doi: 10.1098/rstb.2000.0665

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THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0665

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Deactivation, recovery from inactivation, and modulation of extra-synaptic ion currents in fish retinal ganglion cells

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As is shown magnificently by Heron Island's reef, the visual environment of many fishes includes various light intensities, hues and shapes that can change on large and small scales in space and time. Several articles in this issue address why fishes are sensitive to some of these properties, and how fishes and other aquatic species have acquired or fostered these sensitivities. This article discusses contributions of extra-synaptic ion currents, in a specific population of neurons, to the detection of ambient light levels, the appearance of certain visual stimuli and the disappearance of others.

Keywords: retinal ganglion cells; spikes; voltage-gated currents; leak conductances

1. INTRODUCTION

In fishes, as in other vertebrates, visually driven changes in the synaptic output of retinal photoreceptors and interneurons increase or decrease the probability that retinal ganglion cells—the retina's output neurons—produce action potentials. The production and control of these action potentials, also known as 'spikes', are of interest, because ganglion cells use them to encode different kinds of visual information, and because ganglion cells must do so efficiently (see Levick 1986).

Under what conditions do ganglion cells spike, and at a cellular level, what electrophysiological properties facilitate spiking in these cells? Some ganglion cells spike at rates related to luminance, many ganglion cells spike even in darkness, and certain ganglion cells respond to spatio-temporal differences in specific attributes of light within the visual field. The latter are dependent (at least more so than the former) on integration of excitatory and inhibitory synaptic inputs, as this enables ganglion cells to extract novel and useful information from signals formed at different positions across the distal retina, and to combine information from presynaptic cells that respond differently to changes in stimulus properties (see Barlow 1959; Sakai & Naka 1995). Voltage-gated, extra-synaptic ion currents (i.e. ion fluxes through transmembrane pores the opening and closing probabilities of which are controlled by the membrane potential of ganglion cells themselves, as opposed to being necessarily controlled by neurotransmitters released at synapses) enable ganglion cells to produce action potentials. These currents can contribute to the spike output of ganglion cells in several ways. To begin with, differences in the voltage sensitivity, kinetics, availability and distribution of inward and outward currents (for the most part, cation influxes and effluxes, respectively) will help determine how readily a cell will spike when it is initially depolarized, and when it could spike during repeated or sustained depolarizations. Since (i) these spikes occur at rates that are maintained under certain conditions, (ii) these rates do not increase without limits, and (iii) these spikes occur at rates that fade under certain conditions, the spike-generating mechanisms of ganglion cells also seem geared to shape the dependence of spiking on stimulus intensity, to low-pass filter some types of light-evoked responses, and to high-pass filter others. A brief summary of extra-synaptic current properties that might contribute to these aspects of spiking in ganglion cells, others that probably do not, and related questions that would be of interest to resolve, follows.

2. LINEARIZATION OF INTENSITY–FREQUENCY RELATIONSHIPS

Bipolar cells deliver (or direct) excitatory synaptic input to retinal ganglion cells. If this input is triggered by illuminating photoreceptors with step flashes of light, one finds that the current activated in ganglion cells by neurotransmitter released from bipolar cells becomes larger and faster with light intensity (e.g. Diamond & Copenhagen 1995). Both of these changes in synaptic input will depolarize ganglion cells to spike threshold faster, and in turn, the time between flash onset and the first ganglion cell spike will tend to become shorter. If the same lights are presented for tens of milliseconds or more, one finds that the rate of ganglion cell spiking increases with light intensity. One might, therefore, ask how ganglion cells respond to various amounts of depolarizing current.

It has long been known that exogenous injections of depolarizing current produce 'sustained' (i.e. continuous) firing of spikes in retinal ganglion cells, and that the frequency of these spikes increases linearly with the intensity of the depolarizing current even at low stimulus intensities (Baylor & Fettiplace 1979). This linearity differs from the convex (and at low stimulus intensities,

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discontinuous) relationship calculated from the voltage-gated Na^+ and K^+ currents described by Hodgkin & Huxley (1952). This implies that retinal ganglion cells are equipped with different or additional types of voltage-gated current.

Patch-clamp studies have shown the latter to include Ca^{2+} currents, Ca^{2+}-activated K^+ currents, transient K^+ currents and inwardly rectifying cation current (Ishida 1995, 1998). Some of these currents influence the operating range and spike frequency of ganglion cells. For example, combinations of inward and outward currents that are activated by sustained depolarizations limit the range of membrane potentials at which retinal ganglion cells dwell between spikes, and thus expand the range of light intensities these cells can respond to (Diamond & Copenhagen 1995). Whether different ganglion cells use the same subtypes of current to 'clamp' the interspike voltage to these levels, and what biophysical properties enable these currents to serve this function, remain to be measured.

Modelling studies have inferred that current flow between a retinal ganglion cell's soma and dendrites, and the charging and discharging of dendritic membrane capacitance, helps linearize the dependence of spike frequency on the intensity of current injections into somata (Fohlmeister & Miller 1997). Those studies showed that calculated current–frequency relationships resemble measured ones if resting membrane conductance is negligibly small, or very small and constant, and if voltage-gated conductances are also small at membrane potentials below spike threshold. While whole-cell conductances have been generally found to be small in ganglion cells at and near resting potential (see Ishida 1995, 1998), a resting permeability to Cl^- ions has recently been found to be modulated by a zinc-dependent protein kinase C activity in fish retinal ganglion cells (Tabata & Ishida 1999). The presence of this conductance in different compartments of ganglion cells, and the extent that changes in this conductance affect spiking, remain to be examined.

Toxins that block Ca^{2+}-activated K^+ currents increase spike firing rate in retinal ganglion cells (Fohlmeister & Miller 1997; Wang *et al*. 1998). However, spike firing can be slowed by several agents that reduce voltage-gated Ca^{2+} currents, including D_1 and $GABA_B$ receptor agonists, Co^{2+}, Cd^{2+} and the *Conus* toxins ω-MVIIC and ω-GVIA (Liu & Lasater 1994; Zhang *et al*. 1997; Rothe *et al*. 1999). These results are not easily reconciled at this time, in part because the cytoplasmic Ca^{2+} levels during the recordings cited here, and the Ca^{2+}-sensitivity of K^+ currents in retinal ganglion cells, are not known in detail.

3. LOW-PASS FILTERING

Retinal ganglion cells can fire action potentials as rapidly as $250\,s^{-1}$, particularly during transient flurries of spikes. Sustained firing rates have generally been found not to exceed $100\,s^{-1}$. Several voltage-gated current properties could limit the maximum spike frequencies in these cells.

One is the amount of Na^+ current that can be activated after single spikes. We have found that single spikes reduce the amount of Na^+ current that can be activated in ganglion cells by at least 75% (Hidaka & Ishida 1998), and that most of the Na^+ current that can be activated recovers from this 'inactivation' along an exponential time-course ($\tau = 3$ ms). To a first approximation, this fast rate of recovery from inactivation should not prevent ganglion cells from generating spikes as often as 100 Hz. We found similar rates of recovery in all of the cells we recorded from, suggesting that the Na^+ current in all retinal ganglion cells can support repeated firing of spikes, and that it can do so at similar maximum rates.

We have also found that a fraction of the Na^+ current in the same ganglion cells recovers more slowly from inactivation (Hidaka & Ishida 1998). We do not yet know if multiple spikes change the probability that Na^+ channels recover more slowly from inactivation. In other studies, Na^+ current amplitudes have been measured after terminating large-amplitude, step-wise depolarizations of varying duration. One study reported that Na^+ current recovers rapidly from inactivation in some cat retinal ganglion cells, and slowly in others (Kaneda & Kaneko 1991). Because Na^+ channel isoforms differ in this respect (e.g. Smith & Goldin 1998), one might infer that functionally different ganglion cells are equipped with structurally different Na^+ channels. This possibility is raised independently by the presence of mRNA for multiple Na^+ channel types in ganglion cells (Fjell *et al*. 1997). However, the recovery of Na^+ current from inactivation was found to be rapid in all adult rat retinal ganglion cells, and it was not exclusively slow in any case (Wang *et al*. 1997). Our results agree with those obtained from this latter preparation, but we do not exclude the possibility that slow recovery from inactivation shapes ganglion cell light responses.

In many cells, multiple spike firing is fostered by outward K^+ currents that begin to activate at membrane potentials that are more positive than those that activate inward currents. Because these 'high-threshold' outward currents accelerate spike repolarization, these reduce the time that Na^+ channels dwell at membrane potentials that inactivate them, and also returns them to membrane potentials that favour recovery from inactivation. However, fast spike firing would also require rapid deactivation of outward currents, to minimize the amplitude and duration of the hyperpolarization produced by the K^+ current, and to maximize the net influx of positive charge due to inward currents. K^+ current properties that foster repetitive spiking have been investigated by modelling (Fohlmeister & Miller 1997), and it will be of interest to compare the kinetics and voltage sensitivities of these against measured values.

In some neurons, a transient outward K^+ current reduces excitability because it is 'low threshold', i.e. because it begins to activate at more negative membrane potentials than Na^+ currents do. This effect requires that the outward current be 'primed' for activation (i.e. allowed to recover from inactivation) at suitably negative membrane potentials. If this happened after each spike of a sequence of spikes, then this type of current would reduce spike firing rate, and would also tend to linearize the intensity–frequency relationship alluded to above (§ 2) (Connor *et al*. 1977). However, this mechanism may not operate in retinal ganglion cells, because the activation threshold of the transient outward current has generally

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not been found to be more negative than that of the Na^+ current in these cells (see Ishida 1995).

4. HIGH-PASS FILTERING

Retinal ganglion cells spike transiently in response to various light stimuli. Some of these stimuli depolarize ganglion cells, so that the spike burst occurs at the appearance of these stimuli. Other stimuli hyperpolarize ganglion cells, and the spike burst occurs at the disappearance of these stimuli. Do these light responses reflect transient synaptic inputs, transient responses to that input, excitation abbreviated by inhibition, or transience in the spiking mechanism of ganglion cells? Two observations imply that, under certain conditions and to some extent, ganglion cells introduce transience into their own spike volleys. First, more than one configuration of light and dark can elicit transient spike firing, and thus spike bursts do not seem to require the activation of particular synaptic pathways. Second, a transient flurry of spikes can be recorded in ganglion cells after the termination of exogenous injections of hyperpolarizing current (see Tabata & Ishida 1996).

What voltage-gated current properties might render ganglion cell responses transient? One might be the rate at which Na^+ current recovers from inactivation at the membrane potential that ganglion cells repolarize to after a spike (or a spike burst). This rate could be finite but so slow that spikes would occur just after the onset of a depolarizing stimulus, but would not re-occur for the remainder of a brief stimulus presentation (Kaneda & Kaneko 1991). Alternatively, this rate could be so slow that ganglion cells would spike after the onset of a depolarizing stimulus, but could not spike again regardless of the duration of a maintained stimulus, as proposed for transient retinal amacrine cells (Barnes & Werblin 1986). However, these would not explain the observation that exogenous current injections elicit sustained spiking in ganglion cells even if light elicits transient spiking in the same cells (Baylor & Fettiplace 1979; see also Diamond & Copenhagen 1995; Wang *et al.* 1997).

A second current proposed as the basis for transient spiking during depolarizing light stimuli is the slowly inactivating K^+ current termed 'I_B' (Lukasiewicz & Werblin 1988). This possibility is attractive particularly because this current has been found in fish, amphibian and mammalian retinal ganglion cells (see Ishida 1995; Tabata & Ishida 1999). This possibility has been difficult to test, however, because a pharmacological agent that can block this current selectively has yet to be identified. It is also difficult to incorporate measured values of I_B into spike calculations, because it is not known whether the apparent amplitude and kinetics of I_B are altered by the compounds used to isolate this current (tetraethylammonium and 4-aminopyridine; see Ishida 1995).

Two other voltage-gated ion currents could transiently augment retinal ganglion cell excitability at the termination of hyperpolarizing light stimuli: T-type Ca^{2+} current ('I_T') and the inwardly rectifying, mixed cation current known as 'I_h' (see Ishida 1998). I_T would recover from inactivation while a ganglion cell was hyperpolarized by light, activate upon termination of the hyperpolarization, then inactivate unless the cell was hyperpolarized again (Bindokas & Ishida 1996). I_h would be activated by an initial hyperpolarization, and would continue to flow after termination of the hyperpolarization, until it deactivated (Tabata & Ishida 1996). Both of these currents would hasten the rate at which ganglion cells reach spike threshold (thus shortening spike latency) and briefly boost spike frequency. Because the voltage sensitivities and current densities of I_T and I_h both seem appropriate for producing spike bursts, what advantage would be gained by having both currents unless these were recruited by different stimuli? Salamander and turtle retinal ganglion cells suggest that there is no particular advantage (or that there are other ways to generate suitable responses), as these cells have measurable amounts of I_T and no detectable amounts of I_h. Most fish ganglion cells have both, however, and rat ganglion cells appear to as well (see Ishida 1998). Is it possible that I_T and I_h drive spike bursts after short and long hyperpolarizing light stimuli, respectively? This might occur, if I_T recovered from inactivation as rapidly as does Na^+ current after brief depolarizations (Hidaka & Ishida 1998), because I_h activates slowly (Tabata & Ishida 1996). However, in some neurons (see Huguenard 1996), I_T has been found to recover from inactivation about as slowly as I_h activates in retinal ganglion cells. This might also be true for retinal ganglion cells if the rate at which their T-type Ca^{2+} channels recovered from inactivation became slower with longer conditioning depolarizations (not unlike the effect, alluded to above (§ 3), of prolonged depolarizations on Na^+ channels).

5. CONCLUSION

Like many other central neurons, retinal ganglion cells are equipped with several different extra-synaptic ion currents. These include voltage-gated Na^+ current, three types of voltage-gated Ca^{2+} current, perhaps three types of K^+ current, I_h, a resting cation permeability, and a resting Cl^- permeability (Ishida 1995, 1998). Voltage-sensitive current properties that are likely to contribute to spike frequency and timing have been summarized here. These include the recovery of Na^+ and T-type Ca^{2+} currents from inactivation, and the recruitment of I_h. Also summarized are some current properties that do not appear to contribute in either event. These include the complete loss of Na^+ current at the membrane potentials that ganglion cells dip to between spikes, and the recruitment of a low-threshold K^+ current. Lastly, some current properties whose contributions to spiking remain to be studied in more detail have been summarized. These include the rate of K^+ current deactivation, the possibility that Na^+ current inactivation accumulates, the rate at which T-type Ca^{2+} current recovers from inactivation, and the magnitude of the resting Cl^- conductance. The functional importance of these properties will have to be weighed against the contributions of synaptic inputs and the electrical properties of other retinal neurons. It will also be of interest to compare the contribution of these voltage-gated ion currents to membrane potential changes before and after they have been modulated by messengers.

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The fish retinal ganglion cell recordings discussed here were supported by National Institutes of Health grant EY08120. I thank Vytas Bindokas, Toshihide Tabata, Soh Hidaka, Angela Pignatelli, Cecilia Fernandez-Vaquero, Katharine Munckton, Ming-Hsing Cheng and Gloria Partida, for contributing to these measurements. Also, I thank Shaun Collin and Justin Marshall for a splendid meeting at Heron Island.

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