Availability of Low-Threshold Ca\(^{2+}\) Current in Retinal Ganglion Cells

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Lee, Sherwin C., Yuki Hayashida, and Andrew T. Ishida. Availability of low-threshold Ca\(^{2+}\) current in retinal ganglion cells. J Neurophysiol 90: 3888–3901, 2003; 10.1152/jn.00477.2003. Spiking in central neurons depends on the availability of inward and outward currents activated by depolarization and on the activation and priming of currents by hyperpolarization. Of these processes, priming by hyperpolarization is the least described. In the case of T-type Ca\(^{2+}\) current availability, the interplay of hyperpolarization and depolarization has been studied most completely in expression systems, in part because of the difficulty of pharmacologically separating the Ca\(^{2+}\) currents of native neurons. To facilitate understanding of this current under physiological conditions, we measured T-type current of isolated goldfish retinal ganglion cells with perforated-patch voltage-clamp methods in solutions containing a normal extracellular Ca\(^{2+}\) concentration. The voltage sensitivities and rates of current activation, inactivation, deactivation, and recovery from inactivation were similar to those of expressed α1G (CaV3.1) Ca\(^{2+}\) channel clones, except that the rate of deactivation was significantly faster. We reproduced the amplitude and kinetics of measured T currents with a numerical simulation based on a kinetic model developed for an α1G Ca\(^{2+}\) channel. Finally, we show that this model predicts the increase of T-type current made available between resting potential and spike threshold by repetitive hyperpolarizations presented at rates that are within the bandwidth of signals processed in situ by these neurons.

INTRODUCTION

Low-threshold, T-type Ca\(^{2+}\) current (\(I_T\)) displays transient activation by depolarization when initiated from relatively hyperpolarized membrane potentials and inactivation during sustained depolarization (for review, Huguenard 1996; Perez-Reyes 2003). This combination of properties enables sustained depolarization (for review, Huguenard 1996; Perez-Reyes 2003). Whole cell and single-channel recordings of these clones in expression systems have shown that they differ substantially in their rates of activation, inactivation, and recovery from inactivation, while continuing to demonstrate the voltage dependence that characterizes this channel family. These studies of expressed channels have provided an explanation for the variation in kinetic properties and pharmacology found in studies of natural \(I_T\) (Huguenard 1996), and it has been possible, in a few instances, to match a specific cloned channel with the native channel in a particular cell (Chemin et al. 2002; Satin and Cribbs 2000). Still, accounting for the availability and influence of \(I_T\) during spiking in particular cell types requires measurements of these properties from native channel populations under meticulous physiological conditions.

The best studied of the native systems are the various classes of thalamocortical cells that exhibit low-threshold spiking. \(I_T\) was shown to support the low-threshold spike, both directly and in numerical simulations (Coulter et al. 1989; Crunelli et al. 1989; Huguenard and McCormick 1992; Huguenard and Prince 1992; McCormick and Huguenard 1992; Suzuki and Rogawski 1989; Wang et al. 1991). This and other work (Bal et al. 1995; Thomson 1988) have demonstrated that the ability of \(I_T\) to support this behavior depends critically on the interaction of cell membrane potential and the timing and frequency of hyperpolarizing inhibitory postsynaptic potentials (IPSPs).

To determine the properties that underlie this function and to examine the role of \(I_T\) in another type of central neuron, we report here on the voltage-dependent control and kinetics of \(I_T\) in retinal ganglion cells, a class of central neuron that has displayed this current in every species examined to date (rat: Guenther et al. 1994; Karschin and Lipton 1989; turtle: Liu and Lasater 1994; goldfish: Bindokas and Ishida 1996; cat: Huang and Robinson 1998; salmon: Henderson and Miller 2003). Activation threshold and inactivation kinetics, as well as some pharmacology, have been described for the \(I_T\) in retinal ganglion cells of postnatal rat (Guenther et al. 1994; Karschin and Lipton 1989), turtle (Liu and Lasater 1994) and goldfish (Bindokas and Ishida 1996), but the other biophysical properties of these currents have yet to be studied. To ensure that we obtained the basal properties of the current, we used isolated cells in short-term culture to avoid the influence of surrounding cells; we used perforated-patch methods to maintain cytoplasmic integrity; and we measured currents in normal physiological Ca\(^{2+}\) concentration to avoid surface charge effects and augmented current amplitudes. Under these conditions, we...
have found in goldfish retinal ganglion cells that the voltage sensitivities and rates of current activation, inactivation, and recovery from inactivation resemble those of T-type currents from a number of other tissues, and that deactivation is too to three times faster. We could reproduce the amplitude and kinetics of \( I_T \) activated by typical depolarizing voltage jumps with a kinetic model developed for an α1G (Ca\(_{v}3.1\)) Ca\(^{2+}\) channel clone. Moreover, we show that this model predicts the increase of \( I_T \) that can be recorded between resting potential and spike threshold after volleys of hyperpolarizations, as might occur during recurrent inhibitory input.

**METHODS**

The voltage-clamp currents described here were measured in retinal ganglion cell somata isolated from adult common goldfish (Carassius auratus). The methods used in this study have been described in detail elsewhere (Bindokas and Ishida 1996; Hidaka and Ishida 1998; Tabata and Ishida 1996, 1999). All animal care and experimental protocols were approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis. Cells were dissociated by brief incubation of freshly dissected retinas in either protease-containing saline or, in a few instances, an enzyme-free, low-Ca\(^{2+}\) solution (Hayashida, Partida, and Ishida; unpublished), followed by thorough rinsing in saline supplemented with bovine serum albumin and trituration with a large-bore glass pipette. Cells were identified either by retrograde labeling, via the optic nerve, of rhodamine B isothiocyanate-coupled dextran (Tabata and Ishida 1996) or by the nucleolar expansion observed exclusively in ganglion cells subsequent to crush of the optic nerve (Ishida and Cohen 1988).

We found no statistically significant difference between these cell groups in terms of the current properties reported here (activation threshold, kinetics, voltage sensitivity of steady-state inactivation, rate of recovery from inactivation). Therefore all data from these cells have been pooled. Voltage-gated Ca\(^{2+}\) current was recorded in perforated-patch mode (Horn and Marty 1988), using an Axopatch-1D patch-clamp amplifier (Axon Instruments, Union City, CA) and pCLAMP software (v. 8.1.01, Axon Instruments) for voltage-jump protocol generation and data acquisition.

Patch electrodes were pulled from borosilicate glass capillaries to tip resistances of ca. 5 MΩ. The tips of these pipettes were filled with either of two “pipette solutions.” One formulation (ca. 60% of experiments) contained (in mM) 15 Na methanesulfonate, 120 CsOH, 30 tetraethylammonium chloride (TEA), 1 ethylene glycol tetraacetic acid (EGTA), 0.34 CaCl\(_2\), 2.6 MgCl\(_2\), and 5 HEPES; pH adjusted to 7.40 with methanesulfonic acid. The second formulation (ca. 40% of experiments) was the same except that TEA was replaced with 30 CsOH, 15 sucrose, and additional methanesulfonic acid to adjust pH. We observed no differences between the Ca\(^{2+}\) currents recorded with these pipette solutions. The osmolarity of both solutions was 285–290 mosmol/kg. The pipette shanks were filled with the same solution supplemented with a 1:200 dilution of a mixture of 2 mg amphotericin B and 3 mg Pluronic F-127 (Molecular Probes; Eugene, OR) in 60 μL DMSO.

The extracellular (“bath”) solution contained (in mM) 120 NaCl, 3 CsCl, 2.5 CaCl\(_2\), 1 MgCl\(_2\), 30 TEA-Cl, 10 glucose, and 5 HEPES. The pH was adjusted to 7.40 with NaOH, and the osmolarity was 290–300 mosmol/kg. Just prior to use, the bath solution was supplemented with 1 μM tetrodotoxin.

Reagents were obtained from the following sources: Na methanesulfonate, methanesulfonic acid (Aldrich Chemical, Milwaukee, WI); CsOH, CsCl (ICN, Aurora, OH); NaCl, TEA, MgCl\(_2\), EGTA, HEPES, amphotericin B, and DMSO (Sigma Chemical, St. Louis, MO); CaCl\(_2\) (BDH; Dorset, UK); and tetrodotoxin (Sanko, Tokyo).

Experiments were performed at 20–24°C within 36 h of cell isolation. Recordings were made from cells adhering to a glass coverslip attached to the bottom of a 35-mm tissue culture dish. Cells recorded the day of isolation or after overnight culture gave equivalent results; there was no drift in T-current density or properties with time in culture. The current monitor output of the patch-clamp amplifier was analog-filtered by the built-in 4-pole Bessel filter (\( F_c = 2 \) or 5 kHz) and digitally sampled (usually at 10 kHz). An agar bridge was used to ground the bath solution, and the membrane potentials reported here have been corrected for liquid junction potentials that arose from differences between the bath and pipette solutions. Linear leak subtraction was performed with the P/N protocol in pCLAMP, using holding and command potentials that were never more negative than −100 mV, and never more positive than −60 mV. Series resistance compensation was not used.

Data were analyzed in pCLAMP and Excel (Excel 2000; Microsoft, Redmond, WA), and data curves were fitted in SigmaPlot (version 5.0.5; SPSS, Chicago, IL). Means are presented as ±SE.

Computer simulations were carried out using the simulation software NEURON (ver. 4.3.1 and 5.2 by J. W. Moore, M. Hines, and T. Carnevale) (see Hines and Carnevale 1997, 2000). Because the dendrites and axons of our retinal ganglion cells were typically shorn off by the dissociation procedures, leaving just the isolated somata, we modeled the ganglion cell as a single spherical compartment, 20 μm in diameter.

Current flow through the T-channel in the open state (\( I_{T,\text{O}} \), units of A/cm\(^2\)) was calculated with an equation

\[
I_{\text{O}} = \frac{P_{\text{Ca}} F z}{RT} \cdot \frac{\exp(-\frac{zFV_m}{RT})}{1 \cdot \exp(-\frac{zFV_m}{RT})} \cdot \frac{[\text{Ca}^{2+}]_{\text{in}} - [\text{Ca}^{2+}]_{\text{out}}}{\exp\left(-\frac{zFV_m}{RT}\right)}
\]

as used in other studies (e.g., De Schutter and Smolen 1998; Hille 1984; Huguenard and McCormick 1992). Here, \( P_{\text{Ca}} \) is the maximum permeability (units of cm/s); \( O \) is the fraction of channels in the open state (see following text); \( z \) is the valence (+2); \([\text{Ca}^{2+}]_{\text{in}} \) and \([\text{Ca}^{2+}]_{\text{out}} \) are Ca\(^{2+}\) concentrations inside and outside the cell; \( V_m \) is the membrane voltage; \( F \), \( R \), and \( T \) are the Faraday constant, gas constant, and absolute temperature, respectively. In this equation, the concentration-dependent rectification of current is described by the Goldman-Hodgkin-Katz constant field equation (Lewis 1979). Because our experiments utilized physiological concentrations of internal Na\(^{+}\) (15 mM) and extracellular Ca\(^{2+}\) (2.5 mM) and were confined to a limited range of negative membrane potentials, we could ignore monovalent cation permeability through the T channel (Fukushima and Hagiwara 1985; Lux et al. 1990). Similarly, as we used the same physiological concentration of external Mg\(^{2+}\) (1 mM) throughout, we did not explicitly incorporate the relatively minor effect of Mg\(^{2+}\) block on the T channel (Fukushima and Hagiwara 1985; Serrano et al. 2000). In the present simulations, an estimate of \( \frac{7}{10} \times 10^6 \) cm/s was used for \( P_{\text{Ca}} \) to produce 5 pA/pF of current density (see RESULTS); and \([\text{Ca}^{2+}]_{\text{in}} \) and \([\text{Ca}^{2+}]_{\text{out}} \) were assumed to be constant at 100 nM and 2.5 mM, respectively. Within the time scale of our simulations, the amplitude and kinetics of calculated currents were not markedly altered by changes in \([\text{Ca}^{2+}]_{\text{in}} \) between 10 nM and 1 μM (data not shown).

The fraction of channels in the open state was calculated using a Markovian kinetic model shown in Fig. 8A. The kinetic scheme was adapted from one developed for a cloned T-channel (Serrano et al. 1999). In brief, the channel occupies any of 12 states (5 closed states, 1 open state, and 6 inactivated states). As membrane potential depolarizes, the probability of channel occupancy is higher for the states in the right-hand side of figure. The transitions through closed states (\( C_1 \) to \( C_5 \)) and most of the inactivated-inactivated transitions (\( I_1 \) to \( I_5 \)) are voltage dependent. The closed-open transition (\( C_5 \) to \( O \)) and one inactivated-inactivated transition (\( I_5 \) to \( I_{5,\text{O}} \)) are voltage-independent. The closed/open-inactivated transitions (\( C_5 \), \( I_6 \) to \( I_{6,\text{O}} \); parallel vertical transitions in figure) are also voltage independent. However, for
the first three closed-closed transitions ($C_1$ to $C_3$), the corresponding inactivation process is coupled allosterically to activation (cf. Kuo and Bean 1994). Thus inactivation develops with membrane depolarization, and saturates as the channel reaches the last two closed states and open state.

In this model, there are a total of 10 free parameters to be estimated. The constraints on parameter estimation were as follows: 1) $k_{o0}$, the rate of voltage sensor movement for activation, was selected to match the time-to-peak at a depolarized potential (~37 mV, Fig. 2A); 2) $k_{c0}$, the rate of voltage sensor movement for deactivation, was selected to reproduce $V_{1/2}$ for the current activation curve (Fig. 5B); 3) $V_k$, $V_{c}$, $V_{o}$, the gating charge parameters associated with voltage sensor movement, were selected to generate the slope factor for the $I-V$ relation (Fig. 5B); 4) $k_{o}$, the channel opening rate, was set to match the time-to-peak at an intermediate potential (~52 mV, Fig. 2A); 5) $k_{c0}$, the channel closing rate, was determined directly from the time constant of deactivation at a hyperpolarized potential (Fig. 3B); 6) $k_{o}$, the inactivation rate, was taken directly from the time constant of the current decay at a depolarized potential, extrapolated from Fig. 2B; 7) $k_{c}$, the reverse rate of inactivation, was set to reproduce $V_{1/2}$ for the steady-state inactivation curve (Fig. 5B); 8) $f$, the allosteric coupling factor for inactivation, was selected to yield the time constant of entry to inactivation measured near resting potential (Fig. 4C); 9) $h$, the allosteric coupling factor for recovery from inactivation, was selected to reproduce the time constant of recovery from inactivation measured at a hyperpolarized potential (Fig. 7B). When simulations were repeated to optimize the parameter values, the values of $k_{o0}$, $V_k$, $V_{o}$, $V_{c}$, and $k_{c0}$ were adjusted first, and then $k_{o}$, $f$ and $h$ were tuned. The iteration and annealing were carried out manually until all constraints were reasonably satisfied.

When comparing the model to a specific data set, time constants for the rise and fall of command voltage steps were set equal to the charging time constant measured from that cell. In all simulations, the temperature and the calculation time step were set to 23°C and 2–5 μs, respectively.

Results

We describe here the following properties of low-threshold Ca$^{2+}$ current ($I_T$) in retinal ganglion cells: voltage dependence and kinetics of activation and deactivation, voltage sensitivity of steady-state inactivation, rate of inactivation near resting potential, and rate of recovery from inactivation. To measure these properties, current was elicited by step-wise depolarizations from holding potentials more negative than ~65 mV, to test potentials more positive than ~65 mV (Fig. 1); 2) it inactivated markedly, if not entirely, when the holding potential was shifted to values more positive than ~60 mV (Fig. 5A); and 3) it was kinetically transient, i.e., it decayed during test depolarizations of 100 ms (Fig. 1). This T-type current is greatly reduced by lowering extracellular Ca$^{2+}$ (data not shown), and it can be abolished by replacement of the extracellular Ca$^{2+}$ with a mixture of 2.4 mM Co$^{2+}$ and 0.1 mM Ca$^{2+}$ (Bindokas and Ishida 1996). It is insensitive to the spider toxin ω-Aga-III (Bindokas and Ishida 1996), so it is unlikely that any of it is the R-type Ca$^{2+}$ current that sometimes shows overlapping voltage dependence and kinetic properties (Randall and Tsien 1997). Most (ca. 75%) of the cells recorded from in this study exhibited low-threshold Ca$^{2+}$ current meeting these criteria, as we have reported previously (Bindokas and Ishida 1996).

All of the currents reported here were recorded under conditions designed to minimize contamination by other voltage-gated currents, optimize recording stability, and avoid space-clamp artifacts due to neurites. Voltage-gated Na$^+$ current was blocked by inclusion of 1 μM tetrodotoxin in the bath (Hidaka and Ishida 1998); hyperpolarization-activated cation current ($I_h$) was blocked by the presence of 3 mM Cs$^+$, and the absence of K$^+$, in the bath (Tabata and Ishida 1996); outward K$^+$ currents were minimized by use of a Ca$^{2+}$-based, K$^+$-free pipette solution and by inclusion of 30 mM TEA in the bath (Tabata and Ishida 1999); activation of high-threshold Ca$^{2+}$ current and of TEA-resistant Cs$^+$ efflux were avoided by use of sufficiently negative test potentials (Bindokas and Ishida 1996; Tabata and Ishida 1996, 1999); and leak-like Cl$^-$ current was minimized by use of perforated-patch recording mode (Tabata and Ishida 1999). Perforated-patch mode was also used to avoid drifts in the voltage sensitivity of gating that occurred during ruptured-patch recording (Munckton, Pignatelli and Ishida, unpublished observations) and allay concerns about possible “rundown” (Wan et al. 1996). We restricted our investigation to voltages negative to ~35 mV to avoid contamination by high-threshold Ca$^{2+}$ current (see following text). Recordings showing signs of inadequate space clamp (in particular, delayed activation or distorted $I-V$ relations) were discarded from the data set. For this reason, we did not study cells bearing neurites longer than 10 μm or so, and we did not attempt to compare the properties of currents recorded from neurite-free cell bodies versus cells with significant amounts of neurites. Instead, we limited our analyses to the amplitude and kinetics of currents where we felt our control of membrane potential was the best possible. As such, our study is concentrated on somatically expressed T current, and we cannot say anything about currents derived from other cell locations (Ahlijanian et al. 1990; Baldridge 1996; Henderson and Miller 2003). Even with all these precautions, about 1/2 of all cells with low-threshold current were discarded because of the presence of slower kinetic components in the voltage range we used to characterize $I_T$. This left 103 cells that formed the basis for the following analysis.

![Figure 1](http://jn.physiology.org/DownloadedfromJNeurophysiol-VOL90-DECEMBER2003-WWWJNORG)
Activation range

The range of membrane potentials that activate $I_T$ was measured by the voltage-jump protocol shown at the top of Fig. 1. Holding potential was set to the most negative value at which stable recordings were routinely obtained (-92 mV), and cells were depolarized to test potentials between -62 and -37 mV. Current versus voltage ($I$-$V$) curves were constructed by plotting the peak amplitude of the Ca$^{2+}$ current versus the test potential at which the Ca$^{2+}$ current was activated. To facilitate comparison of data collected from different cells, current amplitudes were normalized to the peak amplitude obtained in response to the step to -37 mV recorded in each cell. The normalized $I$-$V$ relation for the data in Fig. 1 is plotted in the inset and fitted by Marquardt-Levenberg regression to the Boltzmann equation

$$I = \frac{I_{\text{max}}}{1 + \exp \left( \frac{V - V_c}{V_\text{c}} \right)}$$

where $I$ is peak current at each test voltage, $I_{\text{max}}$ is peak current at -37 mV, $V$ is the test voltage, $V_{1/2}$ is the midpoint of the Boltzmann fit, and $V_c$ is the voltage for an e-fold change around $V_{1/2}$ (i.e., slope factor). For this cell, $V_{1/2}$ is -53 mV and $V_c$ is 4.3 mV.

In most cells, we observed the onset of a slowly inactivating inward component at test potentials more positive than ca. -37 mV. This portion of the whole cell current was considered to be distinct from low-threshold Ca$^{2+}$ current because it was high threshold and was only partially reduced in amplitude by depolarized holding potentials (see Bindokas and Ishida 1996). Because high-threshold Ca$^{2+}$ currents were larger when elicited from a holding potential of -92 mV than from -62 mV, even in cells with no demonstrable $I_T$, we could not subtract high-threshold current elicited from -62 mV to remove contamination, by high-threshold current, from $I_T$ elicited from -92 mV. Moreover, we found previously that there was not a satisfactory mix of blockers that could cleanly isolate $I_T$ from the other Ca$^{2+}$ currents in goldfish retinal ganglion cells (Bindokas and Ishida 1996). Hence, we exploited the differential voltage dependence of $I_T$ activation to assess $I_T$ properties by using currents collected only at command potentials that did not elicit noticeable amounts of high-threshold Ca$^{2+}$ current, as in other studies of native cells (e.g., Biagi and Enyeart 1991; Carbone and Lux 1987). In most cells, the current elicited by the step to -37 mV is probably not quite the maximum for $I_T$. The Boltzmann fit in Figs. 1 and 5B suggests the true maximum current would occur between -35 and -30 mV. A more direct measure of channel activation would be to normalize peak current by the instantaneous current (e.g., Herrington and Lingle 1992; Serrano et al. 1999). However, our inability to isolate $I_T$ across a wider voltage range and the inherent limitations of perforated-patch recording prevented us from determining instantaneous currents. Compared with Figs. 1 and 5B, we would expect the channel activation curve to be broader and show attainment of maximum activation at a slightly more positive potential, as shown by the simulated activation curve corrected for the calculated Ca$^{2+}$ driving force (dashed line) in Fig. 9C (see following text). These curves also indicate that the error introduced by our use of the peak $I$-$V$ relation is small at the hyperpolarized potentials where both channel activation and total $T$ current are small.

At the test potentials used here (between -67 and -37 mV), $I_T$ ranged in amplitude up to ~200 pA. However, large currents were unusual, and the median peak current at -37 mV was 35 pA [44 ± 3.3 (mean ± SE) pA; n = 103]. Division of the maximum current amplitude recorded in each cell by the membrane capacitance, yielded current densities of 3–5 pA/PF [5 ± 0.7 pA/PF for cells identified by retrogradely transported dextran (n = 9); 3 ± 0.3 pA/PF (n = 94) for cells identified morphologically (see METHODS)].

Because $I_T$ was small in most cells, background noise could interfere with our ability to measure the voltage at which current first activated. Typically, we needed ca. 3 pA of inward current to detect this point of threshold. To ensure the sensitivity of this determination, we confined our analysis of activation threshold to cells with larger peak current (>60 pA at -37 mV), such that the current required to identify the threshold was not more than 5% of the peak. The activation threshold in such cells was -61 ± 0.5 mV (n = 21). This is consistent with the $I$-$V$ relation for the cell in Fig. 1 and for the average $I$-$V$ relation of nine other well-characterized cells shown in Fig. 5B (for these 9 cells: peak current -66 ± 9 pA at -37 mV; measured threshold -60 ± 0.8 mV). [The Boltzmann fit to the $I$-$V$ plot for these cells meets the abscissa at around -70 mV (Fig. 5B), suggesting that small amounts of $T$ current might activate at membrane potentials more negative than the measured threshold.] The test potential that elicited half-maximum current amplitudes ($V_{1/2}$) in these nine cells was -51 ± 0.5 mV, and near this voltage the current amplitude grew e-fold in amplitude every 4 ± 0.2 mV ($V_c$, see Fig. 5B).

Activation, inactivation, and deactivation kinetics

The rate at which $I_T$ rose in amplitude was measured by the time-to-peak (time between the onset of the command depolarization and the current peak) as a function of voltage as shown in Fig. 2A. This yielded a smoothly increasing rate of rise with stronger depolarization. The rate at which $I_T$ fell in amplitude at these test voltages was estimated by the exponential time constant that fit the declining phase of currents (Fig. 2B). Stronger depolarizations produced faster decay of current until reaching a plateau at ca. -40 mV. The extrapolated limiting time constant for this inactivation was 15.4 ms.

To determine the rate of deactivation, $I_T$ was activated by a brief step to -42 mV, and the repolarization-induced decay of the current was recorded at potentials from -62 to -92 mV (Fig. 3A). Because deactivation proved to be a fairly rapid process, we restricted our analysis to cells in which the charging time of the cell (approximated by the product of access resistance and cell capacitance) was <0.5 ms. To reduce the access resistance and obtain the best quality measurements, some of these data were acquired in ruptured patch configuration immediately after patch rupture and before any significant change in the voltage dependence of $I_T$ properties. Figure 3B was generated from pooled perforated and ruptured patch data (7 ruptured and 5 perforated measurements as in A in 5 cells). Deactivation was faster as the final membrane voltage was made more negative. The average deactivation time constant at -92 mV was 0.82 ms.
potential was maintained for 15 s prior to initiating the test depolarizations, i.e., for a period well in excess of the inactivation time constant measured in Fig. 4. The current at each test potential from each holding potential was normalized to the current elicited by depolarizations from −92 mV to the same test potential, and fitted by least-squares regression to the Boltzmann equation (Fig. 5A, inset).

For the data in Fig. 5A, the conditioning potential that reduced the Ca$^{2+}$ current to half-maximum amplitude (i.e., $V_{1/2}$) was −74 mV. Near this voltage, the current amplitude fell e-fold every 3.9 mV. We found no dependence of the normalized currents on whether the holding potential was varied from −92 to −62 mV or from −62 to −92 mV. The average steady-state inactivation of nine cells (including this one) is plotted in Fig. 5B. Because we found no difference between the normalized currents collected at a test potential of −52 versus −42 mV, the average steady-state inactivation curve in Fig. 5B includes normalized current amplitudes that were measured at both test potentials. The average $V_{1/2}$ for steady-state inactivation was −77 ± 0.9 mV and the average slope factor was 4 ± 0.2 mV. In three cells, $I_T$ was assessed from a holding potential of −102 mV. Currents activated by depolarizations from −102 mV were only ~3% larger than those activated in the same cells from −92 mV. The normalization error resulting from the

**Rate of inactivation near resting potential**

Little or no T-type current is available for activation if the holding potential is more positive than −65 mV. Because this is near the resting potential of spiking neurons (including retinal ganglion cells; see DISCUSSION), we measured the rate at which $I_T$ inactivates (i.e., becomes unavailable for activation) at these voltages. To do this, we depolarized cells from a holding potential of −92 mV to a conditioning potential equal to a typical resting potential (−62 mV) using the voltage-jump protocol shown at the top of Fig. 4. To assess the rate of inactivation at this voltage, we measured the amount of Ca$^{2+}$ current available for activation after conditioning depolarizations of various durations by depolarizations to a fixed test potential (e.g., Fig. 4, A and B). The amplitude of these currents was normalized to the amplitude of current activated from −92 mV and plotted against the duration of the conditioning depolarization to −62 mV. In all of three cells tested, plots of this type were fitted by an exponential decline in amplitude with an average time constant of 146 ± 25 ms (e.g., Fig. 4C).

**Steady-state inactivation**

The voltage sensitivity of inactivation was measured by the voltage-jump protocol shown in Fig. 5A, top. The holding potential was shifted to various values between −92 and −62 mV, and the amount of Ca$^{2+}$ current available for activation was measured by depolarizing cells from each holding potential to a test potential of either −52 or −42 mV. Each holding

**FIG. 2.** $I_T$ activation and inactivation kinetics. A: time to peak current as a function of test voltage. B: time constant of decay of the current as a function of test voltage as determined from a simple exponential fit. •, mean; error bars represent ±1 SE; n = 19 cells. —, simple exponential fits.

**FIG. 3.** $I_T$ deactivation kinetics. A: $I_T$ decay currents activated by the voltage protocol shown (top). Each trace is the average of 3 trials with P/N leak subtraction. A period approximately equal to the charging time of this cell (0.25 ms for 30 MΩ access × 8.4 pF capacitance, perforated patch) was blanked at the beginning of the repolarization. The traces were staggered to avoid overlap, and a sample exponential fit is shown in the lower right (−92 mV). Fitting was done in Clampfit. B: average deactivation time constants as a function of voltage. —, a simple exponential fit.

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have been reported for carp, salamander, turtle, guinea pig, and cat retinal ganglion cells of a variety of functional subtypes (Baylor and Fettiplace 1979; Coleman and Miller 1989; Demb et al. 2001; Mittman et al. 1990; Murakami and Shimoda 1977; O’Brien et al. 2002; Wiesel 1959). In this voltage region, only a few percent of $I_T$ is not inactivated. To estimate how quickly the availability of $I_T$ can increase, we measured the rate at which it recovers from inactivation at a hyperpolarized potential. Because this required us to compare test current amplitudes against control amplitudes, we used two different protocols to guard against effects of the control depolarization on the rate of recovery itself. In both, the holding potential was set to $-92$ mV, and $I_T$ was elicited by a test depolarization that started at various times after the end of a conditioning depolarization. In the first method, shown schematically at the top of Fig. 6A and with representative data in Fig. 6, $A$ and $B$, the

use of $-92$ mV for the maximum current was therefore considered to be negligibly small.

Currents activating at membrane potentials more negative than the voltages that produce complete steady-state inactivation are termed “window” currents (Attwell et al. 1979). Superimposing the average peak $I-V$ relation on the average steady-state inactivation curve provides an estimate of the voltage range for a window-type Ca$^{2+}$ current in retinal ganglion cells (Fig. 5B). In the cells we recorded from, the overlap between these two curves was maximal at $-64$ mV. At this voltage, $\sim 5\%$ of $I_T$ is not inactivated (see DISCUSSION).

Recovery from inactivation

The normal resting potential of these retinal ganglion cells is in the range of $-60$ to $-75$ mV (Vaquero et al. 2001; C. F. Vaquero and A. T. Ishida, unpublished results); similar values
inactivation at −62 mV (119 ± 11 ms, n = 8) or more depolarized potentials (112 ± 9 ms, n = 9). In three cells in which both protocols were performed, the time constant was 116 ± 9 ms when inactivation was induced by depolarizations to −62 mV versus 104 ± 11 ms when inactivation was induced by depolarizations to −52 or −42 mV. Combining all data obtained by both protocols gives an average time constant for recovery of 115 ± 7 ms (n = 17).

We generally observed that the recovery from inactivation was well-described by a single exponential time constant. In some other preparations, the recovery from inactivation of \( I_T \) is best described by two exponentials, with the faster component being most comparable to that observed here (Huguenard 1996). The amount of the slower component is increased by extending the length of the inactivating voltage step. However, we did not see evidence for a second recovery component in experiments comparing inactivation due to a 0.1 s step to −42 mV versus 3 s at −62 mV. We did not test the effect of holding the potential for many seconds at depolarized potentials, and we explicitly avoided any such time dependence in the determination of steady-state inactivation by holding at each potential for a long time.

**Kinetic model for \( T \) current in isolated retinal ganglion cells**

The preceding experiments characterized the fundamental kinetic properties of the goldfish retinal ganglion cell \( I_T \). To assess whether these properties are consistent with those es-

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**FIG. 6.** Recovery from resting potential \( I_T \) inactivation. A: overlaid currents elicited by the voltage protocol (top). From \( E_{hold} \) (−92 mV), a control current was generated by a 50-ms test step to −47 mV; after 2.5 s at −92 mV, the potential was set at −62 mV for 3.0 s and then stepped back to −92 mV for varying lengths of time (\( t_f \)) before testing the recovery of \( I_T \) with another 50-ms test step to −47 mV. These traces are the average of 2 identical protocols performed in this cell, with P/N subtraction. B: at higher resolution, the currents elicited by the test step to −47 mV after the indicated times at −92 mV. C: plot of the peak current activated by each test step, divided by its matched control vs. time at −92 mV (○) for this cell. —, simple exponential fit (\( \tau \) 76 ms), described in the text.

**FIG. 7.** Recovery from depolarized potential \( I_T \) inactivation. A: overlaid currents produced by the voltage protocol (top). From \( E_{hold} \) (−92 mV), \( I_T \) was measured and subsequently inactivated by a 100-ms test step to −42 mV; cell voltage was then stepped back to −92 mV for varying lengths of time (\( t_f \)) before testing the recovery of \( I_T \) with another test step to −42 mV for 30 ms. With P/N subtraction. B: plot of the peak current activated by each test step, divided by its matched control vs. time at −92 mV (○); this is the average of 3 identical protocols, 1 of which is shown in A. —, a simple exponential fit (\( \tau \) 121 ms) described in the text.
tablished for other T currents and to provide a tool for predicting the behavior of I_T in different circumstances, we developed a numerical model for I_T using the simulation program NEURON (see METHODS). Previous studies have shown that Hodgkin-Huxley models are valid as practical descriptions of whole cell I_T (Huguenard and McCormick 1992; Wang et al. 1991; see also Destexhe and Huguenard 2000), although they are not mechanistically accurate, especially in their description of inactivation processes. The recent cloning of T channels allowed detailed studies of their biophysical properties (Cribbs et al. 1998; Lee et al. 1999a; Perez-Reyes et al. 1998) and provided sufficient experimental data to develop plausible kinetic models of their behavior (Burgess et al. 2002; Frazier et al. 2001; Serrano et al. 1999). A similar kinetic model has also been applied to the I_T of a native neuron (Kuo and Yang 2001). In the following sections, we show that, with one specific modification, the Markovian kinetic model previously developed by Serrano et al. (1999) for a cloned T channel can generate satisfactory fits to our experimental data and that these fits extend over a variety of conditions.

Figure 8A shows the kinetic scheme for gating of I_T used in the present simulation. The behavior of this model is determined by 10 parameters (Table 1). The numerical values of some of these parameters were estimated directly from the present experimental data, whereas others were derived from iterative optimization of the overall model within the constraints imposed by our data (see METHODS for the fitting procedure). The qualitative characteristics of these parameters are the same as those described by Serrano et al. (1999; especially their Fig. 14) except that the transition rate from O to C_5 (k_O) was assumed to be voltage independent. This assumption reduced the number of free parameters in the model, while yielding satisfactory fits to our experimental data (see following text). Fits between the simulations and experimental data did not improve when voltage-dependent rates of k_O [e.g., k_O = k_O0 exp(V/(V_k0))] were used in the simulations (results not shown). Hence, only the forward and backward rates of activation (k_V and k_O) are voltage-dependent variables, and the other transition rates (k_O0, k_C0, k_1, and k_3), as well as the allosteric coupling factors (f and h), are constants.

Voltage-clamp simulations

To evaluate the model, calculated T currents were compared with actual data from the present experiments. For each data set, the time course of the current elicited by the appropriate voltage protocol was simulated, with minor adjustment of the values in Table 1 for each individual cell.

Figure 8 shows simulations of the activation and decay of I_T during sustained depolarization (Fig. 8B) and the deactivation of I_T after repolarization (Fig. 8C). The simulation results (black) superimpose well on the corresponding experimental data from Figs. 1 and 3, respectively (reproduced in gray). For Fig. 8, B and C, the value of k_O0 was adjusted slightly, whereas the other parameters were fixed at the values in Table 1. Such adjustments could affect other properties of the current, but the changes were self-consistent. For example, although the adjustment applied in the simulation in Fig. 8C caused a shift in V_1/2 for the I-V relation from −51 to ca. −46 mV for the simulated data, this value of V_1/2 is close to the value measured experimentally in this particular cell (ca. −47 mV, data not shown).

The time course of entry into (Fig. 9A) and recovery from inactivation (Fig. 9B) were also reproduced well by the model. Figure 9, A and B, shows the simulation results (black) corresponding to the experimental data from Figs. 4 and 7, respectively (light red). For these, the values of k_1, f, and h were adjusted, and the same set of parameter values was used in both simulations. A small discrepancy between the simulation and experiment was seen in the amplitude of current available after 32 ms of recovery in B. This might be due to an initial delay in the recovery phase, noted by others (Burgess et al. 2002; Kuo and Yang 2001; Satin and Cribbs 2000). Simulation of the other voltage protocol we employed (see Fig. 6A) reproduced almost the same time constant for recovery from inactivation.
TABLE 1.  Backward and forward rates of activation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated Value (default)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{v,0} )</td>
<td>3.7</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>( k_{v,-v} )</td>
<td>0.02</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>( V_{k,v} )</td>
<td>25.5</td>
<td>[mV]</td>
</tr>
<tr>
<td>( V_{k,-v} )</td>
<td>-15.3</td>
<td>[mV]</td>
</tr>
<tr>
<td>( k_o )</td>
<td>20</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>( k_o )</td>
<td>1.65</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>( k_f )</td>
<td>0.062</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>( k_{-1} )</td>
<td>( 0.12 \times 10^{-3} )</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>( f )</td>
<td>0.305</td>
<td>unitless</td>
</tr>
<tr>
<td>( h )</td>
<td>0.226</td>
<td>unitless</td>
</tr>
</tbody>
</table>

The forward and backward rates of activation \( (k_v \) and \( k_{-v} \) are given by \( k_v = k_{v,0} \exp(V_o/V_{k,v}) \) and \( k_{-v} = k_{v,-v} \exp(V_o/V_{k,-v}) \). All 10 parameter values were derived from the present experimental data.

Because low-threshold current is almost completely inactivated at resting potential, the availability of \( I_T \) under physiological conditions will depend on hyperpolarizing inputs that bring about relief from inactivation (see DISCUSSION below). Because ganglion cells are buffered by a constant stream of excitatory and inhibitory inputs, and real hyperpolarizing influences are almost certainly transient (e.g., Sakai and Naka 1987), the availability of \( I_T \) will depend not only on the magnitude of the net voltage shift but also on the rates of deactivation and inactivation near the resting potential and the rate of recovery from inactivation at hyperpolarized potentials. We therefore asked if the model utilized in this study, which reproduces the amplitude and time course of currents elicited by stepwise depolarizations, can be extended to describe the currents activated after a sequence of brief hyperpolarizations.

Figure 10 presents a comparison of simulated and experimental data examining the availability of \( I_T \) after cycling the membrane potential from \(-62 \) to \(-82 \) mV a variable number of times. The membrane potential was held at \(-62 \) and \(-82 \) mV for the same amount of time during each conditioning cycle (10, 30, or 50 ms in this example), and then \( I_T \) was activated by a step from \(-82 \) to \(-42 \) mV as shown schematically in Fig. 10, top. The frequencies of these conditioning cycles (generally 5–50 Hz) were chosen to be consistent with the time course of psychophysically measured temporal contrast sensitivity (Woodhouse and Barlow 1982), and the kinetics of GABA inhibitory postsynaptic currents (Protti et al. 1997). The simulated data (middle) predict that the amount of \( I_T \) activated by the step to \(-42 \) mV increases with additional cycles until a maximum value is reached and that this value increases as the steps in the conditioning cycle are lengthened. When the step length is shorter, many more conditioning steps are needed to reach 90% of this limiting value, but the cumulative conditioning time to 90% is similar for each step length.

Representative experimental data obtained by this protocol are shown in the bottom row of Fig. 10. There is good correspondence between the prediction and experiment traces in each column, suggesting that the model satisfactorily implements the “priming” of \( I_T \) by recurrent membrane potential oscillation. We recorded such data from three cells with equivalent results. As a fraction of the peak current elicited by a step to \(-42 \) mV from a holding potential of \(-82 \) mV, the average limiting availability of \( I_T \) for these cells increased from 0.29 ±

FIG. 9.  Inactivation profiles of simulated T current. A: entry to inactivated state near the resting potential simulated by the model (black). The corresponding experimental data were taken from Fig. 4B (light red). Time constant for the command voltage was set to 0.4 ms in the simulation. B: recovery from open-state inactivation simulated by the model (black). The corresponding data were taken from Fig. 7A (light red). The time constant for the command voltage was set to 0.33 ms in the simulation. For both A and B, the model parameters were those in Table 1, except that \( k_f \) was 0.082, \( f \) was 0.224, and \( h \) was 0.196. C: steady-state inactivation and normalized I-V curves, simulated by the model (black lines). The corresponding data were replotted with marks (light red) from Fig. 5B. Model parameters were as given in Table 1. A simulated channel activation curve, corrected for the calculated Ca2+ driving force, is shown by the dashed line.
DISCUSSION

We have characterized and modeled the biophysical behavior of \( I_T \) in goldfish retinal ganglion cells, with particular attention to the voltage dependence and kinetics of activation, inactivation and recovery from inactivation. In the following text, we compare the kinetics of ganglion cell \( I_T \) to that of the T-channel clones and discuss the role and interaction of membrane potential and \( I_T \) in ganglion cells at rest and during light responses.

Comparison with cloned T-channel currents

We found that the rate of activation was strongly voltage dependent, that the rate of inactivation reached a plateau with strong depolarization, and that deactivation and recovery from inactivation were fast. These are general characteristics of \( I_T \) in many different native systems (Huguenard 1996) as well as the three primary cloned channels that produce T-type currents in expression systems (Perez-Reyes 2003). The kinetic parameters of \( I_T \) of the goldfish retinal ganglion cell are plotted together with those of cloned rat and human T channels in Fig. 11. Goldfish \( I_T \) kinetic parameters are comparable to these other values, although goldfish values tend to be among the fastest, especially for deactivation. This could reflect some unique property or accessory subunit association of the goldfish T channel (cf. Hobom et al. 2000), or it might simply reflect conditions used to make the different studies. Although the measurements shown in Fig. 11 were made under reasonably consistent ionic conditions, almost all the studies were done at room temperature, which is cold for the mammalian channels, but normal or a bit warm for the goldfish. Because the \( Q_{10} \)'s of T-type current properties are generally \( >2 \) (Coulter et al. 1989; Takahashi et al. 1991), mammalian T channels at appropriate physiological temperature would gate substantially faster, and their deactivation kinetics would be closer to that of the goldfish \( I_T \). The kinetics of both the goldfish and mammalian \( I_T \) would then be comparable.

Figure 11 shows that the kinetic parameters of the \( I_T \) sub-
Faster deactivation kinetics than channel (Frazier et al. 2001). Although this subtype displays used in our simulations were roughly near the values for cloned (Fig. 11), most of the parameter values of the kinetic model parameters needed to be larger to account for the relatively rapid –

Between the retinal ganglion cell and the cloned subtypes, two observations suggest that the goldfish current is produced by a different channel subtype, but both cloned –

T current from goldfish retinal ganglion cells. Although a very small current, this would still bring in the equivalent of ~150 nM/s homogeneously distributed [Ca$^{2+}$] in a 20 µm diameter cell (~12.5 pF, modeled as a sphere). This “window” current calculation formally assumes the independence of activation types tend to cluster in a characteristic manner. The parameters obtained for goldfish $I_T$ show significant overlap with the $\alpha$1G and $\alpha$1H subtypes but not with $\alpha$1I. Between the overlapping subtypes, two observations suggest that the goldfish retinal ganglion cell $I_T$ is of the $\alpha$1G subtype: the kinetics of recovery from inactivation are markedly faster for $I_T$ in this work and for $\alpha$1G than for $\alpha$1H (Fig. 11) and the $\alpha$1H subtype is blocked by Ni$^{2+}$ at low concentrations (IC$_{50}$ 13 µM) (Lee et al. 1999b), but both cloned $\alpha$1G (IC$_{50}$ 250 µM) (Lee et al. 1999b) and this $I_T$ (38% block at 100 µM) (Bindokas and Ishida 1996) are relatively insensitive to Ni$^{2+}$. In the only other retinal ganglion cells where Ni$^{2+}$ sensitivity has been assessed, T-type current was reduced ~85% by 20 µM Ni$^{2+}$ in Xenopus (Akopian and Witkovsky 1996). This higher sensitivity to Ni$^{2+}$ suggests that this T current is produced by a different channel subtype, but the recording conditions of this study were sufficiently different that a direct comparison might not be valid.

Consistent with this similarity of T-current properties between the retinal ganglion cell and the cloned $\alpha$1G subtype (Fig. 11), most of the parameter values of the kinetic model used in our simulations were roughly near the values for cloned $\alpha$1G (compare Table 1 vs. the legend for Fig. 14 of Serrano et al. 1999). The rate constants for channel opening and closing were significantly different from those for $\alpha$1G; these parameters needed to be larger to account for the relatively rapid deactivation of T current in the retinal ganglion cell. The same kinetic model has been applied successfully to the $\alpha$1I T-type channel (Frazier et al. 2001). Although this subtype displays faster deactivation kinetics than $\alpha$1G (Fig. 11), its other rate constants are substantially slower (Frazier et al. 2001). A simulation using rate constants similar to $\alpha$1I exhibits an order of magnitude slower increase in $I_T$ availability during priming and yields a much poorer fit to our data (not shown). Overall, the kinetics and pharmacology of ganglion cell $I_T$ are in agreement with the properties of the $\alpha$1G subtype, although we cannot exclude that other subtypes may be present.

**Physiological role**

$I_T$ may contribute to setting the resting [Ca$^{2+}$] level. In almost all cell types expressing $I_T$, including the goldfish retinal ganglion cell, the extrapolated window current coincides with the approximate resting potential (Huguenard 1996). This suggests that a small residual Ca$^{2+}$ influx due to an $I_T$ window could help set resting intracellular [Ca$^{2+}$]. There are two different approaches we can use to estimate the Ca$^{2+}$ influx due to persistently active $I_T$ in retinal ganglion cells. The “window” current arising from the overlap of the I-V and steady-state inactivation curves would be on the order of 0.1 pA. [i.e., 0.05 (fraction of nonactivated $I_T$ at −64 mV) × 0.02–0.05 (approximate open probability of $I_T$ at −64 mV) × 51 pA (mean peak $I_T$ adjusted for driving force at −64 mV)] in goldfish retinal ganglion cells. Although a very small current, this would still bring in the equivalent of ~150 nM/s homogeneously distributed [Ca$^{2+}$] in a 20 µm diameter cell (~12.5 pF, modeled as a sphere). This “window” current calculation formally assumes the independence of activation.
and inactivation. On the other hand, in the kinetic model we used to simulate our results (Serrano et al. 1999), activation and inactivation are not independent. In this scheme, inactivation is incomplete and 0.1–0.2% (simulated with our parameter values) of T channels remain open at all voltages where \( I_T \) activates. Although different in mechanism and voltage dependence from the “window” current, the magnitude of persistent Ca\(^{2+}\) current calculated by the kinetic model is very similar to the window at \(-64\text{mV}\) (data not shown). In either case, with measured resting intracellular [Ca\(^{2+}\)] of \(-120\text{nM}\) (Ishida et al. 1991), a continuous Ca\(^{2+}\) influx of this magnitude could contribute in setting [Ca\(^{2+}\)] levels in goldfish retinal ganglion cells.

Another possibility is that T window (or persistent) current could contribute directly to a form of input signal amplification as described in thalamocortical neurons (Williams et al. 1997). However, this phenomenon depends on \( I_T \) being relatively large compared with the leak, and satisfying the relation that the maximum slope of \( dI_T/dV \) in the vicinity of the window is greater than the leak conductance (Williams et al. 1997). Estimated leak conductance in goldfish retinal ganglion cells is 0.9 nS (Lee and Ishida, unpublished). A generous estimate of window \( dI_T/dV \) is 0.1 pA/V 5 mV = 0.02 nS, well short of the threshold for this effect. It therefore seems unlikely that the amounts of T current we have recorded could contribute to excitability by this mechanism in retinal ganglion cells. Cells with more T current might be different.

Voltage- and time-dependent changes in the availability of \( I_T \) might also play a role in spike generation. Two issues must be considered when assessing the possible contribution that \( I_T \) could make. First, do ganglion cells hyperpolarize enough to prime \( I_T \)? Second, after priming, how does \( I_T \) compare in amplitude with other subthreshold currents in ganglion cells?

Because the resting membrane potential of isolated goldfish retinal ganglion cells is in the range of \(-60\) to \(-75\text{mV}\) (Vaquero et al. 2001; C. F. Vaquero and A. T. Ishida, unpublished data), and because most \( I_T \) is inactive in this voltage range (Fig. 5), a mechanism to hyperpolarize ganglion cells, and thereby relieve the inactivation, must come into play for \( I_T \) to contribute to excitability. Intracellular recordings have shown that suitably arranged stimuli hyperpolarize ganglion cells in various species by as much as 15 mV beyond their resting potential (Ammermüller and Kolb 1995; Slaughter and Bai 1989), and recent recordings have indicated that the reversible potential for these voltage changes is around \(-95\text{mV}\) (e.g., Demb et al. 2001; Zaghloul et al. 2003). Assuming that these values are not peculiarities of the species used (salamander, turtle, and guinea pig), these results suggest that ganglion cells could hyperpolarize to membrane potentials that increase the availability of \( I_T \). Figure 10 shows that a sequence of transient hyperpolarizations, mimicking repetitive inhibitory input, leads to partial relief from inactivation of \( I_T \) in goldfish retinal ganglion cells. Hence, if ganglion cells hyperpolarized to \(-80\) or \(-85\text{mV}\) during such inhibitory input, our results predict that the availability of \( I_T \) would increase to \(-75\%\) of maximal current.

Activation threshold for the inactivating Na\(^{+}\) current in goldfish retinal ganglion cells is about \(-55\text{mV}\) and is not changed by prior hyperpolarization (Hidaka and Ishida 1998). Negative to \(-55\text{mV}\), \( I_F \), \( I_H \), persistent Na\(^{+}\) and a linear leak are the only known currents; \( I_H \) activates at voltages negative to \(-70\text{mV}\), and persistent \( I_{Na} \) gates positive to about \(-65\text{mV}\) (Hidaka and Ishida 1998; Tabata and Ishida 1996; 1999; Lee and Ishida, unpublished observations). We can therefore ask if \( I_H \) contributes to changes in membrane potential after terminating a volley of hyperpolarizations, by combining the \( I_T \) current properties found in the present study with our measurements of the current density, kinetics, and voltage sensitivity of \( I_T \), and the input conductance around \(-65\text{mV}\) in the absence of any channel-blocking pharmacological agents. Calculation of the voltage expected in a cell body with these properties (Fig. 12A) shows that termination of a single 100-ms hyperpolarization leads to an off depolarization, like that recorded in various retinal ganglion cell preparations (Eng et al. 1990; O’Brien et al. 2002; Tabata and Ishida 1996), and that the off depolarization is \(-30\%\) smaller when \( I_T \) is deleted from the calculation. Explicitly including a term for persistent \( I_{Na} \) had no qualitative effect on these simulated currents; removing \( I_T \) from the simulation still reduces the amplitude and rate of the rebound depolarization (not shown). Figure 12A also shows that the presence and absence of \( I_T \) makes little difference to the depolarization after a single 50-ms pulse but that a contribution appears after a volley of several 50-ms hyperpolarizations (Fig. 12B). This is consistent with the increase in the availability of \( I_T \) with multiple short hyperpolarizations shown in Fig. 10. A fuller understanding of how this contributes to spiking in situ will require additional recording, as well as modeling that incorporates the mix and magnitude of all currents near spike threshold.

In summary, we were able to record \( I_T \) under physiological conditions and develop a model that replicates the behavior of \( I_T \) under these conditions. This suggests that we can use the model to extend the range of \( I_T \) behaviors that can be investigated and incorporate \( I_T \) into broader models of retinal ganglion cell function. Previously published models of nerve impulse generation in retinal ganglion cells (e.g., Fohlmeister and Miller 1997) did not include hyperpolarization-dependent currents (\( I_T \) and \( I_H \)) and did not address the effect of inhibitory hyperpolarizing inputs. To extend these sorts of models, our results suggest that the increased availability of \( I_T \) at the conclusion of a hyperpolarizing sequence, in combination with concurrent activation of \( I_H \) should lead to a depolarizing rebound at termination of the IPSPs. \( I_T \) could thus function as a modulator of the interplay between excitation and inhibition in retinal ganglion cells, and the results of this study provide a specific time course and voltage range over which to look for this modulation.


