The Effect of Intensity and Duration on the Light-Induced Sodium and Potassium Currents in the *Hermissenda* Type B Photoreceptor

Kim T. Blackwell

School of Computational Sciences and the Krasnow Institute for Advanced Study, George Mason University, Fairfax, Virginia 22030

Light duration and intensity influence classical conditioning in *Hermissenda* through their effects on the light-induced currents. Furthermore, the contribution of voltage-dependent potassium currents to the long-lasting depolarization in type B photoreceptors depends on light-induced currents active at resting potentials. Thus, the present study measures the effect of holding potential, duration, and intensity on the light-induced currents in discontinuous single-electrode voltage clamp mode. Three distinct current components are distinguished by their temporal and voltage characteristics and sensitivity to pharmacological agents. One current component is a transient sodium current, I_{NaIgt} ; another is a plateau sodium current, I_{plateau} , which persists for the duration of the light stimulus. Substitution of trimethylammonium chloride for sodium reduces both currents equally, suggesting that I_{plateau} represents

partial inactivation of I_{NaIgt} . The third current component is a prolonged reduction in potassium currents, I_{KIgt} ; it is accompanied by an increase in input resistance, and it appears at potentials close to rest. An increase in light duration or intensity causes an increase in the peak conductance of both I_{NaIgt} and I_{KIgt} . Latency of I_{NaIgt} is decreased by intensity, whereas rise time is increased by duration. An increase in light duration or intensity causes an increase in the time-to-peak and duration of I_{KIgt} . Characteristics of these currents suggest that I_{KIgt} is responsible for the long-lasting depolarization seen after light termination, and thus plays a role in classical conditioning.

Key words: associative learning; K^+ currents; photoreceptors; phototransduction; classical conditioning; leak currents; sodium currents

Hermissenda crassicornis is a model system for studying classical conditioning because many of the behavioral and biophysical properties are similar to those in mammals (Lederhendler and Alkon, 1989; Matzel et al., 1990). Classical conditioning in *Hermissenda* uses light as the conditioned stimulus and turbulence as the unconditioned stimulus. Intracellular recordings demonstrate that classical conditioning causes the following changes to type B photoreceptors: an increase in input resistance (R_N), an enhanced long-lasting depolarization (LLD) in response to light (Crow and Alkon, 1980; Farley and Alkon, 1982; West et al., 1982; Frysztak and Crow, 1994), a reduction in the calcium-dependent, I_{KCa} , and transient, I_A , potassium currents (Alkon et al., 1982, 1984, 1985), a reduction in the calcium current (Collin et al., 1988), translocation of protein kinase C (McPhie et al., 1993; Muzzio et al., 1997), and facilitation of the IPSP in type A photoreceptors by type B photoreceptor action potentials (Frysztak and Crow, 1994, 1997; Schuman and Clark, 1994). Computer models (Sakakibara et al., 1993; Fost and Clark, 1996) demonstrate that a reduction in I_{KCa} can cause the enhanced LLD and increase in spike frequency, and a reduction in I_A can cause spike broadening and an increase in neurotransmitter release at type A to type B photoreceptor synapses.

A limitation of these computer models is that the equations for the light-induced currents are not based on voltage-clamp recordings. The early, transient light-induced current (I_{NaIgt}) is carried by sodium ions (Alkon and Sakakibara, 1985). It is linearly related to holding potential, with an extrapolated reversal potential of 30–40 mV. The later, prolonged light-induced current (I_{KIgt}) is sensitive to the extracellular potassium concentration, and thus is carried by potassium ions (Alkon and Sakakibara, 1985).

A more recent photoreceptor model (Blackwell, 2000) includes equations for I_{NaIgt} based on voltage-clamp recordings. This model does not include an explicit I_{KIgt} because investigators have concluded that I_{KCa} is equivalent to I_{KIgt} based on its sensitivity to calcium, and because both currents are affected by light stimulation. However, simulations show that without an explicit I_{KIgt} , the simulated light response does not resemble the experimentally measured light response, suggesting that I_{KIgt} is distinct from I_{KCa} . In particular, a prolonged light-induced current is required to produce the LLD. Simulations also suggest that, without the “baseline” LLD, a reduction in I_{KCa} cannot cause an enhancement of the LLD, because I_{KCa} is not active at membrane potentials more negative than –40 mV, the potential of the LLD. Thus, the present study characterizes the prolonged light-induced current that may underlie the baseline LLD and allow expression of classical conditioning.

The effect of light intensity and duration on the light-induced currents has not been systematically investigated. Such information is desperately needed because a variety of light durations and intensities are used for behavioral experiments and *in vitro* conditioning. The interaction between the light and turbulence stimuli may depend on the amplitude and time course of the light-

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Correspondence should be addressed to Kim T. Blackwell, School of Computational Sciences and the Krasnow Institute for Advanced Study, George Mason University, Rockfish Creek Lane, MS 2A1, Fairfax, VA 22030. E-mail: avrama@gmu.edu.

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induced currents, which in turn are affected by the duration and intensity of the light. Therefore, the present study measures the effect of duration and intensity on $I_{K_{\text{ligt}}}$ and $I_{Na_{\text{ligt}}}$.

MATERIALS AND METHODS

Hermisenda were obtained from Sea Life Supply (Sand City, CA) and Marinus (Los Angeles, CA). They were housed in groups of five or less in a refrigerated aquarium containing artificial seawater (ASW) chilled to 12°C. The animals were maintained on a 12 hr light/dark cycle, and they were fed a piece of cooked frozen mussel 4 d/week. Experiments were performed during the middle 8 hr of the light cycle.

An *in vitro* preparation was used to measure light-induced currents in voltage-clamp mode. The *Hermisenda* was killed by a razor cut just caudal to the rhinophores, and then the circumesophageal nervous system was removed by cutting the nerves that exit the ganglia and curve around the buccal crest. Pins were laid across the nerves and connectives of the ganglia to fix the nervous system within a shallow chamber made by an oval ring of grease on a glass microscope slide. The optic nerve was cut at the point where it emerged from the optic ganglion and entered the cerebropleural ganglion using an ultrafine dissecting scissor (Fine Science Tools, Foster City, CA) or a razor blade fragment. This had the effect of eliminating Na^+ spikes and removing the spatially extended axon and terminal branches. To facilitate penetration of the microelectrode, connective tissue was dissolved by incubating with Protease (type IX, 10 mg/ml; Sigma, St. Louis, MO) for 12 min at 25°C. The reaction was stopped by rinsing with 20 ml of ASW at 4°C. During the experiment the nervous system was continuously perfused with chilled (18°C) ASW containing (in mM): 430 NaCl, 10 KCl, 10 $CaCl_2$, 50 $MgCl_2$, and 10 HEPES-Na adjusted to pH 7.6 with HCl.

The light stimulus was provided via a fiber optic bundle aimed at the nervous system. The light source was a tungsten bulb filtered through a Kodak Wratten filter #47 (passband 380–520 nm) resulting in an intensity of 400 $\mu W/cm^2$ (measured with a Tektronix J17 photometer) when no neutral density filter (ND 0) was used. Light duration was controlled with a computer-controlled shutter. The intensity of the light was controlled with neutral density filters, the transmission of which ranged from 10 (ND 1) to 0.1% (ND 3).

Type B photoreceptors, identified by their position within the eye, were impaled and then dark-adapted for 10 min before measuring input resistance, resting potential, and peak generator potential. Input resistance was computed from the voltage change measured during the last 100 msec of a series of 400 msec current injection pulses (Fig. 1). Photoreceptors with input resistance of $>5 M\Omega$, resting potential more negative than $-30 mV$, and generator potential $>10 mV$ were accepted for experiments. Capacitance compensation, anti-alias, sample rate, gain, and phase were adjusted for discontinuous, single-electrode voltage-clamp mode (SEVC), as described in the Axoclamp 2B users manual (Axon Instruments, Foster City, CA). By minimizing the fluid level and using an aluminosilicate glass micropipette of tip resistance between 10 and 15 $M\Omega$ (when filled with KCl), a mean sample rate of $15.7 \pm 1.7 kHz$ and mean gain of 4.4 ± 1.23 were achieved. Measurements of light-induced currents were begun no sooner than after 15 min of dark adaptation.

The voltage dependence of the light-induced currents was measured for both 30 msec light stimuli and 3 sec light stimuli. Cells were clamped at potentials ranging from -100 to $0 mV$ for 10 sec before the light stimulus to allow the voltage-dependent currents to reach their steady-state values. To ensure a constant level of dark adaptation, the response to a 30 msec light was measured every 2 min, and the response to a 3 sec light was measured every 3 min. Measurements were performed once in ASW and then repeated while perfusing with $0 Na^+$ -460 mM trimethyl ammonium chloride (TMA) ASW, which blocks $I_{Na_{\text{ligt}}}$, or while perfusing with $0 Ca^{2+}$ -10 mM Ba^{2+} ASW.

The effect of light duration and intensity on $I_{Na_{\text{ligt}}}$ was measured at a clamp potential of $-80 mV$ in ASW. The effect of light duration and intensity on $I_{K_{\text{ligt}}}$ was measured at a clamp potential of $-20 mV$ in $0 Na^+$ -460 mM TMA ASW. Repeated presentation of light stimuli sometimes sensitized the photoreceptor and increased the light-induced currents. This sensitization opposed and obscured the decrease in current expected from a decrease in light amplitude. To minimize this effect, measurements of the effect of light intensity were counterbalanced; half the experiments presented dim lights first, and the other half presented brighter lights first.

Statistical analysis was performed using the software SAS (SAS Insti-

Figure 1. Measurement of input resistance in discontinuous current clamp. *A*, Response to 400 msec current injection pulses between -0.7 and $0.5 nA$. Steady-state voltage is the mean voltage measured during the last 100 msec of current injection. Resting potential for this cell was $-48 mV$. *B*, Plot of steady-state voltage versus injected current. Input resistance, $8 M\Omega$ for this cell, is the slope of the line.

Table 1. Mean physiological parameters of cells that met criteria and were included in the study

Measurement	Mean $\pm$ SE
Generator potential	$16.66 \pm 0.68 mV$
Resting potential	$-44.21 \pm 1.11 mV$
Input resistance (current clamp, at resting potential)	$8.36 \pm 0.37 M\Omega$
Input resistance (voltage clamp, at $-60 mV$)	$8.42 \pm 0.35 M\Omega$

tute, Inc., Cary, NC). The SAS procedure general linear models (GLM) was performed to assess differences among the cell groups. Where the model was significant, the *ad hoc* test least squares means was used to identify which groups differed significantly. The procedure REG was used to estimate reversal potentials; the procedure CORR was used to compute correlations.

RESULTS

Basic physiological parameters of cells included in study

A total of 43 cells met the criteria for R_N , resting potential, and generator potential, and were included in the study. Table 1 lists the overall mean generator potential, resting potential, R_N measured in current clamp (at dark-adapted resting potential) and R_N measured in voltage clamp at $-60 mV$ for these cells. The resting membrane potential ($-44 mV$), generator potential ($16.7 mV$), and R_N ($8.5 M\Omega$) are lower than that measured by others (Matzel and Rogers, 1993) and by the author (Blackwell and Alkon, 1999) using a single-electrode technique. The low values are partly

Figure 2. Currents in response to 30 msec light stimuli at holding potentials between -100 and 0 mV. *A*, Total currents measured in ASW. The early transient current is I_{Nalgt} ; the prolonged current seen at depolarized potentials is I_{Klgt} , which is a reduction in steady-state outward potassium currents. *B*, Currents measured in 0 Na^+ -TMA ASW. TMA blocks most of I_{Nalgt} , emphasizing I_{Klgt} . *C*, I_{Nalgt} returns to its control amplitude after washing with ASW for 5 min. The bars indicate when the light stimulus occurred. Traces are offset by an arbitrary amount.

caused by the axotomy, which decreases the integrity of the membrane. A second cause of the low R_N and membrane potential is the low pipette resistance required to achieve an adequate sample rate and gain for the SEVC. The resting membrane potential, generator potential, and R_N in the present study are comparable with values reported in some two electrode voltage-clamp studies (Alkon et al., 1984; Huang and Farley, 2001) that also use axotomy and low pipette resistance.

Seventeen cells were used for measuring the effect of intensity and duration at a single-clamp potential. Of these, seven were clamped at -20 mV in 0 Na^+ - 460 mM TMA ASW to isolate I_{Klgt} , and 10 were clamped at -80 mV in ASW to isolate I_{Nalgt} .

To evaluate the voltage dependence of the light-induced currents, seven cells were stimulated with 30 msec duration lights at a range of holding potentials, and 17 cells were stimulated with 3 sec duration lights at a range of holding potentials. Two additional cells, plus three of the cells used for the -20 mV protocols, were used to measure the change in R_N during and after a light stimulus. Other than these three cells, all other cells were used for a single protocol only. The only significant difference among these groups was the value of R_N measured after 15 min of dark adaptation in voltage clamp ($F = 5.38$; $p = 0.0036$). *Post hoc* comparisons showed that the mean R_N of the group of cells clamped at -20 mV ($10.2 \text{ M}\Omega$) was significantly different than the

Figure 3. Currents in response to 3 sec light stimuli at holding potentials between -100 and 0 mV. *A*, Total currents measured in ASW. In addition to I_{Nalgt} and I_{Klgt} , a third current component, I_{plateau} , is visible between potentials of -100 and -40 mV. The duration of I_{plateau} corresponds to that of the light stimulus. *B*, Currents measured in 0 Na^+ -TMA ASW. TMA blocks most of I_{Nalgt} and all of I_{plateau} . *C*, After washing with ASW for 5 min, I_{plateau} returns, and I_{Nalgt} increases toward its control amplitude. The bars indicate when the light stimulus occurred. Traces are offset by an arbitrary amount.

Figure 4. Characteristics of I_{Nalgt} . *A*, Mean peak conductance of I_{Nalgt} in response to 30 msec stimuli (solid triangles) and 3 sec stimuli (open circles) as a function of holding potential. The dashed line is the regression line computed between individual peak I_{Nalgt} measurements and holding potential. Error bars indicated 1 SE. *B*, Mean time-to-peak of I_{Nalgt} in

response to 30 msec stimuli (solid triangles) and 3 sec stimuli (open circles). There was no significant difference among groups in resting potential ($F = 0.23$), generator potential ($F = 2.17$), or R_N measured after 10 min of dark adaptation in current-clamp mode ($F = 1.29$).

Three current components revealed by voltage-clamp measurements

Voltage-clamp measurements of the light-induced currents revealed three distinct components that were separated by their temporal and voltage characteristics. Two of the current components were seen at hyperpolarized potentials. The first component was a transient current, I_{Nalgt} , seen with 30 msec (Fig. 2*A*) and 3 sec duration light stimuli (Fig. 3*A*). Previous reports identified this as a sodium current, because it decreased with depolarization and was eliminated when sodium was replaced with TMA. The second component was a plateau current, I_{plateau} , that was seen with 3 sec light stimuli and persisted for the duration of the light stimulus (Fig. 3*A*). An additional current component, I_{Klgt} , was seen at depolarized potentials and persisted for many seconds after light termination. It appeared to be an inward current, but previous reports (Alkon and Sakakibara, 1985) identified this as a reduction in steady-state outward potassium currents, because it increased with depolarization and was sensitive to the potassium concentration in the bath.

Characterization of the light-induced sodium currents

The light-induced currents were measured at holding potentials between -100 and 0 mV in steps of 20 mV to evaluate their voltage dependence and reversal potential. A graph of the mean peak I_{Nalgt} versus holding potential for 3 sec duration light stimuli (Fig. 4*A*, open circles) verified previous reports demonstrating no voltage dependence of this current. The current amplitude measured at -100 mV was similar to that at -80 mV, probably because this first 3 sec duration light stimulus sensitized the cell, making all subsequent currents relatively larger. Linear regression between the peak I_{Nalgt} measurements and holding potential (excluding the first current measurement) revealed a conductance of 47.8 ± 13.6 nS and a reversal potential of 80.2 mV. At depolarized potentials, the mean peak I_{Nalgt} versus voltage for 30 msec light stimuli (Fig. 4*A*, solid triangles) paralleled that seen for 3 sec light stimuli. However, the currents at hyperpolarized levels were smaller than expected. Again, this was likely attributable to a light-induced sensitization, which occurred over several light stimuli because of the 100 times briefer lights. The mean conductance of I_{Nalgt} , averaged over potentials between -60 and 0 mV, was 32.2 ± 3.0 nS for 30 msec light stimuli.

To further evaluate the voltage dependence of I_{Nalgt} , the effect of voltage on time-to-peak was evaluated. The mean time-to-peak was 340 ± 13.2 msec for 3 sec light stimuli and 362 ± 13.3 msec for 30 msec light stimuli (Fig. 4*B*); it was not significantly affected by holding potential. The absence of voltage dependence for both 30 msec and 3 sec light stimuli supports the supposition that the departure from linearity of current amplitude is caused by sensi-

response to 30 msec stimuli (solid triangles) and 3 sec stimuli (open circles). *C*, Effect of 0 Na^+ -TMA ASW on peak I_{Nalgt} in response to 30 msec stimuli (circles) and 3 sec stimuli (squares). Solid symbols show the mean peak current in TMA; open symbols show the mean peak current measured in ASW. *D*, Mean peak I_{Nalgt} in response to 30 msec stimuli in ASW (open squares), TMA (solid circles), and ASW wash (stars) for the four cells held long enough to wash out the TMA.

Figure 5. Characteristics of I_{Klgt} . *A*, Mean peak conductance of I_{Klgt} in response to 3 sec stimuli as a function of holding potential. The solid line is the regression line computed between individual peak I_{Klgt} measurements and holding potential. The dashed line is the best fit to the Goldman–Hodgkin–Katz equation assuming an internal potassium concentration of 240 mM (which yields a reversal potential of -80 mV). *B*, Mean time-to-peak of I_{Klgt} in response to 3 sec stimuli. *C*, Effect of 0 Ca^{2+} – 10 mM Ba^{2+} EGTA ASW on I_{Klgt} . Open squares show the ratio of peak current in barium to peak current in ASW. Solid circles show the reduction of I_{Klgt} caused by barium normalized by the increase in I_{Nalgt} caused by barium, to account for the increase in phototransduction second messengers caused by lack of calcium.

tization of phototransduction and not by voltage dependence of the channel itself.

$I_{plateau}$ was seen at hyperpolarized potentials with light durations of 3 sec (Fig. 3*A*). To characterize the amplitude of and transition to $I_{plateau}$, a single exponential was fit to the sodium current from slightly after the peak time to light termination. In 2 of 17 cases, either the decay time was too long or a resurgent current delayed the start time such that the plateau value was not reached by light offset. For the remaining 15 cases, the mean time to decay to the plateau was 442 msec at -80 and 425 msec at -60 mV. The $I_{plateau}$ amplitude was 22% of the I_{Nalgt} peak. Values are reported at hyperpolarized potentials to ensure that $I_{plateau}$ measurements were not contaminated by I_{Klgt} .

TMA blocks both the transient and plateau currents

On a subset of cells, measurements were repeated with sodium replaced by TMA to demonstrate that the charge carrier for both I_{Nalgt} and $I_{plateau}$ was sodium, and to isolate I_{Klgt} . In response to 3 sec light stimuli, a small residual I_{Nalgt} remained, but $I_{plateau}$ was not evident in the absence of sodium (Fig. 3*B*). Figure 4*C* summarizes the effect of TMA on I_{Nalgt} in response to 30 msec (circles) and 3 sec light stimuli (squares). TMA reduced I_{Nalgt} in response to 30 msec stimuli to $17.1 \pm 7.0\%$ of its control value at -60 mV. For 3 sec stimuli, TMA reduced I_{Nalgt} to $12.5 \pm 2.0\%$ and $I_{plateau}$ to $15.9 \pm 3.9\%$ of their control values at -80 mV. The difference in these values was not significant ($\Delta = 3.4 \pm 2.8\%$; $p = 0.25$), and the reduction of I_{Nalgt} was correlated with the reduction of $I_{plateau}$ ($R^2 = 0.72$; $p = 0.025$), suggesting that these two currents were carried by the same channel. TMA did not have a significant effect on the time-to-peak of the residual current for 3 sec light stimuli (mean difference = 3.4 ± 21.1 msec; $p = 0.87$) or 30 msec light stimuli (mean difference = 13.9 ± 8.2 msec; $p = 0.10$).

The effect of TMA washes out

A subset of these cells was held long enough to wash out the TMA and repeat the measurements of the light-induced currents in ASW. In response to 30 msec light stimuli, the currents measured in ASW-washed cells were similar to control currents (Figs. 2*C*, 4*D*). The mean value of the ratio of conductance after TMA to conductance before TMA was 1.23 ± 0.11 ; the difference between this ratio and 1.0 barely reached significance ($p = 0.046$) because of the large ratios at -100 (1.55 ± 0.22) and -80 mV (1.71 ± 0.32). In response to 3 sec light stimuli (Fig. 3*C*), either the TMA effect did not completely wash out, or the cell exhibited a general run down because of the length of the experiment. The mean conductance was 11.3 nS during the ASW wash, which was significantly smaller than the 23.9 nS observed before TMA ($t = 8.6$; $p < 0.0001$).

Characterization of light-induced potassium currents

I_{Klgt} was the current remaining, other than the residual I_{Nalgt} , in TMA ASW (Figs. 2*B*, 3*B*). Its amplitude increased with depolarization; it developed shortly after light onset and persisted for many seconds after light termination. For the cell illustrated in Figure 3*B*, a small current was apparent at -69 mV. Neither this current, nor the current measured at -49 mV was likely to be caused by I_{KCa} , as previously assumed, because that current requires depolarization to potentials greater than -40 mV for activation. The mean peak I_{Klgt} in response to 3 sec light stimuli (Fig. 5*A*) was measured at least 2 sec after light offset to minimize contamination by I_{Nalgt} . Linear regression revealed a conductance of 36.5 ± 2.5 nS and a reversal potential of -80.2 mV (Fig. 5*A*, solid line). The departure from linearity was accounted for by Goldman–Hodgkin–Katz rectification (Fig. 5*A*, dashed curve). The time-to-peak for I_{Klgt} , illustrated in Figure 5*B*, was considerably later than for I_{Nalgt} .

Figure 6. Effect of 0 Ca^{2+} –10 mM Ba^{2+} EGTA ASW on currents in response to 3 sec light stimuli at holding potentials between -100 and 0 mV. *A*, Total currents measured in ASW, different cell than in Figure 2. *B*, Currents measured in 0 Ca^{2+} –10 mM Ba^{2+} EGTA ASW. $I_{\text{Na} \text{ lgt}}$ is larger, I_{plateau} has become a second peak current, and $I_{\text{K} \text{ lgt}}$ is reduced. The bars indicate when the light stimulus occurred. Traces are offset by an arbitrary amount.

0 Ca^{2+} –10 mM Ba^{2+} EGTA ASW blocks the light-induced potassium current

Measurements of the light-induced currents were repeated in 0 Ca^{2+} –10 mM Ba^{2+} EGTA ASW and revealed changes in all of the light-induced currents (Fig. 6). Both $I_{\text{Na} \text{ lgt}}$ and I_{plateau} were larger in the absence of calcium. In fact, I_{plateau} was converted into a second peak at hyperpolarized potentials. The increase in sodium currents was attributable to the major role played by calcium in light adaptation. Light stimulation causes release of calcium from intracellular stores (Payne et al., 1990; Ukhonov and Payne, 1995; Talk and Matzel, 1996; Payne and Demas, 2000). The resulting elevation in calcium concentration serves to increase the rate of rhodopsin inactivation by arrestin (Dolph et al., 1993; Alloway and Dolph, 1999), decrease the phospholipase C activity (Smrcka et al., 1991), and likely has other roles in terminating phototransduction. Despite the dramatic effect on $I_{\text{Na} \text{ lgt}}$ and I_{plateau} amplitude seen in this figure, the increase did not reach significance, possibly because of the small sample size ($N = 4$). Only four cells were collected under these conditions because the

Figure 7. Effect of light on R_N . *A*, Voltage-clamp current at holding potential of -20 mV in response to 3 sec light stimulus of ND 0. Voltage was stepped to -10 mV for 200 msec every 2 sec before, during, and after the light stimulus. The bar indicates when the light stimulus occurred. *B*, Segment of current trace from 3 sec before light onset and 11 sec after light termination to better illustrate the clamp current during the brief voltage steps. *C*, Fractional change in R_N and $I_{\text{K} \text{ lgt}}$ versus time for the cell illustrated in *A*. R_N measured between 6 and 14 sec after the light stimulus is larger than that measured before the light. The change in R_N mirrors the change in $I_{\text{K} \text{ lgt}}$.

Table 2. $I_{K\text{igt}}$ had an extremely prolonged time course

Holding potential	Decay time	Fraction of remaining current	
		10 sec	15 sec
–40 mV	5.86 ± 1.65 sec	0.715 ± 0.093	0.545 ± 0.109
–20 mV	10.0 ± 2.54 sec	0.852 ± 0.230	0.634 ± 0.206
0 mV	7.86 ± 1.93 sec	0.95 ± 0.278	0.58 ± 0.144

The mean decay time constant and fraction of remaining current at 10 and 15 sec for holding potential between –40 and 0 mV. Fraction of remaining current is the ratio of current at a particular time-to-peak current.

increase in phototransduction products interfered with estimating the effect of 0 Ca^{2+} –10 mM Ba^{2+} EGTA ASW on $I_{K\text{igt}}$.

The second and more significant effect of 0 Ca^{2+} –10 mM Ba^{2+} EGTA ASW was a reduction in $I_{K\text{igt}}$ amplitude, illustrated for depolarized potentials in Figure 5C (*hollow squares*). On average, 0 Ca^{2+} –10 mM Ba^{2+} EGTA ASW reduced $I_{K\text{igt}}$ (measured at potentials between –40 and 0 mV) to $27.5 \pm 8.2\%$ of its control value. To account for the increase in phototransduction second messengers caused by the elimination of calcium-mediated adaptation, the reduction in $I_{K\text{igt}}$ was normalized by the increase in

I_{Nalgt} . This normalized $I_{K\text{igt}}$ reduction (Fig. 5C, *filled circles*) was slightly, but not significantly, smaller than the non-normalized current reduction.

$I_{K\text{igt}}$ is accompanied by an increase in R_N

To confirm that $I_{K\text{igt}}$ was caused by closure of potassium channels, R_N was measured every 2 sec before, during, and after the light stimulus at potentials between –60 and 0 mV in five cells. Figure 7, A and B, shows current traces at –20 mV for one cell. Comparison of post-light measures with pre-light measures using repeated measures ANOVA showed a significant change in R_N over time ($F = 6.85$). Overall, R_N was reduced to <40% of its pre-light value between 6 and 14 sec after the light stimulus. To show that the change in R_N was attributable to a reduction in $I_{K\text{igt}}$, the correlation was computed between $I_{K\text{igt}}$ and R_N . Figure 7C plots the values of R_N and $I_{K\text{igt}}$, determined every 2 sec to show the correlation for the same cell illustrated in Figure 7A. The mean correlation over all cells and all potentials was 0.68 ± 0.085 .

Duration of $I_{K\text{igt}}$ is similar to that of the LLD

$I_{K\text{igt}}$ had an extremely prolonged time course: both the time-to-peak was long, and the current remained for a very long duration

Figure 8. Effect of light intensity and duration on I_{Nalgt} . *A*, An increase in stimulus duration causes an increase in the peak conductance and rise time. *B–D*, The effect of intensity on I_{Nalgt} for 30 msec stimuli (*B*), 300 msec stimuli (*C*), and 3 sec stimuli (*D*). An increase in intensity causes an increase in the peak conductance and a decrease in the latency. The bars indicate when the light stimulus occurred. Traces are offset by an arbitrary amount.

Figure 9. Group effects of intensity and duration on peak conductance, latency, and time-to-peak of I_{Nalgt} . *A*, Peak conductance increases significantly as intensity is increased from ND 3 to ND 0. Peak conductance increases significantly as duration is increased from 30 to 300 msec, but increasing the duration further does not significantly increase peak conductance. *B*, All intensities produce significantly different latencies, but the only effect of duration was a smaller latency for a 300 msec or greater duration as compared with a 30 msec duration. *C*, Rise times for durations <300 msec are all similar, but rise times for 1 and 3 sec stimuli are greater than that for stimuli of ≤ 300 msec.

after the peak time. To quantify the time course of I_{Klgt} , the decay phase was fit with a single exponential. The start time of the exponential was no earlier than 2 sec after the light offset to minimize contamination by I_{Nalgt} . In cases in which the time course was more complex than a single exponential, the start time was delayed by several seconds to produce a good fit visually. The mean decay time constant was between 5 and 10 sec for holding potentials between -40 and 0 mV (Table 2). The decay time was so long that I_{Klgt} was significant 15 sec after light onset (12 sec after light termination), with the fraction of remaining current >0.5 (Table 2). To verify the characteristics of I_{Klgt} extracted in the presence of sodium currents, the exponential fits were repeated on I_{Klgt} measured in TMA ASW. The amplitude of I_{Klgt} in TMA ASW at 10 and 15 sec did not differ significantly from I_{Klgt} in control ASW ($p > 0.3$ for $V \leq -40$ mV). The prolonged time course and voltage dependence (present at -60 mV) makes it likely that this current underlies the baseline LLD.

Effect of light intensity and duration on the sodium currents

The effect of light intensity and duration on light-induced sodium currents was measured at a holding potential of -80 mV. A longer duration light caused an increase in the peak conductance, as shown in Figure 8*A*. This particular cell had two current peaks for long duration lights, although this was not commonly observed. An increase in intensity caused an increase in the peak conductance and a decrease in the latency (the time between light onset and current onset), as illustrated in Figure 8*B–D* for a 30 msec, 300 msec, and 3 sec light (different cell than in Fig. 8*A*).

The effect of intensity and duration on peak conductance, latency, and rise time (the difference between time-to-peak and latency) for the group of cells is summarized in Figure 9*A–C*, respectively. Both intensity ($F = 58.44$; $p < 0.0001$) and duration ($F = 28.55$; $p < 0.0001$) had a significant effect on peak conductance (Fig. 9*A*). Peak conductance increased significantly as in-

tensity was increased from ND 3 to ND 0. Peak conductance increased significantly as duration was increased from 30 to 300 msec ($p < 0.0001$), but increasing the duration further did not significantly increase peak conductance ($p = 0.22$ between 300 msec and 1 sec duration; $p = 0.63$ between 1 and 3 sec light). Latency was significantly affected by light intensity ($F = 50.69$; $p < 0.0001$) but only minimally influenced by light duration ($F = 3.3$; $p = 0.04$). As seen in Figure 9*B*, all intensities produced significantly different latencies, but the only significant effect of duration was a smaller latency for a 300 msec or greater duration as compared with a 30 msec duration ($p = 0.013$). Rise time was significantly affected by duration ($F = 5.95$; $p = 0.0003$) but only minimally by intensity ($F = 2.23$; $p = 0.02$). As shown in Figure 9*C*, rise time for durations <300 msec were all similar, but rise time for 1 and 3 sec stimuli was greater than that for stimuli of ≤ 300 msec.

I_{Nalgt} inactivates for all duration light stimuli

The observation that $I_{plateau}$ is blocked by TMA to the same degree as I_{Nalgt} suggests that both currents are carried by the same channel and implies that the inactivation of I_{Nalgt} is incomplete, and that $I_{plateau}$ represents steady-state equilibrium of both IP_3 concentration and the fraction of open sodium channels. Under this hypothesis, the transition from I_{Nalgt} to $I_{plateau}$ is caused by channel inactivation, whereas the decay of $I_{plateau}$ is caused by IP_3 degradation. The latter predicts that decay of $I_{plateau}$ has a time constant similar to that of IP_3 degradation. No plateau current is seen with very brief light stimuli, leaving open the possibility that the decay of I_{Nalgt} in response to 30 msec stimuli is caused by IP_3 degradation (Blackwell, 2000) or channel inactivation. These two alternatives are evaluated by comparing the decay time constant of I_{Nalgt} in response to a 30 msec stimulus with that in response to a 3 sec stimulus.

The decay time constants of $I_{plateau}$ and I_{Nalgt} in response to 3 sec and 30 msec stimuli were determined by fitting the current

measurements to single exponentials. Results showed that the time constant of decay of I_{plateau} was 3.6 ± 1.0 sec, within the range of IP_3 degradation rates measured in other cells (Allbritton et al., 1992; Wang et al., 1995), supporting the hypothesis that I_{plateau} represents an equilibrium state of the channel that carries I_{Nalgt} . The results also showed that the I_{Nalgt} decay time constant in response to 30 msec light stimuli (427 ± 43 msec) was similar to that in response to 3 sec light stimuli (511 ± 77 msec). There was no significant difference between these two values ($\Delta = 78.8 \pm 67.7$ msec; $p = 0.2782$), whereas there was a significant difference between the decay time constant of I_{Nalgt} in response to 30 msec light stimuli and the decay time constant of I_{plateau} ($\Delta = 3.0$ sec; $p = 0.0001$). This demonstrated that decay of I_{Nalgt} , even for stimuli as brief as 30 msec, was caused by channel inactivation, not IP_3 degradation.

Effect of light intensity and duration on the potassium current

The effect of light intensity and duration on light-induced potassium currents was measured at a holding potential of -20 mV in 0 Na^{2+} -TMA ASW. As shown in Figure 10A, a longer duration light caused an increase in the peak conductance, time-to-peak, and duration. An increase in intensity caused an increase in the peak conductance, time-to-peak, and duration for 30 msec, 300 msec, and 3 sec light stimuli (Fig. 10B–D). In contrast with Figure 8, no current was seen for ND 2 intensity at 30 or 300 msec, suggesting that I_{Klgt} was less sensitive to light than I_{Nalgt} .

The effect of intensity and duration on peak conductance, latency, and time-to-peak of I_{Klgt} for the group of cells is summarized in Figure 11A–C, respectively. Both intensity ($F = 19.05$; $p < 0.0001$) and duration ($F = 5.94$; $p = 0.0003$) had a significant effect on peak conductance, as shown in Figure 11A. Peak conductance was linearly related to the logarithm of intensity (slope = -0.011 ± 0.001 nA/ND) and duration (0.0087 ± 0.002 nA/log sec). Comparison of Figure 11A with Figure 9A confirmed that I_{Nalgt} was more sensitive than I_{Klgt} : the minimum intensity-duration at which I_{Klgt} appeared was 10 times greater than the minimum intensity-duration at which I_{Nalgt} appeared. Latency was significantly affected by light intensity ($F = 8.28$; $p = 0.0006$), but was not significantly affected by light duration ($F = 0.46$; $p = 0.76$). All of the effect of intensity was attributable to the difference between the ND 2 and ND1 intensities: there was no significant difference between the ND 0 and ND 1 intensities ($p = 0.5$). Even at -20 mV, the residual I_{Nalgt} (not completely blocked by TMA) probably obscured the beginning of I_{Klgt} , thus the lack of significance may not be reliable. Both duration ($F = 13.17$; $p < 0.0001$) and intensity ($F = 9.53$; $p < 0.0001$) had a significant effect on time-to-peak (Fig. 11C). Time-to-peak was proportional to the logarithm of intensity (-1.32 ± 0.26 sec/ND) and duration (-1.72 ± 0.28 sec/log sec).

DISCUSSION

Experiments were performed to characterize the light-induced currents active at resting potentials and to evaluate the effect of duration and intensity on the light-induced sodium and potassium currents of the type B photoreceptor of *Hermisenda crassicornis*. Three inward current components were observed: an early transient sodium current, I_{Nalgt} , a plateau sodium current, I_{plateau} , and a prolonged potassium current, I_{Klgt} . The prolonged current was accompanied by an increase in R_{N} , confirming that this current is caused by a reduction in potassium currents.

(A) Effect of duration

(B) 30 msec light

(C) 300 msec light

(D) 3 sec light

Figure 10. Effect of light intensity and duration on I_{Klgt} . *A*, An increase in stimulus duration causes an increase in the peak conductance, time-to-peak, and duration of current. *B–D*, The effect of intensity on I_{Klgt} for a 30 msec stimulus (*B*), 300 msec stimulus (*C*), and 3 sec stimulus (*D*). An increase in intensity causes an increase in the peak conductance and time-to-peak. The bars indicate when the light stimulus occurred. Traces are offset by an arbitrary amount.

Figure 11. Group effects of intensity and duration on peak conductance, latency, and time-to-peak of I_{Klgt} . *A*, Peak conductance increases linearly as the logarithm of either intensity or duration is increased. No current is seen with ND 3 for any stimulus duration tested. *B*, Latency decreases as intensity is decreased from ND 2 to ND 1, but no difference is seen between ND 1 and ND 0. *C*, Logarithm of both duration and intensity cause a linear increase in time-to-peak.

The results confirm that I_{Naigt} is independent of voltage. Its extrapolated reversal potential (E_{Na}) of 80 mV is higher than previously reported estimates of 30–40 mV (Alkon and Sakakibara, 1985). It is possible that I_{Klgt} contaminated the measurement of I_{Naigt} at depolarized potentials and biased the E_{Na} estimate to more depolarized potentials; however, an E_{Na} of 80 mV is consistent with other measurements of E_{Na} (Amar et al., 1992; Johnston and Wu, 1995; Lapied et al., 1999).

$I_{plateau}$, observed for light durations of 3 sec, is blocked by TMA at hyperpolarized potentials demonstrating that it is a sodium current. It persists for the duration of the light stimulus and decays within 2 sec of light termination. Thus, it contributes to the plateau potential during light, but not to the LLD observed after the light. The correlation between the fraction of I_{Naigt} blocked and the fraction of $I_{plateau}$ blocked by TMA suggests that these currents are carried by the same channel. $I_{plateau}$ is analogous to a window current and is caused by incomplete inactivation of I_{Naigt} and a constant level of second messenger-dependent channel activation. The latter suggests that the biochemical reactions activated by phototransduction have reached equilibrium with the rate of second messenger production equal to the rate of degradation.

The time constants governing the decay of I_{Naigt} and $I_{plateau}$ were analyzed to evaluate the role of channel inactivation and second messenger degradation. Inactivation of I_{Naigt} had the same time constant in response to 30 msec stimuli and 3 sec stimuli, which suggested that channel inactivation is important in terminating phototransduction even for very brief light stimuli. The time constant of decay of $I_{plateau}$ was similar to the degradation time constant of IP_3 , the ligand of I_{Naigt} (Sakakibara et al., 1998), suggesting that decay of $I_{plateau}$ is caused by degradation of IP_3 .

The most intriguing results of this study are the characteristics of I_{Klgt} . Previous studies have concluded that I_{Klgt} is equivalent to I_{KCa} because a light stimulus reduces I_{KCa} measured in voltage-clamp mode (Alkon and Sakakibara, 1985), and because both

I_{KCa} and I_{Klgt} have a fairly long time course. However, studies demonstrate that I_{KCa} becomes significant at potentials more depolarized than –30 mV (Alkon and Sakakibara, 1985; Farley, 1988; Sakakibara et al., 1993; Yamoah and Crow, 1995). Thus, I_{KCa} cannot be the source of the non-zero I_{Klgt} current measured at –60 and –100 mV (observed in some cells) or the –1 nA mean current measured at –40 mV. Furthermore, a computational modeling study of the *Hermisenda* photoreceptor evaluated the ability of light stimulation to activate I_{KCa} (Blackwell, 2000). The photoreceptor model included equations for calcium influx through voltage-dependent channels, calcium release from intracellular stores, I_A , I_{Naigt} , I_{KCa} and I_{Ca} , but did not include an explicit I_{Klgt} . Simulations show that without an explicit I_{Klgt} , the simulated light response does not resemble the experimentally measured light response, suggesting that I_{Klgt} is distinct from I_{KCa} .

Because I_{Klgt} is not carried by calcium-dependent potassium channels, it is necessary to identify the channel that does carry I_{Klgt} . One possibility is the potassium leak conductance, I_{Kleak} , a channel that is independent of voltage, modulated by G-protein-coupled neuromodulators (Hsiao et al., 1997; Jafri et al., 1997; Talley et al., 2000) and blocked by barium (Buckler, 1999). The hypothesis that I_{Klgt} is caused by a reduction in I_{Kleak} is supported by the observation that I_{Klgt} is independent of voltage, other than the rectification predicted by the Goldman–Hodgkin–Katz equation and caused by unequal concentrations of potassium on either side of the membrane. Also, the observation that I_{Klgt} is blocked by 0 Ca^{2+} – 10 Ba^{2+} ASW is consistent with this hypothesis, but it is not clear if the block is caused by the lack of calcium or the presence of barium. To better evaluate whether I_{Klgt} is attributable to a reduction in I_{Kleak} , it is necessary to repeat measurements of I_{Klgt} in ASW with normal calcium concentration and 10 mM barium. It is entirely possible that the observed I_{Klgt} consists of two components, I_{Kleak} and I_{KCa} , which cannot be temporally separated. Measurements of I_{Klgt} in the

presence of highly selective calcium-dependent potassium channel blockers will reveal the contribution of $I_{K\text{leak}}$.

The time course of $I_{K\text{igt}}$ is extremely prolonged, as reflected in the time-to-peak (Fig. 5B), the decay time (Table 2), and the fraction of remaining current at 10 and 15 sec (Table 2). The time-to-peak of $I_{K\text{igt}}$ is considerably greater than that of $I_{N\text{algt}}$, which suggests that either the second messengers that activate $I_{K\text{igt}}$ are downstream from those that activate $I_{N\text{algt}}$, or the kinetics of $I_{K\text{igt}}$ are slower than that of $I_{N\text{algt}}$. $I_{K\text{igt}}$ is observed within 1 sec of light stimulation when I_{plateau} is blocked with TMA (Fig. 3B); thus, under physiological conditions, the plateau potential seen with long-duration lights is a mixture of I_{plateau} and $I_{K\text{igt}}$. The involvement of the potassium current (Farley, 1987) explains why the current observed from 1 sec after light onset until light termination increases with depolarization.

Light intensity and duration had several effects on the light-induced currents. An increase in duration and intensity caused an increase in the peak conductance of both $I_{N\text{algt}}$ and $I_{K\text{igt}}$. The increase in $I_{K\text{igt}}$ was linear with the logarithm of duration and intensity, suggesting that the light quantities used were below saturation for $I_{K\text{igt}}$. This conclusion is consistent with the observation that $I_{K\text{igt}}$ was less sensitive to light than $I_{N\text{algt}}$; the latter appeared at combinations of duration and intensity 10 times less than required for $I_{K\text{igt}}$. Other characteristics influenced by intensity and duration included latency of $I_{N\text{algt}}$, which decreased with intensity, rise time of $I_{N\text{algt}}$, which increased with duration, and time-to-peak of $I_{K\text{igt}}$, which increased linearly with the logarithm of duration and intensity. The duration of $I_{K\text{igt}}$ increased in relation to the time-to-peak; thus dim or short light stimuli produced relatively brief $I_{K\text{igt}}$ currents.

$I_{K\text{igt}}$ may play a role in the expression, but not necessarily the induction, of classical conditioning. Although an enhanced LLD is observed in type B photoreceptors of classically conditioned *Hermissenda* (Crow and Alkon, 1980; Farley and Alkon, 1982), neither the LLD nor cumulative depolarization is required for induction of membrane changes in the type B photoreceptor in response to conditioning. *In vitro* conditioning experiments demonstrate that induction requires a calcium elevation, but the primary source of calcium is release from intracellular stores, not influx through voltage-dependent channels (Matzel and Rogers, 1993; Talk and Matzel, 1996). Calcium and other second messengers (e.g., diacylglycerol and arachidonic acid) activate PKC, which phosphorylates potassium channels (Farley and Auerbach, 1986; Neary et al., 1986) and produces the increase in R_N . However, expression of classical conditioning behavior involves suppression of type A photoreceptor activity by an increase in type B photoreceptor activity; thus the enhanced LLD may contribute to expression of classical conditioning by causing a post-light increase in type B photoreceptor activity. The properties of $I_{K\text{igt}}$ (the lack of voltage dependence and the prolonged duration) suggest that this current maintains the cell depolarized to a potential (the baseline LLD) at which the other potassium currents are active. Then, subsequent to classical conditioning, a reduction in voltage-dependent potassium currents causes an enhancement of the LLD. In addition to $I_{K\text{igt}}$ the hyperpolarization activated inward current, I_H , may contribute to the baseline LLD. I_H is active at resting potential and is enhanced by second messengers activated by serotonin (Acosta-Urquidí and Crow, 1993, 1995). If the LLD is required for the expression of classical conditioning behavior, then the sensitivity of $I_{K\text{igt}}$ to light intensity and duration provides limits to the light stimuli that will allow expression of classical conditioning in *Hermissenda*. Specifically,

the light sensitivity of $I_{K\text{igt}}$ leads to the prediction that expression of classical conditioning requires light stimuli of sufficient intensity and duration to cause a prolonged $I_{K\text{igt}}$.

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