Reconstruction of Ionic Currents in a Molluscan Photoreceptor

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ABSTRACT Two-microelectrode voltage-clamp measurements were made to determine the kinetics and voltage dependence of ionic currents across the soma membrane of the *Hermissenda* type B photoreceptor. The voltage-dependent outward potassium currents, I_A and $I_{Ca^{2+}\text{-}K^+}$, the inward voltage-dependent calcium current, $I_{Ca^{2+}}$ and the light-induced current, I_{lgt}, were then described with Hodgkin-Huxley-type equations. The fast-activating and inactivating potassium current, I_A, was described by the equation; $I_A(t) = g_A(\max)\{m_a^\infty[1 - \exp(-t/\tau_{ma})]\}^3 \times \{h_a^\infty[1 - \exp(-t/\tau_{ha})] + \exp(-t/\tau_{ha})\}(V_m - E_K)$, where the parameters m_a^∞, h_a^∞, τ_{ma}, and τ_{ha} are functions of membrane potential, V_m, and m_a^∞ and h_a^∞ are steady-state activation and inactivation parameters. Similarly, the calcium-dependent outward potassium current, $I_{Ca^{2+}\text{-}K^+}$, was described by the equation, $I_{Ca^{2+}\text{-}K^+}(t) = g_C(\max)(m_c^\infty(V_C)\{1 - \exp[-t/\tau_{mc}(V_C)]\})^{pc}\{h_c^\infty(V_C)[1 - \exp(-t/\tau_{hc})] + \exp(-t/\tau_{hc}(V_C)]\}^{pc}(V_C - E_K)$. In high external potassium, $I_{Ca^{2+}\text{-}K^+}$ could be measured in approximate isolation from other currents as a voltage-dependent inward tail current following a depolarizing command pulse from a holding potential of −60 mV.

A voltage-dependent inward calcium current across the type B soma membrane, $I_{Ca^{2+}}$, activated rapidly, showed little inactivation, and was described by the equation: $I_{Ca^{2+}} = g_{Ca}(\max)[1 + \exp[(-V_m - 5)/7]^{-1}(V_m - E_{Ca})$, where $g_{Ca}(\max)$ was 0.5 μS. The light-induced current, with both fast and slow phases was described by: $I_{lgt}(t) = I_{lgt1} + I_{lgt2} + I_{lgt3}$, $I_{lgti} = g_{lgti}[1 - \exp(-t_{on}/\tau_{mi})]^3 \exp(-t_{on}/\tau_{hi})(V_m - E_{lgti})$ $(i = 1, 2)$. For $i = 3$, $I_{lgt}(t) = g_{lgt3}m_3{}^3h_3(V_m - E_{lgt3})\exp(-t_{on}/\tau_{on}) \times \exp(-t_{off}/\tau_{off})$. Based on these reconstructions of ionic currents, learning-induced enhancement of the long lasting depolarization (LLD) of the photoreceptor's light response was shown to arise from progressive inactivation of I_A, $I_{Ca^{2+}\text{-}K^+}$, and $I_{Ca^{2+}}$.

INTRODUCTION

Associative training of the mollusc *Hermissenda crassicornis* with repeated pairings of light and rotation causes long-lasting behavioral (Alkon, 1975; Crow and Alkon, 1978; Lederhendler et al., 1986), biophysical (Alkon et al., 1982; West et al., 1982; Alkon et al., 1985, Collin et al., 1988), and biochemical (Neary et al., 1981; Nelson and Alkon, 1988; Nelson et al., 1990) changes. These changes are pairing-specific in that they do not occur without stimuli, or when the stimuli occur with no fixed temporal interval, or when they are explicitly unpaired. The biophysical changes occur within identified neurons, the type B photoreceptors in the *Hermissenda* eyes. Two K^+ currents, I_A and $I_{Ca^{2+}\text{-}K^+}$, which flow across the type B soma membrane, are reduced by the pairing regimen. It was thought reasonable, though never quantitatively demonstrated, that this training-specific reduction of the K^+ currents could account for the training-specific enhancement of the type B-sustained response to light as well as the prolonged depolarization of the type B cell following the light stimulus. Since this enhanced type B light response has been implicated in the storage and recall of the learned conditioned response of *Hermissenda*, it seemed important to more directly and quantitatively relate the K^+ current changes to the enhancement of the type B light response specific to the conditioned animals. To this end we have developed here a Hodgkin-Huxley description of the time and voltage dependence of the major soma currents. These equations were then used to model the type B photoreceptor voltage response. The model reproduces previously observed light-evoked membrane potential responses that are associated with conditioning-specific transformation of the type B response to light.

MATERIALS AND METHODS

Electrophysiological recording and analysis

Voltage clamp experiments were carried out on axotomized type B medial photoreceptors from *Hermissenda* as described elsewhere (Collin et al. 1988, 1990a).

Two intracellular microelectrodes (filled with 3 M KCl) were used for current injection (tip resistance of 15–20 MΩ) and for measuring intracellular membrane potential (resistance of 20–25 MΩ). A current-to-voltage converter was used to ground (via a Ag/AgCl wire) the saline in the chamber as well as to measure the current. Control of the voltage steps was achieved with a rise time of ~0.5 ms with electrodes of 15 MΩ. Throughout the experiment, the type B cell was held at −60 mV. All experiments were started after 10 min of dark adaptation.

Cells were bathed in artificial seawater (ASW) at 20–22°C of the following composition (in millimolar): 430 NaCl; 10 KCl; 50 $MgCl_2$; 10 $CaCl_2$; 10, Tris buffer (pH 7.4). In test solutions in which Na^+ was lowered, tetramethylammonium was added to achieve the osmolarity of the natural sea water, i.e., 870–890 mOsmol. The solution containing 100 or 300 mM K^+ had the same composition as ASW except, in the 50 mM solution, Na^+ and tetramethylammonium were added to give an approximately equal osmolarity.

Recordings were made with a 7100 small cell current and voltage clamp amplifier (Pellagic Electronics, Falmouth, MA) filtered at 3 KHz. Experimental protocols and data collection were carried out using an IBM PC-XT computer with p-Clamp at a maximum sampling rate of 10 KHz (Version

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5.03; Axon Inst. Inc., Burlingame, CA). Current Transients were leak-subtracted on-line. Each experiment was repeated many times using type B cells from at least four different animals.

Light stimuli were provided by a halogen 12-V, 100-watt bulb (HLS-2100; Hoya Co., Tokyo, Japan). The light was guided to beneath the preparation by a bundle of fiber optics. The light intensity at the preparation was always less than 5.8 mW/cm^2 at 510 nm. At this wavelength, the sensitivity of the type B cell is maximal (Alkon, 1976). For measurement of light induced currents, the intensity was attenuated 100-fold. Light stimuli were presented (10 min after insertion of two microelectrodes) with an interval of at least 2 min.

Simulation

Data collected by p-Clamp were analyzed and simulated by PC Data Master (Durham Technical Images, Durham, NH) on an IBM-AT compatible computer. In order to obtain either a simulated voltage response under current clamp conditions or a simulated current response under voltage clamp, the recording system including the feedback amplifier, and impedance of electrodes was included in the simulation. The total simulation also included a transfer function in the Laplace domain, $G(s)$, of a feedback circuit in the voltage clamp amplifier and was expressed as being a first order delayed system with a gain constant K; $G(s) = K/(Ts + 1)$, where T is the time constant (200–400 ms) and K is a gain constant. A voltage response under current clamp was obtained to set K at 0. A voltage clamp current could be simulated setting K at 10^4. Since the I–V characteristics of an electrode are linear within the range of current injections (±50 nA), variables in the recording system (e.g., impedance of the electrodes) were not corrected during simulation but rather set to be constant. Parameters in Hodgkin-Huxley equations such as m, h, and τ were estimated by the least mean square error within ±5% to experimental data. Simulations were carried out on a IBM compatible PC.

RESULTS

Previous studies (Alkon, 1979; Alkon et al., 1982, 1984; Alkon and Sakakibara, 1985) have demonstrated that there are four major voltage-dependent and two light-induced currents across the type B soma membrane. Table 1 summarizes these currents characteristics.

The delayed rectifying K^+ current (I_{K^+}), previously observed to flow across the type B soma membrane was not considered here because it is not significantly activated within the membrane potential range (Alkon et al., 1984), (±60 mV from a holding potential of –60 mV) used for the analysis of the present study.

Since we attempted to obtain a quantitative description of the voltage and temporal dependence of all the major type B soma currents, re-examination of each ionic current was performed, with particular emphasis on the kinetics of the currents and the relationships of those kinetics to membrane potential.

TABLE 1 Ionic currents in the type B photoreceptor

	Blocker	Effective dose	Reference
		mM	
Voltage-dependent			
I_A	4-AP	5	(1)
$I_{Ca^{2+}\text{-}K^+}$	EGTA		(1, 2)
I_K	TEA	100	(1)
$I_{Ca^{2+}}$	Ba^{2+}	10	(1)
Light-induced			
$I_{lgt\text{-}Na}$	O-Na	0	(1, 2)
$I_{lgt\text{-}Ca^{2+}\text{-}K^+}$	EGTA		(1, 2)

(1) Alkon et al., 1984, (2) Alkon and Sakakibara, 1985

Potassium currents across the type B photoreceptor soma membrane

Fig. 1 *A* shows a family of currents measured across the type B cell soma in response to positive step changes in membrane potential of 1-s duration from a holding potential of –60 mV. I_A and I_{K^+} were eliminated with 5 mM 4-aminopyrinide and 100 mM tetraethylammonium, respectively (Alkon et al., 1984). $I_{Ca^{2+}\text{-}K^+}$ could be blocked by external Ba^{2+} substitution for Ca^{2+} (Alkon et al., 1984). Fig. 1 *B* shows the current-voltage relationship for I_A and $I_{Ca^{2+}\text{-}K^+}$ peak values. Each of these two currents had a threshold of around –30 mV in ASW.

Properties and kinetics of I_A

The time course of activation and inactivation of I_A, which peaks at approximately 20 ms, was analyzed using the

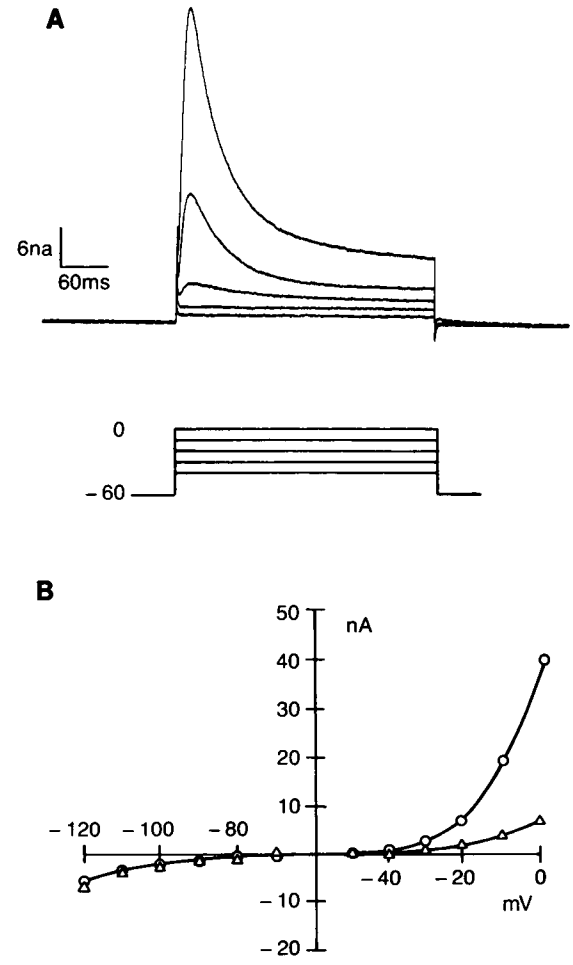


FIGURE 1 (*A*) Voltage-dependent outward currents across the membrane of the isolated type B cell soma in ASW are demonstrated. Absolute membrane potentials are shown at the left. All currents are leak subtracted. (*B*) Current-voltage relations of the peak amplitude of I_A recorded in normal ASW (○) as shown in *A*. The data for $I_{Ca^{2+}\text{-}K^+}$ (▲) was obtained under ASW with 5 mM 4-AP.

Hodgkin and Huxley (1952) model developed for the squid axon Na^+ current.

It is proposed that I_A as a function of time can be adequately described by the equation,

$$I_A(t) = g_A(\max)(m_a\infty(V_C)\{1 - \exp[-t/\tau_{ma}(V_C)]\} + m_a\infty(V_h)\exp[-t/\tau_{ma}(V_C)])^{pa} \times (h_a\infty(V_C)\{1 - \exp[-t/\tau_{ha}(V_C)]\} + h_a\infty(V_h)\exp[-t/\tau_{ha}(V_C)])^{qa}(V_C - E_K) \quad (1)$$

where $g_A(\max)$ is the maximum steady-state conductance in the absence of inactivation (2 μS in ASW), V_C is the clamping potential, V_h is the holding potential, E_K is the potassium reversal potential, τ_{ma} is the time constant for activation, τ_{ha} is the time constant for inactivation, and pa and qa are integers governing the exponential relationships. Although the simulated current may be better fitted to the experimental data under the assumption of pa and qa as free parameters, the power constants were well correlated to the rate constants, so we considered them as integers. If the holding potential is sufficiently negative (e.g., −60 mV), the activation constant ($m_a\infty$) is almost 0, hence the inactivation constant, ($h_a\infty$) is almost 1.Under these circumstances Eq. 1 will be as follows.

$$I_A(t) = g_a(\max)(m_a\infty(V_C)\{1 - \exp[-t/\tau_{ma}(V_C)]\})^{pa} \times (h_a\infty(V_C)\{1 - \exp[-t/\tau_{ha}(V_C)]\} + \exp[-t/\tau_{ha}(V_C)])^{qa(V_C - E_K)} \quad (2)$$

The time constants for activation (τ_{ma}) and inactivation (τ_{ha}) and the best values of the exponents (pa, qa) were obtained from a least square fitting to the experimental data. Fig. 2 *A* (*left*) shows a family of I_A currents elicited by brief command voltages (100 ms) at 22°C. To describe I_A as a function of time in response to various depolarizing steps, pa and qa were determined as 3 and 1, respectively. The values of τ_{ma}, τ_{ha}, pa, and qa obtained with this analysis reproduced the I_A with a satisfactory degree of accuracy as shown in Fig. 2 *A* (*right*) for membrane potentials of 0, −10, −20, −30, −40, and −50 mV at 20°C. The activation time constants, τ_{ma}, derived in this way, were voltage- dependent and varied from 6 ms for a membrane potential of −30 mV to ~2 ms for a level of 0 mV (Fig. 2 *B*) for seven cells. The time constant for inactivation τ_{ha} of I_A was also voltage-dependent and shortened with depolarization from ~200 ms at −30 mV to 100 ms at −10 mV as shown in Fig. 2 *B*.

τ_{ma} and τ_{ha} were described by the following equations.

$$\tau_{ma}(V_m) = 5\{1 + \exp[(V_m + 13)/10]\}^{-1} + 2[\text{ms}] \quad (3)$$

$$\tau_{ha}(V_m) = 120p\{1 + \exp[(V_m + 17)/6]\}^{-1} + 100[\text{ms}] \quad (4)$$

The magnitude of the potassium currents varied with holding potential. The steady-state inactivation $h_a\infty(V_m)$ dependence on membrane potential was obtained by measuring I_A at a fixed membrane potential of −10 mV after a 2.5-s duration pre-pulse to various potentials. The results from seven such experiments illustrated in Fig. 3 *A* show that the current could be fully activated from holding potentials below −40 mV and that inactivation was complete at 0 mV.

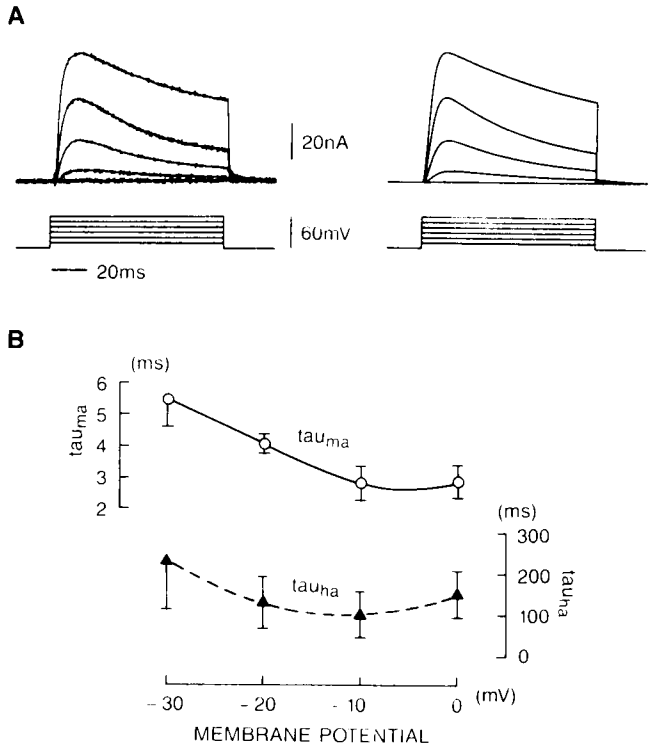


FIGURE 2 (*A*) I_A currents in response to brief (duration, 100 ms) membrane potential changes to 0, −10, −20, −30, −40, and −50 mV (from top to bottom) from a holding potential of −60 mV at 22°C are displayed (left column). Reconstructed I_A currents obtained from Eq. 2 are shown in the right column. Reconstructed currents on right were for conditions used for actual experiments on left except the temperature which was 20°C. All currents are leak subtracted. (*B*) Voltage dependency of activation and inactivation time constants, τ_{ma} and τ_{ha}, for I_A, is shown. τ_{ma} varies from 6 ms at −30 mV to 3 ms at −10 mV. $\tau_{ma} = 5\{1 + \exp[(V_m + 13)/10]\}^{-1} + 2$ [ms]. τ_{ha} ranged from 300 to 100 ms within the membrane level tested. $\tau_{ha} = 120\{1 + \exp[(V_m + 17)/6]\}^{-1} + 100$ [ms]. In *B* experimental data were expressed as mean ± SD for each value ($N = 7$).

The steady-state inactivation of I_A as a function of voltage could be approximated by the Hodgkin-Huxley equation:

$$h_a\infty(V_m) = 0.85\{1 + \exp[(V_m + 42)/n]\}^{-1} + 0.15 \quad (5)$$

where V_m is the membrane potential and −42 mV is the potential for half-maximal inactivation. The best fit was obtained with a slope steepness factor of $n = 9$. Recovery from inactivation was investigated by presenting two identical command voltages from −60 to −10 mV of 200-ms duration with varying interstimulus intervals as shown in Fig. 3 *B*. The relative magnitude of membrane current was plotted against the duration stimulus interval. Recovery occurred exponentially, with a time constant of 47.1 ms.

The relationship between the steady-state activation, $m_a\infty$, and membrane potential is also shown in Fig. 3 *A*. The voltage dependence of $m_a\infty$ was obtained from the relative amplitude change of I_A with depolarizing steps from a holding

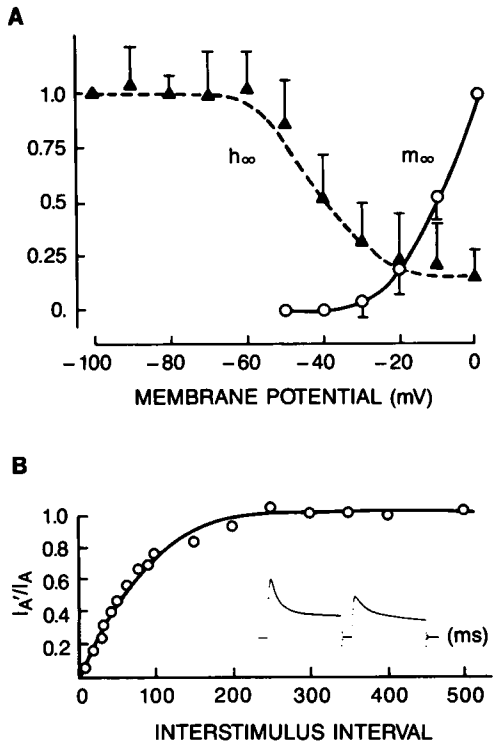


FIGURE 3 Steady-state activation ($m_a\infty$) inactivation ($h_a\infty$) and recovery from inactivation parameters of I_A as a function of membrane potential. (*A*) I_A could be fully activated from holding potentials below −40 mV and the inactivation was complete at 0 mV. Data points represent mean ± SD of seven cells. Continuous curves were obtained from Eqs. 5 and 6. (*B*) Recovery from inactivation of I_A was measured after currents were elicited by twin pulses with varying interstimulus intervals. The stimulus interval was plotted against the amplitude ratio of the two current transients.

potential of −80 mV, where $h_a\infty$ was thought to be 1, to various membrane potentials up to 0 mV (absolute). The potassium conductance through the A channel increased sigmoidally with half-maximal conductance at −11 mV. The steady-state activation, $m_a\infty$, could be approximated with the following equation.

$$m_a\infty(V_m) = \{1 + \exp[-(V_m + 11)/16]\}^{-1} \quad (6)$$

Maximal steady-state conductance (g_A(max)) in ASW was 2.8 μS.

Properties and kinetics of $I_{Ca^{2+}\text{-}K^+}$

Several lines of evidence indicate that the slowly activated voltage-dependent outward current of *Hermissenda's* photoreceptor is largely carried by K^+ ions, and this current is also activated and inactivated by elevation of intracellular Ca^{2+} (Alkon et al., 1984; Alkon and Sakakibara, 1985). Here we analyzed the response of $I_{Ca^{2+}\text{-}K^+}$ to membrane depolarization at three different external calcium concentrations.

The development of $I_{Ca^{2+}\text{-}K^+}$ was slow (Fig. 4), and the average mean time to reach maximal amplitude at 0 mV was 567 ms (mean of seven replications). Inactivation occurred throughout the depolarization steps used (as in Fig. 4). The

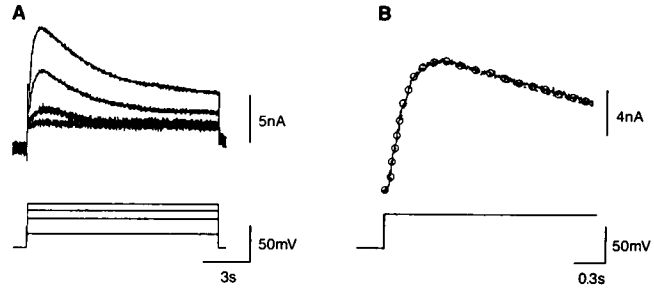


FIGURE 4 The time course of the calcium-dependent potassium current after elimination of I_A by 5 mM 4-AP and 100 mM TEA is shown in *A*. The membrane potential is held at −60 mV. The time to reach the maximal amplitude is 436 ms. (*B*) $I_{Ca^{2+}\text{-}K^+}$ comparison of experimental record and simulation (*open circles* on the curve) on different time scale as *A*. Currents are elicited by voltage command of 60 mV from a holding potential of −60 mV. The simulated response was obtained by the equation: $I_{Ca^{2+}\text{-}K^+}(t) = g_C(\max)(m_c\infty(V_C)\{1 - \exp[-t/\tau_{mc}(V_C)]\})^{pc}\{h_c\infty(V_C)[1 - \exp(-t/\tau_{hc})] + \exp(-t/\tau_{hc}(V_C))]\}^{qc}(V_C - E_K)$.

voltage dependency of the activation and inactivation kinetics of $I_{Ca^{2+}\text{-}K^+}$ were investigated as described above for I_A. The contribution of calcium to the channel's gating was not measured directly, but rather inferred from experiments in which the external calcium concentration was varied, and the resulting parameters incorporated into the equations (Fig. 5). Since the development of $I_{Ca^{2+}\text{-}K^+}$ was slow, it was also possible to estimate the calcium current, which activated rapidly (before activation of $I_{Ca^{2+}\text{-}K^+}$) and showed little or no inactivation. Then, $I_{Ca^{2+}\text{-}K^+}$ was analyzed after subtraction of $I_{Ca^{2+}}$ as well as I_l (leak current). The contribution of $I_{Ca^{2+}}$ will be described later.

$I_{Ca^{2+}\text{-}K^+}$ as a function of time can be described by the equation:

$$\begin{aligned} I_{Ca^{2+}\text{-}K^+}(t) = {} & g_C(\max)(m_c\infty(V_C)\{1 - \exp[-t/\tau_{mc}(V_C)]\} \\ & + m_m\infty(V_h)\exp[-t/\tau_{mc}(V_C)])^{pc} \\ & \times (h_c\infty(V_C)\{1 - \exp[-t/\tau_{hc}(V_C)]\} \\ & + h_c\infty(V_h\exp[-t/\tau_{hc}(V_C)])^{qc}(V_C - E_K) \end{aligned} \quad (7)$$

where g_C(max) the maximum steady-state conductance in the absence of inactivation, was found to be 0.5 μS, $m_C\infty$ and $h_C\infty$ are the steady-state activation and inactivation parameters dependent on external calcium and membrane potential, τ_{mc} and τ_{hc} are the time constants, and *pc* and *qc* are integers governing exponential relations. If the holding potential V_h is negative enough $m_c\infty(V_h)$ is almost 0 and $h_c\infty(V_h)$ is almost 1, then Eq. 7 will be as follows.

$$\begin{aligned} I_{Ca^{2+}\text{-}K^+}(t) = {} & g_c(\max)(m_c\infty(V_C)\{1 - \exp[-t/\tau_m(V_C)]\})^{pc} \\ & \times \{h_c\infty(V_C[1 - \exp(-t/\tau_{hc})] \\ & + \exp[-t/\tau_{hc}(V_C)]\}^{qc}(V_C - E_K) \end{aligned} \quad (8)$$

Equation 8 generated currents which agreed well with those actually observed in Fig. 5, with activation and

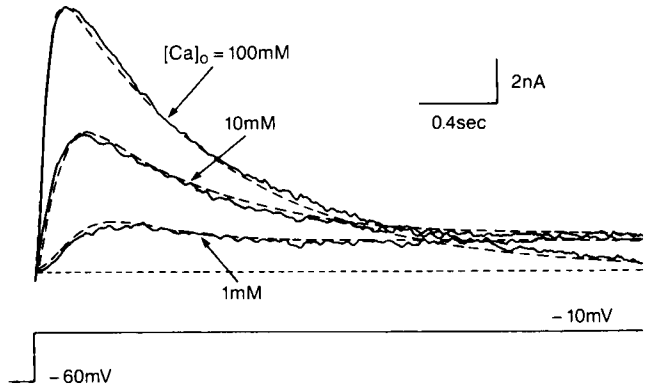


FIGURE 5 Calcium and voltage dependence of $I_{Ca^{2+}\text{-}K^+}$. From a holding potential of −60 mV, cells were depolarized to −10 mV at the three different external calcium concentrations. Note that the inactivation time constant τ_{hc} is much longer than that of I_A (Fig. 2 *B*). Continuous curves were calculated from Eqs. 9 and 10.

inactivation parameters (τ_{mc}, τ_{hc}) of 120 ms and 3s, and the exponent (*pc*, *qc*) of 3 and 1, respectively. Fig. 6 *A* shows the experimental data obtained for τ_{mc} and τ_{hc} dependence on external calcium, and they were described by the following.

$$\tau_{mc}(V_m) = (1100\{1 + \exp[(V_m + 33)/4]\}^{-1} + 170) \times (0.83\{1 + \exp[(\log[Ca] - 0.6)/0.4]\}^{-1} + 0.167)\,[ms] \quad (9)$$

$$\tau_{hc}(V_m) = (10000\{1 + \exp[-(V_m - 5)/10]\}^{-1} + 2000) \times (0.36\{1 + \exp[-(\log[Ca] - 0.8)/0.5]\}^{-1} + 0.12)\,[ms] \quad (10)$$

The integers governing the exponential relations *pc* and *qc* were also the same as those of I_A , i.e., 3 and 1, respectively. Fig. 6 *B* illustrates the calcium dependence of the time constants, $m_c\infty$ and $h_c\infty$. The steady-state activation parameter $m_c\infty$ was evaluated with the experimental data obtained in Fig. 6 *B* and can be fitted with the following equation:

$$m_c\infty(V_m) = \{1 + \exp[-(V_m + 12)/17]\}^{-1} \times \{1 + \exp[-(\log[Ca] + 0.5)/0.6]\}^{-1} \quad (11)$$

where −12 mV is the potential for half-maximal activation.

The time dependence of the activation parameter, $m_c(t)$, was estimated with twin command steps of different interstimulus intervals (<2 s). Since the inactivation time constant, τ_{hc}, is fairly long, $h_c\infty(t) \mid t = 0$ was small. Thus, in 4-AP and TEA ASW, the membrane was depolarized to 0 mV for 1 s, followed by an interval of 2 s or less by a second identical depolarization. That again activates $I_{Ca^{2+}\text{-}K^+}$ so m_c is reset to 0 at time 0 (the timing of cessation

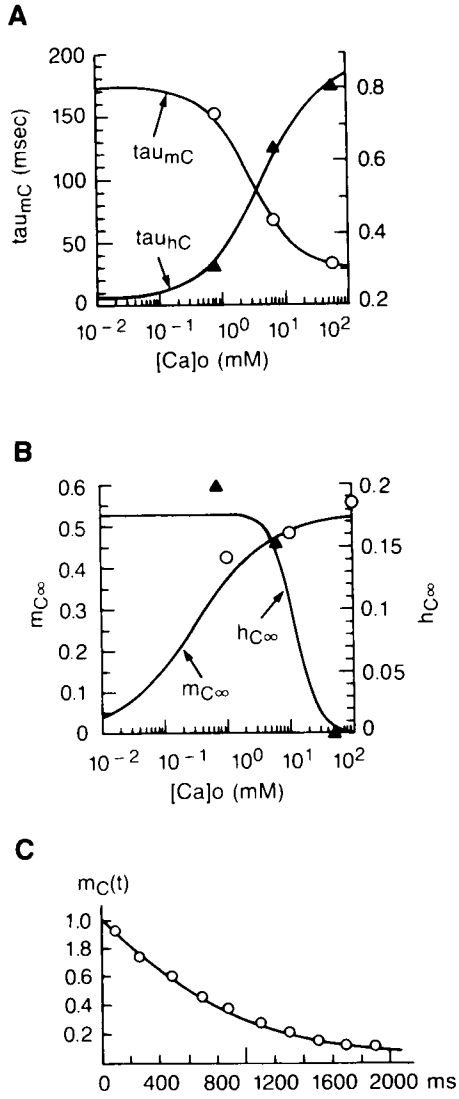


FIGURE 6 Activation and inactivation kinetics of $I_{Ca^{2+}\text{-}K^+}$ isolated pharmacologically by 4-AP and TEA, are calcium- and voltage-dependent. Calcium dependence of τ_{mc} and τ_{hc} (*A*), and $m_c\infty$ and $h_c\infty$ (*B*), were evaluated at the indicated external calcium concentrations. (*C*) Decay of the activation constant of $I_{Ca^{2+}\text{-}K^+}$ (m_c). m_c was determined by the relative amplitude of $I_{Ca^{2+}\text{-}K^+}$ at the steady level at each interstimulus interval, using step command depolarizations to 0 mV from a holding potential of −60 mV, at normal (10 mM) external calcium concentration. Data points are mean ± SD of seven cells.

of the first pulse) and from there we can estimate the dynamics of m_c. That the activation process decayed exponentially is consistent with our observation that the current elicited by the second voltage command became smaller with a longer interstimulus interval (Fig. 6 *C*). With m_c and h_c at time 0 taken to be 1 and 0, respectively, at 0 mV (from a holding level of −60 mV) m_c approached 0 with an exponential decay, while the value of h_c remained 0. Thus, $m_c(t)$ and $I_{Ca^{2+}\text{-}K^+}(t) \mid_{V_m = -60}$ at a 2-s interval following a change of membrane potential from 0 to −60 mV could be described by the following equations.

$$m_c(t) = [\exp(-t/\tau_{mc})]^3 \quad (12)$$

$$I_{Ca^{2+}\text{-}K^+}(t)\,|_{V_m=-60} = g_c(\max) \times [\exp(-t/\tau_{mc})]^3 \times [1 - \exp(-t/\tau_{hc})^1](-60 - E_K) \quad (13)$$

It should be mentioned that Eq. 12 is only valid within the period during which h_c remained 0. For a longer period after the pre-pulse, m_c fully recovers to 1. From Eq. 13, m_c was estimated to be an average value of 2.5 s. This time constant was similar to that measured directly for the actual calcium-dependent potassium currents elicited from a holding potential of −60 mV.

The calcium dependence of the steady-state inactivation ($h_c\infty$) could be obtained from measurements of $I_{Ca^{2+}\text{-}K^+}$ at 0 mV after 5-s pre-pulses (5-s duration) to potentials ranging from −100 to 0 mV. Such measurements taken at three different external calcium concentrations (Fig. 6 *B*) generated the equation:

$$h_c\infty(V_m) = \{1 + \exp[(V_m + 30)/n]\}^{-1} \times [1 + \exp[(\log[Ca] - 1.3)/0.2]\}^{-1} \quad (14)$$

where −30 mV is the potential at half-maximal inactivation and the slope factor of n was estimated to be 13.

Properties and kinetics of $I_{Ca}2+$

A steady inward current carried by Ca^{2+} was found not to have significant temporal dependence (Alkon et al., 1984; Collin et al, 1990) and very little inactivation, unlike a number of Ca^{2+} currents found in other invertebrate neurons (e.g., Eckert and Tillotson, 1981). Elevation of external potassium allowed substantial separation of $I_{Ca^{2+}}$ and $I_{Ca^{2+}\text{-}K^+}$ (Alkon et al., 1984; Collin et al., 1988). Step depolarizations from a holding potential of −60 mV were applied at several external potassium concentrations (from 10 to 300 mM, also with 5 mM 4-AP and 100 mM TEA), and they elicited a sustained inward voltage sensitive current, that was blocked by 1 mM cadmium or 1 mM cobalt (data not shown). The calcium currents obtained in this way are shown in Fig. 7 *A* together with its current voltage relation in Fig. 7 *B*. From its wave form, activation of $I_{Ca^{2+}}$ can be seen to be activated very rapidly, showing no inactivation, unlike the calcium currents of other molluscan neurons (Tillotson, 1979; Brehm et al., 1980; Eckert and Ewald, 1981; Eckert and Tillotson, 1981; Eckert et al. 1981). For the present analysis, therefore, the values of the activation and inactivation parameters were assumed to be always was assumed to be 1. The inward tail current after $I_{Ca^{2+}}$, has been previously characterized as an inward (or reversed) $I_{Ca^{2+}\text{-}K^+}$ under elevated external potassium (Alkon et al., 1984). The magnitude of $I_{Ca^{2+}}$, dependent only on the membrane potential, could then be described by the following equation.

$$I_{Ca^{2+}}(V_m) = g_{Ca}(\max)\{1 + \exp[(-V_m - 5)/7]\}^{-1} \times (V_m - E_{Ca}) \quad (15)$$

Properties and mathematical description of light-induced currents

Previous studies demonstrated complex voltage signals of the type B photoreceptor in response to light (Alkon and Fuortes, 1972; Detwiler, 1976; Alkon, 1976; Alkon and Grossman, 1978; Alkon, 1979). For short duration light steps (~1/30 s) the membrane current, under voltage clamp, is dominated by a light-elicited inward sodium current and the calcium-dependent potassium current resulting from calcium released intracellularly (Alkon and Sakakibara; 1985). Best fits to the light response wave forms and consideration of these two principal light-induced currents generated Eqs. 16–21:

$$I_{lgt} = I_{lgt1} + I_{lgt2} + I_{lgt3} \quad (16)$$

$$I_{lgti} = g_{lgti}(\max)\{m_i[1 - \exp(-t_{on}/\tau_{mi}]\}^3 \times \exp(-t_{on}/\tau_{hi})(V_m - E_{lgti}), \quad (i = 1, 2), \quad (17)$$

where t_{on} = elapsed time after light stimulus is on. Also,

$$m_1 = 0.92\{1 + \exp[(V_m + 70)/40]\}^{-1} + 0.08 \quad (18)$$

$$\tau_{h1} = 100[1 + \exp(V_m + 65)]^{-1} + 150 \quad (19)$$

$$m_2 = 0.92\{1 + \exp[(V_m + 74)/28]\}^{-1} + 0.08 \quad (20)$$

$$\tau_{m2} = 250\{1 + \exp[(V_m + 66)/8]\}^{-1} + 450 \quad (21)$$

where the constants in the equations were as follows: $g_{lgt1}(\max) = 60\ \mu S$, $g_{lgt2}(\max) = 75\ \mu S$, $\tau_{h1} = 80$ ms, $\tau_{h2} = 280$ ms, $E_{lgt1} = E_{lgt2} = 40$ mV.

The last component, I_{lgt3}, was formulated as follows:

$$I_{lgt3} = g_{lgt3}m_3^3h_3(V_m - E_{lgt3})\exp(-t_{on}/\tau_{on}) \times \exp(-t_{off}/\tau_{off})), \quad (22)$$

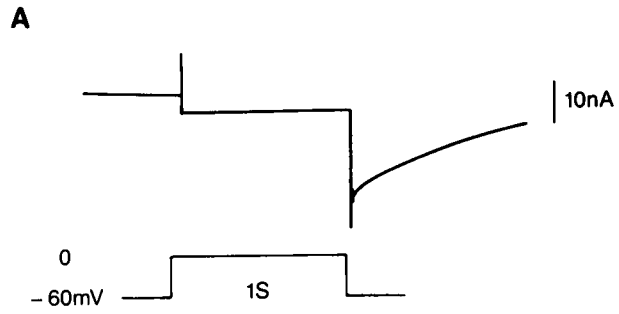
where $g_{lgt3} = 0.5\ \mu S$, $m_3 = 1$, $E_{lgt3} = 0$, $h_3 = \exp(-t_{on}/3)$ s, $\tau_{off} = 1$ s, and t_{off} is the time after light offset.

Because intracellular calcium kinetics during phototransduction have not yet been fully characterized in the *Hermissenda* photoreceptors, and they may involve far more complex intracellular release, buffering, and pumping mechanisms than do the voltage responses (Connor and Alkon, 1984), the light-induced current was modelled here without an explicit formulation of its calcium-dependent component, which was indirectly inferred from its particular voltage dependence.

Fig. 9 *A* demonstrates that the actual current (continuous curve) induced by light at a membrane potential of −60 mV is quite similar to the current simulated by the above equations (open circles).

Modeling of the type B photoreceptor

By adapting the Hodgkin-Huxley description of the action potential of the squid giant axon, we attempted a reconstruc-



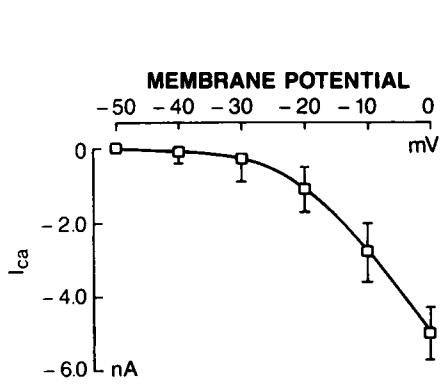


FIGURE 7 (*A*) Voltage-dependent inward calcium current in type B cell is shown after leak substraction, in the presence of 300 mM K^+, 5 mM 4-AP, and 100 mM TEA. The membrane potential is clamped at −10 mV for 1 s. During a depolarizing command the inward current shows no inactivation, and is followed by the large inward tail current, carried by the reversed inward potassium current. (*B*) Current-voltage relation of the inward calcium current is shown. The current is maximally activated at around of 0 mV. A curve is obtained from averages of seven different cells containing 300 mM $[K^+]_o$.

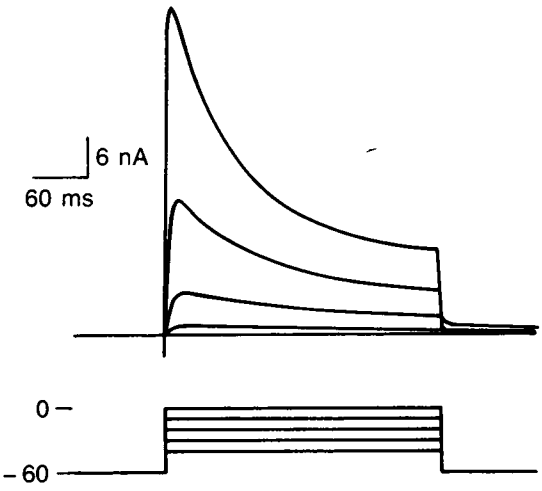


FIGURE 8 Reconstructed voltage-dependent ionic currents are demonstrated for each membrane potential from a holding potential of −60 mV. Compare to actual currents in Fig. 1 *A*.

tion of the type B photoreceptor voltage response to light based on the qualitative and quantitative description of the principal ionic currents as described above. For the type B cell, it is assumed that the total membrane current I_m can be described by,

$$I_m = \frac{C_m \times dV_m}{dt + I_A + I_{Ca^{2+}\text{-}K^+} + I_{Ca^{2+}} + I_l + I_{lgt}} \quad (23)$$

$$I_l = 0.01(V_m + 50) \quad (24)$$

where C_m is the membrane capacitance and I_A, $I_{Ca^{2+}\text{-}K^+}$, $I_{Ca^{2+}}$, and I_{lgt} are given by Eqs. 2, 8, 15, and 16, respectively. The leak current, I_l was considered to be a linear function of membrane potential (i.e., Fig. 3 of Alkon et al. (1984)). The membrane capacitance was obtained from the voltage response elicited by injection of a step current (−0.5 nA in amplitude) through a bridge circuit. The membrane capacitance was calculated as the quotient of the time constant divided by the input impedance (R_i = 9.7 ± 2.5 MΩ) to be 1.18 ± 0.20 nF (average of seven cells).

Finally, the total current elicited by depolarizing step commands from −60 mV, was simulated. In this model, the current flowing through the voltage clamp circuit was included. The family of simulated currents under voltage clamp (Fig. 8) was very similar to the family of actual currents shown in Fig. 1 *A*. In this reconstruction C_m = 1.18 nF; $g_A(max)$ = 2.8 μS; $g_C(max)$ = 0.5 μs; $g_{Ca}(max)$ = 0.1 μS; E_K = −84.6 mV; τ_{h1} = 80 ms; E_{Ca} = 50 mV. The maximal leakage conductance was set at 90 nS in this simulation. A summary of all the simulation parameters is shown in Table 2.

As a function of time, the membrane potential could be represented as a first derivative,

$$\frac{dV_m}{dt} = \frac{(I_m - I_A - I_{Ca^{2+}\text{-}K^+} - I_{Ca^{2+}} - I_l - I_{lgt})}{C_m} \quad (25)$$

Solutions to this differential equation were obtained with the continuous simulator CS2. Fig. 9 shows light responses obtained as a solution for I_{lgt} at the time before (continuous

TABLE 2 Summary of the parameter values

Parameter	Value
$g_{A(max)}$	2.8 (μS)
$g_{C(max)}$	0.5 (μS)
$g_{Ca(max)}$	0.1 (μS)
$g_{lgt1(max)}$	60 (μS)
$g_{lgt2(max)}$	75 (μS)
$g_{lgt3(max)}$	0.5 (μS)
g_l	0.01 (μS)
E_K	−86.4 (mV)
E_{Ca}	50 (mV)
E_{lgt1}	40 (mV)
E_{lgt2}	40 (mV)
E_{lgt3}	0 (mV)
E_l	−50 (mV)
τ_{h1}	80 (mV)
τ_{h2}	280 (ms)
τ_{off}	1000 (ms)
C_m	1.18 (nF)

curve) and after (curves represented by *open circles*) associative conditioning. According to previous studies, I_A and $I_{Ca^{2+}\text{-}K^+}$ are reduced by conditioning (Alkon et al., 1985; Collin et al, 1988) on the order of 70 and 60%, respectively. Taking into account these changes of ionic currents, our simulation showed conditioning-induced enhancement of the LLD following a light flash, as previously observed for actual type B light responses from conditioned animals.

DISCUSSION

The present study provides a mathematical description of the type B photoreceptor voltage response as a function of one voltage-dependent potassium current, I_A, a voltage- and calcium-dependent K_+ current, $I_{Ca^{2+}\text{-}K^+}$, a voltage-dependent Ca^{2+} current $I_{Ca^{2+}}$, and two light-dependent macroscopic conductances, all of which are localized in the cell's soma. In this description the delayed rectifying conductance was ignored because of its negligible activation for membrane potentials < 0 mV (Alkon et al., 1984). This study is mainly focused on the explanation of light response changes induced by associative learning of *Hermissenda*. I_A and $I_{Ca^{2+}\text{-}K^+}$ inactivation can reproduce the prolonged LLD and the increase in the amplitude of light response. Therefore the model should be considered a phenomenological rather than a mechanistic description of the conductance changes.

Alkon et al. (1985) showed that after classical conditioning, I_A and $I_{Ca^{2+}\text{-}K^+}$ are persistently reduced by 70 and 60% compared with control animals. Furthermore, a recent study has demonstrated that the calcium current is also reduced by 40%, with a sufficient number of extra training trials during acquisition of the associative memory (Collin et al., 1988), while neither I_A nor $I_{Ca^{2+}\text{-}K^+}$ is further substantially modified. Conditioning-induced enhancement of the LLD in response to light cannot be clearly understood without considering the dynamic nature of the changes of the ionic currents. The persistent increase of the input impedance (i.e., decrease of the total ionic conductance) which follows conditioning should cause the light response to increase. In order to elucidate the ionic basis of this LLD enhancement, we incorporated into the model previous observations that the three ionic currents, I_A, $I_{Ca^{2+}\text{-}K^+}$, and $I_{Ca^{2+}}$, are progressively decreased after conditioning. Our simulation showed that LLD enhancement could arise from a progressive decrease of these currents. Our model also allows a preliminary estimation of the membrane input impedance changes which occur during a light response before and after conditioning.

The two potassium currents analyzed here are similar to others previously reported in several invertebrate and vertebrate neurons (Connor and Stevens, 1971; Rogawski, 1985; Blatz and Magleby, 1987). The observed calcium current, however, appears to be more unique to the *Hermissenda* type B photorreceptor's soma. Because its contribution to total membrane current is small, the light response is not modified dramatically by changes of $I_{Ca^{2+}}$. Unlike the calcium currents of other *Hermissenda* neurons, such as LP1, the type B $I_{Ca^{2+}}$ is not regulated by second messengers such as protein kinase C or small G proteins (Collin et al., 1990a, 1990b), and only changes marginally with overtraining during classical conditioning of *Hermissenda* (Collin et al., 1988). The function of this channel has not yet been established. Because it does not inactivate, it can contribute significantly to elevation of intracellular calcium at the more depolarized membrane potentials observed with light stimulation (Connor and Alkon, 1984).

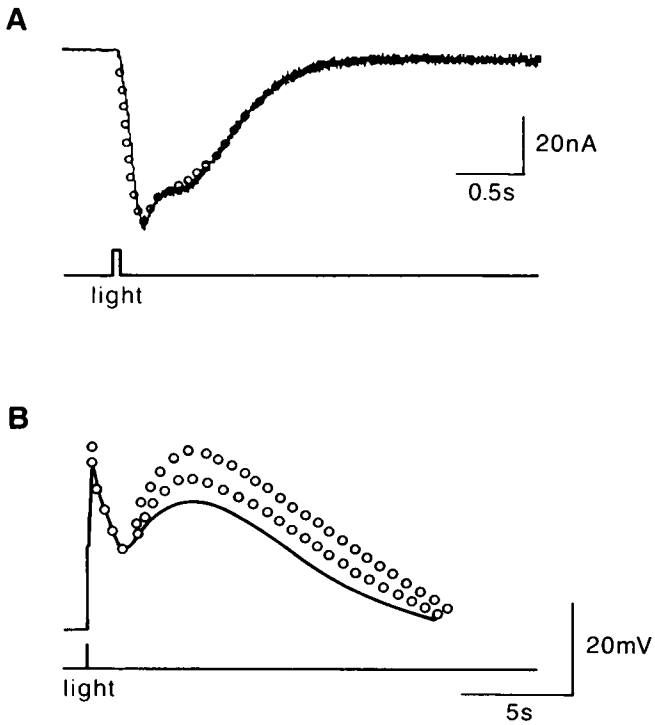


FIGURE 9 (*A*) Light-induced currents under membrane potential clamped at −60 mV. The duration of light flash is 1/30 s. Open circles represent the results of a simulation with the equation: $I_{lgt} = I_{lgt1} + I_{lgt2} + I_{lgt3}$. See Results for details. Calculated light responses of type B obtained from Eqs. 16–21. (*B*) Reductions of I_A and $I_{Ca^{2+}\text{-}K^+}$ after light response in a manner similar to that observed with associative conditioning. The most prominent change is observed as an enhancement of the second component, a LLD of approximately 30 s. The LLD is caused by inactivation of $I_{Ca^{2+}\text{-}K^+}$. For lowest response shown by open circles, I_A and $I_{Ca^{2+}\text{-}K^+}$ and $I_{Ca^{2+}}$ are assumed to be inactivated by 70, 60, and 50%, respectively. In the top response $I_{Ca^{2+}\text{-}K^+}$ decreased by 30% of control value.

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