Calcium Waves and Closure of Potassium Channels in Response to GABA Stimulation in *Hermissenda* Type B Photoreceptors

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**INTRODUCTION**

Classical conditioning, a form of associative learning, requires presentation of paired stimuli, a conditioned stimulus (CS) and an unconditioned stimulus (US), within a specific temporal interval. This implies that both the CS and the US produce signals that interact to cause memory storage. Although the requirement for paired stimuli has been known for decades, the identity of the interacting signals is still unknown.

The sea slug, *Hermissenda crassicornis*, is an animal model of classical conditioning and is an ideal model in which to evaluate the interaction of signals mediating classical conditioning. *Hermissenda* learns to associate light (the CS) with turbulence (the US). The memory of the association is stored in the type B photoreceptors as an increase in input resistance and excitability (Crow and Alkon 1980; Farley 1987; Farley and Alkon 1982). Memory storage critically depends on an elevation of intracellular calcium concentration (Matzel and Rogers 1993; Talk and Matzel 1996), and activation of protein kinase C (Alkon et al. 1988; Farley and Auverbach 1986; McPhie et al. 1993). Light leads to production of diacylglycerol (DAG) (Talk et al. 1997), an activator of protein kinase C (PKC), and inositol triphosphate (IP3), which leads to release of calcium from intracellular stores (Talk and Matzel 1996).

The turbulence US causes hair cells to release γ-amino butyric acid (GABA) onto the terminal branches of the type B photoreceptor (Alkon et al. 1993). The response to GABA stimulation consists of an IPSP followed several seconds later by a small depolarization lasting for several seconds (Matzel and Alkon 1991). The hyperpolarization is caused by opening of GABA_A chloride channels and GABA_B potassium channels (Alkon et al. 1992; Rogers et al. 1994). The cause of the late depolarization has not been determined, but observations are consistent with a G-protein-dependent closure of potassium leak channels (Matzel and Alkon 1991; Rogers et al. 1994).

Because light alone does not cause memory storage but does produce the activators of PKC, it is imperative to identify which critical factors are contributed by turbulence. Evidence suggests that calcium may be an essential second-messenger contributed by turbulence to associative memory storage. Support comes from the observation that dantrolene, which prevents the propagation of calcium waves (Trafford et al. 1995), prevents in vitro classical conditioning of *Hermissenda* (Blackwell and Alkon 1999). Moreover, one experiment demonstrates that turbulence evokes a calcium elevation that propagates...
from the terminal branches to the soma (Ito et al. 1994).
However, another calcium-imaging study has not observed calcium in the terminal branches (Muzzio et al. 1998).

Because the observation of a calcium wave has not been replicated, one purpose of this modeling study was to determine if GABA stimulation can contribute a calcium signal that propagates from the terminal branches to the soma. The potassium channel underlying the late depolarization has not been completely characterized, thus the second purpose of the study was to determine whether the potassium leak channel is responsible for the GABA-induced depolarization.

METHODS

The Hermisenda type B photoreceptor is modeled using the GENESIS simulation software implemented on a UNIX workstation. Chemesis, which consists of two additional libraries of GENESIS objects, was used for simulating the biochemical reactions of the GABA<sub>B</sub> synapse as well as calcium dynamics. One of the Chemesis libraries has objects for simulating general biochemical reactions, pools of molecules, and calcium release from intracellular stores; the other Chemesis library has objects for simulating one- and two-step ligand-gated channels and separable or nonseparable formulations of calcium-dependent rate constants.

Morphology

The geometry of the type B photoreceptor model is an approximation to the morphological features previously described (Crow et al. 1979; Eakin et al. 1967; Stensaas et al. 1969) and is illustrated in Fig. 1B. The rhabdomere is a cylinder 12 μm in diameter and 12 μm in length. The numerous microvilli of the rhabdomere are taken into account by decreasing the membrane resistance and increasing the capacitance proportional to the surface area contributed by 5,000 microvilli of 0.16-μm diameter by 5-μm length. The diameter of the central core of the rhabdomere (the part the microvilli are attached to) is 2 μm. The rhabdomere is connected to the soma which is a cylinder 20 μm in diameter by 24 μm in length. The neurite, which functions as both an axon and dendrite, is 100 μm in length; the elliptical cross section has a long axis of 3 μm and a short axis of 1 μm. The neurite is subdivided into four isopotential elliptic cylinders of 25 μm (Fost and Clark 1996). The neurite’s terminal branches, the site of all synaptic interactions, are modeled as two equivalent cylinders, 15 μm in length. One cylinder represents the set of nonsynaptic branches, and the other cylinder represents the set of synaptic branches. The distal 10 μm compartment of the synaptic branch contains the synaptic channels. Two variations on synaptic connectivity are simulated by using two different radii of the terminal branch cylinders. Under the assumption that 10% of the terminal branches receive synaptic input (used for simulations unless otherwise indicated), the equivalent cylinder radius of the synaptic branch is 0.22 μm and the equivalent cylinder radius of the nonsynaptic branch is 0.93 μm. Under the assumption that 50% of the terminal branches receive synaptic input, the equivalent cylinder radius of both the synaptic and nonsynaptic branches are 0.63 μm. The neurite and terminal branch cylinders are subdivided into 1-μm-long compartments for the purpose of modeling calcium concentration dynamics. Passive membrane resistivity is 10 kΩ-cm<sup>2</sup>; membrane capacitance is 1 μF/cm<sup>2</sup>, axial resistivity is 100 Ω-cm. A somatic shunt of 0.005 μS simulates the effect of a sharp electrode. The resting potential of the cell is −57 mV; the steady-state input resistance is 35 MΩ. These values are comparable to the mean resting potential and input resistance (R<sub>in</sub>) experimentally observed in Hermisenda photoreceptors. The somatic shunt is required to achieve

![Model of type B photoreceptor](http://jn.physiology.org/)

**FIG. 1.** Model of type B photoreceptor. A: biochemical reactions, ionic channels, and calcium regulatory mechanisms included in model. Channels, receptors, and enzymes located within the dashed lines are present only in the synaptic branch. The potassium leak channel, as well as calcium pumps, calcium release channels, and buffers, are located in all compartments of the model. B: morphology of type B photoreceptor model. All compartments are modeled as equivalent cylinders (the neurite is an elliptic cylinder). Membrane surface area of the rhabdomeric microvilli is accounted for by a proportional increase in the capacitance and decrease in the resistance.
the experimentally observed $R_\text{s}$ using a physiologically realistic passive membrane resistivity (Rall and Agmon-Snir 1998). The somatic shunt also has the effect of increasing the resting potential by 5 mV.

### Channels

As illustrated in Fig. 1A, the model contains GABA$_A$ synaptic channels, GABA$_B$$_1$ synaptic channels, and a calcium-sensitive potassium leak channel. Measurements of inhibitory synaptic input are made in dark adapted photoreceptors at resting potential, thus it is not necessary to include channels and second-messenger pathways involved in phototransduction or the voltage-dependent channels that are not active below $-50$ mV, i.e., the transient potassium channel (Acosta-Urquidi and Crow 1995), the calcium-dependent potassium channel (Farley 1988; Sakakibara et al. 1993), or the persistent calcium channel (Yamoh and Crow 1994).

Potassium leak channels are voltage-independent channels that are open and conducting at rest. Neurmodulators coupled to phospholipase C (PLC) cause the channels to close (Bayliss et al. 1994; Hsiao et al. 1997; Jafari et al. 1997; Jones and Baughman 1992; Lee and McCormick 1997), and they are blocked by barium (Buckler 1999). Two sets of experiments support the existence of a potassium leak conductance in *Hermisenda* photoreceptors. First, the late depolarization following GABA stimulation is present at potentials as low as $-70$ mV; and in 30 mM external K$^+$ artificial seawater (ASW), a late phase outward current increases with more negative holding potential (Rogers et al. 1994). Second, light stimulation, which causes an increase in intracellular calcium (Buckler et al. 1996) where $g_L \approx 400 \mu S \text{cm}^{-2}$, and of the light-induced closure of potassium channels in *Hermisenda* photoreceptors (Alkon and Sakakibara 1985; Blackwell 2000a). Second, light stimulation, which causes an increase in intracellular calcium (Buckler et al. 1996) where $g_L \approx 400 \mu S \text{cm}^{-2}$, and of the light-induced closure of potassium channels in *Hermisenda* photoreceptors (Alkon and Sakakibara 1985; Blackwell 2000a).

In the model, the potassium leak channels are distributed uniformly throughout all compartments, with a maximal conductance of 300 $\mu S \text{cm}^{-2}$, and are responsible for 75% of the total leakage conductance. The reversal potential of this channel is $-85$ mV. It is assumed that one calcium ion binds to each of two channel subunits to close the channel

$$K^+ + Ca^{2+} \xrightarrow{\beta} Ca-K_L$$

where $K^+$ is the open state of a channel subunit, $Ca-K_L$ is the closed state of a channel subunit, $\eta = 0.45e-3 \mu M^{-1} \text{ms}^{-1}$, and $\nu = 0.5e-3 \text{ms}^{-1}$. The parameters are adjusted such that 92% of the channels are open at the basal calcium concentration of 0.11 $\mu M$; less than 1% of the channels are open at a 10 $\mu M$ calcium concentration; and the time constant of activation and decay is on the order of seconds, consistent with voltage-clamp data of leak channels in carotid body cells (Buckler 1999) and corticocolosal neurons (Jones and Baughman 1992), and of the light-induced closure of potassium channels in *Hermisenda* photoreceptors (Alkon and Sakakibara 1985; Blackwell 2000a).

Qualitatively, the same results are produced if two calcium ions bind to a single-channel subunit.

In *Hermisenda*, in response to mechanical stimulation, the hair cells depolarize and generate action potentials that cause release of GABA onto the type B photoreceptor terminal branches. In the model, hair cell action potentials are modeled as Poisson distributed random events with an initial rate of approximately 0.15 ms$^{-1}$, and a rate that decreases exponentially with a time constant of 1,000 ms (Alkon and Bak 1973; Schultz and Clark 1997). In the model, for each action potential produced by the hair cell, the GABA receptors are exposed to a 1 mM concentration of GABA for a duration of 1 ms (Destexhe and Sejnowski 1995).

The GABA$_A$ channel is modeled as a ligand-gated receptor channel with two bound states

$$R_X + \text{GABA} \rightleftharpoons \text{GABA-R}_X$$

where $R_X$ is the unbound form, GABA-$R_X$ is the bound and closed form, and GABA-$R_X^*$ is the open and conducting form of the GABA$_X$ channel. The GABA$_X$ channel transitions from the closed state to the bound state with rate constants $k_1 = 0.3e-3 \mu M^{-1} \text{ms}^{-1}$ and $k_2 = 0.06 \text{ms}^{-1}$. A second voltage-independent transition from the bound state to the open state occurs with rate constants, $k_3 = 0.019 \text{ms}^{-1}$ and $k_4 = 0.009 \text{ms}^{-1}$. The open state can return to the closed state either through the bound state or directly with rate constants $k_5 = 0.03e-3 \mu M^{-1} \text{ms}^{-1}$ and $k_6 = 0.165 \text{ms}^{-1}$. These rate constants were obtained from Destexhe et al. (1994) and modified for the colder Hermisenda temperature assuming a Q10 of 1.2 (ffrench-Mullen et al. 1988). The current through the channel equals the fraction of channels in the open state (GABA-$R_X^*$), times the maximal conductance (95 nS), times the driving potential. The reversal potential of chloride permeable GABA$_X$ channels in *Hermisenda* is $-70$ mV (Alkon et al. 1992; Rogers et al. 1994). The maximal synaptic conductance of 95 nS is larger than that demonstrated by Rogers et al. (1994) for two reasons. One reason is the simplified morphology of the synaptic branches and adjusting the parameters to match the voltage response at the soma. Had a more realistic morphology been implemented, the synaptic conductance could have been reduced to a more realistic value with an equivalent voltage response at the soma; however, this would not have changed the overall results. The second reason for the large maximal conductance is that the kinetics of the GABA$_X$ equations produce a small fraction of channels in the open state. The observed GABA$_X$ conductance is $<14$ nS, which is close to the value observed by Rogers et al. (1994).

When the GABA$_B$ metabotropic receptor binds to GABA, it catalyzes the activation of G protein

$$R_B + \text{GABA} \rightleftharpoons \text{GABA-R}_B^*$$

The rate constants describing GABA binding to the GABA$_B$ receptor are $g_1 = 0.06e-3 \mu M^{-1} \text{ms}^{-1}$ and $g_2 = 0.05 \text{ms}^{-1}$. The bound and active GABA$_B$ receptor binds to the inactive G protein (composed of $G_a$ and $G_{p_y}$ subunits) with rate constants $g_3 = 2.0 \mu M^{-1} \text{ms}^{-1}$ and $g_4 = 0.5 \text{ms}^{-1}$; and catalyzes the exchange of GDP for GTP. These rate constants were adjusted from those provided by Destexhe et al. (1994) such that a single vesicle of neurotransmitter does not saturate the receptor in terms of $G_a$-GDP produced or GABA$_B$ postsynaptic current generated (Tempia et al. 1998). The active $G_a$, $G_{p_y}$ GT, is produced with rate constant $g_5 = 0.5 \text{ms}^{-1}$ (Mukhopadhyay and Ross 1999); degradation of $G_a$-GTP (hydrolysis of the bound GDP) occurs with rate constant $g_6 = 0.02 \text{ms}^{-1}$ (Biddulcombe et al. 1996) and is the rate-limiting step for regeneration of inactive G protein, $G_{p_y}$. The total G protein concentration of 100 $\mu M$ is conservatively estimated at 1/10th the concentration measured in photoreceptor membranes (Kahlert and Hofmann 1991; Melia et al. 1997; Nobes et al. 1992).
The active $G_e$ subunit binds to the GABA$_A$ potassium permeable channel

$$K_B + G_e \cdot GTP \xrightarrow{\theta} G_e - K_B \xrightarrow{\xi} G_e \cdot K_B^T$$

(4)

where $K_B$ is the unbound form, $G_e - K_B$ is the bound and closed form, and $G_e \cdot K_B^T$ is the open and conducting form of the GABA$_A$ channel. The GABA$_A$ channel transitions from the closed state to the bound state with rate constants $\theta = 0.018 \mu M^{-1} \text{ms}^{-1}$ and $\xi = 0.05 \text{ms}^{-1}$. A second voltage-independent transition from the bound state to the open state occurs with rate constants, $\psi = 0.01 \text{ms}^{-1}$ and $\delta = 0.002 \text{ms}^{-1}$. These parameter values were modified from Destexhe et al. (1994) using a Q10 of 2 (Otis et al. 1993). The maximal conductance is 9.5 nS and the reversal potential of potassium permeable GABA$_B$ channels in *Hermissenda* is $-85 \text{mV}$ (Alkon et al. 1992; Rogers et al. 1994).

The GABA$_B$ channel transitions from the closed state to the open state when either calcium binding site is occupied (RyR), the inhibitory calcium binding site is occupied (R$_{B2}$), or both calcium binding sites are occupied (R$_{B1}$):

$$R_{B0} \xrightarrow{\lambda} R_{B1} \xrightarrow{\lambda} M_1 \cdot Ca^{2+} \xrightarrow{\lambda} L_1 $$

(7)

where $M_1 = 0.015 \mu M^{-1} \text{ms}^{-1}$, $M_2 = 0.83e-3 \mu M^{-1} \text{ms}^{-1}$, $L_1 = 7.6e-3 \text{ms}^{-1}$, and $L_2 = 0.84e-3 \text{ms}^{-1}$. $R_{B0}$ is the open and conducting state.

The following equation describes calcium flux through the open channels:

$$\Phi_{calc} = F_{max}(RyR)|R_{B1}(Ca^{2+}) - [Ca^{2+}]|$$

(8)

where the equilibrium value of $[Ca^{2+}]_{eq}$ is 20 $\mu M$, units of $R_{B0}$ are fraction of RyR in the open state, and the maximal rate of efflux, $F_{max}(Ry)$, is 0.08 $\text{ms}^{-1}$ unless otherwise specified.

Mechanisms serving to reduce or equilibrate calcium concentration include diffusion (6e-9 cm$^2$/ms), buffers, and pumps. Equations and parameters for the calcium buffer are identical to that described previously (Blackwell 2000b), and are included in the Appendix. Two different pumps, the smooth endoplasmic reticulum ATPase (SERCA) pump and the plasma membrane calcium ATPase (PMCA) pump (Morgans et al. 1998), were implemented in the present model. The equations used to describe calcium flux due to the SERCA pump is:

$$\Phi_{SERCA} = \frac{V_{max}(SERCA) \cdot [Ca^{2+}]^2}{[Ca^{2+}]^2 + K_{D(SERCA)}} - J_{L, \text{calc}}([Ca^{2+}]_1 - [Ca^{2+}])$$

(9)

where $K_{D(SERCA)}$ is 0.1 $\mu M$ (Li and Rinzel 1994), and, unless otherwise indicated, $V_{max}(SERCA)$ is 0.6 $\mu M$/ms. The square power in this equation is the Hill coefficient. The second term on the left hand side is a compensatory leak; $J_{L,calc}$ has units of ms$^{-1}$, and its value is adjusted such that net calcium flux from the ER is zero at the basal calcium value of 0.11 $\mu M$. The equation for calcium flux due to the PMCA pump is:

$$\Phi_{PMCA} = \frac{V_{max}(PMCA) \cdot \text{area} \cdot [Ca^{2+}]^2}{[Ca^{2+}]^2 + K_{D(PMCA)}} - J_{L, \text{calc}}([Ca^{2+}]_1 - [Ca^{2+}])$$

(10)

where $K_{D(PMCA)}$ is 1.0 $\mu M$ (Enyedi et al. 1994). The square area of the cell membrane, vol = volume of the cytosolic compartment, and $J_{L, \text{calc}}$ is the compensatory leak adjusted such that net flux across the plasma membrane is zero at basal calcium concentration. The square power on this equation is the Hill coefficient. For most simulations, $V_{max}(PMCA)$ is 0 $\mu M$/ms/cm$^2$, but this equation is included because one set of simulations evaluates the effect of a nonzero $V_{max}(PMCA)$.

The equations for calcium flux due to diffusion, and the complete equations for calcium concentration in the cytosol and the ER are given in the Appendix.

**Results**

**Calcium waves**

The first issues addressed by this study are whether a calcium wave can propagate from the terminal branches to the soma and which mechanisms are essential for wave propaga-
The contributions of IP$_3$-induced calcium release (IICR) and calcium-induced calcium release through the RyR (CICR) are evaluated by simulations that vary the calcium flux due to each of these. The mechanisms of calcium wave generation due to CICR are further explored by inspecting the dynamics of RyRs during a calcium pulse. The role of the PMCA and SERCA pumps is analyzed with additional simulations and by inspecting the calcium flux terms over time during the calcium wave.

In all simulations, a 3-s-duration mechanical stimulation of hair cells is initiated 2 s after beginning the simulation. As illustrated in Fig. 2A, the stimulus produces an adapting train of action potentials between 2 and 5 s after the simulation is initiated. Figure 2B shows the concentration of G$_\alpha$-GTP produced by exposure of GABA$_B$ receptors to a 1 mM concentration of GABA for a duration of 1 ms in response to each action potential. Due to the dynamics of G-protein activation, the effects of individual action potential. Due to the dynamics of G-protein activation, the effects of individual "vesicles" of GABA are smoothed. The G$_\alpha$-GTP binds to and activates PLC, whose concentration is portrayed in Fig. 2B. Both G$_\alpha$-GTP concentration and active PLC concentration peaks at 0.54 s after the stimulus is initiated. Active PLC catalyzes the production of IP$_3$ (illustrated in Fig. 3), which is required for IICR.

Both IP$_3$ and RyR are required for the generation of calcium waves. Figure 3 shows that a calcium wave propagates from the terminal branches to the soma and that both IICR and CICR are essential for wave propagation. The requirement of CICR is seen by comparing Fig. 3, middle and left columns, which illustrate calcium concentration as a function of time and distance along the neurite for V$_{\text{max}}$(PLC) between 0.01 and 0.1 ms$^{-1}$, values that encompass the range of estimates of PI-specific PLC activity measured in photoreceptors (Mitchell et al. 1995; Rack et al. 1994; Smrcka et al. 1991; Suzuki et al. 1995). A distance of 0 µm corresponds to the distal end of the neurite, connected to the terminal branches; and a distance of 100 µm corresponds to the proximal part of the neurite, connected to the soma. For V$_{\text{max}}$(PLC) = 0.1 ms$^{-1}$, IP$_3$ concentration reaches 0.2 µM (the threshold for IICR) as far as 54 µm from the distal end of the neurite, whereas for V$_{\text{max}}$(PLC) = 0.01 ms$^{-1}$, IP$_3$ concentration reaches 0.2 µM only as far as 8 µm. In all cases for F$_{\text{max}}$(RyR) = 0, the calcium wave propagates to the distance at which IP$_3$ reaches 0.2 µM. In contrast, for F$_{\text{max}}$(RyR) = 0.08, the calcium wave propagates all the way to the soma; thus release through the ryanodine receptor is responsible for calcium wave propagation the remainder of the distance, which is substantial for V$_{\text{max}}$(PLC) = 0.01 ms$^{-1}$.

The mechanism whereby CICR generates a calcium wave is further illustrated in Fig. 4A, which shows calcium concentration and the fraction of RyRs in each state. As calcium concentration increases, the fraction of open channels (in the R$_{10}$ state) increases. This allows calcium to flow from the ER to the cytosol and further increases the calcium concentration. This positive feedback accelerates the rate at which RyRs open and calcium concentration increases, analogous to the activation of sodium channels by depolarization. Similar to sodium channels, the RyRs inactivate when the calcium concentration is too high. This is seen in Fig. 4A by the increase in the fraction of inactive channels (channels in the R$_{11}$ and R$_{01}$ states).

A secondary calcium wave is seen for V$_{\text{max}}$(PLC) = 0.03 and 0.1 ms$^{-1}$. The mechanism generating this secondary wave is analogous to that generating multiple action potentials in response to current injection. The RyR de-inactivates (referred to as adaptation in the cardiac myocyte literature) (cf. Tang and Othmer 1994) once the calcium concentration returns to the basal level. If the calcium influx remains elevated for 6–7 s (due to IICR), the channel will activate again causing another calcium peak and initiating another calcium wave. In the distal part of the neurite for V$_{\text{max}}$(PLC) = 0.1 ms$^{-1}$, the calcium influx due to IICR is so high that calcium concentration does not return to the basal level but remains elevated at 1 µM. The RyR partially de-inactivates, and the secondary wave is of lower amplitude in this part of the neurite.

In addition to CICR, IICR is essential for wave propagation, and V$_{\text{max}}$(PLC) has a dramatic effect on the speed of wave propagation. First, IICR is the initiating stimulus for the calcium wave. If V$_{\text{max}}$(PLC) = 0.003, IP$_3$ concentration is insufficient for IICR (<0.2 µM) and a calcium wave is never initiated (results not shown). Second, IICR is responsible for a portion of the calcium wave because IP$_3$ diffuses much farther than calcium (Allbritton et al. 1992). Wave propagation is faster in regions where IP$_3$ concentration exceeds 0.2 µM; thus the calcium wave reaches the soma within 6.0 s after stimulus onset for V$_{\text{max}}$(PLC) = 0.1 but requires 8.0 s for V$_{\text{max}}$(PLC) = 0.01. The effect on wave speed is seen more clearly in Fig. 4B, which plots wave speed versus distance as a function of V$_{\text{max}}$(PLC). Wave speed is high in the most distal part of the neurite and
decreases to a plateau value of 0.013 μm/ms in the proximal part of the neurite; as \( V_{\text{max}} \) (PLC) decreases to 0.01 ms\(^{-1}\), the region of the neurite in which the calcium wave propagates at 0.013 μm/ms increases from 20 to 80 μm. This suggests that the ryanodine receptor by itself supports calcium wave propagation at a speed of 0.013 μm/ms. The distance at which wave speed drops <0.02 μm/ms corresponds to the distance IP\(_3\) exceeds 0.2 μM, the threshold for IICR reported by Li and Rinzel (1994). A similar effect of \( V_{\text{max}} \) (PLC) is seen in the plots of peak calcium concentration versus distance (Fig. 4C). In regions where IP\(_3\) exceeds 0.2 μM, IICR contributes to a peak calcium concentration >3 μM. The correspondence between calcium concentration and wave speed suggests a possible causal relationship, a concept that is explored further in the next sections.

ROLE OF DIFFUSION, PMCA, AND SERCA PUMPS. In the absence of IICR (e.g., closer to the soma), the calcium wave due to
the calcium concentration increase due to diffusion and the net flux of calcium out of the ER. Figure 5 demonstrates the interaction among diffusion, release, and pump re-uptake by plotting various flux terms versus time for several values of $V_{\text{max}}(\text{SERCA})$. All changes in $V_{\text{max}}(\text{SERCA})$ are accompanied by compensatory changes in the $J_{L-S}$ to maintain a constant basal calcium concentration. Figure 5A shows that calcium flux due to diffusion increases first, at $\approx 3.6$ s, in the compartment $22 \mu m$ from the terminal branches. The concentration increase causes an increase in SERCA flux, which transfers calcium from the cytosol to the ER; thus the net flux out of the ER becomes negative. The concentration increase also activates the ryanodine receptor, and $\approx 0.19$ s after diffusion begins, the CICR flux is large enough to change the net ER flux from negative to positive. The SERCA pump affects this process by its control of the calcium flux. A higher $V_{\text{max}}(\text{SERCA})$ of 0.7 (Fig. 5B) reduces the net flux from the ER and opposes the diffusive flux; calcium concentration is lower and the resulting calcium flux due to diffusion is smaller. The calcium concentration increases more slowly, thereby decreasing the rate of ryanodine receptor activation and increasing the latency between the time of diffusive increase and the time when the net ER flux changes sign.

If $V_{\text{max}}(\text{SERCA})$ is too high relative to $F_{\text{max}}(\text{RyR})$, the calcium wave does not propagate to the soma. This phenomenon is explained in Fig. 5, C and D, which illustrates calcium concentration and flux terms for $V_{\text{max}}(\text{SERCA}) = 0.8 \mu M/\text{ms}$ at two locations along the neurite. In the compartment $22 \mu m$ from the terminal branches, the peak calcium concentration is 2.0 $\mu M$ and the latency is 0.25 s. Although the rate of ryanodine receptor activation is slower than for $V_{\text{max}}(\text{SERCA}) = 0.7 \mu M/\text{ms}$, flux through the receptor reaches the same peak value of $10e-18$ $\mu \text{Mol}/\text{ms}$ at $38 \mu m$, the peak calcium concentration is only 0.44 $\mu M$, and the latency is increased to 0.5 s. The calcium concentration in the adjacent compartment is illustrated to show its relationship to diffusive flux. Such a difference in peak calcium concentration between two adjacent compartments is not seen for lower values of $V_{\text{max}}(\text{SERCA})$. The much lower diffusive flux causes a slow activation of the RyR and allows the inactivation process to proceed at a commensurate pace; thus peak flux through the RyR is lower ($7e-18$ $\mu \text{Mol}/\text{ms}$) than for other $V_{\text{max}}(\text{SERCA})$ values. All flux terms are smaller and increase more slowly as compared with $22 \mu m$.

Figure 5E summarizes the effect of the SERCA pump on peak calcium concentration versus distance along the neurite. For $V_{\text{max}}(\text{SERCA})$ between 0.5 and 0.7 $\mu M/\text{ms}$, peak calcium concentration decreases to a plateau value. For $V_{\text{max}}(\text{SERCA}) = 0.8 \mu M/\text{ms}$, the calcium concentration does not reach a plateau value, instead it decreases with distance, and the calcium wave dies out 40 $\mu m$ from the terminal branches. For all values of $F_{\text{max}}(\text{RyR})$ tested, if $V_{\text{max}}(\text{SERCA})$ is too high, the peak calcium concentration decreases with distance and does not reach a plateau value.

The effect of the SERCA pump on calcium wave propagation is further illustrated in Fig. 6A for $F_{\text{max}}(\text{RyR}) = 0.16 \text{ ms}^{-1}$ and $\text{PLC} = 0.01 \text{ ms}^{-1}$. For both values of $V_{\text{max}}(\text{SERCA})$, the wave from 0 to $20 \mu m$ propagates faster than the remainder of the wave due to IICR in those compartments. The wave from 40 to $100 \mu m$ propagates at a constant speed, with a higher speed in the proximal part of the neurite remaining constant at 0.013 $\text{ms}^{-1}$.

**FIG. 4.** Mechanisms of calcium wave generation. A: calcium concentration and fraction of ryanodine receptor channels (RyRs) in each state in compartment 22 $\mu m$ from terminal branches vs. time for $F_{\text{max}}(\text{RyR}) = 0.08 \text{ ms}^{-1}$, $V_{\text{max}}(\text{SERCA}) = 0.6 \mu M/\text{ms}$, $V_{\text{max}}(\text{PLC}) = 0.01 \text{ ms}^{-1}$. As calcium concentration increases, the fraction of open channels (in the $R_{00}$ state) increases, and the fraction of unbound channels (in the $R_{01}$ state) decreases. Approximately 0.25 s after the initial increase in the $R_{00}$ state, while calcium concentration is still increasing, calcium release peaks and the $R_{01}$ state begins to decrease. The fraction of inactivated channels ($R_{11}$ state and $R_{10}$ state) increases more slowly but remains elevated for almost 2 s after calcium concentration returns to the basal level. B: wave speed vs. distance as a function of $V_{\text{max}}(\text{PLC})$. The wave speed in the proximal part of the neurite remains constant at 0.013 $\text{ms}^{-1}$, and as $V_{\text{max}}(\text{PLC})$ decreases to 0.01 $\text{ms}^{-1}$, the region of the neurite in which the calcium wave propagates with this speed increases from 20 to $80 \mu m$. C: peak calcium concentration versus distance as a function of $V_{\text{max}}(\text{PLC}).$
speed and peak calcium concentration for \( V_{\text{max}} \) (SERCA) of 1.0 \( \mu \text{M/ms} \) as compared with \( V_{\text{max}} \) (SERCA) of 1.4 \( \mu \text{M/ms} \).

The PMCA pump has a lower affinity (\( K_D \approx 1 \mu \text{M} \)) than the SERCA pump, yet it has an effect qualitatively similar to that of the SERCA pump. Figure 6B illustrates the effect of \( V_{\text{max}} \) (PMCA) for \( V_{\text{max}} \) (PLC) of 0.03 \( \mu \text{M/ms} \), a value that produces secondary waves (see Fig. 3). A moderate value of \( V_{\text{max}} \) (PMCA), equal to 4-9 \( \mu \text{Mol/ms/cm}^2 \), slows the propaga-
tion of the primary wave and stops the propagation of the secondary wave. A higher value of $V_{\text{max}}$ (PMCA), equal to $8 \times 10^{-9}$ $\text{Mol}/\text{ms}/\text{cm}^2$, prevents the secondary wave from appearing.

RELATIONSHIP BETWEEN CALCIUM CONCENTRATION AND WAVE SPEED. Wave speed is inversely related to the latency between the influx of calcium and the time when the net ER flux changes sign. Latency is affected by the magnitude of diffusive flux (Tang and Othmer 1994), which depends on calcium concentration in the adjacent compartment. Thus the SERCA pump affects latency by its control of calcium concentration. Table 1, which lists wave speed and peak calcium concentration in the distal half of the neurite, shows that a higher $V_{\text{max}}$ (SERCA) leads to faster wave propagation.

Similarly, $F_{\text{max}}$ (RyR) modifies wave speed through its effect on peak calcium concentration. If $F_{\text{max}}$ (RyR) is doubled, the $V_{\text{max}}$ (SERCA) also is increased to maintain the next ER flux $= 0$ at the basal calcium concentration. When both $F_{\text{max}}$ (RyR) and $V_{\text{max}}$ (SERCA) are doubled, the net calcium flux from the ER is doubled. Thus once the net ER flux becomes positive, the calcium concentration increases faster and reaches a greater peak value. For example, as shown in Table 1, doubling $F_{\text{max}}$ (RyR) and $V_{\text{max}}$ (SERCA) causes an increase in peak cal-

**Figure 6.** Calcium concentration as a function of time and distance along the neurite. A: $F_{\text{max}}$ (RyR) = 0.16 ms$^{-1}$, $V_{\text{max}}$ (PMCA) = 0, $V_{\text{max}}$ (PLC) = 0.01 ms$^{-1}$, and $V_{\text{max}}$ (PMCA) = 0 $\mu\text{Mol}/\text{ms}/\text{cm}^2$. $V_{\text{max}}$ (SERCA) is indicated in top left corner in $\mu\text{M}$. The calcium wave reaches soma at 8 s for $V_{\text{max}}$ (SERCA) = 1.0 and at 12 s for $V_{\text{max}}$ (SERCA) = 1.4. B: $F_{\text{max}}$ (RyR) = 0.08 ms$^{-1}$, $V_{\text{max}}$ (PLC) = 0.03 ms$^{-1}$, and $V_{\text{max}}$ (SERCA) = 0.6 $\mu\text{M}/\text{ms}$. $V_{\text{max}}$ (PMCA) is indicated in top left corner in $\mu\text{M}/\text{ms}/\text{cm}^2$. The higher values prevent or terminate secondary calcium waves.
cium concentration comparable to reducing $V_{\text{max}}$(SERCA) from 0.6 to 0.5 $\mu$M/ms. The change in peak calcium concentration due to a change in $F_{\text{max}}$(RyR) causes a change in latency and wave speed.

Figure 5H shows that the relationship between peak calcium concentration and wave speed is relatively independent of the various mechanisms that affect calcium concentration. The straight line is the best linear fit to all the points, with an $R^2 = 0.96$. For no combination of parameter values is a calcium wave sustained with a peak calcium concentration $<1.3$ $\mu$M. Below this value, the diffusive flux is insufficient to activate the ryanodine receptor channel to the extent required for CICR-mediated amplification of the calcium signal (Tang and Othmer 1994).

### Cause of late depolarization

The second issue addressed by this study is the origin of the late depolarization and increase in $R_N$ observed after GABA stimulation (Matzel and Alkon 1991; Rogers et al. 1994). A 200 ms, −0.5 nA current was injected every 800 ms, before, during, and after simulated GABA stimulation to measure the change in $R_N$ using the formula $\%\Delta R_N = 100 * (\Delta V_{\text{post}} - \Delta V_{\text{pre}})/\Delta V_{\text{pre}}$. Figure 7A illustrates that both a late depolarization and an increase in $R_N$ occur after GABA stimulation. The late depolarization is observed between 4 and 10 s after the beginning of the GABA stimulation and peaks at 6 s. The 1.3 mV change in membrane potential is accompanied by a 4.5% increase in $R_N$; these changes are similar to the 2 mV depolarization and 3% input resistance increase observed by Matzel and Alkon (1991). Figure 7B shows the total conductance of the potassium leak channels in the synaptic branch, nonsynaptic branch, and each of the four isopotential neurite compartments. $V_{\text{max}}$(PLC) is 0.1 ms$^{-1}$, thus two calcium waves are produced (see Fig. 3) and cause two reductions in the potassium leak conductance in the proximal neurite compartments. Comparison of 7B with A, top reveals that the time course of the late depolarization corresponds to the time course of potassium leak conductance decrease. Similarly, the $R_N$ increase during the late depolarization is due to the closure of the leak potassium channels. The onset of conductance decrease in the proximal neurite compartments is delayed relative to that in the distal neurite compartments because of the time it takes for the calcium wave to propagate to the proximal compartments.

The GABA$_A$ and GABA$_B$ conductance underlying the hyperpolarization are illustrated in Fig. 7C. The GABA$_A$ conductance (offset by 7 nS in the figure) consists of multiple brief channel activations; in contrast, the GABA$_B$ conductance shows the slow and prolonged time course characteristic of G-protein-gated channels. The large fluctuations in membrane potential caused by the fast GABA$_A$ current (Fig. 7A) that appear in the branch do not appear in the soma; they are averaged out by the cable properties of the neurite. As previously observed (Matzel and Alkon 1991), $R_N$ decreases by 23% (from 35 to 27 MΩ) during the hyperpolarization because of the increase in conductance of the GABA channels.

The size of both the late depolarization and the increase in input resistance depend on parameters that affect calcium wave propagation. Parameter values that cause a larger reduction in potassium leak conductance (Fig. 8 left) result in a larger late depolarization and increase in $R_N$ (Fig. 8, right). This is shown for $V_{\text{max}}$(PMCA) (A), $V_{\text{max}}$(PLC) (B), $V_{\text{max}}$(SERCA) (C), for $F_{\text{max}}$(RyR) = 0.08 ms$^{-1}$, and $F_{\text{max}}$(RyR) (D), for the ratio of $F_{\text{max}}$(RyR)/$V_{\text{max}}$(SERCA) = 0.133 $\mu$M$^{-1}$. Despite the variation in the magnitude of the effect, all parameters which support release of calcium from intracellular stores also support an increase in membrane potential and $R_N$ following GABA stimulation.

### LATE DEPOLARIZATION IS NOT DUE TO VOLTAGE-DEPENDENT CURRENTS

*Hermisenda* photoreceptors contain two other currents that are partially active at rest. The transient calcium current, $I_{\text{CaT}}$, has a half activation of $-40$ mV and half inactivation of $-48$ mV, and the hyperpolarization-activated current, $I_H$, is active at $-60$ mV. The decrease in $I_{\text{CaT}}$ inactivation caused by hyperpolarization may allow an increase in $I_{\text{CaT}}$ following membrane repolarization. This in turn may cause a small depolarization and consequent inactivation of $I_H$, causing an increase in $R_N$. This possibility was investigated with one additional set of simulations using the following modifications to the model: both $I_H$ and $I_{\text{CaT}}$ were implemented using activation and inactivation parameters presented in (Yamoah et al. 1998). The maximal conductance was adjusted to produce a current amplitude in voltage-clamp mode comparable to that recorded in their experiments: maximal conductance of $I_H = 833$ nS/cm$^2$ and maximal permeability of $I_{\text{CaT}} = 4e^{-7}$ cm/s. Activation of PLC by G$_\alpha$-GTP was eliminated to determine if calcium influx through $I_{\text{CaT}}$ could activate CICR. The results of these simulations did not reveal a delayed depolarization subsequent to GABA stimulation. Examination of the currents showed that the hyperpolarization due to GABA did not produce a significant rebound activation of $I_{\text{CaT}}$. Furthermore, because $I_H$ has a reversal potential of $-30$ mV, any inactivation of $I_H$ sufficient to cause an increase in $R_N$ also would cause a hyperpolarization, which is not consistent with the observations. No significant elevation in calcium, either synchronous or as a wave, was observed; therefore no increase in $R_N$ was observed due to potassium leak channel reduction. Calcium influx through $I_{\text{CaT}}$ channels was not sufficient to activate CICR.

### TABLE 1. Steady-state wave speed and peak calcium concentration in proximal part of neurite

<table>
<thead>
<tr>
<th>$F_{\text{max}}$(RyR)</th>
<th>$V_{\text{max}}$(SERCA), $\mu$M/ms</th>
<th>$V_{\text{max}}$(PMCA), $\mu$M/ms/cm$^2$</th>
<th>Wave Speed, $\mu$m/ms</th>
<th>Peak Calcium Concentration, $\mu$M</th>
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</thead>
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<tr>
<td>0.16</td>
<td>1.4</td>
<td>0</td>
<td>0.0129</td>
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</tr>
<tr>
<td>1.2</td>
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<td>0.0158</td>
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<td></td>
</tr>
<tr>
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<td>0</td>
<td>0.0195</td>
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<td>0.8</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
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<td>0.00931</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
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<td>0.0131</td>
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</tr>
<tr>
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<td>0</td>
<td></td>
</tr>
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<td>0</td>
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<td>1.65</td>
<td></td>
</tr>
<tr>
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FIG. 7. Effect of GABA stimulation on membrane potential, leak conductance, and synaptic conductance. A, top: membrane potential in soma and terminal branches before, during, and after GABA stimulation. Bottom: hyperpolarizing current pulses are used to measure \( R_N \). Both a late depolarization and an increase in \( R_N \) are apparent and last as long as 10 s after the beginning of the GABA stimulation. B: total conductance (channel density multiplied by surface area) of the potassium leak channels in the synaptic branch, nonsynaptic branch, and each of the 4 isopotential neurite compartments. The smaller total conductance value in the terminal branches is due to their smaller surface area, with the synaptic branch being smaller than the nonsynaptic branch. C: \( \text{GABA}_A \) and \( \text{GABA}_B \) conductance underlying the hyperpolarization.
FIG. 8. The change in membrane potential and $R_N$ (right) and potassium leak conductance (left) as a function of $V_{\text{max}}$(PMCA) (A), $V_{\text{max}}$(PLC) (B), $V_{\text{max}}$(SERCA) (C) for $F_{\text{max}}$(RyR) = 0.08 ms$^{-1}$, and $F_{\text{max}}$(RyR) (D) for ratio $F_{\text{max}}$(RyR)/$V_{\text{max}}$(SERCA) = 0.133 µM$^{-1}$. The potassium leak conductance is the sum over all neurite and terminal branch compartments. Parameter values that allow a greater elevation in calcium cause a larger reduction in potassium leak conductance and result in a larger late depolarization and increase in $R_N$. $F_{\text{max}}$(RyR) = 0.08 ms$^{-1}$ for A–C, $V_{\text{max}}$(SERCA) = 0.6 µM/ms for A and B, $V_{\text{max}}$(PMCA) = 0 µMol/ms-cm$^2$ for B–D, $V_{\text{max}}$(PLC) = 0.03 ms$^{-1}$ for A, C, and D.
Size of synaptic branch affects IPSP but not calcium wave

All of the preceding simulations were performed with the size of the synaptic branch much smaller than the size of the nonsynaptic branch (asymmetric). Simulations were repeated in which the synaptic branch and nonsynaptic branch sizes were equivalent (symmetric), implying that GABA synapses occur on 50% of the terminal branches. The maximal conductance of the GABA channels was increased in the asymmetric case to yield the same maximal conductance as in the symmetric case. As illustrated in Fig. 9A, the IPSP measured in the soma is $-8\text{ mV}$, larger than the $-6\text{ mV}$ IPSP measured when the synaptic branch is smaller than the nonsynaptic branch. The GAB$_{A_{i}}$ current in the symmetric case is very close to that in the high-density asymmetric case (Fig. 9B, top). However, the GAB$_{A_{i}}$ current in the asymmetric case is significantly smaller than that in the symmetric case because membrane potential of the asymmetric synaptic branch is close to the GAB$_{A_{i}}$ reversal potential.

Increasing the size of the synaptic branch also has an effect on calcium release, but most of the difference may be explained by the larger concentration of IP$_{3}$. With $V_{\text{max}}$(PLC) adjusted to produce the same quantity of IP$_{3}$ as in the asymmetric case, the size of the synaptic branch has very little effect on whether a calcium wave propagates to the soma. Figure 10 illustrates calcium concentration versus time and distance along the neurite for $V_{\text{max}}$(PLC) of 0.03 ms$^{-1}$. Although the calcium concentration profile is slightly different from in the asymmetric case (the calcium concentration does not return to the basal level in between the two calcium waves), the number of waves and the speed of wave propagation is similar (compare to Fig. 3B, middle). The effect of $V_{\text{max}}$(PLC) on the reduction in potassium leak conductance, the late depolarization, and the increase in $R_{\text{N}}$ are comparable to that observed in the asymmetric case.

DISCUSSION

A model was developed of the effect of turbulence on the type B photoreceptor of H. crassicornis. The model included

FIG. 9. Simulations in which the synaptic branch and nonsynaptic branch are equivalent (symmetric), implying that GABA synapses occur on 50% of the terminal branches. Comparison with the asymmetric case in which GABA conductance is equivalent to that of the symmetric case. $F_{\text{max}}$(RyR) = 0.08 ms$^{-1}$, $V_{\text{max}}$(SERCA) = 0.6 µM/ms, $V_{\text{max}}$(PMCA) = 0 and $V_{\text{max}}$(PLC) = 0.01 ms$^{-1}$. A: membrane potential in the soma and terminal branches. The inhibitory postsynaptic potential (IPSP) measured in the terminal branches is greater in the asymmetric case as compared with the symmetric case. In contrast, the IPSP in the soma is greater for the symmetric case as compared with the asymmetric case. B: the GAB$_{A_{i}}$ current in the symmetric case is very close to that in the high-density asymmetric case. However, the GAB$_{A_{i}}$ current in the asymmetric case is significantly smaller than that in the symmetric case because membrane potential of the asymmetric synaptic branch is close to the GAB$_{A_{i}}$ reversal potential.

FIG. 10. The size of the synaptic branch has very little effect on whether a calcium wave propagates to the soma. $F_{\text{max}}$(RyR) = 0.08 ms$^{-1}$, $V_{\text{max}}$(SERCA) = 0.6 µM/ms, and $V_{\text{max}}$(PMCA) = 0. Calcium concentration versus time and distance along the neurite for $V_{\text{max}}$(PLC) equivalent to 0.03 ms$^{-1}$ is the same as that of the asymmetric case.
GABA<sub>A</sub>, GABA<sub>B</sub>, and calcium-sensitive potassium leak channels, calcium dynamics including release of calcium from intracellular stores, and the second messenger cascade leading from GABA<sub>A</sub> receptor activation to IP<sub>3</sub> production. Simulations were performed to evaluate whether a calcium wave could propagate from the terminal branches to the soma, to identify fundamental mechanisms of wave propagation, and to evaluate the origin of the late depolarization and increased R<sub>N</sub> observed after GABA stimulation. Simulations showed that it is possible for a wave of calcium to propagate from the terminal branches to the soma. The wave requires I<sub>ICR</sub> for initiation and CICR for propagation in the proximal neurite. The speed of propagation is proportional to peak calcium concentration that depends on the balance between rate of release from the ER and rate of pumping; if V<sub>max</sub>(SERCA) is too high, calcium concentration decreases with distance and the wave dies out. Simulations also showed that the late depolarization and R<sub>N</sub> elevation can be accounted for by a closure of calcium-sensitive leak potassium currents but could not be accounted for by I<sub>Ca,T</sub> acting together with I<sub>H</sub>.

Calcium waves in myocytes and neurons

Release of calcium is an excitable process (Berridge 1993), analogous to voltage-dependent activation and inactivation of the voltage-dependent sodium current. The ryanodine receptor channel is activated by calcium, thus an increase in calcium concentration causes release of calcium; this further increases the calcium concentration. The slow, calcium-dependent inactivation of the calcium release channel limits the duration the channel is open.

Because of the importance of calcium for excitation-contraction coupling, calcium waves in cardiac myocytes have been extensively studied. In response to local caffeine application, the wave speed ranged from 40 to 210 μm/s (Cheng et al. 1996; Trafford et al. 1995), and the peak calcium concentration was proportional to wave speed. A similar range of wave speeds have been reported in simulations of calcium dynamics in cardiac myocytes. In one study (Tang and Othmer 1994), a calcium wave with a speed of 81 μm/s was initiated with calcium influx through voltage-dependent calcium channels; IP<sub>3</sub>-sensitive pools were not included. In another study (Dupont and Goldbeter 1994), calcium wave speed ranged from 100 to 270 μm/s, depending on the distance between calcium pools.

Calcium waves also have been observed in a variety of neurons, either initiated by IP<sub>3</sub> production and release through the IP<sub>3</sub>R or initiated by influx of calcium through voltage-dependent channels. In hippocampal neurons (Jaffe and Brown 1994), PC 12 neurites (Lorenzen et al. 1995), and *Hermisenda* photoreceptors (Ito et al. 1994), calcium waves are initiated by production of IP<sub>3</sub> and release through the IP<sub>3</sub>R. Wave speed is as high as 40 μm/s in hippocampal pyramidal cell dendrites and as low as 2 μm/s in PC12 neurites and *Hermisenda* type B photoreceptors. The *Hermisenda* wave speed is estimated from the calculated time for a 5% increase in fluorescence (Ito et al. 1994). A distance between measurement points of 25 μm yields wave speeds of 2.7–5 μm/s; a distance of 50 μm yields wave speeds of 5.4–10 μm/s. In PC12 neurites, wave speed varies with temperature, between 2–4 μm/s at 18°C and 17–30 μm/s at 37°C. Calcium waves independent of release through the IP<sub>3</sub>R have been observed in sympathetic ganglion neurons (Hua et al. 2000; Lipscombe et al. 1988) and cultured telencephalic neurons (Tsai and Barish 1995). Wave speed ranged from 12.5 μm/s for radial spread to 96 μm/s for longitudinal spread along the submembrane region.

In the present study, the speed of calcium wave propagation varied from 143 μm/s at the distal end of the neurite, where the wave was initiated, to 13 μm/s at the soma end of the neurite. This speed is within the range reported for both myocytes and neurons. In particular, the speed at the soma end of the neurite is close to the value observed in *Hermisenda* photoreceptors. Similar to observations in cardiac myocytes, speed decreases with distance from the initiation site, and the peak calcium concentration is proportional to wave speed.

**Significance of potassium leak channels**

This modeling study demonstrates a potentially large role for the potassium leak channel in shaping cell responses. The post-GABA increase in R<sub>N</sub> amplifies any synaptic inputs which occur. Of greater significance, the large calcium elevation caused by light stimulation (Connor and Alkon 1984; Muzzio et al. 1998) affects leak potassium channels in the soma and rhabdomere, generating an increase in R<sub>N</sub> and contributing to the long-lasting depolarization. The R<sub>N</sub> increase amplifies the inhibitory synaptic input from hair cells during classical conditioning and thus may contribute to the rebound depolarization (Werness et al. 1992, 1993). Also, an increase in R<sub>N</sub> amplifies synaptic inputs from other photoreceptors and thus modulates the dynamical behavior of mutually inhibitory photoreceptors of the *Hermisenda* eye. These predicted consequences of the potassium leak conductance will be investigated with future models and experiments.

The GABA<sub>B</sub>-mediated reduction in the potassium leak channel is not the first demonstration of modulation of potassium leak channels. Neurromodulators such as thyrotropin releasing hormone, acetylcholine, histamine, serotonin, and muscarine (Bayliss et al. 1994; Hsiao et al. 1997; Jafri et al. 1997; Jones and Baughman 1992; Lee and McCormick 1997) have all been demonstrated to have an effect on the potassium leak channel. As demonstrated in carotid body cells (Buckler 1999; Donnelly 1999), potassium leak channels are insensitive to the traditional potassium channel blockers TEA, 4-aminopyridine, and charybdoxin, but they are blocked by 2–5 mM Ba<sup>2+</sup>. The current-voltage relationship is linear, or they show weak sensitivity to voltage. Most neurromodulators that inhibit the potassium leak channel are coupled to PLC via G proteins. Although in most cells the GABA<sub>B</sub> metabotropic receptor is not coupled to PLC, there are several experiments that show that GABA<sub>B</sub> acts via PLC (Pfaff et al. 1999) or is coupled to PLC-activating G proteins (Hahner et al. 1991).

The late depolarization in the present simulations is ~1 mV and appears between 4 and 10 s after stimulus onset. This is smaller and earlier than that shown by Matzel and Alkon (1991), which was between 2 and 3 mV and appeared ~120 s after the GABA puff (although it was seen as early as 8 s after the GABA puff when GABA<sub>B</sub> channels were blocked). One source of the discrepancy is the different method of stimulation: a puff of GABA versus release of GABA by hair cell stimulation. The prolonged IPSP (10–20 s) concomitantly observed suggests that puffing GABA onto the terminal branches...
results in a more prolonged stimulation of GABA receptors, which causes a longer duration of increased IP$_3$ and calcium concentration. A prolonged activation in calcium will cause a larger and more prolonged decrease in the potassium leak current, leading to a longer and longer late depolarization. A second source of the discrepancy may be the kinetic model of the leak channel. Calcium may have an additional indirect effect on the leak channel through its activation of protein kinases, such as PKC or calcium-calmodulin protein kinase II. The observation that inhibitors of protein kinases prevent the effect on the leak channel through its activation of protein kinases (cf. Shirai et al. 1998) may explain the late depolarization appearing 120 s after stimulus onset.

What essential element is contributed by the US in classical conditioning?

Although not addressed in the present study, the ultimate goal of the research is to evaluate whether calcium is the essential second messenger contributed by GABA stimulation to classical conditioning. It is important to emphasize that light causes an elevation in calcium, but light alone does not cause memory storage. Because of this latency, it clearly cannot contribute to the immediate light-induced calcium elevation, but it may contribute to the magnitude of a later light-induced calcium elevation, and it may act to prolong the light-induced calcium elevation. An alternative hypothesis is that arachidonic acid (AA) is the essential second messenger contributed by turbulence to associative memory storage. Prevention of in vitro classical conditioning of *Hermisenda* with an inhibitor of phospholipase A$_2$ (Talk et al. 1997) suggests that AA is required. There is some evidence that activation of brain phospholipase A$_2$ (PLA$_2$) by calcium requires a prolonged (10–20 s) elevation of calcium (Hirabayashi et al. 1999). In this case, the role of GABA stimulation may be to prolong the calcium elevation sufficient to activate PLA$_2$, to generate the AA required for PKC activation.

**Appendix**

Calcium flux through the IP$_3$R is given by

\[
\Phi_{\text{IP}} = F_{\text{calc}}(IP_3R)\frac{1}{2}\left[\text{Ca}^{2+} - \text{Ca}^{2+}\right] (A\text{)}
\]

where $F_{\text{calc}}$ is the calcium flux through the IP$_3$R, the maximal permeability of the IP$_3$R, is 0.16 ms$^{-1}$, $X_{\text{calc}}$ is the fraction of channels in the open state, is given by (Li and Rinzel 1994).

\[
dX_{\text{calc}} = (-1)^n \sum \alpha_n \left[\text{IP}_3\right] \cdot \chi_{\text{calc}} + (-1)^n \sum \beta_n \left[\text{Ca}^{2+}\right] \cdot \chi_{\text{calc}}
\]

where $\alpha_n = 400e^{-3} \mu M^{-1} ms^{-1}$, $\alpha_n = 0.052 ms^{-1}$, $\beta_n = 0.37736 ms^{-1}$, $\beta_n = 20e^{-3} \mu M^{-1} ms^{-1}$, $\beta_n = 1.6468e^{-3} \mu M^{-1} ms^{-1}$, $\chi_{\text{calc}} = 0.2e^{-3} \mu M^{-1} ms^{-1}$, and $\gamma_n = 0.0289e^{-3} ms^{-1}$.

Calcium flux due to buffers is given by

\[
\Phi_{\text{buf}} = K_{\text{calc}}[\text{IP}_3] \cdot \frac{[\text{Ca}^{2+}]}{[\text{Ca}^{2+}]} (A\text{j})
\]

where $K_{\text{calc}} = 0.10 \mu M^{-1} ms^{-1}$, $K_{\text{calc}} = 0.5 ms^{-1}$, total buffer in the ER = 12 mM, and total buffer in cytosol = 153 $\mu M$.

Flux into and out of cytoplasmic compartment, $i$, due to diffusion in the axial dimension is given by

\[
\Phi_{\text{calc}} = D_{\text{calc}} \left( \frac{\text{area}_{\text{calc}}}{\text{diff}_{\text{calc}}} + \frac{\text{area}_{\text{calc}}}{\text{diff}_{\text{calc}}} \right) (A\text{f})
\]

where the subscripts L and R refer to compartments to the right and left of compartment $i$; area is the mean surface area between compartments, dist is the distance between the centers of compartments, $\text{vol}_{\text{cyt}}$ is the volume of compartment $i$, and $D_{\text{calc}}$ is the calcium diffusion coefficient, 6.0e-9 cm$^2$/ms.

The complete equation for calcium concentration in a cytosolic compartment is

\[
\frac{d[\text{Ca}^{2+}]}{dt} = \Phi_{\text{calc}} = \frac{\text{vol}_{\text{cyt}}}{\text{vol}_{\text{ER}}} (\Phi_{\text{calc}} + \Phi_{\text{calc}} + \Phi_{\text{calc}}) (A\text{g})
\]

A similar equation, without the diffusion or PMCA flux terms, is used to compute the calcium concentration in the ER.

\[
\frac{d[\text{Ca}^{2+}]}{dt} = \Phi_{\text{calc}} = \frac{\text{vol}_{\text{cyt}}}{\text{vol}_{\text{ER}}} (\Phi_{\text{calc}} + \Phi_{\text{calc}} + \Phi_{\text{calc}}) (A\text{g})
\]

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**References**


