Model for the Pharmacological Basis of Spontaneous Synchronous Activity in Developing Retinas

Pierre-Yves Burgi and Norberto M. Grzywacz

The Smith-Kettlewell Eye Research Institute, San Francisco, California 94115

Spontaneous waves of bursts of action potentials propagate across the ganglion-cell surface of developing retinas. A recent biophysical model postulated that this propagation is mediated by an increase in extracellular K^+ , following its ejection from ganglion cells during action potentials. Moreover, the model hypothesized that bursts might terminate due to the accumulation of intracellular Ca^{2+} and the subsequent activation of a Ca^{2+} -dependent K^+ conductance in the cells' dendrites. Finally, the model proposed that an excitatory synaptic drive causes a neuromodulation of the waves' properties. To test the feasibility of the model, we performed computer simulations of the network of developing ganglion cells under control and pharmacological-manipulation conditions. In particular, we simulated the effects of neostigmine, Cs^+ and TEA, low Ca^{2+} concentrations, and Co^{2+} . A comparison of the simulations with electrophysiological and pharmacological experimental data recently obtained in turtles (Sernagor and Grzywacz, 1993a), and cats and ferrets (Meister et al., 1991; Wong et al., 1993), showed that the model for the most part is consistent with the behavior of developing retinas. Moreover, modifications of the model to allow for GABAergic inputs onto ganglion cells (Sernagor and Grzywacz, 1994) and poor $[K^+]_{out}$ buffering (Connors et al., 1982) improved the model's fits. These results lent further support to important roles of extracellular K^+ concentration and synaptic drive for the propagation of waves.

[Key words: retinal development, biophysical model, spontaneous activity, ganglion cells, Müller cells, extracellular potassium, synaptic neuromodulation]

Spontaneous bursts of synchronous activity occur in the ganglion-cell layer of developing retinas of rats (Galli and Maffei, 1988), cats and ferrets (Meister et al., 1991; Wong et al., 1993), and turtles (Sernagor and Grzywacz, 1993a). By using multielectrode recording, Meister et al. (1991) and Wong et al. (1993) showed that this activity is organized spatially and temporally

to form waves that sweep across the retinal surface. While waves of neural activity are not specific to the developing retinas, as they are also found, for example, in muscular and glandular tissues (Sherman and Rinzel, 1991; Winslow et al., 1993), the speed of propagation of these waves in the retina seems to be uniquely slow. Because bursts last a relatively constant amount of time, the waves' speed affect directly the spatial extent of the synchronization throughout the tissue. Hence, synchronization is much more localized in the retina than in the heart or pancreas. Such a localization might be important, since synchronous activity might have a role in the refinement of topographic maps in the lateral geniculate nucleus and higher visual areas during binocular segregation (von der Malsburg and Willshaw, 1976; Shatz and Stryker, 1988; Montague et al., 1991; Berns et al., 1993; Miller, 1994). What makes synchronization particularly localized in the developing retina? The answer lies in the mechanisms implementing retinal waves.

Following suggestions by Maffei and Galli-Resta (1990) and Meister et al. (1991), Burgi and Grzywacz (1994) proposed a model for the propagation of waves in developing retinas based on the accumulation of extracellular potassium ($[K^+]_{out}$). According to this model, efflux of K^+ from cells during bursts of action potentials would increase $[K^+]_{out}$ and depolarize neighbor cells. Burgi and Grzywacz argued in favor of this K^+ hypothesis, and against gap-junction and synaptic hypotheses. Although gap junctions exist in fetal retinal ganglion cells of mammals (Penn et al., 1992), recent experiments in developing turtle retinas with octanol (Sernagor and Grzywacz, 1993a) and dopamine (Sernagor and Grzywacz, 1994), two gap-junction blockers in the retina, suggest that gap junctions may not be the main mechanism underlying burst synchronization. Moreover, in mammals, burst synchronization occurs (Masland, 1977; Maffei and Galli-Resta, 1990; Meister et al., 1991) prior to the appearance of the first conventional synapses onto ganglion cells (McArdle et al., 1977; Maslim and Stone, 1986; Horsburgh and Sefton, 1987). Burgi and Grzywacz also argued that the immaturity of the glial system in young preparations (Medzihradsky et al., 1972; Rager, 1979) and consequently, a reduced K^+ active uptake, favors a larger accumulation of K^+ in young, but not adult retinas.

Burgi and Grzywacz also emphasized that a refractory mechanism must exist to prevent the waves from propagating backward. They argued against this mechanism being an inhibitory interneuron, the depletion of a neurotransmitter, or synaptic-receptor desensitization. Instead, they suggested that bursts of activity might stop due to a self-inhibitory mechanism in ganglion cells. In particular, they hypothesized a Ca^{2+} -dependent K^+ conductance (g_{AHP}), which would be activated upon accumulation of intracellular Ca^{2+} during the bursts of action po-

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Correspondence should be addressed to Pierre-Yves Burgi, The Smith-Kettlewell Eye Research Institute, 2232 Webster Street, San Francisco, CA 94115.

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tentials. However, they stressed that the data available at the time were not strong in favor of this g_{AHP} hypothesis. One of the goals of the present article is to use more recent data on developing turtle retina to test this hypothesis.

Finally, based on turtle data (Sernagor and Grzywacz, 1993a), Burgi and Grzywacz proposed a role for synapses in the propagation of waves. They suggested that a small synaptic input would have a neuromodulatory role in the control of wave properties.

Although computer simulations showed that the waves generated by the model proposed by Burgi and Grzywacz (1994) have spatial and temporal properties consistent with experimental data (Meister et al., 1991; Wong et al., 1993), stronger tests of the model should come from pharmacological manipulations. In developing turtle retinas, Sernagor and Grzywacz (1993a) showed that the temporal characteristics of synchronous bursts are affected by application of tetraethylammonium (TEA) and Cs^+ , two potassium blockers, and neostigmine, a cholinesterase blocker. Application of TEA and Cs^+ abolished bursts, which during the early phases of recovery had longer duration and longer intervals separating individual spikes. In contrast, neostigmine had the opposite effect on burst duration and interspike interval, and also reduced the quiet period between bursts. Sernagor and Grzywacz also showed a dramatic reduction of activity with low Ca^{2+} and high Mg^{2+} concentrations, or sufficiently high concentration of Co^{2+} . Among other things, these two ionic manipulations should block conventional synaptic activity. A more recent experiment with low concentrations of Co^{2+} (2–3 μM) allowed bursts to occur, and they had shorter duration and longer interspike interval (Sernagor and Grzywacz, 1994). Different than turtles, a low- Ca^{2+} experiment in mammals first performed with normal Mg^{2+} (Meister et al., 1991) and later with high Mg^{2+} concentrations (R. O. L. Wong, personal communication) resulted in shorter burst duration and shorter length of the quiet period between bursts, and did not suppress burst synchronization. We will address the differences between turtles and mammals in the Discussion.

The aim of the work reported in this article was to investigate to what extent the model proposed by Burgi and Grzywacz (1994), which is based on extracellular K^+ accumulation, a Ca^{2+} -dependent K^+ conductance, and an excitatory synaptic drive can account for the pharmacological and physiological findings in reptiles and mammals. Our goal was not to obtain the best fits in a wide parameter space, but rather to use conservative parameters to study whether even under the worst conditions, modulation of $[K^+]_{out}$ could carry the waves or at least affect them. This article also presents untested predictions made by the model on the propagation of waves under different drugs.

Portion of this work appeared in abstract form (Burgi and Grzywacz, 1993).

Materials and Methods

The model consists of ganglion cells arranged in a hexagonal array (Fig. 1A). Each cell comprises two compartments linked by an axial conductance: a soma surrounded by six extracellular spaces and a dendrite (Fig. 1B). Cells have voltage-dependent Na^+ and K^+ conductances at the soma, and voltage-dependent Ca^{2+} and Ca^{2+} -dependent K^+ conductances (g_{AHP}) at the dendrite. This segregation of conductances prevents the rise of $[K^+]_{out}$ around the soma from impairing the burst-stopping (g_{AHP}) mechanism (Burgi and Grzywacz, 1994). Passive-cell channels include leak conductances at the dendrite and soma. The soma leak conductance has K^+ -dependent and -independent components. Besides the leak, Ca^{2+} , and AHP conductances, the dendrite also receives

Figure 1. *A*, Model of a ganglion cell layer. In this model, cells are arranged hexagonally, their respective location is at the center of the gray circles, and the closest membrane-to-membrane distance between two neighbor cells is 12 μm . The circles are drawn to illustrate the extracellular spaces between every three cells. The corresponding physical dimensions of the network are 364 μm for the width and 240 μm for the height. Wave initiation occurs randomly at the border cells (located within the dark gray circles), where an external current is injected at times randomly drawn from a Poisson process. The "electrodes" record the activity of five cells whose burst activity is presented in Figure 2. *B*, Model of a single ganglion cell. Sodium [$Na(V)$] and potassium [$K(V)$] action potentials at the soma eject K^+ into the extracellular space. The extracellular concentration of K^+ affects the Nernst potential of K^+ for voltage-dependent K^+ channels and K^+ -dependent leak conductances (g_{ik}) in the cell's neighborhood. Depolarization produces a Ca^{2+} spike [$Ca(V)$] that triggers inhibition through the calcium-dependent potassium conductance g_{AHP} [$K([Ca]_{in})$]. The excitability of the cell is modulated by synaptic inputs (at least partially cholinergic) represented by I_{syn} . Finally, the model has a dendritic leak conductance (g_d), a K^+ -independent somatic leak conductance (g_{l-k}), and an axial conductance (g_{axial}) linking soma and dendrite.

an excitatory synaptic current, which was constant for the sake of simplicity. Our model of the individual ganglion cell resembles the neural model of Av-Ron et al. (1993).

Equations. The equations, parameters, and boundary conditions used in the model were described in Burgi and Grzywacz (1994). (That article also explains why we used a simplified model for somatic voltage-dependent conductances rather than the detailed model for adult amphibian ganglion cells of Fohlmeister et al., 1990.) Here, we only recapitulate those equations directly related to $[K^+]_{out}$ and I_{AHP} , the main variables controlling wave propagation and burst termination (the Appendix describes the other equations briefly). The voltage-dependent K^+ current flowing into the j th extracellular space from the i th neuron is determined using reduced Hodgkin-Huxley equations (Av-Ron et al., 1991) described as follows:

$$I_{K,i,j} = \frac{g_K}{6} (W_i/s)(V_i - E_{K,i,j}), \quad (1)$$

where g_K is maximal voltage-dependent K^+ conductance, $E_{K,i,j}$ is K^+ reversal potential corresponding to the j th extracellular space (the calculations involve the $[K^+]_{out}$ values in each of the six extracellular spaces surrounding a cell; Burgi and Grzywacz, 1994), and s is a channel parameter controlling the gating of K^+ channel, as does the variable W_i , which is described in the Appendix. Variations of potassium concentration in the j th extracellular space, $[K^+]_{out,j}$, are given by a nonlinear differential equation that takes into account active K^+ uptake by Müller cells (Karwowski and Proenza, 1980):

$$\frac{d[K^+]_{out,j}}{dt} = \frac{\sum_{i=j_1,j_2,j_3} I_{K,i,j}(t)}{Fv_o} - \frac{[K^+]_{out,j}}{\tau_K} - \kappa_K [K^+]_{out,j}^2 + \frac{[K^+]_{rest}}{\tau_K} + \kappa_K [K^+]_{rest}^2, \quad (2)$$

where F is Faraday constant, v_o is extracellular enclosed volume between every three cells, j_1, j_2 , and j_3 are indices of the three neurons surrounding each extracellular space (according to hexagonal geometry), τ_K is a decay time constant, κ_K is a constant, and $[K^+]_{rest}$ is the resting potassium concentration. We assume $[K^+]_{out,j}$ to be homogeneous in each extracellular space between every three ganglion cells; that is, we neglect diffusion within each such spaces, since diffusion would take about 10–25 msec to cover the distance between neighbor cells, a time that is much shorter than the delay (200 msec; Meister et al., 1991) in wave propagation between neighbor cells (see Burgi and Grzywacz, 1994, for more details).

The conductance g_{AHP} , essential to stop bursts in the model presented here, was described by the following differential equation (Borg-Graham, 1991):

$$\frac{d}{dt} g_{AHP,i} = \alpha(g_{AHP} - g_{AHP,i})[Ca^{2+}]_{in,i} - \beta g_{AHP,i}, \quad (3)$$

where g_{AHP} is maximal conductance, α and β are forward and backward rate constants, respectively, and $[Ca^{2+}]_{in,i}$ is intracellular calcium concentration. The variation in $[Ca^{2+}]_{in,i}$ as a function of Ca^{2+} current was

$$\frac{d[Ca^{2+}]_{in,i}}{dt} = \frac{-I_{Ca,i}(t)}{2Fv_d} + \frac{1}{\tau_{Ca}} ([Ca^{2+}]_{rest} - [Ca^{2+}]_{in,i}), \quad (4)$$

where the factor of 2 is for Ca^{2+} valence, v_d is dendritic volume, τ_{Ca} is a decay time constant, and $[Ca^{2+}]_{rest}$ is resting concentration. The Ca^{2+} current that flows into the dendrite ($I_{Ca,i}$) was determined by using a two-state gate model (Borg-Graham, 1991), which is similar to the Hodgkin-Huxley model (see Appendix).

Parameters. We used parameter values that were as realistic as possible (see Burgi and Grzywacz, 1994, for a list of all values). Not all parameters were available for developing retinas. For instance, conductances for embryonic ganglion cells are not currently available, except for Na^+ and K^+ channels, which have been analyzed in dissociated cells (Skaliora et al., 1993a,b). Thus, adult maximal conductances taken from other preparations were used (Yamada et al., 1989; Ekeberg et al., 1991) with their values divided by 3 to take into account smaller membrane surfaces of young ganglion cells and possibly lower ionic channel density (McCormick and Prince, 1987). The only exception was the dendritic Ca^{2+} conductance, which had to be adjusted to give $[Ca^{2+}]_{in}$ in the range of g_{AHP} operation (0.1–1 μM ; Blatz and Magleby, 1986). The (constant) synaptic current was only -2 pA to be consistent with injected currents that generate trains of action potentials in isolated embryonic ganglion cells (Skaliora et al., 1993a). We used a soma diameter D_s of 8 μm (Ramoia et al., 1988). The dendritic volume was approximated by assuming that all dendrites had the same diameter (0.8 μm), their membrane resistivity was the same as the soma's, and that the ratio between their total conductance and that of the soma is unity (Jack and Redman, 1971). We did not take dendritic tapering into account, but the resulting volume turned out to be relatively unimportant since scaling it and the calcium conductance by the same factor left the results essentially unchanged (simulations not shown).

The extracellular-volume parameter critical for K^+ accumulation, and thus for wave's propagation, was determined by using the extracellular volume fraction, defined as the fraction occupied by the extracellular space in the total volume (Nicholson and Phillips, 1981). In adult cerebellum and neocortex, the extracellular volume fraction is about 20%

(Van Harreveld, 1972; Nicholson and Phillips, 1981; Lehmenkühler et al., 1993). In postnatal developing neocortex, the fraction is higher, ranging between 20% and 40% according to the animal's age (Lehmenkühler et al., 1993). In our simulations, we used an extracellular volume fraction of 30%, a value in the middle of the cortical postnatal range (see Discussion for consideration on the effect of volume changes during development). The total volume is composed of the neural volume, the glial volume (Müller cells in the retina), the volume occupied by the extracellular matrix, and the extracellular volume. We assumed that the volume occupied by the extracellular matrix and the Müller cells was negligible. [This assumption was valid, since gliogenesis appears to be the major factor in reduction of extracellular volume fraction during development (Lehmenkühler et al., 1993) and since Müller cells may not be well developed at the stage when synchronized bursts occur.] Hence, the total volume (v_t) was assumed to be partitioned between ganglion-cell volume (v_g) and extracellular volume (v_e), that is, $v_t = v_g + v_e$. Because the extracellular volume fraction is assumed to be 30%, the ganglion cell occupies 70% of the total volume, that is, $v_g = 0.7v_t$. Consequently, we can calculate the extracellular volume in terms of the ganglion-cell volume as $v_e = (0.3/0.7)v_g$. Because a cell was surrounded by six extracellular spaces in our model, the individual volume where one-sixth of the K^+ current was ejected was $v_e/6$ or

$$v_o = \frac{5\%}{70\%} v_g. \quad (5)$$

Another parameter that could affect changes in $[K^+]_{out}$ is the small fraction of cells that do not fire during a wave (Wong et al., 1993). Wong and colleagues (R. O. L. Wong, personal communication) found that this fraction becomes zero by the addition of 2.5 mM of K^+ to the superfusate. Therefore, presumably, cells that fail to fire may not always reach threshold in physiological conditions. In exploratory simulations, we forced up to 10% of the network's cells to be silent during wave propagation without observing substantial effects on the waves' properties. Accordingly, it was not necessary to remove cells from the network in the simulations reported in this article.

Simulations. Currently, the mechanism responsible for the waves' initiation is not known. We assumed in our model a higher excitability for cells situated near the retinal border. In normal physiological conditions, this is probable as neurogenesis occurs most abundantly at the border (Polley et al., 1989), creating cells with small somata, and small or no dendrites, and thus cells that have high input resistance. Moreover, in experimental conditions, possible cuttings of dendritic trees may depolarize cells. Therefore, we modeled border cells by reducing by half the dendritic volume and conductances (the rise in input resistance increased the cells' excitability). Waves were initiated by injecting a small current (-25 pA) for 250 msec into a randomly chosen border-cell pair at times determined by a Poisson process [mean = 10 sec in the drug-free (control) condition]. Such an initiation corresponds to having a critical mass of active cells that can elevate $[K^+]_{out}$ sufficiently high near the border to induce the propagation of waves.

The evolution of a network composed of 241 ganglion cells (Fig. 1A) was analyzed by computer simulation (simulations of networks comprising up to 1921 cells were also performed for exploratory purposes, and behaved in a similar manner to the results presented here). The differential equations were solved by a numerical method based on exponential prediction (as described in Ekeberg et al., 1991), using a time increment of 200 μsec . During the various simulations, average spike frequency, K^+ and AHP currents, $[Ca^{2+}]_{in}$ and $[K^+]_{out}$ were recorded in all cells and extracellular spaces over 200 sec in successive 0.5 sec intervals. Furthermore, over this period of time, the soma potentials of a group of neighbor cells situated in the middle of the network (shown in Fig. 1A), were recorded over successive 200 μsec intervals.

Pharmacology. In addition to the control condition, several drug conditions were simulated. In our model, we simulated the application of neostigmine by increasing the excitatory synaptic current by threefold. This increase was chosen, since it brought the excitatory current close to the maximal value that did not cause chronic, nonbursty, spontaneous firing. The K^+ -blocking effect on spontaneous bursts resulting from the application of TEA and Cs^+ was simulated by reducing the K^+ conductances (including g_{AHP}). A reduction of 60% was used to emulate the early phases of recovery. This reduction was chosen since it was close to the maximal reduction that still allowed propagation of waves (higher reductions produced too little $[K^+]_{out}$). In another simulation, we lowered $[Ca^{2+}]_{in}$ from 4 mM to 1 mM to replicate the low Ca^{2+} /high Mg^{2+} condition in turtle and concomitantly reduced synaptic input to zero.

Finally, we simulated the low- Co^{2+} experiment ($2 \mu\text{M}$ Co^{2+}) in turtle by reducing maximal Ca^{2+} conductance by 5% and synaptic transmission by 20%. To estimate this reduction in Ca^{2+} conductance, we first used that at 10 mM Ca^{2+} , calcium current is half-blocked by about 1 mM Co^{2+} in skeletal muscles (Almers et al., 1984). We then used the two-intrachannel calcium-binding-site model (Almers and McCleskey, 1984; Hess and Tsien, 1984), with the assumption that the binding of a single Co^{2+} ion is sufficient to block the channel, to extrapolate the percentage of blockade to the experimental Co^{2+} concentration. The reduction in synaptic transmission with low Co^{2+} was approximated from the reduction in Ca^{2+} conductance through the fourth power relationship between synaptic calcium current and transmitter release (Dodge and Rahamimoff, 1967). During the simulations, the rate of wave initiation was similar for control and TEA and Cs^+ , was three times higher for neostigmine, and three times lower for low Ca^{2+} /high Mg^{2+} and low Co^{2+} . These threefold modulations took into account variations in excitability at the border due to modulations of excitatory synaptic current (the factor of 3 was arbitrary, but the results indicated that it had little effect).

Results

Normal conditions

The activity of five neighbor cells was recorded over a period of 200 sec. During this period, seven waves propagated across the whole network. This activity is shown in Figure 2*A*, where for every instant an action potential occurred, a vertical line is displayed, and where the traces from top to bottom correspond to the recorded cells from top to bottom in Figure 1*A*. All cells had periods of activity alternating with periods of silence and the activity was synchronized across cells. In addition to this synchronous activity, the direction of wave propagation was apparent in the firing order. The first wave hit the upper cell first, indicating a top-to-bottom wave propagation. This order was reversed during the second wave as the wave hit the bottom cell first. The individual bursts produced by the second wave are shown in Figure 2*B*, where the time interval between 19 and 24 sec was expanded. Propagation of the activity from one cell to a neighbor cell involved a delay of about 500 msec. The bursts' interspike interval ranged from about 130 to 400 msec (the shortest interval being in the middle of the burst, and the longest at the ends of the burst), and lasted about 2.5 sec. The interspike intervals were slightly longer than those recorded in cat and ferret (Meister et al., 1991), but were consistent with average values measured in turtle and rat preparations (Galli and Maffei, 1988; E. Sernagor and N. M. Grzywacz, personal communication).

Waves

The spatiotemporal evolution of waves in the entire network is presented in Figure 3, where a sequence of 112 frames displays the average spike frequency of the 241 cells composing the network. During this sequence, spanning 111 sec, the propagation of seven waves (one of which being a failing wave in the middle-top border of the 17th frame) can be observed, separated by periods of silence during which no wave propagates (this period is about 1 sec between the first and second waves, and about 32 sec between the second and third waves). The wave front is initially circular, but straightens after arriving to the network's borders, as, for example, in the first wave of Figure 3. This is because the higher excitability of border cells causes the waves to propagate faster at the border. The degree of the effect of border cells is probably much higher than what one would predict for the real retina, since the number of cells in our simulations is relatively small, making the wave front more sensitive to border effects.

Figure 2. Burst activity in the middle of the network. The network shown in Figure 1 was simulated over 200 sec and the activity of five neighbor cells (those with the electrodes in Fig. 1*A*), was recorded. The resulting spike activity is shown in *A* as five traces whose order (top to bottom) corresponds to the electrodes' order (top to bottom). Each spike is plotted as an individual vertical line, not discernible in this figure due to its low temporal resolution. Bursts are synchronized across cells, with a cell-to-cell delay varying according to the direction of the wave propagation (as indicated by the two vertical dashed lines). *B*, The period of time from 19 to 24 sec is expanded to show the individual action potentials contained within the second burst in *A*. It corresponds to a wave propagation from the right lower corner to the left upper corner of the ganglion-cell array. For each cell, the action-potential frequency first increases (due to accumulation of extracellular K^+) and then decreases (due to the activation of g_{AHP}).

These results suggest what would happen to the wave front if waves began in the center of the retina. The wave front would be circular until it reached the borders. Lack of spatial uniformity due to the refractory period from previous waves would rarely affect the wave front, because the interwave interval is much longer than the refractory period. Consequently, spiral waves or rotors, a universal behavior of excitable media with refractory period (Winfree, 1990), should be rare in the developing retina. (Such waves can occur if the refractoriness varies across the wave front.) Nevertheless, since for purposes of illustration we used a high rate of wave initiation (more typical values are close to 1/min), Figure 3 contains two examples of

Figure 3. Spatiotemporal evolution of waves in the ganglion-cell network. The average fire frequency of the 241 cells composing the network is shown frame by frame in steps of 1 sec for the period of time between 18 and 129 sec. The numbers to the left are the serial numbers of the first frame of each row. Waves are separated by periods of silence. Apparent in this figure are a failing wave (frame 17), a destructive collision between two waves (from the 87th to the 96th frame), and the linearization of the wave front (e.g., during the first wave). By using the network sizes, it is possible to measure the wave front speed and its associated spatial spread. The wave's speed is in average 33 $\mu\text{m}/\text{sec}$. The wave's spatial spread is about 75 μm . Scale is linear from 0 to 10 Hz.

what may happen in the rare physiological event of two waves beginning in close temporal proximity. In frame 17, a wave starts at the middle top border, but fails to propagate inward due to residual I_{AHP} from the preceding wave. Between the 87th and 96th frames, two waves, one triggered in the left top corner and the other in the right bottom corner, propagate and meet in the middle of the network where they annihilate each other.

Two related properties of the wave front are its speed and spatial spread. Spatial spread can either be measured directly from Figure 3 (about 80 μm) or can be calculated by multiplying the wave front speed with burst duration. For instance, in Figure 3, the first wave crosses the network (from left to right) in about 10 sec. By considering a typical distance between the cell's centers at a relevant stage of development to be 20 μm (Meister et

Figure 4. The excitatory cycle fueling the waves. In this simulation, injection of an external current into two cells situated at the lower left corner of the network triggered the wave. Spikes caused K^+ ejection currents from the cells' somata to their surrounding extracellular space, resulting in an increase in $[K^+]_{out}$. Finally, above a critical $[K^+]_{out}$ (about 5 mM in our model), neighbor cells started firing to continue the cycle that keeps the wave propagating. Frames span 15 sec in steps of 1 sec; the serial number of the first frame of each row is indicated to the left. Scales are $\log(0.1$ pA) to $\log(1$ nA) for K^+ current, 0–12 mM for $[K^+]_{out}$, and 0–10 Hz for average spike frequency.

al., 1991), we get $364 \mu\text{m}$ for the horizontal size of our network. The propagation speed for the first wave of Figure 3 is thus approximately $36 \mu\text{m}/\text{sec}$. By calculating the product speed $\times$ burst duration, one gets $36 \mu\text{m}/\text{sec} \times 2.27 \text{ sec} = 81.7 \mu\text{m}$ as an approximation for the wave's spatial spread.

Besides the average spike frequency, it is interesting to observe the evolution of I_K , I_{AHP} , $[K^+]_{out}$, and $[Ca^{2+}]_{in}$ during a wave's propagation. In Figure 4, the excitatory chain of events that leads to action potentials is illustrated over a period of 14 sec (15 frames). A wave is initiated at the left bottom corner. Ac-

tivation of a group of cells triggers K^+ currents that induce extracellular K^+ accumulation in the cells' neighborhood. This K^+ accumulation causes membrane depolarization in neighbor cells that fire when their associated $[K^+]_{out}$ rises above approximately 5 mM. One can appreciate well the temporal lead of I_K over the rise in $[K^+]_{out}$ in the eighth and ninth frames of Figure 4, but it is difficult from this figure to appreciate the lead of potassium concentration over spike frequency (see Burgi and Grzywacz, 1994, for a demonstration of this latter lead). The figure also shows that the spatial spread of the $[K^+]_{out}$ wave is

Figure 5. Inhibitory chain of events that terminate bursts. Concomitantly to Hodgkin-Huxley spikes at the soma, Ca^{2+} spikes appear at the dendrite where $[\text{Ca}^{2+}]_{\text{in}}$ builds up. An increase in $[\text{Ca}^{2+}]_{\text{in}}$ activates the Ca^{2+} -dependent K^+ conductance (AHP), which eventually becomes strong enough to inhibit the cell. Such an inhibition impedes waves from propagating backward and imposes a refractory period during which no wave can propagate. Frames span 15 sec in steps of 1 sec; the serial number of the first frame of each row is indicated to the left. Scales are 0–10 Hz for average spike frequency, 0–700 nM for $[\text{Ca}^{2+}]_{\text{in}}$, and $\log(0.1 \text{ pA})$ to $\log(100 \text{ pA})$ for AHP current.

wider than that of the I_{K} and spike-frequency waves, due to the slow removal process of extracellular K^+ .

Figure 5 illustrates the inhibitory chain of events, linking spike frequency, $[\text{Ca}^{2+}]_{\text{in}}$, and I_{AHP} that prevents a wave from propagating backward in the model (over the same 15 sec period). Somatic spikes generate Ca^{2+} spikes at dendrites where $[\text{Ca}^{2+}]_{\text{in}}$ can build up. Elevation in $[\text{Ca}^{2+}]_{\text{in}}$ is followed by an increase in I_{AHP} , which eventually prevents cells from firing. The very sluggish $[\text{Ca}^{2+}]_{\text{in}}$ decay prevents the wave from propagating backward or another wave to be initiated right away after a wave

propagated through the network. One can, for example, appreciate the lead of spike frequency over $[\text{Ca}^{2+}]_{\text{in}}$ and I_{AHP} in the 11th frame of Figure 5. Moreover, the sluggish decay of $[\text{Ca}^{2+}]_{\text{in}}$ and I_{AHP} are dramatically illustrated by their wide spatial spread at the end of the wave.

Pharmacological manipulations

The action-potential activity of two neighbor cells situated in the middle of the network was recorded over 200 sec under various drug conditions. The control condition, shown in Figure

Figure 6. Effects of drugs on cells' activity. The spike activity of two cells situated in the middle of the network (third and fourth electrodes from the top in Fig. 1A) was recorded over 200 sec under five conditions: *A*, control; *B*, neostigmine; *C*, TEA and Cs^+ ; *D*, low Ca^{2+} /high Mg^{2+} ; *E*, low Co^{2+} . Whereas neostigmine, a cholinesterase blocker, increased the overall activity, the low Ca^{2+} /high Mg^{2+} condition suppressed it, suggesting a neuromodulatory role for synapses. Longer bursts and longer cell-to-cell delays under application of TEA and Cs^+ , two K^+ blockers, are two effects consistent with the lateral excitation and self-inhibition mechanisms mediated by K^+ currents. There were no significant low- Co^{2+} effects on burst duration and interspike interval (see Discussion).

6A, was compared to four conditions: neostigmine (Fig. 6B), TEA and Cs^+ (Fig. 6C), low Ca^{2+} /high Mg^{2+} (Fig. 6D), and low Co^{2+} (Fig. 6E).

A comparison between control (Fig. 6A) and neostigmine (Fig. 6B) conditions made apparent the increase in activity following the application of neostigmine (a cholinesterase inhibitor). In particular, the simulations showed a 270% increase in the total number of spikes. Furthermore, there was a reduction in burst duration, interspike interval, and activation delay between two neighbor cells, and an increase in the rate of bursts. The reduction in activation delay resulted from faster wave propagation.

Figure 7. Burst characteristics and pharmacology. Two drug conditions, namely, neostigmine, and TEA and Cs^+ , affect the burst duration and the interspike interval in two opposite ways. Whereas the first condition (neostigmine) shortens burst duration and interspike interval, the second (TEA and Cs^+) lengthens them. Drug conditions also affect the depolarization on which the spikes ride on. Whereas in control condition the maximum of such a depolarization is about 8 mV, it becomes 12 mV for neostigmine, and 3 mV for TEA and Cs^+ . These differences stem from the different $[\text{K}^+]_{\text{out}}$ levels, resulting from higher excitability for neostigmine, and blocking of K^+ channels for TEA and Cs^+ conditions.

The effects upon applications of TEA and Cs^+ were the opposite of the effects obtained under neostigmine (Fig. 6C). Partial blockade (60%) of K^+ channels (during the early phases of Cs^+ and TEA wash) resulted in a reduction in the number of bursts (lower rate of bursts), and an increase in burst duration, interspike interval, and activation delay. The increase in interspike interval was small (actually statistically insignificant in our small sample) and will be addressed in the Discussion. The reduction in activation delay resulted from a slower wave propagation. Lowering $[\text{Ca}^{2+}]_{\text{in}}$ and increasing Mg^{2+} concentration (which causes a reduction in excitatory synaptic current) had the effect of suppressing ganglion cell activity almost entirely, as shown in Figure 6D, indicating that under these conditions waves generally do not propagate. (Over several simulations, one wave managed to cross the network. We estimated that with the parameters used here, the rate of such waves was roughly 1/30 min.) Finally, the low- Co^{2+} condition caused insignificant changes in burst duration and rate of bursts (Fig. 6E).

The changes in the bursts' time course resulting from different drug conditions are illustrated in Figure 7. In this figure, a control burst is compared to two other bursts that have been obtained under application of neostigmine and TEA- Cs^+ , respectively. Whereas in all three cases, a voltage envelope on which the action potentials ride can be observed, its amplitude differs from one condition to another. It is about 8 mV for the control condition (a value that is consistent with depolarization measurements obtained in developing retinas of ferrets; Wong et al., 1993), 12 mV for neostigmine condition, and 3 mV for TEA and Cs^+ condition. Such variations in envelope amplitude reflect different $[\text{K}^+]_{\text{out}}$. Under neostigmine, the cell's excitability increases, resulting in a shorter interspike interval and, consequently, a faster and larger K^+ accumulation in the extracellular space. Conversely, Cs^+ and TEA slacken the cell's firing rate by reducing K^+ currents and thus keeping $[\text{K}^+]_{\text{out}}$ low.

Figure 8. Quantitative comparison of bursts' and waves' properties under various pharmacological conditions. Burst duration, interspike interval inside bursts, and wave's speed and spatial spread are compared for four conditions: control, neostigmine, TEA and Cs⁺, and low Co²⁺. Error bars represent 1 SE, and *n* is the number of bursts in a single 200 sec simulation generated by the cell's activity that the fourth electrode from the top in Figure 1*A* recorded. Burst duration (*A*) and interspike interval (*B*) shorten under neostigmine, and lengthen under TEA-Cs⁺ conditions, which is consistent with experimental data. Furthermore, our model predicts a dramatic increase in wave's speed (*C*) and spatial spread (*D*) during application of neostigmine, and a decrease for the TEA and Cs⁺ condition. Scale bar is linear for *A* and *B* and logarithmic for *C* and *D*.

Quantification of pharmacological effects

Figure 8, *A* and *B*, quantifies the simulated effects of neostigmine, Cs⁺ and TEA, and low Co²⁺ on burst duration and interspike interval. Burst duration decreases under application of either neostigmine (−22%) or low Co²⁺ (−3.5%), and increases under blockade of K⁺ channels by TEA and Cs⁺ (+105%) (Fig. 8*A*). Interspike interval inside bursts decreases under neostigmine (−24%) and low Co²⁺ (−3%), and increases under TEA and Cs⁺ (+15%) (Fig. 8*B*). Direction of changes both in burst duration and interspike interval are consistent with turtle data, except for the insignificant decrease in interspike interval under low Co²⁺ (see Discussion). Figure 8, *C* and *D*, quantifies the as yet untested predictions of the model on the effects of neostigmine, Cs⁺ and TEA, and low Co²⁺ on the waves' speed and spatial spread. Application of neostigmine has a dramatic effect on speed, which becomes much higher (+2182%). This contrasts with the TEA-Cs⁺ and low Co²⁺ conditions where waves slow down (−62% and −8%, respectively). The combination of wave speed and burst duration yielded a larger spatial spread for neostigmine (+1796%), and a smaller spread for TEA-Cs⁺ (−21%) and low Co²⁺ (−12%).

We mentioned above that in control condition the refractory period of wave rarely affects the bursts' properties in the next wave. In contrast, under neostigmine, refractory interactions underlie the large variations in speed from one wave to the next. For instance, the wave's speed corresponding to the third burst, in Figure 6*B* was 1060 μm/sec, whereas the speed corresponding to the fourth burst was 200 μm/sec. This speed variation could be accounted for by the interburst interval, which was relatively small between the third and fourth bursts, but relatively large between the second and third ones. The reason for the increased influence of refractoriness under neostigmine is that it causes a high rate of bursts. Therefore, one could use neostigmine to study refractory effects on waves and thus test the validity of our assumption of self-inhibition.

Discussion

We proposed a biophysical model for burst synchronization observed in ganglion-cell layers of developing retinas of various species (rat: Galli and Maffei, 1988; cat and ferret: Meister et al., 1991; Wong et al., 1993; turtle: Sernagor and Grzywacz, 1993a). In this model, ganglion cells communicate via the extracellular milieu that surrounds them. In this milieu, K⁺ ions,

which are extruded during bursts of action potentials, accumulate. Bursts in neighbor cells are initiated when the extracellular K^+ concentration ($[K^+]_{out}$) reaches a critical level (typically 5 mM in our model). Concomitantly to the somatic action potentials, Ca^{2+} spikes occur at the dendrites where intracellular Ca^{2+} concentration ($[Ca^{2+}]_{in}$) builds up. Bursts are terminated when self-inhibition mediated through a Ca^{2+} -dependent K^+ conductance (g_{AHP}) is sufficiently strong, that is, when $[Ca^{2+}]_{in}$ rises above 500 nM in our model.

Besides its ability to reproduce waves with spatiotemporal properties similar to those observed with multielectrodes recording (Meister et al., 1991; Wong et al., 1993), the model was also consistent with a substantial fraction of the pharmacological studies performed on developing retinas of turtles (Sernagor and Grzywacz, 1993a). In these studies, four types of drugs were used: (1) neostigmine, a cholinesterase blocker; (2) tetraethylammonium (TEA) and Cs^+ , two K^+ conductance blockers; (3) Co^{2+} , a calcium-conductance blocker; and (4) octanol and dopamine, two gap-junction blockers. Furthermore, measurements under low Ca^{2+} /high Mg^{2+} condition were performed. In our model, under neostigmine, we obtained shorter interspike intervals, burst duration, and a dramatic increase in activity accompanied by an increase in rate of bursts (Figs. 6, 8). The decrease in burst duration was similar to the decrease in interspike interval. In another simulation, we blocked K^+ conductances by 60% to replicate the TEA- Cs^+ wash condition. This partial blockade resulted in an increase in interspike interval and burst duration (Figs. 6, 8). We also lowered the extracellular Ca^{2+} concentration, while raising Mg^{2+} , to the same values used by Sernagor and Grzywacz (1993a). This condition resulted in a dramatic reduction of activity. All these results were qualitatively consistent with the turtle data.

Quantitative comparison with experimental data

Despite the qualitative similarities existing between our model and the turtle retina's modulations in burst duration and interspike interval under various drug conditions, we did not always find the same magnitude of modulation. For instance, the increase in interspike interval that we obtained under the TEA- Cs^+ wash condition was small (+15%) in comparison to the experimental data (185%). Another example is the lack of significant effect on interspike interval and burst duration in the low Co^{2+} condition. In this section, we will discuss modifications of the model that alleviate these problems, first addressing TEA- Cs^+ and then low Co^{2+} .

The quantitative discrepancies in the TEA- Cs^+ condition may be due to our model being over conservative, since we wanted to know whether even under the worst conditions, modulations of $[K^+]_{out}$ could carry the waves or at least affect them. For instance, one explanation for the small modulation in interspike interval under Cs^+ and TEA conditions might be our use of a too effective K^+ removal process. In our model, $[K^+]_{out}$ is effectively limited to 12 mM, a ceiling observed in all adult nervous tissues (Somjen, 1979) where physiological modulations of $[K^+]_{out}$ have been studied. However, such a ceiling does not appear to exist in developing tissues. In developing, but not adult, rat optic nerve, $[K^+]_{out}$ up to 20 mM have been recorded under low frequency repetitive stimulation (10 Hz) applied for a few seconds (Connors et al., 1982). If our model had a less efficient K^+ removal process, then the waves would result in higher $[K^+]_{out}$ modulations in control condition, and thus allow larger blockades of the voltage-dependent K^+ conductance (g_K)

Figure 9. Effects of weak $[K^+]_{out}$ buffering and inhibitory GABAergic input. The spike activity of a cell situated in the middle of a seven-cell network was recorded over 5 sec under four conditions. Waves were initiated by a 200 pA depolarizing current injected at a border cell. In all four conditions, an extracellular K^+ removal less efficient than in all the other figures in this article was used. The *first trace* shows the control condition. The *second trace* shows the substantially increase in interspike interval (and burst duration) due to the blockade of K^+ channels by TEA and Cs^+ . The *third trace* corresponds to the low- Co^{2+} condition when no GABA mechanism is used. This trace does not conform to experimental results as it does not show significant changes in burst duration and interspike interval. The *fourth trace* corresponds to low- Co^{2+} condition with an added inhibitory GABAergic input to the ganglion cell. Both the decrease in burst duration and increase in interspike interval are consistent with experimental results.

under Cs^+ and TEA, and larger effects on interspike interval and burst duration. (In the present simulations, above approximately 60% K^+ conductance blockade, waves could not propagate due to too little $[K^+]_{out}$).

To investigate whether relaxing the conservative ceiling for $[K^+]_{out}$ would increase the TEA- Cs^+ effect, we repeated the simulations with weaker removal of extracellular K^+ . The weakening of $[K^+]_{out}$ buffering was achieved by reducing κ_K by 60% and increasing τ_K by 25% in Equation 2. Figure 9 shows that this weakening causes a substantially larger interspike interval in the TEA- Cs^+ condition than in the control condition (57% increase). In addition, with less $[K^+]_{out}$ buffering, TEA- Cs^+ blockade could be as high as 80% without preventing wave propagation. Hence, this result shows that the weak TEA- Cs^+ effect on interspike interval in Figure 8 could have been at least partially due to our conservative assumption on $[K^+]_{out}$ buffering.

In itself, a higher ceiling of $[K^+]_{out}$ is not sufficient to account for the experimentally observed low burst duration and high interspike interval under low Co^{2+} , as illustrated in the third trace of Figure 9. On the contrary, the new ceiling appears to have lengthened burst duration under low Co^{2+} . This low Co^{2+} result would challenge a calcium-dependent potassium conductance as the main mechanism for bursts' termination. That was the reason why the low- Co^{2+} experiment was performed in the first place. In its simplest form, an AHP conductance predicts longer bursts under low Co^{2+} , since with reduced Ca^{2+} current (such as caused by Co^{2+}), intracellular Ca^{2+} accumulation would be slower. However, the low- Co^{2+} result does not necessarily rule out a fundamental role for I_{AHP} in the bursts' termination. In the present stimulations, the fall in synaptic excitation under low Co^{2+} is small (20%). Therefore, a reduced I_{AHP} has trouble counteracting the practically unchanged excitation (which is $[K^+]_{out}$ dominated), and thus stopping bursts. If one could make the fall in synaptic excitation larger under low Co^{2+} , then even a small I_{AHP} may compensate excitation to stop bursts, perhaps

even earlier than in control condition. How can the fall in excitation be made larger while keeping the total synaptic input in the control condition at the same level?

One way to obtain lower burst duration and higher interspike interval under low Co^{2+} is to add a direct inhibitory synaptic input to the ganglion cells, while increasing the excitatory input to compensate for the new inhibitory synapse. In this push–pull mechanism, if low Co^{2+} were to affect the excitatory synapse more than the inhibitory one (we discuss this issue below), this would result in a large reduction in net synaptic current to ganglion cells (possibly much larger than 20%). This excitatory–inhibitory synaptic mechanism would also help even if another mechanism, such as a slow calcium-independent potassium current would mediate bursts' stoppage (see Burgi and Grzywacz, 1994, for arguments favoring a self-inhibitory mechanism for the bursts' termination). The addition of an inhibitory synaptic input to the model receives support from the demonstration that bicuculline, an antagonist of GABA_A receptors, augments the spontaneous burst activity of cells in developing retinas (Sernagor and Grzywacz, 1994). Furthermore, support for inhibition being relatively insensitive to Co^{2+} or low Ca^{2+} comes from evidence that in the retina, GABAergic release does not always depend on Ca^{2+} (Schwartz, 1987).

Simulations with GABAergic inhibitory synapses added to individual cells of the network resulted in shorter burst duration and longer interspike interval under low Co^{2+} than in control condition (Fig. 9), confirming the conclusions hereinabove on this push–pull hypothesis. These simulations used depolarizing current of 18.75 pA and a hyperpolarizing current of 16.75 pA (values obtained after a short exploratory search of parameter space). Consequently, the net synaptic current in control condition was the same as in the other simulations (−2 pA), but the current under low Co^{2+} was hyperpolarizing (1.75 pA) due to the 20% reduction in excitatory current. The figure shows that this push–pull mechanism reduced burst duration by 25% and raised interspike interval by 35% under low Co^{2+} .

In conclusion, modifying the model to allow for less efficient buffering of K^{+} and to include an inhibitory input goes a long way to make the model fit the data better. These two mechanisms would work in synergism, since a more efficient buffering of K^{+} would prevent waves from propagating under low Co^{2+} due to low $[\text{K}^{+}]_{\text{out}}$.

Interspecies differences

Although bursts of activity in developing retinas can be found in a wide variety of species, there are some interspecies differences. For instance, in cat and ferret, wave front velocity is in the range of 80–300 $\mu\text{m}/\text{sec}$ (Meister et al., 1991; Wong et al., 1993), whereas our model, based on turtle data, predicts a velocity of approximately 40 $\mu\text{m}/\text{sec}$. Furthermore, interspike interval varies from one animal to another. Whereas in rabbit, cat, and ferret, interspike interval is shorter than 100 msec (Masland, 1977; Meister et al., 1991), in rat and turtle it is typically longer (Maffei and Galli-Resta, 1990; E. Sernagor and N. M. Grzywacz, personal communication). The long average interspike interval in turtle might partially be explained by its relatively low temperature. However, while temperature is reported to have a potent effect on cells' spontaneous activity in developing retinas (Meister et al., 1991; Wong et al., 1993), other factors must be involved to explain interspecies differences as rat and turtle, two animals with different temperatures, have similar average interspike interval. Besides, the range of tem-

peratures over which waves exist or are not too irregular is different in mammals (33–37°C) and turtles (about 25°C), except perhaps in really young mammals (Wong, personal communication).

Another important interspecies difference may be related to the role of synapses in wave propagation. While in turtle, synaptic blockade with low Ca^{2+} or high Co^{2+} resulted in the elimination of bursts (Sernagor and Grzywacz, 1993a), in mammals the same condition yielded higher frequency of bursts, which had short duration (Meister et al., 1991). Although in their original report Meister et al. (1991) described increased burst activity in low Ca^{2+} /normal Mg^{2+} , thus the retina being prone to hyperexcitability (Frankenhaeuser, 1957; Ariel and Adolph, 1985), subsequent experiments using low Ca^{2+} /high (5 mM) Mg^{2+} gave similar results without hyperexcitability (Wong, personal communication). Therefore, different than turtle, it appears that direct synaptic excitation of ganglion cell is not necessary for waves in mammals.

Role of waves

Spontaneous bursts of activity may have an important role in dendritic outgrowth (Wong et al., 1991; Sernagor and Grzywacz, 1993b). In support of this notion, there is a strong correlation between the time in development at which spontaneous bursts disappear and the maturation of receptive-field and dendritic-tree sizes in the retina.

In this section, however, we will focus on possible roles of burst *synchronization* through waves. Synchronous activity in the retina has been suggested to play a role during development in the segregation of monocular layers in the lateral geniculate nucleus (LGN) (Shatz and Stryker, 1988), refinement of the retinotopic organization in the visual system (Montague et al., 1991; Burgi and Grzywacz, 1994; Miller, 1994; Miller and MacKay, 1994), and ocular dominance segregation and development of disparity selectivity in the visual cortex (Malsburg and Willshaw, 1976; Berns et al., 1993). Miller (1994) argued that it is unlikely that synchronization through retinal waves mediate the development of orientation selectivity in cortical simple cells. His main arguments were based on the waves disappearing before the critical period for orientation selectivity and on the large spatial spread of waves as compared to the receptive-field sizes of these cells. Nevertheless, there is not as yet any significant evidence against the role of waves in binocular segregation and retinotopic refinement.

If retinal waves, by locally correlating the activity of neighbor cells, are involved in the refinement of retinotopic organization, then the waves' spatial spread must be considered. For cat (Meister et al., 1991) and ferret (Wong et al., 1993) spatial spread can be evaluated to be about 300 μm and 500 μm , respectively. For turtle, our model predicts a spatial spread of about 80 μm . Therefore, if waves matter for retinotopic organization, one might expect a higher degree of convergence from ganglion cells to LGN's relay cells (and perhaps, to cortical cells) in cat and ferret than in turtle.

Could waves also contribute to the formation of receptive-field properties in the retina? Because the propagation of a wave is spatially asymmetric and slow, it should elicit a more prolonged depolarization in dendrites or axons perpendicular to the wave front than in dendrites or axons parallel to it. This differential depolarization time course could lead to a differential reinforcement of (Hebbian-like) synapses in neural processes parallel or perpendicular to the waves, and thus lead to

a breaking of symmetry or isotropy in the retinal connectivity. Such a Hebbian breaking of symmetry was postulated by Borg-Graham and Grzywacz (1992) for the development of retinal directional selectivity, but is more likely to play a role in the development of retinal orientation selectivity (Grzywacz et al., 1994). Our model suggests that the turtle would be particularly well suited for such a developmental process, since turtle waves might be particularly slow (40 $\mu\text{m}/\text{sec}$ in the present simulations as opposed to 130 $\mu\text{m}/\text{sec}$ in experiments with cats and ferrets).

Synapses and the regulation of waves' properties

Our model indicates that many neural parameters are involved in determining the cell-to-cell transmission delay, and thus the wave speed. For instance, the kinetics of K^+ accumulation, the amplitude of the excitatory synaptic current, and the amount of leak current in cell membrane all affect this delay. And the kinetics of K^+ accumulation is itself dependent on many other parameters, as, for example, volume fraction, cell death (Horsburgh and Sefton, 1987; Galli-Resta and Resto, 1992), g_{K} , and maturity of Müller cells. Some of the parameters controlling K^+ accumulation, synaptic input, and cell input resistance change during development, affecting speed and spread of waves. There are no direct data on these changes for developing retinas. However, in rat neocortex the extracellular volume fraction appears to decrease during development possibly as a consequence of the emergence of glial cells (Lehmenkühler et al., 1993). Because in the retina, glial (Müller) cells also emerge relatively late in development (Polley et al., 1989), one might expect a similar reduction of extracellular volume fraction in the retina as in the neocortex. Reduction in volume would make a $[\text{K}^+]_{\text{out}}$ mechanism for waves more efficient. On the other hand, an increase in the number of mature Müller cells would tend to compensate for the reduction of volume. If our model is correct, one should expect the effect of increased K^+ buffering by Müller cells to dominate the effect of change in extracellular volume, since waves disappear at later stages of development.

Of particular importance in the regulation of the waves' properties are synapses (which might be unconventional; Zucker and Yazulla, 1982; Lipton, 1988). In cat and rat, retinal waves and burst synchronization have been reported (Galli and Maffei, 1988; Meister et al., 1991) prior to formation of synapses onto ganglion cells (Maslim and Stone, 1986; Horsburgh and Sefton, 1987), suggesting that synapses do not underlie wave propagation in these cases. Nevertheless, the progressive development of synapses could help to increase the overall excitability of the ganglion-cell layer to compensate for losses of excitability due to a reduction of $[\text{K}^+]_{\text{out}}$ following the development of Müller cells. This synaptic neuromodulation could explain the constant wave speed observed in ferret through development (from P0 to P21; Wong et al., 1993). Illustration of this neuromodulatory effect in our model is given by the simulation of the neostigmine condition where an increase in the excitatory synaptic current resulted in an increase of the waves' speed and spatial spread (Fig. 8).

Appendix

Action potentials at the soma of any neuron in the network (indices referring to particular neurons have been omitted for simplicity) were determined by integrating the general membrane equation

$$C \frac{dV(t)}{dt} = -(I_{\text{Na}} + I_{\text{K}} + I_{\text{IK}} + I_{\text{I-K}} + I_{\text{border}} + I_{\text{syn}} + \frac{\bar{g}_{\text{axial}}}{g_t + \bar{g}_{\text{axial}}} \sum_n (V - E_n) g_n), \quad (6)$$

where V is somatic voltage, C is membrane capacitance, I_{Na} and I_{K} are currents generated by the Na^+ and K^+ voltage-dependent conductances respectively, I_{IK} is the portion of the soma's leak current dependent on K^+ , $I_{\text{I-K}}$ is the portion of the soma's leak conductance not dependent on K^+ , I_{border} is a current injected into cells situated at the retinal border to initiate a wave, I_{syn} is (constant) synaptic current, E_n is reversal potential (n being for AHP, Ca, and d), $\bar{g}_{\text{axial}}$ is axial conductance, and $g_t = \Sigma_n g_n = g_{\text{AHP}} + g_{\text{Ca}} + g_d$, where g_d is leak membrane conductance at the dendrite, g_{AHP} is calcium-dependent potassium conductance (defined in Eq. 3), and g_{Ca} is transient voltage-dependent Ca^{2+} conductance defined as follows (Borg-Graham, 1991):

$$g_{\text{Ca}} = \bar{g}_{\text{Ca}} y^2 (1 - w)^4, \quad (7)$$

where $\bar{g}_{\text{Ca}}$ is maximal membrane conductance, and the gating particles y and $1 - w$ represent activation and inactivation, respectively. The general equations for these gating particles (hereinbelow denoted by x) are

$$\frac{dx}{dt} = \frac{x_{\infty} - x}{\tau} \quad (8)$$

$$x_{\infty} = \frac{\alpha}{\alpha + \beta} \quad (9)$$

$$\tau = \frac{1}{\alpha + \beta} \quad (10)$$

$$\alpha = \alpha_0 \exp\left[\frac{-z\gamma}{K}(V - V_{1/2})\right] \quad (11)$$

$$\beta = \beta_0 \exp\left[\frac{z(1 - \gamma)}{K}(V - V_{1/2})\right], \quad (12)$$

where V is the membrane potential, z , α_0 , β_0 , γ , and $V_{1/2}$ are parameters of the channel, and $K = 25$ mV at 20°C.

Potassium and sodium currents are described by a minimal cell model (Av-Ron et al., 1991). The current I_{Na} is defined as follows (the definition for I_{K} was given in Eq. 1):

$$I_{\text{Na}} = \bar{g}_{\text{Na}} m_{\infty}^3(V) (1 - W)(V - E_{\text{Na}}), \quad (13)$$

where $\bar{g}_{\text{Na}}$ is maximal conductance and E_{Na} is Na^+ reversal potential. Definitions for m_{∞} and W , the gating particles that control the activation and inactivation of these Na^+ and K^+ channels, are

$$m_{\infty}(V) = \frac{1}{1 + \exp[-2a^{(m)}(V - V_{1/2}^{(m)})]} \quad (14)$$

$$\frac{dW}{dt} = \frac{W_{\infty}(V) - W}{\tau(V)}, \quad (15)$$

$$W_{\infty}(V) = \frac{1}{1 + \exp[-2a^{(w)}(V - V_{1/2}^{(w)})]}, \quad (16)$$

$$\tau(V) = \frac{1}{\bar{\lambda} \exp[a^{(w)}(V - V_{1/2}^{(w)})] + \bar{\lambda} \exp[-a^{(w)}(V - V_{1/2}^{(w)})]}, \quad (17)$$

where $V_{1/2}$, a , and $\bar{\lambda}$ are positive channel parameters.

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