# EFFECTS OF CURRENT PULSES ON THE SUSTAINED DISCHARGE OF VISUAL CELLS OF *LIMULUS*

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ABSTRACT Current pulses were used in the eccentric and retinular cells of the *Limulus* lateral eye to produce changes in the interspike interval of the discharge sustained by a constant light level. The effects on the interspike interval of hyperpolarizing and depolarizing perturbations, applied at various delays from the previous spike, were measured for different intensities and durations of the current pulse. The results show that when the perturbations were applied in the first part of the interval, effects contrary to what is normal were produced (i.e, hyperpolarizing pulses decreased the interspike interval instead of increasing it and vice versa for depolarizing pulses). Here we discuss briefly the implications on neural encoding models.

## INTRODUCTION

A sensory receptor responds to a stimulus in two stages: (*a*) a transduction process by which the stimulus is converted into an electrical current across the cell membrane, (*b*) the generation of nerve impluses by membrane currents. This second stage (*b*) is often referred to as the encoder because the membrane currents are coded as a train of spikes. In the *Limulus* ommatidia the transduction process occurs in the soma of eccentric and retinular cells, while the encoding is usually assumed in the axon hillock of the eccentric cell.

Neural encoding in sensory neurons has generally been studied by using long duration step or periodic stimuli with a periodicity much longer than the interspike interval. This approach was used in the *Limulus* lateral eye (Knight et al., 1970; Ascoli et al., 1974; Ascoli et al., 1977; Angelini et al., 1982), in the crayfish stretch receptor (Fohlmeister et al., 1974; Fohlmeister et al., 1977), and in mammalian muscle spindles (Poppele and Chen, 1972; Poppele and Bowmann, 1970).

Experiments using long-lasting stimuli have characterized the input-output relations of the encode; the operational models developed to account for input-output relations (Knight, 1969; Knight, 1972; Rescigno,1970; Ascoli et al., 1977; Barbi et al., 1975) emphasize the tendency, already present in the experimental approach, to neglect changes in neural excitability in the interspike interval; these, however, are well known (Fuortes and Mantegazzini, 1962), and are also described by the Hodgkin and Huxley equations. To investigate the variations in neural excitability during the interspike interval, a discrete, short stimulus rather than a continous one should be used. A current pulse much shorter than the free-run period can be used to perturb the interspike interval; and it is thus possible to measure the effect of the perturbation as a function of the time elapsed from the previous spike to the stimulus occurrence (delay). Over the last few years short current pulses have been used to stimulate many kinds of excitable tissues and the effects on the discharge have been studied (Hartline, 1976; Jalife and Antzélevitch, 1979; Guttman et al., 1980; Fohlmeister et al., 1974).

Many of these studies investigated very regular cells, such as pacemaker cardiac cells. Since a regular discharge is periodic, many of the experimental results reported make use of a nondimensional variable (phase), which is defined by relating the perturbed discharge to the unperturbed one (Winfree, 1980).

Here the effects of brief current pulses on the discharge of cells in the *Limulus* lateral eye are measured and discussed. As the discharge is not very regular we report our results by presenting the interspike interval variations vs. the time delay of the stimulus.

In *Limulus* visual cells there is a negative feedback of the discharge on itself (self-inhibition) (Stevens, 1964; Purple and Dodge, 1965). Moreover neighboring ommatidia interact through inhibitory synapses. Lateral inhibition acts on the discharge of a given ommatidium as a source of noise, while self-inhibition determines a negative correlation between successive intervals in a steady discharge (Shapley, 1971); thus if a perturbation produces in a steady discharge a longer/shorter interval than the mean interval, the succesive intervals will be shorter/longer before the interval returns to the steady mean value.

In performing the experiments described above, we avoided the effects of self- and lateral inhibition by (*a*) keeping the rate of perturbing pulses low enough to allow the rate of the discharge to adapt to its steady value before the next perturbation and (*b*) comparing the mean values of perturbed and adapted intervals.

## METHODS

Experiments were performed on excised lateral eyes of *Limulus polyphemus*, and the neural activity was recorded by standard techniques (Ascoli et al., 1974; Ascoli et al., 1977). The eye was globally illuminated and the free-run rate was set by adjusting the steady light level. Hyperpolarizing and depolarizing current pulses were injected into the cells through the recording microelectrode. The times at which spikes occurred were measured and stored on line by a Nova 4S mini-computer (Data General Corp., Westboro, MA), which also controlled the amplitude duration and timing of the current pulses.

Two different protocols were used in the experiments. In the first experiment, three intervals were allowed to occur after each current pulse before the perturbation was repeated; this was done to permit the rate of discharge to adapt to its steady value. The pulse delay was set at five different values in succession. Each experiment consisted of ~2,000 spikes; 500 interspike intervals (100 for each value of the delay) were directly affected by the current pulse, 1,000, which were not used in the analysis, were adapting intervals, and 500 were the intervals adapted to the steady value. A first on-line analysis computed the average, the variance, and the interval histogram for the 500-adapted intervals and the five classes of stimulated intervals corresponding to the five delay values.

In the second program we, after each spike, introduced the perturbation at random with a probability of 0.5 and only at one delay. Thus, in collecting 800 spikes we had ~400 intervals affected by pulse stimulation. The variation coefficient, $\sigma_N$, of the mean value of $N$ intervals can be evaluated by $\sigma_N = \sigma/\sqrt{N}$, where $\sigma$ is the variation coefficient of the interval distribution. By using the second program, we could measure smaller variations and so we used it at small delays to measure small effects. Variations exceeding $\sigma/\sqrt{N}$ were considered meaningful; a further check was obtained by comparing pertubed and steady histograms.

## RESULTS

Experiments were performed on 24 visual cells (eccentric and retinular cells). No qualitative difference were found between the responses of retinular and eccentric cells; in particular, hyperpolarizing pulses applied to retinular cells affect the neural discharge and no systematic differences appear between eccentric and retinular cells as regards the amplitude necessary to give a measurable effect.

In almost all the cells analyzed there was an inversion of the stimulus effect (both for hyperpolarizing and depolarizing stimuli) when the current pulse was injected in the first 10–30 ms after the spike. Only four cells did not present this inversion effect. The results obtained in one of these cells are reported in Fig. 1. On the ordinate we plot $\Delta T = T - T_0$ where $T$ and $T_0$ are the mean values of perturbed and unperturbed intervals. Notice that a lenghtening of intervals results in a positive $\Delta T$, whereas a shortening yields a negative $\Delta T$. The results reported in the other figures refer to cells that show the inversion of the stimulus effect described above.

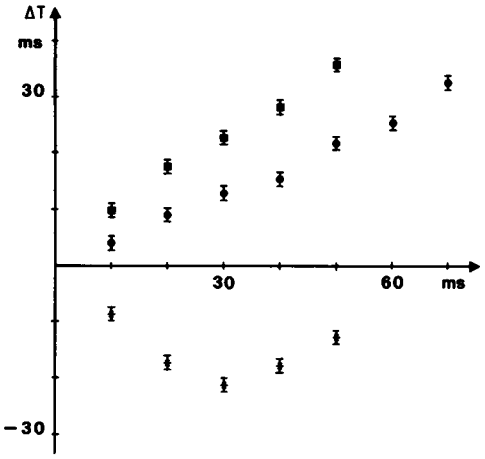


FIGURE 1 Mean difference between perturbed and unperturbed intervals vs. the time delay of stimulating pulses in a retinular cell. $T$ = 90 ms, pulse duration is 5 ms, and the pulse amplitude is indicated by: ●, −1.25 nA; ■, −2.5 nA; and *, +1 nA. Each point corresponds to an average of 100 spikes.

### Hyperpolarizing Perturbations

In Fig. 2 the difference between the perturbed and unperturbed mean interval vs. the delay separating the hyperpolarizing pulse from the previous spike is plotted for four different cells; clearly the hyperpolarizing pulses that occurred in the first part of the interval produced a shortening of that interval, while those that occurred in the second part produced a lengthening.

For a given cell, the degree of shortening at fixed delays depends on the pulse area (i.e., on the total charge injected into the cell). The result (Fig. 3) was, in fact, the same if we used a few pulses instead of a single pulse with the same areas. The dependence of the shortening on the amplitude of the stimulus is shown in Fig. 4. This plot was obtained at a fixed delay in the interval, where the shortening occurred and it shows a saturation of the effect.

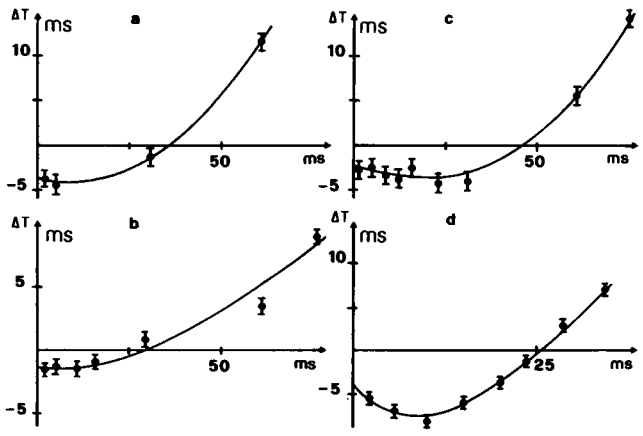


FIGURE 2 Mean difference between perturbed and unperturbed intervals vs. the delay of hyperpolarizing pulses in eccentric (*c* and *d*) and retinular cells (*a* and *b*). The mean unperturbed interval, the pulse amplitude, and duration are, respectively, (*a*) 140 ms, 0.5 nA, 10 ms; (*b*) 145 ms, 0.5 nA, 5 ms; (*c*) 125 ms, 2 nA, 2.5 ms; (*d*) 75 ms, 3 nA, 5 ms.

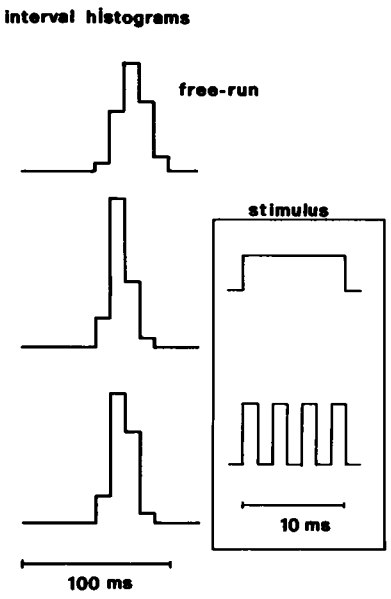


FIGURE 3 Interval histograms for the perturbed and free-run discharges of an eccentric cell. Two kinds of stimuli were used with the same total area (*inset*). The pulse delay was 6 ms. Each histogram comprises 250 spikes.

Lastly, the dependence of the degree of the shortening intensity on the duration of stimulus was investigated (Fig. 5). This shortening increased up to a maximum and fell to zero. This trend may be due to the fact that, as its duration increased, the pulse widened out to cover both the shortening and lengthening zones of the interval, so that the two opposite effects eventually balanced each other.

## Depolarizing Perturbations

Experiments with depolarizing pulses require caution. In fact, as shown in Fig. 6, if the amplitude of the perturbing pulse exceeds a threshold value, a spike is suddenly fired, so a very large shortening of the interval occurs. Moreover, this threshold value changes with the position of the stimulus within the interval. To perform experiments comparable with those described in the previous paragraph, subthreshold depolarizing pulses must be used over

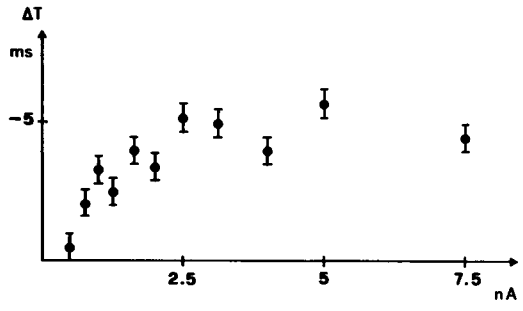


FIGURE 4 Trend of the shortening at a given delay (6 ms) vs. the amplitude of the hyperpolarizing pulse. Same cell as in Fig. 2 *d*. Pulse duration 5 ms.

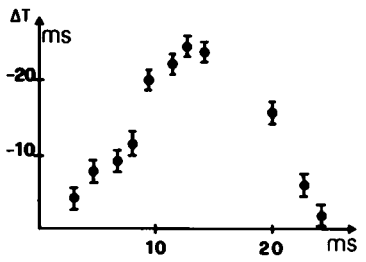


FIGURE 5 Trend of the shortening at a delay of 2 ms vs. the hyperpolarizing pulse duration in an eccentric cell. $T_o$ = 60 ms, pulse amplitude 2 nA.

the whole range of the interval. In this case, the trend of the effect of depolarizing stimuli was symmetrically opposite that of hyperpolarizing stimuli, as shown in Figs. 7 and 8 for two different cells. In the first part of the interval, in fact, depolarizing pulses had a lengthening effect, while in the second part they had a shortening effect.

In spite of the intensity of the injected current (1.25 nA in Fig. 8 against 2 nA in Fig. 7) the effectiveness of the perturbation in Fig. 8 was stronger than in Fig. 7 (greater lengthening and greater shortening). It is a common observation that the real effectiveness of a stimulus is different from a cell to another cell, probably depending on the site of impaling. Fig. 7, which corresponds to a less effective stimulus, shows a smooth curve, while in Fig. 8 there is an abrupt transition between lengthenings and shortenings.

In Fig. 8 two points correspond to the delay that falls right in the transition region. In fact, the interval distribution (shown in Fig. 8) becomes bimodal and the pulse effect can be a shortening or a lengthening; the bimodal shape of the histogram, which occurs only for pulses near the sudden transition between lengthening and shortening, can be ascribed to the noise effecting neural encoding. Note that for all the points reported in Fig. 8 the pulse amplitude was subthreshold (compare with Fig. 6).

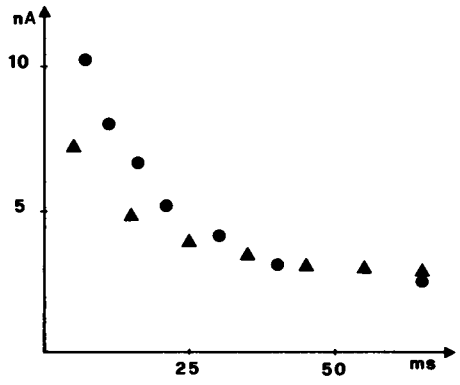


FIGURE 6 Trend of threshold amplitude of depolarizing pulse vs. the pulse delay for two different retinular cells. Pulse duration and free-run intervals are, respectively; 5 ms, 90 ms (●) and 10 ms, 90 ms (▲).

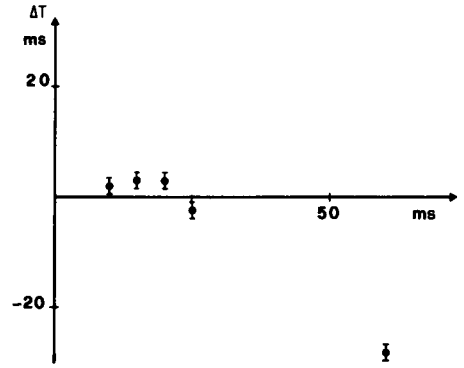


FIGURE 7 Effect of subthreshold depolarizing pulses vs. the pulse delay in a retinular cell. Pulse amplitude 2 nA, pulse duration 10 ms, free-run interval 120 ms.

## DISCUSSION

In *Limulus* visual cells hyperpolarizing currents can decrease the interspike interval, while depolarizing currents can increase it. Similar paradoxical results were found in another sensory neuron, the crayfish stretch receptor (Fohlmeister et al., 1974), in cardiac pacemaker cells (Jalife and Antzélevitch, 1979), and in the squid giant axon (Guttman et al., 1980). Other authors (Perkel, 1964; Hartline, 1976), however, reported a normal trend of excitability in the crayfish stretch receptor (and in the abdominal ganglia of *Aplysia*); we found this kind of trend in a small minority of *Limulus* cells.

Paradoxical results, which seem a rather common response by neurons, only occur if a stimulus is applied in a particular zone of the interspike interval and, with depolarizing pulses, if there is a suitable amplitude. If it is assumed

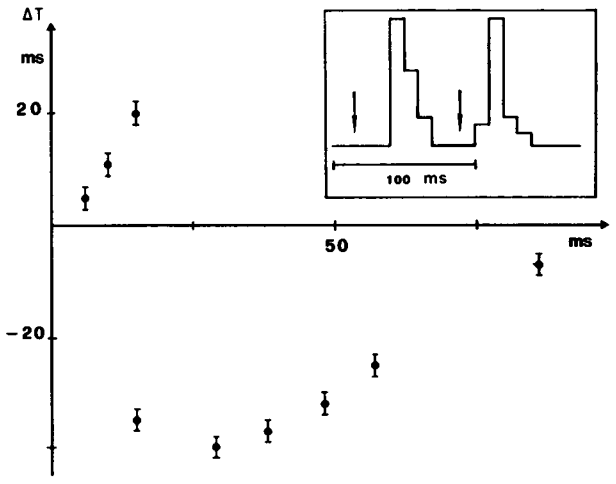


FIGURE 8 Effect of subthreshold depolarizing pulses vs. pulse delay in a retinular cell (same cell as in Fig. 6, ▲). Pulse amplitude 1.25 nA, pulse duration 10 ms, free-run interval 95 ms. Notice the abrupt transition between lengthening and shortening and the double point at a delay of 15 ms. The *inset* shows bimodal interval histogram at delay 15 ms. The *arrows* in the *inset* indicate the stimulus timing and the mean value of the unperturbed intervals.

that the stimulus exerts two different, contrasting influences on cell activity, and that the predominance of one or the other depends on the delay, it becomes possible to explain the inversion of the stimulus effect obtainable by varying that delay.

In fact, the two-parameter model, suggested by Fohlmeister in 1973, qualitatively predicts what we call paradoxical effect. Such a model is like an intergrate and fire system, and is defined by the coupled equations (holding in the interspike)

$$\frac{du}{dt} = -\gamma u + s$$

$$\frac{d\gamma}{dt} = -C\gamma + Du$$

and by the requirement that the firing occurs when $u$ reaches the threshold value. In these equations, $\gamma$ and $u$ are state variables, $s$ represents the driving stimulus, and $C$ and $D$ are two parameters; the spontaneous trend to zero of $\gamma$ depends on $C$, while $D$ is a measure of the coupling between $u(t)$ and $\gamma(t)$.

Note that the first equation is of a leaky integrator with leakage $\gamma$; thus $u(t)$ could represent the membrane potential in the encoder region (the firing occurs when $u[t]$ crosses the threshold), while $\gamma$ could represent, in a cumulative fashion, the ion permeability of the membrane. This last is high after a spike and then decays with a time course depending on $u(t)$. The coupling between the time course of $\gamma$ and $u$ (which is contained in the second equation) is the basic feature of the model and allows it to qualitatively predict the effects of both hyperpolarizing and depolarizing stimuli. In fact, the high value of $\gamma$ after each spike makes the stimulus $s$ ineffective in increasing $u(t)$ towards the threshold in the early part of the interval; a hyperpolarizing current pulse injected just after the spike radically alters this situation, reducing the value of $\gamma$. As a result, and in spite of the negative contribution of the hyperpolarizing pulse to integration, the interspike interval will be shortened (see Fohlmeister, 1973). For the same reason, a depolarizing pulse injected in the early part of the interval will lengthen it.

Now let us consider more specifically the results of Figs. 6 and 8. For stimulus amplitude above a threshold value, a sudden spike was immediately elicited by the current pulse. By decreasing the amplitude, for a suitable delay, a spike may be produced almost immediately, or, if there were no spike, there would be a perturbed interval longer than the unperturbed one (see Fig. 8, in particular the inset of Fig. 8). If the amplitude was decreased still further, the bimodal outcome failed to appear (by inference from Fig. 7).

Perhaps a depolarizing stimulus near the threshold could reach the threshold if it were helped by a bit of noise. Due to the antagonistic increase of the leakage if such a stimulus did not reach the threshold, the next spike occurred later; thus the two-parameter model could

account for the bimodal shape of the interval distribution. With this interpretation however, the spike should occur during the current pulse or immediately after it, while in our data there was a time gap between the current pulse and the new spike (see inset of Fig. 8). This suggests that there is a filtering between the stimulus injected in the cell and the stimulus reaching the encoder site.

In line with this interpretation we used the Fohlmeister model in a computer simulation of the experiments described above, with a filtering between the perturbing current pulse and the pulse that reaches the encoder region. In the simulation a steady input level determined the steady discharge, as did the light level in the experiment. To filter the current pulse we calculated the convolution integral with the function

$$f(t) = t^2 e^{-t/\tau}/G,$$

which is the impulse response of a three-stage cascade filter with time constant $\tau$; $G$ is a normalization factor. The effect of a three stage cascade filter operating between the input of the system and the stimulus $s$ appearing in the differential equations is shown in Fig. 9 in a case of bimodal outcome.

To simulate a noisy discharge we assumed that a slow noise affects the steady input level. In practice, we added Gaussian random numbers with mean value zero to the input level $s_0$; the root mean square of the Gaussian distribution was selected to give a variation coefficient of the steady discharge equal to 0.1. The results of such simulation are shown in Fig. 10 in the same form as the results of the experiments.

The qualitative features of Fig. 8 (experiment) and Fig. 10 (simulation) look very similar; we conclude that the

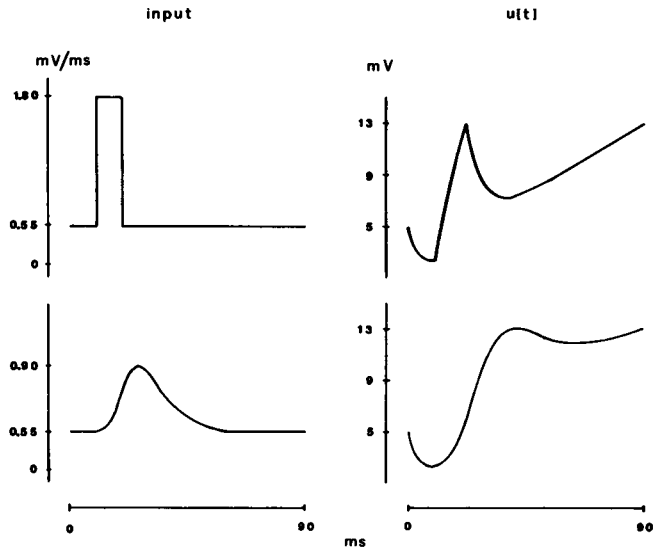


FIGURE 9 The *upper* graphs indicate the time course of the input and of $u(t)$. The *lower* graphs indicate the time course of the filtered input and of the relative $u(t)$. Parameters of the model as in Fig. 10; pulse delay is 10 ms.

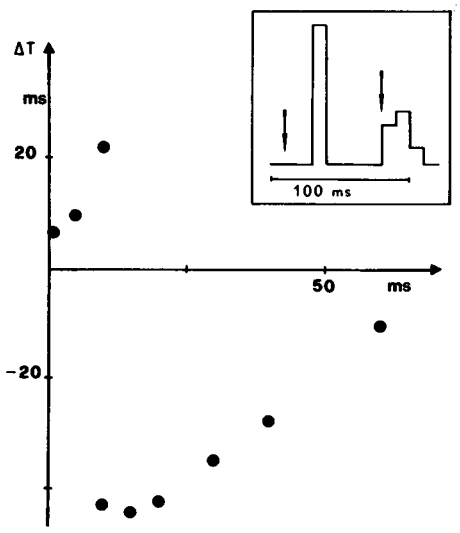


FIGURE 10 Computer simulation of the Fohlmeister model behavior in response to depolarizing stimuli; filtering and noise were added as described in the text. The model parameters were $C = 0.05$ ms$^{-1}$, $D = 0.0015$ ms$^{-2}$ mV$^{-1}$. The threshold for firing and the time constant of the filter were 13 mV and 5 ms, respectively. After each spike the state variables $u$ and $\gamma$ are reset to $u(0) = 5$ mV and $\gamma(0) = 0.34$ ms$^{-1}$. We report the mean difference between perturbed and unperturbed intervals vs. the stimulus delay while the *inset* shows the biomodal interval histogram at a delay of 10 ms. The *arrows* in the *inset* indicate the stimulus timing and the mean value of the unperturbed intervals.

interpretation suggested in this discussion (two competing types of stimulus action, filtering between stimulus in the cell soma and stimulus in the encoder region) works well.

Filtering between the generator potential in retinular cells and the potential in the eccentric cell axon hillock can be inferred from phase-locking experiments. When we sinusoidally modulated the light intensity and recorded the neural activity from retinular cells, the spikes appeared to lock at a phase of the generator potential, which differed from the locking phase when the stimulus was a sinusoidally modulated current (Ascoli et al., 1977); this suggested as a possible explanation a different role for membrane capacitances (and then for filtering) in the case of current and light stimulation. An additional strong effect was found in pacemaker cardiac cells and in the squid giant axon (Mines, 1913; Jalife and Antzélevitch, 1979; Guttman et al., 1980): both hyperpolarizing and depolarizing current pulses can produce an annihilation of the discharge. This result is in accordance with the Winfree theory of responses of periodic cycling systems to patterned perturbations (Winfree, 1977); this theory, based on a topological analysis of the effects of perturbations on the interspike interval, suggests that annihilation could occur at the transition between weak and strong stimuli (Best, 1979).

We did not see an annihilation effect when we scanned the interspike interval with hyperpolarizing and depolarizing pulses of different amplitude, but, as reported herein, we did see bimodal histograms in the effect inversion zone.

Finally, as regards the *Limulus* lateral eye note that hyperpolarizing pulses produced comparable effects in eccentric and retinular cells in spite of the electrical connections between them (Smith and Baumann, 1969), which appeared to be like a rectifier.

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