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The Horseshoe Crab Eye: A Little Nervous System That is Solvable

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THE HORSESHOE CRAB EYE:
A LITTLE NERVOUS SYSTEM THAT IS SOLVABLE

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Biology, like mathematics, is a science in which broad generalizations apply to numerous particular cases. It is a perpetual challenge to the biologist to choose his materials and his methods in such a way that, by studying the particular, he may shed light on the general. In the first few figures we will see several variations on one general theme.

Figure 1 is a picture of brain tissue. The black objects with the interconnecting fibers are individual nerve cells. Wherever you look in the cortex of the brain, you will find organization somewhat similar to what you see here. It is far from random: it is composed of organized layers of cells, interconnected in several highly specific different ways. The picture cries out for explanation: to relate structure to function. We know that this structure collates input information, sifts it for what is important, and outputs appropriate responses. We know that electrical activity in one of these cells will either excite or inhibit electrical activity in cells to which it connects. But except superficially we don't know how this thing works.

The second figure also shows brain tissue. This is a schematic picture of the nerve cells in the human retina. It is brain tissue in the developmental sense: embryologically it is formed by an outpocketing of the brain. The structural similarity is obvious.

In the case of the retina we can say much more about what it does and how it does it. We know the input. The cells in the top layer are sensitive to light. The retina is far more than a camera film. Brain processing begins right here in this network. The receptor cells are one hundred times more numerous than the nerve cells which form the optic nerve. One function of the retinal network is to perform that compression: to abstract from the total input that part which is important.

Unlike most brain tissue, the vertebrate retina is accessible from the outside world, and its input may be experimentally

manipulated conveniently. Thus the retina, which is interesting
in its own right, is also a good place to start studying the brain.

Figure 3 shows another retina, the retina of an invertebrate.
This is a micrograph of the eye of the horseshoe crab, *Limulus
Polyphemus*.   Again we see light-sensitive receptors at the top,
followed by an interconnecting network of nerve fibers, from which
signals flow down an optic nerve which goes to the brain. The
structural similarity to the human retina is obvious, but this is
a much simpler retina. It also has large nerve cells and is probably
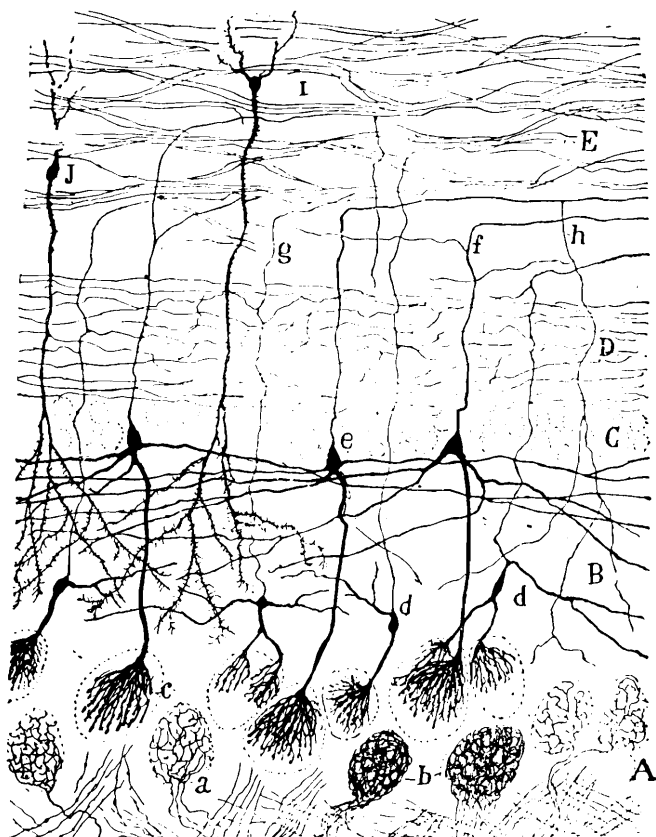the most convenient retina in the world for experimental study.
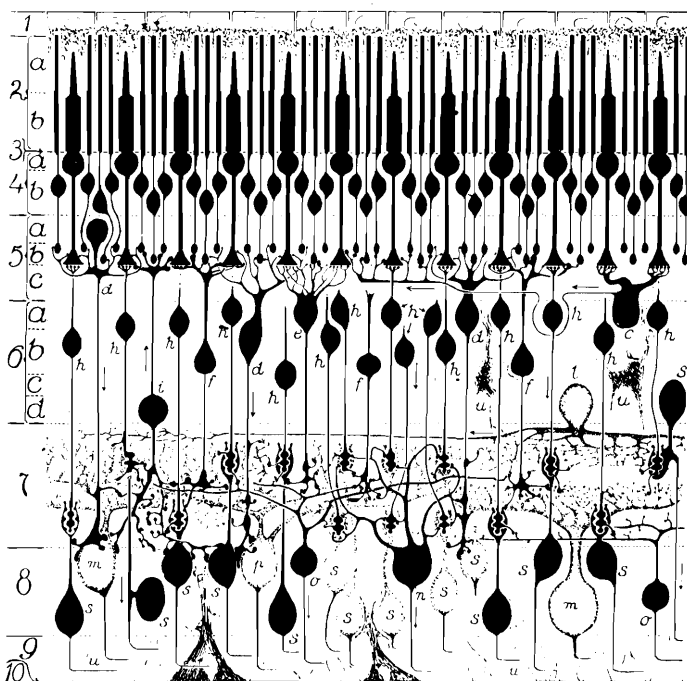

FIGURE 1

FIGURE 2

1MM.

FIGURE 3

FIGURE 4

Figure 4 is the horseshoe crab *Limulus Polyphemus* himself.
Anyone who has been swimming on the Atlantic coast has seen
him. He is a gentle creature and extremely stupid. His eye is a
compound eye, like that of a fly. You see the eye, which may be
excised from the animal, and will, with some care, continue to live
and function by itself for several hours in the laboratory. The
experimental convenience his eye affords was noted by H. K.
Hartline many years ago. I am going to talk about what this eye
has yielded in the last few years, at the laboratory of Hartline
and Floyd Ratliff at the Rockefeller University. It is the work of
people too numerous to list, but I must mention besides Hartline
and Ratliff the names of Fred Dodge and Jun-ichi Toyoda.

Figure 5 shows the basis of electrical activity in nerve cells.
If you dissolve a material such as ordinary salt, NaCl, in water,
it will naturally dissociate itself into charged fragments, with the

sodium, Na, carrying the positive charge. The membrane that surrounds the cell normally is not permeable to sodium, but if momentarily it becomes permeable to sodium, the sodium will rush inside, carrying the positive charge with it. A positive excursion in internal voltage will result, and this may be measured in several different ways.

In nerve cells this voltage response to changed permeability may be exploited in several different ways. First, if it is a sensory neuron, sensory input may stimulate the permeability change. Second, a permeability change may result from activity in other nerve fibers which terminate on the outside of this cell. Third, voltage changes within the cell may themselves cause further permeability change. Evidently this third mechanism may be self-exciting, and in fact can lead to spontaneous periodic oscillations of the limit-cycle variety. This repetition frequency in turn may be modulated by the parametric influence of the first two mechanisms. All of these things happen in the visual nerve cells of the horseshoe crab. (Figure 6.)
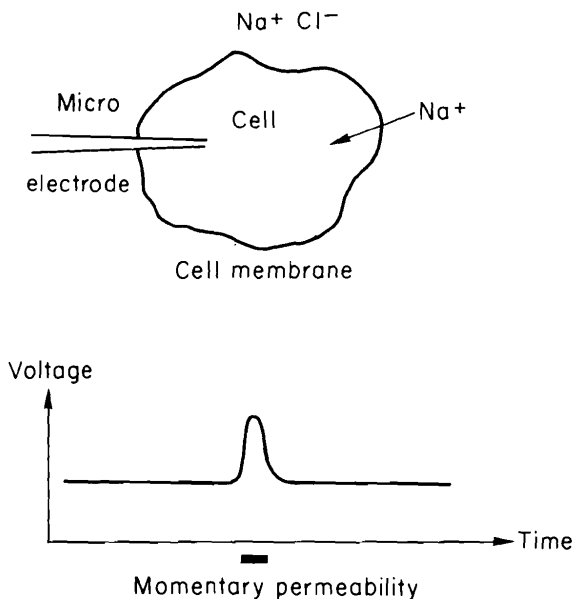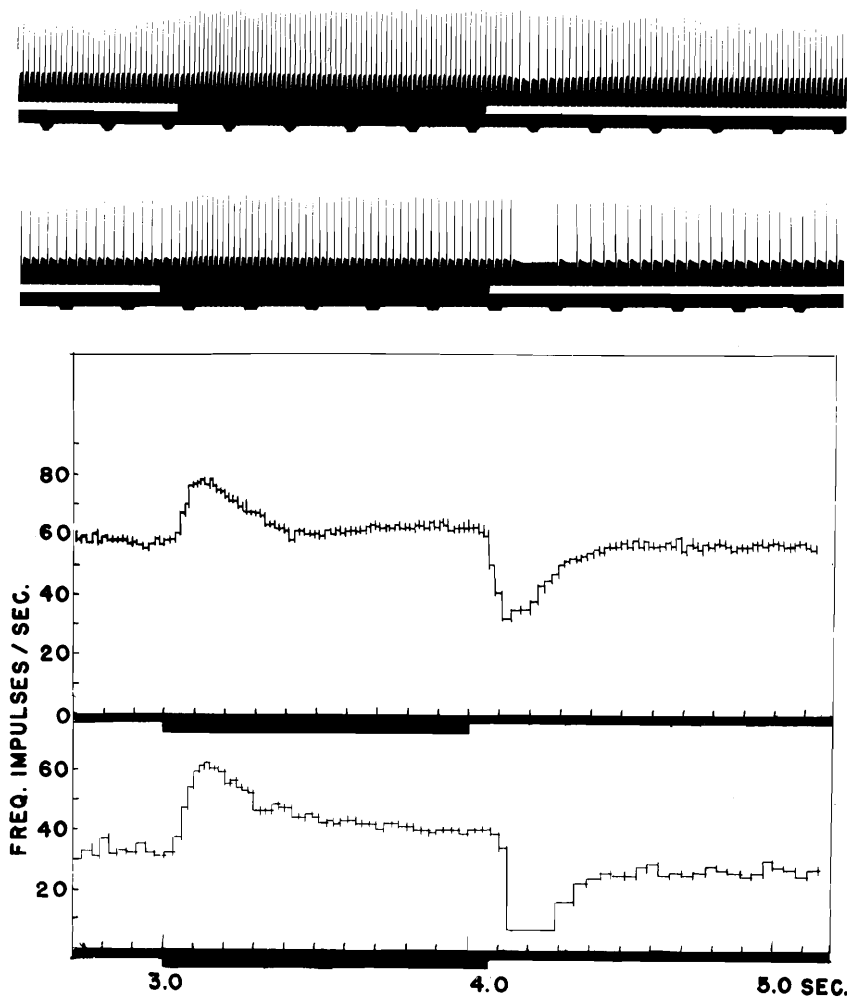

FIGURE 5

FIGURE 6

FIGURE 7

When light is shone on the eye of the *Limulus*, trains of voltage impulses may be observed in single nerve fibers of the optic nerve. These records made by Hartline show a single fiber's response to bright light above and to a dimmer light below.
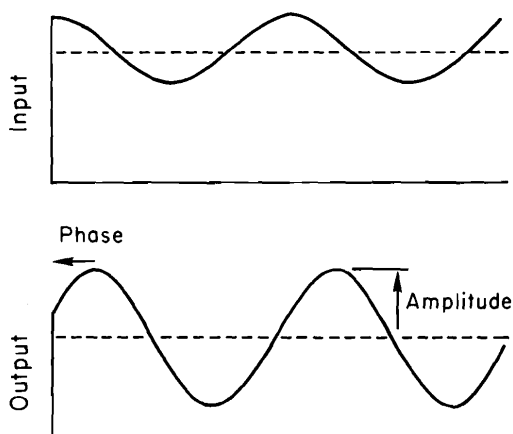
FIGURE 8

In each case an additional light has been turned on and then off in the middle of the record. In each case the rate of impulse generation changes notably in response to the change in light intensity. (Figure 7.)

Here the data of the same experiment are presented in another way. The vertical coordinate is the "instantaneous frequency", which is the reciprocal of the time between consecutive impulses. The instantaneous frequency is a measure of the level of activity within the nerve fiber.

We notice that there is a transient excursion in nerve activity when the brief light comes on, and another when it goes off. Moreover, we make the quantitative observation that the off-transient is the near mirror image of the on-transient. (You must forgive these records for becoming grainy at low firing rates, where impulses are spaced far apart.) The mirror-image transient property is a property of a linear system. Led by this clue, we will spend the remainder of the paper validating and exploiting the fact that the *Limulus* eye behaves as a linear system.

Now if the eye is indeed a linear system, we may make a particular prediction concerning output neural activity in response to input light: namely if, instead of putting in the steps of light as shown here, we had put in a sinusoidal light stimulation, we should observe a sinusoidal response in nerve activity. (Figure 8.) That
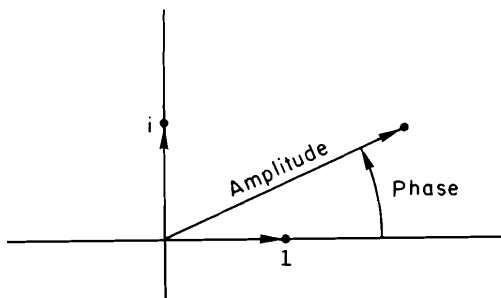
Frequency response
as a complex number

FIGURE 9
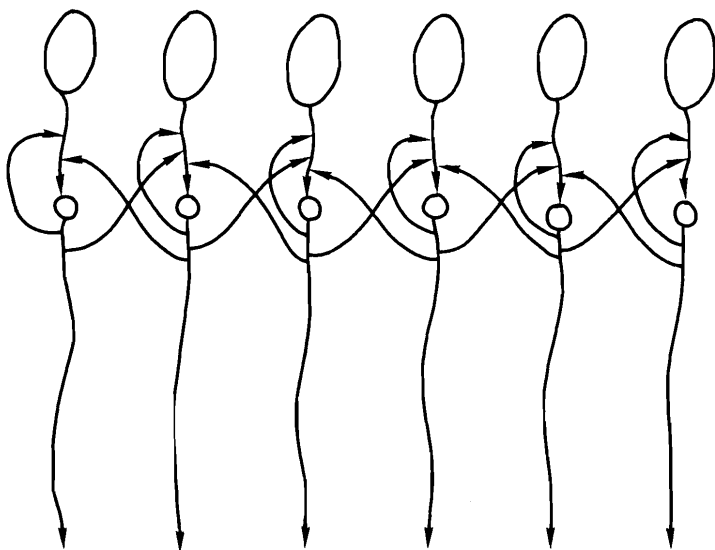
FIGURE 10

is a general property of linear systems. The output sinusoid may
differ from the input in both amplitude and phase. How amplitude
and phase are changed depends, of course, on the nature of the
linear system. Conversely, the system's behavior at that frequency
is characterized by the amplitude and phase. (Figure 9.) It will
prove convenient later on to express amplitude and phase in
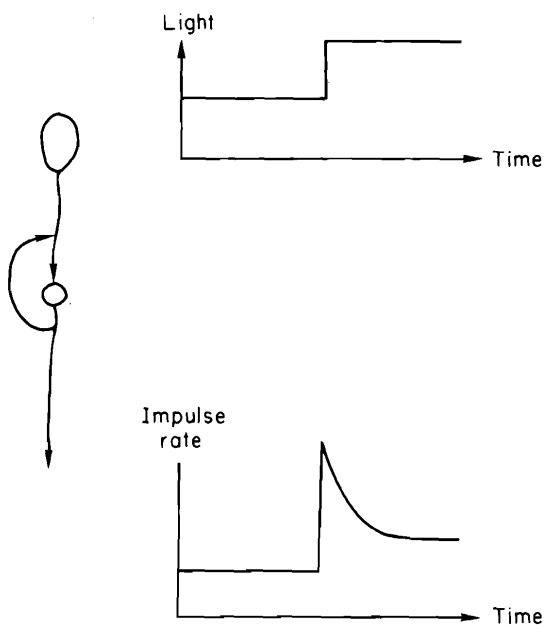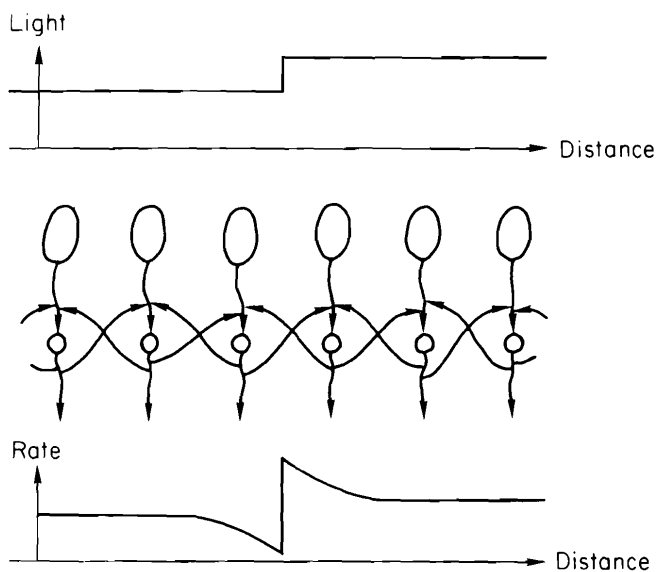terms of a single complex number.

FIGURE 11

FIGURE 12

Before we undertake the job of analyzing the *Limulus* eye quantitatively, we should understand some qualitative things about it. An idealized schematic of the neural network is shown here (Figure 10). The large ellipses on top indicate the light receptors, which respond to light with positive voltage. The little circles indicate the sites of nerve-impulse generation. The more positive the voltage, the faster the firing rate of the impulse generator. The nerve impulses propagate down the nerve fibers to the creature's brain, and also throughout the neural network, as shown by the arrows. Wherever an arrowhead touches a nerve fiber in the schematic, that is a site of neural inhibition: the arrival of nerve impulses at that point causes a *negative* voltage to build up in the nerve cell to which the arrow points. We see both *lateral inhibition* between nerve cells, and *self-inhibition* of a nerve cell back upon itself. These connections, which were originally proposed to account for the observed behavior of the network, all have been found recently by careful electron microscopy. In the real *Limulus* eye the lateral interconnections are not limited to nearest neighbors.

Now what function is performed by this inhibition network? In a qualitative way we can relate structure and function at once. Let us start with self-inhibition. (Figure 11.) If the light increases the firing rate of the nerve cell will likewise increase. But the increased firing rate builds up an inhibitory potential which turns the firing rate down once more. This scheme undoubtedly has been selected by evolutionary pressures: there is a survival value to paying particular note to those features of the visual world which *change*. The *Limulus* eye, like our own, is more sensitive to a flashing light than to a steady one.

Lateral inhibition plays a similar role with respect to changes of illumination in space (Figure 12). A unit well inside the bright part of the field is inhibited from both sides. But a unit near the edge receives less inhibition from the dimly illuminated region, and hence it is more active. The result is contour enhancement. These regions of exaggerated change are called Mach bands, after the celebrated Austrian physicist Ernst Mach who observed the phenomenon in his own eye (Figure 13).
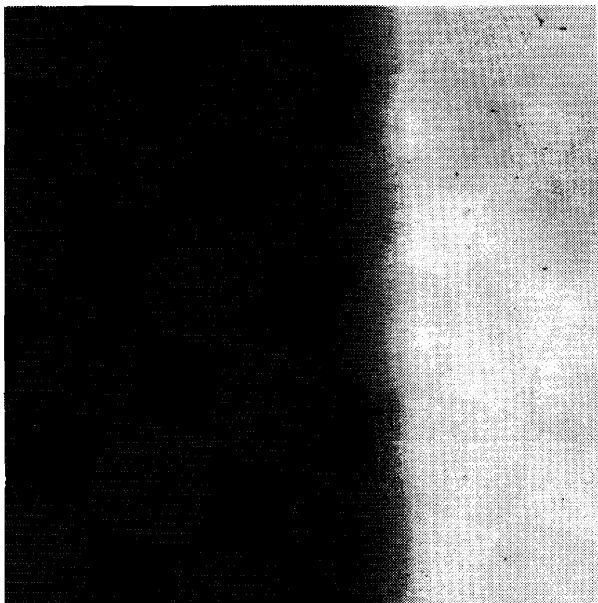
The band of darkness and the band of light you see here are Mach bands and are not up in the picture but rather are in the eye of the beholder, yourself. They are a consequence of the neural network within your eye. The light intensity in the picture in fact increases monotonically from left to right.

We will now explore the neurophysiology of the horseshoe crab's eye in a quantitative way (Figure 14). Here we see another micrograph which shows the receptors, the lateral interconnections, and the optic nerve fibers on their way to the brain. This eye may be excised from the *Limulus*, and will continue to perform all by itself in the laboratory.

The excised eye may be observed and controlled in several different ways (Figure 15). We have seen an electrical recording from a single optic nerve fiber. It is also possible to insert a fine glass micropipette directly into a receptor cell and observe the voltage there. The eye may be stimulated by light. Alternatively the unit may be stimulated by passing current through the micro-electrode and thus directly controlling the intracellular voltage.

FIGURE 14

A third alternative is to deliver electric shocks to the optic nerve: this will cause an artificially created train of nerve impulses to flow backwards up the optic nerve fibers and into the eye, where they invade the lateral interconnections and cause lateral inhibition.

Now I will discuss an experiment in which a flickering light is shone on the receptor, and the sinusoidally modulated intra-cellular voltage (or "generator potential") which results, in the box marked 1, is observed through the microelectrode. And further:

i. An experiment in which the intracellular voltage in the box marked 1 is modulated directly by passing current through the electrode, and the resulting modulation in the rate of impulses, which arise in the box marked 2, is observed.

ii. An experiment in which modulated light is shone on the receptor, and we observe the resulting modulation in the impulse rate.
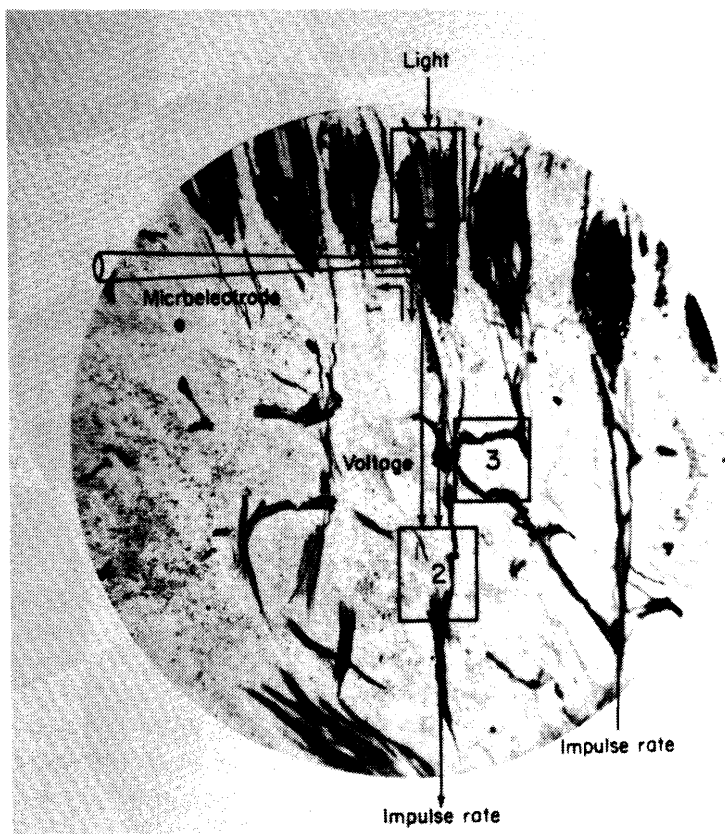
FIGURE 15

iii. An experiment in which we modulate the rate at which impulses are sent backward along the optic nerve, and we observe the resulting intracellular voltage modulation with the microelectrode.

iv. A somewhat similar experiment, in which we put in a modulated train of backward-running impulses, and measure how the lateral inhibition which they cause modulates the rate at which impulses are generated at the box marked 2.

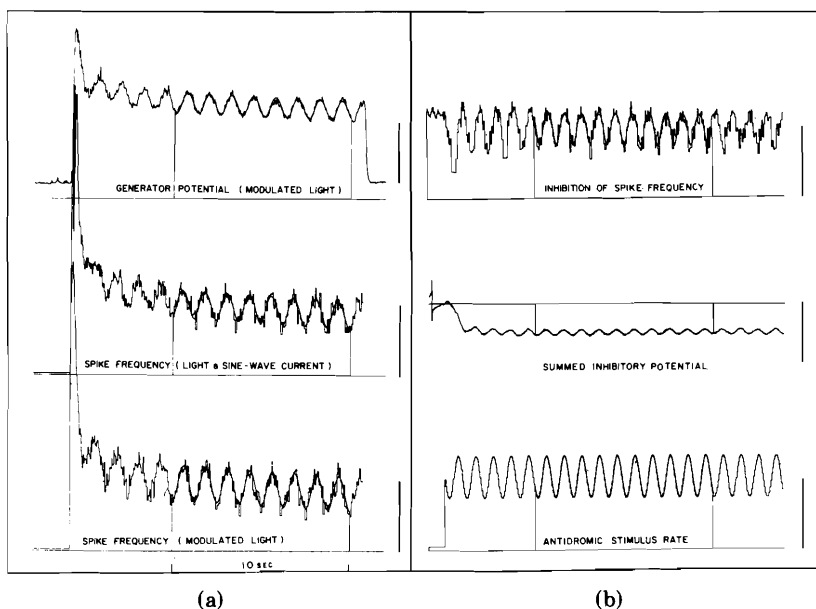A great deal can be learned from these experiments.

FIGURE 16

Here is what the raw data look like (Figure 16 (a) and (b)). The upper section of (a) is a voltage record, the intracellular potential responding to modulated light. Below it is an instantaneous frequency record, impulse rate in response to modulated current through the electrode. The bottom section shows the impulse rate in response to modulated light.

Figure 16(b) should be read from bottom to top. The bottom section shows the modulated rate at which nerve impulses are backfired up the optic nerve toward the eye. The middle section shows the time course of the lateral inhibitory potential that results, and the top section shows the effect upon the firing rate in the same cell.

Let us consider first the experiments in (a). The results of the first two should enable us to predict the results of the third (Figure 17). At a given modulation frequency, the transduction from light to voltage may be characterized by an amplitude ratio between
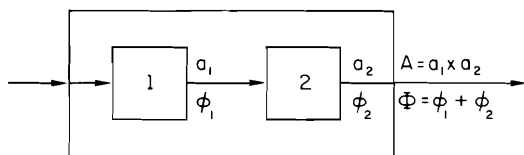
FIGURE 17

output and input, and by a shift in phase. The transduction from voltage to firing rate likewise may be characterized by an amplitude change and a phase shift. For the full transduction all the way from light intensity to firing rate, evidently we should multiply the component amplitudes and add the component phases.

On the left in Figure 18 we plot the experimental values of amplitude and phase, versus modulation frequency, of the two component transductions, from light to voltage and from voltage to firing rate. When the amplitudes are multiplied and the phases added, the solid curves on the right result. The points on the right show the result of direct measurement of the full transduction, and are in good agreement with the prediction.

We've seen that the *Limulus* should be more responsive to a flashing light than to a steady one. The right-hand curves tell us something quantitative about this. The amplitude of the frequency response reaches its maximum at about 3 cycles per second. If you want to attract the attention of a *Limulus* by flashing a light, you should flash that light at 3 flashes per second.

Let us look more closely for a moment at the transduction from voltage to firing rate in Figure 15. The experiment is to run a modulated current through the electrode near box 1, and observe the modulation in impulse rate that results at box 2. Two things are involved here: the generation of impulses in response to voltage, and the production of self-inhibitory intracellular voltages as a result of nerve impulses.

The behavior of voltage-excitable cell membranes is fairly well understood, and leads us to this simple model (Figure 19) for the encoding from voltage to impulses by the *Limulus* nerve cell: the next impulse will be fired when the time integral of the voltage, since the last impulse, reaches a firing threshold value.
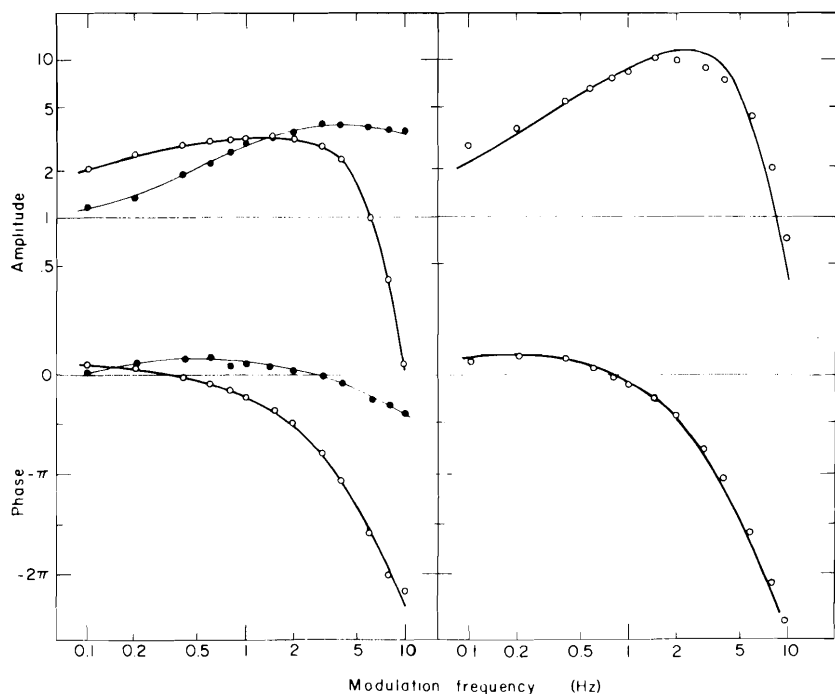
FIGURE 18

Integrate and fire model

$$C = \int_{t}^{t+P} dt\, S(t)$$

C : Threshold
P : Period between impulses
S : Stimulus
Rate 1/P is proportional to mean
    stimulus between impulses

FIGURE 19

After a nerve impulse is fired (Figure 20), the *Limulus* visual neuron feels a self-inhibitory potential, which declines exponentially. If two or more impulses fire, their self-inhibitory effects add, as shown by these intracellular measurements, which were done by Richard Purple.

I spike

6 spikes

26 spikes

5 mV

I SEC
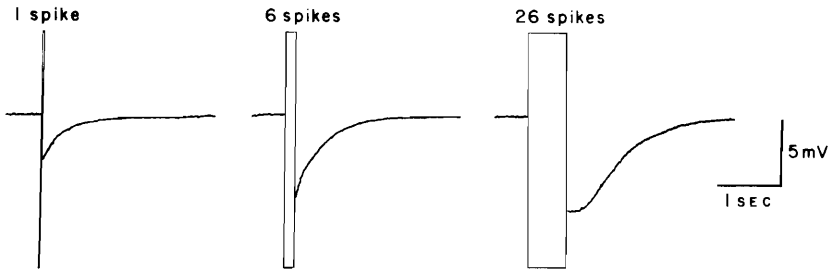
Summation of self-inhibitory p.s.p. s

computer averaged responses
from an experiment of Purple (1963)

FIGURE 20

Transfer Function for the Self Inhibition Model

$$S = (1+K)\left(\frac{1-e^{-i\omega/\nu}}{i\omega/\nu}\right) \bigg/ \left(1+K\left[1-\frac{(1/\tau\nu)(1-e^{-i\omega/\nu})}{(e^{1/\tau\nu}-1)(1-e^{-(1/\tau+i\omega)/\nu})}\right]\right)$$

Relative amplitude

2

1

0.5

0.2

0.1

Σ → FM

SELF IPSP

Phase (radians)

0

−π

0.1 0.2 0.5 1 2 5 10 20
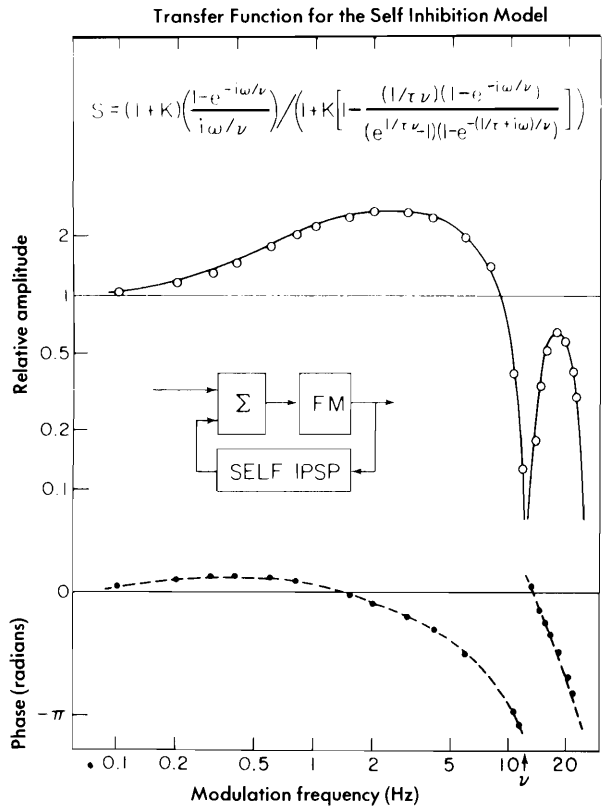
ν

Modulation frequency (Hz)

FIGURE 21

If encoding and self-inhibition act in the ways we have just said, they completely define the transduction from voltage to firing rate, which may be expressed in terms of a mathematical model. Finding the amplitude and phase of the theoretical transduction is simply an exercise in algebra. The theory yields the curves shown here (Figure 21). The points were obtained from an electronic analog device. The expression above is the algebraic rendition of the same information. In it amplitude and phase are expressed as a single complex-number valued function of frequency. It is the "transfer function" for self-inhibition, and we call it "S".

Figure 22 shows a measurement by Robert Shapley of the transduction from intracellular voltage modulation to modulation in impulse rate. The points show the measurements of amplitude and phase at different frequencies, and the lines, which agree quite well, come from the theoretical expression in the previous figure.

Let us now turn from self-inhibition to the process of lateral inhibition (Figure 23). How much a given nerve cell is inhibited by activity in another cell depends on their separation distance. The dependence of inhibition strength upon separation has been measured by Robert Barlow, and his result is shown here in relief. The inhibited neuron lies at the little circle, and it is surrounded by a sort of "volcano crater" of inhibitory coupling from other neurons.

These measurements were made with steady light. However, we may do another experiment to determine whether the spatial distribution of inhibition would be the same if the light were flickering. We record the impulses from the central neuron, which is illuminated by steady light. We then shine a spot of flickering light on the rim of the volcano, and observe how inhibition modulates the impulse rate of the central neuron. We then move that flickering spot away—far down the outer slope of the volcano—and again observe the modulating effect of lateral inhibition. This sequence is repeated at different frequencies. The result is shown on the right-hand side of Figure 24.
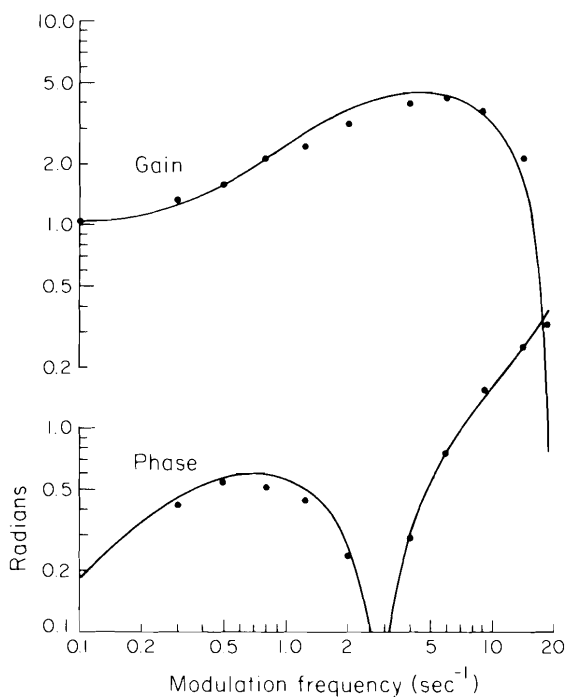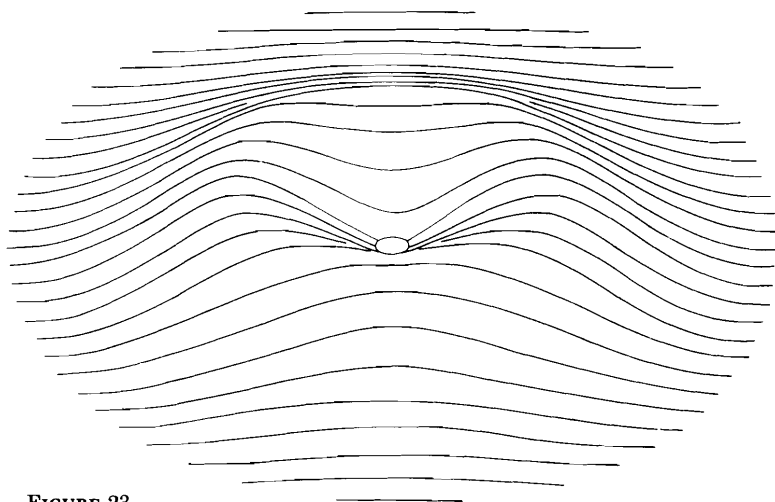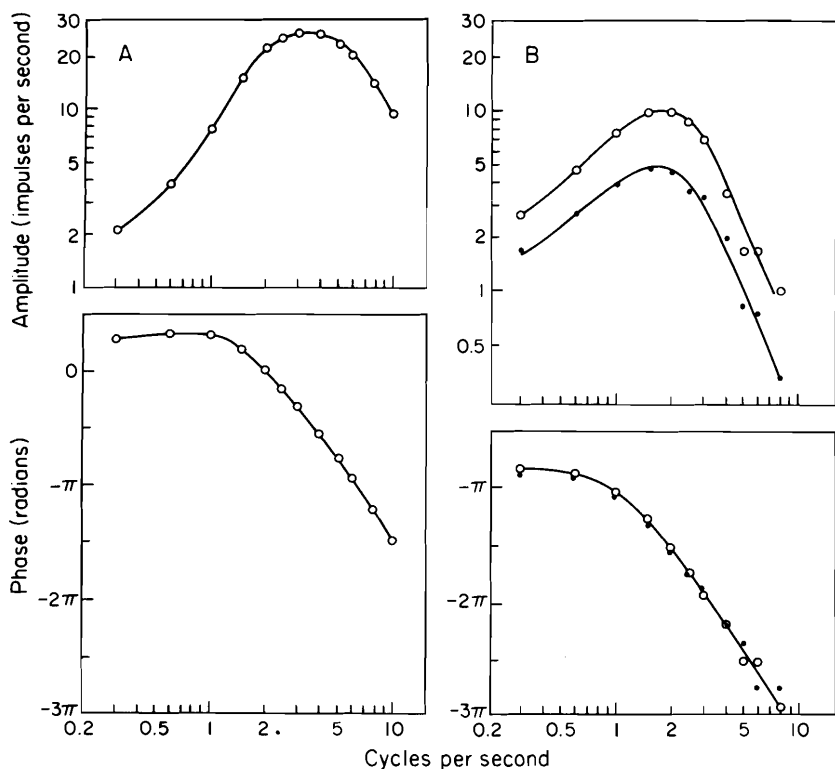
FIGURE 22

FIGURE 23

FIGURE 24

The phases of the two modulation responses are identical at all frequencies. The amplitudes are in a single fixed proportion that does not depend on frequency. Therefore the shape of the volcano is independent of frequency. Moreover, the frequency dependence of lateral inhibition is independent of distance, except for size.

Returning to Figure 15, we may now measure the transfer function for lateral inhibition in the following way: first, by running modulated current through the microelectrode, we measure the modulation of the neuron's firing rate in response to a known modulation in intracellular voltage. We have already discussed this measurement in some detail. Next we fire backwards — through
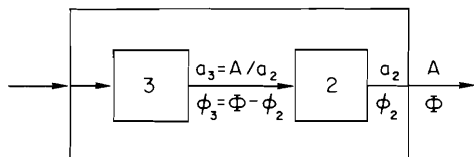
FIGURE 25

all the fibers of the optic nerve *except* the fiber of that nerve cell from which we are recording — we fire backwards a modulated train of nerve impulses. This modulated train of impulses arrives at our recording cell at the box marked 3 where it proceeds to transduce a modulated inhibitory potential within our cell. Our recording cell in turn responds to this inhibitory potential by modulating its own impulse train.

This information we may unravel for the lateral inhibitory transfer function (Figure 25). We divide the amplitude of the total transduction by the known amplitude of the voltage-to-rate transduction. Similarly, we subtract phases. The result is shown in Figure 26.

The partial transduction from voltage to impulse rate is marked B. The total transduction from neighbors' firing rate to inhibitory potential and back to our cell's firing rate is marked A. To the right, the curve C is the lateral inhibitory frequency response which we deduce.

Now if lateral inhibition is linear, and if we know how it responds to sinusoidal inputs at all frequencies, we can predict its response to an arbitrary input. In particular, we can predict how it will respond to a single impulse. We have made this prediction, using Fourier analysis, and the result is shown in the inset. It is also possible, by averaging many runs, to measure the impulse response directly, in the same cell. The next figure (27) compares the direct measurement (the dots) to what Fourier analysis predicts.

We believe this result validates our assumption that lateral inhibition is a reasonably linear process.

Now we will check that the *Limulus* retina combines excitation and inhibition in a linear way (Figure 28). This photograph was taken after the experiment I am about to describe. We are looking
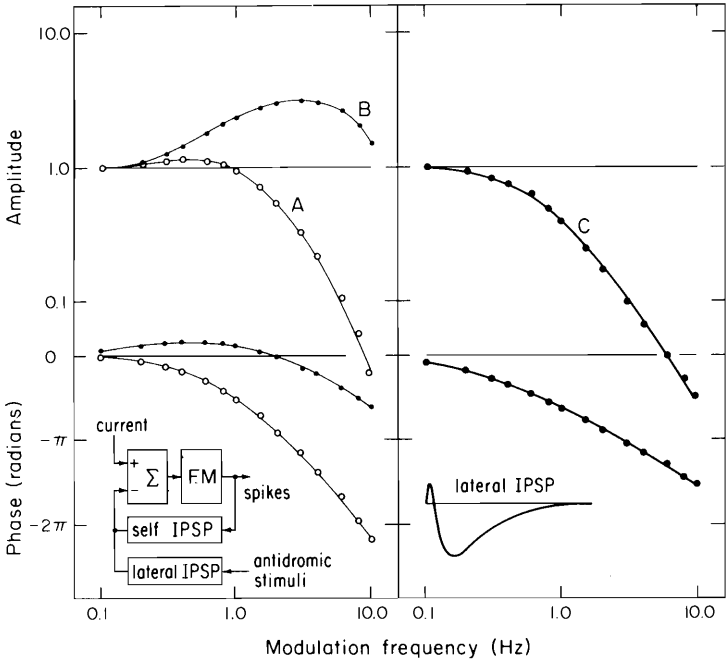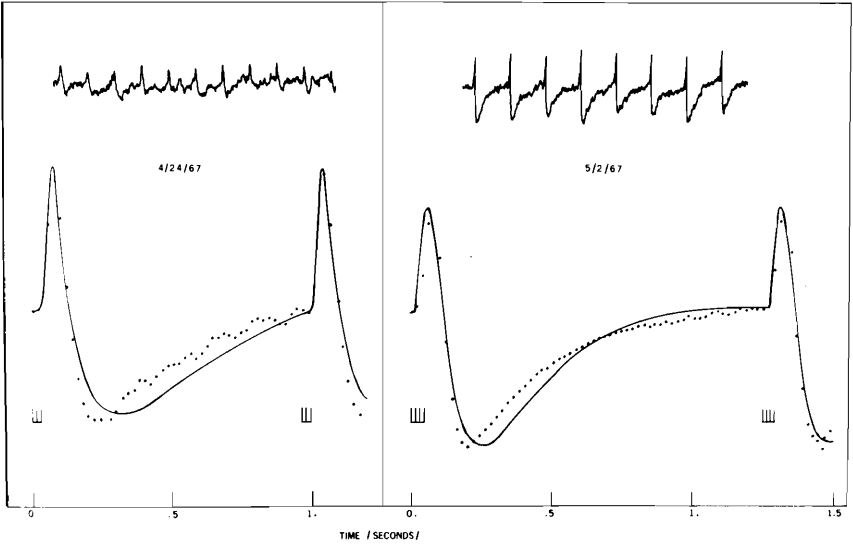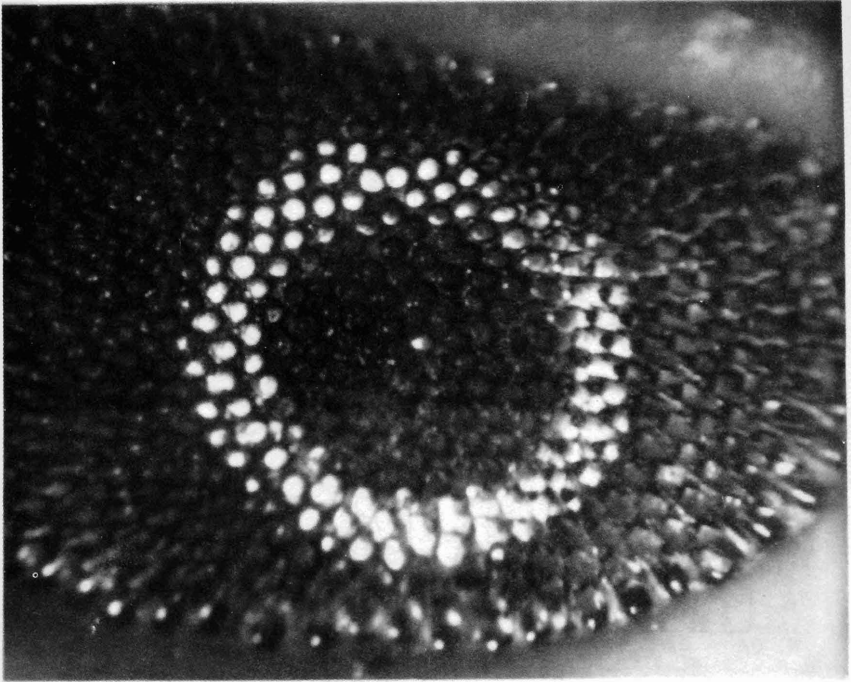
FIGURE 26

FIGURE 27

FIGURE 28

out from the inside of the *Limulus* eye. The neural tissues have been removed and we are looking out through the *Limulus'* faceted optics. Outside are two independent light sources, which cause the central spot of light and the surrounding ring. The firing rate of the central illuminated unit was recorded. The light sources were flickered in different ways. In some runs the ring was held steady and the spot was flickered. In other runs the spot was held steady and the ring was flickered. In other runs the spot and ring were flickered in unison. In yet other runs the flicker cycle of the ring was delayed behind that of the spot by a fixed delay time.

In Figure 29, on the left, we have the frequency response to excitation, obtained by flickering the central spot. On the right is the frequency response to inhibition, obtained by flickering the ring. We notice that it has a phase-shift of pi radians at low fre-
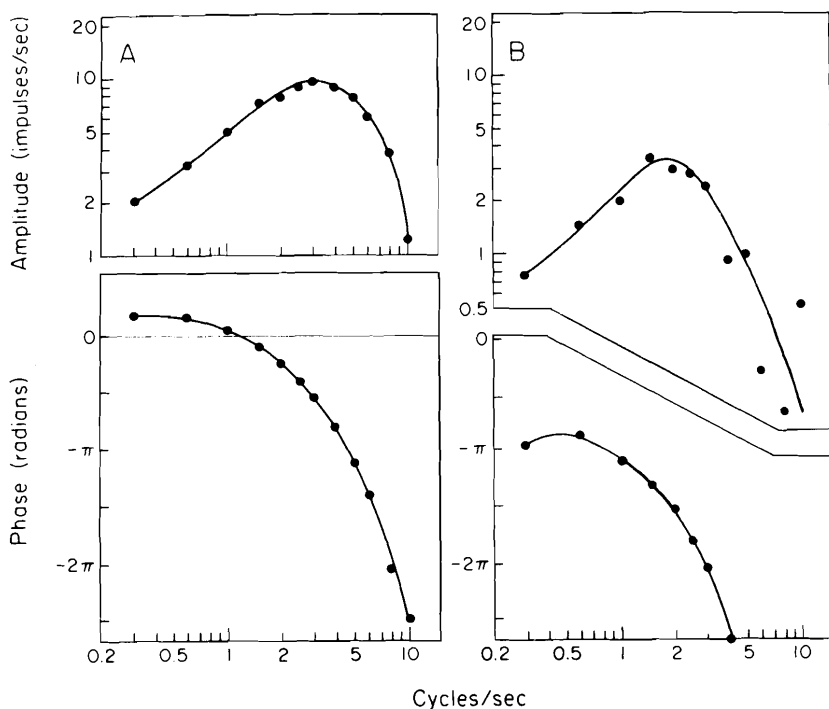
FIGURE 29

quency, as inhibition should. We should be able to superimpose these frequency responses and predict the outcomes of the runs in which both light sources were flickered.

The right-hand side of Figure 30 shows the result when the two light sources are flickered synchronously. The left-hand side shows the result when the ring is time-lagged. The points are experimental measurements and the lines are predictions made from the previous figure.

We have characterized the dynamics of excitation, self-inhibition, and lateral inhibition, and we have shown how they combine (Figure 31). We are now in a position to formulate mathematically the dynamics of the entire eye, performing as an interacting nervous system. With a couple of small simplifications, here is the *Limulus* eye reduced to equations.
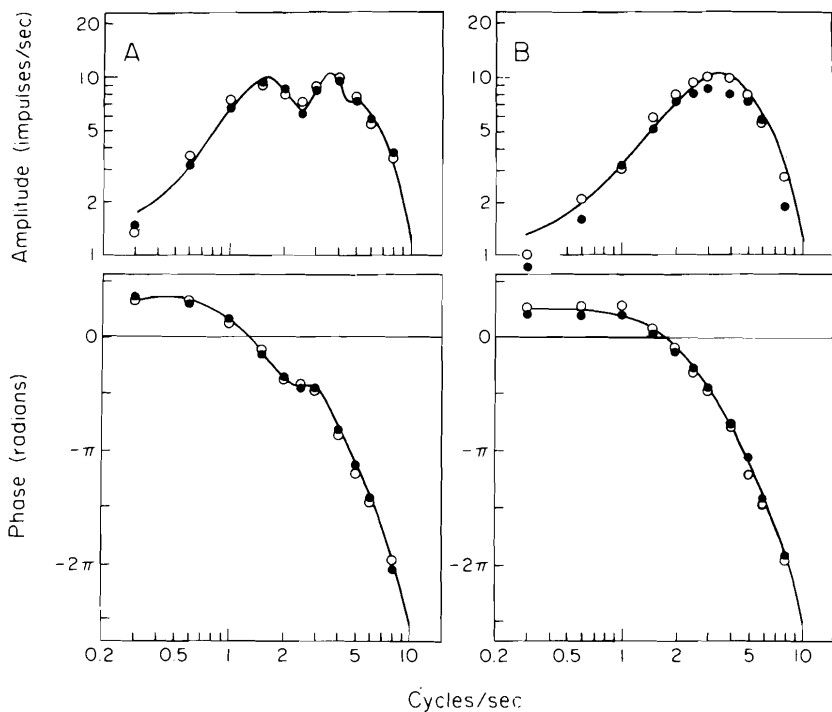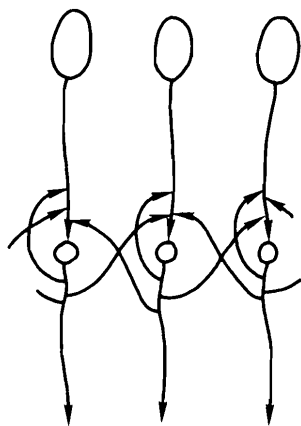
FIGURE 30

$$I_m = I_m(o)e^{i\omega t}$$

$$\epsilon_m = G(\omega) I_m$$

$$r_m = \epsilon_m - T_s(\omega) k_s r_m - T_l(\omega) \sum_n k_{mn} r_n$$

FIGURE 31

The light intensity causes an excitatory intracellular potential through a transduction that we know because we have measured it. The firing rate of a visual nerve cell depends on the excitatory potential and also on the inhibitory effects of self-inhibition and of lateral inhibition. The self-inhibition is transduced from the cell's own firing rate in a known way, and the lateral inhibition is transduced in a known way from the activity of other connecting nerve cells. At a given frequency of flicker, this becomes a set of linear simultaneous equations which may be solved for the firing rates of all the cells.

We did this for a circular array including 19 active cells. The predicted frequency response for the central nerve cell is given by the dotted line in Figure 32.

The corresponding experiment, flickering a large spot of light on the eye and recording from the central nerve cell, was also done. The open circles show the result of the experiment.

At this point we have still to demonstrate that we can predict the response of the whole eye to a stimulus whose time course is not sinusoidal. A frequency response like this one was measured on an eye which was also stimulated with a step in illumination. The directly measured step response is shown in the lower part of Figure 33. The upper part shows the step response predicted from flicker measurements and Fourier analysis. One can tell from the quality of the figure that this project has just reached its final stage.

I have almost finished, but would like to make a few more points. There were a few approximations in the dynamical equations which I have demonstrated. In particular, the instantaneous frequency is not a perfect indicator of what is going on in a population of neurons.

The top curve and open circles of Figure 34 show the density of nerve impulse firings of a nerve population, in response to flickering light. The lower curve shows the modulation in the instantaneous frequency of a single member. The discrepancy only becomes important if the flicker frequency f approaches or exceeds the nerve cell's mean firing rate f_0. The discrepancy can be taken into account on a theoretical basis. In Figure 35 the average is taken over the stochastic scatter in inter-pulse intervals.
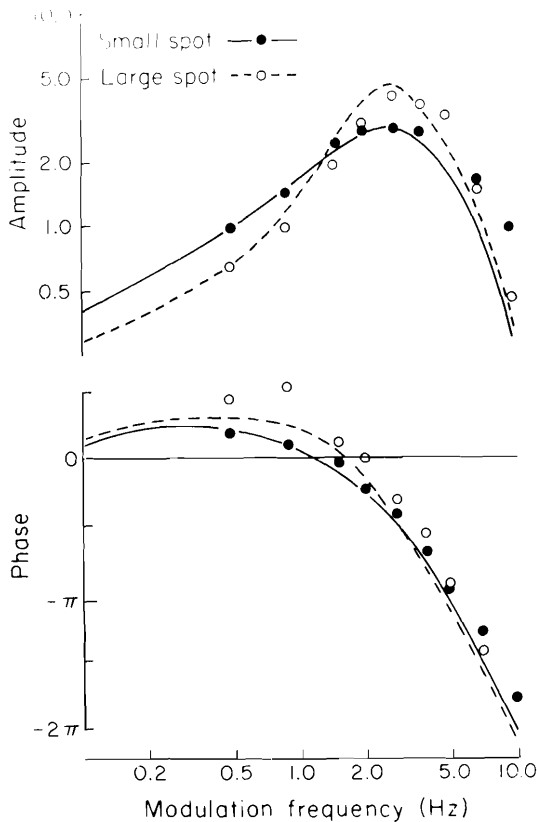
Small spot ──•──
Large spot ──○──

FIGURE 32

Modulation frequency (Hz)

It is possible (Figure 36) to solve the *Limulus* neural dynamics for a running sine wave of illumination. One interesting feature is that the total lateral inhibition at a given unit is obtained from a sum weighted according to the phase of the sine wave at different points on the eye. Thus the spatial dependence of the wave in effect takes the spatial Fourier transform of the volcano-crater profile which we saw earlier.

Figure 37 is an exact deduction of how a running sine wave of light intensity leads to modulation in single unit firing rate. The steps are very simple and straightforward, so let us only look at the last line.

FIGURE 33

In the numerator the single unit response depends on the self-inhibition transfer function S, which we discussed before, and upon the excitatory light-to-voltage transfer function, G. The denominator has a form that is typical of negative feedback systems, and involves everything: $1/B$ is the transfer function from single unit to population, S again is the self-inhibitory transfer function, T_l is the lateral inhibitory transfer function, and $\tilde{k}$ is the Fourier transform of the volcano crater profile.

Now any light intensity pattern, which changes arbitrarily in space and time, may be expressed as a superposition of running sine waves, and in that sense the final formula here may be regarded
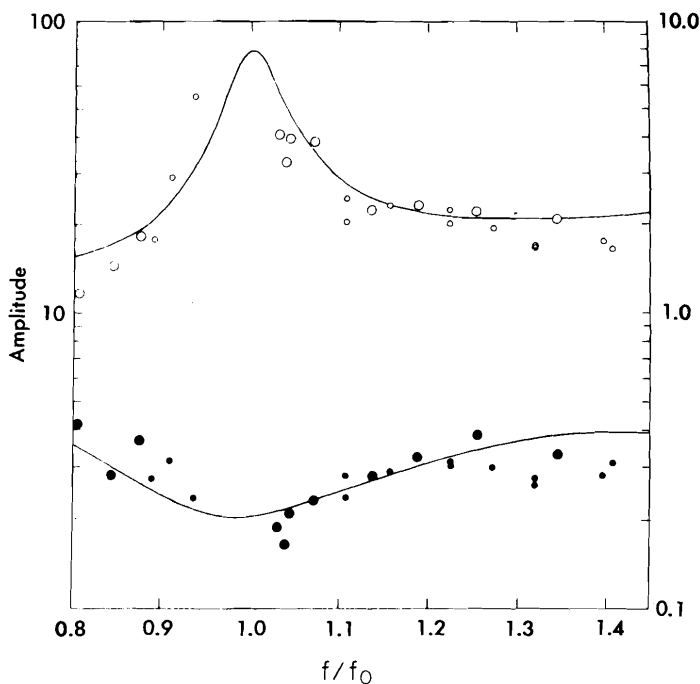
FIGURE 34

f/f_0

Transduction between
single unit rate and population rate:

r : Population rate

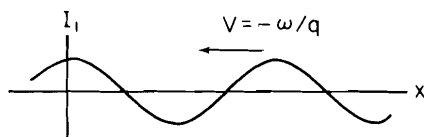u : Single unit rate

$$\cdot u = Br$$

where

$$B = \frac{1 - \langle e^{-i\omega T_0} \rangle}{i\omega \langle T_0 \rangle}$$

FIGURE 35

Stimulation by running sine-wave:

$$I_1(\vec{x}, t) = I_1(0,0) e^{i(\vec{q} \cdot \vec{x} + \omega t)}$$

I_1 $V = -\omega/q$

Total lateral inhibition on a given
unit must be weighteded according to
the phase of the wave at other units:

$$\widetilde{k}(\vec{q}) = \sum_{\vec{x}} e^{i\vec{q} \cdot \vec{x}} k_{\vec{x}}$$

FIGURE 36   ($\vec{x}$ is position of inhibiting unit)

Single unit response to running wave:

V : Intracellular voltage modulation
    from excitation and lateral inhibition

G: Excitation transfer function

ϵ : Excitation voltage modulation

$$r = \frac{1}{B}\, SV \quad V = \epsilon - T_l \widetilde{k} r \quad \epsilon = GI_1$$

$$r = \frac{\dfrac{1}{B}\, S}{1 + \dfrac{1}{B}\, ST_l \widetilde{k}}\, GI_1$$

$$u = \frac{S}{1 + \dfrac{1}{B}\, ST_l \widetilde{k}}\, GI_1$$

FIGURE 37

V = 0.6 mm/sec

V = 0.2 mm/sec

FIGURE 38   V = 0.0 mm/sec
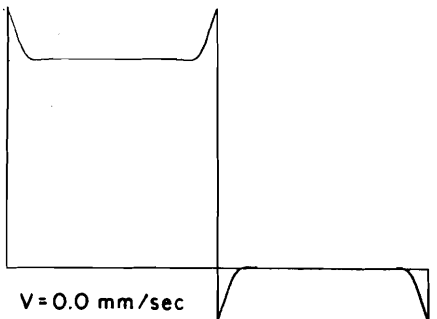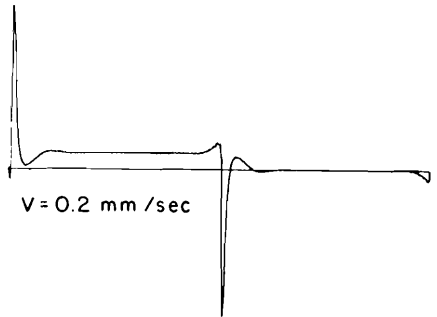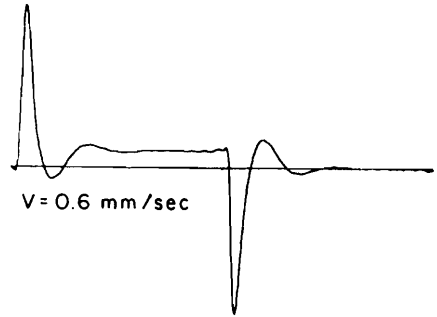
as a complete solution to the dynamics of the *Limulus* retina. We have used that complete solution to obtain Figure 38.

A step in light intensity, moving from right to left, was Fourier analyzed into running sine waves, which were multiplied by the transduction of the previous slide and then summed. What we see here is a snapshot of the instantaneous frequencies of nerve cells across the eye. The step up in light intensity has just reached the left-hand edge. The step down which follows has just crossed the middle. If the step moves very fast, (which is 6/10 millimeters per second for the *Limulus*) we expect an activity profile in space which looks like the profile we would get in time if the light were stepped simultaneously on the whole eye, and that is indeed what we find in the calculation. If the step of light is not moving, the discontinuity is flanked with Mach bands of neural activity. That likewise has been verified in the laboratory.

If the step is moving at a modest rate, we predict a hybrid form, showing both the motion transient and the Mach band. The experiment is still a few months off.

It would certainly be incorrect to assert that these theoretical techniques can give an equally complete accounting for the dynamics of the other neural networks that I showed to you at the start. But we do like to hope that they may have some further usefulness in man's struggle to understand himself.

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