

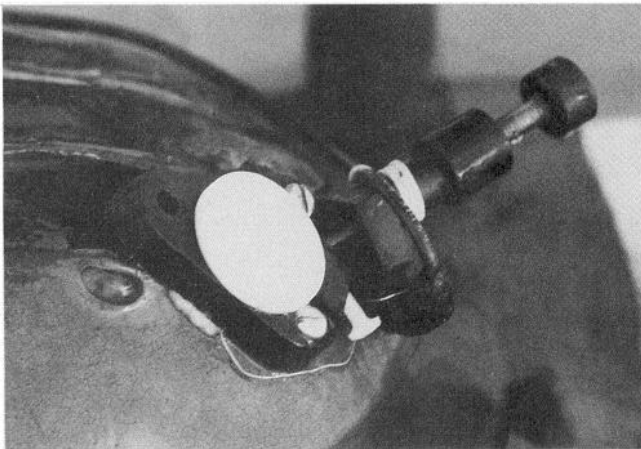
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***Limulus* Vision in the Ocean: Comparing Neural and Behavioral Thresholds**  
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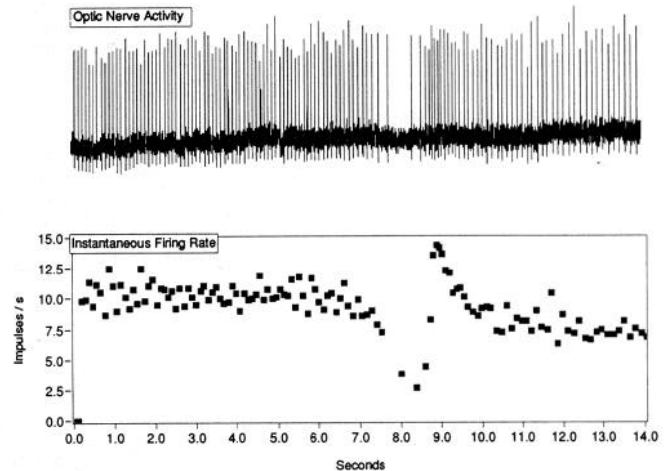
The horseshoe crab, *Limulus polyphemus*, uses vision to locate mates (1, 2). What information must the eye send to the brain for the animal to see a mate? Building on the extensive knowledge regarding the animal's visual system (3, 4) and its visually guided behavior (2), we are investigating this question with a wide range of techniques (5, 6). Here we report our technique for recording optic nerve activity *in situ* from animals moving past behaviorally significant scenes in the ocean. We also report preliminary data comparing neural and behavioral thresholds.

We conduct all experiments in about 0.5 m of water on a sandy, gently sloping beach in Woods Hole, Massachusetts, under ambient conditions. Adult male *Limulus*, about 20 cm across the carapace, are collected and maintained at the Marine Biological Laboratory. We gain access to the lateral optic nerve by cutting a 2-cm hole in the carapace, then we gently slide the uncut nerve into a sealed, nylon recording chamber affixed to the carapace (Fig. 1). We tease a single active fiber from the optic nerve and suck it into a microsuction electrode built into the recording chamber. Nerve impulses are amplified ( $10^4$  gain AC preamplifier, Electronics Shop, Rockefeller University), digitized, and recorded on a portable 80486 personal computer with Labview Software (National Instruments, Austin, Texas). In the laboratory, we select optic nerve fibers from ommatidia that view the world near the animal's horizon, and we use an underwater goniometer to measure their precise directions of view (optic axes). We then mount the animal on a sled and take it to the ocean.

Our strategy is to record visual responses from the "wired" animal under field conditions approximating those observed during the mating season. Behavioral experiments have shown



**Figure 1.** Chamber for recording optic nerve activity *in situ* from *Limulus* underwater. The lateral optic nerve exits the eye on the left, travels under the carapace and runs through the chamber on the right on its way to the brain. The microsuction electrode is shown inserted into the chamber from the right.



**Figure 2.** (Top). Impulse discharge recorded from a single optic nerve fiber as the animal moved past a 9" cylindrical target at a distance of 0.25 m. The animal passed the target between 7.5 and 8.5 s. (Bottom). Instantaneous firing rate of the spike train above. The nearby black target produced a robust modulation of the discharge, reducing the firing rate to about 2.5 impulses/s followed by a burst of activity at about 15 impulses/s.

that males will turn towards and make contact with targets that have been placed in the water and that are about the size of a female (1). For these experiments, we selected a black cylinder (9" diameter; 6" tall) as a visual target because we know from previous experiments how close an animal must be to this type of target to see it (7). The cylinder is a convenient target because its profile appears the same regardless of the direction of approach. We suspend the sled (with the animal) on a 2-m-long underwater track so that the lateral eyes are about 9 cm above the bottom; then we pull the sled past the visual target which is placed at various distances from the track. Optic nerve activity is recorded simultaneously on an FM tape recorder and a VCR, as well as on the portable computer. We pull the animal at about 10 cm/s (which approximates the average speed of *Limulus* locomotion), determining the precise velocity from the videotape after the experiment is completed. To measure the temporal variation in stimulus intensity during each run, a photodiode is attached to the carapace above the eye with an acceptance angle of an ommatidium and an optic axis parallel to the recorded unit.

As the animal, moving along the track, comes within 1.25 m of the target, we detect a clear modulation of the optic nerve discharge (Fig. 2). We define the neural threshold as the distance at which the target no longer evokes an audible modulation in the spike discharge. When the target is placed more than 1.25 m from the eye, the modulation in firing rate decreases to the back-

ground level recorded in control runs without a target. This neural threshold of 1.25 m is slightly less than the behavioral threshold of 1.4 m. Behavioral experiments show that males do not turn toward the 9° target when it is placed more than 1.4 m away from the animal (7).

We have previously shown that, at any given moment, as the animal moves along the track, the target at 1.4 m subtends approximately 6–12 of the 1000 receptors in the lateral eye (5). This report suggests that the modulated activity of a small population of retinal neurons transmits to the brain the information necessary for the animal to see the target.

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## Equivalent Sine Wave Frequency for Interpretation of Responses to Frequency-Swept Sinusoids Defined: an Algorithm From Studies in Skate Ganglion Cells

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The frequency-swept sinusoid (FSS) has proven useful in the presentation and qualitative interpretation of responses to physiological stimulation (1–4). It also played an extensive role in early sonar applications because the echo of sweep-frequency modulated signals exhibited a recognizable chirp (5). An example of responses to such stimulation from our studies of OFF pathways in the skate (*Raja erinacea*) retina (Fig. 1A) shows that extracellularly recorded (6) responses of a decrementally responding ganglion cell to FSS stimulation are a function of light adaptation time. Although intuitively helpful, results from such experiments have been difficult to quantify because the frequency is continuously changing as a function of time; thus, the time for which the frequency is determined must be carefully specified. Furthermore, the driving frequency is not the same as the apparent frequency that might be estimated by simply measuring the time between peaks, as for a regular sinusoid; nor is the driving frequency directly comparable to the frequency of regular sinusoids used in the rich literature of flicker-fusion frequency studies. Therefore, the results of FSS and regular sinusoidal stimulation have been difficult to compare. Consequently, we have derived relationships that we have used to define an equivalent sine wave frequency,  $f_E$ , representing the instantaneous slope of an FSS in terms of the frequency of a regular sinusoid of equivalent instantaneous slope having the same peak-to-peak amplitude.

Consider an FSS described by a sinusoidally driven function for which the command frequency,  $f_c$ , increases linearly with time,  $t$ , from some initial frequency,  $f_0$ , such that the command frequency is given by the relation:

$$f_c = At + f_0 \quad (1)$$

where  $A$  is a constant having the units of  $\text{Hz}^2$ , and the general form of the stimulus as a function of time,  $y(t)$ , is given by:

$$y(t) = \sin(2\pi f_c t) \quad (2)$$

where the amplitude of the stimulus is taken as 1 for convenience. Substituting for  $f_c$  from Eqn. (1) we have:

$$y(t) = \sin(2\pi(At^2 + f_0 t)). \quad (3)$$

Differentiating, we obtain:

$$dy/dt = 2\pi(2At + f_0) \cos(2\pi(At^2 + f_0 t)). \quad (4)$$

Observing that  $(2At + f_0)$  can be written  $(2(At + f_0 - f_0))$ , and substituting  $f_c$  from Eqn. (1), we have:

$$dy/dt = 2\pi(2f_c - f_0) \cos(2\pi f_c t) \quad (5)$$

as the instantaneous slope of the FSS at any time,  $t$ .

We note that for a regular sinusoid having a fixed frequency,  $f_c$ , which is not a function of time, the comparable expression for its slope would be simply:

$$dy/dt = 2\pi f \cos(2\pi f t). \quad (6)$$