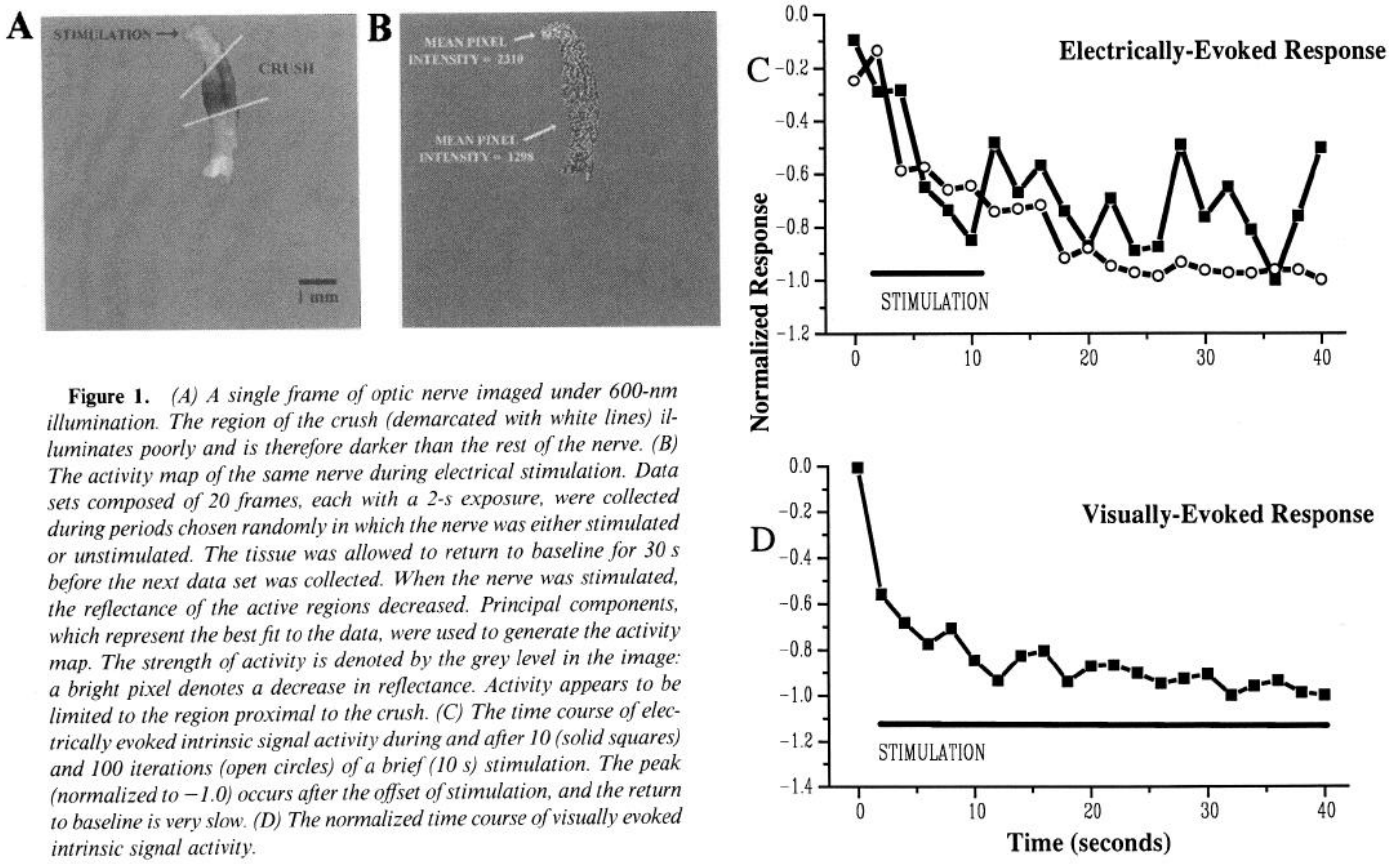

Figure 1. *(A) A single frame of optic nerve imaged under 600-nm illumination. The region of the crush (demarcated with white lines) illuminates poorly and is therefore darker than the rest of the nerve. (B) The activity map of the same nerve during electrical stimulation. Data sets composed of 20 frames, each with a 2-s exposure, were collected during periods chosen randomly in which the nerve was either stimulated or unstimulated. The tissue was allowed to return to baseline for 30 s before the next data set was collected. When the nerve was stimulated, the reflectance of the active regions decreased. Principal components, which represent the best fit to the data, were used to generate the activity map. The strength of activity is denoted by the grey level in the image: a bright pixel denotes a decrease in reflectance. Activity appears to be limited to the region proximal to the crush. (C) The time course of electrically evoked intrinsic signal activity during and after 10 (solid squares) and 100 iterations (open circles) of a brief (10 s) stimulation. The peak (normalized to −1.0) occurs after the offset of stimulation, and the return to baseline is very slow. (D) The normalized time course of visually evoked intrinsic signal activity.*

Our results indicate that optical intrinsic signals can be recorded from visual pathways in the invertebrate nervous system. These intrinsic signals should be fruitful for studying spatially localized regions of activity and for differentiating the variables which contribute to the intrinsic signal.

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Limulus Is Tuned into Its Visual Environment

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Every spring millions of horseshoe crabs invade the shallow waters along the eastern coast of North America in search of mates. Behavioral studies show that male crabs use vision to find mates, whereas female crabs use vision to avoid other nesting crabs (1, 2, 3). Horseshoe crabs reliably detect one another under a variety of environmental lighting conditions despite differences in the contrast of their carapaces. They must also cope with visual interference from water turbidity, seaweed, fish, sandbars, etc. Under these conditions, it is remarkable that male crabs can detect black and grey cylindrical targets of similar size and contrast as females equally well day and night (3). *Limulus* appears to achieve such visual performance by tuning into the natural fluctuations of light in its environment.

How do males detect low-contrast objects such as a grey target or a female crab of light carapace (Fig. 1A)? We approach this question by recording the spike discharges of single optic nerve

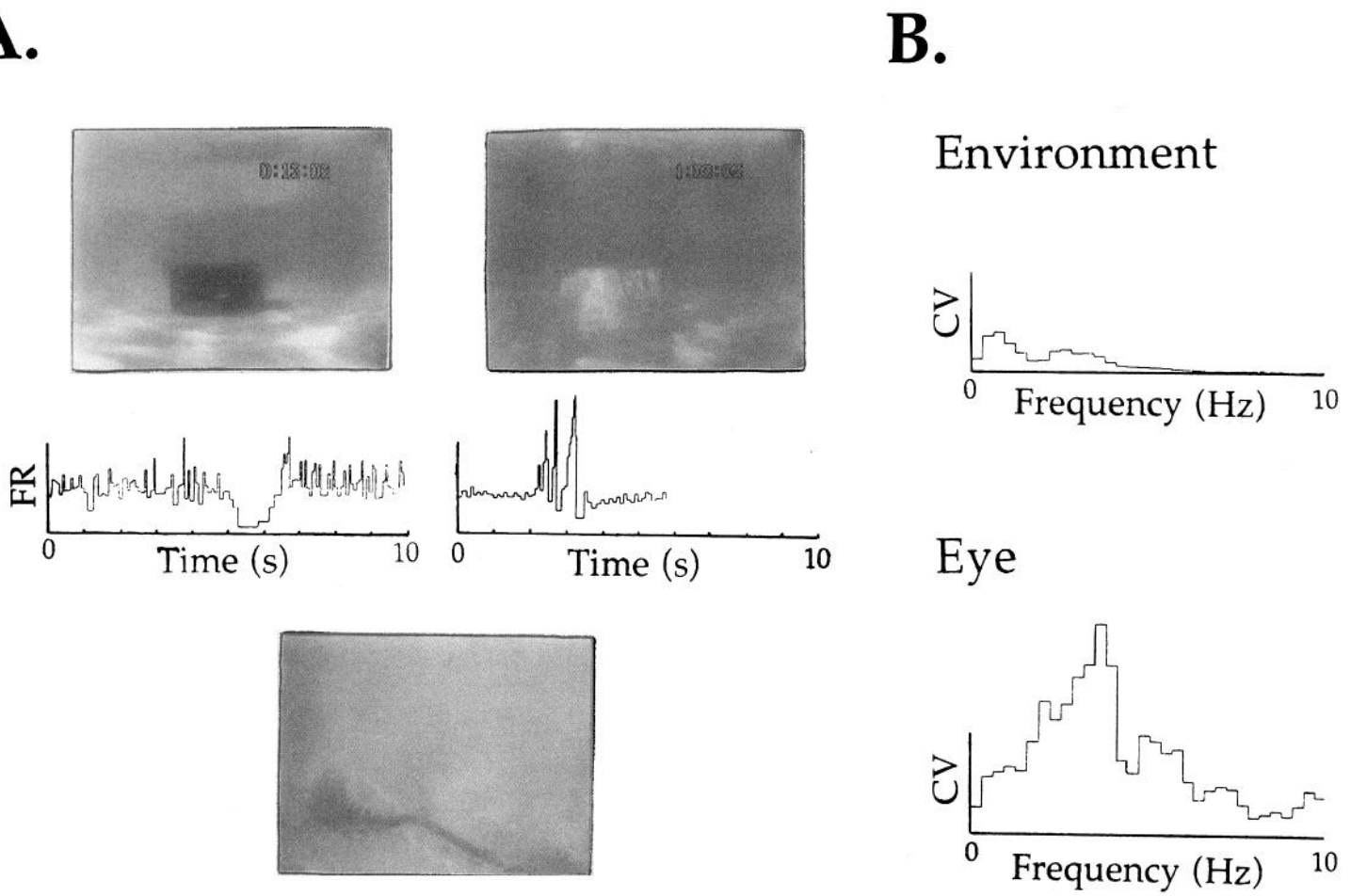

Figure 1. *(A) Top: Images of a black target, grey target, and female horseshoe crab taken with an underwater videocamera mounted on a crab and aligned in the direction of view of the recorded ommatidium. Notice the bands of light reflected from the grey target and female. Bottom: The optic nerve responses recorded from single ommatidia as the crab moved past the above targets. The average instantaneous firing rates (FR) when the ommatidium was not viewing the target were ~14 and ~11 impulses per second for the left and right experiments respectively. (B) Top: Power spectrum of the light signal reflected from the grey target. Bottom: Power spectrum of the train of optic nerve impulses recorded from a single ommatidium viewing the grey target. Notice that the eye amplifies the environmental light signals in the 2–4 Hz range while attenuating those at lower frequencies. For the purpose of comparison, the power spectra of the light signal and spike train were plotted (bin width of 0.3 Hz) on a common ordinate scale after normalization by the square of their respective means.*

fibers while a crab is moving freely in the ocean or is pulled along a track (4). We mount a small underwater camera above the eye, align it with the optic axis of the recorded unit, and record what the crab sees as it passes by an object of known contrast. We store the images and spikes on videotape for subsequent analysis with NIH-Image and other software installed in a 660AV Macintosh computer.

Black and grey targets modulate the firing rate of optic nerve fibers as they move past the eye (Fig. 1A). Despite their difference in contrast, both targets evoke sizeable responses consistent with the male's ability to find them (2). High-contrast black targets decrease the firing rate of optic nerve fibers, whereas low-contrast grey targets generally increase the firing rate in a quasi-periodic manner. Such peculiar responses to grey targets in the ocean are unlike those evoked by uniform large-field stimuli in the laboratory.

The source of the large periodic responses to low-contrast grey targets appears to be the natural fluctuations of light in the animal's underwater environment. As waves move overhead, they focus light onto the sandy bottom that sweep across the scene at a rate of 2–4 Hz. The flickering light highlights reflective surfaces against the murky background that scatters light and degrades the appearance of objects. As a result, grey targets and horseshoe crabs generate bright, quasi-periodic signals that stimulate units in synchrony with the traveling overhead waves (Fig. 1A). Because the periodic signals also move through space, the

activity of nearby receptors in the eye that see an object becomes correlated (5).

The lateral eye is highly sensitive to the flickering light from the overhead waves. A large component of the light reflected from grey targets centers at ~2–4 Hz which is the peak of the temporal transfer function of the eye (Fig. 1B; 6, 7, 8). Phototransduction mechanisms set the underlying shape of the transfer function, which two inhibitory processes then sharpen and amplify (6, 7). As a result, the power spectrum of the spike train recorded from receptors viewing the grey target grows considerably in the range of 2–4 Hz (Fig. 1B).

In this paper we show that a consideration of the natural environment of an animal can lead to a better understanding of its visual system. The *Limulus* eye appears to be adapted to a particular feature of its environment—the flickering light reflected off the carapace of a potential mate. These light signals help males detect a female irrespective of the contrast of her carapace. Frequency tuning of vision is not unique to horseshoe crabs and has been observed in many animals, such as cats (9) and humans (10). Perhaps their lighting environment also deserves a closer look.

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Flutter-Like Response in Visual Cortex of the Semi-Isolated Turtle Brain

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There is increasing evidence that high-frequency synchronization of specific, distributed neuron populations is a reliable correlate of some forms of sensory processing (1, 2) and of some attentive sensori-motor behaviors (3, 4, 5). The function and fine structure of such responses is, however, still unclear. In the turtle, every salient change in retinal input, whether due to a stimulus or self-induced by visual orienting, is correlated with widely distributed 20-Hz field potentials in its visual cortex (6). The oscillation has a spindle form and its amplitude and frequency modulates with a slow potential of <4 Hz. Although this response represents increased coherence in the 15- to 25-Hz band between spatially separated loci, recent analyses with linear electrode arrays show that, along the rostro-caudal axis, systematic phase lags are observed between most waves (7). The phase lags change from cycle to cycle and represent velocities between 0.05 and 0.3 m/s. Synchronous cycles also occur in each response. Although this response has been called an oscillation, its complex temporal and spatial features suggest that a better descriptor would be the term "flutter" (*i.e.*, a rapid, nonstationary undulation). Here I report that a flutter-like response is also observed with visual stimulation in a semi-isolated brain preparation (epipial 11-electrode linear array, 250 μm spacing). The flutter-like response is contrasted with a more regular and synchronous type of oscillation that was induced in three of the preparations with DC electrical stimulation.

The semi-isolated brain is prepared by sectioning cranial nerves V–XII and the spinal cord under anesthesia (NIH guidelines), followed by intravascular perfusion with oxygenated artificial cerebrospinal fluid (8). Although this preparation lacks the tonic somatic and visceral afference of an intact animal, moving stimuli (black bar, 20-cm distant, 8.5 cm/s) still induce a flutter response.

Figure 1 (upper traces) shows a high-amplitude segment of visually induced responses recorded, 2 mm apart, from the rostral (solid line) and caudal (dotted) poles of the visual cortex. Annotated time intervals indicate wave lags; intermediate lags are also recorded from the middle seven electrodes (data not shown). The lower superimposed oscillatory responses in Figure 1, recorded with the same electrodes, were induced after 9 s of anodal DC stimulation with a blunt surface electrode placed on the