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Introduction to Featured Article: The Lateral Eyes of Two Species of Horseshoe Crabs Are Similar, but Not Quite the Same

The lateral eye of the horseshoe crab has been a useful and productive model for vision research: it is complex enough to be interesting, yet simple enough to be understood. Extensive studies of this eye have yielded fundamental insights about how eyes of other animals, including humans, encode and process visual information. Lateral inhibition, light adaptation, and efferent-mediated circadian rhythms are just a few of the numerous physiological properties that were first uncovered through experiments on the horseshoe crab eye.

We always speak about "THE horseshoe crab," and indeed, all of the studies and advances have been made with the eye of a particular species, *Limulus polyphemus*, which inhabits the waters along the eastern shores of North and Central America. But there are actually four extant species of horseshoe crabs—one of them, *Tachypleus tridentatus*, inhabits the shores of Japan. In the following article, T. Saito and his colleagues compare the properties of the eyes of *Tachypleus* with those of the standard model, *Limulus*.

Saito *et al.* have found that, although the retinal sensitivity of the lateral eyes of *Tachypleus* and *Limulus* shows nearly the same circadian rhythms, the underlying mechanisms are different. The *Limulus* eye increases in sensitivity at night primarily by catching more photons, whereas the *Tachypleus* eye shows, in addition, a substantial increase in photoreceptor gain: *i.e.*, an enhanced response of the photoreceptors to absorbed photons.

This difference could be explained by the fact that the lateral eyes of *Tachypleus* are smaller than those of *Limulus* (see Fig. 1); moreover these smaller eyes contain fewer, smaller ommatidia that cannot catch as many photons as those of *Limulus*. So the increased response of the *Tachypleus* photoreceptors to each photon they absorb may be a compensatory adaptation. In short, what the small *Tachypleus* eye lacks in photon catch, it may make up in gain.

Once again we find that although the functions of homologous tissues and cells are similar, they are never exactly the same, and that important and unexpected lessons can be learned in even small species differences.

—Robert B. Barlow
August 1997

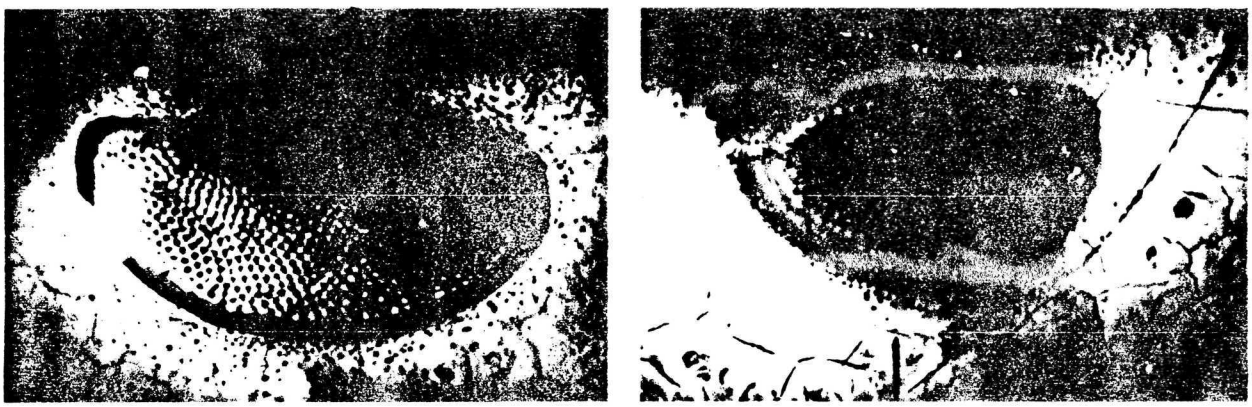

Figure 1. *The lateral eyes of* Tachypleus *(right) and* Limulus *(left). The photographs are at the same magnification; the* Limulus *eye is 1 cm wide.*

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Circadian Rhythms in the Lateral Eye of the Japanese Horseshoe Crab

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The visual system of the "American" horseshoe crab, *Limulus polyphemus,* provides a clear example of the brain modulating a sensory input. At night, efferent optic nerve fibers transmit neural signals to the lateral eyes from a circadian clock located in the brain (1). The efferent signals change the structure and function of the lateral eye (2), increasing its sensitivity to such a level that the animals can find mates underwater at night (3,4). These circadian rhythms in retinal sensitivity persist in constant darkness and can be phase shifted by exposure to light pulses at various times during the circadian cycle (5). Although four extant species of horseshoe crabs inhabit various oceans of the world (6), circadian rhythms have been detected only in *Limulus polyphemus,* which inhabits the eastern shores of North and Central America. Here we report that circadian rhythms occur in the visual system of the Japanese horseshoe crab, *Tachypleus tridentatus,* and differ in at least one property from those in *Limulus.*

Three adult *Tachypleus* males were shipped to the Marine Biological Laboratory (Woods Hole, Massachusetts) from the Horseshoe Crab Museum (Kasaoka, Okayama, Japan). We were fortunate to receive these animals because they are protected by the Japanese government as a result of the recent precipitous decline in their population. Before experimentation, both *Tachypleus* and *Limulus* were entrained to natural environmental lighting for at least five weeks in aquaria at the Marine Biological Laboratory. Following techniques described elsewhere (5), we mounted a crab on a rigid platform in an aquarium contained in a lightproof, shielded cage and, using corneal electrodes, recorded electroretinograms (ERGs) from their dark-adapted lateral eyes in response to 20-ms light flashes generated by a green LED. We measured the peak-to-peak response of the ERG, which provides a convenient measure of the summed response of photoreceptor cells to brief flashes of light (5). We measured circadian rhythms in the photoreceptor response by recording ERGs every 15 min over several days as the animal remained in constant darkness. We assessed changes in photoreceptor sensitivity by measuring the intensity-response function of the ERG response. To avoid noncircadian changes in sensitivity (5), we recorded the intensity-response functions and other circadian properties after the animals were kept in darkness >24 h.

The *Tachypleus* lateral eye exhibits a circadian rhythm as does that of *Limulus.* The ERGs recorded from both species increase in amplitude at about the time of dusk, remain high throughout the subjective night, decrease near dawn, and remain low during the subjective day. As reported elsewhere (5), the period of the endogenous rhythm in *Limulus* eye ERG ranges in duration from 22.2 to 25.5 h with a mean value of 23.9 ± 0.7 h (n = 75). Using the same measurement method (5), the periods of the circadian oscillations recorded from the eyes of three *Tachypleus* were 24.3 h, 24.3 h, and 23.5 h in duration. That of the *Limulus* eye was 24.1 h. As reported elsewhere, the phase of the circadian rhythm in *Limulus* can be advanced up to 3 h and delayed up to 4 h by exposing the animal to brief periods of illumination at various times during the circadian cycle (5). We found that exposing *Tachypleus* to 1 h of light at CT 15 (see legend for definition of CT) delayed the phase of the ERG rhythm by 4 h. In sum, the endogenous rhythm in the ERG of the *Tachypleus* eye free runs with a period of about 24 h and can be phase shifted. We conclude that, as in *Limulus,* a circadian clock is a fundamental component of the *Tachypleus* visual system.

How do changes in ERG amplitude relate to changes in photoreceptor sensitivity? Figure 1 plots the amplitudes of ERGs recorded from dark-adapted *Tachypleus* and *Limulus* eyes on log scales as a function of log light intensity. The "Day" data (open circles) for *Limulus* were fitted by eye with a smooth curve. This same curve shifted 0.9 log units to the left approximately overlays the "Night" data (filled circles), indicating that

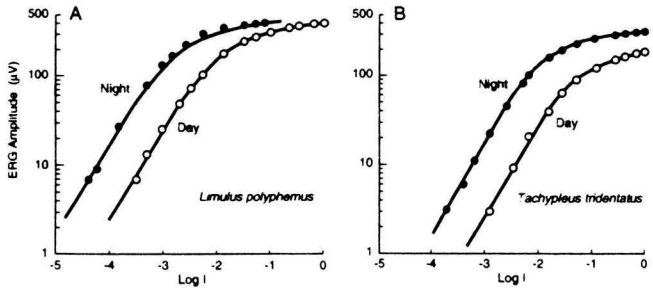

Figure 1. *Intensity-response functions for ERGs recorded from dark-adapted lateral eyes of* Tachypleus *and* Limulus. *The ERG amplitude is plotted on a log scale on the ordinate as a function of log light intensity plotted on the abscissa. The "Day" data were recorded near the middle of the second subjective day (CT 8 for* Tachypleus *and CT 7 for* Limulus*), and the "Night" data were recorded near the middle of the second subjective night (CT 17* Tachypleus *and CT 15 for* Limulus*). Retinal sensitivity had reached steady levels at each of these circadian times. The circadian time of 0 h (CT0) is defined as the animal's subjective dawn and CT 18 is the middle of its subjective night. Refer to (5) for the technique for measuring circadian times for ERG rhythms. Repeated measurements 24 h later yielded nearly identical functions, with ERGs greater than 50 μV varying <5% and those less than 50 μV varying <30%. The maximum intensity (Log I = 0) incident on the surface of the cornea was 19.5 μwatts/cm² at λ = 520 mn* (Model 262 photodiode, Graseby Corp., Orlando, FL).

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the increased sensitivity of the nighttime state caused a lateral shift of the intensity-response function. Such a shift is consistent with the idea that clock-induced structural changes in the ommatidia at night allow photoreceptor cells to absorb more photons than during the day (2). The shift of 0.9 log units corresponds to ~8-fold increase in photons absorbed at night and is consistent with previously reported results (5). We conclude that responses of *Limulus* photoreceptors to brief flashes increase at night, not because they are intrinsically more sensitive to light, but because they absorb more photons.

The intensity-response functions for the *Tachypleus* lateral eye appear similar to those for *Limulus*, but there are important differences. The "Day" and "Night" functions are similar in the sense that they can be fitted by the same curve used for the *Limulus* data in Figure 1A, indicating that increases in photoreceptor responses to increases in light intensity are about the same in both species. At low flash intensities, the ERG amplitudes for both species are linearly related to light intensity over ~2 log unit range; that is, a 10-fold increase in intensity produces about a 10-fold increase in response amplitude. One clear difference between the *Tachypleus* and *Limulus* intensity-response functions is that those of *Limulus* are shifted to the left on the intensity axis; that is, the *Limulus* eye generates significantly larger ERGs in response to the same stimulus intensities.

Another significant difference between *Tachypleus* and *Limulus* intensity-response functions is that the "Day" curve for *Tachypleus*, unlike that of *Limulus*, cannot be shifted horizontally to match the "Night" curve. However, shifting the "Day" curve 0.6 log units to the left and then 0.23 log units vertically provides a match to the "Night" curve. The 0.6 log unit horizontal shift corresponds to only a 4-fold increase in photons absorbed by photoreceptors at night; this is half that of *Limulus* photoreceptors. However, the 0.23 log unit vertical shift corresponds to a 1.7-fold increase in gain of *Tachypleus* photoreceptors. *Limulus* photoreceptors show no comparable increase in gain in their responses to brief flashes. A previous study shows that *Limulus* photoreceptors attain an increased gain at night only for steady-state responses (7).

Examination of the lateral eyes of adult *Tachypleus* males shows that they contain fewer and smaller ommatidia than the *Limulus* eye. They are also smaller than *Limulus* eyes: the *Tachypleus* and *Limulus* eyes recorded in Figure 1 contained 452 and 733 ommatidia, respectively; the ommatidia were 143 and 193 μm in diameter; and the eyes had retinal areas of 16.6 and 27.8 mm^2.

Both *Tachypleus* and *Limulus* exhibit circadian rhythms in lateral eye sensitivity at night. Although the nighttime increase in sensitivity is about the same for both species, the underlying circadian mechanisms are not. Both *Tachypleus* and *Limulus* photoreceptors catch more photons at night, but *Limulus* photoreceptors are twice as effective, exhibiting an 8-fold increase in photon catch to only a 4-fold increase for *Tachypleus*. Circadian changes in retinal structure mediate the nighttime increases in photon catch in *Limulus* (9). Because photon catch is proportional to the cross-sectional area of ommatidial corneal facets, a single *Limulus* ommatidium (0.029 mμ^2) should catch ~1.8 times more photons than a *Tachypleus* ommatidium (0.016 mμ^2). *Tachypleus* photoreceptors, on the other hand, acquire greater gain at night, generating responses to brief flashes that are about 1.7 greater in amplitude. The nighttime increase in gain of *Tachypleus* photoreceptors appears to compensate for their smaller increase in photon catch.

Higher visual sensitivity at night helps *Limulus* males find mates (3). No behavioral studies have been carried out thus far with *Tachypleus*.

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