

Literature Cited

1. Kelley, D. B. 1996. Pp. 143–176 in *Biology of Xenopus*, R. C. Tinsley, and H. R. Kobel, eds. Clarendon Press, Oxford.
2. Tobias, M., and D. B. Kelley. 1987. *J. Neurosci.* 7: 3191–3197.
3. Yamaguchi, A., and D. B. Kelley. 2000. *J. Neurosci.* 20: 1559–1567.
4. Kelley, D. B., S. Fenstemaker, P. Hannigan, and S. Shih. 1988. *J. Neurobiol.* 19: 413–429.

Reference: *Biol. Bull.* 199: 176–178. (October 2000)

Optic Nerve Responses of *Limulus* in its Natural Habitat at Night

Jillian L. Atherton¹, Matthew A. Krutky², James M. Hit³, Frederick A. Dodge, and Robert B. Barlow
(Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

What information does the eye send to the brain when an animal sees? We are exploring this question with the relatively simple visual system of the horseshoe crab, *Limulus polyphemus*. By combining cell-based computational models of the retina with single-cell electrophysiology, we have examined the optic nerve code underlying *Limulus* vision during the day in the animal's natural habitat (1).

Field studies during the animals' mating season show that male horseshoe crabs use vision to find mates and do so about equally well day and night (2). We attribute their remarkable nighttime vision to a circadian modulation of the sensitivity of their lateral eyes (3). At night, efferent optic nerve fibers carry signals from the circadian clock in the animal's brain to its eyes, increasing nighttime retinal sensitivity as much as 1,000,000 times. The increased sensitivity nearly compensates for the average 1,000,000-fold decrease in ambient light intensity after sundown. Here we investigate optic nerve activity recorded from the animal in its habitat, both day and night, with emphasis on signals that convey information about potential mates at night.

A convenient method for recording what the horseshoe crab sees underwater is to mount a miniature video camera, "CrabCam," on the animal (1). This documents the crab's eye view during the day, but not at night when light levels fall below the camera's sensitivity. To investigate the optic nerve responses of an animal in its natural habitat at night, we used a repetitive, artificial stimulus that simulates the movement of a potential mate within the animal's visual field. The stimulus is a rotating grey cylinder (30 cm in diameter, 15 cm in height) with a black sector (30 cm in width) that simulates the size of a typical female horseshoe crab. The cylinder was placed 1 m from the crab and rotated by hand (4–8 rpm), moving the black sector horizontally at 7–13 cm/s, which simulates the average speed of a horseshoe crab. Our strategy is as follows: first, during the day and under water, we record the optic nerve response of a stationary crab to the rotating cylinder while simultaneously videotaping the eye's input with the shell-mounted CrabCam. This allows us to document the visual input when ambient light levels are sufficient for CrabCam operation. We then leave the animal and cylinder in place underwater until after sundown, when we repeat the experiment carried out during the day, but of course without the Crabcam. This method allows us to

record the response of the eye to a known visual stimulus at night in the animal's natural habitat.

We recorded the response of single optic nerve fibers following a procedure developed in this laboratory (1). In brief, we trephine a hole in the carapace about 2 cm anterior to the right lateral eye, expose the optic nerve trunk, and draw it into a chamber that is then attached to the carapace. We tease away a single active optic nerve fiber corresponding to a single ommatidium and pull it into a micro-suction electrode. The chamber is sealed to make it water tight, and then a small point light source is used to locate the optic axis of the recorded ommatidium. We then mount the CrabCam on the carapace and align it in the direction of view of the recorded ommatidium. The CrabCam (72° by 54° field of view) encompasses about a quarter of the hemispheric view of the lateral eye, which is seen by about 250 ommatidia, each viewing about a 6° region during the day and about a 12° at night (4). The animal is firmly attached to a weighted platform that is placed on the sandy bottom of the animal's habitat and oriented so that the optic axis of the recorded ommatidium intersects the axis of rotation of the cylinder located about 1 m away. Experiments were carried out at depths of 0.5–1 m in estuaries near the Marine Biological Laboratory in Massachusetts. Signals from the microsuction electrode and the CrabCam are led *via* shielded cables (13 m in length) to a portable camcorder on shore or in a nearby boat.

Figure 1 (right) shows two video frames taken with the CrabCam during the day. These frames show the rotating cylinder at two distances (.87 m and 1 m) from the horseshoe crab. On the left are 14 s samples ("Day") of the responses of a single optic nerve fiber to rotations of the cylinder at the two distances from the crab. The video frames were taken 6 s after the beginning of the response records (arrows), when the grey-black edge of the cylinder began to enter the field of view of the recorded ommatidium from the right. In both cases, the black sector evoked clear decreases in response, with the larger decrease recorded when the cylinder was closer to the animal and water turbidity was minimal. The top "Day" response was recorded at 1630 h and the second was recorded at 1800 h. In both cases, the setup was bathed in direct sunlight. After the second record was recorded, the animal and cylinder were left underwater as nightfall approached.

The experiment was then repeated several hours later after sundown (2030–2100 h) but without CrabCam recordings because of insufficient lighting. Figure 1 ("Night") displays the responses of the same single optic nerve fiber to nine sequential rotations of the cylinder (thin black traces). The heavy black trace gives the average of the nine responses. Note that the individual responses

¹ Allegheny College, Meadville, PA.

² Syracuse University, Syracuse, NY.

³ Upstate Medical University, Syracuse, NY.

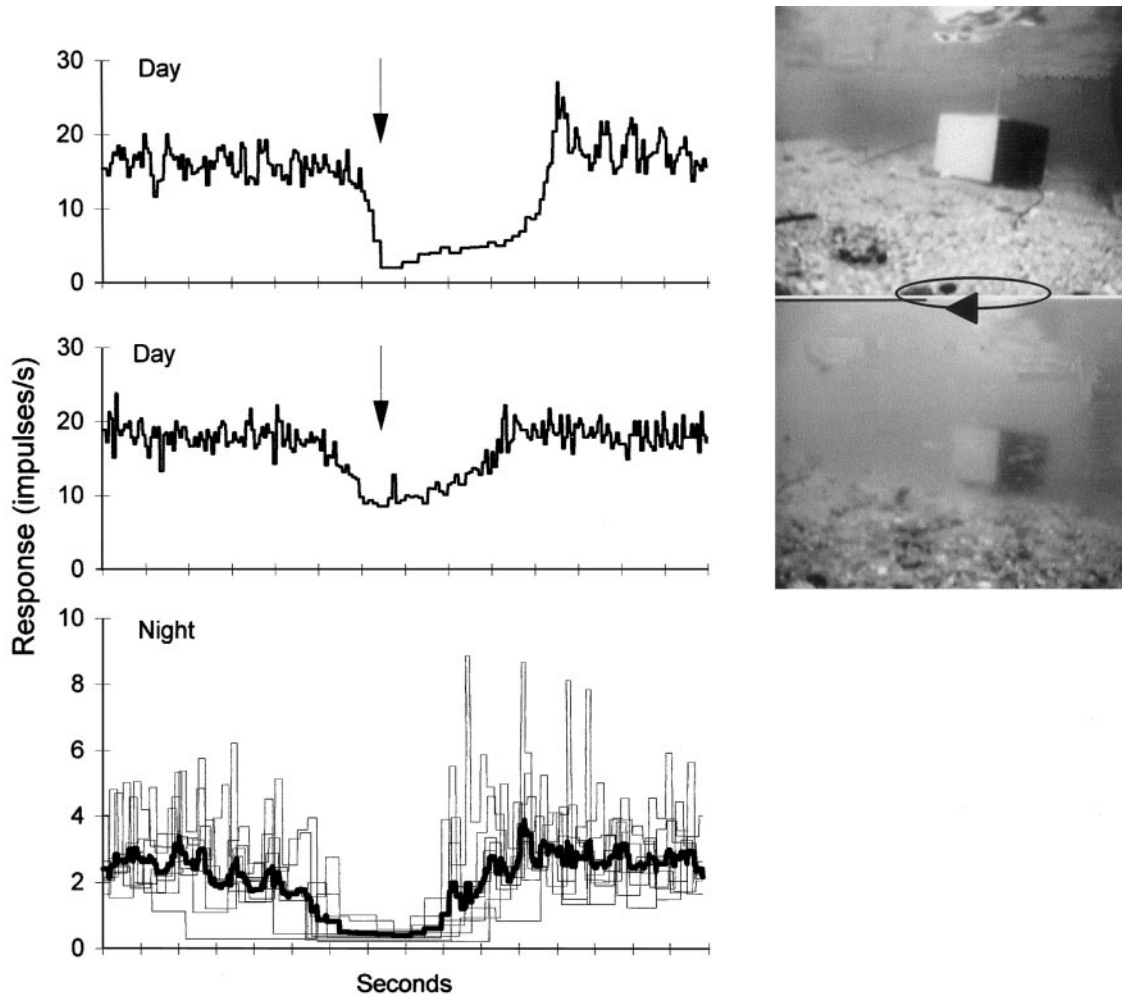


Figure 1. The responses of a single ommatidium to an underwater rotating visual stimulus. The records on the left plot the instantaneous frequency (reciprocal of the interval between adjacent optic nerve impulses) as a function of the rotation of the stimulus shown on the right. The speed of rotation is approximately the same for all records. The visual stimulus is aligned so that the optic axis of the recorded ommatidium is centered on the rotating cylinder with its field of view lying within the black sector. At a distance of 1 m the black sector intercepts the optic axis of about 9 ommatidia. The “Day” responses were recorded between 1630 and 1800 h, and the “Night” responses were recorded between 2030 and 2100 h. The top video frame was taken in the clear water of Great Harbor, Woods Hole, Massachusetts, where the grey/black sectors of the cylinder had a contrast of 69% [contrast = $(L_{Grey} - L_{Black}) / (L_{Grey} + L_{Black})$]. The second video frame was taken near Stoney Beach, Woods Hole, where turbid water reduced the contrast of the grey/black sectors to 26%. Arrows indicate the times at which the underwater scenes to the right were videotaped. At these times the black sector begins to enter the field of view of the recorded ommatidium, reducing its response rate. The “Day” records are responses to a single rotation of the cylinder in a right to left direction (loop with arrow). The “Night” records show responses to nine consecutive rotations of the cylinder (thin black traces; period of rotation ~ 16 s) and their average (thick black trace). The peaks and valleys of the thin black traces reflect the highly variable rate of discharge of the single optic nerve fiber under low nighttime levels of illumination.

are highly variable relative to those recorded during the day, and that the average response rate to the grey sector is about 3 impulses/s which is 6-fold lower than the mean daytime response rate of about 18 impulses/s (middle trace). We attribute the highly variable response rates to random photon events occurring at the very low nighttime levels of illumination. The nighttime sky during this experiment was heavily overcast and lacked moonlight. From radiometric measurements we estimate that ambient light decreased by about 10^6 to 10^7 relative to daytime levels. The circadian increase in lateral eye sensitivity cited above nearly compensates for such large reductions in ambient lighting. Experiments in the laboratory (R. Barlow and F. Dodge, unpub. obs.)

indicate that the average response to the grey sector of ~ 3 impulses/s is about 50% lower than expected for the low nighttime levels of illumination. The surgery performed to isolate the single optic nerve fiber may have partially damaged the fragile efferent fibers that carry the circadian clock’s signal from the brain to the eye; as a consequence, the lateral eye may not have received the normal efferent input and thus the retina may not have shifted completely to its fully sensitive nighttime state. Nevertheless the eye’s circadian increase in sensitivity was sufficient to detect the rotating black sector of the cylinder, which mimics a moving mate.

Computational analyses of visual processing in the *Limulus* brain indicate that retinal inputs may sum at the first synaptic level

(5). Spatial summation across a matrix of 5–10 ommatidia significantly increases the signal-to-noise properties of responses recorded at night. Indeed summing seven sequential optic nerve responses to the rotating cylinder yielded a relatively noise-free response.

These experiments represent our first attempts to analyze lateral-eye responses of *Limulus* at night in the animal's natural habitat. The use of a periodic stimulus obviated the need for video documentation of the visual stimulus, which is not feasible under nighttime lighting conditions. With this technique, we successfully recorded visual responses in the animal's habitat and found that the lateral eye transmits information to the brain about mate-like objects at night under dark overcast skies. Under such conditions *Limulus* could see what we could not.

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Literature Cited

1. Passaglia, C., F. Dodge, E. Herzog, S. Jackson, and R. Barlow. 1997. *Proc. Natl. Acad. Sci.* **94**: 12649–12654.
2. Barlow, R. B., L. C. Ireland, and L. Kass. 1982. *Nature* **296**: 65–66.
3. Barlow, R. B. 1983. *J. Neuroscience* **3**: 856–870.
4. Barlow, R. B., S. C. Chamberlain, and S. C. Levinson. 1980. *Science* **210**: 1037–1039.
5. Hitt, J., C. Passaglia, F. Dodge, and R. Barlow. 2000. *Ninth Annual Computational Neuroscience Meeting*. Brugge, Belgium. p. 75.

Reference: *Biol. Bull.* **199**: 178–180. (October 2000)

Do the Properties of Underwater Lighting Influence the Visually Guided Behavior of *Limulus*?

Matthew A. Krutky¹, Jillian L. Atherton², Spence Smith, Frederick A. Dodge and Robert B. Barlow
(Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

In the spring, horseshoe crabs, *Limulus polyphemus*, migrate to the water's edge along the East coast of the United States to pair off and build nests (1). As they enter a nesting area, males use their lateral eyes to locate mates both day and night (2). They approach females and objects resembling them, such as rocks, patches of seaweed, or mate-like objects. What does a male see in a female? Her size and contrast are two important factors. Males are attracted to objects that approximate the size of females. They orient toward mate-like objects at distances up to 1.2 meters, detecting higher contrast objects better than lower contrast ones (3). How do the properties of underwater lighting in the animal's natural habitat influence whether a crab finds a mate during the day or at night? The approximate 1,000,000-fold reduction in ambient lighting after sundown has no appreciable effect. Their remarkable visual performance results in part from a circadian increase in lateral eye sensitivity of as much as 1,000,000 times at night (4). In this paper we consider another property of the animal's underwater habitat, termed "strobic lighting."

In the shallow waters of nesting areas, overhead waves act like lenses, creating moving beams of sun- and moonlight that reflect off the sandy bottom and submerged objects. On average, the peak intensity of these beams is about three times that of ambient illumination. The fields of view of single ommatidia are wider than the moving beams of light. Because ommatidia sum the illumination within their field of view, the amplitude of modulation of the light beams reaching the underlying photoreceptor cells decreases to about 70% contrast. The strobic illumination by the beams strongly modulates the firing rate of an ommatidium, with peak firing rates reaching three times the mean (5). Such strobic illumination might be expected to enhance the detectability of under-

water objects, such as potential mates. Indeed, an earlier study suggested that strobic conditions enhance the visibility of low contrast mate-like objects, and that without strobing, *Limulus* is attracted to higher contrast objects (6). We have further explored the influence of strobic lighting by carrying out more field studies and combining the results with those collected over the past five years.

We investigated the visual performance of *Limulus* during their springtime mating seasons at Mashnee Dike, Bourne, and North Monomoy Island, Chatham, both located in Massachusetts. Our study and those of previous years were carried out day and night under various weather conditions ranging from dense cloud cover to clear skies, yielding 10^5 to 10^7 -fold diurnal changes in the intensity of ambient illumination, with an average change of about 10^6 . In all our studies, a modified two-alternative forced choice technique adapted from human psychophysics was used (7). As shown in Figure 1, we placed on the sandy bottom a clear Plexiglas chute with a funnel at one end and a narrow chute at the other. Crabs entered the funnel and, upon exiting the chute, were presented with the choice of a black or gray female-sized object; these were located 1 m from the exit of the chute and 1 m from each other, creating an equilateral triangle. The objects were either a hemisphere (diameter of 0.3 m) or a cylinder (height of 0.15 m; diameter of 0.3 m), both approximating the size of an adult female horseshoe crab. The objects were switched periodically during an observation period to avoid any effects of directional bias in behavior. Their black and gray tones represent the greatest range of contrast of the female carapace (8). The black object has a negative contrast of 37% against the background of sand and seawater, and the gray object has a positive contrast of 35%. Animals exiting the chute either approached and contacted one of the two targets or swam by them. In 1999 and 2000, about 60% of animals exiting the chute did not approach or contact either submerged object. The animals passing by both objects were not recorded in the years

¹ Syracuse University, Syracuse, New York.

² Allegheny College, Meadville, Pennsylvania.