

in steady state response in the absence of inhibition is one piece of evidence. The other is the hyperpolarization of the membrane potential following the first action potential in the second trace; it represents the impulse-initiated IPSP of self inhibition. Such hyperpolarizations are minimal in trace 3 when the effects of cimetidine are maximal.

The relatively high concentrations of histamine and the long periods of treatment needed to mimic the effects of lateral inhibition are not easily explained. Contributing factors for the slow responses may include replacement volume of the recording chamber, and diffusion barriers caused by retinal tissue clogged with clotted blood. Regarding concentration, Hardie reported that 0.2 to 0.5 mM histamine was required to exert physiological effects in the fly eye (6), which is a much smaller piece of tissue than the *Limulus* eye.

The biochemical and immunocytochemical work carried out by Battelle and her colleagues shows that histamine is a major biogenic amine in the *Limulus* visual system. Histamine antibody intensely labelled cell bodies and axon collaterals of eccentric cells in the lateral eye and eccentric cell projections in the brain. Photoreceptor (retinular) cells were also labelled, but much less intensely than eccentric cells (9). Retinular cells have no known role in lateral inhibition. Rather, they transmit light-evoked current electronically to eccentric cells which then medi-

ate lateral inhibition with their neighbors (3). These results, combined with our studies with histamine and cimetidine, strongly support histamine as the transmitter of lateral inhibition in the *Limulus* lateral eye.

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Visual Performance of Horseshoe Crabs: Role of Underwater Lighting

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For millennia, horseshoe crabs have migrated toward shore during the spring to build nests and deposit eggs. Behavioral studies show that vision plays an important role in their mating behavior. Male crabs use their lateral eyes to locate mates (1). Female crabs appear to use their eyes to avoid nesting crabs (2). The visually guided behavior of male crabs is particularly striking and has been the basis for detailed analyses of the visual performance of the animal (2, 3).

Male crabs swimming along a mating beach turn and approach horseshoe crabs and objects resembling them, such as rocks, patches of seaweed, or cylindrical targets. The animal's ability to see such objects was evaluated in previous studies by measuring the distances at which males orient to targets of different size and contrast (2, 3). These studies showed that most crabs turn to hit black or grey crab-size targets (9–12" in diameter) at distances of 0.4 to 1.2 meters. Moreover, the decrease in probability of target detection with distance was nearly the same day and night. Horseshoe crabs can thus detect objects having the range of contrasts of their carapace almost equally well under a variety of lighting conditions.

We further explored the ability of horseshoe crabs to see behaviorally relevant objects using the method of two-alternative forced choice. Figure 1 illustrates the experimental setup.

During mating seasons 1995–1997, we anchored a clear Plexiglas chute to the sandy bottoms of Mashnee Dike, Mashnee, and Stage Harbor, Chatham, both in Massachusetts. The flanges of the chute guided animals toward its narrow passageway, which forced them to exit straight ahead. Upon leaving the chute, they encountered black (B) and grey (G) targets of equal size, either 9" or 12" in diameter, positioned 1 m from the exit and from each other, forming an inverted isosceles triangle. Crabs thus had the choice of turning left, right, or proceeding straight ahead. The crabs that turned either hit one of the targets, or missed to the left or right of it. We tallied the number of crabs whose behavior fell into these categories, excluding those that proceeded straight. The hit tallies of each experiment are given in Table I.

We analyzed the results by testing the null hypothesis that the black and grey targets are equally visible to horseshoe crabs. Equal numbers would then hit the two targets if animals turn left or right without preference. The probability $P(X \geq n)$ that n or more of N crabs respond in manner X is thus given by a binomial distribution $\beta(n, N, p)$, with p equal to 0.50. To control for directional biases caused by underwater currents, nonuniformities in the sand, shadows, and other unknown factors, we switched the locations of the two targets periodically during an

experiment. Experiments showing a statistically significant ($P < 0.05$) bias for turning left or right independent of target contrast are not included in the table. Analyzing the overall tallies, we find that equal numbers of crabs hit the black and grey targets in this visual task (202 black hits out of 395 total hits for $P < 0.34$). Equal numbers of crabs also missed the two targets (191 grey misses out of 380 total misses for $P < 0.48$) either because the animals did not see them or were unmotivated to approach them. These results are consistent with previous behavioral studies showing that horseshoe crabs can see black and grey targets almost equally well (3).

We next sorted the hit tallies into two categories according to the underwater lighting environment. We defined one category as "strobic," which included data collected on sunny days or moonlit nights when overhead waves created moving beams of light that reflected off the sandy bottom and the submerged targets (4, 5). We defined the other category as "nonstrobic," which included data collected under overcast skies or in the absence of overhead ripples or waves. We noted the strobic or nonstrobic lighting conditions as each crab left the chute because these conditions can change during an experiment, especially on partly cloudy days. The rightmost block of columns in Table I separate the hits of black and grey targets into these two categories. The columns show that greater numbers of crabs hit the grey target under strobic conditions (115 grey hits out of 199 total hits for $P < 0.017$) and the black target under nonstrobic conditions (118 black hits out of 196 total hits for $P < 0.003$). The animals' preference for the black target under nonstrobic conditions indicates that wave-induced glitter helps horseshoe crabs detect the grey target. The reversal of their target preference under strobic conditions was unexpected and may reflect a natural bias of horseshoe crabs for bright flickering objects. Indeed, their lateral eyes are maximally sensitive to light flickering at the characteristic frequencies (2–6 Hz) of wave-induced glitter in their natural habitat (6). Another possi-

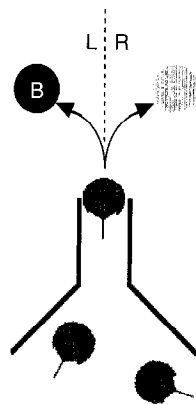


Figure 1. Horseshoe crabs swimming near the water's edge were guided into a transparent Plexiglas chute weighted to the sandy bottom. Upon leaving the chute, horseshoe crabs encountered black (B) and grey (G) cylindrical targets of the same diameter (either 9" or 12"). The targets were positioned 1 m from the exit of the chute and from each other. Crabs could thus turn either left (L) or right (R). After turning, they either hit or missed one of the targets which were switched periodically during an experiment.

Table I

Number of crabs that hit the black (B) and grey (G) targets in each experiment during the 1995–97 mating seasons. Experiments showing a statistically significant bias for turning left or right independent of target contrast are not included. In the rightmost block of columns, hits were sorted into "strobic" and "nonstrobic" categories based on the underwater lighting conditions during an experiment. These categories indicate that wave-induced underwater glitter was present (strobic) or absent (nonstrobic) as crabs exited the chute

Exp	Time	Black B	Grey G	Strobic		Nonstrobic	
				B	G	B	G
060495	night	13	1			13	1
060595	night	13	10	13	10		
061295	dusk	5	4			5	4
052896	day	1	1	1	1		
053096	dusk	9	5			9	5
053196	dusk	6	9	6	9		
060196	night	6	20	6	20		
060296	night	8	7	8	7		
060396	night	10	11	2	9	8	2
060396	day	9	6			9	6
060596	day	11	9	4	8	7	1
060696	day	11	16	11	16		
060896	day	9	7			9	7
060996	day	19	18			19	18
052997	day	0	4			0	4
053197	day	17	6			17	6
060197	dusk	11	7			11	7
060297	dusk	1	5			1	5
061597	night	17	15	17	15		
061697	night	10	12			10	12
062397	night	16	20	16	20		
overall tallies		202	193	84	115	118	78

bility is that the bright light reflected off the grey target gives it more contrast than the black one.

We conclude that the visual performance of horseshoe crabs depends strongly on underwater lighting. Strobic light created by waves on sunny days and moonlit nights enhances the visibility of low-contrast objects that are otherwise difficult for crabs to see. More animals are thus attracted to high-contrast objects in the absence of wave-induced flicker and to low-contrast ones in its presence. The visual system of horseshoe crabs and other marine animals appears highly sensitive to this prominent feature of their visual environment (4–7). Strobic underwater lighting may play an important role in their visual ecology as well.

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Squids (*Loligo pealei* and *Euprymna scolopes*) Can Exhibit Polarized Light Patterns Produced by Their Skin

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Cephalopods can see polarized light (1–3) and may use this sensory capacity for object detection and recognition (1, 4). Only recently was it discovered that the light reflected from the skin of cuttlefish, *Sepia officinalis* (5), and possibly octopus (6) is partially linearly polarized (termed here- “polarized reflection”), generating specific optical patterns (termed here- “polarized patterns”). In cuttlefish, the polarized patterns have been suggested to be produced by dermal reflecting cells such as those found in the “Pink iridophore arm stripes” (7). Cuttlefish are diurnal animals that interact within small groups, and the polarized patterns may be playing a part in intraspecific communication (5). Therefore, we were interested in examining the polarized patterns of other cephalopod species, namely *Euprymna scolopes* and *Loligo pealei*, that possess similar skin structures, but have a different social structure and time of activity.

The Hawaiian sepiolid *Euprymna scolopes* is a predominantly nocturnal, solitary predator (7, 8). Mating occurs at night, and apart from this brief event, the animals do not seem to engage in complex social interactions. The long-finned squid *Loligo pealei* is active day and night. It exhibits a complex range of social interactions, accompanied by various body pattern displays (7).

Sexually mature animals were examined with an imaging polarimeter capable of measuring the intensity, partial polarization (also termed “percent polarization”), and direction of linear polarization (also termed “plane of polarization,” “orientation of polarization” and “e-vector orientation”) at each pixel in an image (9, 10). In summary, the polarimeter consists of two twisted nematic liquid crystals (TNLCs; provided by the Liquid Crystals Institute, Kent State Univ., Ohio) placed in series with a linear polarizing filter (Polaroid, HN38S, serving as an analyzer) fixed at the horizontal (0°) orientation. When TNLCs are relaxed, they rotate the direction of polarization of light by a predetermined angle. When electric current is applied, the molecules within the crystals reorient and no longer rotate the light’s direction of polarization. By using one TNLC set to produce a 90° rotation, and a second for a 45° rotation, the direction of polarization of the incoming light is rotated by 0°, 45°, and 90°. The overall effect is similar to rotating the analyzer to these positions. Additional technical details can be found in references 9–12. Each transmitted image is recorded with a Hi-

8 video recorder. Images (single fields) taken from the green video channel are digitized by a frame grabber and sent to a computer where they are analyzed pixel-by-pixel. From consecutive images collected at three settings of the TNLCs, we calculate the partial polarization and direction of polarization. The sensor is checked and calibrated by examining a radial polarization filter (Oriel # 25328) in air and submerged in the experimental tank. Animals were examined through a glass window, verified not to create polarization aberrations from various angles and under different illumination conditions. In all cases, the sensor was set so that the TNLCs were parallel to the glass window.

Both *E. scolopes* and *L. pealei* exhibited polarized reflection during all measurements (Fig. 1). Polarized reflection with a partial linear polarization of up to 0.8 (80%, Fig. 1B) was measured from all body parts of *E. scolopes*, but no specific pattern could be identified (Figs. 1B, C). This polarized reflection did not change with the animal’s behavior. *L. pealei* presented polarized reflection at the location of the iridescent stripes in the center of its arms (Figs. 1E, F). Partial polarization here reached 0.75 (Fig. 1E). The direction of polarization was predominately horizontal (0°; Fig. 1F); however, in several cases, the direction of this polarized reflection changed by up to 30° within 1 s, without the animal exhibiting any movement or change in coloration, nor any detectable changes in the light regime in the tank. Unlike cuttlefish (5), the polarized reflection of *L. pealei* varied between the arms. In general, when a squid was sitting calmly on the bottom, polarization was strongest from the first pair of arms. While the squid was in the head-down posture, all arms showed a polarized pattern, with maximal polarized reflection recorded from the third pair. We do not know whether these changes arise from structural changes in the skin structures controlling the polarized reflection, or from specific directionality in the polarized reflection. Other areas on the squid that reflected partially linearly polarized light were the “Dorsal iridophore splotches” on the mantle, but this reflection had partial polarization of less than 0.5.

Like other cephalopods, polarized reflections are part of the body patterning repertoire of *L. pealei* and *E. scolopes*. Like cuttlefish, the polarized reflections of the social long-finned squid *L. pealei* consist of distinctive patterns that can change rapidly. The solitary *E. scolopes* exhibits polarized reflection