between every other field, calculating the angular velocity of the eye, and subtracting the drum velocity.

Figure 1A shows the ERG amplitude in μV as a function of LED flash frequency (left-hand axis). The ERG amplitude modulation became vanishingly small at 32 Hz and 50 Hz, for dark- (dashed line) and light- (solid line) adapted animals, respectively. Figure 1B shows the ERG amplitude as a function of flash duration. The value at which the response saturates is about 65 ms in a dark-adapted crab. This estimate of T_s is corroborated by the frequency response data in Figure 1A. If we Fourier-invert the frequency data to get the impulse response, we find that this is well fitted by a 2nd order transfer function, which corresponds to an impulse function of the form $t(\exp(-t/\tau))$ (see Fig. 1A inset). From this we can estimate a time constant (τ) of about 14 and 21 ms in light- and dark-adapted crabs, respectively. The great majority of the energy in the impulse response occurs within 3τ, giving an estimate for dark adapted animals of 63 ms. This value, along with $\Delta\phi_h \cong \zeta = 2.1°$, means that the crab should not lose spatial resolution with a retinal slip velocity $\leq$ 33.3°/s. Figure 1C shows a representative plot of retinal slip in a crab walking inside a drum (upper trace). The peaks in slip velocity represent saccadic eye movements, in which the animal moves its eyes to a new position as it turns. Clearly, the retinal slip that is tolerated rarely exceeds 3°/s, even during fast locomotory movements, indicated in the lower trace (right-hand axis).

The flicker fusion value is indicative of the retinal slip speed at which *all* spatial information is lost, whereas 33.3°/s is the velocity at which spatial information *begins* to be lost. This far exceeds observed retinal slip speeds, suggesting some other reason for image stability unrelated to spatial acuity. Indeed, this maximum slip speed limit is only approached in animals that actively scan the environment with their eyes, primarily to enlarge the visual field [spiders, planktonic mollusks and crustaceans (6), but see stomatopods (2)]. As for fiddler crabs, which do not scan, we can only speculate that the image on the retina must be extremely stable for successful motion vision, especially the detection of optic flow; image slip would pollute the optic flow pattern, and reduce the information that might be gained therefrom.

This project was funded by the Grass Foundation, and by grants to R. Barlow from NSF and NIH.

Literature Cited

1. **Srinivasan, M. V., and G. Bernard. 1975.** *Vision Res.* **15:** 515–525.
2. **Land, M. F., J. N. Marshall, D. Brownless, and T. W. Cronin. 1990.** *J. Comp. Physiol.* **167:** 155–166.
3. **Land, M. F., and J. E. Layne. 1995.** *J. Comp. Physiol.* **177:** 81–90.
4. **Land, M. F. 1981.** *Handbook of Sensory Physiology,* vol. VII/8B. H. Autrum, ed. Springer-Verlag, Heidelberg.
5. **Fuortes, M. G. F., and A. L. Hodgkin. 1964.** *J. Physiol.* **172:** 239–263.
6. **Land, M. F. 1995.** *Eye Movement Research.* J. M. Findlay *et al.*, eds. Elsevier Science B. V.

Reference: *Biol. Bull.* **193:** 203–205. (October, 1997)

Histamine: Putative Transmitter for Lateral Inhibition in *Limulus* Eye

Karen M. Stewart, Darrell M. Porcello, Maireade E. McSweeney, Takehiko Saito, Christopher L. Passaglia, Frederick A. Dodge, and Robert B. Barlow
(Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

Lateral inhibition is an integrative mechanism found in many nervous systems. In retinas, it enhances borders and edges in the visual field (1). Since its discovery in the lateral eye of the horseshoe crab, *Limulus polyphemus,* by H. Keffer Hartline and colleagues in 1952 (2), lateral inhibition has been studied extensively (3). It is now the foundation of a comprehensive model of retinal function (4). One question, however, remains unanswered. What is the neurotransmitter that mediates lateral inhibition in the *Limulus* eye? Histamine has been implicated as a photoreceptor neurotransmitter in Uniramia and Crustacea, two of the major groups of arthropods (5, 6, 7). In preparations that study the postsynaptic effects of histamine, histamine has been shown to be inhibitory (7, 8). Biochemical studies show that histamine and the enzymes that synthesize it are present in the *Limulus* retina (9), but provide no physiological evidence that histamine is the transmitter of lateral inhibition. In this paper, we report electrophysiological and pharmacological studies that test the role of histamine in lateral inhibition in the *Limulus* eye.

In the first experiment, we tested whether histamine mimics the action of lateral inhibition on optic nerve activity generated by eccentric cells in individual ommatidia. To gain access to eccentric cells, we excised the lateral eye and sectioned it into three or four slices. One slice was placed in a Lucite perfusion chamber (volume 0.5 ml). A glass microelectrode filled with 3 *M* KCl (25 MΩ) was connected to a DC bridge amplifier (Electronics Shop, Rockefeller University) and advanced into an ommatidium using a Burleigh Inchworm (Burleigh Instruments, Fishers, NY) attached to a manipulator. A fiber optic light pipe was aligned with the optical axis of the ommatidium for maximal stimulation. In this manner, we recorded intracellular responses of eccentric cells to 8-s light flashes delivered every 5 min. After a series of control runs in which a modified *Limulus* Ringer was perfused over the slice (rate: 1 ml/min), a 1 m*M* solution of histamine in saline was applied to the slice at the same rate.

Figure 1A shows responses of an eccentric cell before treatment, during the application of histamine, and after washout

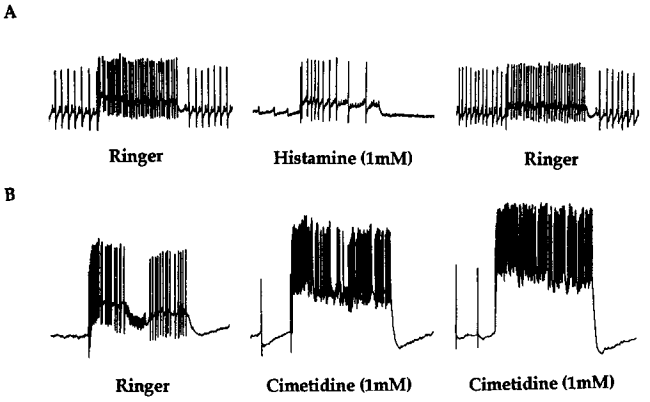

Figure 1. *The action of histamine and cimetidine on eccentric cells of the* Limulus *lateral eye. (A) Eighty minutes of perfusion with 1 m*M *histamine reduced both the spontaneous and light driven response of the eccentric cell (57 mV resting potential). Washout with modified* Limulus *Ringer reversed the effect of histamine. The amplitude of the largest spontaneously evoked action potential was 45 mV, but appears in these traces as 18 mV because of the low pass characteristics of the Gould Brush recorder. Note that the intracellular recordings contain action potentials of different amplitudes. The smaller one is generated by a second eccentric cell in the same ommatidium and is detectable because of partial coupling among ommatidial cells. The existence of multiple eccentric cells in single ommatidia is not uncommon and can be as high as 20% (R. Barlow, unpub. obs.). (B) Twenty minutes of perfusion with 1 m*M *cimetidine reduced the action of lateral inhibition and forty minutes of perfusion abolished it. Forty-five minutes of Ringer washout partially reversed the cimetidine effect (data not shown). Artifacts caused by antidromic shock delivered to the optic nerve trunk are detectable in the middle of the first trace. The amplitude of the spontaneously evoked action potential in the second trace was 65 mV, but was attenuated to 24 mV by the pen recorder. All traces are 18 s in duration.*

with modified *Limulus* Ringer. The cell fired large (45 mV) action potentials in response to dim background illumination (~1.8 impulses/s) before the light flash. A second eccentric cell located in the same ommatidium generated much smaller action potentials (~2.5 mV) with the same firing rate (see legend). While perfusing the slice with modified *Limulus* Ringer (first trace), the light flash evoked a burst of ~7 action potentials at the leading edge of the generator potential, followed by 34 action potentials yielding a steady-state firing rate of ~4.5 impulses/s for the last 4 s of the response. The second trace was recorded after 80 min of perfusion with histamine and shows its maximal effect on this cell. Notice that the cell's activity decreased both in response to the dim background and to the light flash. Only 2 spikes fired at flash onset, followed by 9 spikes for a steady rate of ~0.75 impulses/s. Thus the response rate of the cell was reduced by > 80%, or 3.75 impulses/s. Note that the response of the second eccentric cell to background illumination declined by > 50%, but we could not assess its response to the light flash because of masking by the larger action potential. Ringer washout reversed the action of histamine. The third trace shows that after a 55 minute washout, the light flash evoked 33 action potentials with a steady state rate of ~4.0 impulses/s. The response rate of the cell thus returned to ~90% of its original level. Reapplication of histamine decreased the steady state response rate of the cell again to ~0.75 impulses/s after 180 min of perfusion (data not shown). As before, the number of action potentials rose after washing out the histamine. In general, the initial effects of histamine were detected about 20 min after application, while maximal effects occurred about 50 min later. In all experiments in which histamine was tested (n = 3), it reduced the optic nerve activity generated by eccentric cells.

In a second type of experiment, we tested whether cimetidine, a blocker of histaminergic receptors, could reduce the effects of lateral inhibition on eccentric cell responses. To do this, we excised the lateral eye from the animal in such a way as to retain ~0.5 cm of the optic nerve attached to the back of the eye. We gained access to ommatidia by slicing away the ventral half of the eye without cutting the optic nerve trunk. The eye was then mounted in a chamber, and the optic nerve was pulled into a suction electrode. Following the procedures described above, we impaled single eccentric cells and recorded their responses to light with and without lateral inhibition exerted by antidromic stimulation of the optic nerve trunk. Current pulses applied through the suction electrode exerted massive lateral inhibitory effects in the cell impaled by our electrode. After control runs in which the eye was perfused with modified *Limulus* Ringer, 1 m*M* cimetidine was applied to the preparation.

Figure 1B shows the response of an eccentric cell to both light and lateral inhibition before and during the application of cimetidine. The light flash was 10 seconds in duration. Four seconds after light onset, optic nerve shock was delivered for 2 seconds at the rate of 10 shocks/s. As can be seen in the first trace, the antidromic stimulation completely inhibited the discharge of action potentials (65 mV in amplitude) during Ringer perfusion. The decrease in steady state response, from 4.0 to 0 impulses/s, was associated with a partial hyperpolarization of the generator potential from 10 to 5 mV depolarization. After 20 min of perfusion with cimetidine (second trace), the steady state response in the absence of inhibition increased from 4.0 to 9.3 impulses/s, and the generator potential nearly doubled to ~20 mV depolarization. After an additional 20 min of cimetidine perfusion, the inhibitory effects of antidromic inhibition were completely abolished, the generator potential was further depolarized, and the steady state response increased to 12.5 impulses/s. Washout by Ringer perfusion partially, but not completely, reversed the effects of cimetidine before the cell was lost (data not shown).

For a molecule to be the putative transmitter of lateral inhibition, it must mimic the effects of lateral inhibition. Histamine satisfies this criterion because it decreases the rate of action potentials fired by an eccentric cell in response to light. Another criterion is that an antagonist of the putative lateral inhibitory transmitter must block its effects. The anti-histamine, cimetidine, satisfies this criterion by abolishing the inhibitory effects evoked by antidromic stimulation of the optic nerve trunk. These physiological effects of histamine and cimetidine support the hypothesis that histamine is the neurotransmitter of lateral inhibition in the *Limulus* eye.

Figure 1B also contains evidence that histamine is the mediator of self inhibition, which is a negative feedback of an eccentric cell on itself initiated by nerve impulses (6). The increase

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 m*M* 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 mediate 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.

Supported in part by REU Fellowships from the National Science Foundation and NSF grant IBN9696208 and NIH grants MH49741 and EY00667.

Literature Cited

1. **Ratliff, F. 1965.** *Mach Bands:* Quantitative studies on neural networks in the retina. Holden Day, San Francisco, CA
2. **Hartline, H. K., H. G. Wagner, and E. F. MacNichol, Jr. 1952.** *Cold Spring Harbor Symp. Quant. Biol.* **17:** 124–141.
3. **Ratliff, F. (ed).** 1974. *Studies on Excitation and Inhibition in the Retina.* The Rockefeller University Press, New York.
4. **Passaglia, C. L. 1997.** Doctoral Dissertation. Syracuse University, Syracuse, New York.
5. **Elias, M. S., and P. D. Evans. 1983.** *J. Neurochem.* **41:** 562–568.
6. **Hardie, R. C. 1987.** *J. Comp. Physiol. A.* **161:** 201–213.
7. **Callaway, J. C., and A. E. Stuart. 1989.** *Visual Neurosci.* **3:** 311–325.
8. **Hardie, R. C. 1988.** *J. Exp. Biol.* **138:** 221–241.
9. **Battelle, B.-A., B. G. Calman, A. W. Andrews, F. D. Grieco, M. B. Mleziva, J. C. Callaway, and A. E. Stuart. 1991.** *J. Comp. Neuro.* **305:** 527–542.

Reference: *Biol. Bull.* **193:** 205–207. (October, 1997)

Visual Performance of Horseshoe Crabs: Role of Underwater Lighting

Christopher L. Passaglia (Marine Biological Laboratory, Woods Hole, Massachusetts 02543), Maireade E. McSweeney, Karen M. Stewart, Edward Kim, Edward J. Mole, Maureen K. Powers, and Robert B. Barlow

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