founding with the nonassociative processes of sensitization and pseudoconditioning (*14, 15*). Moreover, previous findings (*16*) suggest that the expectancy procedure may provide a more sensitive index of association than classical conditioning. In view of these considerations, the expectancy procedure may prove valuable to the study of associative processes in normal and brain-damaged infants.

Our results, providing what we believe to be the first evidence of associative learning in the hydranencephalic infant, bolster the growing view that subcortical networks are capable of mediating complex behavioral processes. The progressive expansion of cerebral cortical systems through phylogenetic development has undoubtedly contributed to the elaboration of behavioral capacities. Nevertheless, complex psychological processes, such as learning, do not appear to be exclusively within the domain of the cerebral hemispheres.

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17. We dedicate this report to Neal E. Miller, a pioneer in the study of brain-behavioral function and learning processes. This research was cleared by the Human Subjects Committee of Children's Hospital and Ohio State University. Parental permission was obtained before testing. We gratefully acknowledge the cooperation of the attending physician, G. Morrow, and the counsel of D. D. Wickens and C. D. Wickens.

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Limulus Brain Modulates the Structure and Function of the Lateral Eyes

Abstract. *At night efferent optic nerve activity generated by a circadian clock in the* Limulus *brain changes the structure of the photoreceptor and surrounding pigment cells in the animal's lateral eyes. The structural changes allow each ommatidium to gather light from a wider area at night than during the day. Visual sensitivity is thereby increased, but spatial resolution is diminished. At daybreak efferent activity from the clock stops, the structural changes reverse, and the field of view of each ommatidium decreases. The cyclic changes are endogenous and continue in the dark. Thus, under the control of a circadian clock, the* Limulus *eye exchanges its daytime acuity for greater sensitivity at night.*

Visual sensitivity exhibits a circadian rhythm in *Limulus*, the horseshoe crab (*1*). At night a clock in the brain transmits nerve impulses via efferent fibers to the photoreceptor cells of the lateral compound eyes. The efferent input increases the response and decreases the spontaneous activity of the photoreceptors (*2*) and optic nerve fibers (*1*). In addition, the efferent input is essential for the daily turnover of the rhabdom structures of the photoreceptors (*3*). We report here that the efferent input also changes the morphology of the ommatidial cells (*4, 5*). The circadian changes in morphology increase the light quantum catch and the field of view of single ommatidia. At night the acceptance angle of an ommatidium doubles, allowing the photoreceptors to view a larger region in visual space.

An ommatidium in the *Limulus* eye is composed of 8 to 12 photoreceptors (retinular cells) clustered tightly around the dendrite of an eccentric cell. Light collected by the corneal lens passes through an aperture formed by surrounding pigment cells and enters the photosensitive rhabdom of the retinular cells. The rhabdom is composed of microvilli, which contain a rhodopsin-like visual pigment. The responses of the retinular cells to light are conducted to the eccentric cell by electrical coupling with its dendrite. The eccentric cell, in turn, generates optic nerve impulses and transmits them to the brain.

During the day the aperture formed by the distal pigment cells is small (17 μm in diameter) and the retinular cells are separated from the lens by about 30 μm (Fig. 1). The light entering the retinular cells is thereby restricted. As shown in the cross-sectional view, processes of proximal pigment cells containing large granules run between adjacent retinular cells at the ends of the rhabdomeral rays. Smaller pigment granules in the cytoplasm of the retinular cells are concentrated near the edge of the rhabdom.

At night, during the period of peak efferent optic nerve activity, the ommatidial structure changes (Fig. 1). The pigment cell processes move radially away from the ommatidial axis, increasing the diameter of the aperture to 60 μm (*5*). The retinular cells shift position and lie within 4 μm of the corneal lens. Also, they appear to be compressed against the base of the lens. The rhabdom is shortened by 36 percent and widened by 34 percent. In cross section the individual rays of the rhabdom appear folded; in the longitudinal reconstruction they are seen as loops. The deep folds in the rhabdom appear to induce bends in the distal portion of the eccentric cell dendrite.

These morphological changes continue in complete darkness, following the circadian rhythm of efferent optic nerve activity (*6*). Cutting the optic nerve abolishes the cyclic changes and leaves the morphology of ommatidia in the daytime state. Delivering pulses of current to the distal end of the cut optic nerve during the day changes the morphology to the nighttime state. We conclude that efferent fibers in the optic nerve mediate

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circadian rhythms in the morphology of the lateral eye.

The circadian changes in morphology parallel changes in the field of view of single ommatidia. We measured the effect of the circadian clock on the field of view by recording the spike discharge from a single ommatidium in situ without cutting the optic nerve trunk. An animal that had been maintained in a natural light-dark cycle was clamped to a rigid platform in a seawater aquarium located in a lightproof cage. We exposed the optic nerve trunk by making a hole in the shell, dissected free a single active nerve fiber, and recorded its activity with a glass suction electrode (*1, 7*). A fiber-optic light pipe attached to the arm of a vernier protractor was aligned with the optic axis of the ommatidium, 20 cm from the cornea. The sensitivity of the dark-adapted ommatidium to 0.1-second flashes was then determined with the light pipe located at various angular positions along the anteroposterior axis of the eye. Figure 1C gives the relative sensitivity as a function of angle for a single ommatidium. The acceptance angle (width at half-maximum) was 6° during the day and 13° at night.

Cutting the lateral optic nerve abolishes the circadian changes in acceptance angle, leaving it in the narrow daytime state. The mean daytime acceptance angle of 6.0° ± 1.3° (N = 11) corresponds well to measurements in excised eyes (*8*). Shocking the distal end of the cut optic nerve to mimic the efferent input to the retina increased the acceptance angle to 12.3° ± 1.5° (N = 7), which closely matches the nighttime value. We conclude that efferent optic nerve activity mediates the circadian changes in acceptance angle.

The circadian rhythm in the internal structure of ommatidia appears to modulate both the sensitivity and field of view of the retinal photoreceptors (*9*). The size of the acceptance angle is largely determined by the refractile properties of the lens, the location of pigment cells, and the configuration of the photosensitive rhabdom of the retinular cells (*10*). During the day, separation of the rhabdom from the base of the lens and formation of a small aperture by surrounding pigment cells reduce the light incident on the rhabdom and restrict the incident rays to those near the optic axis of the ommatidium. At night enlargement of the aperture, distal movement of the rhabdom, and its distribution across the base of the lens increase the incident light intensity and allow more divergent rays to reach the photosensitive membrane. The average increase in sensitivity at night to a point source of light on the optic axis of an ommatidium was 1.0 ± 0.5 log unit (N = 6). The wide field of view at night yields an additional 0.4 log unit of sensitivity to extended light sources. Thus the sensitivity of a dark-adapted ommatidium may be as much as 30 to 100 times greater at night.

The circadian changes in retinal morphology increase the light quantum catch of the eye at night but reduce its spatial resolution. The fields of view of adjacent ommatidia overlap considerably at night; in the central region of the retina the acceptance angle of a single ommatidium, 13°, is about twice the angle separating the optic axes of adjacent ommatidia, 6°. During the day the acceptance angle is about equal to the angle between ommatidia, so each ommatidium "sees" approximately its proportional share of visual space.

In sum, the acuity of the *Limulus* lateral eye during daytime is exchanged for greater sensitivity at night. Many vertebrate visual systems achieve this by shifting from cones to rods and changing the pupil diameter. *Limulus*, possessing just one type of photoreceptor cell, has evolved several mechanisms for modulating visual sensitivity. One is to change the size of the aperture between the corneal lens and the photoreceptors. Another is to change the morphology of the rhabdom to increase the number of light quanta absorbed by the photoreceptor cells (*11*). Both mechanisms are controlled by a circadian clock located in the brain.

The *Limulus* visual system thus provides an admirable example of central control of a sensory input. The circadian clock in the brain changes the structure and function of retinal cells and thereby modulates the visual information transmitted back to the brain. The morphological results presented here suggest that the efferent input to the retina may in-

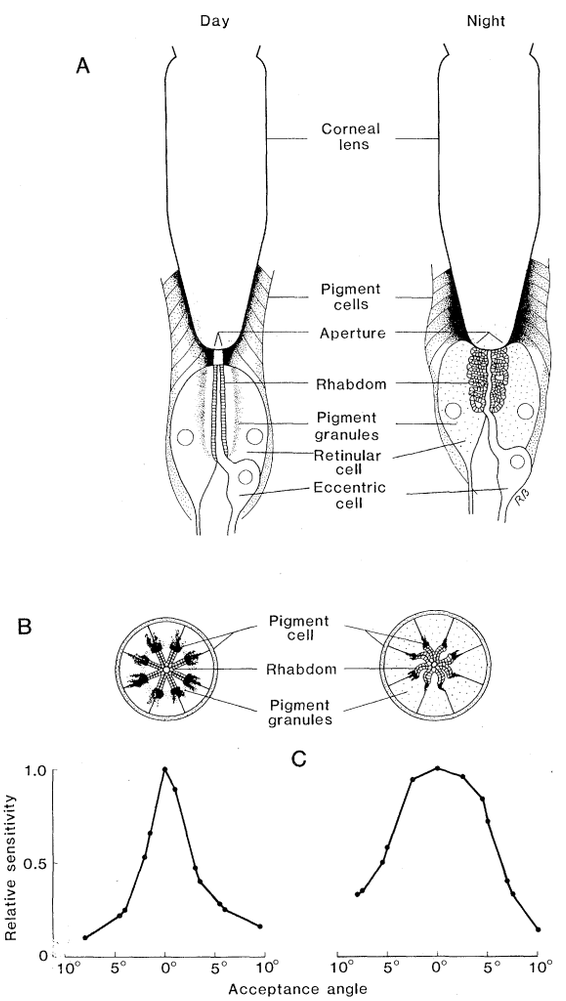

Fig. 1. Circadian changes in the morphology of a *Limulus* ommatidium. (A) Longitudinal reconstructions from micrographs of dark-adapted ommatidia fixed during the day and at night (*3*). The plane of the section on the left is through the central ring of the rhabdom and shows the microvillus structure extending the entire length of the eccentric cell dendrite. (B) Cross-sectional views taken halfway between the corneal lens and the eccentric cell body. Each view shows eight retinular cells with their rhabdomeres surrounding the eccentric cell dendrite. Pigment granules at the ends of the rhabdomeral rays are located in pigment cell processes that run between adjacent retinular cells. The width of the microvillar structure was increased for diagrammatic purposes. The diameter of the corneal lens is 200 μm. (C) Circadian changes in the field of a view of a *Limulus* ommatidium. The graphs show the relative sensitivity of the optic nerve discharge from a dark-adapted ommatidium in situ as a function of the angle of incidence of test flashes from a point light source (left, daytime state; right, nighttime state). The light source was located at various positions in a plane along the anteroposterior axis of the eye. Relative sensitivity is the reciprocal of the light intensity required to elicit a threshold response from the discharge of the single optic nerve fiber. The dark-adapated ommatidium was 1.1 log units more sensitive at night, as measured with the point light source aligned along the optic axis of the receptor (0°). For comparison the relative sensitivity along the optic axis was normalized to 1.0 for the daytime and nighttime states. The field of view of the ommatidium (total acceptance angle at half-maximal sensitivity) increased from 6° during the day to 13° at night.

duce cytoskeletal changes in the photoreceptor cells. Indeed, preliminary experiments in which we used microtubule and microfilament inhibitors indicate that the circadian rhythm in retinal sensitivity requires the integrity of cytoskeletal structures (*12*). The regulation of cell motility may therefore play an important role in determining the response characteristics of this sensory system.

In addition to the daily morphological changes described here, the photoreceptors break down and rebuild the rhabdom structure at the first light of day. Such dynamic mechanical effects are not unique to *Limulus* photoreceptors, however. The photosensitive membranes of other invertebrate and vertebrate photoreceptors are periodically broken down and renewed (*13*), although the mechanisms may differ from those in *Limulus* (*3*). Other mechanical effects in the retina include migration of pigment granules (*14*), movement of rod and cone outer segments (*15*), and changes in the synaptic structure (*16*). These mechanical effects, controlled by either light or an endogenous circadian clock, appear to adapt the retina to its photic environment. Thus, in addition to developing neural and biochemical mechanisms of adaptation (*17*), many retinas have evolved mechanical processes for controlling visual sensitivity.

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9. When nerve shock was used to mimic the efferent input, the increase in acceptance angle required the same shock rate (two per second) and duration (1.5 hours) as the increases in retinal sensitivity (*1*) and the changes in morphology (*2*). Thus the increase in acceptance angle and at least part of the increase in sensitivity appear to result from changes in ommatidial morphology.
10. Analysis of the optical properties of the corneal lens, together with numerical calculations and measurements of the acceptance angle (S. C. Chamberlain and R. B. Barlow, Jr., in preparation), indicate that the refractile mechanism originally proposed by S. Exner [*Die Physiologie der Facetterden Augen von Krebsen und Insecte* (Deuticke, Leipzig, 1891] and recently revived by M. F. Land [*Nature (London)* **280**, 396 (1979)] largely determines the acceptance angle of an ommatidium. Reflective properties of the corneal lens [R. Levi-Setti, D. A. Park, R. Winston, *ibid.* **253**, 115 (1975)] appear to play a minor role.
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Classical Conditioning: Induction of Luteinizing Hormone and Testosterone Secretion in Anticipation of Sexual Activity

Abstract. *A classical conditioning paradigm was used to demonstrate that male rats can learn to secrete luteinizing hormone and testosterone in anticipation of sexual activity. Sexually naïve males were exposed to a neutral stimulus and then to a sexually receptive female once daily. After exposure to the paired stimuli for 14 trials, the neutral stimulus was as effective as the female in triggering luteinizing hormone and testosterone secretion. These findings provide two novel perspectives on the control of reproductive hormone secretion in male rats: (i) environmental cues, which males learn to associate with sexual activity, induce the secretion of hormones that regulate pituitary-testis function, and (ii) classical conditioning may be used as a noninvasive method to evoke functional alterations in the secretion of luteinizing hormone and presumably the neuroendocrine pathways that mediate its release.*

Short-term exposure of males to females evokes temporary elevations in the systemic concentration of testosterone in numerous male mammals (*1, 2*). These findings have attracted widespread investigative interest because testosterone supports certain structural and functional aspects of the male reproductive system and plays a pivotal role in the expression of male sexual behavior (*3, 4*). Investigation of the endocrine basis for female-induced increments in circulating testosterone titers has focused on the secretion of luteinizing hormone (LH) since this pituitary gonadotropin is the primary hormone regulating the production of testicular steroids (*5*). Unexpectedly, the outcome of such investigations is equivocal: some studies have demonstrated that LH secretion is elevated after exposing males to females (*6*), but others have failed to detect alterations in LH release despite profound increments in blood testosterone levels (*7*). The controversy surrounding LH-mediated testicular endocrine responses may be due to the lack of conclusive information about stimuli that trigger LH release. Indeed, neither the effects of previous sexual experience nor the relative functional importance of visual, olfactory, auditory, and tactile cues are known in detail for any mammalian species.

Given the ambiguity surrounding the nature of the provocative stimulus, and the suggestion, based on indirect evidence, that the mere anticipation of coitus stimulates testosterone secretion in human and rat males (*8*), we hypothesized that the stimulus that evokes LH secretion during sexual encounters need not originate with a female. Rather, LH secretion could be elicited by ambient cues that males learn to associate with a

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