light. The latter typically shows a 3-minute latency, a 4- to 5-minute period of rapid growth, and a final decrease in growth rate to baseline levels (*1*)—a pattern that coincides with the light-induced period of interference we have observed.

Our evidence for interference between the light growth response and the avoidance response does not contradict the findings of Ortega and Gamow (*4*), because they did not apply the avoidance stimulus until 12 minutes after the light stimulus. Our experiments show that a light stimulus is capable of causing interference only for a period of 3 to 8 minutes after the beginning of the light stimulus. Our results are likewise compatible with those of Cohen *et al.* (*3*), since they used steady illumination, which does not cause a light growth response.

Why does a bilateral light stimulus cause a reversal of avoidance bending in addition to simple inhibition? The phenomenon of temporary tropic reversals induced by light stimuli has previously been noted for the phototropic and geotropic bending response (*5*). Castle (*6*) has proposed a theory to explain such reversals, and his model can be extended to include reversals of the avoidance response.

Castle postulates that there is a substance, M, that is continually supplied and is distributed equally on both sides of the sporangiophore growing zone, forming M pools. The M substance is consumed during growth, being depleted equally on both sides during straight growth. When a sporangiophore bends, the M substance is depleted faster on the convex side because that side is growing more rapidly. When a light stimulus is applied, it triggers a period of rapid growth, and, since the rate of growth is proportional to the size of the M pools, the side with the larger M pool grows faster, causing a reversal of the original bending.

Castle's model implies that the magnitude and the speed of onset of the reversal should be proportional to the degree of imbalance in the two M pools, the imbalance being in turn directly related to the amount of bending that has occurred prior to the light stimulus. Our results are in accordance with this prediction. The latency of the reversal decreases and the magnitude of reversal increases as the elapsed time increases between the avoidance and the light stimuli (Table 1). This more rapid onset and greater magnitude may be a consequence of the greater M pool imbalance. No reversal is observed when a light stimulus is applied before avoidance bending has occurred

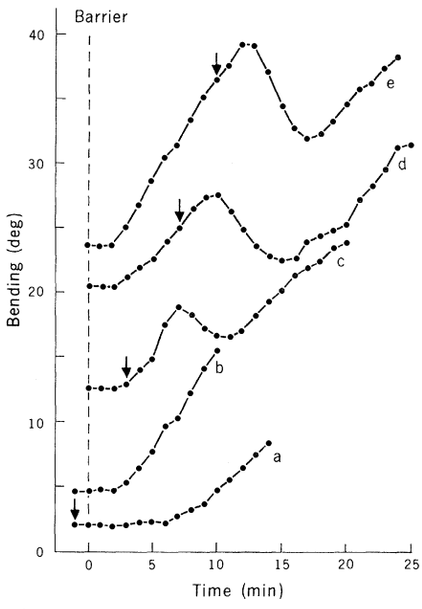

Fig. 1. Stage IV sporangiophores are placed 1 mm from a barrier stimulus at $t = 0$ minutes. A blue light step at 315 μW/cm^2 was applied 1 minute before the barrier (a), at $t = 3$ (c), at $t = 7$ (d), and at $t = 10$ (e), as shown by the arrows. No light step was applied in (b). Curves a through e represent the average of seven, six, four, four, and four experimental runs, respectively. All curves are displaced along the bending scale.

(Fig. 1, curve a). In this case there has been no previous bending to cause an imbalance in the M pools, and the interference period is seen as an extension of the latency of the avoidance response.

The significance of this work lies in the discovery of the light-induced delay of onset, a phenomenon unique to the avoidance response. The existence of an element common to both phototropic and avoidance pathways has been shown by the isolation of mutants in which both pathways are blocked (*2*). The delay of onset indicates that this common element is nonlinear and cannot function simultaneously in both pathways when they each carry saturating stimuli.

Our results raise the question of whether this interference is reciprocal. If so, one would expect a bilateral avoidance reponse to interfere with phototropism.

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Hydrostatic Pressure Reversibly Blocks Membrane Control of Ciliary Motility in *Paramecium*

Abstract. *A hydrostatic pressure of only 68 atmospheres prevented swimming* Paramecium caudatum *from "avoiding" or reversing direction; 170 atmospheres stopped or decreased forward velocity by more than 75 percent. A decompression of 40 atmospheres invoked a single reversal, even at 280 atmospheres. In contrast, 170 atmospheres did not significantly affect swimming behavior of paramecium "models" that were reactivated in a solution containing adenosine triphosphate and magnesium ions after their membranes had been disrupted by Triton X-100.*

In a survey of pressure effects on the activity of ciliates and flagellates, Kitching (*1*) reported that 100 to 200 atm slowed or stopped swimming paramecia. Our studies were designed (i) to characterize more fully the pressure-related changes in the swimming behavior of paramecia and (ii) to determine whether pressure induces alterations in the ciliary machinery or in the electrically excitable plasma membrane that controls the frequency and direction of ciliary beat. The bioelectric properties of the paramecium's surface membrane are closely coupled to the activity of its locomotory cilia (*2*). A depolarizing influx of calcium ions causes ciliary reversal and backward swimming; with hyperpolarization, the frequency of ciliary beating and rate of forward swimming increase (*2*). By comparing the effects of pressurization and decompression on normal paramecia with their effects on paramecium "models" [paramecia treated with Triton X-100 so that their membranes were no longer functional barriers (*3*)], we deter-

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mined that the pressure-sensitive components of the paramecium's motility system primarily reside in the plasma membrane. Further studies with "pawn B" mutants of *Paramecium aurelia* suggested more specifically that changes in hydrostatic pressure alter locomotion by affecting calcium channels in the plasma membrane.

Paramecium caudatum (Carolina Biological Supply) and pawn B mutant (*4*) of *P. aurelia* strain d4-500 (*5*), were grown as described by Sonneborn (*6*) in Cerophyl medium inoculated with bacteria. Paramecium models were prepared with Triton X-100 (Calbiochem) and reactivated according to methods described by Naitoh and Kaneko (*3*). The microscope pressure chamber and pressure-generating equipment were similar to those described by Salmon and Ellis (*7*), except that the pressure chamber was twice as large as the one originally described to permit objective and condenser port apertures of 10 mm with windows 16 mm in diameter, made of 5-mm-thick optical glass.

For the experiments with wild-type and mutant paramecia we transferred animals from the culture medium to standard buffer (4 m*M* KCl, 1 m*M* $CaCl_2$, 10 m*M* tris-HCl, 0.1 m*M* EDTA, *p*H 7.2), washing three times by gentle hand centrifugation at 4°C. Paramecia were confined in an enclosure created by sandwiching a silicone rubber washer (0.5 mm thick; diameter 10 mm outside and 6 mm inside) between an 18-mm circular cover slip and a 10-mm square fragment of cover slip, and sealing the preparation with silicone grease. All air was excluded. Satisfactory preparations were placed in the pressure chamber, which had been filled with standard buffer. Experimental temperature was 22° ± 1°C. Triton-extracted paramecium models were similarly prepared for observation in the chamber, except that reactivation medium was used instead of the standard buffer (*3*). The reactivation medium consisted of 4 m*M* $MgCl_2$, 50 m*M* KCl, 4 m*M* adenosine triphosphate (ATP; P-L Biochemical, Milwaukee), 10 m*M* tris-maleate, *p*H 7.0, and either 3 m*M* EGTA or 50 μM $CaCl_2$.

We observed paramecia in the chamber with a Leitz Diavert microscope equipped with an X2.5 Zeiss Planachromatic objective and No. 1 condenser phase annulus to produce dark-field illumination. Swimming behavior was determined from photomicrographs (Kodak Plus X film developed in Microdol X) taken with a stroboscopic dark-field illumination system, which produced a predetermined number of flashes (typically ten) per frame at precise intervals (usually 0.3 second for whole organisms and 1.0 second for Triton-extracted models). Swimming velocity was determined by measuring the distance an organism traveled during several flash intervals.

Pressurization caused the velocity of forward-swimming paramecia to decrease rapidly to a new steady-state value (Fig. 1, a_1 and a_2, and Fig. 2). The higher the pressurization, the lower the steady-state velocity (*8*). At 170 atm the average velocity decreased by more than 75 percent and many paramecia had completely stopped moving forward, although their cilia still appeared to be beating vigorously. When pressure was increased to 400 to 500 atm, most of the stationary paramecia again moved forward, but slowly and with continuous violent jerking. Irreversible damage occurred within 30 seconds at such high pressures.

Pressurization to 68 atm immediately blocked the "avoiding" reaction, but swimming velocity decreased by only 50 percent. (An avoiding reaction occurs when a forward-swimming paramecium suddenly backs away from a stimulus, turns, then resumes forward swimming in a new direction.) The higher the pressure, the fewer avoiding reactions occurred (*8*). At pressures above 68 atm paramecia did not reverse their ciliary beat. For instance, at 68 atm, when a paramecium swam to the edge of the specimen enclosure, it continued to swim forward until it eventually ended up swimming in a circular path defined by the enclosure wall.

Abrupt decompression induced a transient reversal response similar to the avoiding reaction: the paramecia quickly backed up before resuming forward

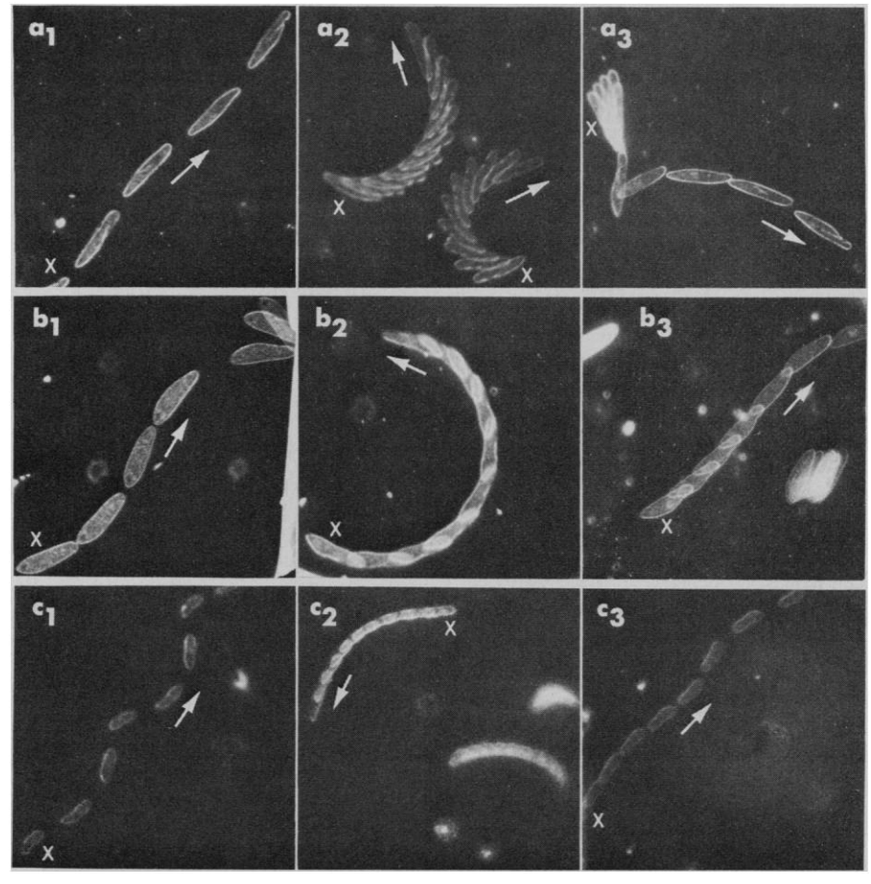

Fig. 1. Stroboscopic time-lapse photomicrographs comparing (a) typical swimming behavior of *P. caudatum* in standard medium, (b) forward-swimming Triton-extracted paramecium models in reactivation medium containing EGTA, and (c) pawn B mutants of *P. aurelia* in standard medium; (1) an atmospheric pressure, (2) under pressurization to 170 atm, and (3) during decompression from 170 to 13.6 atm. The anterior of the paramecium is narrow and shows a groove at the location of the gullet. Arrows indicate the forward swimming direction. On each frame x indicates the earliest flash in a sequence. For (a) and (c) the time between flashes was 0.3 second; for (b) it was 1.0 second. Whole organisms were pressurized for 5 minutes, but the models were pressurized for only 1 minute because they swam most actively during the first 5 to 10 minutes after addition of ATP. Later, many became stuck to the cover slip. In (a_3), (b_3), and (c_3), pressure was reduced after the fourth flash.

swimming (Fig $1a_3$ and Fig. 2). If the initial pressurization was less than 280 atm, rapid decompression of 40 atm or more caused even stationary paramecia to back up quickly.

The effects of pressurization on swimming speed and the frequency of avoiding reactions appeared to be completely reversible up to 280 atm. After the initial reversal following decompression, paramecia resumed forward swimming with the characteristic velocity and frequency of avoiding reactions for the lower pressure.

In contrast to their effects on normal paramecia, pressurization and abrupt decompression did not significantly affect the swimming of Triton-extracted paramecium models. At atmospheric pressure in reactivation medium containing EGTA, the models swam forward at 0.2 to 0.4 mm/sec (compared with 1.0 to 1.5 mm/sec for normal paramecia in standard buffer), and no avoiding reactions ever occurred (Fig. $1b_1$). Pressurization of the models to 170 atm caused their forward velocity to decrease only 25 percent (Fig. $1b_2$). After decompression there was no reversal response; the animals simply accelerated to the velocity at atmospheric pressure (Fig. $1b_3$). When 50 μM $CaCl_2$ was included in the reactivation medium instead of EGTA, the models all swarm backward. The swimming direction of the models, unchanged by decompression, depended solely on the calcium concentration of the surrounding medium (*13*). Since a functioning plasma membrane is the major attribute of normal paramecia that the models lack, it appears that pressurization and decompression affect swimming behavior in normal paramecia by inducing changes in the plasma membrane's control of ciliary motility.

Normally, the membrane maintains a concentration gradient with a Ca^{2+} concentration outside the cell of about 1.0 mM, while inside the ciliary membrane the Ca^{2+} concentration is less than 0.1 μM. A mechanical or chemical stimulus to the anterior of the organism causes depolarization, which opens Ca^{2+}-selective, voltage-sensitive channels in the ciliary plasma membrane. The resulting influx of Ca^{2+} produces a regenerative depolarization, the calcium response. Efflux of K^+ through separate voltage-sensitive channels repolarizes the membrane. An increase in the intraciliary calcium concentration to more than 1.0 μM causes the ciliary axonemes to reorient, reversing the direction of the power stroke and causing the paramecium to back up. When calcium is pumped back out of the cell, the ciliary orientation returns to the normal direction for forward swimming (*2*).

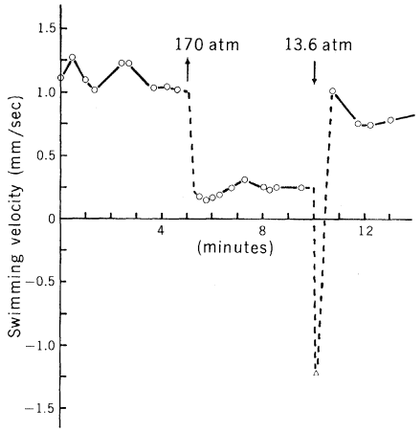

Fig. 2. Changes in swimming velocity of a single, typical *P. caudatum* during pressurization to 170 atm and decompression to 13.6 atm in standard buffer. (○) Forward swimming velocity during the exposure intervals. (△) Swimming velocity determined by measuring the distance the organism traveled backward just after decompression (see Fig. $1a_3$).

The experiments just described indicate that in normal paramecia increased pressure blocks the entry of calcium into the cilia, and rapid decompression causes a rapid, transient rise in the intracellular calcium concentration, which results in backward swimming. We studied the response to decompression of pawn B mutants of *P. aurelia* to determine whether the source of the calcium responsible for the reversal on decompression was intra- or extracellular. Pawn B is a mutant paramecium that has defective calcium channels in its ciliary membrane (*4*). Nonfunctional calcium channels prevent spontaneous avoiding reactions in pawn B mutants, but pawn B cilia remain capable of reorienting if sufficient calcium levels are attained (*9*). In control experiments with wild-type *P. aurelia*, strain 51-S, the responses to changes in pressure were essentially identical to those of wild-type *P. caudatum*. *Paramecium aurelia* pawn B mutants slowed down in response to pressurization (Fig. $1c_1$ and c_2), but after decompression no transient reversal occurred, only the expected increase in forward velocity (Fig. $1c_3$). Apparently, then, the reversal of wild-type paramecia in response to decompression is caused by a transient influx by Ca^{2+} through specific membrane channels, those genetically blocked in pawn B mutants.

Because neither pawn B mutants nor Triton-extracted models backed up when decompressed, the effect of pressure on swimming direction is apparently mediated by the calcium channels located in the ciliary plasma membrane (*10*). It is not yet clear, however, what molecular features of calcium channels make them sensitive to such low levels of pressure. Schein (*11*) suggested that the ciliary membrane channels open and close by changing their conformation. Perhaps the closed or inactivated channels occupy a smaller volume than open channels. Alternatively, pressurization may increase the size and strength of the Ca^{2+} hydration layer and so inhibit calcium ion passage through the membrane channels. Possibly, the effect of pressure on calcium movement is more general; for example, the lipid bilayer may be restructured by pressure so that the membrane channels are physically restricted (*12*). It also may be that the mechanoreceptors located at the anterior of paramecia are affected directly by pressure.

The relatively small reduction in forward speed of Triton-extracted models pressurized to 170 atm, compared with normal paramecia, suggests that specific changes in the plasma membrane are important in regulating velocity as well as swimming direction. Although hyperpolarization of the membrane causes velocity to increase (*2*), the molecular events corresponding to velocity changes are not yet understood. Pressure might produce decreased beat frequency and reorientation of the cilia by affecting ion movement so that the membrane depolarizes somewhat (*2*). Perhaps pressure simply packs the membrane molecules closer together, stiffening the membrane so that it becomes more resistant to deformation and thus restricts the cilia's power stroke. Direct electrophysiological measurements in pressurized paramecia would help to characterize the mechanism of speed alterations.

Although many details of membrane function in paramecium remain to be clarified, we feel that the excitable membrane of paramecium may be a good model system for studying the general question of how hydrostatic pressure affects excitable tissues. The bioelectric properties of the paramecium's membrane resemble those of the membranes of nerve and muscle cells in many respects (*2*), and specific changes in the electrogenic activity of the paramecium membrane are reflected in its swimming behavior, which is easily observed and quantified.

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8. Pressure was increased from atmospheric in steps of about 34 atm at 5-minute intervals to 170 atm, then returned to 13.6 atm. At each pressure, average swimming speeds for five to ten organisms were determined from photographs taken during each 5-minute interval. The experiment was repeated four times and the data for each pressure level were pooled and averaged. In separate experiments, avoidance frequency was determined by visual counts of the number of reversals, jerks, or hesitations of four or five organisms during each 5-minute pressure interval. The experiment was repeated five times and the data were pooled and averaged for each pressure level. At atmospheric pressure, the average forward swimming velocity was 1.4 ± 0.4 mm/sec and the average frequency of avoiding reactions was 100 ± 25.
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13. We thank D. Oertel for comments and criticisms, N. Salmon for editorial assistance, and M. Spillane for photographic help. This work was supported by NSF grant 77-07113.

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Light and Efferent Activity Control Rhabdom Turnover in *Limulus* Photoreceptors

Abstract. *Photosensitive membrane structures in the retinular cells of the* Limulus *lateral eye are broken down and renewed daily. The first light onset causes a rapid, synchronous disassembly and buildup of the rhabdom in each photoreceptor cell. The entire process is complete within 30 minutes. Blocking the efferent input to the retina from the brain blocks the turnover of the rhabdom, and mimicking the efferent input by optic nerve stimulation restores it.*

Vertebrate photoreceptors periodically shed and renew the parts of the cells that transduce visual stimuli. Rods shed packets of membrane discs from the tips of the outer segments soon after the onset of light (*1*), and cones shed packets of outer segment discs soon after the onset of darkness (*2*). Disc shedding is followed by renewal, a process of disc assembly that occurs at the base of the outer segment and generally requires a longer period than shedding (*3*). Diurnal changes in retinal illumination appear to control the turnover of outer segment membrane (*1*, *4*), although in constant darkness disc shedding exhibits an endogenous, circadian rhythm in several species (*1*, *3*). No hormonal controlling factors have been found in rats (*5*). Indeed, recent evidence from frogs suggests that control of the turnover process in vertebrates is endogenous to the retina (*6*).

We report here an analogous turnover of photosensitive membrane structures in an invertebrate, *Limulus polyphemus*. We show that the onset of light triggers a rapid breakdown and buildup of the rhabdomeres of all retinular cells in the lateral compound eyes. In addition, we report that an essential requirement for rhabdom turnover is efferent activity transmitted from the brain to the retina by fibers in the lateral optic nerve.

Turnover in *Limulus* retinular cells was first detected in our experiments with freshly collected horseshoe crabs in Woods Hole, Massachusetts. During the afternoon of the day of collection, we placed seven animals in seawater aquaria and kept them in the dark throughout the night. The following day we transferred six of the animals to aquaria in direct sunlight and fixed the lateral eyes after various durations of exposure (*7*). The eyes of the seventh animal were fixed in the dark.

Figure 1 shows the sequence of light-induced changes in the rhabdomeres of retinular cells. In each electron micrograph the plane of section is perpendicular to the optic axis of the ommatidium, and the view is near the central region of the rhabdom (*8*). Figure 1A shows the rhabdom of the animal that was not exposed to light. Included are portions of the well-defined microvillar arrays from the rhabdomeres of three neighboring retinular cells and a portion of the central ring surrounding the dendrite of the eccentric cell. Figure 1B shows that a 15-minute exposure to sunlight triggered an extensive breakdown of the light-sensitive rhabdomeres. The microvilli were almost completely disassembled, and whorls of microvillar membrane formed lamellar bodies in the nearby palisade region. After 30 minutes of exposure (Fig. 1C), the rhabdom was nearly restored to its original state (Fig. 1A). By this time no lamellar bodies were present, and numerous small multivesicular bodies (MVB) lined the rhabdom rays (Fig. 1D). After 1 hour of sunlight the small MVB began to coalesce, forming larger MVB (Fig. 1E), which moved away from the rhabdomere. After 4 hours, several large MVB formed along the peripheral margin of the rhabdom (Fig. 1F). After 8 hours, large dense inclusion bodies were present in the peripheral region of the photoreceptor cells (Fig. 1G).

The onset of light causes a rapid, synchronous turnover of rhabdom in all the photoreceptor cells of the lateral eye. Because of the high rate of breakdown and buildup of the microvillar array, it has not yet been possible to determine the exact percentage of rhabdom involved in a given experiment. Nonetheless, all experiments exhibit evidence of turnover after light onset, but not all show the extent indicated in Fig. 1 (*9*). A useful indicator of turnover is the appearance of MVB near the rays of the rhabdom (*10*). We have no direct evidence that the MVB contain all of the membrane from the disassembled microvilli; in fact, the size and density of MVB appear inadequate to account for all membrane removed from the rhabdom. However, we conclude from the sequence in Fig. 1 that the disassembly and reassembly of the rhabdom most likely represents a process of membrane turnover.

Rhabdom turnover is not solely an effect of light exposure. Under natural conditions of illumination, the first light of dawn triggers turnover; a subsequent period of darkness and light on the same day does not (*11*). Efferent optic nerve activity that modulates lateral eye sensitivity exhibits a circadian rhythm (*12*). The efferent fibers discharge impulses only during the animal's subjective night. They are not active during the day, even in darkness. Our results show that rhabdom turnover occurs at the first light onset after a normal nighttime period of enhanced lateral eye sensitivity. However, no turnover was detected with animals that exhibited little or no circadian rhythm in visual sensitivity.

We further investigated the possible influence of efferent activity on rhabdom turnover by cutting one lateral optic nerve in each of five animals. The animals had been entrained to normal diurnal changes in illumination. Nerve transection was performed shortly before dusk, and the animals were stored in the dark overnight. The following day the lateral eyes were fixed after 15 minutes of light exposure. In each case the eyes

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