planation, melatonin synthesis and, hence, possibly the function of the pineal, is dramatically reduced in the aging Syrian hamster.

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6. All animals were purchased from Lakeview Hamster Colony, Newfield, N.J. The old animals were maintained in our facility in San Antonio until the young animals were purchased. Thereafter, they were kept under identical environmental and caging conditions. The vaginal cycles of both young and old females were checked by means of post-estrous discharge [M. W. Orsini, *Proc. Anim. Care Panel* **11**, 193 (1961)] for 4 weeks before they were killed. All the young animals exhibited regular 4-day estrous cycles. Somewhat unexpectedly, 20 of 29 old animals also had nearly normal vaginal cycles on the basis of the post-estrous discharge. Some of these had a prolonged cycle; however, most of them had a postovulatory discharge every 4 days. The remaining nine animals were acyclic (at least 10 days without a postestrous discharge).
7. In the case of the young animals, there were eight females and males killed at each time point. For the old males and females, eight each were killed at the two points during darkness (2400 and 0400 hours) while the groups killed during the light phase (0800 and 2000 hours) contained either six or seven females and the same number of males. The total number of hamsters used in the study was 155. Animals killed at night were exposed to a dim red light (25-W tungsten bulb behind a Kodak 1A safe light filter) for 6 to 10 seconds before decapitation. All animals were killed during the same 24-hour period.
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13 June 1980; revised 4 August 1980

Neural Organization Predicts Stimulus Specificity for a Retained Associative Behavioral Change

Abstract. *Paired, but not random, presentations of light and rotation produced long-term changes in* Hermissenda*'s response to light. The nature of this change depended on the orientation of the animals with respect to the center of rotation and was predicted by known organizational features of* Hermissenda*'s nervous system. When rotation that excited caudal hair cells was paired with light, a significant increase in response latency to test lights resulted. Rotation exciting cephalic hair cells when paired with light decreased the response latencies compared with latencies produced by random presentation of light and rotation.*

Hermissenda will normally move toward a light source in an otherwise unilluminated environment (*1*). This movement can be modified by pairing discrete presentations of light with rotation of the organism (*1*). Paired (but not unpaired or randomly paired) presentations result in significantly longer latencies for the animals to enter illuminated areas when tested immediately and several days after training (*2*). This long-term associative behavioral change is due, at least in part, to primary conductance changes within the somata of type B photoreceptors (*3–5*). The neural organization (*6–8*) of *Hermissenda* (Fig. 1) and stimulus-specific cumulative depolarization of the type B cells (*9, 10*) predict, and this report confirms, that these associative behavioral changes will depend on the intact animal's orientation with respect to the center of rotation.

Hermissenda ($N = 70$) were maintained individually in 15°C seawater (*11*). Each animal was fed daily until satiated on mussel gonad (*Mytilus edulis*) and exposed to 6 ($N = 35$) or 12 ($N = 35$) hours of light daily. Training and testing of animals began after at least 3 days of these maintenance conditions. Training consisted of three phases: (i) baseline assessment of behavior in response to light; (ii) light and rotation regimens; and (iii) multiple reassessments of responses to light. Training and testing techniques have been described (*2, 12, 13*).

The light and rotation schedule began shortly after the end of phase 1 (*14*). Each animal was randomly assigned to one of four treatment conditions. Two groups of animals (paired/caudal and paired/cephalad) received 50 contiguous and completely overlapping pairings of 30 seconds of light and rotation (*15*) on each of three consecutive training days. The time between the initiation of each pairing was, on the average, 2 minutes. Two other groups of animals (random/caudal and random/cephalad) also received 50 30-second presentations of both light and rotation during each of three consecutive daily training sessions. For these animals, light and rotation presentations were presented randomly and independently of one another, although at the same rate (average interstimulus interval for both light and rotation was 2 minutes) as for paired groups. One group of both paired and random animals were secured throughout training with heads oriented toward the center of rotation (paired/caudal and random/caudal) to ensure that the caudal hair cells would be those stimulated by rotation. Similarly, both of the remaining groups (paired/cephalad and random/cephalad) were secured throughout training with heads oriented away from the center of rotation to ensure that the cephalic hair cells would be those stimulated by rotation. After training session 3, each animal was tested for both short- and long-term modifi-

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cation of latency to enter the area of test illumination. Immediately after the session, each animal was removed from its tube, the lens paper wrapping was removed, the animal was returned to its tube, and its latency to enter the center of the illuminated turntable was recorded. Animals were then returned to their home cages for 24 hours and were tested again at 48, 72, 96, and 120 hours after the beginning of session 3 of acquisition training.

No significant differences existed among groups for absolute baseline latencies. Furthermore, no significant differences were apparent among any of the groups when tested immediately after the end of acquisition training (unweighted means, repeated measures analysis of variance; $P < .05$) (*16*). All groups were slower to enter the test area (Fig. 2). When tested for retention of behavioral change, however, marked differences emerged among groups. Scheffé multiple comparisons ($\alpha = .05$) indicated (*17*) that the paired/caudal animals were slower to enter the test area of illumination than random/caudal animals 48 and 72 hours after training. Paired/cephalad animals were quicker to enter the test light than random/cephalad animals 48 and 72 hours after training. Paired/cephalad animals were also faster relative to baseline at 72 hours. Neither of the random groups differed from one another at any time.

Caudal versus cephalad hair cell stimulation, then, clearly produces different long-term behavioral changes after training with paired (but not random) light and rotation. This difference is predicted by orientation-specific features of the *Hermissenda* nervous system (Fig. 1) and the hypothesis that (i) short-term cumulative depolarization of the type B cell (*10*) leads to the observed long-term depolarization (*4*) for animals trained with paired (but not random) stimuli and (ii) this long-term depolarization causes, at least in part, the associative behavioral changes retained during the days after training.

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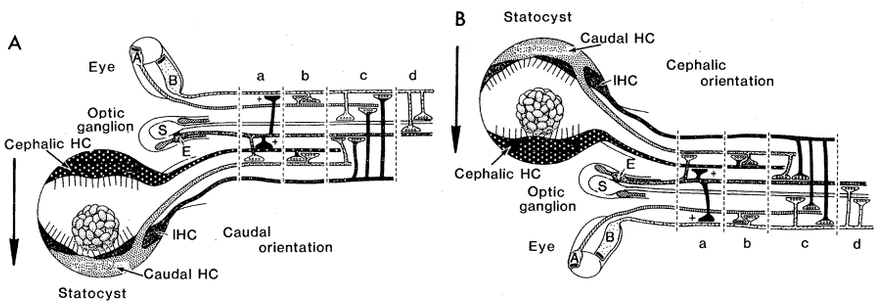

Fig. 1. Schematic diagram of interactions between *Hermissenda* visual and statocyst systems. The neural interactions (intersection of vertical and horizontal processes) identified to be reproducible, from preparation to preparation, are based on intracellular recordings from hundreds of pre- and postsynaptic neuron pairs as well as light and electron microscopic studies. (A) Caudal orientation. When caudal hair cells are depolarized by rotation (as they are for the caudal orientation) their inhibition of the E optic ganglion cell increases. Following inhibition by these hair cells as well as inhibition by the ipsilateral type B photoreceptor, the E cell undergoes rebound depolarization. During this depolarization the E cell increases its synaptic excitation of the type B cell. The E cell is also responsible for synaptic inhibition of the caudal hair cell. During rebound depolarization of the E cell, the inhibition of the caudal hair cell is also increasingly inhibited. The resultant of these synaptic effects is increased synaptic excitation of the type B cell after rotation (for caudal orientation) alone and after light alone, but particularly after light paired with rotation. This is so because during stimulus pairing (for the caudal orientation), the caudal hair cell (or cells) depolarizes both in response to rotation and because of decreased inhibition from the E cell, which is now inhibited both by the caudal hair cells and the type B photoreceptors. (B) Cephalic orientation. When cephalic hair cells are depolarized by rotation, the resultant synaptic effect is slight inhibition after stimulus pairing. The arrow indicates direction of the centrifugal force vector produced by rotation. The darkened vertical processes with plus signs represent the only excitatory synaptic interactions within this network.

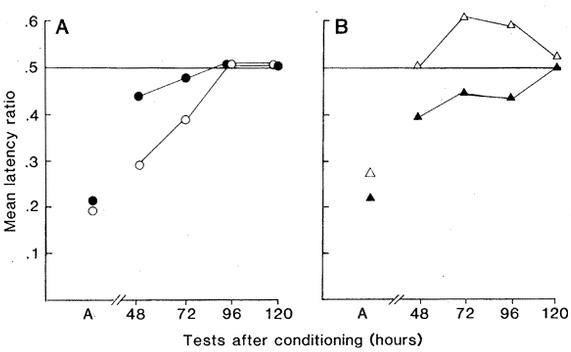

Fig. 2. Mean latency ratios indicating changes in *Hermissenda*'s response latencies to enter an illuminated area after various schedules of light and rotation pairings. Since individual *Hermissenda* differ greatly in their absolute latencies to enter the test area of illumination, all latency scores obtained during testing were converted to relative scores by means of a ratio comparing test to baseline latencies (*16*). With this measure, values less than .50 indicated that test latencies are longer than in baseline, and values greater than .50 indicate shorter latencies. Symbols: ○, paired/caudal; ●, random/caudal; △, paired/ cephalad; and ▲, random/cephalad. The latency ratio is of the form $A/(A + B)$ where A denotes baseline response latency and B denotes latency during test (cutoff score of 180 minutes). Group data consist of three independent replications for all experimental and control groups. Handling during restriction of orientation may have produced a nonspecific decrease in locomotor activity which masked other differences in test latencies previously observed by Crow and Alkon (*2, 4*) on day 3.

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11. This experiment consisted of three separate and independent replications. In the first, animals were kept individually within glass Pyrex beakers filled with 250 to 300 ml of 0.44 μM filtered seawater, inside a 15°C incubator. In subsequent replications, animals were maintained in flow-through plastic cages, submerged in aerated seawater (12° to 14°C; flow rate of 1 liter/min). *Hermissenda* were provided by M. Morris of the Sea Life Supply Co., Sand City, Calif.
12. Light intensity was 4.6×10^3 erg cm^{-2} sec^{-1}, as measured by a radiometer (Yellow Springs International, 65A) at the level of the tubes (39 cm normal to the lamp). Additional measurements were also made with a narrow-band-pass filter (centered at 400 nm—the wavelength of peak sensitivity for *Hermissenda*'s A and B photoreceptors)—in front of the light source. Intensity was attenuated by a factor of 4.3.
13. Four of the original 80 animals failed to traverse the tube during baseline tests and were discarded.
14. This period of time was variable (range 20 to 50 minutes) since each animal was removed by hand and secured for replacement into its tube in a fixed orientation. Each tube was also cleaned and filled with fresh seawater.
15. All animals were snugly wrapped in small pieces of wet translucent lens paper and replaced in the tubes. This method successfully maintained animals in the desired orientation; observations before and after each acquisition session revealed no deviations from assigned orientation for any animal. Six animals had either died, or appeared noticeably sick, at the end of acquisition training and were excluded from further

study. Subject loss was not related to training group condition.

16. This measure is similar to the suppression ratio commonly used as an index of conditioning in conditioned emotional response procedures [Z. Annau and L. J. Kamin, *J. Comp. Physiol. Psychol.* **54**, 438 (1961)]. During testing, cutoff scores of 180 minutes were used.
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18. We thank J. Harrigan and I. Lederhendler for their helpful advice, T. Crow for discussions of this work, and A. Gelperin and J. Rudy for comments on an earlier version of this report. Supported in part by a research grant from the Marine Biological Laboratory "Steps Toward Independence" program to J.F.

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Membrane Depolarization Accumulates During Acquisition of an Associative Behavioral Change

Abstract. *Long-lasting electrical changes of identified* Hermissenda *neurons, the type B photoreceptors, can account for concomitant associative behavioral changes. Depolarization of the type B cells after paired light and rotation accumulates (as monitored with intracellular electrodes) with repetition. This accumulation was specific to stimulus pairing (versus light alone or explicitly unpaired stimuli) and to the orientation of the nervous system with respect to the center of rotation; it provides a neural step in the acquisition of associative behavioral changes for gastropod mollusks and possibly other species.*

Type B photoreceptors of the nudibranch mollusk *Hermissenda crassicornis* undergo long-lasting depolarization (LLD) after a light step (*1*) of moderate ($\geq 10^3$ to 10^5 erg cm^{-2} sec^{-1}) intensity (Fig. 1 and Table 1). This LLD is a nonsynaptic process originating in the type B cell body. It arises at least in part from a light-induced, voltage-dependent Ca^{2+} conductance (*2*). The LLD and other nonsynaptic electrical characteristics of the type B cell show long-lasting changes (*3*) after exposure of intact *Hermissenda* to 3 days of light paired with rotation (compared with randomized and explicitly unpaired control tests). This paired-stimulus regimen produced short- (*4*) and long-term (*5*) behavioral changes, the latter having defining features of associative learning (*6*). In this experiment, the same sensory stimuli that produced the behavioral and neural (*5*, *7*) changes were presented to the isolated circumesophageal nervous system with intact eyes and statocysts through the use of an apparatus for continuous intracellular recording (*8*, *9*).

When the circumesophageal nervous system was rotated in the caudal orientation (with the statocysts' caudal poles oriented away from the center of rotation) during the light step, the LLD was increased in amplitude and prolonged (Fig. 1 and Table 1). Accumulation of this depolarization, measured instantaneously at 20 and 60 seconds after the light step, was apparent when these paired sensory stimuli were repeated 90 seconds after the first paired stimulus presentation. Cumulative depolarization after two stimulus pairs was greater than that after two lights alone (at 90-second intervals) or that after light and rotation in an explicitly unpaired sequence (Fig. 1 and Table 1). The same number of explicitly unpaired stimuli were presented over the same total time period as for the paired stimulus regimen. For the caudal orientation, with repeated stimulus pair presentations the cumulative depolarization progressively increased (Fig. 2A) and persisted for many minutes after the stimulus pairs (Fig. 2B). Cumulative depolarization after stimulus pairing did

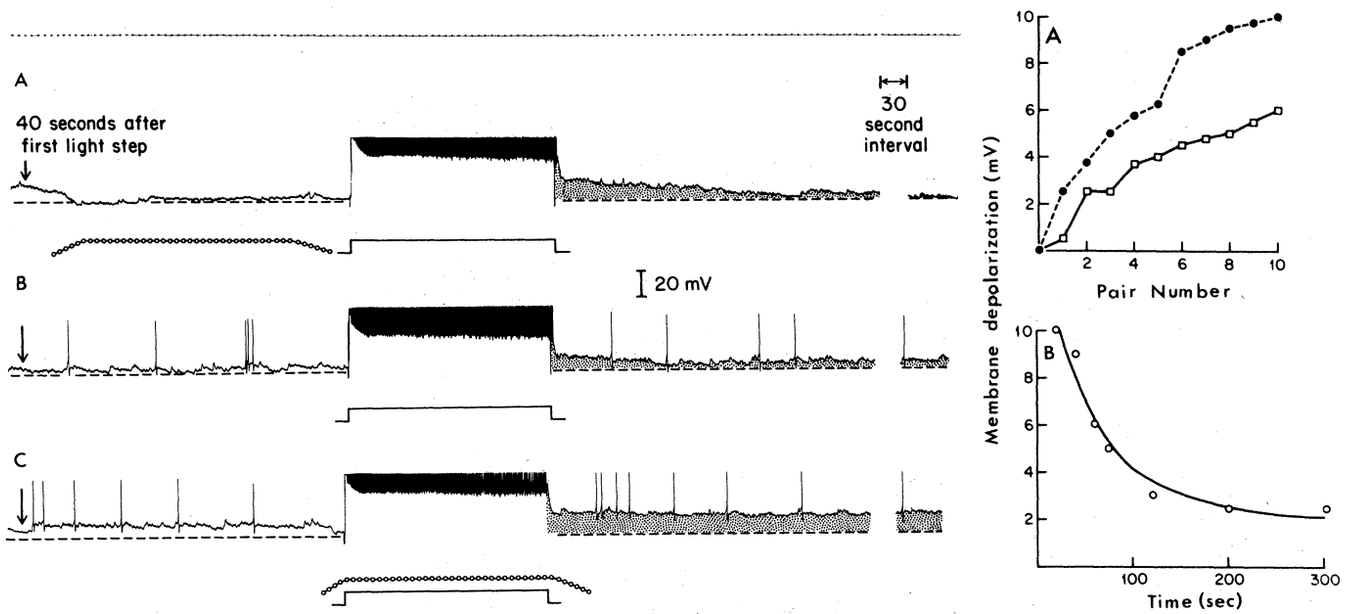

Fig. 1 (left). Intracellular voltage recordings of *Hermissenda* neurons during and after light and rotation stimuli. (A) Responses of a type B photoreceptor to the second of two succeeding 30-second light steps (with a 90-second interval intervening). The cell's initial resting potential, preceding the first of the two light steps in (A), (B), and (C), is indicated by the dashed lines. Depolarization above the resting level after the second of the two light steps is indicated by shaded areas. (A) Light steps (~ 10^4 erg cm^{-2} sec^{-1}) alternating with rotation (caudal orientation) generating ~ 1.0 g. The end of the rotation stimulus preceded each light step by 10 seconds. (B) Light steps alone. (C) Light steps paired with rotation. By 60 seconds after the first and second light steps, paired stimuli cause the greatest depolarization and unpaired stimuli the least. The minimal depolarization was in part attributable to the hyperpolarizing effect of rotation. Depolarization after the second presentation of paired stimuli was greater than that after the first. Fig. 2 (right). (A) Increase of type B membrane depolarization with repetition of the stimulus pairs. Membrane potential was measured instantaneously 20 seconds (filled circles) and 60 seconds (open squares) after successive presentations of light steps paired with rotation. (B) Decrease of type B membrane depolarization after repeated presentation of stimulus pairs as described in (A).

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