Pradhan served as interpreter and solicited volunteers.

5. The EPC mini-stimulators were obtained through the generosity of A. F. Battista, New York University Medical Center, and M. L. Mashburn, president, Stimulation Technology, Inc.
6. This binary decision criterion, which was used in place of a pain rating scale to simplify the decision task, bears no relation to the criterion for reporting pain. In designing this study, we were aware that the one-interval pain rating scale procedure with six response categories would measure the pain report criterion directly. However, we were concerned that such judgments would prove too complex for poorly educated subjects. Experience suggests that we probably were mistaken; we recommend that the multicriteria pain rating scale (*2*) be tried for a more direct assessment of the pain criterion locus.

4 March 1980

Associative Behavioral Modification in *Hermissenda*: Cellular Correlates

Abstract. *Three days of training consisting of trials of light paired with rotation produces a long-term modification of photopositive behavior in* Hermissenda crassicornis. *The behavioral modification depends on the temporal association of light and rotation. For animals that received light paired with rotation, significant increases in the spontaneous activity of type B photoreceptors were correlated with changes in photopositive behavior after training. A persistent tonic depolarization of type B photoreceptors can explain the cellular changes correlated with the long-term behavioral modification produced by the temporal association of light and rotation.*

Cellular analysis of long-term behavioral modifications in invertebrates may elucidate neural mechanisms of learning (*1–3*). Conditioning procedures have produced long-term behavioral modifications in gastropod mollusks in which some progress has been made toward defining the neural circuitry and the neural activities involved in behavior (*2*). The eyes, optic ganglia, and statocysts of *Hermissenda* consist of relatively few cells whose synaptic relations and cellular organization have been examined in detail (*4*). Stimulation of the animals' eyes and statocysts with light during rotation resulted in a short-term nonassociative change in photopositive behavior (*5*). Recently, Crow and Alkon (*3*) reported long-term modification of a photopositive response—approaching and moving into an illuminated area (*3, 5*)—in the nudibranch mollusk *Hermissenda crassicornis*. Response latencies of individual *Hermissenda* to enter an illuminated area were significantly longer after training than before in animals receiving training with diffuse light paired with rotation on a modified turntable. Trained animals were significantly different from groups that received random control procedures (*3*). The behavioral modification persisted for several days after training, depended on the temporal association of the training stimuli, increased with practice, reversed to levels before training with repeated testing, and may have exhibited some savings (*3*). The response was specific to the light test and not a change in general activity: When the trained animals were tested in both light and dark, only latencies to enter the illuminated area increased (*6*). The increase can be accounted for by a significant increase in the latency to initiate movement in response to light (*6*). Changes in hair cell responses to light stimuli have been observed after short-term training with light paired with rotation, and stimulation of the isolated nervous system with trials of light paired with rotation resulted in a change in type A photoreceptor responses to light (*7*). We have now found that nervous system modification of the photopositive response in *Hermissenda* is correlated with cellular changes in the type B photoreceptors.

Animal maintenance and automated training (*8*) and testing procedures were used (*3*). The animals were placed on a cycle of $6^1/_2$ hours of light in 24 hours for 3 days before the start of behavioral training. Training, behavioral testing, and intracellular recording from the isolated nervous systems took place during the animals' light cycle. For three consecutive days trained animals ($N = 25$) received 50 trials per day of light (30 seconds) paired with rotation (30 seconds) (average intertrial interval, 2.0 minutes). Control groups (total $N = 25$) received identical trials of random light and random rotation or random rotation alone. Immediately after the last training trial on the third training day, response latency to enter an illuminated area was measured. The circumesophageal nervous systems were then removed and pinned to the Sylgard (Dow Corning) bottom of a recording chamber filled with seawater maintained at 15°C during the recording session. The nervous system was incubated in a solution of digestive enzyme prior to microelectrode penetration of the photoreceptors (protease, type VII, Sigma). Photoreceptors were impaled with single microelectrodes filled with 4*M* potassium acetate for conventional intracellular recording and current injection. A bridge circuit was used to pass current through the recording electrode for input resistance measurements. Current was monitored by a virtual ground current-to-voltage converter or by the voltage drop across a resistor in the ground path. Measurements of spontaneous activity of the photoreceptor, responses to brief light flashes, and input resistance were taken after 15 minutes of dark adaptation. Frequency of B activity was determined for all groups by counting the number of spikes in a continuous 20-second period immediately after dark adaptation. Illumination was provided by a quartz-iodide incandescent lamp. The light was attenuated by neutral density filters. The measurements were taken from one type B cell in an eye, and data from only one eye per preparation were used in the statistical analysis. We found the spontaneous activity in the dark of type B photoreceptors from the group receiving paired light and rotation was significantly higher than that of the two random control groups (Fig. 1). A Cochran test for homogeneity of variance (*9*) showed that the sample variances were not significantly different. The spontaneous activity of the B photoreceptors between the groups differed significantly [$F_{(2, 47)} = 7.32$; $P < .01$]. The a posteriori tests showed that the spontaneous activity of the B photoreceptors from the paired light and rotation group was significantly higher than that of the two random control groups (Newman-Keuls test, $P < .01$). The two random control groups were not significantly different from each other. A replication of the experiment was conducted with a blind procedure. The spontaneous activity of B photoreceptors in trained animals ($N = 6$) was compared with random controls ($N = 5$) under identical maintenance conditions. Trained animals [mean ($\bar{X}$) = 2.84 spikes per second] were significantly different from random controls ($\bar{X} = 2.09$ spikes per second) ($t = 2.47$; $P < .025$).

As before (*3*), the group that received light paired with rotation took significantly longer to enter the illuminated area after training than the random light and rotation control group ($z = 1.79$, $P < .03$) and the random rotation control group ($z = 2.12$, $P < .01$) (Fig. 1B). The latencies to enter the light were not significantly different for the two random control groups. The relationship be-

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tween the activity of B photoreceptors and the behavioral modification was indicated by a significant positive correlation (Spearman $\rho = .63$; $P < .01$) between B spike frequency and the behavioral response latencies to enter light for animals in the paired light and rotation group. In order to control for possible effects due to variation in photoreceptor activity during the light phase of the cycle, some animals were selected at random from the group receiving light paired with rotation ($N = 7$) and random

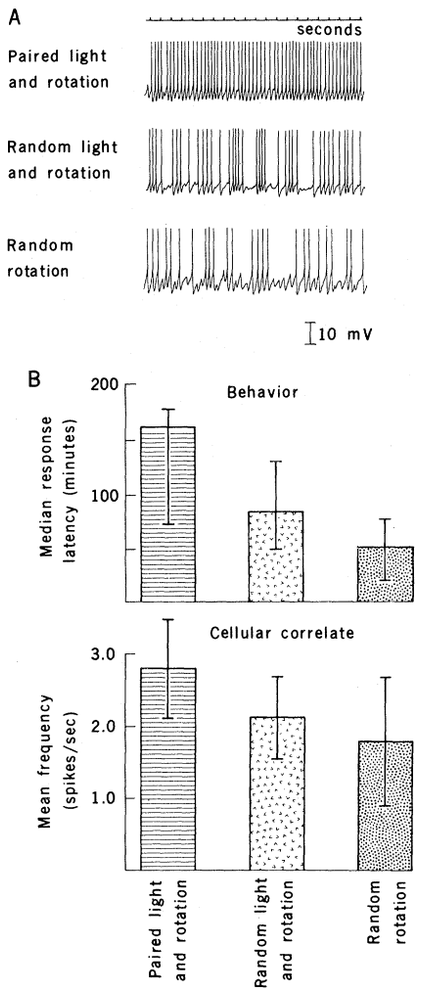

Fig. 1. Effect of training procedures on the spontaneous activity of dark-adapted type B photoreceptors and photopositive behavior. (A) Examples of intracellular recordings from dark-adapted B photoreceptors. (B) Histograms comparing behavioral changes in response to light immediately after the last training trial with the cellular correlate (spike frequency of B photoreceptors) from experimental ($N = 25$) and random control animals (total $N = 25$; random rotation, $N = 7$; random light and rotation, $N = 18$). Behavioral data are expressed as medians ± interquartile ranges. Intracellular recordings were taken after behavioral response. Spike frequency data are shown as mean spikes per second ± standard deviations. Cutoff scores of 180 minutes were used for all groups during behavioral testing.

control group ($N = 8$) at the same time in the light cycle immediately after training but before testing their responses to light. Recordings from type B photoreceptors were then taken at the same time of the light cycle for both experimental and control groups. We again found a difference in the spontaneous spike frequency of dark-adapted B photoreceptors ($t = 3.28$; $P < .01$) between the groups. Therefore, the difference in spike frequency between trained and random controls cannot be explained by variations in activity of the photoreceptors during the light phase of the light-dark cycle.

The increase in spike frequency of type B photoreceptors after training is not the result of disinhibition or an increase in the frequency of excitatory postsynaptic potentials (EPSP's) in the B photoreceptors. To show this, we hyperpolarized the photoreceptors with steady negative current to block spike activity. This revealed a series of inhibitory postsynaptic potentials (IPSP's) in the photoreceptors from both the paired group and the control group. The IPSP's are typical of the synaptic interactions between photoreceptors and are the result of direct inhibitory input from other photoreceptors (*4*). The IPSP frequency was typically greater for cells from paired animals than from random controls. Thus, a decrease in inhibitory synaptic input cannot account for the increase in spike frequency. Hyperpolarizing the photoreceptors to the approximate reversal potential of the IPSP's did not reveal any differences between the paired group and control groups in the frequency of EPSP's. The B photoreceptors from the paired light and rotation group required a significantly greater hyperpolarization, from the resting potential, to block spike activity ($\bar{X} = -13.9$ mV) than did those from random controls ($\bar{X} = -7.6$ mV) ($t = 4.66$, $P < .005$). These results could be explained by a tonic depolarization of the type B photoreceptors as a result of the training procedure. Tonic changes in the membrane potential of locust motoneurons following a conditioning procedure may explain the modification of motoneuron pacemaker activity (*10*). To further investigate the depolarization of type B photoreceptors, we compared the input resistance of the B photoreceptors from the paired group ($\bar{X} = 88.5$ megohms) and the random control group ($\bar{X} = 62.7$ megohms) (Mann-Whitney U test, $U = 4$, $P = .026$).

We then examined the cellular changes after training in a preparation from which impulse activity and synaptic input were eliminated by cutting the optic nerve before its entry into the cerebropleural ganglion (*11*) (Fig. 2A). In the cut nerve preparations, B photoreceptors from animals in the experimental group ($N = 9$) had a higher input resistance than cells from the random control group ($N = 6$) ($U = 10$, $P = .025$) (Fig. 2, B and C). The membrane potential of isolated photoreceptors from cut nerve preparations from trained animals was more depolarized ($\bar{X} = -56.5$ mV) than the random control group ($\bar{X} = -62.25$) ($U = 5$, $P = .024$). The electrophysiological results from the isolated photoreceptors of cut nerve preparations are consistent with the observations

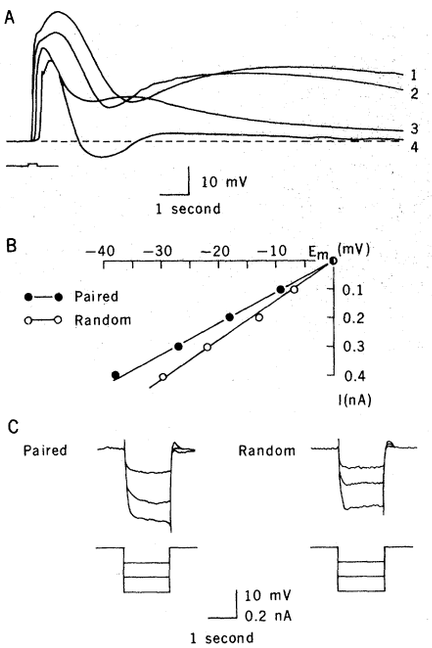

Fig. 2. Cellular changes in cut nerve preparations from experimental and random control animals. (A) Receptor potentials of dark-adapted type B photoreceptor from an experimental animal (light paired with rotation). Responses evoked by brief light flashes of increasing intensity (4), −log 3.0; (3), −log 2.0; (2), −log 1.0; (1), −log 0.5. Dashed line indicates resting membrane potential and lower trace indicates duration of light flash. The absence of spikes and synaptic potentials indicate that the photoreceptor soma was successfully isolated from the area of spike initiation and synaptic input. (B) Representative linear current-voltage relationship of dark-adapated isolated (cut nerve) type B photoreceptors from experimental (paired) and random control groups. (C) Examples of changes in membrane potential of dark-adapted isolated B photoreceptors from experimental and random control animals. Electrotonic potentials evoked by hyperpolarizing square current pulses (bottom traces) through a balanced bridge. Resistance measurements taken with the single electrode-bridge circuit are consistent with data from experiments in which the photoreceptors were impaled simultaneously with two microelectrodes for current injection and voltage recording (*15*).

from intact B photoreceptors after training. The increase in input resistance and the persistent tonic depolarization of the B photoreceptor could be explained by a decreased potassium conductance.

Specific ionic conductances invoked during a light stimulus (step) have been recently examined (*12–15*). The initial depolarizing transient arises mainly from an inward Na^+ current. The hyperpolarizing phase of the light response arises from an outward K^+ current. A sustained depolarizing light response during stimulation arises from an inward voltage-dependent Ca^{2+} current (*15*). After a light step, the slowly decreasing depolarization, long-lasting depolarization (LLD), arises from a slowly decreasing inward Ca^{2+} current and probably a decrease of resting K^+ current (*15*).

The amplitudes of the hyperpolarizing phase (measured with respect to the peak amplitude of the initial depolarizing transient ($U = 5$, $P = .024$) and the depolarizing tail (LLD) of the light response ($U = 7$, $P = .053$) were significantly increased in cut nerve preparations from trained animals as compared with random controls. These differences in generator potential waveforms are consistent with the other cellular changes, indicating that training results in a persistent depolarization of type B photoreceptors. Namely, for a more depolarized cell the amplitude of the initial depolarizing transient would decrease as the cell membrane potential is moved closer to the sodium equilibrium potential (E_{Na}), and the hyperpolarizing phase would increase as the membrane potential is further from the potassium equilibrium potential (E_K). The LLD after the light step would increase in the more depolarized cells of trained animals because of the voltage-dependence of the light-induced Ca^{2+} current.

All of the results can be explained by a persistent decrease of a voltage-dependent K^+ conductance across the type B photoreceptor membranes. Such a decrease of K^+ conductance in darkness would produce an increase in input resistance. It would also cause the observed changes in the light-induced voltage responses. These findings, in turn, may be a consequence of long-term changes of intracellular Ca^{2+} to which dark K^+ conductances are sensitive (*15*).

TERRY J. CROW
DANIEL L. ALKON

Laboratory of Biophysics, National Institute of Neurological and Communicative Disorders and Stroke, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

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Chemical Feeding Deterrent Mobilized in Response to Insect Herbivory and Counteradaptation by *Epilachna tredecimnotata*

Abstract. *Experimentally damaged leaves of* Cucurbita moschata *mobilize substances to the damaged region within 40 minutes. These substances stimulate feeding by* Acalymma vittata *(Coleoptera: Chrysomelidae) and inhibit feeding by* Epilacha tredecimnotata *(Coleoptera: Coccinelidae). Under natural conditions,* Epilachna *cuts a circular trench in the leaf before feeding on the encircled leaf material, thus preventing mobilization of the deterrent substances to the feeding area.*

Levin (*1*) has divided chemical defenses of plants into two broad classes: those present before herbivore attack and those that change in response to herbivore attack. The latter class of defense, termed induced resistance, is commonly used by higher plants in response to infections by microorganisms but is considered to be less commonly used to counterattack insects.

Most examples of insect-induced resistance are characterized by long response times. Wallner and Walton (*2*) demonstrated that gypsy moth female larvae that fed on oak leaves from previously defoliated trees weighed less than moths reared on leaves from trees with no history of defoliation. Röttger and Klingauf (*3*) found that physiological changes in beets that had been attacked by the beetfly *Pegomya betae* caused a 29 percent increase in beetfly mortality. Developmental time increased in the geometrid larva *Oporinia autumnata* when fed on undamaged leaves of branches from which a single leaf had been torn 2 days earlier (*4*). In general, the time response for an insect-related, induced resistance appears long, relative to the feeding times of individual insects. Rhoades (*5*) lists plant response times that range from 12 hours to several years.

We now present experimental evidence for rapid, induced resistance and a behavioral counterresponse by *Epilachna tredecimnotata* (Coleoptera: Coccinelidae) when feeding on leaves of squash, *Cucurbita moschata* (Cucurbitaceae), in southeastern Mexico (*6*). *Epilachna tredecimnotata* and perhaps other cucurbit-feeding species in this genus (for example, *E. borealis*) engage in characteristic solitary feeding. Enlarged apical mandibular teeth are used to cut a circular trench in a squash leaf. The leaf tissue is almost completely cut through; only a few veins and bits of lower epidermal tissue hold the encircled leaf section in place. The beetle then feeds on the encircled material. Trenching behavior takes approximately 10 minutes while complete feeding on the leaf disk takes between 1 and 2 hours. After a morning feeding period the beetle crawls away from the damaged leaf and does not feed until the following morning. We propose that this trenching behavior is an adapt-

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