

dins of the E series. These novel compounds, therefore, are apparently the most abundant prostaglandins in the richest known source of mammalian prostaglandins.

Since this work was completed, the occurrence of 19-hydroxy prostaglandins  $E_1$  and  $E_2$  has been found by Taylor and Kelley (10), who also obtained the dehydration products (11).

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3. To minimize the possibility of oxidation of the prostaglandins, solvents were freshly distilled and purged with dry nitrogen. All apparatus was flushed with dry nitrogen.
4. Parallel studies with [ $^3H$ ]prostaglandins  $B_1$ ,  $E_1$ , and  $F_{1\alpha}$  showed that these compounds were all eluted in the "prostaglandin" fraction.
5. An LKB 9000 instrument, equipped with a glass column (2.7 m by 0.6 cm) containing 1 percent SE-30 on Gas-Chrom Q (100 to 120 mesh); flash heater, 270°C; the column temperature was programmed from 140° to 290°C at 2 degrees per minute; molecular separator, 250°C; electron energy, 22.5 eV.
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## Localized Desensitization of Limulus Photoreceptors Produced by Light or Intracellular Calcium Ion Injection

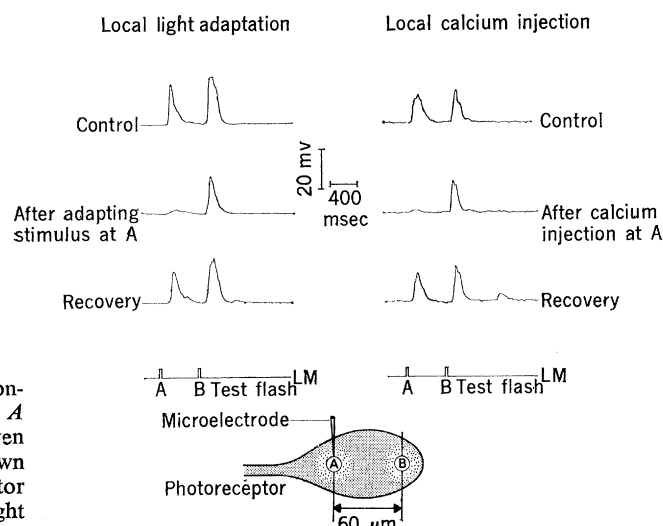
**Abstract.** Spots of light were used to measure the light sensitivity of spatially separated regions of single *Limulus* photoreceptors. The desensitization caused by irradiating part of the cell was largest in the irradiated region. The desensitization caused by intracellular calcium ion injection was largest near the injection site. The spread of desensitization away from the injection site suggests that calcium ion can diffuse over neuronal dimensions, but that the effective rate of diffusion is not so high as to abolish calcium gradients. The results are compatible with the previously proposed hypothesis that a rise in the intracellular calcium ion concentration mediates light adaptation.

Photoreceptors have the ability to adapt to light and dark, that is, to change their sensitivity. Some photoreceptors are sufficiently large so that small areas of the transducing membrane can be illuminated. The adaptation caused by irradiating part of a cell tends to be localized to the region of illumination (1).

Lisman and Brown (2) showed that injection of  $Ca^{2+}$  reduced the response of *Limulus* ventral photoreceptors to spatially uniform illumination and proposed that a light-induced increase in the intracellular calcium concentration ( $Ca_i^{2+}$ ) (3) is a factor controlling light adaptation. Experiments by Fein (4) indicate that in these cells light adaptation is localized to the region of illumination. Therefore, if the  $Ca^{2+}$  hypothesis is correct, local changes in sensitivity would be caused by local changes in  $Ca_i^{2+}$ . This would imply that cytoplasmic  $Ca^{2+}$  gradients can occur over neuronal dimensions ( $< 100 \mu m$ ); however, such gradients have not previously been described.

We examined the possibility of cytoplasmic  $Ca^{2+}$  gradients by injecting  $Ca^{2+}$  into one end of a *Limulus* ventral photoreceptor (schematized in Fig. 1) and monitoring the change in the responses to two spots of light, one placed at the injection site (A) and the other at the opposite end of the cell (B). The photoreceptors are located on the lateral olfactory nerve, which was dissected, desheathed, and mounted in a small chamber with a transparent base. Single photoreceptors ( $\sim 50$  by  $150 \mu m$  in cross section) (5) were observed through a compound microscope ( $\times 400$ ). The cell was illuminated from below by two spots of light that could be positioned on it. All experiments were carried out under visual control. Cells were penetrated with a  $Ca^{2+}$ -containing electrode (6), which was used both for iontophoretic injection of  $Ca^{2+}$  and for recording the responses evoked by the two spots. Despite efforts to minimize light scatter (7), the spots, which were nominally  $10 \mu m$  in diameter, appeared considera-

Fig. 1. Localized desensitization produced by a local adapting light or  $Ca^{2+}$  injection. The lower part of the figure is a schematized version of the photoreceptor, showing the two stimulus spots labeled A and B, with the intracellular  $Ca^{2+}$ -containing electrode aligned with the spot at A. Two test flashes of constant intensity, one at A and one at B, were given in succession, as shown by the light monitor (LM). In the local light adaptation experiment (left, middle trace) the test flashes occurred 3 seconds after termination of a local adapting stimulus at A (5 seconds in duration). In the  $Ca^{2+}$  injection experiment (right, middle trace), the test flashes occurred 3 seconds after termination of the injections (2 na for 10 seconds). Note that there was a small change in the amplitude of the photoreponse between the local light adaptation and the  $Ca^{2+}$  injection experiments.



bly larger (15 to 20  $\mu\text{m}$ ). The centers of the spots were typically separated by 60 to 80  $\mu\text{m}$ , and therefore there was little overlap of the two stimuli.

Under the experimental conditions described above, local light adaptation could be demonstrated (Fig. 1). Cells were irradiated for several seconds with a dim adapting light at *A*. The responses to subsequent test flashes at *A* were greatly attenuated, whereas the responses to flashes at *B* were nearly unaffected. Injections of  $\text{Ca}^{2+}$  were then made in the same cell. An injection current of 2 na for 10 seconds reduced the response at *A* but had little effect at *B*. The peak amplitude of the responses at *A* (local site) and *B* (distant site) are plotted as a function of time before, during, and after  $\text{Ca}^{2+}$  injection in Fig. 2a. The curve connecting the data points is not smooth because of variation in the number and amplitude of the quantal events which summed to give the responses (8) to the dim stimuli used. Figure 2a shows that the response decrement at the local site was present for several minutes after the injection and that during this period there was little effect at distant parts of the cell. For larger  $\text{Ca}^{2+}$  injections (Fig. 2b) desensitization spread to distant regions of the cell; however, the desensitization was larger and lasted longer at the local site. Differences in sensitivity between the two sites lasted for up to 5 minutes after the injection. The effects of  $\text{Ca}^{2+}$  injection were not artifacts of the injection process, since similar injections of  $\text{K}^+$  had no significant effect.

Changes in threshold can be estimated from the amplitude changes in Fig. 1 by using the (nearly linear) intensity response curves from that cell. We estimate that after both  $\text{Ca}^{2+}$  injection and light adaptation, the decrease in threshold was 0.9 log unit at *A* and less than 0.2 log unit at *B* (9). In other cells we have directly measured localized threshold changes of this magnitude produced by local adapting stimuli (10). In the four cells examined, an adapting spot at *A* chosen to raise the threshold at *A* by 1.1 log units caused a rise of the threshold at *B* of  $0.3 \pm 0.1$  log unit. Spatially uniform illumination of the same cells which raised the threshold at *A* by 1.1 log units raised the threshold at *B* by  $1.2 \pm 0.3$  log units.

Localized effects of light adaptation and  $\text{Ca}^{2+}$  injection were observed in cells where the absolute thresholds at *A* and *B* were nearly the same (within 1 log unit) before injection. In many

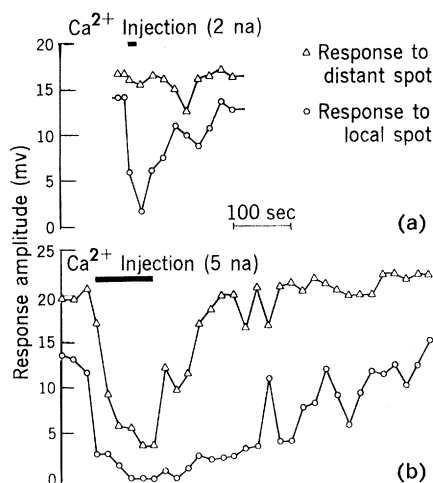


Fig. 2. Photoreponse amplitude as a function of time before, during, and after  $\text{Ca}^{2+}$  injections. Responses are to stimuli of constant intensity at the local site (*A* of Fig. 1) and the distant site (*B* of Fig. 1). To reduce some of the response variability from flash to flash, two consecutive peak amplitudes were averaged and the result plotted. Responses "during" the injection period were recorded during brief interruptions of the current. In (b) the absolute change in response amplitude due to  $\text{Ca}^{2+}$  injection is greater at the distant site ( $\sim 17$  mv) than at the local site ( $\sim 13$  mv). However, it is the proportional change in response amplitude that is related to sensitivity changes. The sensitivity decrease at the local site is more than a factor of 30, whereas that at the distant site is no more than a factor of 6.

cells impaled with a  $\text{Ca}^{2+}$  electrode, the threshold at the impalement site was about 2 log units higher than the threshold at distant regions of the cell, and localized effects of light adaptation or  $\text{Ca}^{2+}$  injection could not be demonstrated. We suspect that sensitivity near the electrode at *A* was lowered because  $\text{Ca}^{2+}$  leaked out of the electrode. Under these circumstances, the threshold intensity of the stimulus at *A* is so high that the response may be generated by the more sensitive region near *B* responding to light scattered from the insensitive region. If the responses to stimuli at *A* and *B* are both generated at or near *B*, local effects would not be expected.

We assume that the desensitization of the photoreponse brought about by  $\text{Ca}^{2+}$  injection is due directly to the injected  $\text{Ca}^{2+}$  ions (11). Given this assumption, the results of our experiments indicate that  $\text{Ca}^{2+}$  can diffuse from one end of the cell body to the other. However, the effective rate of diffusion is not sufficiently high to eliminate  $\text{Ca}^{2+}$  gradients (12).

It might be thought that local light adaptation (Fig. 1) is related to pig-

ment bleaching in the illuminated region of the cell. A component of adaptation that is correlated with pigment concentration is well known in the vertebrate retina (13). However, recent work has shown that adaptation in the ventral photoreceptors is unrelated to the recovery of visual pigment [measured either photometrically (14) or by using the early receptor potential (15)].

Our results appear to be consistent with the hypothesis that a light-induced rise in  $\text{Ca}_i^{2+}$  is a factor controlling light adaptation (2). The results may be interpreted to indicate that local illumination leads to a localized rise in  $\text{Ca}_i^{2+}$  and that the effective rate of diffusion of  $\text{Ca}^{2+}$  is sufficiently low that adaptation tends to be localized to the region of illumination.

Our experiments have implications for other neuronal functions thought to be regulated by  $\text{Ca}_i^{2+}$  [transmitter release (16), repolarization in snail neurons (17), and excitation in vertebrate photoreceptors (18)]. Our results suggest that  $\text{Ca}^{2+}$  can serve as an intracellular messenger that carries information from a local  $\text{Ca}^{2+}$  source to a distant ( $\sim 80 \mu\text{m}$ ) site. Furthermore, spatially nonuniform entry of  $\text{Ca}_i^{2+}$  into the cytoplasm could lead to non-uniform activation of the  $\text{Ca}^{2+}$ -controlled processes, a situation which could add considerable complexity to neuronal function.

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6. Micropipettes were filled with a solution containing 0.09M  $\text{Ca}(\text{OH})_2$ , 0.1M tris, and 0.1M ethyleneglycol-bis( $\beta$ -aminoethyl ether)-*N,N'*-tetraacetic acid (2). Only a fraction of the current passed through these electrodes is carried by  $\text{Ca}^{2+}$ . J. P. Reuben, P. W. Brandt, and H. Grundfest [*J. Mechanochem. Cell Motility* **2**, 269 (1974)] have determined that for these electrodes the transport number for  $\text{Ca}^{2+}$  is 0.2. These electrodes had resistances of 100 to 150 megohms when measured in the artificial seawater that bathed the preparation. The artificial seawater consisted of 435 mM NaCl, 10 mM KCl, 10 mM  $\text{CaCl}_2$ , 25 mM  $\text{MgSO}_4$ , 20 mM  $\text{MgCl}_2$ , and 10 mM tris and had pH 7.8.

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10. The photoreceptors were light-adapted with repetitive flashes (40 msec) given once every 10 seconds. Thresholds were measured 1 to 2 seconds after an adapting stimulus by determining the relative energy of a 20-msec flash which elicited a criterion photoresponse. The results were independent of the particular criterion chosen in the range of 2 to 6 mv. These experiments were done on cells which did not exhibit the large regenerative responses sometimes observed in *Limulus* photoreceptors.
11. The rate-limiting step in the recovery from  $\text{Ca}^{2+}$  injection might be dissociation of  $\text{Ca}^{2+}$  from a regulatory site rather than reduction of  $\text{Ca}_i^{2+}$ . If so, the amplitude change during the recovery period would no longer serve as a measure of the instantaneous value of  $\text{Ca}_i^{2+}$ .
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## Predation and Aversive Conditioning in Coyotes

The control of coyote predation is a problem of interest to behavioral biologists, students of wildlife management, and ranchers alike. Indeed, the report by Gustavson *et al.* (1) that suggests that coyote predation may be controlled by aversive conditioning involving bait laced with lithium chloride (LiCl) does contain some interesting ideas. Unfortunately, their data and behavioral criteria do not support adequately their hypothesis that scattering baits "that smell like sheep, taste like sheep, and contain a nonlethal emetic toxin" will control coyote predation. Consequently, further reference to this report (1) in a subsequent article by Garcia *et al.* (2, p. 830) is unjustified.

Briefly, the data of Gustavson *et al.* for their first test with lamb bait laced with LiCl involved only three of their subjects, and two of the three immediately killed a lamb after receiving aversive conditioning. The fact that both of these animals showed an increased latency to feed and decreased feeding rate is quite meaningless, since a dead lamb is a dead lamb! The fact is, prey was killed. When these two "killers" were subjected to a second session of aversive conditioning, the methodology was changed. However, this fact was not taken into account in the hypothesis offered in either of the reports by this research group (1, 2). The methodological alteration consisted of giving an intraperitoneal injection of LiCl after

the animals had been unsuccessfully conditioned by mere exposure to LiCl-laced bait. Therefore, the appropriate conclusion should be that two trials with LiCl-laced bait and one injection of LiCl were necessary to stop attack by two of three animals. The fact that an injection was also given is of paramount importance when considering the hypothesis that is proposed by these authors (1, p. 583; 2, p. 830), and this is not evident in table 1 of Gustavson *et al.* (1).

The hypothesis that coyote predation may be controlled by some type of aversive conditioning is interesting but, as yet, unsupported. The apparent requirement for LiCl injection makes the proposed method of control impractical because of the obvious difficulties involved in performing this operation in the field.

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Gustavson *et al.* (1) tested these hypotheses: "(i) Can . . . aversions be . . . readily established in a feral carnivore which preys principally on animals? (ii) Will gustatory aversions

inhibit attack behavior . . . [on] . . . living prey? (iii) Can the inhibitory effect be limited to a specific prey . . . ?" Thus injections were entirely appropriate. The data were affirmative, so it was suggested post hoc that field trials with toxic emetic baits and lithium-perfused sheep carcasses were now in order. Research is under way on these methods without injections.

Our progress was recently reported to the Coyote Research Workshop, Denver, Colorado, 14 to 17 November. Films demonstrated that consumption of a single sheep flesh bait treated with lithium chloride (LiCl) was sufficient to block a subsequent attack upon a sheep by a pair of hungry sheep-killing wolves. Coyote research, carried out on rabbits, since small lambs were not yet available, indicated that oral consumption of either baits or carcasses treated with LiCl blocked predatory attacks in one or two trials. Furthermore, a single meal of deer meat treated with LiCl caused a hungry cougar to have an aversion to deer meat but left its appetite for cow and horse meat intact. More research is needed, but the results so far are very promising.

Bekoff presents an alternative post hoc suggestion that the injection of LiCl was of "paramount importance." We disagree. He presents no data, but Garcia *et al.* (2) reviewed the related research. Potent variables for food aversions are (i) flavor strength, (ii) illness intensity, and (iii) time between consumption and illness. Route of toxin administration is a relatively trivial variable. Food aversions have been obtained in a wide variety of species in many laboratories with oral administration of LiCl. Perhaps Bekoff thinks the jab of a needle will deter the coyote, but research indicates that peripheral pain is not very effective for establishing food aversions. Illness is required. We would welcome Bekoff's research on his hypothesis.

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