References and Notes

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8. Supported by PHS grant HD-01253.

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Laser Stimulation of Nerve Cells in Aplysia

Abstract. *Laser radiation at 488 nanometers selectively stimulates neurons in the abdominal ganglion of the marine mollusk* Aplysia californica. *The laser radiation can be scanned over the surface of the ganglion and can be effectively utilized in mapping cellular interconnections. The laser appears to cause these changes through some mechanism other than damage.*

A method for stimulating selectively and reversibly any of a large array of neurons within a short time period would be highly useful in studies of individual cells and especially in the mapping of cellular interconnections. I report here the development of such a technique in which laser radiation is applied to the abdominal ganglion of the marine mollusk *Aplysia californica*. This preparation possesses some cells that have light-sensitive properties (*1*) and has a number of identified neurons as well as some unidentified neurons whose locations have not yet been specified (*2*). [The notation identifying various cells is given in (*2*).]

Intracellular recordings were obtained from identified cells by impaling them with conventional microelectrodes filled with 2*M* KCl and then illuminating either those cells or the surrounding regions of the ganglion with a blue (488 nm), green (515 nm), or near-infrared (1060 nm) laser beam having a minimum spot size (*3*) of about 10 μm. The beam penetrated the ganglion with an attenuation of ~50 percent. The experimental arrangement is shown in Fig. 1.

The laser stimulation of the cells with blue or green light produced firing with the light pulse "on" in some cases and with the light pulse "off" in others. An example of light-induced firing in a silent cell (cell R2) is shown in Fig. 2. The irradiation caused an initial depolarizing component which gradually returned to near rest potential. With the light pulse "off" the membrane potential dropped momentarily and then moved above the critical firing threshold, causing spiking. The change in membrane potential was typical of the responses seen in seawater at room temperature (~ 21°C). In some instances, particularly with the addition of ouabain, firing occurred during the light pulse whereas in others firing occurred only as a rebound to turning the laser beam off. These effects could be reproducibly obtained at levels of intensity which did not cause significant damage (abrupt irreversible depolarization) (*4*). With this technique it was possible to obtain firing in each of a representative sample of 12 identified cells located on the dorsal surface of the ganglion (cells L1, L2, L3, L4, L6, L7, L8, L9, L11, R1, R2, and R15). None of these cells was selected for any unusual photoreceptive properties; presumably this technique will be effective in stimulating almost any cell in the ganglion.

The capacity to activate individual cells without impaling them could pro-

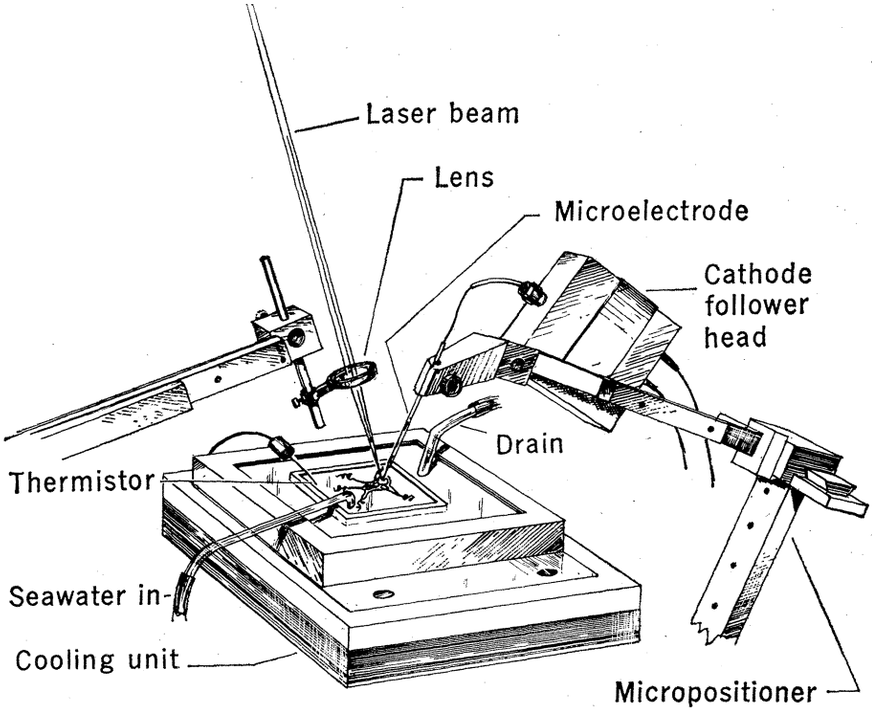

Fig. 1. Experimental arrangement. The lens is mounted on a micropositioner which permits scanning of the beam across the ganglion as well as focusing in the vertical direction.

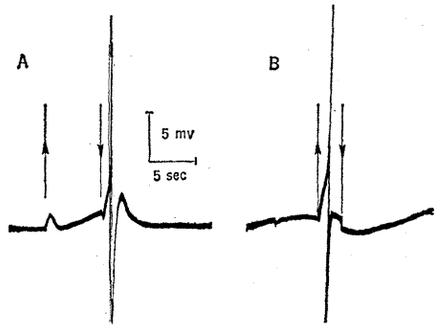

Fig. 2. Typical response of a silent cell (cell R2) to 488-nm light (A) in normal seawater (12.5 mw of beam power), and (B) in seawater and 1.37 m*M* ouabain (4.5 mw of beam power). The arrow pointing upward indicates that the light pulse is "on," and the arrow pointing downward indicates that the light pulse is "off." In normal seawater the initial depolarization was followed by an approximate return to rest potential with firing with the light pulse "off." In ouabain solution less than half the light intensity causes firing with the light pulse "on." The full nerve spikes (not shown) were ~ 90 mv peak-to-peak for the various recordings presented.

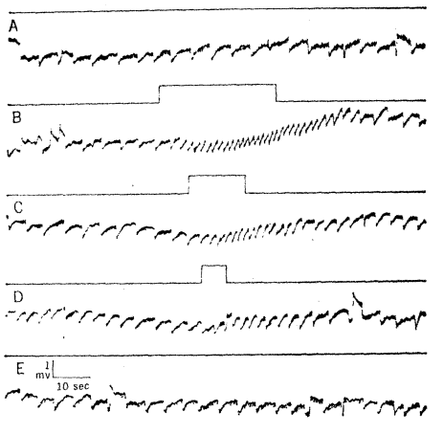

Fig. 3. Response of a spontaneously firing follower cell (cell L7) on directing the blue (14-mw, 488-nm) beam with a 10-μm spot size at the ganglion surface, onto the center of the left hemiganglion. The IPSP's correspond to input from interneuron II. The traces show (A) a control (no illumination) followed by [(B) through (D)] three periods of illumination of decreasing duration followed by (E) a return to control. The latency of the response was typical. Here the principal effect has been to speed up the response of interneuron II. In other cases, interneuron II was silent and was stimulated into a burst by the illumination.

vide a rapid and highly useful scanning technique for mapping connections between cells. In a preliminary study, I examined two neurons, one of which is known to receive synaptic input from an identified interneuron and the other from an unidentified interneuron. First I observed cell L3, which received distinctive inhibitory postsynaptic potentials (IPSP's) from an identified interneuron (cell L10), and I found that I could trigger these IPSP's by stimulating a region in the left caudal quarter ganglion known to contain the interneuron. I then searched for the location of interneuron II, whose position in the ganglion was unknown at the time (*5*) and whose existence within the ganglion had been inferred from the characteristic IPSP's it produces in cell L7 and the simultaneous postsynaptic potentials produced in a number of other cells. The laser beam was scanned over the ganglion and the location was noted where laser stimulation was most effective in producing the characteristic burst of IPSP's in cell L7 (see Fig. 3). In repeated experiments in over ten preparations, this interneuron could always be activated by directing the light onto the central area of the left hemiganglion, a result which suggests that the interneuron is located within this area. Although scattering of the laser beam in the tissue renders the localization of the unidentified interneurons rather crude (within a circle with a diameter of about 300 μm), this technique nonetheless holds considerable promise for unraveling connections within this ganglion. Attempts are being made to improve this technique by limiting the area of stimulation still further (*6*).

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3. For this work the spot size is defined as twice the radius of the 1/*e* circle of the amplitude distribution, where *e* is the base of the natural logarithmic system. See, for example, G. D. Boyd and J. P. Gordon [*Bell Syst. Tech. J.* **40**, 489 (1961)] for a discussion of laser-beam spot size.
4. As a means of testing the thermal character of the stimulation, the response of the cells to infrared radiation at 1060 nm was compared with that at 488 nm. Very weak hyperpolarizing responses as expected from warming [see D. O. Carpenter, *J. Gen. Physiol.* **50**, 1470 (1967)] were observed with 1060-nm radiation at a level ranging up to 20 times that required for maximum responses at 488 nm. Although selective absorption of 488-nm radiation by small, highly pigmented regions could, by some unusual effect, cause the observed responses, the most reasonable interpretation of this evidence suggests a nonthermal stimulation mechanism.
5. In a recent experiment performed in collaboration with J. E. Blankenship, of New York University Medical Center, we were able, using the laser beam as a guide, to locate and impale interneuron II in the area of the left hemiganglion indicated by earlier experiments.
6. A considerable effort was also made to identify the specific mechanisms of the light-induced stimulation by means of blocking agents and ion-free solutions. Although the results strongly suggested that the light acted by opening the Na gate and stimulating activity of the Na-K exchange pump, the occasional persistence of a residual inhibitory response in the presence of ouabain cast some doubt on this simple picture of the light response.
7. I thank J. E. Blankenship and E. R. Kandel for advice and assistance in carrying out this work, as well as H. Wachtel, who suggested the possibility of stimulating *Aplysia* neurons with the laser beam. L. J. Heilos and Mrs. C. R. Leavens assisted ably with the apparatus construction and sample preparation.

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Cell-Free Protein Synthesizing System from Yeast Mitochondria

Abstract. *An active, cell-free protein synthesizing system has been obtained from yeast mitochondria. The system is stimulated by both polyuridylate and R17 RNA and is sensitive to inhibitors of bacterial protein synthesizing systems. A comparison is made between this system and that found in the cytoplasm of yeast.*

It is now apparent that mitochondria possess a protein synthesizing system (*1*). Ribosomes (*2*, *3*), transfer RNA (*4*), and polymerization enzymes (*5*) have been demonstrated in mitochondria of a variety of organisms. In addition, inhibitors of bacterial protein synthesizing systems significantly decrease the rate of amino acid incorporation into mitochondria of *Saccharomyces cerevisiae* in vivo and in vitro, but are without effect on the cytoplasmic incorporating system (*6*). This suggests that yeast, like *Neurospora* (*5*), contains a bacterial-like protein synthesizing system in the mitochondrion, but a mammalian-like system in the cytoplasm (*7*). Similar results have been found with mitochondria from rat liver (*8*) and mammalian cells (*9*).

We have developed a cell-free protein synthesizing system from yeast mitochondria enabling, for the first time, a comparison with that of the cytoplasm (*10*).

Mitochondrial ribosomes were isolated from *Saccharomyces cerevisiae* strain IL46 or Y55 (*3*). Supernatant enzymes were prepared as an 80 percent ammonium sulfate precipitate supernatant resulting from centrifugation at 105,000*g*; 50 μg of mitochon-

Table 1. Characterization of mitochondial cell-free protein synthesizing system.

Additions or omissions	Percentage of complete system	
	Poly(U) directed	R17 RNA directed
Complete	100	100
Minus ribosomes	0	0
Minus poly(U) or R17 RNA	67	22
Plus 100 μg of poly(U)	360	
Minus ATP, UTP, CTP, GTP	25	13
Minus nucleotides and PEP, PEP kinase	0	12
Minus PEP, PEP kinase		40
Minus 1.0*M* NH_4Cl ribosomal factors		29
Magnesium optimum (m*M*, magnesium acetate)	16	12