Phycomyces: Stimulus Storage in Light-Initiated Reactions

Abstract. *The sporangiophores of* Phycomyces blakesleeanus, *a unicellular fungus, increase their growth rate temporarily when given a symmetric light pulse. When a sporangiophore is cooled until normal growth stops, the light pulse can still be perceived, and, after the sporangiophore is warmed to room temperature, the normal light-growth response is observed. Thus stimulus information can be "stored" at low temperatures in this sensory system.*

The sporangiophores of the fungus *Phycomyces* show two classes of light responses (*1*). The first, phototropism, is a consequence of asymmetrical illumination, that is, a spatial inhomogeneity of light. The second, the light-growth response, is a consequence of a change in the intensity of symmetric illumination, that is, a temporal inhomogeneity of light. When light is arranged about the sporangiophore symmetrically (for instance bilaterally), and the light intensity is given a short (1 minute) steplike increase, then there is a temporary increase in the growth rate. If the increase in light intensity is maintained rather than cut off as in a pulse, then the growth rate will, nevertheless, return to normal. Thus, in continuous illumination, the growth rate is independent of the background light intensity.

One of the intermediate steps in the chain of events between stimulus and response can be stabilized at a given temperature. Thus, when a sporangiophore is cooled and growth stops, a light stimulus can nevertheless be perceived and stored so that, when the *Phycomyces* is returned to room temperature, the light reaction will take place (*2*).

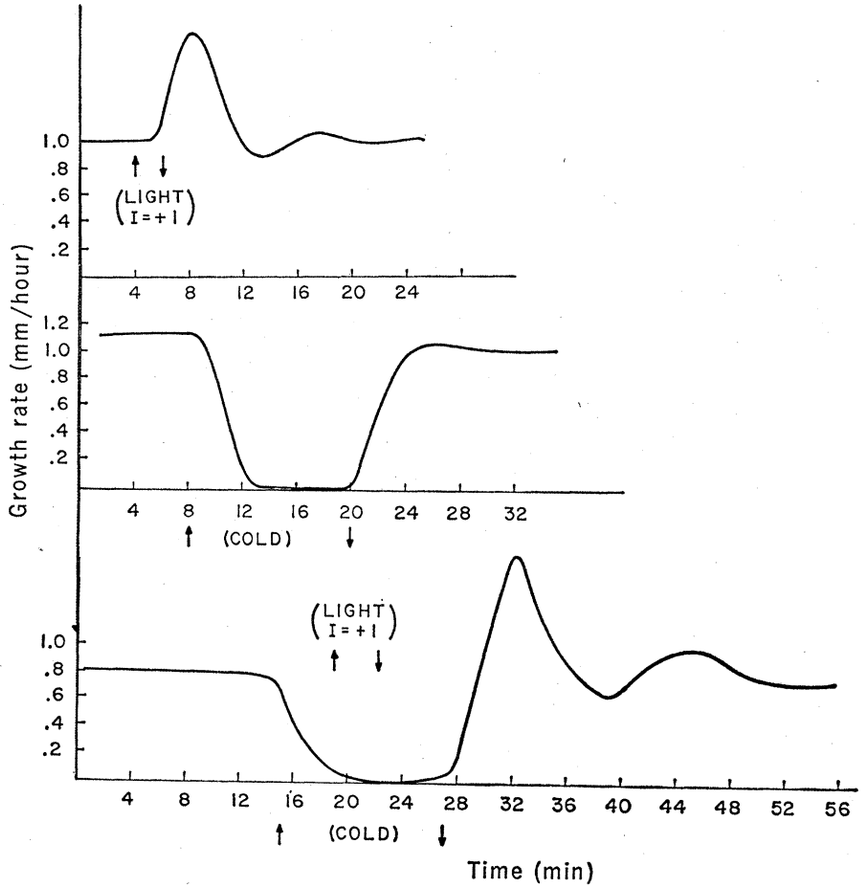

Fig. 1. (Top) Typical light-growth response of a *Phycomyces* sporangiophore. The time from when the light is turned on till the onset of the response is about 2.5 minutes and is independent of the size of the stimulus. The time to the peak is about 4.5 minutes. (Center) Effect of gently cooling a *Phycomyces* sporangiophore and then allowing it to return to room temperature. (Bottom) Stimulus storage in a *Phycomyces* sporangiophore. After the sporangiophore was cooled, and it had stopped growing, a 5-minute light pulse is given. After 8 minutes the sporangiophore is returned to room temperature and a light-growth response is observed.

Phycomyces blakesleeanus was grown on potato dextrose agar. The sporangiophores develop through four distinct stages (*1*). Our experiments were done with stage 4 sporangiophores. The rate of growth of the sporangiophores was measured with a microscope and graticule modified according to Dennison (*3*).

To obtain symmetrical illumination the sporangiophores were illuminated with blue light by two lamps placed opposite each other. Red light was used as the "working" safe light.

The best type of device to cool the air around the sporangiophores consisted of a glass enclosure for the sporangiophore around which ice could be placed. It was found that if the temperature changes were to affect the growth rate reversibly, the temperature decrease must be gradual and the ice was found to give a sufficiently slow rate of cooling.

In these experiments we observed the light-growth response rather than phototropism, because unwanted movements due to temperature changes were less pronounced for the light-growth measurements. The temperature of the cooled sporangiophores was between 1° to 5°C. At these temperatures the sporangiophores, which normally grow at about 1 mm per hour, stopped growing.

Several combinations of light and temperature were used. Figure 1 shows a typical light-growth response, that is, one without any temperature changes made. The light-growth response (Fig. 1A) was elicited by a moderate pulse increase of light intensity (*4*). Without cooling, the time from when the light was turned on until the maximum growth rate was achieved was about 4.5 minutes. When a 5-minute bilateral pulse was given 5 minutes after the start of a 12-minute cold period, there was no light-growth response. Then the cooling device was removed so that the sporangiophore was exposed to air at room temperature, and a normal light-growth response was observed. These experiments were repeated several times.

THOMAS G. EBREY*
R. K. CLAYTON
Section of Genetics, Development, and Physiology, Cornell University, Ithaca, New York 14850

References and Notes

1. M. Delbrück, *Ber. Deut. Bot. Ges.* **75**, 411 (1963); W. Shropshire, *Physiol. Rev.* **43**, 38 (1963); E. S. Castle, *Science* **154**, 1416 (1966).
2. "The postilion hung his great coat on a peg and sate down near the kitchen fire, to forget and drown his cares. I sat down on the other side doing the same. Suddenly we heard a tereng! tereng! teng! teng! We looked round, and now found the reason why the postilion had not been able to sound his horn. His tunes were frozen up in the horn, and came out now by thawing, plain enough, and much to the credit of the driver, so that the honest fellow entertained us for some time with a variety of tunes, without putting his horn to his mouth."—*The Singular Adventures of Baron Munchausen.*

   There have been several reports of stimulus storage at low temperature. G. E. Fogg [in *The Growth of Plants* (Penguin Books, Baltimore, Md., 1963)] states that if Mimosa leaflets are mechanically stimulated at 10°C, they do not fold up. When, however, the plant is placed at room temperature, the leaflets fold. Similarly Brauner and Hager [*Planta* **51**, 115 (1958)] report that bean plants, when placed on their side in the cold and later placed upright at room temperature, show a delayed geotropic response. Indeed, it might be speculated that stimulus storage at low temperature can be observed in all sensory systems. However, the rapidity of some responses to a sensory stimulus may make detection of the responses to some types of stored stimuli difficult.
3. D. Dennison, thesis, California Institute of Technology (1958).
4. M. Delbrück and W. Reichardt, in *Cellular Mechanisms in Growth and Development*, D. Rudnik, Ed. (Princeton Univ. Press, Princeton, N.J., 1956). By the system of Delbrück and Reichardt $I = +1$ and $t = (2)$.

5. An account of this work was given at the 12th Biophysical Society Meeting at Pittsburgh (1968). We thank R. A. Cellarius (University of Michigan) for his suggestion of the story of Baron Münchausen and M. Delbrück (California Institute of Technology) and J. R. Platt (University of Michigan) for advice. Much of this work was done at the Cold Spring Harbor Laboratory of Quantitative Biology in 1965 during Prof. Delbrück's course on *Phycomyces*. Supported by NIH grant GM-14035-02 and NSF grant GB-3149.

* Present address: Department of Biological Sciences, Columbia University, New York 10027

5 December 1968, revised 30 January 1969

Octopamine: Normal Occurrence in Sympathetic Nerves of Rats

Abstract. *Octopamine has been identified in several organs of normal rats by means of a sensitive enzymatic assay. It is localized within the sympathetic nerve endings.*

Octopamine, first identified in the posterior salivary glands of *Octopus vulgaris* (*1*), has been demonstrated in mammalian organs and urine after inhibition of monoamine oxidase (MAO), and in small amounts in urine from normal humans (*2*). It has not been identified previously in any tissue of normal animals. We developed an enzymatic assay which measures 0.5 ng of octopamine in a 0.5-ml tissue extract. Using this method, we found octopamine in several sympathetically innervated organs of the rat.

Male and female Sprague-Dawley rats (100 to 225 g) were killed by a blow to the head, and the organs were removed and homogenized in 0.01 *M* tris (hydroxymethyl)aminomethane buffer, *p*H 8.6. The homogenate was heated to denature proteins, and, after centrifugation, the supernatant was assayed for octopamine. Bovine adrenal phenylethanolamine *N*-methyltransferase was used to transfer the methyl group from C^{14}-*S*-adenosylmethionine to the nitrogen of octopamine to form C^{14}-*N*-methyloctopamine (synephrine). This enzyme is specific for those phenylethylamines having a β-hydroxyl group (*3*). The synephrine formed was extracted into a mixture of toluene and isoamyl alcohol (3:2) at *p*H 10. Under these conditions, epinephrine formed from norepinephrine was not extracted.

Octopamine assays were performed on several organs of the normal rat. The apparent concentration of octopamine varied from 5 ng/g in the brain to about 500 ng/g in the adrenal gland (Table 1).

To identify the radioactive product extracted, samples of the extraction mixture were subjected to thin-layer chromatography; *N*-butanol saturated with 1*N* HCl or a mixture of isopropyl alcohol, NH_4OH, and H_2O (80 : 10 : 19) were used as developing agents. Similar results were obtained with both solvents.

In the heart, synephrine and dimethyloctopamine, formed by the further methylation of synephrine, were the only radioactive compounds present (Fig. 1, solid lines). In the salivary gland and spleen, however, synephrine was only one of several C^{14}-methylated compounds extracted. From such chromatographic separations, the percentage of total radioactivity represented by C^{14}-*N*-methyloctopamine (synephrine) was estimated for six sympathetically innervated organs (Table 1). Authentic octopamine calculated from these percentages varied from 2.4 ng/g in the brain to 461 ng/g in the adrenal gland. Inhibition of MAO with catron (β-phenylisopropylhydrazine) resulted in a five- to tenfold increase in the concentration of octopamine in most organs examined. Under these conditions, synephrine comprised at least 90 percent of the radioactivity extracted.

Experiments were performed to determine whether octopamine was present within the sympathetic nerve endings. 6-Hydroxydopamine, a compound which selectively destroys the sympathetic nerve endings in most tissues of the rat (*4*), was administered, and the tissues were examined for octopamine 2 days later. Almost all of the octopamine disappeared from the heart, spleen, and salivary gland (Fig. 1, dashed lines). Similar results were ob-

Table 1. Endogenous octopamine in rat tissues. Tissues were obtained from six male Sprague-Dawley rats (100 to 125 g). The percentage of apparent octopamine has been estimated from four separate chromatograms in which *N*-butanol saturated with 1*N* HCl or a mixture of isopropyl alcohol, NH_4OH, and H_2O (80:10:19) were used as developing agents.

Tissue	Octopamine		
	Apparent (ng/g)	Authentic (% of apparent)	Authentic (ng/g)
Adrenal gland	461 ± 49	100	461
Brain	4.7 ± 0.93	50	2.4
Heart	49.6 ± 2.8	100	50
Salivary gland	95.7 ± 8.8	50	48
Spleen	23.7 ± 3.8	60	14
Vas deferens	75.6 ± 2.9	40	30

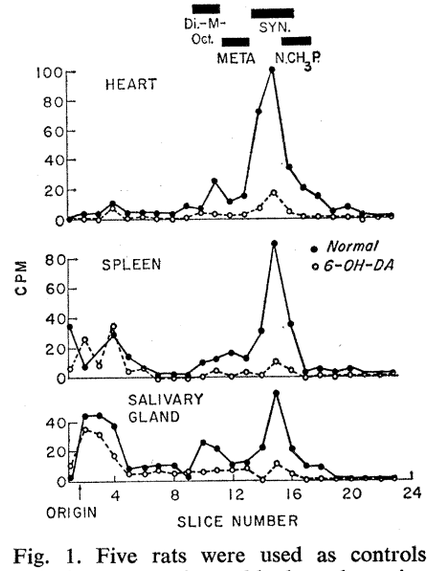

Fig. 1. Five rats were used as controls and five were given 6-hydroxydopamine (100 mg/kg, intravenously) 24 and 48 hours before death. Assay for octopamine was performed as described in the text. A sample of the extract was evaporated to dryness and subjected to thin-layer chromatography (silica gel), butanol saturated with 1*N* HCl being used as developing agent. Nonradioactive dimethyloctopamine (Di-M-Oct), metanephrine (Meta), synephrine (Syn), and *N*-methylphenylethanolamine (NCH_3P) were cochromatographed with each sample. The compounds were made visible by staining with diazotized *p*-nitroaniline followed by ninhydrin. After staining, sections (1.5 by 0.6 cm) were transferred to vials for scintillation spectroscopy.