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Growth Responses of Phycomyces to Polarized Light Stimuli

Abstract. The effectiveness of stimuli in air depends upon the direction of polarization. For sporangiophores immersed in a medium of refractive index similar to that of the protoplasm this dependence disappears. These facts indicate that one is dealing with simple Fresnel reflection losses and not with dichroism of oriented photoreceptors.

For the tropic response of *Phycomyces* sporangiophores, Castle (*1*) observed that light polarized horizontally (electric vector perpendicular to the long axis of the sporangiophore) was 10 to 15 percent more effective than equal incident flux polarized vertically. Castle concluded that the differential response to polarized light was due to Fresnel reflection losses at the front surface of the cylindrical sporangiophore. Reflection losses are larger for vertically polarized light. Recently, however, Jaffe (*2*) stated, without explanation, that differential reflection cannot explain the observation.

Table 1. Average values of intensity (arbitrary units) necessary for growth response null. "Polarized vertically" denotes that the electric vector was parallel to the long axis of the sporangiophore; 15 to 18 determinations were averaged for each 450 mμ value and only 7 and 4 for 380 mμ value. Standard errors of the mean are given.

Direction	In air ($\lambda = 450$ mμ)	In air ($\lambda = 380$ mμ)	In FC-43 ($\lambda = 450$ mμ)
Polarized vertically	1.24 ± 0.02	1.21 ± 0.01	1.06 ± 0.04
Polarized horizontally	1.00 ± 0.03	1.00 ± 0.06	1.00 ± 0.05

He suggested that since the photoreceptors of *Phycomyces* are likely to be dichroic, the differential response might be due to an alignment of photoreceptors within the sporangiophores.

To test this hypothesis the relative effectiveness of horizontally and vertically polarized light in producing growth responses was measured by a null method. The apparatus, intensity units, and growth response measurements have been described previously (*3*). Sporangiophores were rotated continuously (2 rev/min). A standard blue stimulus of fixed intensity impinging bilaterally 60° from the vertical long axis of the sporangiophore was alternated every 5 minutes with a test stimulus impinging unilaterally 90° from the vertical. The monochromatic test stimulus (wavelength 450 or 380 mμ) was plane polarized by a glass laminated linear polarizer (Polaroid Corp., HN22). The dispersing elements of a Beckman DU spectrophotometer were used as a monochromator, and the intensity of the test beam was controlled by varying the slit width. Intensity as a function of slit width was calibrated against a thermopile standard with an RCA 935 phototube-amplifier system.

The increase in sporangiophore length was measured to the nearest micron for each 5-minute period. The difference in growth during two consecutive 5-minute stimuli (blue followed by test) was ideally zero if the test stimulus matched the blue stimulus. If the test signal was too large, a growth response maximum occurred during the blue stimulus (response occurs 5 minutes after stimulus) and a positive difference, blue minus test, was observed. If it was too small, a minimum occurred during the blue, and a negative difference was observed. For a given intensity and polarization of the test stimulus the sum of the differences for three cycles was plotted against log slit width. The intensity necessary for a growth response null was determined graphically.

In air (Table 1), the horizontally polarized beam is about 20 percent more effective than the vertically polarized beam, as would be expected from Castle's observation for the tropic response. The growth response has the advantage of requiring no assumptions about the spatial distribution of the light within the sporangiophore in producing a response, except that the light must enter to be effective.

If the observed effect were due to dichroism of oriented receptors, the magnitude of the effect might be expected to be different for a second strong absorption band. However, the response differences for the two directions of polarization at two action spectrum maxima, 380 and 450 mμ (*4*), were identical.

To eliminate reflection losses, sporangiophores were stimulated while immersed in totally fluorinated tributylamine (FC-43, Minnesota Mining and Manufacturing Co.). The FC-43 has an index of refraction at 25°C of 1.29, which is close to the average value for intact sporangiophores (1.38) (*5*), and is optically inactive. Thus, the reflection losses are negligible, since they are a function of the square of the difference in index of refraction at the interface. In FC-43, growth rates are normal and a usual time course of response to a large pulse-up is observed. Since there is little focusing effect, continuous rotation of the sporangiophores was unnecessary. In FC-43 (Table 1) there is no significant difference in growth response for the two directions of polarization.

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On the Instrumental Conditioned Reaction Evoked by Electrical Stimulation of the Hypothalamus

Abstract. Weak electrical stimulation of the hypothalamic lateral area of satiated goats elicited the previously established conditioned reflex of putting the left foreleg on the food tray and then eating the food given as reinforcement. When, during stimulation, food was not given, an extinction of the conditioned reaction took place.

Experiments on alimentary conditioned reflexes performed several years ago by one of us (*1*) led to the conclusion that an already established instrumental reaction is due to the excitation of a hypothetical "alimentary center" caused by the conditioned stimulus. The discovery of the feeding center in the lateral hypothalamic area (*2*) has suggested that it may correspond to our hypothetical center. Therefore, to test our conclusion, we performed experiments with electrical stimulation of the hypothalamic feeding center of satiated animals.

An instrumental conditioned reflex of putting the left foreleg on the food tray was established in four goats; every movement was reinforced by a small amount of oats. After a period of training, the hungry animal, on entering the

experimental pen, started immediately to perform the learnt movement; after some minutes of repeated conditioned reactions, the movements became less frequent and then ceased to appear. A kymogram of a usual experiment is shown in Fig. 1*A*. If the learnt movements were not reinforced by food, the usual course of extinction of the conditioned reflex was observed: at first the movements grew more frequent and energetic, then they gradually disappeared; sometimes the movements returned for a while, then disappeared definitely (Fig. 1*C*). When the food was given again the reflex was immediately restored.

After the training series an experiment with electrical stimulation was performed in each goat. According to Hess' method (*3*) adapted to goats by Andersson (*4*), three unipolar electrodes were unilaterally placed in the lateral hypothalamic area of the animal under local anesthesia. Immediately after the operation the animal was placed in the experimental pen and, usually after several minutes, it began to perform the learnt movement and eat food. After some minutes the goat, completely satiated, ceased to perform the movements and did not eat the offered food. Then the electrical stimulation (0.5 to 1.5 volt; 10 to 50 pulses per second) was applied.

At first the animal usually showed a kind of orientation-examination reflex; then licking frequently appeared, and afterward (10 seconds to 1 minute or more after the beginning of the stimulation) the animal came to the food tray and performed the learnt movement which was reinforced by food. If the stimulation lasted several minutes the movements were repeated and food was eaten as in the usual experiment; this is shown in Fig. 1*B*. After the interruption of stimulation, one or two movements still appeared sometimes; the animal, however, refused to eat the food, remained for a while with the hoof on the food-tray, then, as if very surprised, it went slowly away.

In three goats an extinction of the conditioned reflex was performed in such a way that the food was not given for some minutes during the electrical stimulation. As is seen on the kymogram of this experiment (Fig. 1*D*) the course of extinction is very similar to that occurring under normal conditions (Fig. 1*C*). At the beginning the learnt movements become more frequent and then, after about 1 minute, they disappear; several minutes later a few movements appear again, then the animal goes away from the food tray and, despite continuing stimulation, does not show any interest in the food. After about 7 minutes the goat was called to come to the food tray and the food was given. After eating the food the goat began to repeat the learnt movement which was now every time reinforced by oats. A similar result was obtained in two other goats.

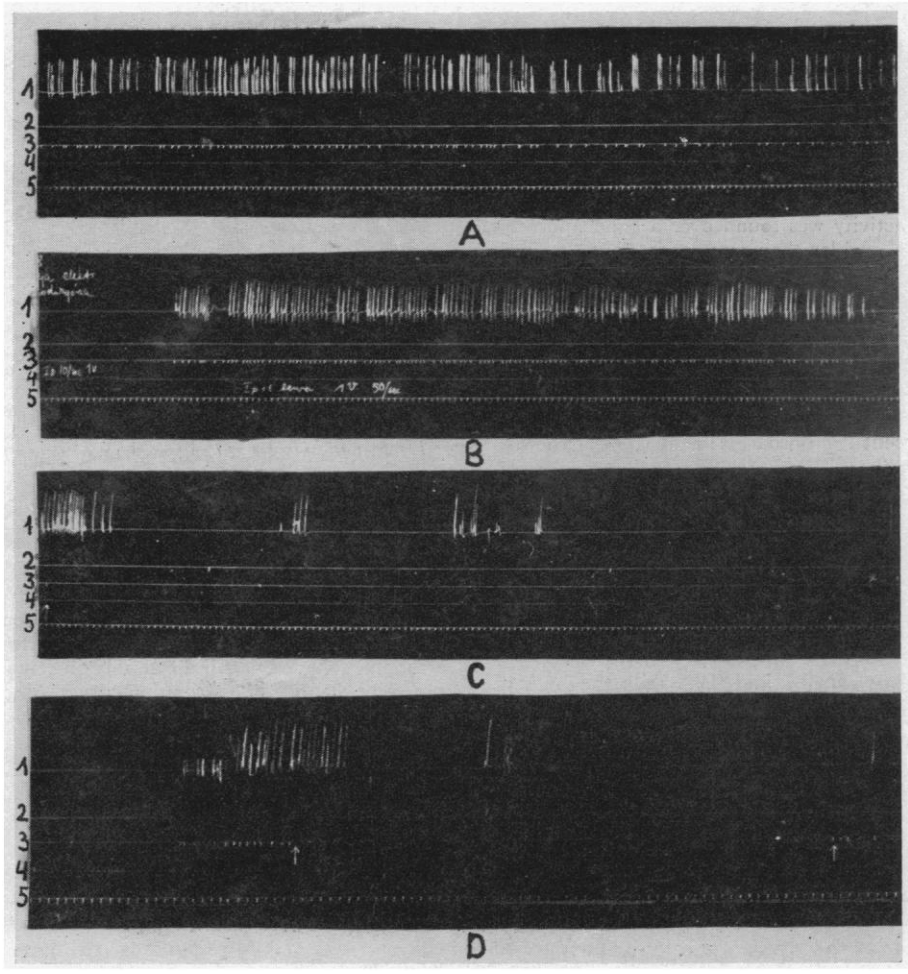

Fig. 1. Kymograms of experiments with goat No. 5. *A*, Course of a usual training experiment. *B*, Experiment with electrical stimulation of the lateral hypothalamic area. *C*, Extinction of the conditioned reflex under usual conditions. *D*, Extinction of the conditioned reflex during electrical stimulation. 1, Learnt movements of foreleg; 2, (not pertinent to this study); 3, food given; 4, electrical stimulation; 5, time (5 seconds). The arrows in *C* and *D* show the period in which the food was not given.

When the experiment lasted several hours and the animal was more and more satiated with food, the movements evoked by stimulation were less and less frequent and only a part of food offered was eaten every time.

The animal was killed the same day and its brain was removed for anatomical control.

The elicitation of the conditioned instrumental reaction by electrical stimulation in these experiments is in harmony with the results obtained by other investigators (*5*) and confirms our previous conclusion, mentioned at the beginning of this report. Some points of our results ought, however, to be explained. The performance of one or two learnt movements after the interruption of stimulation seems to be caused by inertia of the conditioned movement's center. The similar course of extinction in usual conditions and during the stimulation seems to indicate that in both cases some inhibitory agents intervene; they are perhaps connected with the lack of impulses deriving from the reinforcement.

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