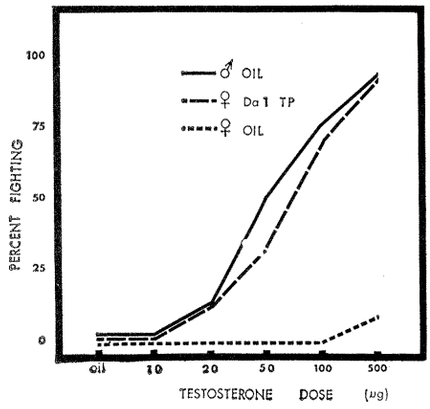

Fig. 1. Effects of neonatal stimulation by testosterone on adult fighting in mice. Represented are the percentages of pairs in each group fighting at various dosages of testosterone during adulthood.

birth (OM), (ii) females treated with oil at birth (OF), and (iii) females treated with testosterone propionate at birth (TPF). During the 1st week of isolation all mice were injected subcutaneously daily with 0.05 ml of oil. On the 6th and 7th days of the 1st week, all mice were tested for fighting; none fought. Successively during each of the subsequent 5 weeks, all subjects received daily injections of 10, 20, 50, 100, and 500 μg of testosterone; on the 6th and 7th days of each week they were tested for fighting. Thus during successive weeks they were tested under progressively higher doses of testosterone.

For testing, two mice from the same group were paired in a neutral cage. If they fought within 10 minutes, the latency from the start of the test to the beginning of the fight was recorded to the nearest minute, and the test was terminated by restoration of each mouse to its home cage. A "fight" was scored if one mouse (or both) persistently attempted to bite the other. The same individuals were paired for each test.

More than 90 percent of the pairs of virilized females showed fighting during at least one test. One hundred percent of the pairs of males fought. Only one pair of group OF ever fought. Once fighting occurred, it persisted in most subsequent observations under conditions of increased dosage of testosterone.

For each pair in the OM and TPF groups, a mean latency to fighting was calculated for those tests on which fighting occurred. Analysis of variance of the data on latency (Table 1) failed to indicate any significant difference between males and virilized females.

When the dose of testosterone during adulthood was increased, the proportions of pairs of males and of virilized females showing fighting were increased (Fig. 1). The close correspondence of the curves for the OM and TPF groups indicates that these groups were little different in their response to testosterone; at no dose level was the difference in proportion of pairs fighting statistically significant. In constrast, females treated with only oil at birth failed to fight even when given large doses of exogenous testosterone in adulthood. Chi-square comparisons of group OF with group TPF are significant beyond the 0.05 level at all dose levels greater than 20 μg per day.

These data clearly indicate that administration of testosterone to the neonatal female mouse facilitates the display of testosterone-aroused fighting in adulthood; they suggest that the usually observed difference between normal male and female mice, with respect to fighting, is due to the fact that males are stimulated by testicular androgens early in life. One may presume that the stimulation by endogenous testosterone in the male (and exogenous testosterone in the female) can "organize" or cause the differentiation of a neural substrate for fighting.

The ability of early androgenic stimulation to affect adult patterns of behavior has been clearly documented in several species for a variety of sexually dimorphic behaviors (*3*). In these instances, androgenic stimulation affected behavioral differentiation only when this stimulation occurred during a particular and limited period during maturation. It is not yet clear to what extent the "organizing" capacity of androgens with respect to adult fighting in mice is similarly time-limited. Data now being collected in our laboratory (*4*) suggest that the period, during which administration of testosterone promotes fighting in the female mouse, may extend well beyond the 10th day after birth.

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Red and Far-Red Light Effects on a Short-Term Behavioral Response of a Dinoflagellate

Abstract. *Cessation of movement (stop response) is used as a criterion for light reception by the dinoflagellate* Gyrodinium dorsum *Kofoid. Brief irradiation (2 seconds at 470 nanometers) elicits a stop response in cells any time during the 6-minute interval after removal from growth lights. This stop response is inactivated by exposure for 4 minutes to 470-nanometer light prior to stimulation. Red light (620 nanometers) reactivates this stop response, and far-red light (700 nanometers) reverses this reactivation. This red–far-red photo reversibility is taken as evidence for phytochrome involvement.*

Phytochrome mediates several long-term growth responses (flowering, seed germination, stem elongation) in higher plants (*1*). Studies of leaf movement in *Mimosa* (*2*) and in *Albizzia* (*3*), and of algal chloroplast movement (*4*), suggest the participation of this pigment in short-term light responses, not mediated by way of effects on RNA metabolism. Phytochrome, however, has not been implicated in the control of movement in motile lower plants, perhaps because the effects of prior irradiation on the light-induced behavioral response have been ignored. We now report the possible involvement of phytochrome in a short-term response of the dinoflagellate *Gyrodinium dorsum* Kofoid.

Our culture procedure and experimental apparatus remain as reported (*5*) with the following modifications. Experiments were only conducted 8 hours after the beginning of a light period, on 5-day-old cultures grown on a 16-hour-light, 8-hour-dark cycle. Stimulation sources were a Bausch and Lomb No. 33-86-02 grating monochromator with a 20-nm bandpass between 400 and 740 nm, and a Toyoda microscope lamp in combination with

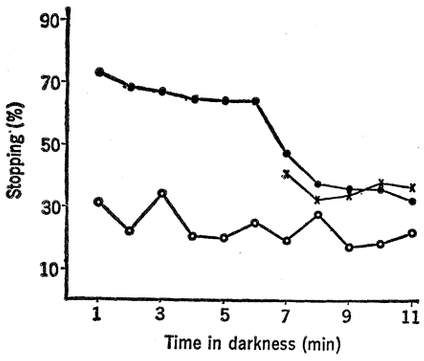

Fig. 1. Time course of typical stop response. The cells are removed from the culture lights (one 60-watt incandescent lamp plus seven daylight fluorescents) and placed in darkness at time zero. Solid circles, percentage stopping upon repeated blue stimulation (2 seconds at 470 nm from the monochromator); ×'s, response to repeated stimulation after 7 minutes in darkness; open circles, random stopping without light stimulation.

a 470-nm interference filter (Optics Technology, Inc.) having a 20-nm bandpass. Intensity from the monochromator was controlled by a continuously graded neutral-density filter (Optical Coating Laboratory). Both stimulation sources were calibrated for wavelength energy as described (*5*).

The response studied was the cessation of movement (stop response) upon stimulation with light of the proper wavelength and intensity. The peak in the action spectrum for this response and for oriented phototactic movement is 470 nm (*5*).

The stop response was recorded photographically. Since its latency was 0.4 to 0.6 second (*5*), the picture was taken mechanically by a solenoid-driven Robot Recorder-24 camera, 0.75 second after beginning of stimulation, to record the maximum numbers of responding cells. Stopped cells appeared round, and those moving appeared as blurry lines in ¼-second exposures. The sample size was 150 to 200 cells.

Table 1. Effectiveness of red and far-red light at reactivation and reinactivation of stop response, respectively. (A) Time in darkness, afer red exposures, until response drop-off. (B) Time in darkness after far-red exposures (same energy level as 620 nm) until response drop-off.

Wavelength (nm)	A (min)	B (min)
580	0	
600	4	
610	6	
620	7	
630	6	
640	4	
660	0	6
680	0	3
700	0	2
720	0	3
740	0	6

The response becomes meaningful as a behavioral assay if, under varying experimental conditions, one considers change in the percentage of cells responding plotted against time. A level of stopping above 50 percent is a positive stop response, and a level of stopping from 35 to 50 percent (as typical of response from 7th minute on) is a response drop-off (Fig. 1).

Response to 2 seconds of 470-nm light was inactivated by a longer exposure at this wavelength prior to stimulation. Cells were removed from the culture box, irradiated for 4 minutes at 470 nm from the monochromator and microscope lamp, and then placed in darkness; responsiveness was then tested as before. The response for the length of the experiment (10 minutes) was not different from random stopping. The 4-minute blue irradiation was chosen because a series of increasing exposure times showed that it always completely abolished responsiveness.

A positive stop response could be restored if the blue irradiation was followed by red irradiation. To test for the effective wavelength of reactivation, the cells were first given 4 minutes of blue, and then a 45-second exposure to light of equal energy at various wavelengths in the red. Thereafter, the cells were left in darkness and stimulated every minute at 470 nm. Exposure for 45 seconds was selected because we found that at least 30 seconds was required for maximum reactivation. The most effective wavelength for reactivation is 620 nm since it allows the positive stop response to persist for the longest time (Table 1).

Reversibility with far-red radiant energy was tested by a 45-second irradiation at wavelengths between 660 and 740 nm after 4 minutes of 470 nm and reactivation with 45 seconds at 620 nm. Cells were then left in darkness and stimulated every minute with 470 nm light. Shortening the time in darkness until blue light fails to produce a positive stop response implies reversibility. The activating effects of 620 nm were most effectively reversed by exposure to 700 nm (Table 1).

In another experiment, cells were given 4 minutes at 470 nm, and then alternate 45-second exposures of red and far-red. Repeated reversibility between responsiveness and nonresponsiveness occurs, and the duration of the response is solely governed by the last red or far-red irradiation (Table 2). This reversibility by red and far-red light indicates that phytochrome is a pigment in this photoresponse of *G. dorsum*.

Table 2. Reversibility of stop response activation and inactivation by red (620 nm) and far-red (700 nm) irradiation.

Combinations of red (R), far-red (FR) after blue exposure	Time in darkness until response drop-off (min)
FR	0
R	7
R, FR	2
FR, R	7
FR, R, FR	2
R, FR, R,	7
R, FR, R, FR	2
FR, R, FR, R	7

Under conditions of our experiments, prior irradiation with red or far-red light affects the stop response of *G. dorsum*. The effective wavelengths (620 and 700 nm) are shorter than those reported for phytochrome-mediated responses in higher plants (660 and 730 nm), and resemble the absorption regions of chlorophylls c and a. Reversibility, however, implicates a phytochrome as the active pigment rather than a chlorophyll; and the shorter wavelengths may not be unexpected in that other lower plant phytochromes show a similar shift (*6*).

Since red and far-red light do not produce any overt behavioral response, and the shorter wavelength absorption peaks of phytochrome in higher plants are in the long ultraviolet (*7*), phytochrome may be acting in combination with a blue-absorbing pigment to control the observed response. The alternative remains that there may be a phytochrome which has a blue absorption peak at 470 nm.

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