The horseshoe crab, *Limulus polyphemus* (L.), is of particular interest to biologists because of its primitive phylogenetic position (Patten, 1900; Loeb, 1893; Ley, 1948). This crab also has been used widely in physiological research because of its large lateral compound eyes. Hartline (1927) found that the size and consistency of the nerve fibers from the lateral eyes made them useful in electrophysiological research involving the visual process. The correlations between the resulting electrophysiological data and human psychophysical data are very good. Particularly in dark and light adaptation (Hartline, 1930; Hartline & McDonald, 1947) and with the difference threshold (Riggs & Graham, 1945) does the electrical response of the crab eye parallel the data from human Ss. Little is known, nevertheless, about how the *Limulus* uses these visual functions as part of its behavior.

Early investigations of photic behavior of *Limulus* have yielded confusing results. Loeb (1893) noted that the larvae of *Limulus* were positively heliotropic in cold water and negatively heliotropic in warm water. Northrop and Loeb (1922) found that some older specimens reacted negatively to light at room temperature, but more animals failed to react at all to the light stimulus. In their investigation, they found that only 10 of 48 animals gave a response reliable enough to measure.

Cole (1922) investigated the responses of *Limulus* to a light stimulus and found his adult animals to be basically positively phototropic. He also found, however, a high percentage of irregular reactions to photic stimulation including positive, negative, and indifferent responses. He claimed that *Limulus* was positively phototropic and the other reactions were brought about by fear, hunger, stereotropism, photokinesis, and unknown causes. In a later paper, Cole (1923) demonstrated that the laboratory age of the animal had a definite effect on the photic response. Most freshly collected animals were positively phototropic, but after only 24 hr. this tropic response was lost. Although few freshly collected animals exhibited a negative response to light, this response increased rapidly for two days and then decreased. He noted that most of the animals would be indifferent to the light stimulus by the end of one week of laboratory confinement.

Wolf and Wolf (1937) found young *Limuli* to be positively phototropic and reported no change in photic response when the animals were kept in an aquarium supplied with fresh sea water.

The phototropic response is not clear. In order to determine whether the behavior of this animal is correlated with the discharge of its optic nerve, as is generally assumed in the electrophysiological studies, a more consistent behavioral response must be established to light. The parameters which are known to govern the discharge of the optic nerve then can be manipulated to determine their effect upon the animal’s behavior.

The purpose of the present investigation was to explore the possibility of obtaining a more reliable response to a light stimulus from the *Limulus* through a classical conditioning technique.

**METHOD**

**Subjects**

Eighteen male *Limuli*, with a mean width of 14 cm., were studied. They were obtained from the Gulf of Mexico and stored in a large outdoor aquarium at the Florida State University Marine Biological Laboratory, Alligator Harbor, Florida. During the experiment, the animals were kept in an indoor water tank which was supplied with fresh running sea water.

**Apparatus**

The S was rigidly fixed in a holder and placed in a 10-gal. aquarium with sea water covering the gills but not the lateral eyes. The aquarium was placed in a dark box equipped with an exhaust fan. Approximately 8 in. above the animal holder was a cylinder containing a 200-w. bulb, which constituted the CS. This light presented 94 ft-c of illuminance on the eye of the S. The US was a 9-v. dc electric shock administered to the animal by means of two alligator clips attached to the posterior carapace on opposite sides of the tail spine. The presentation of the CS and US was monitored by a

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tape programmer. Tail movement was graphically recorded by means of a thread clipped to the tail spine and leading to a kymograph outside the dark box. The kymograph recorded the US, CS, and tail-spine movement on the same record. An additional box, partially filled with wet sand, was used for dark-adapting the animals prior to the experimental sessions.

Procedure

On the initial day of experimentation, each animal was placed in the dark-adaptation box for 20 min. Then the animal was moved to the experimental apparatus, and its anterior carapace was bound to the holder with two heavy rubber bands. The recording thread was attached to the tail spine, and the alligator clips were fastened to the posterior carapace. The container was then closed, the exhaust fan started, and the animal was allowed to dark-adapt for 5 min. This was followed by 20 presentations of the light stimulus, each of which was 10 sec. in duration. The time interval between light stimulations was at least 100 sec. to maintain the animal's dark adaptation. The interval was varied randomly between 100 and 250 sec. Two animals which gave more than a criterional number of responses of tail-spine movement to the light stimulus alone during this period were eliminated from the study. The criteria (12 responses) was established to exclude any animals whose high rate of responding might make it difficult to observe a rate increase.

The animals were then divided into three groups designated as experimental (E), light-control (L-C), and shock-control (S-C). The procedure for each of these groups was as follows:

Experimental group. This group was composed of eight animals. Once a day they were placed in the dark-adaptation box for 20 min. and then removed to the experimental apparatus. After 5 min. of adaptation, the S was given 20 conditioning trials with a 10-sec. light stimulus and a 1-sec. shock. The shock overlapped with the last second of light. The procedure was continued daily until each animal reached a criterion of three consecutive days of 17 or more responses to the light alone. The animals were then given extinction trials until they reached a criterion of two consecutive days of 8 or less responses. This criterion represented a return to the approximate average number of responses to the light alone before conditioning began.

### Table 1

<table>
<thead>
<tr>
<th>Vincent Tenths</th>
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<tbody>
<tr>
<td>1</td>
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<td>10</td>
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Light-control group. The four animals in this group received 10 days (200 trials) in the same manner as the experimental group except that the US (shock) was never presented.

Shock-control group. To determine if the shock sensitized the animal to the light stimulus, a shock-control group of six animals was run. (Owing to a failure in the water supply, two of these animals died after 120 trials.) After these animals had been dark-adapted and placed in the experimental apparatus, they received a shock of 1-sec. duration every 30 sec. until 10 shocks had been administered. These shocks were each 9 v. dc. After 3 min. of rest, the animals were given 20 light trials exactly like the light-control group. This procedure was repeated daily until each animal had received 200 trials.

Results

The response to the electric shock was one of rapid tail-spine movement. The CR was tail movement, but usually of lesser magnitude. The mean number of responses to the light stimulus alone on the initial day of the experiment was 7.4 for the experimental group, 5.8 for the light-control group, and 9.5 for the shock-control group. Because of lack of homo-

![Fig. 1. A comparison of the tail-spine responses of Limulus for an experimental conditioning group and two control groups.](image1)

![Fig. 2. The curve of extinction for Limulus after light-shock classical conditioning of the tail-spine response.](image2)
geniety of variance the Kruskal-Wallis (1952) nonparametric test was employed to check for a significant difference between these means. This yielded an $H$ of .47, which is not significant at the .05 level of confidence.

The number of trials taken to reach the learning criterion ranged from 80 to 480 with a mean of 220. The data for the three groups were converted to Vincent Tenths (Hunter, 1917) and are presented in Figure 1.

In order to determine the significance of the difference in response frequency for the three groups, the Kruskal-Wallis (1952) one-way analysis of variance was employed for each Vincent Tenth. These data are summarized in Table 1. It can be seen that the differences between the curves become significant beyond the .05 level of confidence at the fourth Vincent Tenth. The one reversal is in the ninth Vincent Tenth, where the value of $H$ must be 5.99 to be significant at the .05 level, and the value obtained was only 5.92.

The number of trials to reach the extinction criterion varied from 40 to 140 with a mean of 85. These data were converted to Vincent Fifths and are seen in Figure 2.

**DISCUSSION**

Figure 1 clearly shows that a reliable response to the light stimulus can be obtained from Limulus by a classical conditioning technique. The performance of the light-control group indicates that the increased tail-spine response is not merely the result of increased sensitivity to the photic stimulus. The shock-control group provides evidence that pseudoconditioning was not probable in the performance of Group E, since repeated shock stimulation did not cause an increase in response frequency to the light stimulus. The performance of the S-C group also indicated that the shock does not increase the general activity level of the animal.

In the electrophysiological work with the Limulus eye it would make no difference if the crab were a positive, negative, or indifferent responder to the light stimulus. On the other hand, it would be necessary to have an unequivocal response before visual functions could be studied behaviorally. If visibility data are to be collected from the intact Limulus it seems that the phototropic responses are not the responses to observe. This experiment indicates that a much more reliable response to a light stimulus can be obtained by utilization of the conditioning technique.

**SUMMARY**

The purpose of the present experiment was to study the acquisition of a classical light-shock conditioned response by Limulus polyphemus. Eighteen male animals were studied. They were divided into an experimental and two control groups. The response frequency for the experimental group rose significantly while the response frequency for the control groups showed no increase. Classical conditioning is presented as a technique for eliciting a reliable response to a light stimulus from Limulus.

**REFERENCES**


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