Conditioning in the Horseshoe Crab[1]

W. L. MAKOUS, *University of Washington, Seattle, Wash. 98105*

The results of classical, instrumental, and operant conditioning in the horseshoe crab are summarized. Although some conditioning occurs, a useful technique has yet to be developed.

The horseshoe crab, *Limulus polyphemus*, is an attractive subject for behavioral investigation because of a recent increase of interest in learning in simple organisms (Bullock, 1966), the great amount known about the senses of Limulus, its large size for an arthropod, and the development of techniques for studying physiological correlates of behavior in Limulus (Corning, Feinstein, & Haight, 1965). The purpose of this note is to help others to avoid the blind alleys explored in a research project, now ended, extending over two years while data was collected on over 40,000 trials with some 75 horseshoe crabs.

OPERANT CONDITIONING

Methods

Operant techniques usually give the best control over behavior. Here they gave the worst. The behavior of the horseshoe crab suggests few good prospects for either operant responses or reinforcers. Attempts were made here to reinforce movements at joints between segments and, in other Ss, positions of various segments, by passing cool, aerated sea water over the gill books of an S suspended in air. Other attempts were made to reinforce movements of the *chelicerae* (the first pair of appendages, used in eating) by applying to the taste buds a drop of sea water in which various sea foods had been soaked. It was hoped that the flavored sea water would act like saccharin does in some mammals, for Limulus survives months of complete food deprivation without noticeable effects, and its stomach is quickly filled.

Results

Both "reinforcers" cause S to struggle or move about generally; thus, any response measured increases. The effect is only transient, however, and attempts to form a discrimination between darkness and flashes from a Strobotac at 1 Hz were unsuccessful. Daily sessions before the "reinforcer" lost its effects were not tried long enough, however, to exclude them definitely as a possibly effective technique.

CLASSICAL CONDITIONING: EXPERIMENT 1

Smith & Baker (1960) have reported classically conditioned telson (tail) movement using 1-sec dc shock coinciding with the last second of 10-sec illumination from a 200-W incandescent source positioned 8 in. above S. Thus, the CS could have had its effect through heat or light. This experiment was a pilot attempt to replicate their results and to obtain a hint as to the mode of action of the CS.

Methods

The procedure used here differed from Smith and Baker's in four ways: (a) Ss were stored at 15-deg C in filtered, aerated, artificial sea water instead of fresh sea water; (b) telson movement was monitored here by changes in capacitance between the telson and a metal plate folded at right angles to form two perpendicular planes parallel to the telson; (c) after one of the two Ss had responded on 18 out of 20 trials for three consecutive days, the CS for him was changed for one day to a 10-ft-c, heat-filtered spot of light directed on a small area containing a compound eye; (d) on the next day the spot of light was shifted to the median eyes.

Results

The rate of conditioning of the two Ss was well within the range reported by Smith and Baker. S also gave 16 out of 20 CRs to illumination of one compound eye alone, but only two questionable CRs to illumination of the median eyes. Thus, light entering the compound eyes was probably sufficient to elicit the CR in Smith and Baker's experiment whereas light entering the median eye probably was not, and storage in fresh sea water is not necessary, as it had at first seemed (Baker, personal communication).

CLASSICAL CONDITIONING: EXPERIMENT 2

Methods

In order to test 10 Ss at a time in a semi-automated apparatus, the following changes were made: (a) juveniles were used instead of adults; (b) the CS was 10 ft-c of heat-filtered illumination of the entire dorsal surface of S; (c) CS duration was 5 sec; (d) ac instead of dc shock was used as US to minimize polarization; (e) to eliminate the chance of cueing S by a change in capacitance-measuring current when the metering instrument was switched to him, the response was observed by a change in potential difference between the alligator clips attached to the posterior carapace on opposite sides of the telson. The potential was generated by muscles working to move the telson against restraints that held the telson in place. The shock was administered through the same clips. Control observations showed that the threshold for detectable potentials was the same as that for observable telson movements when the telson was free to move.

To study the roles of the median and compound eyes in conditioning, paraffin with a heavy suspension of lamp black was used to cover the median eyes of one group, and the compound eyes of another. A third group had no eyes covered, and a control group ("blind") had both kinds of eyes covered. Another control group (pseudoconditioned) with uncovered eyes were presented the CS and US at random times with respect to one another. The eye covering reduced illumination by only three log units because of leakage of light through the carapace and interior of S, but the alternative of excising the eyes was abandoned when Ss with excised eyes in earlier attempts to do the experiment showed signs of bad health.

Results

A positive response was defined as a detectable potential change during the 4 sec of CS prior to the US and no potential change during the 4 sec preceding the CS; and a negative response was defined as a detectable potential change during the 4 sec prior to the CS and no change during the 4 sec of CS prior to the US. In the 16,000 trials of the experiment, there were only 427 responses. The spontaneous level of response, i.e., the number of negative responses, totalled 251, or once every 64 trials. The number of positive responses, however, was only 176, or once every 91 trials. This is significantly less than the spontaneous rate; the effect of the CS was to decrease response, not increase it.

Specifically, both Ss with only median eyes covered and one of the Ss with no eyes covered showed a significant decrease in activity during the CS, but both the pseudoconditioned and the "blind" control groups made very nearly their spontaneous rate of response during the CS. The binomial statistics on which these conclusions are based do not permit comparisons between groups here, however, nor generalization to other Ss, nor test of any hypothesis except that of complete randomness of response with respect to the CS. Thus it is only possible to say that some Ss in this experiment responded to the light (by an inhibition of movement) but not that this response was conditioned.

INSTRUMENTAL CONDITIONING

Locomotion is the last potentially useful behavior category in the horseshoe crab not yet treated here. Others have

(Continued on page 6)

Ss trained on orientation in Stage 1, those trained with 0 deg positive selected the 45-deg lines on 58.5% of trials, while those trained with 90 deg positive selected the 45-deg lines on 50% of trials. Neither of these differences approached significance (in both cases, $p > .20$).

In conclusion, therefore, pigeons, like rats and humans, learn IDS problems faster than EDS problems. Although the effect was a relatively small one, the results suggest that transfer between problems may occur which cannot be explained simply in terms of differential response tendencies. It appears that learning to attend to the relevant dimension is part of what is involved in learning a discrimination problem.

REFERENCES

ISAACS, I. D., & DUNCAN, C. P. Reversal and nonreversal shifts within and between dimensions in concept formation. Journal of Experimental Psychology, 1962, 64, 580-585.

LAWRENCE, D. H. Acquired distinctiveness of cues: I. Transfer between discriminations on the basis of familiarity with the stimulus. Journal of Experimental Psychology, 1949, 39, 770-784.

LOVEJOY, E. *Attention in discrimination learning.* San Francisco: Holden-Day, 1968.

SHEPP, B. E., & EIMAS, P. D. Intradimensional and extradimensional shifts in the rat. Journal of Comparative & Physiological Psychology, 1964, 57, 357-361.

SIEGEL, S. Overtraining and transfer processes. Journal of Comparative & Physiological Psychology, 1967, 64, 471-477.

SUTHERLAND, N. S. The learning of discriminations by animals. Endeavour, 1964, 23, 148-152.

TRABASSO, T., DEUTSCH, J. A., & GELMAN, R. Attention in discrimination learning of young children. Journal of Experimental Child Psychology, 1966, 4, 9-19.

ZEAMAN, D., & HOUSE, B. J. The role of attention in retardate discrimination learning. In N. R. Ellis (Ed.), *Handbook of mental deficiency: Psychological theory and research.* New York: McGraw-Hill, 1963. Pp. 159-223.

NOTE

1. This research was supported by Grant APA-259 from the National Research Council of Canada.

(Continued from page 4)

reported limited success with unconditioned locomotory responses to light (Loeb, 1893; Cole, 1922; Northrup & Loeb, 1923; Wolf & Zerrahn-Wolf, 1937; Waterman, 1953; von Campenhausen, 1966), but these responses appear to vary uncontrollably with a myriad of variables, including the emotional state of the animal (Cole, 1923). In order to achieve some consistent control over the animal, avoidance conditioning was attempted.

Method

A modified shuttlebox was made out of a plastic tray 12 x 14 in. with just enough sea water in it to permit juveniles to swim. A sufficient dc potential could be applied between two carbon rods spanning opposite ends of the tray to cause S to swim in the direction away from the anode. S was given 10 sec after onset of one of two light sources at opposite ends of the tray, in order to approach (or avoid, in some cases) the light. If, at the end of that time S was not in the correct end of the tray, a potential was applied across the tank that was of sufficient voltage and in the direction necessary to drive S into the correct end of the tray. This was kept on until S actually had moved to the correct location. Light sources and hence correct ends of the tray varied randomly, independently of the location of S so that mere light-contingent activity did not serve to avoid the shock.

Because this response conflicted with a phototaxis, it was necessary to move the lights to the sides of the tray at right angles to the direction of movement required of S, after 200 trials. The training with the direct approach or avoidance of the light seemed necessary for S to "learn" the complicated response of determining the correct end of the tray on the basis of which side of the tray the source was on.

Results

A response was defined as a crossing from one end of the tray to the other, in either direction. Passive avoidances, or passive failures to avoid were not counted as responses. Responses occurred on 20% of the trials. Of these, 60% were in the correct direction, when 50% was expected by chance alone. More active Ss did no better than inactive ones.

Although "conditioned" responses thus occurred on only about 1½ to 2% of the trials, any level of significance could be obtained by running enough trials; t-scores greater than 12 were obtained for individuals, and it was possible to generalize, on the basis of the five Ss used in the last experiment, to the population of Ss at the 0.05 level.

Two Ss showed, in addition to the instrumental responses, statistically significant phototaxic behavior during the conditioned stimulus, one positive in sign, the other negative. Four of the five Ss showed very strong phototaxic behavior during shock (up to 90% in the same direction), but again two were positive and two negative in sign.

DISCUSSION

The significance and utility of any of the behaviors studied here is at best marginal. They are not, however, out of line with previous results from other invertebrates (Warren, 1965). The most promising line of investigation probably is to instrument general activity in the "shuttle-tray," for observation of S during avoidance training gave a distinct impression that S anticipated the shock although being unable to respond appropriately to it very often.

REFERENCES

BULLOCK, T. H. (Ed.) Simple systems for the study of learning mechanisms. Neurosciences Research Program Bulletin, 1966, 4, 105-233.

COLE, W. H. Circus movements of Limulus and the tropism theory. Journal of General Physiology, 1922, 5, 417-426.

COLE, W. H. Effect of laboratory age upon the phototropic reactions of Limulus. Journal of General Physiology, 1923, 6, 295-297.

CORNING, W. C., FEINSTEIN, D. A., & HAIGHT, J. M. Arthropod preparation for behavioral, electrophysiological, and biochemical studies. Science, 1965, 148, 394-395.

LOEB, J. Über kuenstliche Umwandlung positiv heliotropischer Thiere in negativ heliotropische und umgekehrt. Archiv für die Gesamte Psychologie, 1893, 54, 81-107.

NORTHRUP, J. H., & LOEB, J. The photochemical basis of animal heliotropism. Journal of General Physiology, 1923, 5, 581-595.

SMITH, J. C., & BAKER, H. D. Conditioning in the horseshoe crab. Journal of Comparative & Physiological Psychology, 1960, 53, 279-281.

von CAMPENHAUSEN, C. The ability of Limulus to see visual patterns. Journal of Experimental Biology, 1967, 46, 557-570.

WARREN, J. M. The comparative psychology of learning. Annual Review of Psychology, 1965, 16, 95-118.

WOLF, E., & ZERRAHN-WOLF, G. Reactions of Limulus to illuminated fields of different areas and flicker frequency. Journal of General Physiology, 1937, 20, 767-776.

NOTE

1. This research was done while the author was on the staff of the IBM Research Center, Yorktown Heights, N. Y. Analysis of the data and writing were partially supported by U. S. Public Health Service Grants MH14955-01 and NB08070-01. The author is indebted to Jean Kirby for her assistance.