Perceptual and Motor Skills, 1967, 24, 727-730.

ESTABLISHMENT OF A CLASSICALLY CONDITIONED RESPONSE AND TRANSFER OF TRAINING VIA CANNIBALISM IN PLANARIA

ROBERT A. WILSON[1] AND GORDON D. COLLINS

The College of Wooster

Summary.—To show that a conditioned response could be established in Planaria using massed training trials and this conditioning could be transferred via cannibalism, this experiment utilized 5 training groups, an experimental group with light-shock paired (LSP) and control groups of light-shock not paired, light, shock, and naive (neither light nor shock). All groups were given 225 massed trials and then tested under 25 trials of paired light and shock. These groups were then cannibalized by a second group of *Ss* and this second group tested under 25 trials of paired light and shock. The data indicated that a classically conditioned response was obtained only in the LSP training group and training was transferred in all groups via cannibalism without significant change.

Since Thompson and McConnell's (1955) experiment in the classical conditioning of planaria (*Phylum Platyhelminthes*), there has been much debate in the literature concerning the results obtained in this type of experiment. Studies showing the transfer of classical conditioning in planaria via cannibalism have also been severely criticized. Studies by VanDeventer and Ratner (1964), Halas, James, and Knutson (1962), Best (1960), Best and Rubenstein (1962), and others have pointed to the various methodological problems which were uncontrolled in planarian research. It was the purpose of this experiment to attempt classical conditioning in planaria using a modified version of the procedure established by McConnell, Cornwell, and Clay (1960) and to evaluate transfer of training through cannibalism. The control groups and control procedures were designed partially to eliminate the methodological problems described by the above authors.

METHOD

Subjects

Ss were 125 *Dugesia Dorotocephala* purchased from the General Biological Supply House, Inc. (Chicago, Ill.). Twenty-five *Ss* were eliminated during the course of the experiment. *Ss* were kept in a dark refrigerator (42°F) until experimentation and were never fed.

Materials

Training apparatus consisted of a trough, light source, shock source, timers, and control boards. The trough was a clear plastic block (40.5 cm. × 20.5 cm. × 4.5 cm.). Four semicircular troughs were routed from the surface of the block, each 25 cm. long, 2 cm. wide and 1.5 cm. deep. The troughs were evenly spaced and each had two brass screws placed about 1.4 cm. from the ends which acted as electrodes. All electrodes on

[1]Now attending the University of Akron, Department of Psychology. Any correspondence should be addressed to this author.

one side were positive. The light source utilized a U-shaped aluminum hood placed over two narrow, clear 40-w bulbs and was 12 cm. above the surface of the trough. The shock system[2] for the unconditioned stimulus consisted of a circuit which produced 20 VDC at 450 μamp. when conducted across two water-filled troughs. The timing system consisted of (a) 3 Hunter timers (Model 115) wired in series to present a continually cycling sequence of intertrial interval (45 ± 15 sec.), light (3 ± .1 sec.), and shock (1 ± .03 sec.) which occurred in the last second of light and (b) a Hunter timer (Model 111-C) set at 1 ± .03 sec. to present the shock to the light-shock-not-paired group.

An automatic control system allowed for presentation of the light-shock cycle to the various groups. For manual presentation of shock *E* held a 3-position toggle switch and a normally open push switch away from the troughs. This arrangement allowed *E* to present the not-paired-light-shock group with 1 sec. of shock. All troughs and related systems were cushioned by 2 in. foam rubber to prevent vibration. A system of diffuse red lights was designed to simulate darkness as red light is beyond the planarian's visual spectrum (Marriot, 1958).

Procedure

There were two conditions. Condition 1 was the training and testing of the Sacrifice *Ss* (SS). *Ss* ($n = 10$) were isolated individually in white light at room temperature for 72 hr. before training. During the last hour of this 72-hr. period, *Ss* were placed in red light. They were then randomly divided into 5 equal subgroups designated as: (a) LSP or light-shock paired, (b) LSNP or light-shock not-paired, (c) L or light only, (d) S or shock only, and (e) N which received neither light nor shock. Subgroups (2 *S* each) LSP, LSNP, and L were then trained simultaneously using different troughs. The S and N groups were then trained using different troughs. All troughs were preslimed for all groups. The LSP group received 3 sec. of light and 1 sec. of shock (in the last second of light) per trial with a varied intertrial interval (45 ± 15 sec.). The LSNP and L groups received 3 sec. of light per trial with the same intertrial interval. *E* presented the LSNP group with 1 sec. of shock at random times during the intertrial interval and never paired the light and shock for the LSNP group. The S subgroup received 1 sec. of shock per trial (same intertrial interval) and the N group received neither light nor shock. All subgroups received 225 massed trials on the first day. All training utilized red background light.

After 24 hr. in darkness at room temperature, each of the subgroups was given 25 testing trials of light-shock paired using the same procedure referred to in the LSP subgroup, with all responses scored. *Ss* were always tested in pairs with the procedure suggested by Cornwell (1959). Condition 2 consisted of cannibalizing the SS subgroups by corresponding subgroups of the Cannibalistic Group (CG). Fifteen CG *Ss* were isolated 10 days before use in white light at room temperature. Three hours before use, the CG *Ss* were placed 3 to a dish in 5 Pyrex dishes in red light. Each *S* of each subgroup of the SS was cut to 5 to 7 pieces and placed into a Pyrex dish containing 3 CG *Ss*. The first 2 CG *Ss* in any dish to be seen eating a piece of the SS were designated as the CG subgroup corresponding to that SS subgroup. The third CG *S* was destroyed. In the event 2 CG *Ss* would not eat within 2 hr., the entire procedure was rerun. The subgroups of the CG were then placed in darkness for 48 hr. at room temperature. CG subgroups were then tested on 25 trials, using the same procedure referred to in the SS testing above,

[2]Schematic diagrams of the shock apparatus and analysis of variance Table A are available through American Documentation Institute, Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington, D. C. 20540. Order Document No. 9384, remitting $1.25 for 35-mm. microfilm or 6- × 8-in. photocopies.

with all responses recorded. All of the above procedures were repeated five times so that each subgroup of the SS and CG contained 10 *Ss*.

The following controls were incorporated: (a) all *Ss* came from the same large container and the selection was designed to prevent the selection of specific *Ss* for specific subgroups, (b) *Ss* were given long periods of rest in white light to prevent sensitization, (c) *Ss* for any given repetition of the experiment were matched for size to minimize the effect observed by VanDeventer and Ratner (1964), (d) the occurrence of the light and shock for the LSNP subgroup cannot be considered random, but an attempt was made to space the light presentations randomly throughout the intertrial interval, (e) the training, feeding, and testing were coordinated in that these were all conducted at the same time each day (9 P.M.—1 A.M.), (f) the training, feeding, and testing of the subgroups were rotated in order, for each repetition of the experiment.

Results and Discussion

Table 1 shows the mean conditioned contractile responses for the testing periods for all SS and CG subgroups. A 2 × 5 analysis of variance with 10 *Ss* per cell indicated a significant difference among different training conditions ($F = 29.72$, $df = 4/90$, $p < .001$) but no difference between the SS and CG groups and no significant interaction.

TABLE 1

Mean Conditioned Contractile Responses of Each Subgroup of the SS and CG Groups for the Two Testing Periods of 25 Trials

Subgroup	M_{SS}	M_{CG}
LSP	18.4	19.9
LSNP	12.6	13.9
L	10.7	12.6
S	11.3	11.5
N	10.6	11.2

Scheffe's test showed that the LSP subgroup for SS and CG was significantly different ($p < .01$) from all other subgroups. All data were tested for homogeneity of variance, with F_{MAX} not significant. The data indicated that the LSP subgroup obtained significantly higher mean responses than any other subgroup and that this was due to the classical conditioning effect of the paired light-shock; variation among the control subgroups was not significant. The statistical tests indicated that the training of each subgroup transferred from the SS subgroup to the CG subgroup without significant gain or loss. Since the different subgroups were subjected to very similar conditions, it is doubtful that the results are a product of reflex sensitizing as suggested by Halas, *et al.* (1962) or due to the variables mentioned by VanDeventer and Ratner (1964). All groups were trained, fed, and tested at the same time each day and given sufficient time for environmental adjustment shown necessary by Best (1960) and Best and Rubenstein (1962). The LSNP, L, S, and N subgroups did not obtain significant dif-

ferences in responses and this indicated that conditioning probably did not occur in these groups. The slightly higher mean response rate of the LSNP subgroup could be interpreted as sensitization, but this is not known. All CG subgroups had a slightly higher mean response rate than their corresponding SS subgroups which could be due to dark adaptation as mentioned by VanDeventer and Ratner (1964) or to the effects of cannibalism as shown by Hartry, Keith-Lee, and Morton (1964). Long periods of light and temperature adaption are necessary to control for sensitization in this type of experiment. Effect of *E* bias was tested by use of a naive observer who independently judged contractile responses for 8 *S*s over 200 trials. The correlation obtained between *E*'s judgments and those of the naive observer was .75 ($p < .05$). This test was run to check on possible observer bias since blind running was not feasible. The correlation indicated that observer bias was not a significant variable. Rotation of each repetition of the experiment controlled for *E*'s fatigue and experience.

The data confirmed both hypotheses, that the conditioning of the LSP subgroup produced significantly higher mean responses than any other subgroup and that the training of each subgroup was transferred via cannibalism without significant change.

REFERENCES

BEST, J. Diurnal cycles and cannibalism in planaria. *Science*, 1960, 131, 1884-1885.

BEST, J., & RUBENSTEIN, I. Environmental familiarity and feeding in the planaria. *Science*, 1962, 135, 916-918.

CORNWELL, P., & CORNWELL, G. Planarian running procedure. *Worm Runners Digest*, 1959, 1, 4.

HALAS, E. S., JAMES, R. L., & KNUTSON, C. An attempt at classical conditioning in planaria. *J. comp. physiol. Psychol.*, 1962, 55, 969-971.

HARTRY, A. L., KEITH-LEE, P., & MORTON, W. D. Planaria: memory transfer through cannibalism reexamined. *Science*, 1964, 146, 274-275.

MARRIOT, F. H. C. The absolute light-sensitivity and spectral threshold curve of the aquatic flatworm. *J. Physiol.*, 1958, 143, 369.

MCCONNELL, J. V., CORNWELL, P. R., & CLAY, M. An apparatus for conditioning planaria. *Amer. J. Psychol.*, 1960, 73, 618-622.

THOMPSON, R., & MCCONNELL, J. V. Classical conditioning in the planaria *Dugesia dorotocephala*. *J. comp. physiol. Psychol.*, 1955, 48, 65-68.

VANDEVENTER, J. M., & RATNER, S. C. Variables affecting the frequency of responses of planaria to light. *J. comp. physiol. Psychol.*, 1964, 57, 407-411.

Accepted March 17, 1967.