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DISCRIMINATION LEARNING IN PARAMECIA (*P. caudatum*)

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Previous attempts to condition a 1-celled organism, paramecium, by either classical or instrumental procedures, have yielded equivocal results. The present experiments were designed to determine whether the use of positive reinforcement provided by DC electrical stimulation at the cathode, which had previously been shown to be attractive to paramecia, could be used to train these organisms in a discrimination learning task. The results indicate that such learning did take place.

Prior investigations into the possibility of either instrumental or classical conditioning in paramecia have reported both positive and negative findings, with some serious questions later raised about the possible lack of proper controls in some of the studies. Day and Bentley (1911) observed turning behavior of paramecia in a capillary tube. They found that the number of turns and the elapsed time before the paramecia reversed their direction of movement decreased with the number of trials. Their results were similar to those reported earlier by Smith (1908), but sensitization, rather than conditioning, could have accounted for both; there was no control for this.

French (1940) reported that time to escape from a liquid-filled capillary tube decreased with trials, a finding later supported by Hanzel and Rucker (1971) and by Huber, Rucker, and McDiarmid (1974). However, Applewhite and Gardner (1973), after similar findings, presented substantial evidence leading to the conclusion that these positive results might have resulted from a change in the liquid medium, rather than from learning. Unlike these instrumental procedures, Hennesey, Rucker, and McDiarmid (1979) reported classical conditioning of spinning and jerking responses in paramecia.

Another approach dealt with attempts to condition stimuli such as brightness or vibration to electric shock or heat. Examples of such work are experiments by Bramstedt (1935) and Soest (1937), which reported successful conditioning, and those of Best (1954), Mirsky and Katz (1958),

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and Sqonina (1939), which did not. It was possible that the earlier positive findings resulted from either sensitization or from uncontrolled factors. A more complete treatment of these and other early studies may be found in Applewhite (1979), Corning and Von Burg (1973), and Thorpe (1963).

Other relatively recent experiments that have dealt with this issue are those of Gelber (1952, 1956, 1957, 1958, 1962a, 1962b), Jensen (1957a, 1957b), and Katz and Deterline (1958). Gelber reported successful conditioned approach behavior to a sterile platinum wire inserted into a reservoir containing paramecia after the paramecia had received training with the wire coated with food (bacteria). She carried out a number of studies that used variations on this basic procedure. Jensen as well as Katz and Deterline, however, provided evidence to support the contention that Gelber's positive results did not indicate conditioning, but were artifactual. It thus seems that there has been no unequivocal evidence presented that conditioning is possible in paramecia.

The present research does not deal with classical or simple instrumental conditioning, but with the possibility that paramecia can learn a brightness discrimination based upon reinforcement. Previous research has shown that paramecia (*P. caudatum*) respond differently to DC electrical stimulation at the anode (+ electrode) from that at the cathode (- electrode). Anode stimulation was shown to have aversive properties, whereas cathode stimulation was attractive (Armus & Montgomery, 2001).

Experiment 1

Method

Subjects and Apparatus

Subjects were 288 paramecia (*P. caudatum*) taken from the colony maintained in the laboratory. The colony was housed in a violet-colored glass dish, 15 mm in diameter, filled with a mixture of Ward's cerophyll culture medium and distilled water, according to the directions provided by the supplier, to a depth of approximately 9 mm and loosely covered with violet-tinted transparent plastic wrap. The illumination level at the top of the colony dish was approximately 12 ft-c, and the room temperature varied from 70 - 75 ˚F. Each week three organically grown wheat grains were added to the culture medium to provide bacteria on which the paramecia fed. The parent stock of paramecia was obtained from Ward's Natural Science Establishment, Rochester, NY 14692.

The apparatus was a 22-mm long transparent glass trough made from a heated microscope slide cover glass and bent into a V shape. The trough was glued to a microscope slide at the apex of the V. A 2-ml trail of filtered culture medium was placed along the bottom of the V, with a stainless steel wire electrode projecting horizontally 2 mm into the water at each end of the trough through a plastic end cap. Electrical stimulation (shock) was provided by a Mallory power supply, model 12RS6D and was set at 6.5 V DC with a duration of 60 ms and an intershock interval

of 500 ms. Subjects were observed through a microscope under 10X magnification. The trough was divided into a light (30 ft-c) and a dark (7 ft-c) side by means of a dark gray transparent plastic filter placed under the microscope stage so as to darken half the trough. These parameters were selected on the basis of pilot work. Illumination of the trough was provided by an incandescent microscope lamp fitted with a blue filter and was measured with a Gossen Tri-Lux foot candle meter. A multilayered sandwich of clear plastic strips with air spaces between them was placed between the light source and the microscope stage to reduce the heat at the trough level. The temperature difference in the medium between the light and dark sides of the trough was less than 2 °F.

Procedure

A small drop of culture medium was removed from the colony using an eyedropper, was placed on a microscope slide, and was observed under 10X magnification. The blunted needle of a syringe was then inserted into this drop, and, when a single paramecium had attached itself to the needle, the paramecium was transferred to the trough, which was on the stage of a second microscope. Individual paramecia were observed in the trough for ten 90-s periods. Periods 1 to 7 were training sessions, during which shock was delivered or not delivered, as described below. Periods 8 to 10 were test sessions, during which there was no shock. Training and test phases were run sequentially with no interphase interval. The trough was thoroughly rinsed with distilled water four times between subjects. Three groups of 96 subjects each differed according to the shock procedures used during training, as follows:

1. Gp E (experimental) - A paramecium received a train of shocks (one 60 ms shock every 500 ms) as long it was in the cathode half of the trough.

2. Gp C-NS (no shock control) - Subjects received no shock at any place in the trough.

3. Gp C-PS (paired shock control) - A train of shocks was delivered regardless of whether the subject was in the anode or cathode half of the trough, with the number and temporal distribution of shocks identical to that received by its paired Gp E "partner."

The timing and recording were by means of electromechanical program apparatus. The dark and light halves of the trough were counterbalanced with respect to location on the right or left sides.[1] The insertion points of the paramecia were also counterbalanced, with half the subjects being inserted into the light side and half, into the dark side, but always as close as possible to the light-dark boundary. Furthermore, the cathode was on the dark side for half of these counterbalanced subjects, and on the light side for the other

[1]The functional stimulus difference between the two halves of the trough was a combination of illumination and temperature. For the sake of simplicity, this is referred to as illumination level.

half. The data were the times that each subject spent in the cathode half of the trough on each of the seven training and three test sessions, recorded in multiples of 500 ms.

Results and Discussion

Figure 1 shows the mean time spent in the cathode half of the trough during training sessions for Gps E and C-NS and during test sessions for the experimental group and both control groups. No shock was delivered to any subject during the test sessions. A 2 (group)[2] x 7 (training sessions) ANOVA showed that, during training, Gp E spent significantly more time than Gp C-NS in the cathode half of the trough, $F(1, 190) = 13.73$, $p < .001$. The decline in the time spent in the cathode half over the seven training sessions, possibly resulting from habituation, was not significant, nor was the interaction of groups x trials, $p > .05$ in both cases. The difference between these two groups on Session 1 might have been the result of

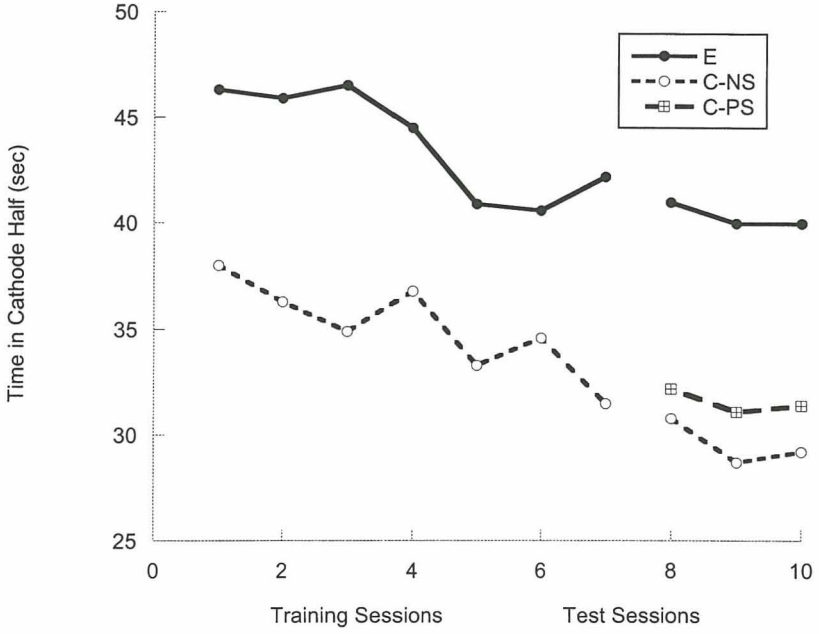

Figure 1. Experiment 1. Mean time spent in the cathode half of the trough during training and test. Training: Gps E & C-NS. Test: Gps E, C-NS, & C-PS.

[2]As shock was programmed for delivery to each Gp C-PS subject during training sessions, paired with the shock distributions of its Gp E "partner" and regardless of location in the trough, the time spent in the cathode half of the trough was not recorded for Gp C-PS during those sessions. It was recorded during the test phase, however, where it was relevant for the control function of this group.

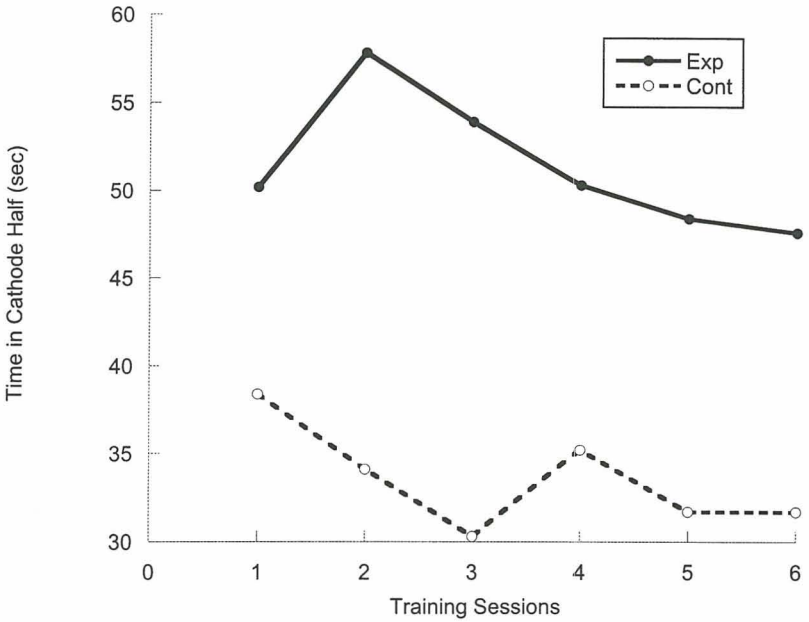

Figure 2. Experiment 2. Mean time spent in the cathode half of the trough during training.

rapid learning during this session, or it might represent an initial group difference. A similar difference was observed in Experiment 2 (see Figure 2), perhaps lending more weight to the former interpretation. However, as the data recorded were the total times spent in the cathode half of the trough in each 90-s session, it is not possible to determine whether there was rapid learning during the first few seconds of Session 1 or whether there was an initial group difference.The main data of interest, however, stem from the test sessions (8-10), during which no shock was delivered. During training, Gp E received only cathode shock, while the other two control groups received either no shock (Gp C-NS) or shock matched to that of Gp E, but not dependent upon location of the subject in the trough (Gp C-PS). If Gp E showed a greater preference for the cathode half of the trough during these nonshock test trials (based on the illumination of the cathode side, light and dark sides being counterbalanced) than did either Gp C-NS or Gp C-PS, this could be taken as evidence that the paramecia *did* learn a brightness discrimination based on cathode shock reinforcement. This is precisely what occurred. After an ANOVA showed a significant group effect for the test phase, $F(2, 285) = 8.38$, $p < .001$, Fisher's PLSD revealed a significant preference by Gp E for the former cathode side over that shown by either Gp C-NS or Gp C-PS, $p = .0002$ and $p = .0013$, respectively. As expected, there was no significant difference between Gps C-NS and C-PS, $p > .05$.

These results confirm the previous findings about the relative attractive

nature of cathode shock for paramecia compared to control groups and within the parameters employed. More important, however, is the fact that these results can be taken as evidence that paramecia can exhibit discrimination learning based on reinforcement by cathode shock.

Experiment 2

Although Experiment 1 seemed to demonstrate discrimination learning in paramecia, it is possible that there might have been confounding factors that resulted in this apparent learning. If the composition of the liquid in the trough had changed as a result of the electrical stimulation, this hypothetical change might have resulted in the cathode portion being made more attractive to the paramecia. Such an outcome would have favored Gp E over Gp C-NS during testing, but would not have favored Gp E over Gp C-PS, as both of these groups received the same number of shocks during training. As the subjects of Gp E spent significantly more time during test sessions in the former cathode half, this possible explanation is not supported by the data.

However, there is a second possibility. If the subjects that received positive reinforcement (electrical stimulation) only when they were in the cathode half of the trough exuded a substance that remained in the liquid during the no-shock test sessions, and if this hypothetical substance had attractive properties, then these paramecia might have spent more time in the cathode half of the trough during test sessions because of the attraction offered by this substance, not because they had learned to associate a given level of brightness with shock reinforcement. This would not have been the case for the no-shock control group (Gp C-NS), nor, to the same extent, for the paired shock control group (Gp C-PS). Experiment 2 was conducted to control for this possibility.

Method

Subjects, Apparatus, and Procedure

The subjects were 128 paramecia *(P. caudatum)* from the same colony as in Experiment 1, divided into two groups of 64 each. The apparatus was identical to that of Experiment 1, as was the training procedure, except for the following:

1. There were two groups of subjects, experimental (E) and control (C), rather than the three groups of Experiment 1, as the crucial question concerned Gps E and C.

2. There were six training sessions and four test sessions, rather than the seven training and three test sessions of Experiment 1.

For the testing sessions, the locations of the light and dark portions of the trough were reversed from right to left and left to right. Thus, the side, either right or left, that was dark during training was light during test, and vice versa. The shock electrodes remained in place, but the

electrodes were disconnected from the shock source, so that no shocks were delivered during the test phase. As in Experiment 1, the times that each subject remained in the (former) cathode half during test sessions constituted the data of interest.

Results and Discussion

The training data are presented in Figure 2. As was true for the training phase in Experiment 1, a 2 (group) x 6 (trials) ANOVA of the training data showed a significant group effect, $F(1, 126) = 19.26$, $p < .001$, Gp E showing a greater preference for the cathode area than did Gp C. There was no significant effect of trials, and the groups x trials interaction was also not significant, $p > .05$ in both cases. However, the results during the test phase, especially those for Gp E, are the focus of interest. The illumination *reversal* during test ensured that any substance exuded into the cathode half of the trough, light or dark, would now be paired with the *opposite* level of illumination. If it was this hypothetical substance that attracted the Gp E subjects of Experiment 1 to the former cathode side (light or dark) during test, then, as a consequence of the illumination reversal in the test phase of Experiment 2, this substance should now attract them to the same side (right or left) as during training, even though the illumination level was now reversed. If, on the other hand, the Gp E subjects had associated the shock reinforcement with a given level of illumination, they should then go predominantly to the side having *that* level of illumination, even though the location of that side was reversed during test (right to left and vice versa). As the Gp C subjects received no shock reinforcement during training, they should exhibit little or no change in their side preferences.

The crucial aspect of this experiment deals with the behavior following reversal of the location of the cathode-associated illumination stimulus during the test phase. Therefore, the behavior of only those subjects who had had substantial exposure to that stimulus during training was relevant to the test. Those were the paramecia that had at least *entered* the cathode area on more than three of the six training sessions. As a result, 53 subjects remained in Gp E and 45 in Gp C.[3] The test phase results are shown in Figure 3. They indicate that the subjects of Gp E *did* reverse their side preferences during the test phase, and those of Gp C did not.

It should be noted that, following the reversal of the location of the cathode associated stimulus, whether light or dark, subjects with a low preference for the cathode half of the trough and its associated stimulus would now seem to exhibit a strong preference for that stimulus, and vice versa. This is merely an artifact of the method of graphic presentation. However, Gp C showed no significant change in preference over the four test sessions, as contrasted to the behavior of the subjects of Gp E (see below).The change in location of the cathode-associated stimulus initially disrupted the preference for this stimulus for the experimental subjects;

[3]Also as a result, the values for Training Session 6 are different in Figures 2 and 3, as they are based on different numbers of subjects.

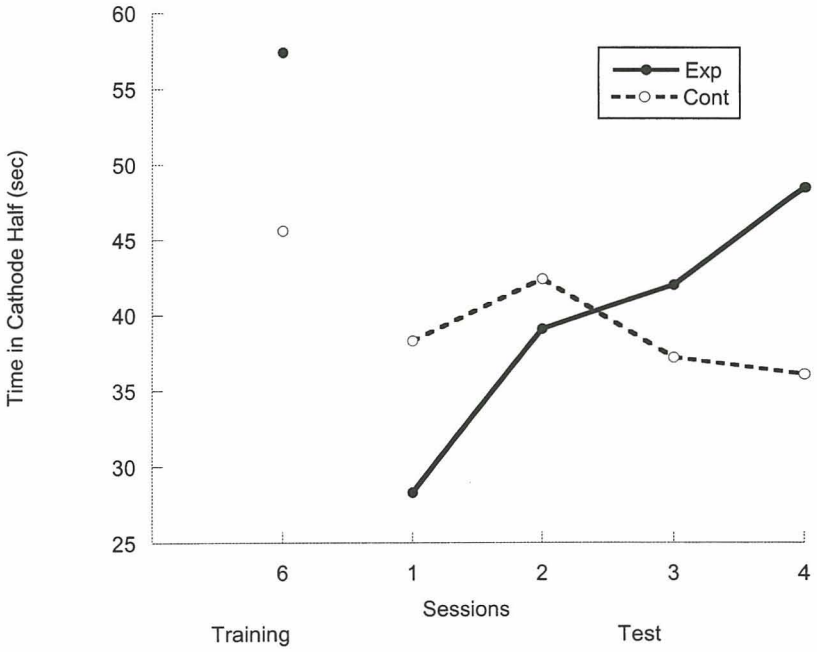

Figure 3. Experiment 2. Mean time spent in the half of the trough containing the cathode-associated stimulus during the last training session and the four test sessions. Location of the cathode-associated stimulus was reversed for the test sessions.

they tended to remain in the previous cathode area of the trough even though the cathode-associated stimulus had been switched to the opposite side. This is demonstrated by the significant drop in preference for the cathode-associated stimulus from Training Session 6 to Test Session 1, $t(52) = 3.34$, $p = .002$, as indicated by the time spent in association with that stimulus. There was no such drop for Gp C, $t(44) = .68$, $p = .50$. However, the preference for the cathode-associated stimulus showed a significant recovery over the four test sessions, indicating that the experimental subjects did learn the new location of that stimulus. Following the determination of a significant groups (2) by test sessions (4) interaction, $F(3, 288) = 3.48$, $p < .02$, simple effects tests were conducted. These showed that the time spent in association with this stimulus significantly increased for Gp E over the four test sessions, $F(3, 159) = 9.24$, $p < .001$. By contrast, there was no significant change for Gp C, $p > .30$. The alternate explanation for the results of Experiment 1 was based on the possibility that paramecia receiving cathode shock might have exuded some substance into the cathode half of the trough, and that this hypothetical substance might have accounted for the preference for this part of the trough even in the absence of cathode shock. The initial disruption of the preference for the cathode-associated stimulus shown by the experimental subjects when the location of this stimulus was switched, followed by the subsequent recovery of this

preference over the remaining test sessions, does seem to eliminate that explanation. Thus, the results of these two experiments offer evidence that paramecia (*P. caudatum*) can and did learn a brightness discrimination and that illumination level acted as an acquired or secondary reinforcer.

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