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Classical conditioning in paramecia

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Single *Paramecium caudatum* were conditioned by pairing ac-generated electric shock (US) with a vibratory stimulus (CS) produced by an auditory speaker. Naive paramecia subjected to shock reliably exhibited a backwards jerk and axial spinning similar to the avoiding reaction described by Jennings in 1904. Such responses did not occur initially to CS alone, but increasingly appeared during the CS period preceding shock pairing (delayed conditioning paradigm). Control subjects given the CS and UCS at the same intervals, but explicitly unpaired, did not show a sustained increase of responses to the CS alone. Short-term memory was demonstrated by subjects first conditioned and then presented CS alone during extinction. These subjects were readily reconditioned. Paramecia trained and stored for 24 h showed reliable memory savings as compared to stored control subjects. Other paramecia were differentially conditioned by training with two CSs. Following the recommendations of Rescorla (1967), a procedure was designed for truly random presentation of the CS and UCS as an additional control for pseudoconditioning. Single paramecia were conditioned with intervals between CSs randomly ranging from 8 to 32 sec. Control subjects received the same number of CSs and UCSs, which were administered independently and randomly during the same total session duration. Thus, CS and UCS were occasionally paired for control subjects. The responses to CS in the conditioned group were anticipatory conditional responses due to the pairing contingency and not wholly due to pseudoconditioning.

After a century of sporadic investigation, the question remains whether protozoa are capable of behavioral change that in higher organisms would be described as learning (reviews of the issue include: Corning & von Burg, 1973; Eisenstein, 1975; Jensen, 1965; McConnell, 1966; Poskocil, 1966; Thorpe, 1968). Using a method originated by French (1940), we have recently shown that single paramecia sucked by capillary action into a glass tube escape back into a drop of culture medium more rapidly over repeated trials, and that this acquisition is not related to time in the drop, time in the tube, the inner diameter of the tube, the intertrial interval, or the pH or temperature of the medium (Hanzel & Rucker, 1971, 1972; Huber, Rucker, & McDiarmid, 1974). Activity in the drop following escape also increased over trials, though in a manner not obviously related to escape speed, and both changes were retained at least 150 min (Huber et al., 1974). Subjects whose tube capture was made contingent on activity in the drop, however, decreased their swimming speed in the drop while increasing their speed of escape from the tube; controls whose tube capture was yoked to the time of capture of the experimental subjects did not increase their rate of swimming in the drop (Benson, Rucker, & McDiarmid, 1974).

While these studies bear a remarkable resemblance to instrumental learning, the relevant stimuli are not easily identified. We therefore initiated the present studies of classical conditioning in paramecia.

These investigations, supported in part by the Mankato State University Research Council and an award through the Mankato State University President's Research Lectureship to the second author, were presented in part at the 5th Annual Meeting of the Society for Neuroscience, New York City, November 2-6, 1975, and in part at the Midwestern Association for Behavioral Analysis, 2nd Annual Meeting, Chicago, May 1-4, 1976. We appreciate C. Cullen's help with time-lapse photography and E. Lamont's help in preparing a video summary of work to date presented at the latter meeting. Todd Hennessey is currently at the Department of Neuroscience, University of Wisconsin, Madison, Wisconsin 53076. Reprints may be obtained from W. B. Rucker, Department of Psychology, Mankato State University, Mankato, Minnesota 56001.

EXPERIMENT 1: CLASSICAL CONDITIONING

Method

Subjects. The subjects were *Paramecium caudatum* supplied by Turtox (Chicago, Illinois) and were maintained in culture medium from the same source. The 40 subjects were randomly chosen from cultures so that they were of varying ages, sizes, and activity. The cultures were attended daily by adding distilled water and were recultured at 2-week intervals.

Apparatus. Single paramecia were captured by capillary action into 10-mm-long glass tubes cut from 10 lamda Microcaps (standard tube from Bolab, Inc., Reading, Massachusetts). The subjects were observed through a Nikon Stereomicroscope with fluorescent light provided overhead, 31.52 cd/m^2 at the microscope stage. A 4-in. speaker (Zenith 490959) was mounted on the stage. Across the speaker was glued a microscope slide in the manner of Rucker and Huber (1973). A piece of dark paper was taped on the underside of the slide to facilitate viewing. On top of the slide was taped a plastic cover slip to which was taped a permanent stainless steel electrode approximately 2.5 cm long and .5 mm in diameter. The capillary tube with a single paramecium was plugged onto this electrode and a similar electrode was

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inserted in the other end. This removable electrode was lightly coated with paraffin up to the 1 mm entering the tube. The paraffin, along with the plastic cover slip, helped to maintain the tube in position during the presentation of vibrations ("tones"). The electrodes were slightly smaller than the inner diameter of the capillary tube to prevent compression of its contents. To protect against evaporation, a bacteriological loop was touched to the tube ends, releasing filtered medium whenever necessary. The liquid was cleaned out of the tube as required by a water-powered aspirator. The suction end of the aspirator was a short length of polyethylene tubing attached to the base of the microscope near the work. When all was assembled and viewed through the microscope, the paramecium was easily seen trapped in the capillary tube between the inserted electrodes.

Procedure. Each tube was cleaned three times before training by sucking up distilled water and removing it by the aspirator. This tube was then used to select a subject from a paramecia-rich drop of culture medium. The tube was examined and refilled until only one paramecium was present. A training trial began as soon as the tube was connected to the testing apparatus and the ends of the tube doused with filtered media to protect against evaporation. Occasionally, a smaller subject would escape between the electrode and capillary tube, and this subject would be replaced in the experiment by a new paramecium.

Each training trial consisted of a 4-sec vibration (CS), the last 2 sec of which were paired with a 2-sec shock (UCS). The electrodes and speaker were connected to interval timers which provided ac shock (5 V, 7.75 mA) and vibration (approximately 1.6-V intensity at the speaker), respectively, with a 10-sec intertrial interval (ITI). Ten subjects were trained with a CS of 300 Hz, 10 with 500 Hz, and 20 with 400 Hz. Distinct relay clicks began the CS and separated the CS-only period from the CS-UCS period. The relays were on a floor rack, so that the clicks were not translated to vibrations of the experimental table. Shock always produced a response similar to that described by Jennings (1904) as "an avoiding response" observed when a paramecium swam full speed into a physical barricade or was subjected to ac shock. The full avoiding response consists of an immediate backwards jerk (ciliary reversal) and subsequent axial spinning, turning toward the aboral side, and resumption of forward motion (cf. Bullington, 1938). Such a response rarely occurred in naive paramecia exposed to the CS alone in our experiments. As training proceeded, the experimenter recorded the occurrence of either an obvious backwards jerk, axial spinning, or the jerk followed by the spin as a single anticipatory conditioned response (CR) if one (or infrequently more) response occurred during the CS-alone period. Subsequently, responses were independently videotaped and rescored without significant deviation (Hennessey, Cullen, & Rucker, Note 1).

Since only the presence or absence of a CR was recorded by a Hunter counter, the score was translated into a linear trend score for each subject by multiplying the score for each trial by the appropriate coefficient of the orthogonal polynomial for the total number trials. For the 10 trials in this experiment, the coefficients, respectively, were: -9, -7, -5, -3, -1, 1, 3, 5, 7, and 9. The 10 products of score and coefficient were summed and divided by the square root of the sum of the squared coefficients. This calculation defined a learning score, or L-score, for each subject, normalized for number of training trials conducted. Responses early in training have the effect of lowering the L-score, and a high L-score signifies acquisition of the anticipatory CR over trials. The criterion for classical conditioning was that the mean L-scores be positive and significantly greater than zero.

Results and Discussion

Timed exposure photographs (taken from the videotape used to monitor Experiment 3 described below) are presented in Figure 1 in order to illustrate the URs and CRs observed in this and other experiments.

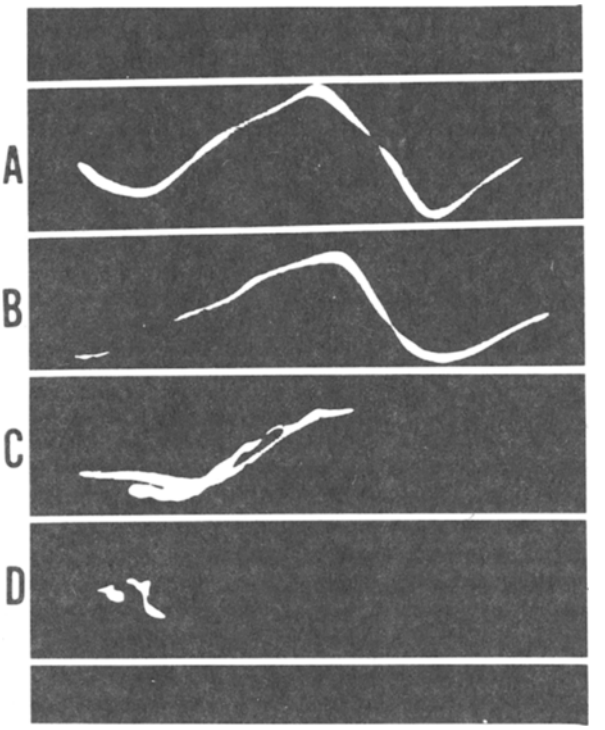

Figure 1. In these time-lapse photographs (exposure time of 4 sec), the paramecia are initially seen swimming from the left, the white line of their trail proceeding to the right unless interrupted by shock or by avoiding reactions. (A) Normal swimming in the tube. (B) Normal swimming is not interrupted by CS in this untrained subject. (C) Shock reliably produces the avoiding reaction lasting the full 2 sec of shock, presented to this subject after a 2-sec delay. (C) After 10 pairings of a 2-sec shock in the last half of a 4-sec CS, this subject shows avoiding reaction during the entire 4-sec presentation of CS without any shock on the first trial of extinction.

As can be seen from Figure 2, the three groups reached the same level of final performance, but at different rates, depending on the frequency of the training tone. The asymptotic performance of all three groups appears to be about 40%, a rather low level of conditioning, and indeed far lower than that demonstrated in following experiments.

Subjects trained with 300 Hz as the CS had a positive mean L-score which, however, failed to reach statistical significance [$t(9) = .91$, $p > .5$]. Pairing shock with 400 or 500 Hz did produce a rise in CRs compatible with the criterion for classical conditioning [$t(19) = 2.99$, $p < .01$, and $t(9) = 11.1$, $p < .001$, respectively]. The mean L-scores increased with higher frequencies (.143, .275, and .489 for 300, 400, and 500 Hz, respectively), a difference that was significant between the 400- and 500-Hz groups [$t(28) = 2.71$, $p < .02$]. Although the mean L-scores had been higher for the 400- and

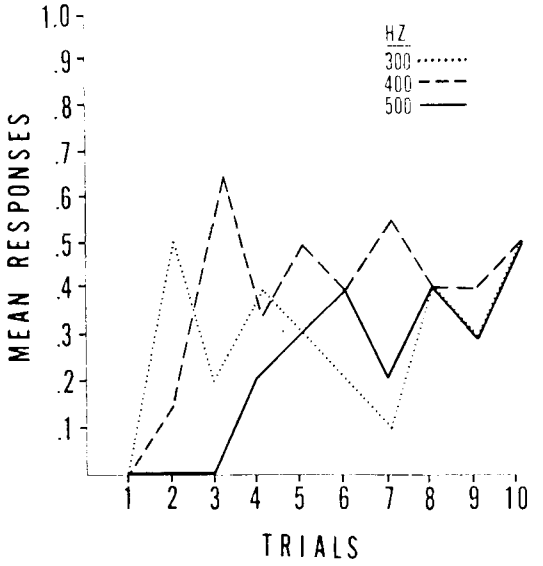

Figure 2. Acquisition of anticipatory CR with various CSs paired with shock. Mean responses were calculated by dividing the total number of responses per trial by the n of the subjects for each group, and thus provide an estimate of response probability.

500-Hz groups, the responses appeared to involve faster jerks and spins over shorter distances and consequently were harder to discern than responses at 300 Hz. With the 300-Hz CS, the mean L-score was lower than that due to training with 400 or 500 Hz, but the responses were well defined. As pointed out by Patterson, Cegavske, and Thompson (1973), rapid acquisition of a CR in simple preparations may be due to a lack of competing responses. In their experiments and in those of Durkovic (1975) as well, conditioning in the spinal cat was mostly acquired during the first 10 trials of training.

EXPERIMENT 2: ACQUISITION WITH PSEUDOCONDITIONING AND SENSITIZATION CONTROLS

Method

Subjects and Apparatus. Thirty paramecia were selected as subjects and were trained using the apparatus described for Experiment 1.

Procedure. The protocol for this experiment followed that of Experiment 1 with the following exceptions. A CS of 350 Hz was selected for all subjects as a compromise between the good response definition at 300 Hz and the more reliable CR acquisition at 400 and 500 Hz observed in Experiment 1. The interval between CS offset and onset was increased to 16 sec (making a total intertrial interval of 20 sec). For 10 subjects, the US came on during the last 2 sec of the 4-sec CS. For 10 control subjects, the 2-sec US came on 7 sec after the offset of the preceding CS. Both of these groups received 10 CSs and 10 USs. An additional 10 subjects were first trained for 10 trials under the control condition of explicitly unpaired stimuli, followed by 10 trials of paired stimuli. Responses during the first 2 sec of CS under the control condition were counted as a measure of pseudoconditioning. Responses during the 2 sec preceding unsignaled shock in these subjects was taken as a measure of sensitization or increased reactivity of subjects submitted to the two stimuli. Clearly, an increase in responses indicated by a significantly positive L-score for either control measure would imply that a similar increase in responses observed in subjects receiving paired stimuli could not depend on the pairing contingency and would not permit the description of these responses as anticipatory CRs.

Results and Discussion

The results of the two groups tested over 10 trials of explicitly paired or explicitly unpaired stimuli are shown in Figure 3. For the paired group, the anticipatory CRs rose reliably, as indicated by a mean L-score of .699 [$t(9) = 6.41$, $p < .001$]. This effect could not be attributed to either pseudoconditioning or sensitization, in view of the failure of the first control group to increase responsivity either during the first 2 sec of the CS or during the 2 sec just preceding unsignaled shock, as shown, respectively, by mean L-scores of $-.110$ [$t(9) = 1.10$, $p > .3$] and .110 [$t(9) = 1.30$, $p > .2$].

The conclusion that classical conditioning is, indeed, responsible for the rise in anticipatory CRs in subjects trained with paired CS and UCS is again reinforced by the performance of the second control group in this experiment, which went through two phases of training. In the first phase of explicitly unpaired stimuli, neither the L-score for pseudoconditioning ($-.020$) nor the L-score for sensitization ($-.071$) was significant [respectively: $t(9) = .445$, $p > .5$; $t(9) = 1.12$, $p > .5$]. Again, it is apparent that the steady rise in CRs depends on the pairing contingency and not on the application of the stimuli or on the interval between like stimuli. Rescorla (1967) has criticized the use of explicitly unpaired stimuli to control for pseudoconditioning on the grounds that the CS may be used

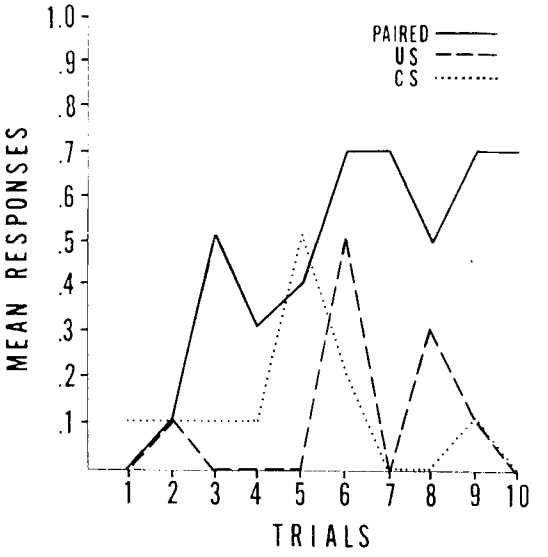

Figure 3. Acquisition controlled for pseudoconditioning and sensitization, respectively, by responses of the first control group to CS alone or during the 2 sec preceding shock.

by the subjects to signal the nonoccurrence of the unconditional stimulus, i.e., safety from shock in our experiments. If so, then the control subjects that went on to training with paired stimuli should not have acquired the response. Because these subjects *did*, in fact, acquire the response, as shown by a mean L-score of .385 [$t(9) = 2.65$, $p < .05$], two conclusions may be drawn: (a) These paramecia may not have learned that the CS signaled safety from the UCS, and (b) the use of explicitly unpaired stimuli in this experiment supports the conclusion that acquisition of the CR is not due to pseudoconditioning.

EXPERIMENT 3: TRULY RANDOM PSEUDOCONDITIONING CONTROL FOR CLASSICAL CONDITIONING

In these experiments, two types of controls for pseudoconditioning and sensitization have been conducted. First, control subjects in Experiments 2 and 5 were administered explicitly unpaired CSs and USs, presented alternately and at the same intervals used for the subjects receiving explicitly paired stimuli. These subjects showed no reliable increase in responsivity either during the first 2 sec of the CS alone or during the 2-sec period just preceding US alone. Secondly, other subjects in Experiments 6 were presented CSs of either 300 or 500 Hz on alternate trials, only one of which was paired with the US. Regardless of the CS selected for US pairing, conditioned responding increased reliably to this CS but not to the CS presented alone.

Rescorla (1967) has criticized both of the foregoing types of controls on the grounds that the CS may be learned as a signal for safety from the US and thus would not rule out pseudoconditioning, defined as a rise in responsivity not specifically due to the pairing contingency during training. Given the logic of this argument, it is not possible to state whether the experimental subjects (with pairings) had learned that the CS signaled safety. There is indirect evidence in Experiment 2 that the controls administered both US and CS explicitly unpaired did not learn safety; they subsequently went on to training with paired stimuli and acquired the response.

Rescorla (1967) has recommended a procedure of truly random presentation of the CS and UCS as a control for pseudoconditioning. Under this design, the CS and US would occasionally be paired, but tone would predict neither shock nor safety. Thus, a rise in responsivity under this condition would indicate that the responsivity of subjects given paired training was not due to the pairing contingency. This is the control procedure used in the present experiment.

Method

Subjects and Apparatus. Thirty *paramecium caudatum* were selected as subjects and trained with the apparatus described in Experiment 1.

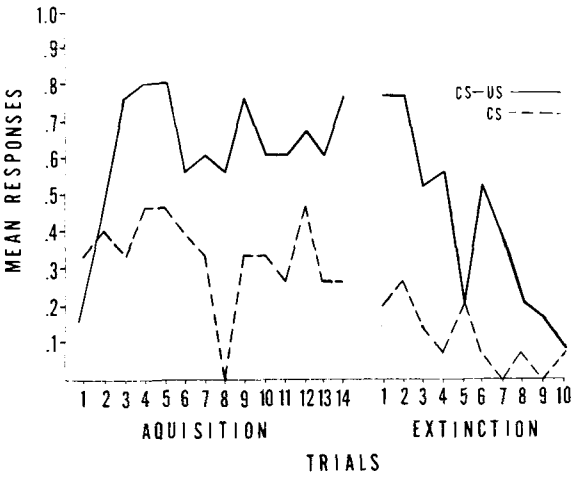

Figure 4. Acquisition and extinction of anticipatory conditioned responses observed in the first 2 sec of a 4-sec CS which was paired in the last 2 sec with shock during acquisition (CS-US). Pseudoconditioning controls (CS) received the same CSs and shock in a truly randomized fashion with occasional pairing and made substantially fewer responses. The ITI was randomized for both groups during acquisition but was set at 20 sec during extinction where no shock was used.

Procedure. Presentations of the CS and US were controlled by two tape readers so that the CS and UCS could be timed independently while still retaining common time for the readings. Each tape independently randomized the order of 14 intervals, two each of 8, 12, 16, 20, 24, 28, and 32 sec. The tape for the CS was randomly accessed to determine the first interstimulus interval for each subject. For the 15 experimental subjects, shock (5 V, 7.75 mA, ac) was administered continuously during the last 2 sec of the 4-sec CS (1.6 V at the speaker, 350 Hz). For the 15 control subjects, shock was administered according to the pattern of intervals on the other tape, randomly accessed at the beginning of training. Thus, the number of chance CS-UCS pairings varied among the subjects and there were episodes of backward pairings as well. The data for the control subjects was therefore corrected for responses to the CS that had been accompanied by shock. Responses were also videotaped and independently rescored (see Figure 1).

Immediately after the 14 presentations of CS and US, subjects in both groups were given a 30-sec pause followed by 10 trials of CS alone presented at 20-sec intervals as a test for extinction.

Results and Discussion

The mean number of responses during the first 2 sec of CS during training was substantially greater for the group receiving explicitly paired US than for the group receiving randomly paired US [$t(28) = 4.64$, $p < .01$]. These responses are shown on the left side of Figure 4 and must be described as anticipatory CRs in the experimental group as they cannot be attributed wholly to pseudoconditioning.

During subsequent extinction with CS alone, the experimental subjects made substantially more responses than the controls in the first 2 sec of the CS (shown on the right side of Figure 4), the last 2 sec of the CS, and during the entire CS presentation [$t(28) = 4.1, 3.4, 4.2$, respectively, $ps < .01$].

EXPERIMENT 4: EXTINCTION AND REACQUISITION

Method

Subjects and Apparatus. Ten paramecia were selected as subjects and were trained using the apparatus described for Experiment 1.

Procedure. The protocol for this experiment followed that of Experiment 1, except that each subject was trained with a 350-Hz CS paired in the last 2 sec with US over 10 trials of acquisition, followed immediately by 10 trials of the CS alone for extinction, and followed finally by 10 trials of CS-US presentations for reacquisition. For all 30 trials, the interval between the offset of one CS and the onset of the next was 16 sec, for a total intertrial interval of 20 sec.

Results and Discussion

Acquisition of the anticipatory CR was again shown by a mean L-score of .601 [$t(9) = 8.01$, $p < .001$]. There was a reliable decrease in CRs during extinction, as shown by a negative L-score of $-.771$ [$t(9) = 7.20$, $p < .001$]. This typical curve of extinction of a classically conditioned response implies that: (a) the results of conditioning are not due to an irreversible change in the organism, and (b) paramecia are capable of at least short-term memory of their conditioning. That extinction itself did not occur through fatigue or some similar process is shown by the ability of the subjects to reacquire the response during the final phase of paired training, producing a mean L-score of .584 [$t(9) = 3.13$, $p < .02$]. Reacquisition did not, in fact, differ from acquisition [correlated $t(9) = .056$]. Acquisition, extinction, and reacquisition are shown in Figure 5. The demonstration of short-term memory during extinction suggested the possibility of long-term memory, which was tested in the next experiment.

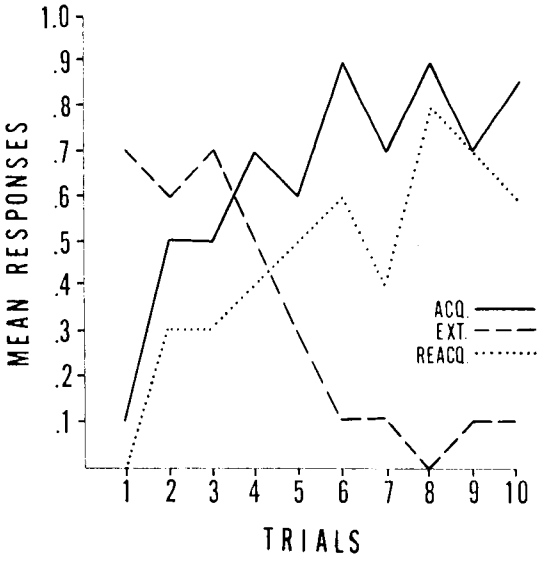

Figure 5. Acquisition, extinction, and reacquisition of anticipatory CRs in Experiment 4.

EXPERIMENT 5: LONG-TERM MEMORY

Method

Subject and Apparatus. Twenty-five paramecia were selected as subjects and were trained with the apparatus described for Experiment 1. Whether trained or not, on Day 1, all subjects were transferred individually to a drop of filtered medium on a depression slide which was covered by a cover-slip taped in place to prevent evaporation. Each slide was given a code number. All animals were tested after 24 h of storage on Day 2. The few animals which divided during this period were replaced by similarly stored substitute subjects.

Procedure. The procedure generally followed that of Experiment 1. A 350-Hz CS was used with 16 sec between offset of one CS and the onset of the next. The first 10 subjects were given 15 conditioning trials on Day 1, stored individually for 24 h, and then given 15 trials of retraining. This retention interval is quite long given the life span of paramecia (Huber et al., 1974). This procedure was repeated for 5 more subjects, which were to be compared with two sets of 5 control subjects each. Neither control received training on Day 1. Instead, they were coded and stored for 24 h. One group then received 15 presentations of paired tone and shock, the other 15 presentations of explicitly unpaired tone and shock.

A memory score, or M-score, was developed similar to the L-score described in Experiment 1. The M-score was the sum of the products of the responses on each trial multiplied by appropriate coefficients, divided by the square root of the sum of the squared coefficients. For Day 1, the coefficients were -15, -14, -13, ... -3, -2, -1; and for Day 2, they were 15, 14, 13, ... 3, 2, 1. In this fashion, if a subject had responded with the same pattern on the 2 days, the M-score was 0. If the subject performed better during early trials on Day 2, than on Day 1, the M-score was positive, indicating savings. Since the control groups had no Day 1 scores, the mean value of the Day 1 part of the M-score of the trained group was substituted in the formula to calculate the M-scores of the subjects in the control groups.

Results and Discussion

For the original 10 subjects trained and then retrained 24 h later, significant memory savings were shown by a mean M-score of .779 [$t(9) = 5.09$, $p < .001$]. The rate of continued acquisition on Day 2 (L-score of .424), however, could not be distinguished from that on Day 1 (L-score of .754) [correlated $t(9) = 2.20$, $p > .1$]. To test for the possibility that the conditions of storage had simply produced more reactive animals on Day 2, rather than memory savings, we compared the additional subjects trained on Day 1 with subjects that were stored without such training. These results are plotted in Figure 6. Significantly greater memory savings were shown by the paramecia given training on both days than by those trained for the first time on Day 2 [$t(8) = 2.49$, $p < .05$]. As can be seen from Figure 6, storage alone did not promote reactivity (in those trained for the first time on Day 2) or

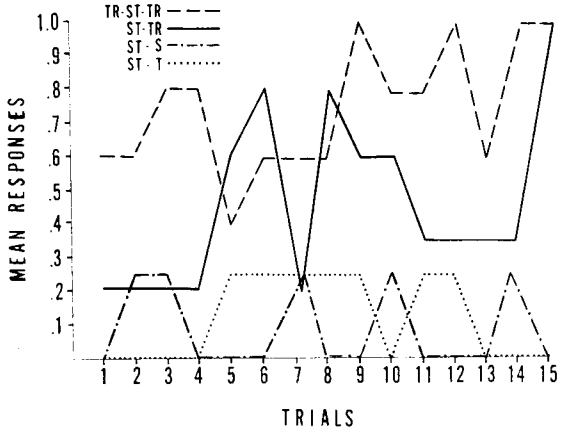

Figure 6. Memory savings shown by paramecia trained, stored for 24 h, retrained (TR-ST-TR). Controls were stored without training and either given paired stimuli (ST-TR) or given explicitly unpaired stimuli. For the latter, responses are shown for the first 2 sec of CS (ST-T) and for the 2 sec preceding shock (ST-S).

pseudoconditioning or sensitization (in the subjects tested with explicitly unpaired stimuli on Day 2).

EXPERIMENT 6: DIFFERENTIAL CONDITIONING

Pavlov (cf. Kimble, 1961, p. 362) used the method of contrasts or successive presentation of CSs, only one of which was paired with the UCS, to establish discrimination between stimuli in the same modality. In Experiment 1, the acquisition curves and characteristics of the CR differed between subjects trained with 300- and 500-Hz CSs. This suggested the possibility that the two stimuli could be discriminated by paramecia.

Method

Subjects and Apparatus. Twenty paramecia were selected as subjects and trained with the apparatus described for Experiment 1.

Procedure. Each subject was trained with 4-sec CSs of 300 and 500 Hz alternating over 20 trials, with a 20-sec ITI. For 10 subjects, shock was administered during the last 2 sec of the 300-Hz CS, but not during the 500-Hz CS. For the remaining 10 subjects, shock was administered during the 500-Hz CS but not during the 300-Hz CS. For all subjects, shock was administered on odd-numbered trials. L-scores were obtained separately for the two groups under both the paired (CS+) and unpaired (CS−) conditions and were submitted to a mixed analysis of variance.

Results and Discussion

In the between-subjects part of the analysis, the 300- and 500-Hz CS+s were equally effective in acquiring the anticipatory CR [$F(1,18) = 2.13$, $p > .25$]. This is in contrast to the results of Experiment 1 and may be attributed either to the use of a longer intertrial interval in this experiment or to other conditions involved in the use of alternating stimuli.

In the within-subject part of the analysis, the anticipatory CR was acquired under the paired condition, but not under the unpaired condition [$F(1,18) = 5.00$, $p < .05$]. Again, the choice of CS+ made no difference [$F(1,18) = .32$]. Since there was no difference in the reactivity to the two frequencies, the data were combined for the two CSs for the paired and the unpaired conditions and are shown in Figure 7. The mean L-scores were .363 and .005, for the combined CS+ and CS−, respectively.

It is possible that the paramecia were predicting the nonoccurrence of shock on alternate trials rather than discriminating CS+ and CS−. But if paramecia had this ability, then the control subjects in Experiment 2, who had a full 10 trials to learn safety with no conflicting contingencies prior to their acquisition test, would have been likely to demonstrate learned safety. As they did not, the alternating response interpretation of the present experiment is less likely. Thus, from the present experiment, it appears that paramecia are capable of discriminating two arbitrarily chosen vibratory stimuli, thus fulfilling part of Neal Miller's (1967) criteria for what he calls "Grade-A certified learning."

GENERAL DISCUSSION

Analysis of the physiology of learning at the cellular level will proceed more rapidly as model systems are developed in which all of the cells involved in the learning can be studied simultaneously in a setting in which all of the relevant stimuli can be controlled. Classical conditioning in invertebrates may offer such models (Lee, 1976; Mpitsos, 1976), particularly since the relevance of the stimuli involved is deter-

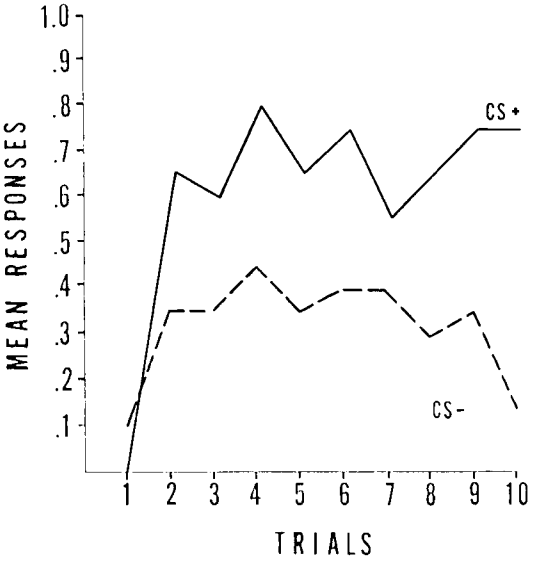

Figure 7. Differential conditioning shown with responses to CS+ and responses to CS−. Data pooled for CSs 300 and 500 Hz.

mined experimentally only by the temporal association of the CS and the US. In studies of learning in single protozoa, there is no question which cells may be involved, and any properties of behavioral change that can be observed cannot be attributed to synaptic interactions.

In this investigation of classical conditioning in paramecia, it has been shown that (a) the anticipatory CR increases in response strength over paired presentations of CS and UCS; (b) this change is relatively permanent, though it can be reversed by extinction; (c) the change is based on the pairing contingency between CS and UCS and not on either pseudoconditioning or sensitization; and (d) the learned response may be specific to arbitrarily chosen stimuli. Demonstration of learning in protozoa is hard to accept because it is inconsistent with the dominant assumption that learning is a property of synaptic interactions and not of the cells themselves. While the present study does not establish beyond a shadow of doubt that protozoa are capable of learning, the burden of proof now lies with the contrary point of view. It seems far easier to study the cellular physiology of learning and memory at the level of the isolated single cell than at the simplest metazoan level (Halstead & Rucker, 1967), and the conduct of such studies may be encouraged by the demonstration of classical conditioning in single paramecia.

In the last decade, paramecia have become a standard model for excitability at the cellular level. Ciliary beat and reversal in response to electrical and mechanical stimuli have well-defined characteristics controlled parametrically by cation concentration (Hildebrand & Dryl, 1976; Machemer, 1976; Naitoh & Eckert, 1969; Nelson & Kung, 1978). Although some speculation has begun relating membrane changes to learning mechanisms in our laboratory (Huber, 1972) and elsewhere (Eisenstein, 1975), more precise control of behavioral changes in conjunction with ongoing measures of physiological changes are prerequisite for a more complete understanding. This new phase of research seems feasible in light of the behavioral results reported herein.

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