Acquisition and retention of active avoidance in *Xenopus laevis**

RALPH R. MILLER†, ALVIN M. BERK
and ALAN D. SPRINGER††
Brooklyn College of the City University of New York
Brooklyn, N.Y. 11210

Adult male South African clawed frogs, *Xenopus laevis*, were trained in an aquatic shuttlebox on one-way active avoidance of electric shock. Acquisition was observed within daily sessions (30 trials) and between days (4 days). Control groups indicated that learning, rather than pseudoconditioning or sensitization to the CS, was responsible for the observed change in behavior. A retraining measure yielded appreciable savings after a 28-day retention period. Some of the advantages of this species for behavioral research are discussed.

The South African clawed frog, *Xenopus laevis*, is an animal frequently used in biological experiments but rarely seen in psychology laboratories. As a species that has been successfully cloned (Elsdale, Gurdon, & Fischberg, 1960), it is a plausible S for many innovative psychological studies, particularly in behavioral genetics. Moreover, being a fully aquatic animal during all stages of its life, *Xenopus laevis* is well suited to serve in experiments concerned with the effects of vertebrate metamorphosis upon innate and acquired behavior. Yet to date, Haubrich (1966) provides the only report of successful laboratory training of *Xenopus laevis*, and that study suffers from a lack of control for changes in activity, response bias, and pseudoconditioning. Haubrich's task was an air-filled T-maze with water immersion used as a reward. Given that water is the animal's natural habitat, the present study was designed to permit the S to perform in water. Because adult *Xenopus laevis* are basically inactive and prefer the dark, we sought to train animals to avoid actively a darkened chamber.

METHOD

Subjects

Fifteen adult male *Xenopus laevis* between 18 and 30 months old were obtained from Nasco (Fort Atkinson, Wis.). Animals were maintained in 20°C aged tap water in two 20-gal glass aquariums with recirculating filter systems and 12 h per day of light. Food in the form of live goldfish was provided for 1 h per day twice a week. Animals were identified by select clipping of claws several days prior to the start of the experiment.

Apparatus

Training was given in a two-compartment Plexiglas tap water-filled (20°C) shuttlebox consisting of a black start chamber, 12 cm wide x 16 cm long, and a white safety chamber, 15 cm wide x 25 cm long. Stainless steel plate electrodes covered the 16-cm walls of the black compartment for the full 10-cm water depth. The electrodes were attached, when activated, to a 25-V ac source through 12,000 ohms. To minimize tetanization, shock was pulsed 0.1 sec on, 1.0 sec off. The two chambers were separated by a 12-cm-wide black gate that could be raised (opened) 10 cm by the E, and by an equally wide clear Plexiglas gate that was hinged at the surface of the water for the frog to push open when it shuttled. The latter gate was used to add to the distinctiveness of the shuttle response, and it contained numerous holes 0.6 cm in diam to reduce water resistance to the swinging of the gate. Illumination was provided by a 7.5-W incandescent light bulb positioned 25 cm above center of the white compartment. The black compartment was shaded from the bulb and was illuminated only by transmission from the white compartment when the black door was open. Noncontingent shock was given in a green Plexiglas box of the same dimensions and electrode configuration as the black compartment of the shuttlebox. This box was filled with 10 cm of tap water and was illuminated by two 15-W fluorescent tubes located 50 cm above the center of the box. The shock source servicing the training apparatus was switched to the green box when needed.

Procedure

On Day 1 each frog was given a black-white preference test in the training apparatus with both gates removed. Testing consisted of placing the animal in the black compartment facing away from the white compartment and recording the frog's movements for 30 min. On this and all subsequent days, the animals were transported the few feet between home tank and apparatus in a fine mesh cloth net to minimize stress. The gates were reinstalled after the preference tests.

On Day 2 each frog received 10 massed pseudotraining trials. A trial consisted of placing an animal in the black compartment facing away from the gates. After 10 sec the black gate was opened, permitting light to enter the black compartment. Entry by the frog into the white compartment within 5 sec of the gete's being opened was recorded as an "avoidance response" (despite the lack of shocks on Day 2). Shuttling during a second 5-sec interval was recorded as an "escape response." The black gate was closed as soon as the frog shuttled or after the gate had been open for 10 sec, whichever came first. Following each trial, animals were left wherever they were for a 30-sec intertrial interval (ITI) and were then netted, even if they were already in the black chamber, and returned to the black chamber for the next trial. Since high activity levels can produce pseudolearning, to prevent a bias toward acquisition, the most active animal was assigned to one control group (PT) and the next most active to the other control group (PT + NCS). The remaining animals were assigned to one of the three treatment groups on the basis of a rough counterbalancing of both black preference on Day 1 and avoidances (activity) on Day 2.

On Days 3-6 frogs in the training group (T) received 30 massed training trials per day identical to the pseudotraining trials of Day 2 except for the administration of electric shock in the black compartment for the second 5 sec that the black gate was open. Thus the animal that shuttled during the first 5 sec that the gate was open avoided shock, and one that shuttled during the second 5 sec escaped shock. The pseudotraining group (PT) received 30 massed pseudotraining trials per day identical to those of Day 2. The third group of animals (PT + NCS) also received pseudotraining trials but spent all ITIs in the green box in which noncontingent shock was administered. Each animal in the PT + NCS group was paired with a different S from the T group and received a stream of noncontingent shock pulses in the green box which was identical to the shock received by the paired T animal on the equivalent training trial. The noncontingent shock was administered in the middle of the

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†Reprint requests should be sent to Ralph R. Miller, Department of Psychology, Brooklyn College of CUNY, Brooklyn, N.Y. 11210.

††Now at the Mental Health Research Institute, University of Michigan.

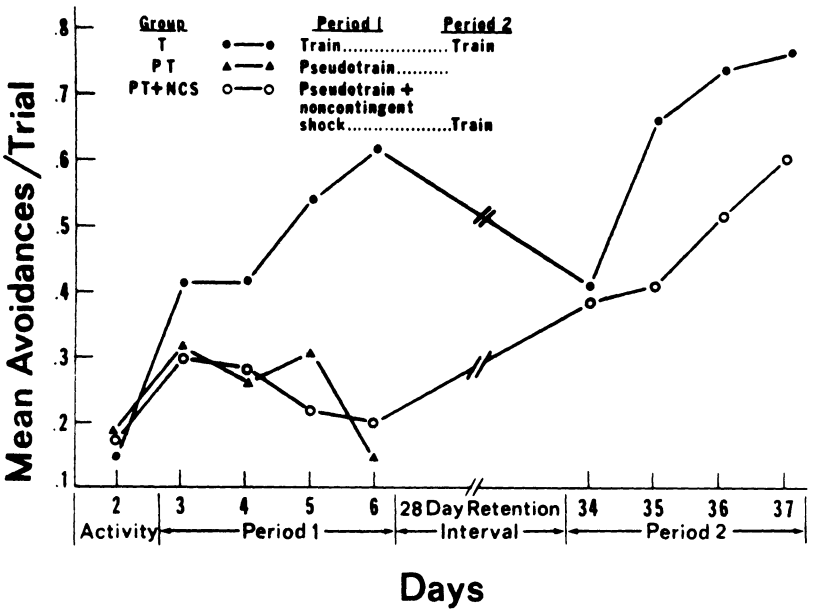

Fig. 1. Mean number of avoidance responses per trial over days. Each animal received 10 massed trials on Day 2 and 30 massed trials on Days 3-6 and 34-37. Each group contained five animals.

30-sec ITI. The need to analyze the T group shock sequences and rewire the shock source to the green box made it convenient to run the PT + NCS group some days after the T and PT groups; however, the high similarity in behavior of the PT and PT + NCS groups on Days 1-6 suggests that this did not introduce any significant bias. Moreover, because of this similarity, we felt it unnecessary to train both groups after the retention interval. Consequently, the PT group was terminated after Day 6.

On Day 34, after a 28-day retention interval, frogs in the T and PT + NCS groups were "retrained" by a procedure identical to the initial training of the T group on Days 3-6.

RESULTS

The T group displayed acquisition on Days 3-6 as compared to the two control groups, which did not differ from one another in their lack of acquisition. Significant retention by the T group was seen 28 days later. Group means over days are illustrated in Fig. 1.

On the initial preference test, 14 of the 15 animals displayed a preference for the black side. Of the 14 black-preferring animals, 12 entered the white compartment within the first half of the 30-min test period, indicating that the observed black preference was not an artifact of starting the animals in the black compartment. The white-preferring frog was assigned to the PT group so as to prevent a bias toward acquisition of dark avoidance by the T group. Analysis of variance of black compartment times showed no differences between the groups ($p > .50$), consistent with the groups' being assigned with intent to counterbalance for dark preference.

Avoidance responses on Day 2, the pretraining activity test, were few in number, which is consistent with the lack of shock on Day 2. Because there were so few pretraining avoidance responses over the 10 trials of Day 2, comparisons between groups necessitated the use of nonparametric statistics. Mann-Whitney U tests between all pairs of the three groups found no significant differences between groups (each $p > .50$), which is in accord with group assignments made in an effort to counterbalance for Day 2 avoidances (activity).

Analysis of variance of daily avoidances on Days 3-6 found a significant treatments effect ($F = 49.91$, $df = 2,12$, $p < .001$) and Days by Treatments interaction ($F = 4.38$, $df = 6,36$, $p < .005$), but not a days effect ($p > .50$). As is evident in Fig. 1, the lack of a days effect is due to the fact that the improvement over days of the T group is paralleled by a decrement in avoidances by the PT and PT + NCS control groups. Individual two-tailed t tests on total avoidances over Days 3-6 showed that the T group differed from the PT group ($t = 10.12$, $df = 8$, $p < .001$) and from the PT + NCS group ($t = 4.59$, $df = 8$, $p < .002$), while the two control groups did not differ from each other ($p < .50$). The first of these t values was particularly large owing to the small variance of the PT group. Examination of T group scores within days found that performance on the second half of each day always exceeded that of the first half and almost always exceeded that of the first half of the next day. This latter point suggests either partial forgetting or a decrement in motivation overnight.

Analysis of variance of daily avoidances on Days 34-37 found a significant treatments effect ($F = 11.01$, $df = 1,8$, $p < .02$) and days effect ($F = 19.25$, $df = 3,24$, $p < .001$) but not a Treatments by Days interaction ($p > .05$). Comparison of the T group on Days 3-6 with the PT + NCS group on Days 34-37, i.e., initial training for each group, yielded no difference in total number of avoidances ($p > .50$). However, comparison within groups between Days 3-6 and Days 34-37 found increased avoidances in the latter period for the T group ($t = 6.85$, $df = 4$, $p < .005$) and for the PT + NCS group ($t = 10.55$, $df = 4$, $p < .001$).

DISCUSSION

The improvements in performance within days and between days of the T group over Days 3-6 and the PT + NCS group over Days 34-37, compared to the PT and PT +NCS groups over Days 3-6, serve to demonstrate the acquisition of active avoidance in *Xenopus laevis*. The PT and PT + NCS control groups void arguments for pseudolearning and other nonassociative effects. In order for acquisition over trials to occur, there must of course be retention over trials. The improvement over days of the groups receiving training indicates retention over days; however, the need for warm-up suggests that some limited forgetting may have taken place. Performance on Day 34 of the T group barely exceeded that of Day 3, but the rapid improvement of the T group over Days 35, 36, and 37, compared to either the PT + NCS group over the same days or to the T group itself over Days 4, 5, and 6, argues that there must have been considerable retention by the T group over the 28-day retention period.

This demonstration of acquisition and retention of active avoidance by *Xenopus laevis* should be of particular interest to researchers interested in the effects of metamorphosis on learning, memory, and innate behavior. First, *Xenopus laevis* is a vertebrate species that undergoes metamorphosis without changing its aquatic habitat. Second, a reinforcer was used that should be similarly motivating to adult and larvae. This similarity is not available using food as an appetitive reinforcer because of the change in diet that occurs over metamorphosis. Third, retention has been demonstrated over intervals that are comparable to the duration of metamorphosis.

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