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Appetitive Conditioning and Discriminatory Learning in Toads

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The consummatory response of the water-uptake behavior in toads was used as reinforcer in two experimental procedures: (1) operant conditioning in an alleyway and its extinction, and (2) discriminative learning and its reversal. In Experiment 1 toads dehydrated to 78% of their initial weight were trained in a runway using water as reinforcement. Running time was reduced from 62.56 sec on the 1st trial to 26.23 sec on the 35th trial. Extinction was obtained in 10 trials. In Experiment 2 toads dehydrated to the same degree were trained in a Y-maze with water reinforcement. Animals reached the learning criterion in 30 trials during acquisition and in 50 trials during reversal. These results show the capability of toads to learn when a biologically relevant motivation is used.

The study of learning in anurans has not been greatly developed compared to the vast neurophysiological literature available on these species. Escape and avoidance learning from an electric shock have been attempted with contradictory results (Boice, 1970; Crawford & Langdon, 1966; McGill, 1960; Yaremko, Jette, & Utter, 1974). Appetitive learning has been successfully demonstrated by Van Bergeijk (1967), who trained a group of bullfrogs (*Rana catesbeiana*) to gather in a feeding area of a terrarium at a certain time of day using mealworms as reinforcers. Brzoska and Schneider (1978) failed to train toads (*Bufo B. Bufo*) to turn toward a prey in response to a tone. Difficulties in selecting the appropriate motivation, one which can be experimentally triggered and controlled, may explain these unfruitful results.

Terrestrial varieties of amphibia display a water uptake behavior to restore the water lost by evaporation through their skin (Schmajuk, Segura, & Reboreda, 1979). The consummatory response of this behavior,

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i.e., hydration through the belly skin, was studied as reinforcer in two experimental procedures.

EXPERIMENT 1

In Experiment 1 operant conditioning in an alleyway and its extinction was attempted.

Methods

Subjects. Six male toads, *Bufo arenarum* Hensel, weighing 128.5 to 175 g, captured in the vicinity of Buenos Aires, were used. Experiments were performed at the beginning of autumn (March, 1979). Temperature oscillated between 20 and 25°C.

Apparatus. The apparatus consisted of a black Plexiglas runway (100 × 12 × 20 cm high). The floor was covered with an acrylic grid with 1 cm^2 square holes. The top of the alley was covered with transparent Plexiglas which served as a one-way screen and provided acoustic isolation. Two guillotine doors were placed 25 cm from either end of the alley forming the start- and goalboxes. The floor of the startbox was covered with an absorbent cloth in order to dry the animal's belly.

In the goalbox there was a plastic container (20 × 10 × 3 cm) filled with distilled water. The water surface was covered by a pivoting grid. During acquisition the position of the grid was maintained by a rubber band. When a toad walked onto the grid, it moved downward under the toad's weight, allowing the animal to submerge. During extinction the grid remained fixed above the liquid. This mechanism controlled whether the animal was rewarded or not, and provided constant visual or olfactory clues. A 15-W light was placed at the end of each box. The experimental room was an acoustically isolated chamber.

Procedure. Toads were habituated to their cages for at least 2 weeks. They were group housed in an environment saturated with moisture with available running tap water. They were force fed once a week. Animals were dehydrated to 78% of their initial weight. They were rehydrated daily, 1 hr after training, until they attained 86% of their initial weight. Animals moulted once or twice during the experimental period. During moulting, toads remained motionless for several hours and so no trials were carried out. Two or three days before preliminary training, the toads were brought to their maintenance schedule by being hydrated once a day in the goalboxes.

Preliminary training of the animals consisted of habituation to handling. Each animal was allowed to explore the maze for 20 min on 2 consecutive days. No water was in the goalbox during these trials. Training trials began on the next day. Each animal was placed into the startbox and 1 min later the door was opened. Running time to reach and totally enter the goalbox was recorded. Once the animal entered the goalbox the guillotine door was gently closed. Following entry into the goalbox, toads were reinforced by being allowed to lie in the water for 2 min. Each reinforcement resulted in an average of 1.5 g rehydration. The animals were then gently removed from the water or the grid and placed once again in the startbox to initiate the next trial.

To interrupt water uptake from its wet belly skin the toads had to be dried, and since they seemed fearful and could not be dried by hand, an absorbent dry cloth was placed on the floor of the startbox. When placed into the startbox the toads pressed down their bellies as if they were trying to absorb the water wetting their skin. The result, however, was to dry their bellies since the floor was covered with an absorbent cloth. This procedure allowed the toads to be dried with minimal handling. Hydration time and the number of daily trials were limited by two factors: (a) the total rehydration throughout the daily trials should not be so great as to alter the level of drive, and (b) the reward should be great enough to overcome the aversiveness of handling to obtain a positive balance in the reinforced trials. Five trials per day were given for 7 days (between 2 and 5 PM).

For extinction trials, the water was rendered inaccessible by locking the grid above the water in place. The criterion for extinction was that the animal not reach the goalbox within 2 min on each of three consecutive trials. Extinction trials were continued for each animal until criterion was achieved.

Results

Figure 1 shows the mean running times for both acquisition and extinction. Running time decreased from 62.56 to 26.83 sec (2.33 times) during acquisition. Analysis of variance for these data revealed a significant acquisition effect ($F[6, 30] = 5.82, p < .001$) and extinction effect ($F[2, 10] = 7.02, p < .05$). Even though the mean running time on the last day of training appears to differ from that of the first day of extinction, these values are not significantly different from one another ($t\ [7] = 0.89, p > .3$).

The toads remained quiet for a brief period when placed in the apparatus, but shortly thereafter they tried to escape by climbing the cage walls. They seemed frightened when the door was opened on the first trials, but habituation occurred during the experiment. On later trials, toads waited for the door to open, sometimes pushing and leaning against it. When the toads entered the water they lay down submerging their bellies.

EXPERIMENT 2

In Experiment 2, a discrimination learning in a Y-maze and its reversal was attempted.

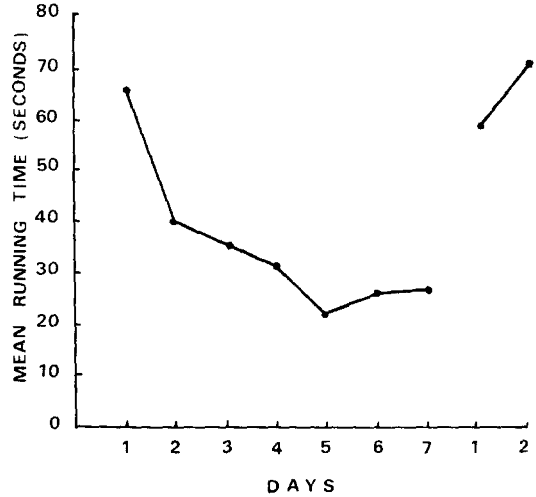

FIG. 1. Mean running time during acquisition and extinction of the runway response in Experiment 1.

Methods

Subjects. Eight male toads, *Bufo arenarum* Hensel, weighing 89.5 to 115.5 g were used. Experiments were performed during summer months (December 1978–March 1979).

Apparatus. The apparatus consisted of a black Plexiglas Y-maze. Each arm was 40 cm long, 12 cm wide, and 20 cm high with a stainless-steel grid floor (rods: 0.2 cm diameter, 1 cm apart). The maze was placed over a tank filled with water to avoid any olfactory clues. The top of the maze was covered with transparent Plexiglas which served as one-way screen and provided acoustic isolation. Three guillotine doors were placed 35 cm from the end of each arm so as to form two goalboxes and one startbox. The floor of the startbox was covered with an absorbent cloth in order to dry the animal's belly. A rectangular aluminum dish (20 × 10 × 3 cm) containing 2 cm distilled water was placed in one arm of the maze. A similar dish containing a sheet of glass to simulate 2 cm level of water was placed in another arm. A 15-W light was placed at the end of each arm providing uniform illumination. The experimental room was acoustically isolated.

Procedure. The procedure was the same as in Experiment 1 with respect to dehydration, maintenance schedule, feeding, and preliminary training. After the preliminary training, the toads were given five trials with water in both arms of the maze to establish whether there is any spatial preference (tendency). The object was to counter any arm preference, or else to divide the group arbitrarily if no preference was observed. Two subgroups were obtained; for one group, the right arm was correct, while the left arm was correct for the other.

Toads were placed in the startbox 1 min before the door was opened. Since preliminary experiments with a correcting procedure produced no learning, a training procedure not permitting correction was employed. Once an animal entered the goalbox, the guillotine door was gently closed to prevent retracing of the Y-maze.

Reinforcement parameters were as described in Experiment 1. If the correct arm was selected, the animal received a water reinforcement for 2 min. In the case that the animal selected the incorrect arm, it was kept for 2 min on the dry glass plate. The animals were then gently withdrawn, either from the water or from the glass plate, and placed again into the startbox. The maze floor was carefully cleaned after each trial.

Five training trials per day were given between 2 and 5 PM. The learning criterion was 3 consecutive days with no more than one incorrect choice each day. All animals attained criterion performance within 8 days and all animals received 8 days of training. Reversal training was given for 50 trials, over 10 days. Statistical differences were estimated with analysis of variance.

Results

Figure 2 shows the percentage of correct responses for acquisition and the percentage of incorrect responses for reversal. Analysis of variance of these data revealed significant *F*'s for acquisition (*F*[7, 49] = 8.27, $p <$ .001) and for reversal (*F*[9, 63] = 10.68, $p < .001$).

The behavior of the toads in the startbox was described in Experiment

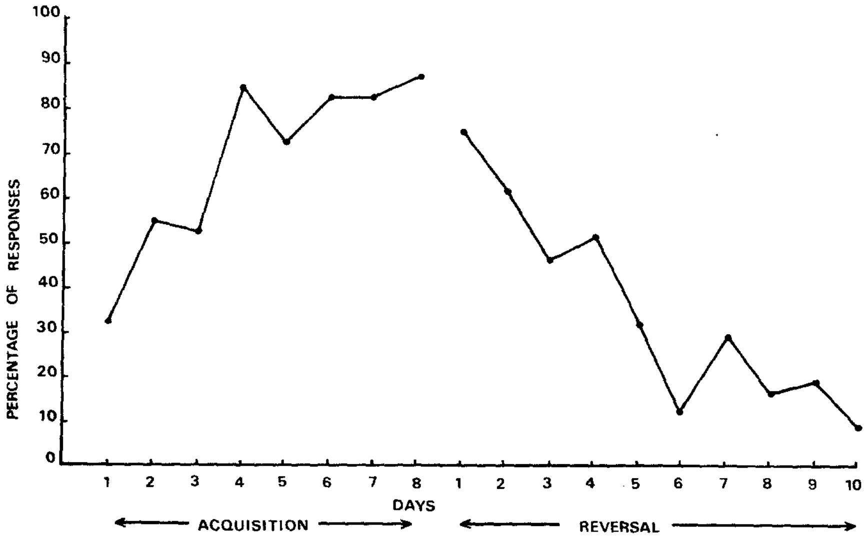

FIG. 2. Percentage of correct responses during acquisition and percentage of errors during reversal training in the Y-maze apparatus in Experiment 2.

1. At the beginning of reversal training, when the positions of the water and glass were interchanged, the animals entered the wrong container without hesitation, and then tried to escape from the goalbox. After some trials the quick jumps over the glass changed to a cautious exploration of the container's content with the forelimbs.

DISCUSSION

As was already mentioned, the only positive reference to learning in toads is that of Van Bergeijk (1967), who studied groups of animals. Our experiments, performed with individually tested animals, demonstrate the capability of this species to learn.

These results show that the consummatory response of water uptake behavior, corresponding to drinking behavior in mammals, can be successfully applied in toad learning experiments. Evidently, learning of the location of water is very valuable for these animals, which are exposed to dehydration by evaporative loss, and which use ponds as breeding places.

The use of water as reinforcer for dehydrated toads seems to offer several advantages over other stimuli. Criticisms can be leveled against the use of electrical shock in avoidance learning because of its biologically unnatural characteristics. Water also appears to be preferable to living food as reinforcers in these animals since it is easier to manage.

These data may help toward realizing the vision of an evolutionary and comparative learning analysis, in which, according to Bitterman (1975), clear-cut results in amphibians are lacking.

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