Classical and avoidance conditioning of the nictitating membrane in frogs (*Rana pipiens*) and toads (*Bufo Americanus*)¹

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Classical and avoidance conditioning of the nictitating membrane, using a tactile CS and UCS, was studied in two experiments with frogs and one experiment with toads. In Experiment 1, a classical conditioning procedure did not produce acquisition in frogs although presentation of the CS to the nostril area produced greater responding than did a CS delivered to the cephalic region. In Experiment 2, frogs failed to acquire either classical or avoidance conditioning of the same response, and mode of housing the animals (refrigeration or aquarium) was ineffective in altering response rates. Experiment 3 demonstrated rapid and reliable classical and avoidance conditioning of this response in toads, when the same procedures were employed. It was suggested that the findings might reflect marked differences in degree of passivity of frogs and toads.

The literature on learning in salientians presents some interesting, albeit perplexing results. Attempts to condition toads apparently have met with little difficulty. Thorpe (1956) lists five early studies in which classical and instrumental conditioning of toads was obtained, and recent findings (Crawford & Langdon, 1966; Boice, 1968) demonstrate that some species of *Bufo* are capable of acquiring the relatively difficult shuttle avoidance response.

By contrast, attempts to condition frogs have not been uniformly successful. Yerkes (1903) noted little or no learning in trial and error and active avoidance conditioning situations. Schaeffer (1911) reported that three species of *Ranidae* learned passive avoidance of noxious food objects. Bajandurrow & Pegel (1932) found evidence for the conditionability of breathing and jumping responses, but Kleerekoper & Sibabin (1959) and McGill (1960) were unable to obtain successful conditioning of the shuttle avoidance response.

Recently, Goldstein, Spies, & Sepenwall (1964) reported successful classical condi-

tioning of the nictitating membrane in *Rana pipiens*. A conditioned stimulus (CS), consisting of a light touch to the region just behind the nostril, was paired with an unconditioned stimulus (UCS) consisting of a light touch to the cornea of the same side. An investigation of the tactile sensitivity of this organism by the present authors revealed that the postnostril region was potentially reflexogenous: Application of light-to-moderate tactile stimulation directly on, dorsal to, or ventral to the nasal bridge resulted in lid closure of varying magnitude and frequency. The question was raised as to whether the acquisition reported by Goldstein et al was "true" conditioning of an instance of reflex sensitization (Kimble, 1961). Thus, it was decided to conduct further studies of this procedure and variations of it in order to gain additional information on the feasibility of this technique with frogs, and to collect directly comparable data on the conditionability of the nictitating membrane in toads.

EXPERIMENT 1

In this experiment the procedure reported by Goldstein et al was compared to one in which the CS produced a lower operant blink rate. It was reasoned that if true conditioning had occurred, the application of a tactile CS to an adjacent, but nonreflexogenous, zone would produce comparable acquisition performance.

Subjects and Apparatus

Ten frogs (*R. pipiens*) were obtained from the Ohio University Zoology Department and housed in individual plastic boxes containing approximately 1 in. of water. Between conditioning sessions they were kept refrigerated at a temperature of 7 deg C, and were force-fed small bits of ground beef once each week.

Four wooden blocks (3.5 x 1 x 3 in.) mounted on a detachable base, and covered with clear plastic, served as a platform on which the frogs were secured. Two 0.25-in.-wide rubber-band straps placed behind the forelimbs and in front of the hindlimbs held the frog in place ventrally on the platform. An ordinary stainless-steel probe was used to deliver the CS, and a soft rubber cone fixed to a wire handle was used to deliver the UCS.

Procedure

The frogs were divided at random into two groups of five frogs each. Prior to each

day's conditioning session the frogs were removed from their containers and placed in a pan of water at room temperature for 10 min in order to normalize body temperature. Thirty conditioning trials were given each day for 7 consecutive days. For one group the CS was a light touch to the cephalic region just anterior to the fronto-parietal area. For the other group, the CS was a light touch directly behind the nostril. The UCS for both groups was a touch to the cornea just sufficient to evoke membrane closure. In the event of an antedating lid closure, the UCS was delivered to the lid itself. Trials were initiated only when the frog was quiescent. The intertrial interval was always at least 30 sec, and trials were delayed for 10 sec when the frog struggled or moved about in the apparatus. CS duration was 2 sec (i.e., contact was maintained for that period) and was timed by counting "one-thousand one," etc. UCS onset occurred simultaneously with CS offset, and UCS duration was variable. A 0.5-sec maximum duration was set; however, closure usually occurred with initial contact. Twice during each session the frogs were doused with water to prevent drying.

Results

A conditioned response (CR) was defined as a partial or complete lid closure that occurred within the CS-only interval. Figure 1 shows the mean number of CRs in the nostril and cephalic CS groups on each day of acquisition. It is clear from the figure that the two groups differ widely in their respective rates of responding but that neither group showed any systematic increase in responding over trials. No changes in the relative frequency of partial to complete lid closures were observed.

Analysis of variance indicated that the difference between groups was significant ($p < 0.005$), but that neither the trials nor groups by trials effects were significant. Since some tendency toward a decrease in responding within each day was observed in the nostril CS group, these data were divided into blocks of first 15 vs second 15 trials of each day to test the reliability of this observation. The chi square for overall differences between first and second half days across all days approached significance ($p < 0.10$).

EXPERIMENT 2

Since the "cephalic" CS more closely approximated the traditional criterion of a neutral CS, it was decided to conduct further investigations of the efficacy of this technique. The purpose of Experiment 2 was (1) to determine whether refrigeration of the frogs was an effective performance variable, and (2) to explore the possibility of utilizing the blink response in an instrumental avoidance conditioning paradigm.

Fig. 1. Mean number of CRs for each day of conditioning (Experiment 1).

Subjects and Apparatus

Twenty-four frogs (*R. pipiens*) were obtained from a biological supply house. Between conditioning sessions 12 frogs were maintained in an aquarium (20 x 10 x 12 in.). Approximately 1 in. of water covered approximately 50% of the bottom surface of the aquarium. The remaining 12 frogs were refrigerated as in Experiment 1. The apparatus was essentially the same as in Experiment 1 with the exception that the number of platforms was increased to accommodate eight frogs for each conditioning session, and all platforms were covered with sponge.

Procedure

The refrigerated and aquarium frogs were divided at random into subgroups of six frogs each, to receive either classical or avoidance conditioning. All frogs received 30 conditioning trials each day for 5 consecutive days. For all frogs the CS was a touch to the cephalic region as in Experiment 1 with the exception that, in order to insure a more intrusive CS, sufficient pressure was applied to the frog's head to produce a very slight downward movement. The UCS was exactly as that in Experiment 1, and was delivered simultaneously with CS termination for both classical conditioning groups. For the avoidance conditioning groups the UCS was delivered simultaneously with CS termination, and only in the absence of a clearly observable partial or complete closure of the nictitating membrane. In all other respects the conditioning procedure was exactly as that used in Experiment 1.

Results

For all groups a CR was defined as a partial or complete membrane closure during the CS-only interval. Table 1 shows the mean number of CRs for all groups for each day of conditioning. Analysis of variance showed that the differences among

groups were not significant, and that there were no systematic changes over trials.

EXPERIMENT 3

Since there was some reason to expect that frogs and toads might respond differentially to the same conditioning procedures, it was decided to replicate portions of Experiment 2 with a species of *Bufo*.

Subjects and Apparatus

Eighteen toads (*Bufo americanus*) were gathered in the vicinity of Athens, Ohio, and maintained in individual plastic containers with moist sand at room temperature. Otherwise the apparatus was exactly as that in Experiment 2.

Procedure

The toads were divided into three groups of six toads each to receive either classical conditioning, instrumental avoidance conditioning, or a series of temporally unpaired CSs and UCSs as a control for nonconditioning effects. Thirty trials were given each day to all groups. For the classical and avoidance conditioning groups the procedure was exactly as that in Experiment 2. The control group received 30 CSs and UCSs each day in a random sequence with an intertrial interval of at least 30 sec.

Results

In all groups a CR was defined as a partial or complete membrane closure within the CS-only interval. Figure 2 shows the mean number of CRs for all groups for the five

Table 1
Mean Number of CRs for Each Day of Conditioning (Experiment 2)

Condition	1	2	3	4	5
Avoidance					
Refrigerated	3.0	3.0	2.2	5.3	4.0
Aquarium	3.5	2.8	1.0	2.0	2.5
Classical					
Refrigerated	2.3	3.5	2.3	4.3	3.7
Aquarium	4.3	1.8	3.7	2.0	1.8

days of conditioning. It is clear from the figure that both conditioning groups show systematic increases in responding over training, and that there appears to be no systematic differences between these two groups. By contrast, the control group appears to have maintained an extremely low level of responding throughout.

Analysis of variance revealed that both conditioning groups significantly outperformed the unpaired control group ($p < .001$), that the increase in responding of these two groups over trials was significant ($p < .001$), and that the Groups by Trials interaction was also significant ($p < .001$).

DISCUSSION

Experiment 1

The significantly higher rates of responding to the nostril CS lend support to the notion that this procedure does not meet the criterion of a "neutral" CS as well as the cephalic CS procedure. Since neither group revealed any systematic tendency toward increased responding as training progressed, there is some basis for hypothesizing that any acquisition effects realized with this (nostril CS) procedure may, in fact, be due largely to nonconditioning effects (sensitization or pseudoconditioning). In this respect it is well to consider the results of Franzisket (1963) and Kimble & Ray (1965) on reflex potentiation in the frog. Specifically, it was found that variation in the locus of stimulation from trial to trial resulted in an increased frequency of wiping reflexes, i.e., that a lowered threshold of responding generalized to adjacent areas. In the present study the failure of the nostril CS group to show acquisition effects may, in fact, be due to the care taken to consistently stimulate the same locus. Certainly the possibility of reflex potentiation of the membrane closure response via nostril stimulation should be further investigated.

Experiment 2

First, the results of this experiment offer no evidence for the argument that refrigeration was in any way responsible for the absence of response increments in Experiment 1. This is consistent with the observation that refrigerated frogs, although initially sluggish, quickly became as active as aquarium-housed frogs and, in fact, maintained a somewhat higher level of activity (e.g., struggling) than did aquarium frogs.

Second, the lack of differences between the two major groups in this study (classical and instrumental avoidance conditioning) fails to shed light on the question of which learning procedure is more effective in modifying the membrane response, and

Fig. 2. Mean number of CRs for each day of conditioning (Experiment 3).

needless to say, offers no support for the notion that either type of conditioning is possible in this organism. It should be noted that in spite of the fact that CS intensity (pressure on the cephalic region) was increased in this study, thereby raising, presumably, the probability of effective and reliable sensory input, the response rates of all groups were at the same general level of the cephalic CS group in Experiment 1.

Experiment 3

The highly significant increase in responding for both conditioning groups leaves little doubt that acquisition of a conditioned response did occur. Because of the remarkably and consistently low response rates in the unpaired control group, there seems little basis for arguing that nonconditioning effects were present in any appreciable degree in the two conditioning groups. It is interesting, however, to note the lack of differentiation between the two conditioning groups, since studies of eyelid conditioning in humans, and nictitating membrane conditioning in rabbits, have clearly demonstrated the superior performance obtained with classical as opposed to instrumental avoidance conditioning procedures (Gormezano, 1965). This finding surely warrants further comparisons of the two procedures at this and other evolutionary levels.

General Discussion

It is apparent that the present experiment offers little evidence for the conditionability of the nictitating membrane of the frog. The observed differences in responding to cephalic and nostril CSs suggests only that the latter procedure is highly questionable. The observations also suggest that classical and instrumental conditioning procedures are equally ineffective in producing behavioral modification in the frog.

By contrast, toads appear to exhibit relatively rapid and reliable conditioning with either classical or instrumental procedures. Although not surprising in view of previous findings, these results, nevertheless, pose an interesting problem for students of comparative behavior modification. Van Bergeijk (1967) has suggested that behavioral passivity in some salientians (*R. pipiens* would certainly qualify here) is related to their apparent inability to perform when the learning task is arbitrarily selected by the E. This notion is supported by the fact that Van Bergeijk (1967) clearly demonstrated the modifiability of pre-feeding aggregations (classifiable as a natural behavior) in *R. catesbeiana*, and Boice (1968) found that *Scaphiopus hammondi*, a passive desert amphibian, never learned to avoid shock, while *Bufo woodhousei*, an active hunter, readily acquired the shuttle

avoidance response. Perhaps an explanation of the results of this and related studies more likely will be found in the differences in interspecies natural behavior than in the morphological differences that form the basis of conventional taxonomy.

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NOTE

1. The authors wish to thank Carol Boice, Grady Poe, and Dave Baumer for their assistance.