A Learning Paradigm and the Behavioral Demonstration of Audition for the Lizard *Anolis grahami*

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Audition in *Anolis grahami* was investigated using classical conditioning of subjects within a simulated "natural" habitat. The conditioned stimulus (CS) was a tone which matched the dominant frequency of the species' agonistic type A vocalization; shoving a subject from its perch with a rod was the aversive unconditioned stimulus (UCS).

Before conditioning experiments took place, baseline observations showed no observable responses by the lizards to the tone. Furthermore, during the conditioning experiments, evidence of learning (i.e., orienting and avoidance behavior during presentation of the tone) was more evident when visual cues were reduced. These results indicate that the species will respond to sounds, but that it has a greater reliance on visual rather than auditory information.

*Anolis* species utilize visual cues to a large extent in their social displays (Jenssen, 1977) and when feeding (Andrews, 1971; Schoener, 1969). In the field, they show little indication that sound is used to avoid potential predators. It seems that audition is relatively inconsequential for most anoline species within their present adaptive zone. However, there is some evidence that anoles hear. Electrophysiological recordings made by Werner (1972) on *Anolis lionotus* showed a physiological response to sound stimuli at the cochlear level. Of the 16 or more anoline species which vocalize, at least *Anolis grahami* produces sounds during aggressive intraspecific encounters (Milton and Jenssen, 1979). One would suspect that anoles which vocalize in social contexts would also respond to these and other sounds, but the evidence remains equivocal (Milton and Jenssen, 1979).

It has not been proven that anoles will actually respond to auditory cues. The purpose of this research was 1) to develop an effective model for learning by anoline lizards based on aversive classical conditioning, and 2) to demonstrate that anoles are capable of utilizing auditory information to influence their behavior.

**Methods and Materials**

Seven male and three female adults were housed in three 1.3 × 0.7 × 0.6-m enclosures having glass front panels, wire screen tops and overhead fluorescent lights. Inside the home enclosures were simulated natural habitats of tree trunks, limbs, dense artificial foliage and a moss-soil substrate. Food (mealworm larvae, crickets and insects collected from grassy fields) and water were provided daily. The animals were held on a 9 h light-15 h dark cycle at 21–23°C.

Classical conditioning was used to determine whether *A. grahami* would respond to sound alone. The unconditioned stimulus (UCS) was the act of "shoving" the subject from its perch with a 3-mm diameter rod. The conditioned stimulus (CS) was a tone with a 1775-Hz dominant frequency, the same as that of the species' type A sound which is emitted during intraspecific attacks (Milton and Jenssen, 1979). The CS was produced by a Hewlett Packard 200 AB Audio-oscillator and modulated by an Amtrons-Programmed Sequential Controller. The CS was delivered 2.6 times/sec in 190-msec pulses, with a sound level of 92 db at the speaker.

Three learning experiments were devised, each using a male subject. The protocol of each subsequent experiment was modified to reduce behavioral suppression. All experiments, however, shared: 1) a baseline period when only the CS was presented; 2) a training period when the subjects were exposed to pairing of the CS with the UCS; 3) a test period when it was observed whether the subject would respond to the CS; and 4) "catch trials" during which all procedures were followed, but the CS and the UCS were not presented.

*Baseline protocol.*—Baseline observations were conducted on 10 lizards while they were in their...
home enclosures. The speaker delivering the CS was suspended 5 cm over the enclosure. A 10 sec burst of the CS was broadcast 10 times to each observed lizard. Any change in its behavior, posture or body color during or just after tone presentation was recorded.

Training and testing protocol.—The enclosures used for training and testing had their glass panels covered so that a subject inside the enclosure could not see out. A subject was viewed through a wide-angle lens and a small slit through which the rod was introduced. The speaker delivering the CS was mounted on the training and test enclosures with a foam rubber gasket. This eliminated vibration so that the subjects were "hearing" the stimulus and not "feeling" it. A sensitive vibration meter (Gen. Radio Co., type 1553-A) registered no response from the enclosures' surfaces when the CS was broadcast.

The test enclosure was 0.8 x 0.6 x 0.8 m, illuminated by overhead fluorescent lights, and contained habitat like that of the home enclosures. During testing the CS was delivered only when the male lizard was on top of the prominent tree trunk in the enclosure, and exhibiting his bright green and aquamarine coloration (characteristic of an alert and territorial male). This had two results. First, the subject's behavior determined the inter-trial-interval (ITI). Second, when the subject left the top of his conspicuous perch, he was not exposed to the next trial of testing. This simulated the natural situation where lizards in the field have less exposed perches to which they can escape from aversive stimuli.

Subject #1 was trained in a barren 0.3 x 0.3 x 0.3-m cage. Two blocks of 10 CS-UCS pairings (20 trials) were delivered each day for four days. Within a block, the CS-UCS delivery was on a variable schedule, with an average interval of 45 sec. Each tone CS was presented in a 10-sec burst. As with all CS-UCS pairings, the UCS was delivered 1-5 sec after the tone. The lizard was then placed in the test enclosure. After two days, he exhibited bright coloration and commanded the elevated, exposed perch site. With the trunks and rich foliage available, the male could now make use of its species-typical escape behaviors (i.e. "squirreling" around the back of trunks and hiding under foliage) which were prevented by the barren interior of the pretraining cage. Seven trials were run in which only the CS was presented.

Subject #2 was trained in the barren left half of the test enclosure, separated from the habitat in the chamber's right half by a removable partition. The training period was shortened from that of subject #1. Only two blocks of 10 CS-UCS pairings each were delivered in the same manner as to subject 1, but using a CS of 5-sec duration. At the end of the 20th trial, the divider was raised and the animal entered the habitat.

Testing followed the next day. Three blocks of 10 and one block of six CS-UCS pairings (total of 36 trials) were then presented over the next four days.

Subject #3 was trained in his home cage. Training consisted of 10 exposures to only the UCS within a 5-min period for each of three days.

The lizard was then placed in the test enclosure and testing begun when the animal demonstrated normal, dominant behavior after two days.

Testing consisted of CS-UCS pairings presented in blocks of 10 trials per day for eight days. Each tone CS was presented in a 10-sec burst.

Catch trials.—Throughout training and testing, frequent "catch trials" were included during which the experimenter made movements outside the apparatus similar to actions made when the CS and UCS were presented; however, the CS and UCS were not actually delivered during the catch trials. This procedure was to control for the lizard using cues not in the experimental design. The lizard's behavior was recorded during these catch trials as well as during a 10-sec interval just before the test period CS was presented.

Response criteria.—Responses which occurred after CS onset and prior to UCS presentation were categorized as: 1) orienting responses, defined as eye, head or small body movements in the direction of the CS (i.e., speaker), and 2) avoidance responses, defined as any movement which placed the animal at least one body length away from his previous position. If both orienting and avoidance behaviors were observed, the behavior was simply scored as avoidance.

Results

Baseline and catch trials.—No response was made by any of the 10 subjects to the baseline CS
Subject #1 remained brown (indicating a subordinate or wary animal) throughout the training period. He also showed rapidly increasing behavioral suppression in training which was relieved by transfer to the foliated test situation. Audition was demonstrated by six orienting responses to the tone CS in seven test trials (Fig. 1a), with the mean ITI (inter-trial-interval) being 3.3 min. Between each test trial, the male became darker brown. He also left his elevated perch site after each CS presentation, though not soon enough to be scored as avoidance. However, after trial 7 the subject virtually took up residence beneath the leaves at the base of the perch. Since the animal remained there for several hours, test trials were terminated.

Subject #2 showed a trend of increasing orienting responses to the tone CS as trials continued (Fig. 1b); a total of 26 orienting responses were elicited over 36 trials. The mean test ITI was 27.8 min and the male remained green and aquamarine for much longer periods of time than subject #1. Color changes from green and aquamarine to brown were also observed in 23 of the 36 trials. Testing was terminated because the animal remained predominantly brown throughout the last five trials and took progressively longer to return to the perch stop, increasing the subject-determined ITI to over an hour.

Subject #3 was the only animal to show avoidance responses to the tone CS during test trials (Fig. 1c). Total responses (orienting plus avoidance) gradually increased over time, reaching 100% in the last block. A total of 52 responses (37 orienting and 15 avoidance) were recorded over 80 trials. The mean test trial ITI was 13.0 min, and the male remained predominantly green and aquamarine throughout testing. This latter point is significant as it indicates that the conditioning protocol was not interfering with the subject expressing his normal dominant and territorial state.

**DISCUSSION**

Lizards in their home cages showed no response to the test sound, establishing a baseline response of zero. However, under the three test conditions, the subjects all demonstrated that they could perceive the sound. That these responses were a direct consequence of presentations of the auditory CS during visually reduced conditions is evidenced by the absolute lack of observable responses during both catch
trials and the 10 sec observation periods preceding CS presentations. The responses observed during the CS presentation were, therefore, not due to increased random activity. It is concluded that *A. grahami* is capable of audition and that auditory cues can have behavioral significance, at least in the absence of visual information.

Although all three lizards oriented to the tone CS, it is interesting that the auditory cue elicited consistent avoidance responses only from subject #3 (Fig. 1). It may be possible that this comparatively low level of avoidance is related to the species’ extreme reliance on visual cues in their natural environment. In the learning experiments described above, responses were only designated as “orienting” or “avoidance” if they occurred during the tone CS and prior to insertion of the rod. Such a designation was necessary in order to determine whether the lizards were capable of audition. Although the animals perceived the sound (as evidenced by orienting and avoidance responses), they were far more likely to run into the foliage after the rod was inserted and prior to physical contact by the rod.

Because the interval between the tone CS offset and physical contact by the rod was variable (1-5 sec), it is very possible that the actual sight of the inserted rod tip provided a more salient cue for escape behavior than did the auditory stimulus. Evidence for this occurred in all three subjects. After less than 10 initial contacts from the rod, the lizards consistently scampered from their pretrial positions at the moment the rod made its appearance into the enclosure following CS offset. If one re-examines the experimental paradigms in terms of the visual reliance characteristic of this species, it becomes plausible that the inserted rod tip may well have served as an effective visual CS, and contact with the rod remained as the UCS. Defined in this manner, the above observation indicates a rapidly learned, consistent avoidance of the aversive stimulus.

The avoidance elicited by the postulated visual CS (rod tip), as well as the avoidance demonstrated for the sound CS, contradict Powell & Mantor’s (1969) conclusion that lizards are incapable of avoidance learning. A possible reason for this disagreement is that Powell (1967, 1968) and Powell and Mantor (1969) used experimental setups designated to “eliminate incompatible responses, such as climbing” (Powell, 1968:509). Our study used a simulated “natural” situation in which Species-Specific Defense Reactions (Bolles, 1971:185) such as leaping from a perch, “squirreling” around the back of a trunk and running to the shelter of nearby foliage could be performed.

The problems of evaluating reptilian learning or using behavioral responses to monitor reptilian perceptual phenomena can be best met with an appreciation for the species’ particular physiology, ecology and behavioral repertoire. For the most part, studies on these topics are only now taking this orientation (Brattstrom, 1972, 1978; Greenberg, 1978; Northcutt and Heath, 1971; see Burghardt, 1977, review).

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**Literature Cited**


A shift from aerobic to anaerobic metabolic pathways may result from failure of the oxygen supply system in meeting the energetic demands of tissues. In reptiles, this shift occurs to varying degrees in response either to high energy utilization during intense muscular activity (Bennett and Dawson, 1976) or to anoxia during diving (Hochachka and Storey, 1975). Until recently, various studies have concentrated on the capacities of reptiles for aerobic and anaerobic metabolism under artificial laboratory conditions of forced activity or submergence. These studies have supplied important information about the capabilities of reptilian metabolic machinery, but it is now desirable to know the natural conditions under which reptiles rely significantly on aerobic and anaerobic pathways.

This study approaches the problem by examining blood lactate in sea snakes diving naturally at sea. Although other metabolites such as pyruvate, succinate and alanine may increase slightly during anaerobiosis, lactate accounts for more than 95% of anaerobic products in the squamates so far examined (Bennett, pers. comm.). Some sea snakes may show a diving response during forced submergence and the lactate may remain in the muscle temporarily. In the few minutes following the first breath, however, the lactate is rapidly released into the blood where its subsequent disappearance may be measured over a period of several hours (Seymour and Webster, 1975). The persistence of lactate in the blood makes this metabolite a useful indication of anaerobiosis which may have occurred hours before sampling.

**Material and Methods**

In September, 1975, 18 specimens of four sea snake species were netted by hand at night (ca. 2000–0400 hrs) as they surfaced and swam near the research vessel, Alpha Helix, in the Visayan and Camotes seas, Philippine Republic. Blood was taken from seven of these snakes by heart puncture within two min of lifting them from the water. Because this procedure sometimes caused struggling, these animals were manually compressed on both sides of the heart to prevent venous blood, which might contain lactate produced after capture, from reaching the sample. Eleven other snakes were gently placed on a board and decapitated within 30 sec of capture. The first few drops of blood from the wound were collected in test tubes. In some cases two or three samples from the same snake were taken and treated separately. The lactate concentration in 20 μl of each sample...