

## Conditioned Learning in a Katydid

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### Abstract

Visual conditioning is a mechanism of learning where an animal forms an association between a visual stimulus and a reward or punishment. Conditioning in insects is a convenient way to establish behavioral patterns that can be compared with neural activity. Studies on visual conditioning in insects are wide ranging, however, there is little or no research on conditioning in katydids. Because katydids are close relatives of crickets, a group in which conditioning has been shown; the hypothesis that katydids exhibit conditioned responses was investigated. Using a visual conditioning paradigm, 39 katydids (*Conocephalus brevipennis*) were trained to associate a square line pattern or a circular bull's eye pattern with either water (reward) or saline (punishment). In tests prior to training, no preference to either visual stimulus was observed in trials using visual cues without the reinforcement stimuli. Training lasted 5 days and the test arenas were rotated daily to control for any position effects. Tests for learning and retention were conducted at 1 and 7 days. One day after training, 11 individuals moved to a visual stimulus. Of those, 9 (significantly more than expected at random) chose the visual stimulus that was associated with positive reinforcement. Similarly, 8 of 10 individuals moved to the correct visual stimulus 7 days after training. These results suggest that katydids can be conditioned and that they retain learned information for as long as a week.

**Keywords: Katydid, Learning, Conditioning**

### 1. Introduction

Conditioning is a process where an animal learns to associate a certain stimulus with a reward (appetitive), such as food or water, or a punishment (aversive), such as electric shock. Some insects can be conditioned and may retain memories for as long as 6 weeks<sup>1</sup>. Studies on conditioning include visual and/or olfactory learning in honeybees<sup>2</sup>, cockroaches<sup>3</sup>, crickets<sup>4</sup>, flies<sup>5</sup>, and wasps<sup>6</sup>. While neural plasticity (capacity to alter neural pathways with experience) is widely accepted in humans, it is rarely attributed to insects<sup>7</sup>. Interestingly, recent research indicates that juvenile crickets can learn, and then reverse and relearn information<sup>1</sup>.

Although extensive research has been performed on katydids' mating and communication behavior<sup>8</sup>, there is little or no research investigating their ability to learn. Katydids are ideal subjects for such studies because they are abundant, they can be bred in a laboratory, and they live for long periods when in captivity. They are also easily located and captured in the field using their conspicuous acoustic songs.

The first goal of this study was to determine whether katydids can learn through appetitive and aversive conditioning. Crickets and katydids are closely related and classified in a group called Ensifera. Because crickets can be conditioned, it was expected that katydids would show the same conditioned responses. If katydids could learn, then the second goal of this research was to determine the persistence of katydid memory.

## 2. Methodology

### 2.1. insects

Adult katydids (*Conocephalus brevipennis*, N=46) were collected with nets, in the wild, in Buncombe County, NC. Individuals were housed in separate holding containers (10x10x10cm; Tri-States Plastics, Inc.) and fed and watered daily. Katydids were fed a diet of apples, green leaf lettuce, and insect food. To increase the desire to search for water, katydids were deprived of water, apple, and lettuce for 2 days prior to training and testing. All katydids were housed, trained, and tested separately.

### 2.2. preference testing

A preference test was given before training to determine if katydids instinctively preferred one visual cue over the other. Two visual patterns were used; a black and white square line pattern and a black and white circular bull's eye pattern (Figure 1). The visual cues were placed opposite each other, without the corresponding stimuli, in testing arenas that were modified versions of those used by Unoki et al.<sup>4</sup> and Matsumoto and Mizunami<sup>1</sup> (Figure 2). The arenas were clear plastic cylindrical containers (Tri-State Plastics, Inc.) (25.2cm dia × 9cm high). The testing arenas were cleansed after each testing session to control for chemical and olfactory cues from previously tested individuals.

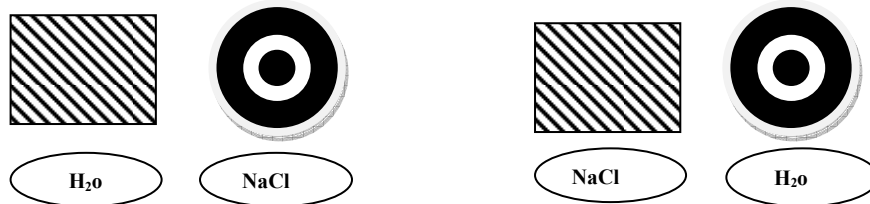


Figure 1.

Visual cues and

corresponding stimuli.

Figure 1 Two groups of katydids were conditioned to associate reward (H<sub>2</sub>O) or punishment (NaCl) with either a square-lined or circular bull's-eye pattern.

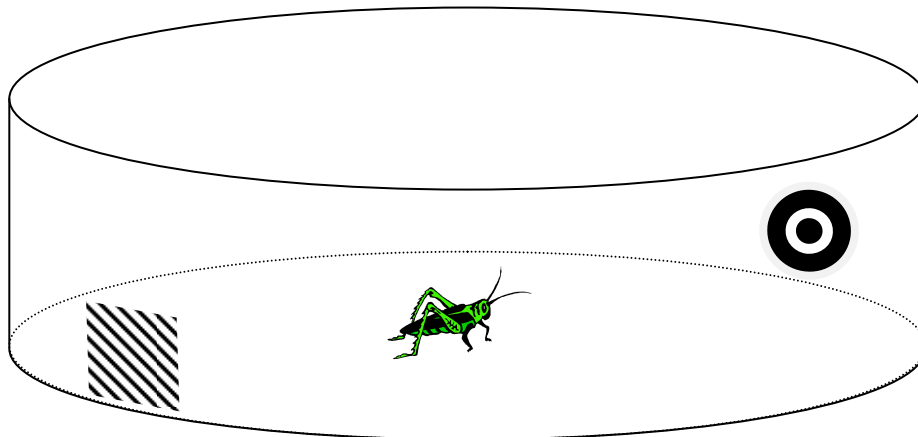


Figure 2. Testing arena with visual cues.

Figure 2 Katydids were released in the center of the testing arena and observed for movement toward the two training cues (no H<sub>2</sub>O or NaCl present). A correct response was noted when the animal touched the training pattern associated with the appetitive stimulus.

To increase the katydids desire to search for water, wet paper was placed on the outside of the arena behind holes in both visual cues. This enabled the water vapor to be sensed inside the arena, but prevented the katydids from drinking. The katydids were placed in the testing arena and given 2 minutes to acclimate before testing started. Once testing began, katydids had a total of 4 minutes to visit the visual cues. The arena was rotated 180° 2 minutes into testing so that each visual cue spent equal time on each side of the arena. A successful visit was considered when any part of the katydid's body touched the visual cue. The number of visits to each visual cue was quantified. A 2-category log-likelihood ratio goodness of fit test was used to analyze the data<sup>9</sup>.

### 2.3. training

Each katydid was placed inside a training chamber along with the visual cues and corresponding water and saline. To control for preference to a certain visual cue, 19 of the katydids were trained to associate water with a square line pattern, and 20 were trained to associate water with a bull's eye pattern. Visual cues remained paired with their designated stimuli throughout the training. Because each katydid had their own isolated drinking platforms, chemical and olfactory cues from others were controlled for. Katydids were trained for 1 day to determine 24 hour memory retention and 5 days to determine 7 day memory retention. Katydids remained in the training chamber for the duration of the training interval and the chambers were rotated 180° daily to control for katydid preference to either side of the chamber due to sunlight, temperature, or olfactory effects.

### 2.4. retention testing

To determine 24 hour retention, katydids were tested 24 hours after their 1-day training session concluded and the visual cues and corresponding stimuli were removed. To determine 7 day retention, katydids were tested 7 days after their 5-day training session concluded and the visual cues and corresponding stimuli were removed. The same procedure used in preference testing (above) was applied in retention testing. The number of visits to each visual cue was quantified. A successful visit was considered when any part of the katydid's body touched a visual cue. The data were analyzed using a 2-category log-likelihood ratio goodness of fit test<sup>9</sup>.

## 3. Results

### 3.1. preference testing

Twelve of the 39 katydids moved to a visual cue. Of those, 42% (N=5) chose the circular bull's eye pattern and 58% (N=7) chose the square line pattern (Figure 3). There is no significant difference in the preference to either cue (G=0.335, P>0.05).

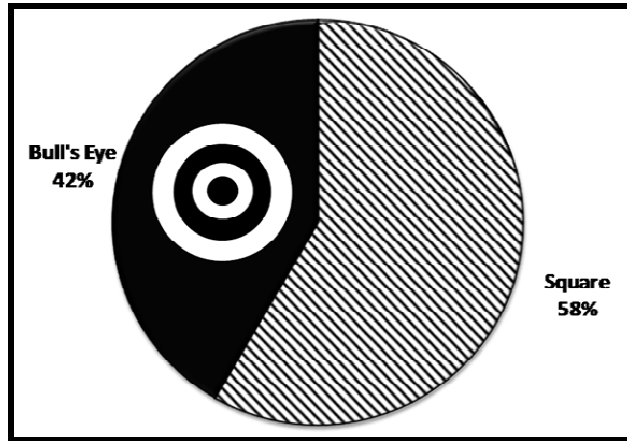


Figure 3. Preference test results.

Figure 3 Katydids (N=12) showed no preference for either training pattern prior to conditioning.

### 3.2. retention testing

In 1 day retention testing 11 katydids visited a visual cue. Of those, significantly more ( $G=4.818$ ,  $P < .05$ ) visited the correct visual cue (N=9) as opposed to the incorrect visual cue (N=2) (Figure 4). In 7 day retention testing 10 katydids visited a visual cue. Of those, significantly more ( $G=3.855$ ,  $P < .05$ ) visited the correct visual cue (N=8) as opposed to the incorrect visual cue (N=2) (Figure 4).



Figure 4. Retention test results.

Figure 4 Bars represent the number of individuals that chose the correct (dark green) or incorrect (light green) visual cue, 24 hours and 7 days after training. Asterisks indicate that the number moving to the visual cue associated with the appetitive stimulus during training (i.e. a correct response) was significantly greater than expected at random.

## 4. Discussion

The results of the 1 and 7 day retention tests show that katydids can retain a memory for up to 7 days. While short by human standards, seven days may represent a significant portion of the lifespan of a katydid, which can range from hours to 4 months in nature. Since katydids are herbivorous and travel to find food and mates, it is probably useful for them to remember appetitive and aversive food sources when moving from one environment to the next. The behavior exhibited when visiting a visual cue was obvious. It was clear to the observer that the katydids knew which cue would lead them to a reward and approached it robustly. Although insect conditioning and behavior have been widely studied<sup>1,2,3,4,5,6</sup>, these data are the first to show conditioning and learning in katydids. By controlling for spatial, olfactory, and environmental effects, my study was restricted to visual learning only; demonstrating the need for only one paradigm to be present for learning and retention to be achieved.

The results from the preference test show a lack of visual preference in katydids to square or circular geometric shapes and also to black and white bull's eye patterns or line patterns. This suggests that there is no environmental need for katydids to innately distinguish between these shapes and patterns. These results agree with previous findings on crickets by Unoki et al. that show no visual preference between different bull's eye patterns of black and white stimuli<sup>4</sup>.

My study introduces a novel training paradigm in insect conditioning. Previous research paired the stimuli with the reward or punishment within minutes of each other, removing the stimuli directly after the pairing<sup>1,4</sup>. Unoki et al. found that crickets need a minimum of 6 training sessions (within minutes of each other) to form 1 day memory retention<sup>4</sup>. However, I left the visual cues and corresponding stimuli in the training chamber with the katydids for the entire training interval (24 hours or 5 days). Since the katydids remained in the training chamber and could freely visit the cues and reinforcement stimuli, there was most likely an absence of stress during conditioning sessions. This approach can be used to alleviate stress caused by insect transfer before conditioning.

In addition, the training paradigm allowed the katydids to associate appetitive and aversive stimuli simultaneously. The results suggested that both aversive and appetitive stimuli can be present at the same time, for a continual duration, and produce learning and memory retention. Interestingly, both the aversive and appetitive memories were still present at 7 day retention tests. Previous insect studies have shown that aversive memories decay at a faster rate than appetitive when conditioned separately<sup>4</sup>. It is possible that linking the memories together leads to a longer retention rate in both appetitive and aversive conditioning; however, there is little or no research on using both stimuli at the same time.

Future research includes determining how long katydids can retain their memory. It is likely that they can retain memories for longer than 7 days because previous research on crickets has shown that juveniles can retain memories for up to 6 weeks<sup>1</sup>. It is also of interest to examine memory rewriting in katydids. Juvenile crickets can reverse and relearn new information<sup>1</sup>. Research on conditioning in insects is important because it helps to establish behavioral patterns that can be compared with neural activity. Insects are model subjects for studying neural processing for several reasons. First, they have a small number of neurons that have been mapped to specific regions and neural circuits<sup>7</sup>. Second, electrodes can record from or stimulate specific neurons and the correlated behavioral outputs studied. Third, because mechanisms of neural processing are constant across animals and because the insect brain performs many of the same functions as the vertebrate brain, results should relate to other animals<sup>7</sup>.

Katydid are ideal subjects for studying learning and memory retention and the corresponding neural mechanisms. They are abundant in nature and easily captured which provides for a profuse sample size. They are small in size and therefore easily maintained in a laboratory. Since they are capable of learning and memory retention, the mechanisms behind these processes can be evaluated. Also, because they have a neural system that can be easily accessed, it is possible to examine the neural mechanisms behind learning and memory retention.

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