USING FREE FLYING HONEY BEES TO TEACH PRINCIPLES OF LEARNING

Öğrenme Prensiplerinin Öğretiminde Açık Alanda Özgür Olarak Uçan Arıların Kullanılması

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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ABSTRACT

This paper outlines procedures for using free flying honey bees in a choice discrimination task suitable for the classroom. Procedures for acquisition and extinction are described. The exercise is suitable for all levels of the educational system ranging from elementary school to college. We describe the details of the basic experiments. From this the possible experimental variations are vast and present students with the opportunity to explore many intriguing questions in the fields of animal behavior, behavioral ecology and psychology.

INTRODUCTION

This paper describes the use of free flying honey bees to teach basic principles of learning. It is a companion piece to the classical conditioning of proboscis extension in harnessed bees described earlier in the *Uludag Bee Journal* (Abramson*, et all*., 2007). The exercise is appropriate for all levels of the education system including elementary school, middle school, junior high school, high school, and college. The exercise can be used in a wide variety of classes such as biology, ecology, and psychology. It is also a useful technique for beekeepers seeking behavioral demonstrations for children and adults visiting their apiaries.

One of the most widely used and simple techniques to study honey bee learning is to train free flying forager honey bees to fly back and forth from the hive to a table where they drink sucrose solutions from targets distinguished by color, odor and/or position. There are many variations of this exercise that can be used and your students are only limited by their imaginations.

In the basic exercise, two targets differing in color will be used. One target contains sucrose solution and the other tap water. The ability to discriminate among objects is one indication of cognitive ability. Discrimination is also used in learning experiments to set the stage for more complex experiments such as reversal learning. In reversal learning the reward value of the targets are switched such that the one previously rewarded is no longer rewarded, and the target previously associated with no reward is now rewarded. The ability to adjust to this changing circumstance is another indication of cognitive processes. Discrimination learning is also used as a control in learning experiments to estimate the effect of stimulation *per se*. Conditioning is revealed not by comparing two groups of subjects but by comparing the choice made between two stimuli.

We recommend that before you try this exercise with students that you learn about honey bee behavior. There are several good books and articles (*e.g*. Wenner and Wells 1990; Sanderson and Wells 2005). Variations of this exercise can be found in Abramson (1990).

CONDUCTING THE EXPERIMENT

Below are some recommended materials. The targets to be discriminated can be wide ranging and need not be those suggested. That is all equipment can be made of alternative materials. What is needed, however, is a gray training target of some type. The purpose of this target is to get the bee to associate some point with a nectar source and return several times to this same target. Once the bee is returning regularly, say, for example, three consecutive visits, replace the gray target with your two choice targets to begin your experiment. One of the more interesting target variations is the use of a "mirage" machine that produces holographic-like images of stimuli placed within. Bees respond not to the actual physical target but to the image of it (Abramson *at al.,* 1996). Another target variation is to paint a Petri dish gray, drill small holes around its circumference, and tape a plastic or paper color splotch in the center. These splotches can be obtained from any store that sells household paint. By placing a few drops of an odorant, such as a natural, oil on a cotton ball which is placed inside of the Petri dish you have a compound stimulus consisting of color and odor. For construction details see Abramson (1990).

MATERIALS

1. **Three targets.** Make your own honey bee conditioning set. The set contains three targets of equal shape but differing in color. One of these targets must be gray. We have used targets that are 3.5 cm diameter discs, one orange and one blue made of painted plexiglass. Many different shapes and colors can be used. Figure 1 provides some examples.

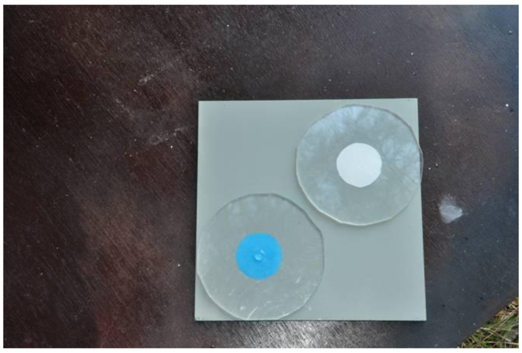

Figure 1: Examples of simple targets.

2. **One honey bee colony** (*Apis mellifera* L.). You will need access to a colony. If you do not have a colony you can contact a local beekeeper or beekeeping club and ask if your class can come out to do this experiment. It has been our experience that beekeepers are always glad to help students.

3. **One container of 50% sucrose solution.** To prepare the solution use equal amounts of sugar and water. Before using make sure the sugar has dissolved into the water. An interesting variation of the basic exercise is to vary the amount of sugar. For example after training a bee for several visits with 50% sucrose, switch to a 5% solution. When the bee first makes contact with the lower concentration solution you will notice some interesting behavior.

4. **Two eyedroppers**. The eyedroppers are used to apply the solutions; one eyedropper for each solution. Eyedroppers can be obtained from any pharmacy or hobby store.

5. **Two bowls filled with tap water.** The bowls are used to wash the targets after each visit. The purpose here is to remove any chemical cues the bee may have left behind. Another interesting variation is to leave the chemical cues on the target to explore whether bees need learning to solve the discrimination.

6**. One roll of paper towels.** The towels are used to dry off the target after being washed. Washcloths can substitute for the towels. Whatever you use must be scent free so not to confuse the bee with additional scents that may contaminate the target.

7. **One bottle of nail polish.** The rationale behind the nail polish is that you will use it to mark your bee. The mark you make will distinguish your bee from other bees that may "intrude" the experimental situation. When marking your bee you have a choice between head, thorax, and abdomen. If you have more than one bee different colors may be used and/or different locations. For example, you can have a bee that is marked with white nail polish on the head and thorax and another marked with a combination of red and green. It is important that you do not use cheap nail polish. We found that L'Oreal works well.

8. **One mechanical or electronic counter.** The counter is used to record how many times a bee lands on a target during the extinction phase of the experiment. The experiment is in two parts, the first part known as acquisition is the choice phase in which one target is rewarded with a drop of sucrose solution and the other target with a drop of tap water. During the second part of the experiment known as the extinction phase, both targets now contain tap water. Learning is measured during this phase by the number of times the bee will land on both targets during a 10 minute extinction interval. If it is difficult to find a counter a tally sheet will do.

9. **Experimental table.** The table is used to place your targets. Nothing elaborate is needed and a chair or stool will work nicely. The table should be at least 6 meters from the artificial feeder. The targets should be separated by at least 10 cm.

10. **One empty wooden matchbox.** The matchbox is used to capture a bee from an artificial feeder.

11. **Artificial feeder.** There are several ways to create an artificial feeder. One of the most efficient to use the methods described by Wells (Hill *et al*., 1997, 2001; Sanderson *et al*., 2006). Bees are trained to fly 50 m to a clear dish containing clove-scented 50% sucrose solution (2 drops clove oil to 1 liter 50% sugar solution) set on top of the gray training target. This feeding station can be place on a small stool for convenience. Set up the feeder close to the study colony (1 or 2 meters, or if you are brave on the hive entrance). Spay a very small amount of clove-scented sucrose solution on the hive entrance every 20 minutes until bees find the feeding dish. It is important that you let the sugar-water on the hive entrance be consumed so that bees search for the nectar source between feedings. Once the artificial feeder has several bees regularly visiting, move it several meters at a time until you reach the location where you plan to perform the experiment. Figure 2 provides an example of an artificial feeder.

Figure 2: Example of a feeding station.

12**. Data sheet.** The data sheet will consist of two parts. The first part is the acquisition phase and the second is the extinction phase. Prior to running the experiment take a piece of paper and include the following: 1) Your name, 2) date, 3) time, 4) temperature, 5) type of stimuli and 6) miscellaneous information.

METHODS

Approach the feeder with the match box halfway open. Select a bee that has just landed and capture it in the matchbox. Figure 3 provides an example. The rationale for selecting a bee that has just landed is that it will more readily feed than one that is full. In addition, since it is taking more time to feed you will have more opportunity to mark the bee with nail polish.

When the bee is inside the matchbox go to the experimental table located 5 to 10 meters from the feeding station. The gray target with a large drop of 50% sucrose should be in the center of the table. Place your match box over the drop while gently and slowly opening the match box. You will see the proboscis come out. When the proboscis makes contact with the drop the bee will begin to feed. While feeding, slowly open the match box and the bee will come out. Once the bee is out of the box and its proboscis is in contact with the sucrose drop, mark it with the nail polish (bees are oblivious to the environment when they are drinking).

Figure 3: A bee in the process of being captured.

The procedures of capturing the bee in the box, having it leave the box while still in contact with the sucrose drop, and applying the nail polish will take some practice. We suggest that you capture several bees – one per box – and practice. Figure 4 illustrates the procedure.

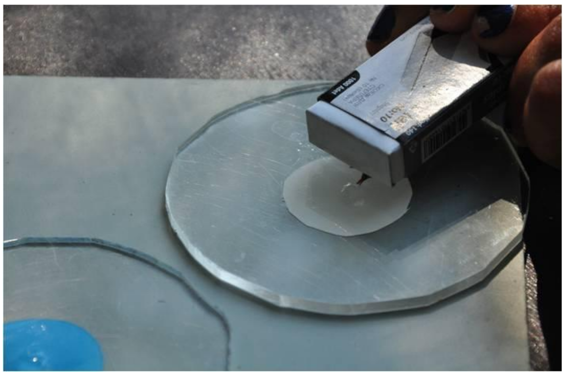

Figure 4. A captured bee drinking from a target in preparation for marking.

When the bee is marked it will continue feeding until she is full at which time she will return to the hive. If the bee does not return to the experimental table look for it at the artificial feeder. If you see your marked bee, recapture it and place it on the drop. You will not need to mark it again since you have already done so. Often, several placements are necessary before the bee returns on its own. When the bee returns to the gray target three consecutive times you are ready to begin the choice phase of the experiment. It is not necessary to wash the gray target after each placement or visit. Any chemical cues left behind by the bee will help it to find the experimental table and the gray target.

Since two stimuli will be presented simultaneously, you will need some way of randomizing the left-right orientation of the two targets. We will call the target containing the sucrose solution S+ and the target containing the water as S-. One method is to use a pseudorandom sequence of LRRLRLLR where the L refers to the S+ target being on the left (and the S-target on the right) and R refers to the S+target being on the right (and the S-target on the left). If this method is used, create a column labeled consisting of three consecutive repetitions of the eight characters in the suggested sequence (representing the 24 acquisition trials of phase one). The rationale behind the use of the pseudo-random procedure is to ensure that the bee has learned to associate the color with the reward and not its location. For example, if you placed the S+target always on the left you have no way of knowing whether the bee learned the color of the target or simply learned to always pick the target on the left.

During the acquisition phase, your data will consist of the first target the bee lands on. In addition to this information, it is more interesting to record the number of errors also. To do this, use the letter C to represent a correct choice and the letter E to represent an error. Using this system the data for trial one might look like "EEC" which represents that the bee landed on the incorrect target twice before landing on the correct target. Typically when the bee lands on the correct target it will stay there until it has completed feeding.

Another column should be created for what is known as return time. Return time is the interval from when the bee leaves a target for its return trip to the hive until in lands on a target on its next visit. The rationale for recording return time is both practical and theoretical. Practically speaking it allows you to determine whether the bee hovering above your experimental situation is your marked bee or an "intruder" bee. A trained bee will generally return to your experimental situation at regular intervals. If, after several consistent returns, a bee returns in a short time chances are that it is an intruder bee. Theoretically, return time is another indication of choice. You might think of return times as “decision times.

During the extinction phase of the experiment both targets should contain tap water. Leave the targets in place for 10 minutes. During each 30 second period record the choices the bee makes using the C and E system described earlier. Over the course of the 10 minute interval you will notice that the bee will land more on the target previously associated with the reward. A sample data sheet is provided in Table 1.

Table 1A: Sample Data Sheet (Acquisition Phase)

Name: ________________________________

Date: ______________ Time: ______________ Temperature: ______________

Miscellaneous information ____

____

Trial	Position of S+	Choice	Return Time (sec)
1	Left		
2	Right		
3	Right		
4	Left		
5	Right		
6	Left		
7	Left		
8	Right		
9	Left		
10	Right		
11	Right		
12	Left		
13	Right		
14	Left		
15	Left		
16	Right		
17	Left		
18	Right		
19	Right		
20	Left		
21	Right		
22	Left		
23	Left		
24	Right		

Table 1B: Sample Data Sheet (Extinction Phase, 10 minutes/30 second intervals)

Interval	Choices	Cum R S+	Cum R S-
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

RESULTS

The results of the acquisition phase can be analyzed in a number of ways. One way is to plot the number of correct responses across the 24 training trials. You will notice as the number of trials increase so do the correct number of responses. The number of repetitive errors will also decrease as the bee gains experience with the training situation. Return times can also be plotted and you will find that after the first few visits they will become a bit shorter. Figures 5 and 6 show the results of a bee landing on the correct target in a situation where blue is the S+ and white is the S-. Notice the intruder bee in Figure 6.

Figure 5: A bee landing on the blue target in a blue/white discrimination task. Blue is the S+ target.

Figure 6: The same bee landing on the blue target in another visit. Notice the presence of the intruder bee hovering nearby.

The data during the extinction phase can be plotted cumulatively for both the previously correct target and the previously incorrect target during each 30 second period. For example, if the bee lands on the correct target 6 times and the incorrect target 2 times these values will be plotted for the first 30 second period. If during the next 30 second interval the bee lands on the correct target 7 times and the incorrect target 2 times the values plotted for the second 30 second period will be 13 (6+7) and 4 (2 +2), respectively.

DISCUSSION

This simple exercise can stimulate a wide variety of discussion. For example, Can learning be considered the same in all individuals? How do you define intelligence? How are proper comparisons made? In addition to stimulating discussion, the methods described here can provide a spring board for students to design and implement their own experiments. Once they have mastered the techniques they can explore questions of their choosing. Presenting opportunities for students to ask their own questions is particularly important in light of the emphasis being placed on inquiry-based learning. For example, the changes in the Advanced Placement Biology curriculum that are being implemented in the fall of 2012 will emphasize students developing their own questions for open-ended laboratory experiences.

In regards to the specific exercise students could explore the following questions:

1. Do the number of correct choices increase over the 24 training trials?

2. Are there any differences in the S+ and S- curves during extinction?

3. How do your results compare with those of your classmates?

4. What is the biological significance of choice learning for honey bees?

5. How would you apply this training method to problems in agriculture?

6. How does learning in free flying bees compare to learning in harnessed bees?

In conclusion the exercise is easy to use and is considered fun and informative by students. Students learn about the generality of learning and are often surprised at how fast the honey bees learn. The importance of experimental design and the use of controls to isolate instances of learning from such non-associative effects as position and sensitization is also emphasized in this exercise. It is important to note that because honey bees can sting it is important for instructors and students alike to make sure that precautions are taken to minimize risk. Perhaps the best precaution you can take is to be well prepared in advance, detail planning with your students and working closely with a local beekeeper. It is also important to know whether you or any of the participants are allergic to insect venom.

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