THE USE OF HONEY BEES TO TEACH PRINCIPLES OF LEARNING

Bal Arılarından Yararlanarak Öğrenim İlkelerinin Anlatılması

(Extended Summary in Turkish can be found at the end of this article)

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ABSTRACT: Experiments are described with harnessed and free-flying forager honey bees suitable for classroom exercises and zoological/botanical park demonstrations. The experiments require bees to discriminate between two scents or two colored targets. Both experiments are easy to perform with minimal training, and the apparatus is inexpensive and constructed from common items such as plastic straws. Suggestions are provided on how the study of learning can be used to educate the general public and students about the importance of honey bees.

Key Words: Teaching, Honey bees, *Apis mellifera*, Conditioning, Learning

INTRODUCTION

This paper describes the use of honey bees (*Apis mellifera*) to demonstrate principles of learning. Two experiments are described. The first uses honey bees harnessed in plastic straws and the second uses foragers trained to fly from the colony to the experimental situation. Background and historical information on these procedures can be found from many sources including Byrne (2003). A review of the economic importance of honey bees can be found in articles by Çakmak (Çakmak & Wells 2001; Çakmak, 2004).

Our goal in this paper is three-fold. First, we wanted to develop a honey bee preparation useful to educators and beekeepers to demonstrate conditioning. Many members of the public are familiar with the dance language, various aspects of defensive behavior, and the role of honey in agriculture but few realize that honey bees also have a great capacity to learn. We believe that by familiarizing people with the learning ability of honey bees a new appreciation of bees can be developed. There are many examples in the literature of honey bees learning to associate a stimulus with feeding, and this is known technically as Pavlovian conditioning. Such a preparation is easy to use, versatile, and encourages student participation and discussion. Second, we wanted to stimulate interest in the general public on the economic and ecological importance of honey bees by showing how readily these animals learn and how sensitive they are to pesticides and other forms of pollution. Finally, we wanted to develop a preparation that can be used in botanical and zoological gardens to increase public awareness of the importance and value of honey bees as a natural resource.

MATERIALS AND METHODS

Preparation 1: Pavlovian discrimination in harnessed foragers

The purpose of this exercise is to demonstrate the ability of honey bees to discriminate between two odors–one of which is paired with a feeding. The materials are a plastic drinking straw with an internal diameter of 6 mm, two 20-cc syringes, scents (perfume, essential oils), timer, toothpick, honey, cotton balls, duct tape, and a data sheet (Figure 1).

Steps in conditioning test of honey bees;

Figure 1. Syringes and essential oils to be used in learning experiment.

The easiest way to do this is to ensnare a single bee in a wooden match box or glass vial and place the box or vial into the freezer compartment of a refrigerator or, alternatively, in a bucket of ice (Figure 2 & 3).

Figure 2. Honey bees brought to the laboratory in vials with perforated caps.

Figure 3. Bees are briefly anesthetized by placing on ice.

Cut the straw into 2.5 cm long sections. Capture several honey bees and place them individually in the straw tubes, which acts as a harness–one bee per harness with the bee's head protruding from the tube. When the bee becomes inactive, put the bee into the harness and secure it with a thin piece of duct tape placed between the head and the thorax with the ends of the tape secured to the sides of the harness. A strip 15 mm long and 4 mm wide is effective (Figure 4).

Figure 4. One bee placed in to a holder for harnessing. The holder is metal (e.g. a bullet shell cut with a dremmell). The strip of duct tape cut to about 2mm thickness is placed over the "neck" of the bee, under the head capsule and above the thorax.

Care must be taken to allow the proboscis to extend. The harnessing procedure takes a bit of practice and can be done within 10 seconds (Figure 5).

Figure 5. Four bees harnessed for a conditioning experiment. The first and the last bee in the photo are properly prepared. The bees in the middle may not be able to extend proboscis in learning trials, Tape is placed over the head capsule.

Once active, feed the harnessed bees with honey until satiated and the bee will be ready to use the next morning. Alternatively, a bee can be used as soon as harnessed but the danger here is that it may not be motivated to feed. We recommend harnessing at least 10 bees over what is needed. If, for example, the demonstration involves 10 students each working with their own bee we recommend harnessing 20 bees.

On the day of the demonstration pre-test your sample bees by touching a honey tipped toothpick to an antennae–do not allow the bee to feed. Only use those bees whose proboscis rapidly extends. If the bee does not extend its proboscis it may not be motivated to feed, or more likely, is placed in the harness in such a way that the proboscis is physically unable to extend and only the mandibles will be observed to move. If this happens, repositioned the bee in the straw and stimulate the antennae again.

Prepare the scents by placing a drop or two of one of the odorants onto a cotton ball placed within a syringe. To prevent confusion, label the syringe with the name of the scent. Follow this procedure for the second scent. Many different types of scents can be used. For example, we have used the scents of cinnamon and lilac. If two scents cannot be found, one syringe can remain odorless in which case the honey bee will learn to discriminate between air only and scented air.

Once the syringes containing the scents are prepared, the data sheet can be created. The preparation of this sheet can be done at anytime during the preparation phase of the experiment. To create the sheet space is needed for general information such as the name of the student, date, time, weather condition, and location. The main part of the sheet will consist of 24 rows labeled 1-24. These numbers represent experiences with the scent and are technically known as a trial. Each bee will be exposed to 12 trials of a scent each followed by a feeding (known as the CS+ trials) and 12 trials of a scent none of which are followed by a feeding (known as the CS- trials). The use of two scents requires that a way must be found to randomize their presentations. One way to do this is two present the two scents in a pseudo-random order. We recommend the following order be used: CS+ CS- CS- CS+ CS- CS+ CS+ CS- CS+ CS- CS- CS+ CS- CS+ CS+ CS- CS+ CS- CS- CS+ CS- CS+ CS+ CS-. For example, if the scent of cinnamon is followed by a feeding and the scent of lilac is not, the first four trials would be: Cinnamon (feeding), Lilac (no feeding), Lilac (no feeding), Cinnamon (feeding).

When the experimental preparations are completed trial 1 of the experiment can begin. A harnessed bee is exposed to a three second presentation of the CS+ scent. The syringe containing the CS+ odor is rapidly depressed near the head of the bee and during the last moments of the presentation the hand containing the tooth pick dipped in honey is touched to the mouthparts. When this is done the proboscis will extend and the bee is permitted to feed for two seconds. It is critical for the correct operation of the experiment that the time between the presentation of the CS+ scent and the feeding be short as possible. The dependent variable is proboscis extension which should be observed on each of the 24 trials. When the CS+ is presented, for example, note whether the proboscis extended during application of the scent but before the feeding. If so, mark down a “1” on the data sheet, if it does not extend mark a “0.” When the first trial is completed return the syringe to the extended position and wait 5 minutes before beginning the second trial and each subsequent trial. The time interval between trials is technically known as the “intertrial interval.” For this experiment the inter trial interval is 5 minutes. When the five minute interval has elapsed begin trial 2. This trial is similar to trial 1 with the exception that it is a CS- trial in which the second scent is now used and there is no feeding. Again, note whether the bee responded to this CS-scent by extending its proboscis. Continue this procedure for the 24 trials of the experiment. In two hours the experiment will be completed. The bee will have been exposed to 12 CS+ trials and 12 CS–trials with the results being that they will have readily learned to discriminate the two scents by extending their proboscis significantly more times to the CS+ scent than to the CS–scent. The CS–trials are technically known as an "experimental control" and demonstrate that the bee is actually learning to respond to the food.

Preparation 2: Free–flying experiments

One of the more widely used techniques to measure learning in honey bees is to train free-flying foragers to discriminate between two plastic targets one of which contains a drop of reward. The targets can be discriminated on the basis of color, scent, or both. Unlike the previous experiment, the free-flying procedure is conducted outdoors.

The purpose of this demonstration is to teach the bee to discriminate between two targets on the basis of color. The procedure is very similar to what was done in the harnessed situation. Materials needed are a bee colony, a feeder containing 8-12% sugar water, two differently colored plastic tiles 4 x 4 cm square (we recommend yellow and orange), one 4 x 4 cm gray plastic tile, a small jar of 50% by weight sugar solution (equal parts water and sugar), paper towels, wooden match boxes, timer, eye dropper, nail polish, portable table and two bowls of water.

Prior to the experiment a feeder must be established where foragers regularly visit. There are several ways to do this and beekeepers have much practical experience getting bees to visit a particular location. One way is to fill a jar with 50% sugar water and place it in such a way where the fluid slowly seeps out (see Hill & Wells 2002 for an alternative method). Locate this feeder near the colony and capture several bees in a match box and place the match box near the sugar solution and slowly open it. When the bee's proboscis comes in contact with the solution it will extend and the bee can be removed from the box. We recommend that 20 or more bees be placed on the feeder. When this is done properly there will be many bees regularly visiting the feeder and the sugar solution will soon deplete. Refill the jar with 25% sugar water and move the location of the feeder about 5 meters from the colony in the direction where you want the feeder to be. The 25% solution is still highly attractive. Within an hour or so this feeder will also be depleted. This time re-fill the feeder with an 8–12% solution and move it an additional 5 meters to its final location. When this is done 5–10 foragers will be regularly visiting. Replenish the feeder with 8–12% sugar water as needed.

Establishing the feeder is important because the bee used in the experiment will come from this feeder. Once the feeder is established let it sit for a day or two being sure to re-fill the feeder as needed. On the day of the experiment capture an individual bee, which has just landed on the feeder, in a match box and bring it over to the experimental "arena". This arena can be as simple as a portable table, or a chair–any flat surface with a minimum usable space of 75 cm x 35 cm. With the gray target in the center of the arena place a large droplet of 50% sugar water in the center of the target. Open the match box slowly and when the proboscis comes in contact with the solution open the box wider and the bee will crawl onto the target. While on the target mark the thorax with the nail polish. In this way a specific bee can be identified. Within a minute or so the bee will have filled-up, marked the target, and returned to the colony. If the bee has not re-visited the target within 10 minutes return to the feeder, wait until the marked bee has returned, recapture it, and place it again on the gray target. Eventually the bee will return not to the feeder containing 8-12% sugar water, but to the gray target containing the 50% solution. When the bee returns twice on its own accord the experiment can begin.

As in the harnessed experiments the bees will be taught to discriminate scent or color cues. Whereas the harnessed bees learned to discriminate a scent presented one at a time (called a successive discrimination), this experiment requires the bee to discriminate targets placed side by side (called a simultaneous discrimination). Decide which of the targets will be the CS+ and the CS-. The CS+ will have a large drop of 50% sugar water and the CS- an equally sized drop of water. For example, if yellow and orange targets are used with orange being the CS+ place the sugar water in the center of the orange target and a drop of water in the center of the yellow target. Place each target side by side with an approximately 20 cm separation from center to center. When the bee returns it will find the two colored targets rather than the single gray target. Eventually the bee will land on one of the two colored targets. If it landed on the correct target (the CS+ target) allow it to feed until satiated and record a correct response. A bee landing on the incorrect target (CS- target containing a large drop of water) should be allowed to correct its mistake by flying to the correct target. After the bee leaves for its return flight wash and dry both targets, place them in their proper position, and put the appropriate solution in the center of the targets.

As in the previous experiment a method must be found to randomize the position of the targets. Use the pseudo-random order of the previous experiment. This time the order will represent whether the CS+ is on the left (CS- on the right) or right (CS- on the left). The data sheet can be the same with the number of visits 24. The bee will learn to discriminate quickly and 24 trials will not be necessary to show learning. In contrast to the harnessed experiments, the free-flying experiments may take longer to perform in large part because the bee determines how fast it returns to the experimental arena. In other words, the inter trial interval is determined by the bee and not by the experimenter.

DISCUSSION

The harnessed and free-flying experiments are easy to perform and often are considered astounding to students of all ages. These new “citizen scientists” are often surprised that honey bees learn and do so rapidly. Discussions of the data can focus on the importance of learning in the life of a honey bee, and how learning can be used as an environmental monitor.

There are many variations that can be run on these two paradigms. For example, harnessed bees can be exposed to pesticides to provide a vivid demonstration of the potential dangers pesticides and environmental pollutants can cause. Different types of scents and food can also be used to determine honey bee preferences. In the free-flying situation persistence (known as extinction) can easily be studied by substituting water for the sugar solution after the bee has demonstrated it has learned to discriminate the two targets. When both targets now contain water the number of landings will be greater on the target previous associated with food. Another interesting variation is to convert the CS+ to a CS- and the CS- to a CS+ and discover how fast the bee can adjust to the new situation. This is known as reversal learning. A third variation is to examine the effect of pheromones by not washing the targets. Additional variations and how to design low cost apparatus for Invertebrate learning experiments can be found in Abramson (1990) and Hill and Wells (2002).

A unique use of these paradigms is to conduct the experiments in zoological and botanical parks to increase the general public’s awareness of the importance of honey bees and the dangers they face. All such parks contain educational facilities for the public and these experiments are ideal. Moreover, educational exhibits can be created with the assistance of local beekeepers and beekeeping associations describing how bees learn and, if possible, contain video clips of experiments such as those presented here and the names and addresses of local scientists working with honey bees.

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ÖZET: Bu çalışmada bal arıları kullanılarak öğrenme ilkeleri anlatılmaktadır. Bal arılarından hem arıcılar ve hem de eğitimciler koşullu öğrenme konusunda yararlanabilirler. Zaten arıların beslenme yerini bir uyarıcı ile öğrenebilmesi Pavlov koşullanması olarak bilinmektedir.

Bu makalede 3 hedef amaçlanmıştır. Birincisi, eğitimciler ve arıcılar tarafından şartlanmanın gösterilebilmesi için bal arısı hazırlığını geliştirmek istedik. Zaten birçok kişi bal arıları dansı, savunma davranışı ve balın rolü hakkında bilgisi vardır fakat bal arılarının öğrenme konusunda büyük bir kapasiteye sahip olduklarını bilmezler. İkincisi, ekonomik ve ekolojik açıdan oldukça önemli olan bal arılarının hızlı bir şekilde öğrenmesini, pestisit ve diğer kirleticilere hassas olmasının halka gösterilmesi ile ilgi uyandırmayı amaçladık. Üçüncü olarak da Hayvanat bahçelerinde ve Zooloji parklarında, bal arılarının doğal kaynaklar içerisinde ne kadar önemli ve değerli olduğu konusunda ilerleme sağlamak istedik.

Denemeler plastik kamışa sabitlenmiş ve serbest uçan arılarla, hedef olarak iki farklı koku ve renk kullanılarak yapılmıştır. İki denemede çok basit ve az malzeme ile yapılabilir. Birinci denemede, arılar buzlukta kısa bir süre tutulup hareketi yavaşlayınca plastik kamışlar içinde bantla kafa ve göğüs arasından sabitlenir. Dilini çıkarabilecek durumda olması gerekmektedir. Bu şekilde bağlanan arılara tesadüfi şekilde iki koku birbiri arkasından verilmiştir. Seçilen bir kokunun hemen ardından şeker solüsyonu verilerek toplam 24 kez tekrarlanır ve arıların belli bir koku ile şeker solüsyonunun geleceğini öğrenmesi denenmiş olur. İkinci denemede, dışarıda uçan arılarda iki farklı renkte hedef ve bunlardan birinde arılara benzer şekilde şeker solüsyonu verilmiştir. Bu denemede arılar iki hedeften birini rengine göre öğrenip seçmesi amaçlanmıştır. Renklerden birine şeker solüsyonu diğerine bir damla su verilmiştir. Önceki denemede olduğu gibi hedefin yerleri tesadüfi olarak değiştirilmiştir. Yine 24 kez bu işlem devam etmiştir. Bu denemede arıların sabitlendiği denemedeki gibi yine şeker solüsyonu olan hedefi öğrenmesi ve ona göre ayırım yapması beklenir. Sabitlenen denemede arılara arka arkaya tesadüfi sırada verilen bir kokuyu ayırması, ikinci denemede aynı anda yan yana duran iki hedeften rengine göre arıların ayrım yapması gerekmektedir. İkinci çalışmada deneme daha uzun süre alabilir, çünkü denemenin her sıra arasındaki zamanı araştırmacı değil arı belirler. Farklı koku, renk ve besin bal arılarının tercihlerini tespit etmede bu şekilde kullanılabilir. Üçüncü olarak şeker solüsyonu ve su damlası vermede kullanılan renkli plastik kareler yıkanmadan feramonların etkileri incelenebilir.

Bu şekilde hem sabitlenen ve hem de havada serbest uçan arılarla yapılan bu öğrenme çalışmaları her yaştan öğrenciler için çok şaşırtıcıdır. Genel olarak halk arasında bilime yatkın insanlar tarafından arıların bu şekilde hızlı öğrenmesi sürpriz bir durumdur. Bu sunumların ışığında arıların yaşamında öğrenmenin ne kadar önemli olduğu ve bu öğrenmenin çevrenin izlenmesinde kullanılabileceği tartışılabilir. Bu şekilde bal arıları aynı zamanda zooloji ve botanik parkları ve eğitim amaçlı gösterilerde arıcılar ve arıcılık konusunda çalışan araştırmacılarla birlikte yapılabilir.

Bu denemeler basit malzemelerle ve az bir çalışma ile yapılabilir. Burada halka ve öğrencilere öğrenmenin öneminin anlatılmasında bal arılarının nasıl kullanılabileceği incelenmektedir.

Anahtar Kelimeler: Öğretme, Bal arıları, *Apis mellifera*, Şartlanma, Öğrenme.