

# Further Studies of the Cap Pushing Response in Honey Bees (*Apis mellifera*)

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## ABSTRACT

The Cap Pushing Response (CPR) is a free-flying technique used to study learning and memory in honey bees (*Apis mellifera*). The series of experiments outlined in this paper aimed to test whether honey bees exhibit the cognitive concept of “expectancy” utilizing the CPR in a weight differentiation paradigm. Five previous experiments in our laboratory have explored whether the concept of expectancy can account for honey bee performance and have all failed to support the cognitive interpretation. The first experiment examined if bees could differentiate between the two caps in the amount of force they used to push the cap and the distance the cap was pushed when the caps were presented one at a time. The second experiment explored cap weight preference by presenting bees with a choice between the two caps. The third and fourth experiments tested the bee’s ability to expect reward or punishment based on cap weight. Results revealed that bees were found to have a strong preference for the light cap and therefore were not able to expect reward or punishment based on cap weight. These experiments contribute to the debate on whether bees have “cognitive” representations and continue to support the behaviorist interpretation.

## 1. INTRODUCTION

The purpose of this experiment is to continue a line of investigation examining whether honey bees have the cognitive ability of “expectancy.” Expectancy is defined by the American Psychological Association as “the internal state resulting from experience with predictable relationships between stimuli or between response and stimuli” [1] (np). Expectancy is a cognitive concept that is explored in opposition to conditioning techniques from the behaviorist perspective.

We would like to note at the outset that the honey bee experiments we conduct in our laboratory are from a behaviorist position. We may be the only laboratory that approaches honey bee learning from a “non-cognitive” perspective. For over 30 years we have conducted various types of experiments designed to tease apart the behaviorist and cognitive explanations. In each class of experiment, we have found that honey bee learning (and other invertebrates) can be accounted for by a straightforward application of traditional behaviorist principles [2].

We believe that the reliance on cognitive explanations of honey bee behavior is unfortunate. We do not know if many or any at all even consider alternative explanations to the current cognitive zeitgeist – the lack of alternative explanations or their consideration is always a dangerous enterprise. As has been pointed out many times [e.g. 2, 3], the term cognition has no consistent definition, and there are no consistent rules to tell a researcher what behavior is considered cognitive from those that are not. These and other issues related to cognition are seldom discussed in the literature or the classroom with the result that an entire generation of behavioral scientists are uninformed of behaviorism and its various forms such as neo-behaviorism. It would not be surprising that many of the models developed during the golden age of learning theory in the 1950s, while not always appropriate to higher vertebrates, would provide perfectly reasonable accounts of “cognition” in an invertebrate [4]. In the experiments reported here, we once again look for cognition in honey bees.

Before we discuss the present experiments, we will review previous attempts in our laboratory to uncover “cognition” in honey bees. Our first line of investigation focused on signaled avoidance learning. The central question of avoidance learning is “How can the absence of an event be reinforcing?” The general answer is that the aversive event must be “expected” [5]. Honey bees were trained to fly off a specially designed target in which vibration and/or air could be used as cues and electric shock could be administered when the bee was feeding. After a number of cue/shock pairings, the bee flew off the target in “expectation” of the shock. It is important to note and is often overlooked, that until the organism begins to prevent or postpone the aversive stimulus, the first few trials of any signaled avoidance experiment are actually a classical conditioning procedure. The results of the bee avoidance experiment were interpreted in terms of straightforward applications of classical conditioning principles. There was no need to invoke any expectancy-like process to account for the behavior of flying off the target to a stimulus predicting shock [6]. What controlled the behavior was not the avoidance of an expected event but the fact that it was paired. After a few trials where the bee would fly off the target in response to the cue, it would remain on target when the cue was subsequently presented. The response of flying off the target to the cue constitutes extinction in that the bee does not receive the shock on trials in which it responds to the cue. This pattern of acquisition, followed by extinction is exactly what is predicted with straightforward applications of basic Pavlovian principles. There is no need to postulate a “cognitive” process to account for the results.

In addition to the classical conditioning interpretation of avoidance learning, successful signaled avoidance learning can also be accounted for, not by an expectancy-like process but by a two-process theory in which classical conditioning establishes “fear” in the organism. Fear can then be reduced, postponed, or removed by making an instrumental response, *i.e.* the avoidance response. The organism is not avoiding an aversive stimulus *per se* but escaping from fear. This view was proposed by O. H. Mowrer [5], in his famous two-factor theory of avoidance. While this theory may not account for all signaled avoidance designs with vertebrates, it has seldom been postulated as a mechanism of avoidance in an invertebrate but should be considered as a suitable mechanism.

Signaled avoidance experiments were also performed with green crabs [7]. In these experiments, animals were compared when receiving either classical conditioning or avoidance learning. Animals receiving classical conditioning could not avoid the stimulus while those receiving signaled avoidance training could. Contrary to the expectancy-like interpretation, the results did not differ between the groups. In addition, animals were given classical conditioning followed by signaled avoidance training and those given signaled avoidance training were followed by classical conditioning. The results showed that the manipulation from one paradigm to the other did not influence performance. The expectancy-like interpretation would lead one to predict that animals transitioning from signaled avoidance to classical conditioning (*i.e.*,

where a previously successful avoidance response now has no effect) would produce extinction, and avoidance performance would deteriorate. Their performance was not affected. The expectancy-like interpretation would also predict that the avoidance paradigm would produce better conditioning than the classical conditioning paradigm. Once again, performance was not affected.

In summary, the signaled avoidance experiments with honey bees, and crabs, suggest that it is the pairing of the aversive event with the cue and not its “expected” omission that is creating successful avoidance learning. There was no need to postulate an expectancy-like process when the simpler non-cognitive explanation accounted for the data.

In a second series of experiments, we studied proboscis extension response conditioning (PER) in honey bees. Bees were confined to tubes [8] and given an olfactory conditioned stimulus (CS) followed by a sucrose unconditioned stimulus (US). As predicted, based on previous experiments, bees readily acquired the association between odor and food by extending their proboscis [9]. The novel question we asked was whether bees would respond to a CS when the CS was the termination of the odor which is then followed by the US. The data showed that the bees only responded when the CS was presented, and not when the CS was the absence of the odor. This finding was especially revealing because the bees were given a within-subject discrimination task in which two CSs were used—both paired with a US. One CS was the addition of the odor, and the other CS was the absence of the odor. An expectancy-like account would predict that bees would respond to both CSs. Our results, similar to our signaled avoidance data, showed that the bees responded only to the onset of a stimulus, not its absence [10].

The ability of honey bees to represent time was the focus of our third attempt to find expectancy-like processes in honey bees [11]. To successfully estimate an arbitrarily selected duration of time, there must be some type of expectancy-like process. It is well known that honey bees can be trained to visit a flower at a particular time of day [12, 13]. However, will they still tell “time” when confronted with traditional operant fixed intervals (FI) schedules of reinforcement? Bees were trained to fly into an automated operant chamber where they were given various FI schedules [14]. Each of the FI schedules studied failed to show evidence of timing at the individual level even when all seven of the traditional methods of analysis were used [11]. In contrast, a study of timing in horses readily revealed evidence of timing with all seven measures [15]. Thus far, the data of signaled avoidance, responding to the absence of a CS, and timing all suggest that honey bees do not have expectancy-like processes.

Our fourth attempt was to determine whether free-flying honey bees could solve a discrimination task when the defining feature was the presentation or absence of a cue [16]. The results of these “feature positive/feature negative” experiments [16] revealed that bees could readily solve a discrimination problem when a feature was added but found it difficult to solve the problem when a feature was absent. The results of this experiment were again consistent with our previous efforts in that bees responded to the presentation of a cue and not its absence.

Our last attempt—the fifth—before the current series of experiments, was to use a within-subject discrimination task to determine if bees can “expect” the type of US in a traditional PER experiment with harnessed foragers [17]. Bees will readily extend their proboscis to honey and to low-molarity sucrose. Those bees feeding on honey will keep their proboscis extended for longer periods of time than bees feeding on low-molarity sucrose. The question we asked was whether a CS associated with honey will elicit longer extension times than a CS associated with low-molarity sucrose in a test trial when the CSs are presented without the US. The answer was they could not. When the test trial was presented, the bees extended their proboscis for the same amount of time whether the US was honey or low-molarity sucrose. If honey bees had an expectation of the US we would have predicted that a CS associated with honey would produce longer extension times than a CS associated with low-molarity sucrose.

Our previous experiments on signaled avoidance, absence of a CS, timing, feature positive/negative, and representation of a CS all suggest that honey bees do not show expectancy-like processes. It is the presentation of a stimulus, not its absence that controls the honey bee’s behavior. We now turn our attention to a sixth attempt to find expectancy-like processes by using the recently developed free-flying technique where bees are trained to push a cap to reveal a hidden food source [18-21]. Previous research on

shaping the honey bees to push the caps [21] was used in each experiment presented in this paper. The cap pushing response (CPR) is used in the following four experiments to determine if bees can use the weight of a cap to “expect” a reward.

There are many human examples where weight can be used to study expectancy-like processes. Consider a situation, where you are working at your desk and begin to drink from a metal water bottle that you are not able to see through. When you lift this water bottle, you may have expected the bottle to be a certain weight when it is actually another. Therefore, you may use the incorrect amount of force needed to lift the water bottle because you were distracted by your work. As you lift the water bottle, you are able to adjust the amount of force you are using to drink from the water bottle. The next time you lift the water bottle you will be able to expect the weight of the water bottle and use the proper amount of force because you now know the water level. In this series of experiments, we are using a situation much like the water bottle, with honey bees to see if they can expect force when pushing two different weighted caps. The cap pushing response is an ideal situation to test whether bees can use weight as a cue for a reward.

## 2. MATERIALS AND METHODS

### 2.1. Subjects

There were 80 honey bees selected at random from a group of foragers from a feeding station in Lesvos, Greece. The bees were captured from a maintained three maintained hives using a common feeding station [8]. The feeding station consisted of 8% sucrose by volume and was located 4.572 m (15 ft) from the hives and 4.572 m (15 ft) from the feeder to the research tables. Bees were collected from a feeder station captured in a matchbox and brought over to a research table. The bees consumed 50% sucrose (by volume) found in each of the platform’s wells (10 mm diameter, 6 mm height), located in the center of each platform. Different subjects were used for each of the four experiments described.

### 2.2. Apparatus

The training apparatus consisted of a circular 3D printed platform (88 mm) and an upside-down 3D printed cap (12 mm diameter, 10 mm height). The four experiments all utilized a 3D printed platform (88 mm) and two caps (12 mm diameter, 10 mm height). There were two caps, a light 0.456 g and a heavy 3.376 g cap filled with lead, which were also 3D printed (see [Figure 1](#) to see the two caps).



**Figure 1.** The platform was painted with white nail polish around the well so that the researcher could place the cap in the same place for every trial. This made measuring distance more accurate and kept the apparatus the same for every trial. The caps in this picture are both flipped upside down. The heavy cap was filled with lead and the light cap was empty. When turned right side up, the two caps were indistinguishable from each other.

## 2.3. Calculations

### 2.3.1. Distance

The distance the cap moved after every trial was measured with a ruler. The end of the cap was measured to the end of the nail polish painted around the well on each of the platforms (see [Figure 1](#)). The distance between the two markers represented the distance the bee pushed the cap, measured in mm on each trial.

### 2.3.2. Force

The formula for force is  $F = (\Delta d / \Delta t^2) * \text{mass}$ , where  $(\Delta d / \Delta t^2)$  equals acceleration [22]. The total force the bee used to push each cap was determined by using the total distance millimeters (mm) divided by the overall time squared multiplied by the weight grams (g). Units were converted for consistent measurement accuracy (millimeters to meters & grams to kilograms). In order to solve for force, the distance the cap was pushed, the weight of the cap, and the time (s) it took the bee to push the cap were collected. The time the bee took to push the cap was collected from a video recording using researchers' iPhones mounted with a phone holder which did not move for all trials. The distance the caps traveled was collected in person after each trial.

### 2.3.3. Pre-Training Procedure

Honey bees were collected in the morning between 7:00 am and 12:00 pm and then again from 4:00 pm until 9:00 pm to avoid the heat in the middle of the day. Bees were collected in a matchbox from a feeder containing 8% sucrose by volume and brought to the training platform. The first time they returned they were marked with various colors of "LBK" nail polish. Once they returned to the platform twice, resistance shaping began [20]. Resistance training was conducted using the light cap flipped upside down. To shape the bees, researchers applied force on the opposite side of the cap with more and more force until each bee pushed the cap when the well was completely covered. Once the cap was pushed twice with the well completely covered (shown in [Figure 2](#)), the experimental phase began. The experimental phase utilized two different weighted caps. After each trial, the platform and caps were cleaned with water and a paper towel to prevent chemical or odor signaling [23]. When a bee reached 20 min without returning, a similar criterion we used in our previous research [16, 19], the bee did not continue to the next trial and was considered finished or excluded from the experiment. Once the experimental phase was completed, bees were sacrificed so that they were not used again. Variables such as intertrial intervals (ITI), duration of cap pushing seconds (s), the distance the cap is pushed (mm), and the weight of the cap (g) were recorded.

## 2.4. Experiment 1: The Effects on Different Weighted Caps in a CPR Paradigm

The purpose of experiment 1 was to determine if honey bees could distinguish between two differently weighted caps. It was predicted that honey bees would push the light cap farther than the heavy cap and would be unable to change the amount of force they used to push each cap. Typically, bees are trained to fly to targets distinguished by color, odor, and/or position. This was the first time that bees were trained to distinguish between weights. There was a total of 20 honey bees split into two groups, where half ( $N = 10$ ) started with the light cap first and the other half started with the heavy cap first, to account for counterbalancing.

### 2.4.1. Apparatus

The apparatus consisted of a circular 3D-printed white platform with a well in the middle that held 50% sucrose by volume [24]. A 3D-printed right side-up cap consisting of various weights was placed over the well, one cap per trial (10 mm diameter, 6 mm height).

### 2.4.2. Procedure

Once the experimental phase began, one platform was on the research table with one right-side-up cap. For every fourth trial, the bees were given a test trial, and the weight of the cap was changed. Once the honey bee had left the research table to fly back to the hive, that trial was concluded. When the honey bee



**Figure 2.** Honey bees can push the cap two ways. They can either lift the cap or push the cap with their heads.

returned and touched the platforms, a new trial began for a total of 16 trials. In between every trial, both the caps and the targets were cleaned using standard methods [6, 25]. Video footage was also recorded so that the force to push each cap could be calculated for each honey bee. The dependent variables included ITIs, total time pushing the cap, the distance the cap was pushed, and cap weight.

## **2.5. Experiment 2: CPR Paradigm Utilizing Weight Differentiation in Choice Preferences**

The purpose of experiment 2 was to determine if honey bees will have a preference for which cap they push or if they will push the first cap that they return to. The prediction was bees would push whichever cap they approached first. The only difference between the two platforms was the weight difference of each of the caps. Honey bee weight preference was analyzed by touches made to each of the platforms. Five bees were randomly assigned to each of the four groups. Half of the groups with discriminative stimulus (Sd) light cap and the other with Sd heavy cap totaling four groups: heavy on the left side, heavy on the right side, light on the left side, and light on the right side.

### **2.5.1. Apparatus**

The experimental phase utilized two platforms instead of a single platform as in experiment one. The platforms included one with a light cap and the other with a heavy cap. The platforms both had a 50% sucrose by volume underneath each cap. Bees had the choice between the two platforms.

### **2.5.2. Procedure**

Bees had the choice between two platforms, one with a heavy (3.376 g) and one with a light (0.456 g) cap following the ABBABAAB paradigm [26, 27]. There were a total of 16 trials. The dependent variables were the number of times each bee landed on the target, number of pushes, number of drinks, and distance pushed (mm) were recorded for each trial.

## **2.6. Experiment 3: Discrimination with Water and Sucrose Using CPR in Weighted Caps**

The purpose of experiment 3 was to manipulate honey bee weight preference by adding water to one of the wells instead of a sugar solution. The prediction was that bees would not be able to expect rewards based on cap weight. Honey bees have been found to prefer sugar solutions over drinking water [28]. Following experiment 2, since a preference was found, the central question was to determine if substituting water for sucrose in one of the wells changes the honey bee's ability to "expect" reward based on cap weight.

Twenty bees were randomly divided into four groups of five subjects each to account for counterbalancing. The groups consist of two light discriminative stimuli and two heavy discriminative stimuli. Half of the groups with discriminative light caps and the other with discriminative heavy caps totaling four groups: Group 1: heavy on the left side, Group 2: heavy on the right side, Group 3: light on the left side, and Group 4: light on the right side. Groups one and two reinforced the heavy cap and three and four reinforced the light cap.

### 2.6.1. Apparatus

The third experiment used the same apparatus as experiment two. The only difference was the solution that was put into the wells. One had 50% sucrose by volume solution and the other consisted of water.

### 2.6.2. Procedure

In the experimental phase, honey bees had the option of two platforms each with a cap either light or heavy. One of the platforms was filled with water and the other with a 50% sucrose by-volume solution. Honey bees landed on the platforms and coding began. Once the bee left the research table, the next trial began, and so on until all 16 trials were completed.

## 2.7. Experiment 4: The Effect of Punishment on Weight Discrimination

The purpose of this, like all of the experiments, is to look for expectancies. The prediction was that bees would not be able to expect punishment based on cap weight. This experiment is based on Smith and colleagues' [27] experiment where honey bees received two CS that both consisted of 50% sucrose but one was also associated with shock. Similarly, in this experiment, both targets contained sucrose, but one of the targets was also paired with shock. The first paper to study aversive conditioning in honey bees was conducted by Abramson [6]. Since then, aversive learning has been found to be a very effective learning technique and a way to gain better insight into the cognitive abilities of honey bees [25, 29].

Just as in experiment two (CPR paradigm utilizing weight differentiation in choice preferences) and three (discrimination with water and sucrose using CPR in weighted caps), four groups of bees with five bees in each of the groups were used. The groups consisted of two light discriminative stimuli and two heavy discriminative stimuli. Half of the groups with discriminative light caps and the other with discriminative heavy caps totaling four groups: heavy on the left side, heavy on the right side, light on the left side, and light on the right side.

### 2.7.1. Apparatus

This apparatus consisted of one platform with two wells consisting of 50% sucrose by volume. The wells were each covered by the two different weighted caps following the ABBABAAB paradigm. One wire was attached to a metal ring surrounding the food well and a second wire was connected to the bottom of the food well. When the bee was standing on the ring and its proboscis was in contact with a sucrose-filled food well, the circuit was completed by the experimenter when a switch was thrown. Once thrown, the bee received a 9-volt direct current (VDC) shock. The duration of the shock was about one second. A picture of the apparatus can be found in [Figure 3](#).



**Figure 3.** This apparatus has a manual switch that controls what side and for how long the shock is administered. The switch and base are connected by wires to one platform consisting of two separate wells filled with 50% sucrose by volume solution.

### 2.7.2. Procedure

During the experimental phase, honey bees landed on the platform, whether they got shocked depended on which side they landed on. If they landed on the platform with the cap associated with punishment, a quick shock was administered, and if they landed on the platform with the cap not associated with punishment, no shock was administered. Every time the bee touched a side of the platform, its behavior was coded. Every time the bee landed on the  $S^A$  within a trial, a shock was administered.

## 3. RESULTS

### 3.1. Observation Orientated Modeling

All results utilized Observation Oriented Modeling (OOM) [30-32]. OOM is an alternative to traditional null hypothesis significance testing (NHST) statistics. This software allows researchers to create descriptive models to determine the accuracy of one's theoretical model [30]. This software was used to look at the behavior of each individual bee and each group within each experiment. The individual bees' pattern of behavior was used to see if the bees fit each hypothesis. This method does not take into account assumptions, like those found in traditional statistics.

### 3.2. Experiment 1: The Effects on Different Weighted Caps in a CPR Paradigm

The prediction was confirmed. Honey bees pushed the light cap farther than the heavy cap and were unable to alter their force used to push each cap. Using OOM, an *Efficient Cause—Blocked Orderings* procedure was conducted to determine the difference between the caps over the 16 trials. The analysis was set up so that each block consisted of one of the cap weights. This allowed us to directly compare each cap based on the force they used to push the cap and the distance the cap was pushed at the individual level. For instance, if the first three trials included the light cap, they constituted the first block and the heavy cap that followed constituted the second block. Each time a weight change was made, a block change was made, resulting in eight blocks for the efficient cause analysis. Bees in group one started with the light cap while bees in group two started with the heavy cap. **Table 1** shows example distance measures for bee nine from the first group. As can be seen and consistent with expectation, from one block to the next this bee tended to push the light cap farther distances than the heavy cap.

The efficient cause analysis yields two statistics: the Percent Correct Classifications (PCC) index and the chance value ( $c$ -value). The PCC is computed by comparing the distances between the light and heavy caps in adjacent blocks of trials (cf. [33]). Each light cap trial is compared to each heavy cap trial in an adjacent block, and the number of instances in which the light cap is pushed farther than the heavy cap is counted as a correct classification. The result from the 21 comparisons is converted into a percentage, and for the bee in **Figure 4**, the PCC index equals 80.95% ( $17/21 \times 100$ ). In only four instances was the light cap not pushed farther than the heavy cap in an adjacent block (viz., Trial 12 vs. Trials 9, 11, and 15; Trial 16 vs. Trial 15).

In addition to the PCC, a  $c$ -value is computed from a randomization test in which each bee's responses are randomly shuffled, repeatedly ( $k = 1000$  randomizations). The number of instances in which the PCC from the randomized data is greater than or equal to the observed PCC and is then tallied and converted to a proportion, the  $c$ -value. For bee nine, the result was .033. Such a low  $c$ -value indicates that behavioral theory, rather than physical chance, should be chosen as the more plausible explanation of the observed pattern of behaviors [30]. In other words, a high PCC index and low  $c$ -value indicate that a given bee's behavioral pattern is consistent with theoretical expectations.

The results for all bees are reported in **Table 1**. As can be seen, 16 of the 20 honey bees yielded PCCs greater than 80% with  $c$ -values less than 0.04, thus pushing the lighter cap greater distances than the heavy cap in the vast majority of adjacent trial comparisons. The patterns for the remaining four bees were also consistent with expectation, but with lower PCCs that ranged from 52.38% to 76.19%. Three of these four bees were in the second group (heavy cap first). Aggregating the distance results from all of the bees in group one yielded a PCC of 93.33% ( $c < 0.001$ ), whereas aggregate results from bees in group two yielded a



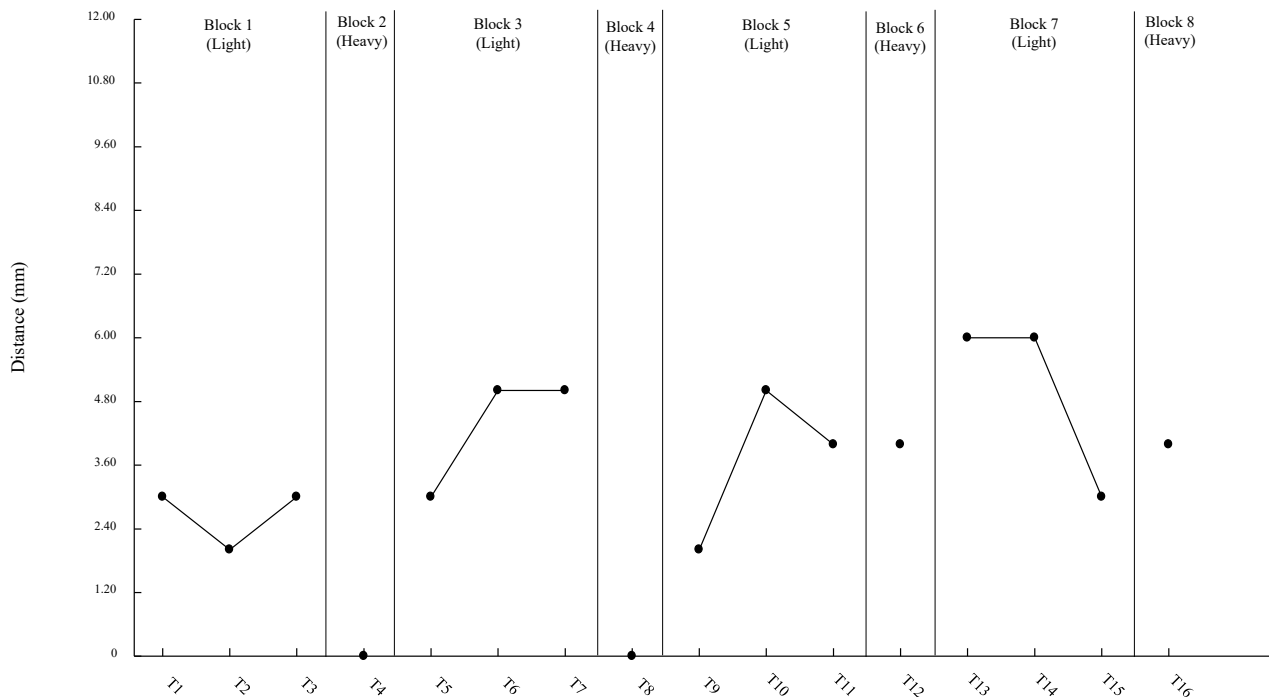
PCC of 85.71% ( $c < 0.001$ ). **Figure 5** shows these aggregate results as medians plotted across the 16 trials and eight blocks for each of the two groups. As can be seen, the average response patterns are mirror images of one another, consistent with expectation given the change in order of presentation of the light and heavy caps.

Force was computed from the weight of the cap, the distance the cap traveled, and the total time the bee used to push the cap. The *Efficient Cause—Blocked Orderings* analysis with eight blocks was then used; however, the expected pattern was set so that the heavy cap would be pushed with greater force than the light cap. **Table 1** shows the individual force results for the two groups and the 20 individual honeybees. As can be seen, 10 of the 20 bees yielded PCCs greater than 80% with  $c$ -values less than 0.05, thus indicating more force being applied to the heavier caps. The PCCs for six bees were less than 50%, indicating a poor fit to the expected pattern of responses. Overall, bees in group one yielded a PCC of 63.81% ( $c = 0.003$ ), whereas bees in group two yielded a PCC of 74.76% ( $c < 0.001$ ). The results for force were therefore not as consistent with expectation compared to the results for distance.

**Table 1.** Individual honey bee distance and force classification results.

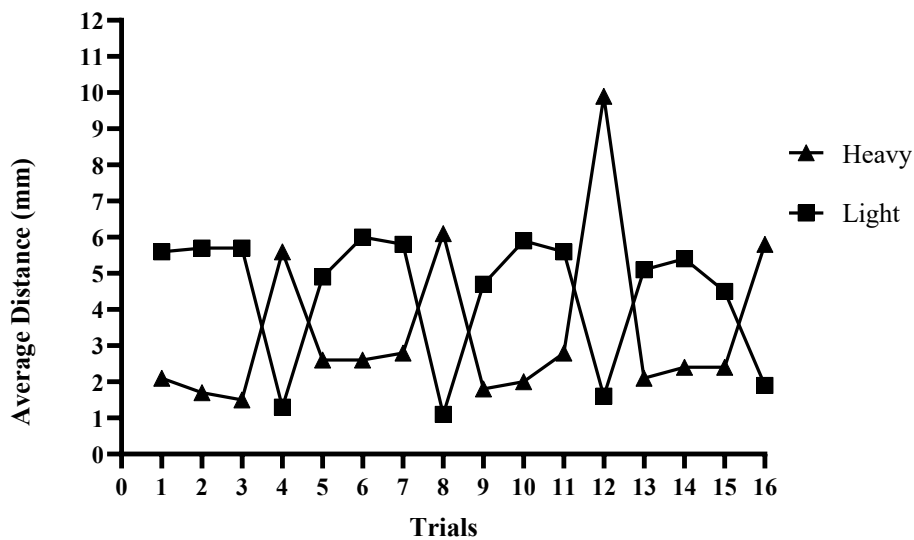
Group	Bee ID	Distance		Force	
		PCC	C-value	PCC	C-value
Light Cap First		93.33	<0.001	63.81	0.003
	1	95.24	0.002	47.62	0.59
	2	71.43	0.078	28.57	0.87
	4	95.24	0.003	0.00	1.00
	5	90.48	0.006	23.81	0.94
	8	100.00	0.002	71.43	0.14
	9	80.95	0.033	66.67	0.23
	11	100.00	<0.001	100.00	<0.001
	15	100.00	0.001	100.00	<0.001
	16	100.00	0.001	100.00	<0.001
Heavy Cap First		85.71	<0.001	74.76	<0.001
	3	52.38	0.382	66.67	0.20
	6	100.00	0.002	95.24	<0.001
	7	80.95	0.022	38.10	0.73
	10	80.95	0.028	80.95	0.05
	12	100.00	0.002	80.95	0.02
	13	76.19	0.031	28.57	0.89
	14	100.00	<0.001	100.00	<0.001
	17	100.00	0.001	100.00	<0.001
	18	66.67	0.066	61.90	0.05
20	100.00	<0.001	95.24	<0.001	

<sup>1</sup>PCCs and  $c$ -values listed for distance the cap was pushed and force for each bee separated by group.



<sup>1</sup>Bee #9 distance pushing each cap separated by each block.

**Figure 4.** Efficient cause block orderings pattern for bee #9.



<sup>1</sup>The heavy and light first group medians across 16 trials.

**Figure 5.** Median across both groups.

### 3.3. Experiment 2: CPR Paradigm Utilizing Weight Differentiation in Choice Preferences

The results did not support our prediction. Honey bees were able to differentiate cap weight and showed a preference towards the light cap. A *Concatenated Pattern Analysis* in the OOM software was utilized to examine the differences between the heavy and light cap push distances from the ABBABAAB design. This analysis is conducted to allow researchers to test a specific pattern of observations across several orderings. Two groups were created to counterbalance the positioning between the two caps. Group

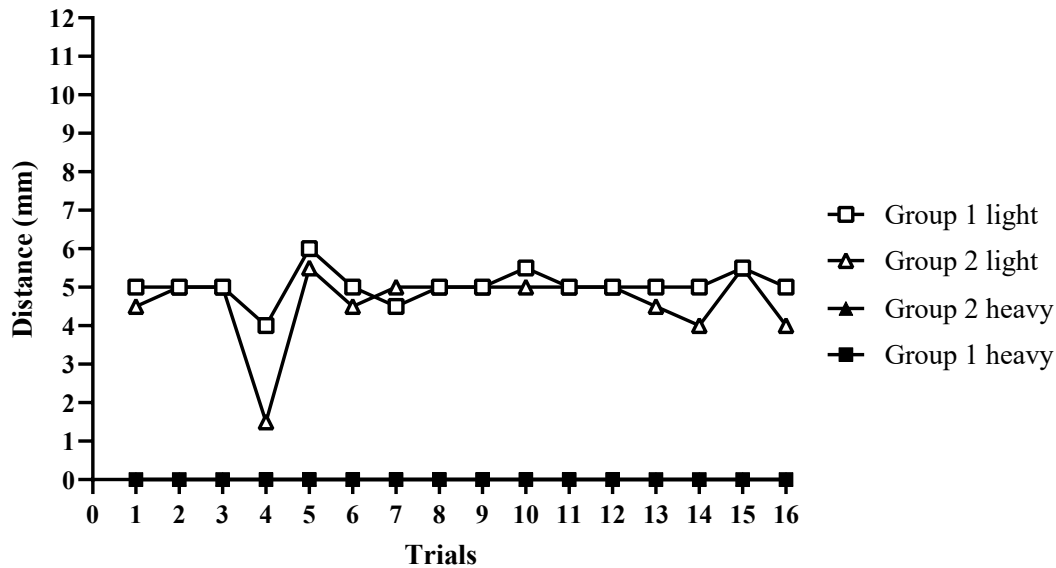
one represented the heavy cap on the left and the light cap on the right side of the platform. Group two represented the heavy cap on the right and the light cap on the right.

For each bee the number of trials in which the light cap was pushed a greater distance than the heavy cap was tallied and recorded as a PCC value. The results in [Table 2](#) show that sixteen bees yielded PCCs of at least 80% with low  $c$ -values ( $<0.014$ ), thus indicating a clear preference for pushing the lighter cap farther distances than the heavy cap. The PCCs for the four remaining bees (one from group one, and three from group two) ranged from 68.75% to 75.00%, showing greater distances for the light caps across the trials, but the corresponding  $c$ -values were not as impressively low (0.027 to 0.097). Aggregating across bees within each group yielded PCCs of 93.13% and 83.75% ( $c$ 's  $< 0.001$ ) for groups one and two, respectively, and average distances across the 16 trials are shown in [Figure 6](#). You can also see that honey bees preferred the light cap over the heavy cap for all 16 trials.

**Table 2. Individual honey bee distance difference classification results.**

Group	Bee ID	PCC	C-value
Heavy Cap Left		93.13	$<0.001$
	3	100.00	$<0.001$
	4	100.00	$<0.001$
	5	81.25	0.014
	6	93.75	0.001
	10	87.50	$<0.001$
	11	100.00	$<0.001$
	12	100.00	$<0.001$
	14	75.00	0.051
	17	100.00	$<0.001$
	19	93.75	$<0.001$
Light Cap Left		83.75	$<0.001$
	1	87.50	$<0.001$
	2	81.25	0.007
	7	100.00	$<0.001$
	8	81.25	0.007
	9	87.50	0.001
	13	75.00	0.039
	15	75.00	0.027
	16	87.50	0.003
	18	93.75	$<0.001$
	20	68.75	0.097

<sup>1</sup>PCCs indicate percentage of instances across the 16 trials each bee pushed the light cap farther than the heavy cap.



<sup>1</sup>Group 1 indicated light was on the left and Group two indicated light was on the right. The light and heavy key determined which cap weight was presented within the groupings.

**Figure 6.** Medians across both groups when provided a choice

### 3.4. Experiment 3: Discrimination with Water and Sucrose Using CPR in Weighted Caps

Our prediction was confirmed, bees did not expect a reward based on cap weight. Four groups were created to counterbalance position and reward association for each weighted cap in an ABBABAAB design in the OOM *Concatenated Pattern Analysis*. For each bee the number of trials in which the reward-associated cap (light or heavy) was pushed a greater distance than the other cap was tallied and recorded as a PCC value. The results in [Table 3](#) show the resulting PCCs for the twenty individual bees and the four counterbalanced groups. As can be seen, all but one of the bees yielded a PCC of 100% ( $c$ 's < 0.001) when the light cap was associated with the reward indicating that the light cap was pushed farther than the heavy cap in every trial. The PCC for bee #15 was also very high (87.50%,  $c < 0.001$ ).

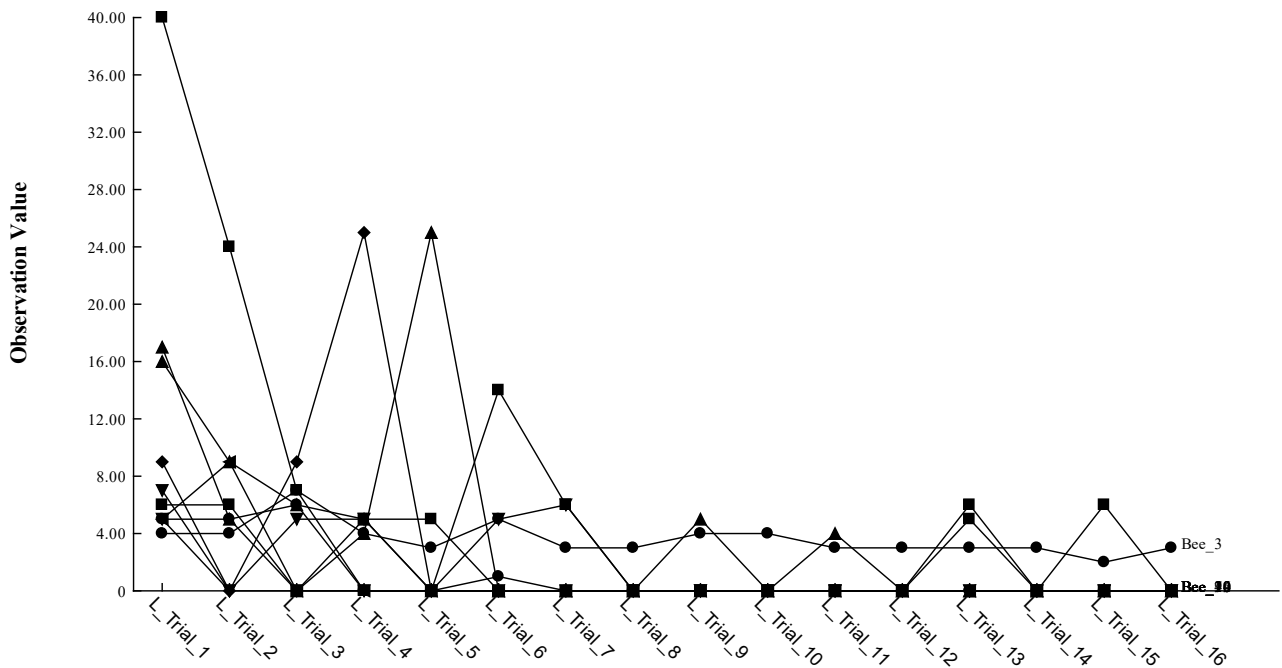
By contrast, when the heavy cap was associated with the reward, only one bee (#16) pushed the heavy cap a greater distance than the light cap for a large majority of trials (PCC = 93.75,  $c < 0.001$ ). The PCC for two bees from these groups were slightly above 50% while the PCCs for six bees were 0% (see [Table 3](#)), indicating that not a single bee pushed the reward-associated heavy cap farther than the light cap. It is important to point out that five of these six bees did not push the heavy cap a measurable distance for any of the sixteen trials (*i.e.*, distance = 0 mm). Moreover, opposite of expectation, one bee (bee three) pushed the light cap, which was not associated with the reward, a farther distance than the heavy cap for 11 of the 16 trials (PCC = 68.75%,  $c = 0.100$ ).

As can be seen in [Figure 7](#), all ten of the bees in the heavy cap groups pushed the light cap at least 4 mm for the first trial. This behavior was largely extinguished by trial seven, however, for all but one bee (bee three). The bees in the light cap groups can be found in [Figure 8](#). As shown in [Figure 9](#), honey bees clearly prefer the light target and performed best when the light target was associated with the reward. Even when the heavy cap was associated with a reward, bees still pushed the light cap more until the fifth trial. This shows that honey bees had a strong preference for the light cap.

### 3.5. Experiment 4: The Effect of Punishment on Weight Discrimination

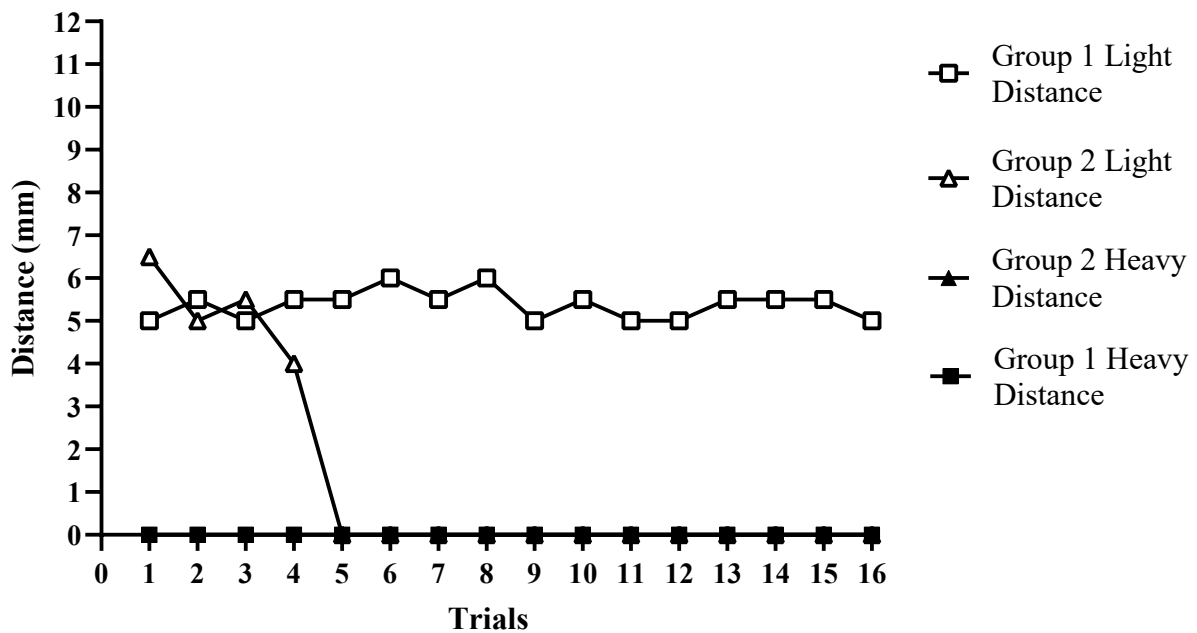
Our prediction was confirmed, honey bees were not able to expect punishment based on cap weight.

The *Concatenated Pattern Analysis* was utilized to analyze the distance each cap was pushed. The four groups were used to counterbalance the position and reward associated with each weighted cap. Applying the logic ordering function in OOM, the system marked ones for each time light was greater than heavy for each group for all 16 trials.



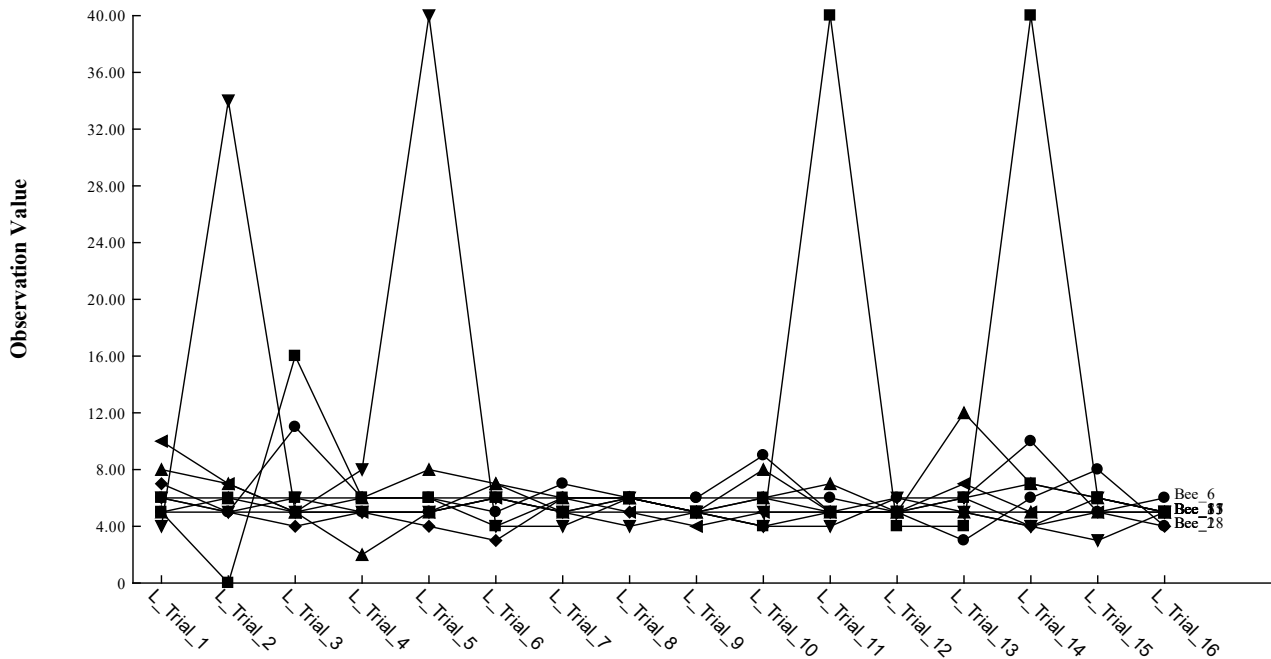
<sup>1</sup>Each shape represents a different bee in the heavy cap group (10 bees) distance pushed over the 16 trials.

**Figure 7.** Heavy reward associated with cap.



<sup>1</sup>Group 1 light is reinforced, and Group 2 heavy is reinforced.

**Figure 8.** Median distance across 16 trials when one is associated with reward.



<sup>1</sup>Each shape represents a different bee in the light cap group (10 bees) distance pushed over the 16 trials.

**Figure 9.** Light reward associated with cap.

**Table 3.** Individual honey bee distance difference classification results.

Group	Bee ID	PCC	C-value
Heavy Cap Left		26.25	1.00
	3	18.75	0.996
	9	56.25	0.418
	14	56.25	0.362
	19	0.00	1.000
	20	0.00	1.000
Heavy Cap Right		18.75	1.000
	4	0.00	1.000
	7	0.00	1.000
	10	0.00	1.000
	12	0.00	1.000
	16	93.75	<0.001
Light Cap Right		100.00	<0.001
	2	100.00	<0.001
	5	100.00	<0.001
	11	100.00	<0.001
	13	100.00	<0.001
	18	100.00	<0.001

Continued

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Light Cap Left		97.50	<.001
	1	100.00	<.001
	6	100.00	<.001
	8	100.00	<.001
	15	87.50	<.001
	17	100.00	<.001

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<sup>1</sup>PCCs indicate the percentage of instances across the 16 trials where each bee pushed the reward-associated cap (either heavy or light) farther than the other cap.

Four groups were again created to counterbalance position and punishment association for each weighted cap in an ABBABAAB design. For each bee the number of trials in which the punishment-associated cap (light or heavy) was pushed a *shorter* distance than the other cap was tallied and recorded as a PCC value. The results in [Table 4](#) show the PCCs for the 20 individual bees and the four counterbalanced groups. When the heavy cap was associated with the punishment, a majority of the bees pushed the light cap farther than the heavy cap across the 16 trials. However, as can be seen in the table, there was a good deal of variability among the bees. Whereas six bees yielded PCCs greater than 80% with low *c*-values, two bees yielded PCCs of approximately 50% (*c*'s > 0.35) and one bee (bee seven) yielded a PCC of only 37.50% (*c* = 0.904, PCCs > 75%). When the cap was positioned on the left side of the platform, the PCC was higher (92.50%) than when it was positioned on the right (70.00%).

In contrast, when the light cap was associated with the punishment a majority of the bees did not push the heavy cap farther than the light cap. As shown in [Table 4](#), The PCCs for the individual bees in these two groups were less than 38% (*c*'s > 0.90). When the heavy cap was positioned on the left side of the platform, the PCC was higher (10.00%) than when it was positioned on the right (8.75%). Examination of the individual responses clarifies these low PCC values; specifically, across all 10 bees in these two groups and all 16 trials, only in 20 instances (13%) did a bee push the heavy cap a noticeable distance. In other words, most of the recorded values were greater than 0 mm. In 57 instances (36%) the recorded distances for the light cap were greater than 0 mm. In [Figure 10](#), we can see that the heavy caps' medians are non-existent compared to the light caps, even when the heavy cap was associated with reward. The light cap was the highest when it was associated with reward and was still pushed even when the heavy cap was associated with reward and then dropped off until around the 10th trial. This shows that honey bees were not able to expect punishment based on cap weight.

#### 4. DISCUSSION AND CONCLUSION

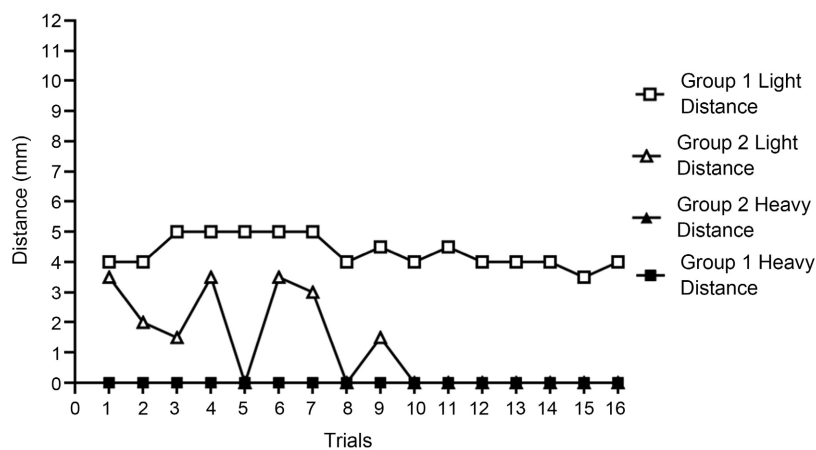
Honey bees were not able to expect a reward based on cap weight. Results revealed that bees had a strong preference for the light cap, even when the heavy cap was associated with a reward, or the light cap was associated with a punishment. These four experiments identified the amount of force used to push each cap and the choice preference measured by the distance the cap traveled. In every experiment, the light cap was pushed farther than the heavy cap, suggesting that the light cap was preferred over the heavy cap. This means that the bee was not choosing the correct cap based on where the reward was held and therefore was not able to expect a reward or a punishment based on their ability to discriminate between the weights.

In experiment 1, we tested honey bees' ability to push the heavy and light cap individually, as well as the force each bee used when pushing each cap. Results revealed that the light cap was pushed farther than the heavy cap under both conditions (heavy first and light first). Specifically, 80% of the bees scored a PCC higher than 80%, but all scored above 50%. This means that it did not matter in which order the caps were presented, the honey bees still pushed the light cap farther than the heavy cap.

**Table 4.** Individual honey bee distance difference classification results.

Group	Bee ID	PCC	C-value
Heavy Cap Left		10.00	1.000
	3	0.00	1.000
	9	6.25	1.000
	12	6.25	1.000
	15	0.00	1.000
	18	37.50	0.902
Heavy Cap Right		8.75	1.000
	4	0.00	1.000
	6	0.00	1.000
	11	31.25	0.965
	19	0.00	1.000
	20	12.50	1.000
Light Cap Right		92.50	<0.001
	1	100.00	<0.001
	5	100.00	<0.001
	13	75.00	0.036
	14	100.00	<0.001
	17	87.50	0.002
Light Cap Left		70.00	<0.001
	2	100.00	<0.001
	7	37.50	0.904
	8	56.25	0.395
	10	100.00	<0.001
	16	56.25	0.418

<sup>1</sup>PCCs and c-values for the 20 individual bees and the four counterbalanced groups.



<sup>1</sup>Group 1 light was reinforced, and Group 2 heavy was reinforced.

**Figure 10.** Median distance across 16 trials when one is associated with an aversive stimulus.



The results on the amount of force were not as conclusive as the distance results. Only half of the bees fit the expected pattern of the heavy cap being pushed with more force than the light cap. Therefore, we were not able to conclude that bees were able to change the amount of force they use based on the cap weight. Consequently, distance followed the expected pattern and force did not. Honey bees pushed the light cap farther yet were not able to alter the amount of force they used to push each cap when discriminating between cap weights.

Experiment 2 tested whether honey bees had a preference towards one of the cap weights when provided with a choice between the heavy and light caps. In this experiment, both caps were presented at the same time, and both platforms contained a sucrose reward. Results revealed that honey bees preferred the light cap over the heavy cap. Overall, the position of the caps did not matter because both groups picked the light cap over the heavy cap. Bees were able to differentiate weight.

Similarly, to experiment 2, bees in experiment 3 were presented with both caps at the same time; however, one cap consisted of a sucrose reward, and the other contained water. Bees preferred the light cap even when the heavy cap was associated with the reward. When the light caps were associated with the reward, the bees did not make many errors. However, when the heavy cap was associated with the reward the bees selected the light cap until around the 5<sup>th</sup> trial. These results indicate that bees were not able to expect a reward based on the weights of the caps.

Lastly, in experiment 4, honey bees chose between a cap associated with being shocked and one without a punishment. The light and heavy caps both contained sucrose rewards. Results were similar to those from experiment 3. Bees selected the light cap over the heavy cap no matter which cap was associated with the punishment. When the heavy cap was associated with the punishment, the bees did not make many errors. However, when the light cap was associated with shock the bees still selected the light cap until the 10th trial. This provides evidence that the bees were not able to anticipate the punishment based on cap weight and therefore again shows that expectancy is not present in honey bees.

These results match previous experiments outlined in “Five Classes of Experiments Used to Search for Cognition in This Laboratory” [6, 10, 16, 17, 34]. Abramson [6] explored honey bees’ cognitive ability through avoidance of shock using a CS of vibration. Honey bees left the platform when they felt the vibration instead of receiving the shock. These results follow simple classical conditioning principles and thus do not fit the cognitive interpretation.

Likewise, Abramson and colleagues [10] explored if the absence or termination of a stimulus could elicit a response. Honey bees yet again were not able to elicit the appropriate response therefore not showing signs of cognitive processes. Next, Craig and Abramson [34] wanted to know if bees possess temporal conditioning. Experiments involving five FI schedules showed no evidence of expectancy-like processes in honey bees. The study that is the most similar to our experiments is Stauch *et al.*'s [17] results on molarity. Results showed that honey bees were not able to differentiate between different viscosities, thus not showing signs of expectancy. Lastly, Abramson and colleagues [16] researched feature effects on bees’ ability to expect a reward. Bees were not able to expect the different backgrounds or features of a conditioned stimulus as a cue for the location of the reward. These five experiments did not find the cognitive representation of expectancy in honey bees. Our results follow these previous findings.

In these experiments, honey bees showed a strong preference for the light cap (shown in [Table 5](#)). Therefore, they were not able to distinguish reward or punishment based on cap weight or alter the amount of force they used to push each cap. These four experiments provide further validation of the CPR as an operant conditioning paradigm and are the first to study the possibility of cognition using the CPR [18]. As shown in the results, the CPR further validates the behaviorist interpretation and continues to show a lack of the cognitive representation of expectancy in honey bees.

Cognition is a term that is frequently used, but the field of Cognitive research is riddled with inconsistencies in the definitions used and the application of the theory. Furthermore, the broad definition of cognition is described as the “science of the mind” [2] (p. 64). However, the lack of clearly defined operational definitions makes it impossible to make these scientific claims. The field of cognition houses an array of 79 definitions as opposed to behaviorism which is comprised of 27 definitions [35]. The wide array

**Table 5. Interpretation of each experiment.**

Experiment	Results	Interpretation	Was Expectancy Present?
Experiment 1	Honey bees pushed the lighter cap farther than the heavy cap and did not push the heavier cap with more force.	Bees were not able to expect weight by altering the amount of force they used to push each cap.	No
Experiment 2	When provided with a choice, bees pushed the light cap over the heavy cap.	Honey bees showed a weight preference and therefore were able to differentiate weights.	Yes
Experiment 3	Bees chose the light cap over the heavy cap even when the heavy cap was paired with reward.	Bees were not able to expect reward based on cap weight.	No
Experiment 4	With the addition of punishment, bees still preferred the light cap over the heavy cap.	Honey bees were not able to expect punishment based on cap weight.	No

<sup>1</sup>Interpretation of each experiment separated by results, interpretation, and if expectancy was present.

of definitions makes it difficult to determine what cognition researchers aim to explore. The use of the term cognition has also increased in use over the years and is still not properly defined [36]. Psychology research articles have increased in length, with less focus on replicability, and are now more focused on attracting attention to gain readers [36].

To test the cognitive perspective of expectation, these researchers aimed to use the CPR to employ weight discrimination with honey bees in four different experiments. Based on past research in the laboratory, cognitive conclusions were not found, and results can instead be interpreted using conditioning techniques and reliable terminologies. This work was important to show that behaviorism is a more effective paradigm for honey bee research compared to the less consistent area of cognition.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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