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Aversive learning in *Bombus terrestris* Audax (Hymenoptera: Apidae): responses to electric shock in a simulated environment

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Abstract. The study of animal behavior, particularly in insects, is crucial for understanding their biological and evolutionary aspects, with wide-ranging applications in agricultural science, pest management, conservation biology, and neuroscience. Investigating cognitive characteristics, specifically aversive learning, plays a pivotal role in comprehending the success of insects. This adaptive ability enables animals to efficiently cope with the stressful factors in their environment. In this study, we investigated the aversive learning capabilities of *Bombus terrestris* Audax workers, crucial pollinators across diverse ecosystems. Bees were trained and tested in a flight arena using artificial flowers equipped with electric shocks to simulate conditions wherebees could associate punishment alongside food resources with available cues. The result suggested that bees possess the ability to simultaneously detect potential threats and food resources, indicating a dual aversive-appetitive memory. Furthermore, comparing groups trained with aversive (electric shock) and neutral (distilled water) stimuli showed that danger cues led to faster learning and stronger memory formation. This conditioning setup aimed to simulate real-life foraging situations, exploring bee responses when confronted with potential dangers. These findings provide insights into the survival strategies of insects in challenging environments that negatively impact bee populations.

Keywords: Aversive learning, bumblebees, pllination, pollinator, insect behavior, free-flying bee, conditioning

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Introduction

Learning is a fundamental aspect of insect behavior that plays a crucial role in their survival, adaptation, and overall success within the animal kingdom (Jones & Agrawal, 2017). Through learning, animals acquire knowledge and skills, leading to behavioral changes that facilitate navigation through their environment, resource acquisition, danger avoidance, and interactions with other species (Jones & Agrawal, 2017). Associative learning is a basic cognitive function that involves forming a connection between two or more stimuli or between a stimulus and response (Pearce & Bouton, 2001). It can take different forms, including appetitive and aversive learning (Itzhak *et al.*, 2014). Appetitive learning occurs when animals learn to associate a particular stimulus with a positive outcome such as food or a reward (Itzhak *et al.*, 2014). Aversive learning, on the other hand, occurs when animals learn to associate a particular stimulus with a negative outcome, which could be related to factors such as the presence of a predator or a natural enemy, toxic food, or the absence of food, prompting the animal to avoid the cues or display defensive behaviors (Ings & Chittka, 2008; Litvin *et al.*, 2008; Roussel *et al.*, 2009; Itzhak *et al.*, 2014).

Aversive learning allows animals to recognize and respond to potential threats, thereby increasing their chances of survival (Itzhak *et al.*, 2014). Insects, as a highly diverse and successful group of animals, exhibit remarkable

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cognitive abilities (Giurfa, 2015; Chittka, 2017). Appetitive learning in insects has been extensively studied, particularly in species like fruit flies (Schipanski *et al.*, 2008), honeybees (Menzel, 2001), and bumblebees (Laloi *et al.*, 1999). Through appetitive learning, a conditioned stimulus (CS), such as color, odor, or location, is paired with an unconditioned stimulus (US) as a reward, such as food, host, or mate (Menzel *et al.*, 1993; Vinauger *et al.*, 2011; Reser *et al.*, 2012; Lenschow *et al.*, 2018; Cholé *et al.*, 2019). Consequently, this reward motivates the insect to persist in the learning process and actively participate in the experiment (Menzel *et al.*, 1993). One of the most renowned experiments in the field of appetitive learning focuses on conditioning of the proboscis extension response (PER) in bees (Giurfa & Sandoz, 2012). These experiments require restraining and closely monitoring bees in small apparatus or cages (Urlacher *et al.*, 2010; Giurfa & Sandoz, 2012; Delkash-Roudsari *et al.*, 2020). During the experiment, the bees are repeatedly exposed to a particular CS, which is usually a specific odor or color (Giurfa & Sandoz, 2012). At the same time, their antennae are stimulated by US, typically sugar water (Giurfa & Sandoz, 2012). As a result, bees develop PER, leading them to extend their proboscis and lick sugar water when presented with the CS (Giurfa & Sandoz, 2012). After undergoing multiple stages of training, the conditioned stimulus alone triggers PER in the bee, demonstrating Pavlovian conditioning (Menzel, 1993; Laloi *et al.*, 1999; Aquino *et al.*, 2004; Giurfa & Sandoz, 2012).

One example of appetitive learning that allows insects to choose and make decisions more freely includes experiments in which the CS is presented outside the hive, paired with a reward. In these experiments, bees have the opportunity to travel from the hive to the test site. This setup creates a natural foraging environment, enabling the learning process to occur in a more realistic and ecologically relevant context (Ravi *et al.*, 2016). However, there have been few reported examples of aversive conditioning in natural or semi-natural settings. One prominent example of such studies in insects is the sting extension reflex response (SER) observed in restrained bees (Núñez *et al.*, 1983; Guiraud *et al.*, 2018). In this experimental setup, the bee is entirely immobilized and positioned on an electric shock device or hot plate and, subsequently, a CS is presented in that setup (Forman, 1984; Vergoz *et al.*, 2007; Tedjakumala and Giurfa, 2013; Junca *et al.*, 2014). Recent studies have used honey bees in a maze with electric shocks to explore how walking insects avoid specific stimuli and move towards the opposite arm of the maze, which is marked with a different CS (Agarwal *et al.*, 2011; Dinges *et al.*, 2013; Nouvian & Galizia, 2019). However, the main problem with restrained bees and maze-based methods is that they may not accurately replicate the complex and dynamic nature of an insect's natural habitat and only offer limited spatial complexity. Consequently, such experiments may not fully capture the entire spectrum of navigational strategies and decision-making processes utilized by insects in the wild. Tethering insects, keeping them in small cages, or confining them within the maze can induce stress and result in altered behavior.

Artificial and restricted environments may lead to behavioral changes that do not reflect natural behavior, potentially causing biased outcomes in experimental studies. It is important to note that insects encounter different risks in their foraging environment, including predators, parasites, climate change issues, and the frequent use of agrochemicals in ecosystems, which expose pollinators to environmental stressors (Klein *et al.*, 2017). This raises the question of how pollinating bees in situations similar to a foraging environment recognize dangers and adjust their responses accordingly. Using a novel conditioning apparatus, this research attempts to study bumblebees' aversive behavior in a situation that is more similar to their natural habitat by allowing them to forage and fly freely in a simulated environment. The aversive stimulus presented here is the electric shock, which can represent the presence of danger and is delivered exactly at the location where the insect anticipates receiving the reward, which is the artificial flower. In this study, *Bombus terrestris* workers were used to examine how aversive stimuli affect their ability to learn and recall related visual cues. Because of their relatively large size, limited flight height, and impressive cognitive abilities (Goulson, 2008; Riveros & Gronenberg, 2009; Chittka, 2017), bumblebees have become ideal subjects for insect behavioral research.

Studying aversive learning in bumblebees, especially in a simulated foraging setting that closely resembles their ecological environment, provides insights into the cognitive abilities of these important pollinators and can enhance our understanding of the mechanisms underlying their behavior, memory formation, and decision-making processes. By identifying factors that negatively affect bumblebees' foraging behavior, such as exposure to pesticides or specific floral cues, this knowledge can contribute to the development of effective conservation strategies and the promotion of sustainable agricultural practices. By creating pollinator-friendly environments, these efforts ensure the continued well-being of these beneficial insects.

Materials and methods

Experimental setup and insects

Three colonies of bumblebees (*Bombus terrestris*) were provided by the BIOBEST commercial company. The nutritional requirements of the colony, including pollen and 50% sucrose solution, were supplied *ad libitum*. All three colonies were maintained at standardized room temperature and a photoperiod of 12 hours of light followed by 12 hours of darkness. All the experiments were conducted at the Ecology and Behavior Laboratory of Plant Protection Department, University of Tehran. Each colony was housed in a two-partite wooden nest box (40x20x20 cm). Two sections of the nest were interconnected through a two-centimeter diameter hole located at the bottom.

The reason for using a two-partite nest was to ensure that all bees and broods, including eggs, larvae, and pupae, were kept in darkness (Fig. 1-A-1). Meanwhile, in the other part, it was possible to control the light exposure using a lid (Fig. 1-A-2). Consequently, food could be provided in this part to encourage bees to exit and forage for food when the experiment was not in progress, and bees exhibiting stronger foraging motivation were identified before entering the flight arena. Using a transparent plexiglass corridor (25×5×5 cm) (Fig. 1-A-3), the nest was connected to a wooden flight arena (60×50×50 cm) which was covered with a transparent plexiglass lid (Fig. 1-A-4).

The corridor was equipped with sliding doors that allowed controlling the bee's access to the flight arena. This arena allowed the insects to be exposed to different stimuli, resembling a simulated foraging environment, enabling the investigation and study of their behavior under controlled conditions. Feeders installed on a platform (hereafter referred to as artificial flowers) were placed inside the flight arena. The bees' visits to the artificial flowers were recorded using an Action Camera (YI Technology, China) and captured in slow motion mode (240 frames per second).

Artificial flowers

An artificial flower consisting of two metal discs was placed on a 4 cm high plastic platform inside the flight arena. The outer disc, a hollow circular plate with a 5.5 cm diameter, was connected to a wire as the positive pole, and the inner disc, a circular plate with a 1.5 cm diameter, was connected to another wire as the negative pole. A layer of plastic separated the two metal plates. This allowed electric current to be delivered when a bee touched both of the metal discs. Each artificial flower was separately connected with wires to a device comprising an AC-DC power transformer (converting 220 V to 24 V, 50 Hz, 3 Amps), a DC-DC converter, a voltage reducer module, and switches allowing for manual control of the electric shock, enabling users to turn it on or off as needed (Fig. 1-B). Blue or yellow laminated hollow discs positioned on top of the outer metal plate were used as the CS (Fig. 1-E).

When the bees landed to feed on the food solution placed at the center of the inner metal disc, their contact with both metal discs completed the circuit, allowing the generation of an electric current (Fig. 1-D). In restrained honey bees, a voltage of 7.5 - 8 induces the SER response (Núñez *et al.*, 1997; Vergoz *et al.*, 2007, Carcaud *et al.*, 2009). To find the best situation for free-flying bumblebees in this setup, before conducting the main experiment, different voltages ranging from 3 V to 24 V were tested on the bees. A voltage of 20 volts was selected because it elicited the most intense behavioral response without causing harm to bees. If immediately after landing on the flower and making contact with the food solution, the bees displayed a brief tremor, moved their legs and antennae away, fell on their backs on the arena floor, or flew away, it could be confirmed that they had been subjected to an electric shock.

Experiments

The experiment consisted of three phases: pre-training, training, and the memory test.

Pre-training phase

Prior to the learning phase, a pre-training phase was conducted to acclimate the bees to the artificial flowers and landing platforms. During this phase, all bees were given unrestricted access to the artificial flowers to forage and find the reward location, which was a 10-microliter droplet of 30% sucrose solution, without experiencing any aversive stimuli or color cues (Fig. 1-C). By using a lower sucrose solution concentration during the pre-training phase, the bees were motivated to learn and perform better during the subsequent training phase when the concentration was increased. This approach maximizes the efficiency of the conditioning process. Among the actively visiting bees, 36 individuals were selected from different colonies and labelled on their thorax with a small number tag (12 bees from each colony).

Training phase

In order to study the impact of aversive stimuli on the avoidance learning process of workers, previously marked bees from the pre-training phase were individually trained to establish an association between color and either reward or punishment during the learning procedure. In the flight arena, a total of eight artificial flowers were arranged, each containing a 10-microliter droplet of the desired solution. Among these flowers, four were associated with reward, while the other four were associated with punishment.

The bees were divided into four groups of nine individuals each. In the first group, yellow flowers were associated with a 20-volt electric shock, while blue flowers in this group did not have any electric shock. Conversely, the second group experienced the opposite condition, where blue flowers were paired with the electric shock, and yellow flowers had no electric shock. Therefore, if there was a preference for a specific color among the bees, this factor could be considered in the statistical analyses. In both the first and second groups, both types of flowers (with and without electric shock) contained a 10-microliter droplet of a 50% sucrose solution. For the third and fourth groups, instead of using the electric shock, only a droplet of distilled water was provided to examine the effect of the absence of reward as a control group, versus punishment. Therefore, among the eight flowers, four contained a 10-microliter droplet of a 50% sucrose solution, while the other four contained a 10-microliter droplet of distilled water. The color arrangement for these flowers followed the same order as that of the first two groups. Training consisted of five bouts, with each bee undergoing five foraging trips individually. At each bout, the bee was allowed to enter the flight arena and visit the flowers as long as she desired. Afterwards, the bee could freely return to the nest through the corridor. A visit was considered only when the bee landed on a flower and the solution (either sucrose or water) was tested with her proboscis and antennae. After each visit by the bee, the empty flowers were immediately refilled. At the end of each training bout, all flowers were cleaned with 70% ethanol. The color cues and aversive/appetitive/neutral stimuli were then randomly rearranged to ensure that the bee choices were not influenced by visual signals or olfactory markers.

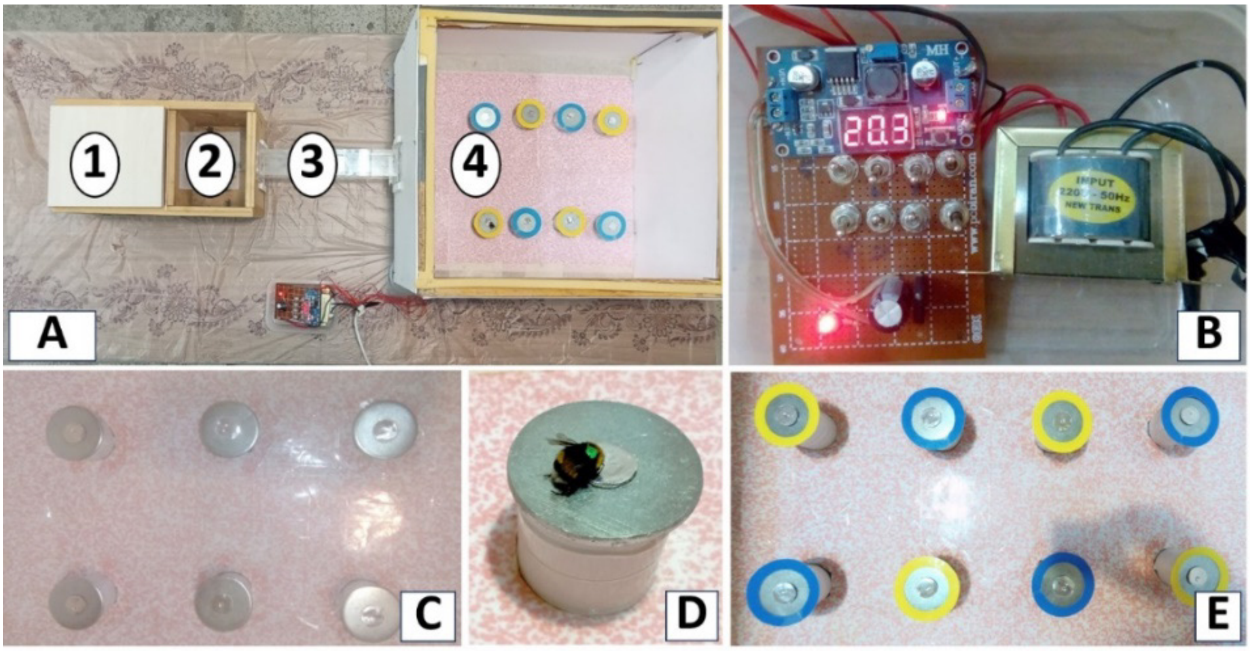

Fig. 1 - Insect Conditioning Apparatus. **A)** A two-partite nest box connected to the flight arena via a transparent corridor, with the flight arena being equipped with electrified artificial flowers; **B)** The device used to equip artificial flowers with an electric shock mechanism; **C)** A metal artificial flower with the outer disc wired as the positive pole and the inner disc wired as the negative pole; **D)** A *Bombus terrestris* worker feeding sucrose solution on the artificial flower; **E)** Artificial flowers with colored signs for conditioning purposes.

Memory test

Twenty-four hours after each bee completed all five training bouts, she was allowed to enter the flight arena once again for the memory test. During this phase, the colored cues were presented in a new random pattern, and none of the flowers were associated with reward or punishment (sucrose solution and electric shock were removed). Instead, all flowers contained only a 10-microliter droplet of distilled water. Each bee was given six minutes to forage and the number of visits to each color was recorded.

Statistical analysis

The statistical analysis for both learning and memory data was performed in R software version 4.3.0, using Generalized Linear Mixed Effect Models (GLMM) from "lme4" package (Bates *et al.*, 2015). To test the effect of the response variables, an ANOVA with the Anova () function of the "car" package was used. The simplest model was identified using the AIC criterion and backward stepwise elimination. Because the response variable consisted of binary data, a binomial distribution with a logit link function was used for the analysis. The model assumptions were verified by examining histograms and Q-Q plots, and a significance level of $p<0.05$ was considered (Russell & Burch, 1959). In the model used for learning, the effects of colony and color were found to be non-significant. As a result, these two factors were removed from the model through the simplification process. The dependent variable in the analysis was the response of bees to the stimulus. The independent variables, including the stimulus and learning bouts, were treated as fixed effects. The bee ID was considered a random effect in the model to account for individual variability among bees. In the model used for memory performance, the condition was similar to that of the first model, but the only independent variable was the stimulus. Graphs were made using the "ggplot2" package from R 4.3.0.

Results

Learning performance

The response of the bees was investigated during the five training bouts. The electric shock was used as the aversive stimulus, while distilled water served as the neutral stimulus during the learning phase. The response of the bees varied significantly depending on the bout ($\chi2= 87.5954$, df=4, $P<0.001$), revealing that the probability of visiting a rewarded flower increased with bout number. Specifically, the responses of the bees increased from the third to the fifth bouts for both stimuli. However, the increase in the response was greater for the aversive stimulus compared to the neutral stimulus (Fig. 2). The post-hoc test indicated that the effect of the electric shock as an aversive stimulus on learning performance was stronger compared to the distilled water as a neutral stimulus. This difference was particularly significant during the fourth and fifth bouts (Table 1).

The findings indicate that the bees' responses in identifying rewarding colors are modulated by the stimulus type. Aversive stimuli elicit a more distinct reaction compared to neutral stimuli in the context of color recognition by bees. Consequently, the model proposes that, in general, bees exhibit a higher tendency to visit the rewarded color when exposed to aversive stimuli as opposed to neutral stimuli.

Table 1 – Comparison of aversive stimulus (electric shock) versus neutral stimulus (distilled water) in different learning phases of *Bombus terrestris*.

Comparison of aversive stimulus versus neutral stimulus	*z ratio*	*p value*
First Bout	-0.350	0.7260
Second Bout	1.043	0.2967
Third Bout	1.319	0.1873
Fourth Bout	2.288	0.0222*
Fifth Bout	3.643	0.0003**

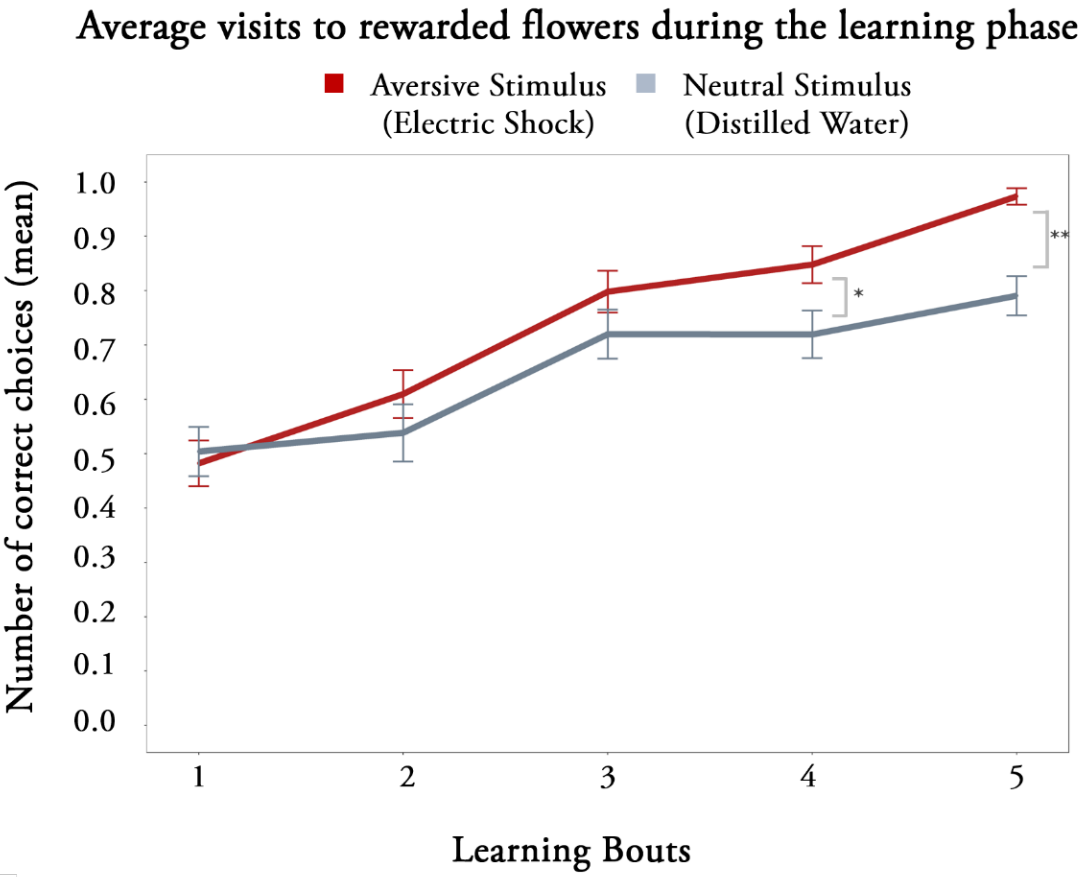

Fig. 2 - Learning curve of *Bombus terrestris* exposed to an aversive stimulus (electric shock) compared to a neutral stimulus (distilled water), during five training bouts. * p=0.0222, ** p=0.0003

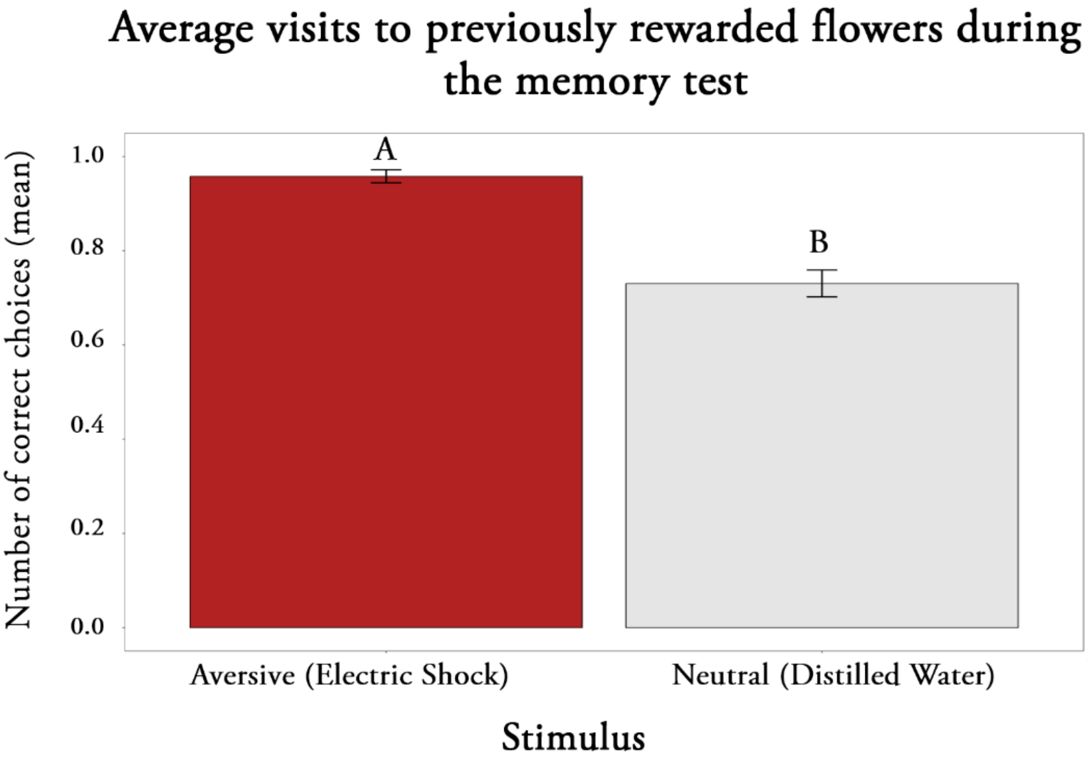

Fig. 3 - Memory performance of *Bombus terrestris* 24 hours after training, comparing the aversive stimulus with the neutral stimulus. Different letters indicate significant differences. ***p < .0001.

Memory

The results revealed a significant difference between the aversive and neutral stimuli, regarding the memory performance of the bees 24 hours after training ($\chi2= 33.28$, df=1, $P<0.001$). Bees in the aversive group displayed a preference for previously rewarded flowers, selecting them 2.13 times more frequently compared to bees exposed to the neutral stimulus (Fig. 3).

The model did not find any significant effect on the colony or color. This suggests that these factors did not significantly influence the bees' ability to respond accurately to the stimuli, and after the training process, bees did not exhibit a specific preference for any particular color. Therefore, the aversive stimulus leads to the formation of a strong long-term memory in bees to avoid the electric shock.

Discussion

The investigation of animal behavior is of considerable significance across different scientific fields, including ecology, evolution, and neuroscience (Mench, 1998; Smith & Kennedy, 2009). Insects have evolved to occupy diverse ecological niches and, as well-adapted organisms within various ecosystems, possess notable information-processing capabilities that facilitate their effective adaptation to their environment (Smith &Kennedy, 2009). Importantly, insects demonstrate the capacity to learn and adjust their behavior in response to different situations (Ritzmann & Büschges, 2007; Grueter & Leadbeater, 2014). Discovering how insects acquire and apply knowledge from past experiences to overcome potential threats is crucial to understand their decision-making abilities (Guerrieri *et al.*, 2005; Hadar & Menzel, 2010; Devaud *et al.*, 2015).

Recent advances in the study of insect behavior have provided opportunities to explore the underlying mechanisms that drive these adaptive responses. However, avoidance learning in insects under semi-natural conditions remains poorly studied. It is important to note that honey bees and bumblebees play a significant role in pollination and contribute to the improved functioning of ecosystems (Goulson, 2008; Khalifa *et al.*, 2021). Despite their ecological significance and crucial role in advancing our understanding of the cognitive abilities of insects, we have limited knowledge about aversive learning in these species under comparable simulated conditions (Dukas, 2008; Hollis *et al.*, 2017). Previous studies have explored aversive learning in honey bees and bumblebees by subjecting them to various aversive conditions, including pesticides (Tan *et al.*, 2014; Delkash-Roudsari *et al.*, 2020), toxic food resources (Black *et al.*, 2021), predators (Craig, 1994; Jones & Dornhaus, 2011), and changes in oxygen (O2) and carbon dioxide (CO2) levels (Gholami, 2023; Gholipour Faramarzi, 2020).

These investigations have been conducted either entirely within natural farm environments (Black *et al.*, 2021; Dukas & Morse, 2003) or entirely under controlled conditions, often involving restrained insects within cages (Vergoz *et al.*, 2007; Carcaud *et al.*, 2009; Tedjakumala & Giurfa, 2013; Delkash-Roudsari *et al.*, 2020). It is worth highlighting that, while it is necessary to control certain experimental conditions to investigate the mechanisms underlying the observed behaviors, we also need to study the behavior of insects in conditions similar to their natural habitat. Therefore, to investigate aversive learning in these insects, it is crucial to design experiments that replicate their natural habitat in a related ecological context while controlling the experimental conditions. This approach facilitates the exploration of complex interactions and underlying neural circuits, providing ecological validity and unbiased observations (Simons & Tibbetts, 2019; De Bruijn *et al.*, 2021; Thiagarajan & Sachse, 2022).

Researchers can replicate real-life conditions in experiments by combining factors, such as flower structures, simulating insect predators or threats, and incorporating environmental cues. This approach guarantees that observed behaviors, to some extent, remain uninfluenced by completely artificial laboratory elements but instead reflect the responses of insects in their ecologically relevant context. In this study, we used a novel apparatus to investigate aversive learning in insects within an arena that closely resembled their foraging environment. *B. terrestris* workers underwent training and were tested within a flight arena containing artificial flowers equipped with electric shocks. Upon leaving the nest, bees could enter the foraging environment, and by flying and observing the available artificial flowers, they could freely evaluate and choose flowers based on their preferences.

The unique design of the artificial flowers allowed bees to locate them easily and, once landed, they could evaluate the danger only when they fed on the flower nectar (sucrose solution). Bees were successfully trained to recognize visual cues, and their learning process became stable after approximately three training bouts. After undergoing all five bouts of training, particularly in the group exposed to electric shocks as an aversive stimulus, the establishment of long-term memory occurred within 24 hours. In accordance with the findings of other studies which demonstrated the ability of insects to learn and avoid different unpleasant situations (Desneux *et al.*, 2007; Gill *et al.*, 2012; Schneider *et al.,* 2012; Williamson & Wright, 2013; Moret & Schmid-Hempel, 2000; Mayack & Naug, 2010; Iqbal & Mueller, 2007; Schafer *et al.*, 2011), bees in this study could also learn to associate specific colors with danger (aversive learning). Additionally, they were capable of recognizing stimuli that indicated the absence of the electric shock and, conversely, the presence of a reward (appetitive learning).

Previous studies have demonstrated that different insects, including cockroaches (Horridge, 1962), mantids (Zabala *et al.*, 1984), grasshoppers (Foreman, 1984), wasps (Santoro *et al.*, 2015), and social insects such as honeybees (Vergoz *et al.*, 2007; Giurfa *et al.*, 2009; Tedjakumala & Giurfa, 2013), exhibit avoidance learning when confined to restricted conditions and subjected to punishment. However, it should be noted that insects placed under these conditions not only experience aversive stimuli, but also encounter additional stress due to their confinement and the lack of freedom to make independent choices (Van Huis, 2019). Thus, in subsequent stages of experiments, scrutinizing molecular interactions in the brain or other body organs might not be reliable, and the investigation of social insect behavior when interacting with fellow nestmates or other insects in the foraging environment could become challenging. The present conditioning apparatus offers an opportunity for more balanced experiments, allowing us to assess the behavior of free-flying insects in a simulated aversive context.

The results of the experiment showed strong evidence of the existence of a dual aversive-appetitive memory in *B. terrestris* workers. Comparing two groups of bees subjected to a noxious stimulus and a neutral stimulus showed that cues representing life-threatening dangers enhanced rapid learning and resulted in more robust and persistent memory, as opposed to cues associated with lower food quality. The absence of food or lower food quality can be compensated by expending additional energy to locate new resources in the environment (Boyd, 1999). However, warning signals that threaten an individual's survival require immediate avoidance.

As indicated by the findings of this study, after 24 hours, during the memory test, bees that experienced electric shocks exhibited complete avoidance of danger-predicting color cues. Evidence indicates that numerous environmental stressors can affect bees, even if they do not directly result in mortality. For instance, exposure to pesticides or climate change issues, such as elevated carbon dioxide levels and changes in oxygen levels, can influence the mobility, memory, orientation, and foraging efficiency of bees (Desneux *et al.*, 2007; Gill *et al.*, 2012; Schneider *et al.*, 2012; Williamson & Wright, 2013; Delkash-Roudsari *et al.*, 2020; Gholipour Faramarzi, 2020; Gholami, 2023). Parasites can also subject bees to stress, leading to learning disabilities and disruptions in temperature regulation (Moret & Schmid-Hempel, 2000; Mayack & Naug, 2010; Iqbal & Mueller, 2007; Schafer *et al.,* 2011). Although these non-lethal effects may not necessarily result in the death of every individual bee, they can significantly affect the colony dynamics and performance. By investigating how these organisms respond to environmental stressors and potential risks, we can develop more appropriate solutions to help their conservation. Integrating behavioral ecology, cognitive neuroscience, and evolutionary biology facilitates the development of effective strategies for conserving and managing pollinators in different ecosystems.

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یادگیری اجتنابی در *Bombus terrestris* Audax (Hymenoptera: Apidae): پاسخ به شوک الکتریکی در یک محیط شبیه‌سازی شده

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چکیده

مطالعه رفتار حیوانات، به ویژه حشرات، برای درک جنبه‌های بیولوژیکی و تکاملی آن‌ها، کاربردهای گسترده در علوم‌کشاورزی، مدیریت آفات، زیست‌شناسی حفاظتی و علوم اعصاب دارد. بررسی ویژگی‌های شناختی، به‌ویژه یادگیری اجتنابی، نقشی اساسی در درک موفقیت حشرات دارد. این توانایی تطبیقی حیوانات را قادر می‌سازد تا در محیط خود به طور موثر با عوامل استرس‌زا مقابله کنند. در این مطالعه، توانایی یادگیری اجتنابی زنبورهای کارگر *Bombus terrestris* Audax، گرده‌افشان مهم در اکوسیستم‌های مختلف، مورد بررسی قرار گرفت. زنبورها در یک محفظه‌ی پرواز با استفاده از گل‌های مصنوعی مجهز به شوک الکتریکی برای شبیه‌سازی شرایطی که بتوانند تنبیه را در کنار منابع غذایی موجود شناسایی و با نشانه‌ها مرتبط کنند، آموزش داده و آزمایش شدند. نتایج نشان داد که زنبورها توانایی شناسایی همزمان تهدیدات بالقوه همراه با منابع غذایی را دارند، که نشان‌دهنده یک حافظه دوگانه اجتنابی – اشتیاقی است. علاوه بر این، مقایسه گروه‌هایی که با محرک‌های آزاردهنده (شوک الکتریکی) و خنثی (آب مقطر) آموزش داده شدند، نشان داد که نشانه‌های خطر منجر به یادگیری سریع‌تر و شکل‌گیری حافظه قوی‌تر می‌شود. هدف این دستگاه شرطی‌سازی بررسی واکنش زنبورها در مواجهه با خطرات احتمالی، شبیه به موقعیت‌های جستجوگری در زندگی واقعی بود. این یافته‌ها بینشی در مورد استراتژی‌های بقای حشرات در محیط‌های چالش‌برانگیزی که تأثیرات منفی بر جمعیت زنبورها دارد، ارائه می‌دهد.

کلمات کلیدی: رفتارشناسی حشرات، زنبور مخملی، یادگیری اجتنابی، توانایی‌های شناختی، یادگیری شرطی، گرده‌افشانی، گرده‌افشان‌ها

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