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RESEARCH ARTICLE

Aversive memory and extinction learning for noxious stimuli and aversive tastants in bumblebees

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ABSTRACT

Rapid learning of aversive stimuli is adaptive, but the persistence of the avoidance response in the absence of further reinforcement might depend on the severity of the adverse experience. For example, an experience involving injury would be expected to lead to more durable memory than the mere exposure to an unpleasant tastant, especially when new experiences indicate that the aversive stimulus is no longer present. We investigated how bumblebees (*Bombus terrestris*) learn and retain associations between flower colours and two types of aversive stimuli: electric shock and saturated salt (NaCl) solution. Using a conditioning paradigm, we examined how these stimuli influence avoidance learning across foraging bouts and tracked the process of extinction learning, the formation of new memory in response to the absence of the reinforcement, over two weeks. Our results show that bees rapidly learn to avoid both stimuli, and reach >90% accuracy of avoidance after six foraging bouts. We then examined how bees modified their avoidance behaviour in the absence of further aversive stimulation. Testing extinction learning on days 1, 3, 5, 7 and 14, we found that electric shock as a nociceptive stimulus induces a more persistent avoidance response, whereas exposure to the salt by engaging gustatory aversion pathways leads to a three times faster extinction rate. This suggests that although the initial training leads to equal levels of avoidance for both stimuli, bumblebees might display greater behavioural flexibility when updating the association between a colour and an unpleasant taste in comparison to a potentially injurious stimulus.

KEY WORDS: Aversive learning, *Bombus terrestris*, Cognitive flexibility, Decision-making, Gustatory aversion, Nociceptive aversion

INTRODUCTION

Making advantageous decisions in an ever-changing environment is challenging for animals and requires behavioural and cognitive flexibility (Scott, 1962; Wang et al., 2013, 2018; Brown and Tait, 2014; Chittka, 2022). Learning to associate environmental cues with the occurrence of rewards, such as food or a potential mating partner, and punishments, such as the absence of a positive outcome of a behavioural investment or even a negative experience (e.g. with a competitor or predator), can improve future gains (Rescorla and Holland, 1982; Speed, 2000; Ings and Chittka, 2008). However, when environmental conditions change, the memory formed and recalled for a specific behaviour in a previous situation may not be adaptive any longer. Thus, for long-term benefits, animals must be able to update or modify memories in response to new conditions (Chittka et al., 1999; Dunlap et al., 2009; Dunlap and Stephens, 2012).

When the aversive stimulus is no longer present, not having the possibility to make flexible decisions imposes costs. For example, continued avoidance of a previously dangerous but now harmless stimulus may reduce foraging efficiency or mating opportunities, ultimately affecting survival and fitness. In some species, inflexible decision-making because of safety may result in post-traumatic stress disorders (Effting and Kindt, 2007; Diamond and Zoladz, 2016), a reduction in feeding time (Lima and Dill, 1990; Lima, 1998; Brown et al., 1999; Preisser et al., 2005) and a reduced probability of finding a mate, leading to lower fecundity (Zanette et al., 2011; Dudeck et al., 2018).

Within the framework of classical conditioning, repeated encounters with a conditioned stimulus without experiencing the unconditioned stimulus result in extinction learning (Pavlov, 1949; Bouton, 2004; Bouton and Moody, 2004). Extinction of learned avoidance behaviours can promote behavioural flexibility and adaptation to changing environmental conditions (Eisenhardt and Menzel, 2007; Eisenhardt, 2011). However, in the case of highly aversive or life-threatening experiences, such as predatory attacks or any harmful stimuli that threaten an animal's body integrity, such extinction of the information can be detrimental or even fatal and come at an irretrievable cost to the animal (Fraser and Duncan, 1998; Paul et al., 2005; Ings and Chittka, 2008; Gibbons and Sarlak, 2020; Gibbons et al., 2022a). In such instances, strong and persistent memory retention may have evolved as a protective mechanism to prevent future injury or death. The role of pain and nociception in shaping these indelible associations is documented across taxa (Ledoux et al., 1989; Ohman and Mineka, 2001; Fanselow and Gale, 2003; Elwood, 2011; Adamo, 2019). However, whereas many different aversive stimuli, such as predation risk, extreme temperatures, noxious mechanical stimuli or unpleasant tastants, have been used to explore cognition in insects (Chittka and Leadbeater, 2005; Dawson et al., 2016; Gibbons et al., 2022b), less is known about how insects prioritize these threats and whether all aversive stimuli lead to equally persistent avoidance responses. In particular, the effect of different aversive stimuli on acquisition and extinction learning, and behavioural flexibility in insects remains unclear.

In this study, we investigate how free-flying bumblebees (*Bombus terrestris*) learn and retain associations between flower colours and two types of aversive stimuli, including electric shock and saturated sodium

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chloride (NaCl) solution, both known to induce aversive responses in bees (von Frisch, 1934; Bateson et al., 2011) and whether these conditioned responses persist or extinguish over time. Electric shocks directly activate nociceptive pathways (Nunez et al., 1997; Beck et al., 2000; Balderrama et al., 2002; Seymour et al., 2005; Kalisch et al., 2006; Vergoz et al., 2007; Giurfa et al., 2009; Andreatta et al., 2012; Diegelmann et al., 2013; Tedjakumala et al., 2013; Kirkerud et al., 2017; Springer and Nawrot, 2021; Sarlak et al., 2023; Lai et al., 2025), leading to immediate avoidance responses, whereas saline solutions induce aversive gustatory experiences, which may influence decision-making through different sensory and neural pathways (Bhagavan and Smith, 1997; Chandra and Smith, 1998; Cook et al., 2005; Rodrigues et al., 2010; de Brito Sanchez et al., 2015; Bestea et al., 2021). We thus explore the trade-off between adaptive avoidance and behavioural flexibility, demonstrating how different aversive experiences shape decision-making and extinction learning to understand how insects prioritize threats while foraging in a complex habitat.

MATERIALS AND METHODS

Experimental setup

Forty bumblebees (*Bombus terrestris* Linnaeus 1758) from two colonies provided by BIOBEST (Biobest Belgium) were used in this study. The colonies were housed in a bipartite wooden nest box (40 cm×20 cm×20 cm) connected to a wooden flight arena (60 cm×50 cm×50 cm) with a transparent ceiling. The floor of the flight arena was covered with a randomly patterned pink dot background to enhance the contrast between the bees' bodies and the background, ensuring accurate video tracking and providing sufficient optic flow to support their natural flight behaviour. A transparent corridor (25 cm×5 cm×5 cm) with sliding doors connected the nest box and flight arena, allowing controlled individual access. Flower visits by bees were recorded using a camera at 120 frames s^{-1}, positioned above this foraging box (YI Technology, China) (Fig. 1A). The colonies' daily nutritional requirements were provided with sufficient pollen and sucrose solution. All experiments were conducted under standardised conditions (24±2°C; 12 h:12 h light:dark cycle) at the Plant Protection Department, University of Tehran, Karaj, Iran. All experiments were conducted in compliance with relevant ethical regulations.

Artificial flowers

The artificial flowers were made of two metal discs on a 4 cm plastic platform. The outer disc, a hollow circle (diameter=5.5 cm), was connected to the positive pole of the wire, while the inner disc (diameter=1.5 cm) was connected to the negative pole. A non-conductive plastic layer separated these discs to enable the generation of an electric current to deliver a shock once bees touch the artificial flowers. Blue and yellow laminated hollow discs were placed on top of the outer surface of the metal plates as visual cues (Fig. 1A). Solution droplets were carefully positioned directly on the surface at the centre of the inner disc for the bees to access, requiring the bee to contact both metal components of the artificial flower to access the solution. Each artificial flower was connected to an electrical setup to deliver controlled shocks. The apparatus consisted of an electric power transformer (220 V to 24 V, 50 Hz, 3A) AC–DC, DC–DC converter, a voltage reducer regulator module with the output voltage adjusted to 20 V, 1.5 mm diameter wire cables and toggle switches. The device was placed outside of the arena, allowing the electricity for each artificial flower to be manually turned on or off as needed (Fig. 1A).

Pretraining procedure

Before starting the experiment, a pretraining phase was conducted to familiarise the worker bees with the setup. During this phase, all the bees were allowed to freely forage from artificial flowers and identify the landing platforms with rewards (30% sucrose solution). No colour cues or shocks were present during this phase (Fig. 1B). During the experiment, active foragers were marked with number tags on their thorax for individual recognition.

Training procedure

To investigate the effect of a noxious stimulus, bees were individually trained to learn the associations between the electric shocks (20 V) and the colours. Eight artificial flowers (each filled with 10 µl 50% sucrose solution) were placed in the flight arena. Bees were assigned to two independent groups. Group A: four blue flowers (without shock) and four yellow flowers (with shock) were pseudorandomly distributed throughout the flight arena at each bout. Bees received an immediate electric shock upon landing on and tasting the solution from the electrified flowers. Group B: the colours were reversed, with blue flowers delivering shock and yellow flowers presented without shock. To investigate the effect of an aversive tastant, bees were trained in two additional, independent groups. Group C: four blue flowers were rewarded with 10 µl of 50% sucrose solution and four yellow flowers contained 10 µl of saturated NaCl solution. Group D: the colours were reversed, with yellow flowers rewarding and blue flowers containing NaCl solution. Each group consisted of 10 bees. During training, each bee completed 10 foraging trips within a single day, with inter-trial intervals of 10–15 min. A foraging trip (bout) was defined as the period from when a bee entered the arena until she stopped visiting the flowers. A choice was considered valid only when a bee landed on the flower and probed the solution. The number of landings on any flower was recorded. Once a flower was emptied, it was refilled after the bee moved to another flower. Between foraging bouts, all the flowers and the arena floor were cleaned with 70% ethanol and then rearranged to a new random location, to ensure that flower selection was only based on visual cues. To this end, the influence of chemical cues and scent marks was ruled out.

Extinction procedure

Individual bees were given access to the flight arena 24 h after the last training bout to assess memory retention and extinction of aversive responses. During each extinction bout, bees were allowed to forage freely for 6 min in the arena. The flowers were placed in a new pseudorandom arrangement and no aversive stimuli were presented. All flowers were filled with 10 µl distilled water instead of sucrose or NaCl solution. The number of visits to previously rewarded or previously punished flowers was recorded. Flowers were cleaned with 70% ethanol to remove olfactory cues and positioned in re-randomised locations. After completing the first extinction bout, bees returned to the nest for 48 h. This process was repeated every other day for 7 days. Fourteen days after the last training bout, a final extinction bout was conducted to record the bees' choices (Fig. 1B). Between the training phase and memory tests, the bees were maintained within the nest in controlled colony conditions. The colony was fed 30% sucrose solution, a lower concentration than the 50% sucrose used for training. To motivate bees to resume foraging behaviour, we placed a single feeder (a soda bottle cap containing 10 ml of 30% sucrose solution) in the second part of the nest near the corridor door, 1 h before memory (extinction) bouts. This allowed bees to access food and stay motivated to leave the nest and forage again. During the extinction phase, bees did not experience any additional punishment or reward related to the conditioned associations during the acquisition phase.

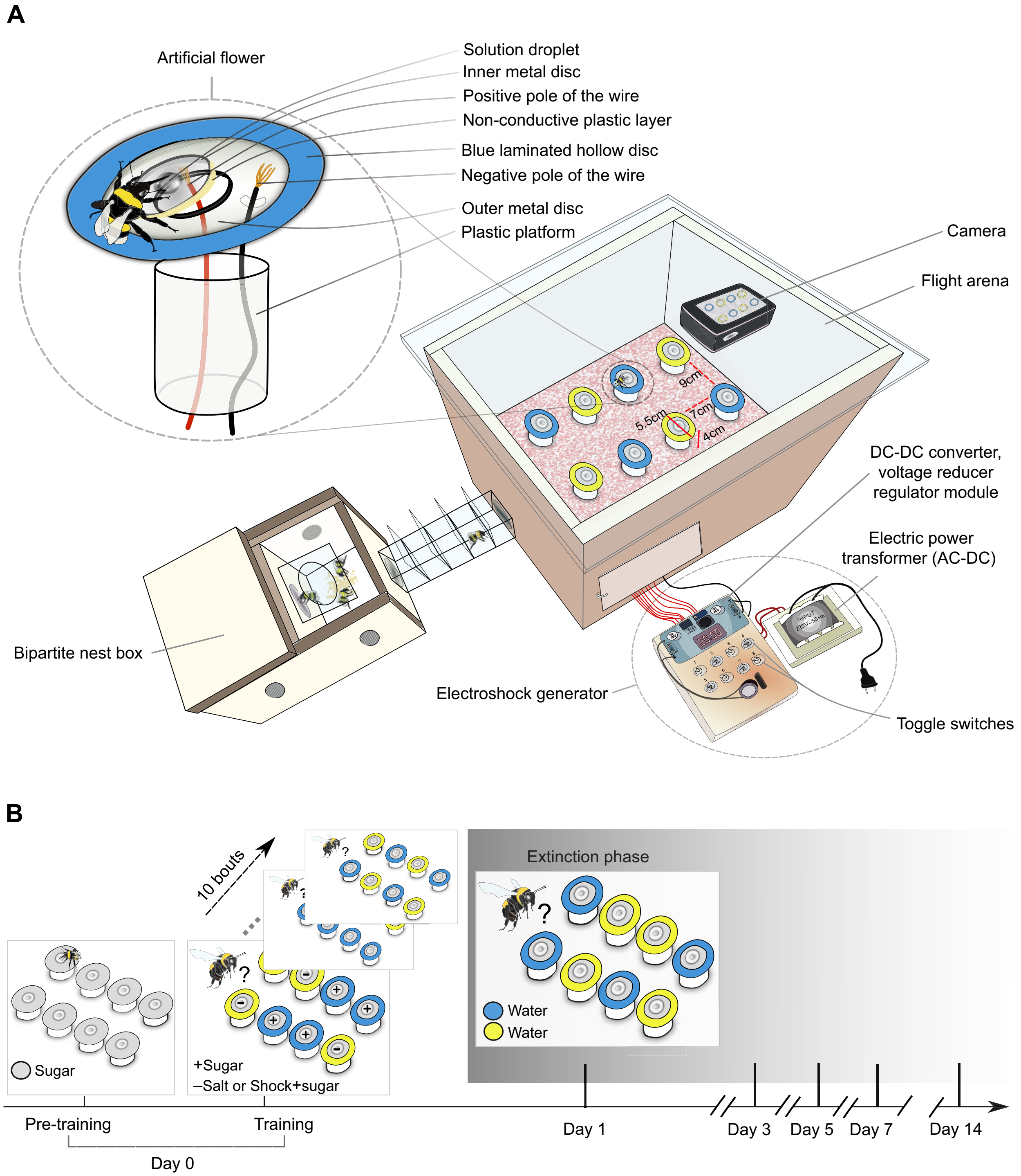

Fig. 1. Schematic view of the experimental setup and timeline, illustrating pretraining, training and extinction phases. (A) The nest box (40 cm×20 cm×20 cm) was connected to the flight arena (60 cm×50 cm×50 cm) via a transparent corridor (25 cm×5 cm×5 cm) with sliding doors. The arena contained artificial flowers connected to an electric shock generator. Each artificial flower consisted of a 4 cm plastic platform and two metal plates: an outer hollow disc (5.5 cm diameter) connected to the positive pole of a wire and an inner disc (1.5 cm diameter) connected to the negative pole (note that the central disk is lifted to show the red wire underneath). A non-conductive plastic layer separated the discs to enable shock delivery. Laminated blue and yellow discs were placed on the outer surface of the flowers, allowing bees to associate colour cues with different stimuli. The electric shock system included a power transformer and a voltage reducer regulator module, with manual switches controlling each flower individually. (B) The experiment began with a pretraining phase on Day 0, during which bees were allowed to freely forage on artificial flowers containing 30% sucrose solution to familiarize them with the setup without any aversive stimuli or colour cues. This was followed by the training phase, where bees completed 10 training bouts to associate flower colours (blue or yellow) with either reward or aversive stimuli. In the shock–colour groups (groups A and B), one flower colour delivered a 20 V electric shock in addition to sucrose, while the other colour provided only sucrose reward. In the NaCl–colour groups (groups C and D), one flower colour contained saturated NaCl solution while the other provided sucrose. Flower configurations were pseudo-randomized, and all surfaces were cleaned with 70% ethanol between bouts to eliminate olfactory cues. The extinction phase began on day 1 and was repeated on days 3, 5, 7, and 14. During this phase, all flowers were filled with distilled water, and no aversive stimuli were presented. N=20 bees (electric shock), and N=20 bees (NaCl).

Statistical analysis

Using a generalized linear mixed-effects model (GLMM) we assessed the acquisition and extinction process in bumblebees that were exposed to 'electric shock' in comparison to 'salt solution'. Individual bees (Bee_ID) were considered as random effects to account for repeated measures within subjects. We used Akaike Information Criterion (AIC) comparisons to select the best balance between model complexity and goodness of fit in describing the data. Models initially included the variables 'Colony' and 'Colour'; however, both variables were excluded from the final models, based on the AIC values.

For the acquisition phase, we assessed differences between stimuli across training bouts using a GLMM with a binary response variable indicating whether a bee visited a rewarded flower (1=visited, 0=not visited) (Response~Stimulus*Bout+(1|Bee_ID)). During the extinction phase, a similar binomial GLMM was used to analyse visits to previously rewarded flowers. Both models were fitted using the glmer function (*lme4* package; https://CRAN.R-project.org/package=lme4) with a logit link and BOBYQA optimizer (https://cran.r-project.org/package=minqa) for convergence control. To compare acquisition rates and extinction rates, linked to the two types of aversive stimuli, we fitted an exponential function with a single parameter to scaled data (scaled avoidance=$1-\exp^{-k*t}$ and $\exp^{-k*t}$, respectively). The response, e.g. the percentage of choices to the punished colour was scaled to 0–1 with 0 corresponding to an unbiased choice of 50% [scaled avoidance=2*(relative avoidance–0.5)].

To compare the number of visits to punished flowers across all bouts, as well as for rewarded visits, a Wilcoxon rank-sum test was used. The same test was applied to compare blue and yellow flower visits at the first acquisition bout, to analyse the number of previously rewarded visits before bees made their first visit to a previously punished flower during extinction, and to compare the probability of visiting previously punished flowers after the first extinction visit.

We checked model assumptions through residual and *QQ* plots. Pairwise comparisons were performed using the *emmeans* package (https://CRAN.R-project.org/package=emmeans) to examine significant effects within each bout when interactions were significant, and statistical significance was defined at $P<0.05$. Plots were generated using *ggplot2* (https://cran.r-project.org/package=ggplot2). All statistical analyses were conducted in R software (version 4.5.1; r-project.org).

RESULTS

Bumblebees learn to avoid different aversive stimuli at a similar speed

At the beginning of the acquisition phase, the very first visit was unbiased, with 20 bees choosing blue and 20 bees choosing yellow flowers, and within the first training bout, colour preference also did not differ significantly (Fig. S1). There was a significant main effect of bout ($\chi^2=139.8550$, $P<0.001$), indicating overall learning across foraging bouts, but the effect of stimulus ($\chi^2=0.3688$, $P=0.5437$) was not significant. During training, the effect of each training bout was highly significant, with increased visits to rewarded flowers and avoidance of flowers containing aversive stimuli (Fig. 2A, Table S1A). The acquisition of avoidance behaviour shows that bees effectively learned to distinguish between rewarding and aversive stimuli. The type of stimulus (electric shock versus salt solution) did not influence the acquisition process significantly (Table S1B), indicating that both aversive stimuli led to comparable and very high avoidance responses (Fig. 2A).

Visits to the rewarded (sucrose-only) flower did not differ significantly between our experimental groups ($Z=0.67679$, $P=0.506$) (Fig. 3A) during the acquisition phase. However, when looking at the total number of visits to punished flowers across all training bouts (treating the acquisition phase as a whole), stimulus type did have a significant effect ($Z=-2.598$, $P=0.0071$). Bees exposed to the salt solution made more visits to the punished flowers during reinforced learning (median=13.5, IQR=9.75–17.5) compared with those exposed to electric shock (median=9, IQR=7.75–11) (Fig. 3B).

Nociceptive aversion is more resistant to extinction learning compared with gustatory aversion

All bees successfully learned the association, with 100% choosing the rewarded flowers at the end of the training phase. Accordingly, the extinction phase began with all bees visiting previously rewarded flowers. During the extinction phase, bees reduced their visits to previously rewarded flowers over time and shifted their

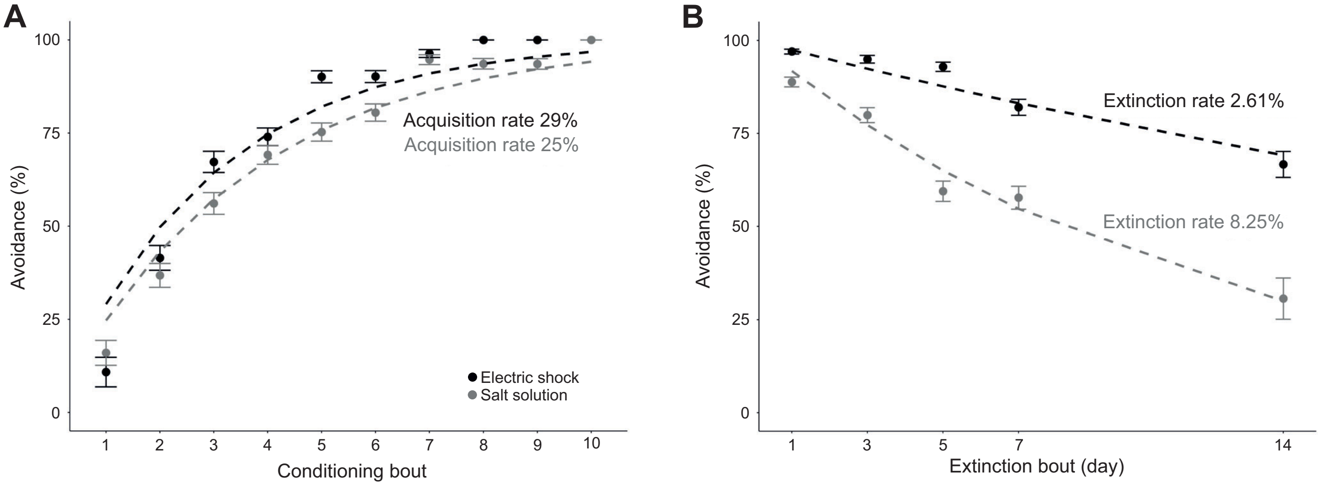

Fig. 2. Scaled avoidance response of bumblebees to flowers paired with electric shock or salt solution. (A) Avoidance across bouts where maximum avoidance is 100% and unbiased colour choice is 0. (B) Extinction of avoidance response across 14 days with extinction bouts on day 1, 3, 5, 7 and 14. Scaled avoidance=2*(relative avoidance –0.5). *N*=20 bees (electric shock) and *N*=20 bees (NaCl). Data are means±s.e.m.

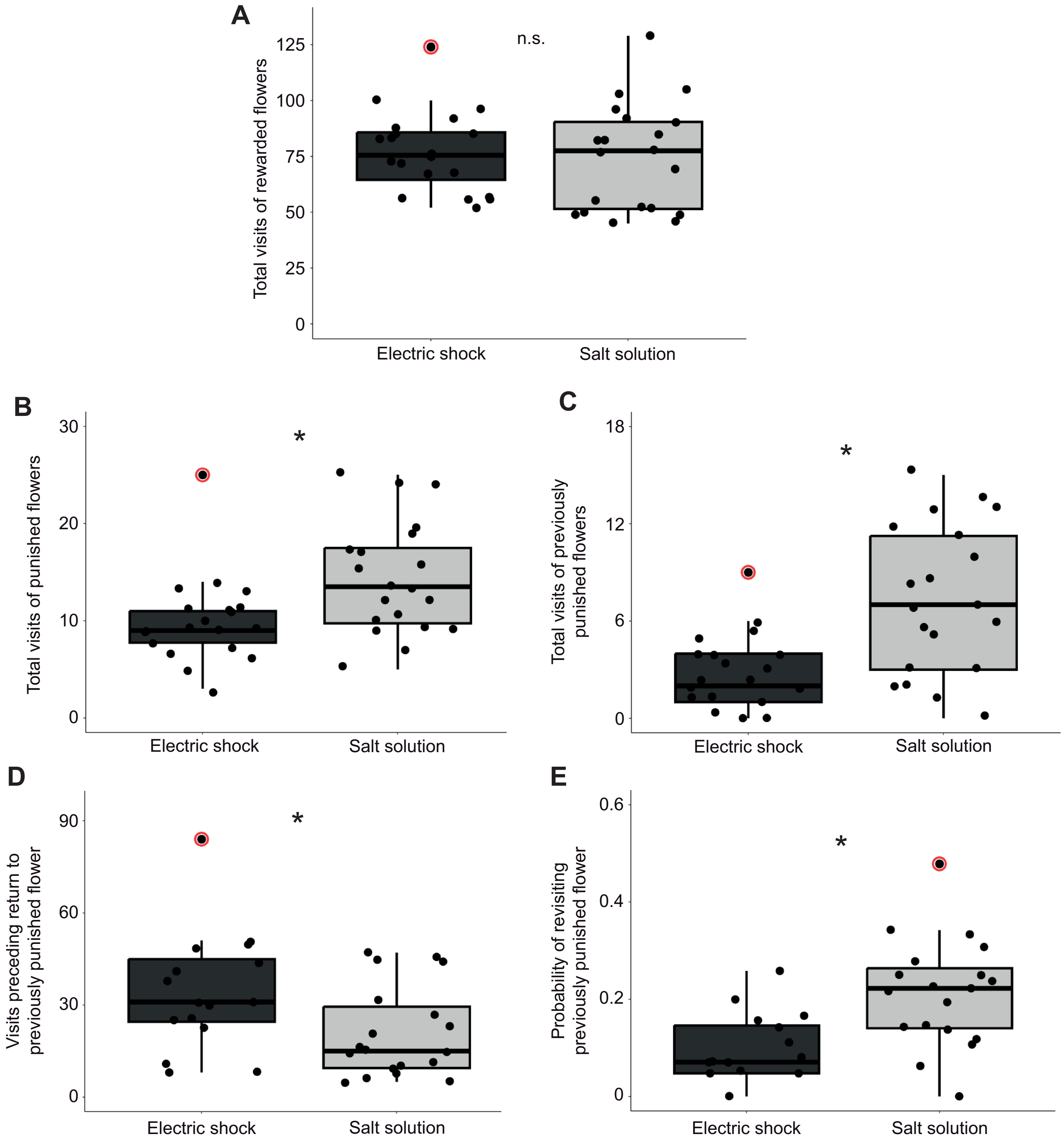

Fig. 3. Response of bumblebees to electric shock versus salt solution during learning and extinction bouts. (A) Total visits to rewarded flowers over all learning bouts (Z=0.67679, P=0.506). (B) Total visits to punished flowers over all learning bouts (Z=−2.5978, P =0.0071). (C) Total visits to previously punished flowers over all extinction bouts (Z=−3.2598, P=0.00007). (D) Delay in visiting the previously punished flower (Z=2.1042, P=0.035). (E) Probability of revisiting a previously punished flower after the first extinction visit, with higher values indicating a greater likelihood of revisitation (Z=−3.1489, P=0.00006). Statistical significance (P<0.05) is indicated by asterisks; n.s., not significant; red circled dots indicate outliers beyond the boxplot whiskers. N=20 bees (electric shock) and N=20 bees (NaCl).

choices toward those that had been punished during the acquisition phase (Fig. 2B). This effect varied by stimulus type, as bees exposed to salt solution during acquisition began changing their preferences earlier, by day 5, whereas those exposed to electric shock showed significant changes starting at day 7 (Table S2). Across all extinction days, bees trained with electric shock made fewer visits to the previously punished flowers (median=2, IQR=1–4) than those exposed to salt solution (median=7, IQR=3–11.2), indicating stronger resistance to extinction in the electric shock group (Z=−3.2598, P=0.0007) (Table S3, Fig. 3C). The time it took until the bees revisited the previously punished flowers depended on the type of punishment. By considering the rank of the first visit to a previously punished flower during extinction, bees that experienced electric shock had a longer delay in switching to previously punished flowers compared with those exposed to salt solution (Z=2.1042, P=0.035, Fig. 3D). Despite the absence of punishment, bees were also significantly less likely to revisit these flowers after their first extinction visit ($Z=-3.1489$, $P=0.0006$; Fig. 3E), with mean probabilities of 0.09 for electric shock and 0.21 for salt solution. This suggests that bees trained with nociceptive stimuli were less likely to return to previously punished flowers even after extinction occurred (here, we considered extinction to have occurred at the individual level starting from the first visit to a previously punished flower). This decline persists even in the absence of extinction trials, e.g. from day 7 to day 14 (Fig. 2B).

The balanced experimental design, the unbiased colour choice at the beginning of the training and the high avoidance response at the last training bouts allowed us to fit an exponential function with a single parameter to the data ($1-\exp^{-k*t}$ for aquisition and $\exp^{-k*t}$ for extinction). The rate of change from bout to bout was comparable during the acquisition phase (25% and 29%; estimate=0.2836, s.e.=0.0163, $t=17.38$, $P<0.001$ and estimate=0.3439, s.e.=0.0329, $t=10.45$, $P<0.001$, respectively, for salt and shock punishment). However, extinction of the avoidance response was about three times faster when salt was used as the reinforcer during acquisition, compared with the noxious stimulus electric shock (Fig. 2B, estimate=0.0861, s.e.=0.0054, $t=16.09$, $P<0.001$ and estimate=0.0264, s.e.=0.0026, $t=10.18$, $P<0.001$, respectively for salt and shock punishment). Acquisition rate and extinction rate of individual bees might be related to each other; however, the analysis of individual behaviour did not reveal any significant relationship (Figs S2 and S3).

DISCUSSION

Decision-making in changing environmental conditions often involves trade-offs, where immediate needs must be balanced against long-term benefits (Stephens, 2008). When cues predict danger, animals should prioritize actions that ensure their immediate safety or consider future needs, such as access to breeding sites or care for offspring (Monkkonen et al., 2009; Himeidan et al., 2013; Hale et al., 2019) and providing food for their colony in social species (Lima and Dill, 1990). However, when the predictions are unreliable in the same context, extinction learning allows organisms to adapt by updating or replacing previous associations with new information (Pavlov, 1949; Bouton and Moody, 2004).

In this study, we hypothesized that the persistence of aversive behaviour and the willingness to gamble on a formerly punished option depends on the type and severity of the initial experience. We examined how bumblebees (*B. terrestris*) associated flower colours with two different aversive stimuli, specifically electric shocks (nociceptive) and saturated salt solution (gustatory aversion). Our experimental design represents ecologically relevant scenarios in which bees encounter complex and sometimes conflicting stimuli, such as a rewarding flower harbouring a predator (nociceptive group) versus a non-rewarding flower (tastant group). While bees rapidly learned to avoid both stimuli at a similar speed, those trained with nociceptive stimuli learned the association with fewer visits to the punished flowers. Results suggest that bees subjected to electric shock showed more persistent avoidance, demonstrating significant resistance to extinction of aversive behaviour compared with those exposed to gustatory aversion induced by salt. In bees, gustatory and mechanosensory systems are capable of detecting differences in fluid properties, including viscosity, through contact with the proboscis and tarsal sensilla. The higher viscosity of sucrose solutions might be perceived as a more rewarding or distinct stimulus compared with salt solutions (Pattrick et al., 2020). This highlights the differential efficacy of sensory modalities in shaping associative learning outcomes. While sucrose and shock were presented together in the nociceptive group, potentially causing devaluation of the sucrose reward, our behavioural data show that visits to rewarded flowers did not differ between groups during acquisition. In fact, preliminary tests prior to the experimental setup presented here showed that a reward in combination with the noxious stimulus is necessary to sufficiently motivate the bees to revisit the flower. Moreover, although the aversive treatments differed in sugar reward, if sugar had an effect on preference, we would expect slower extinction for salt (no sugar) and faster extinction for shock (with sugar). However, extinction was slower and more persistent for shock, indicating that differences in extinction are driven by the nature of the aversive stimulus rather than the reward accompanying it.

In the appetitive domain, studies on honeybees have demonstrated that different unconditioned stimuli such as fructose, sucrose and glucose affect not only acquisition speed but also the persistence of the learned response (Simcock et al., 2018). Moreover, metabolizable but tasteless sugars can support long-term memory, showing the importance of nutritional value beyond taste (Mustard et al., 2018). Therefore, the valence and sensory properties of the unconditioned stimulus modulate learning processes across sensory modalities. Evidence from human studies indicates that, relative to appetitive learning, aversive learning often leads to faster acquisition, reflecting a 'better-safe-than-sorry' approach to threat, with no difference in extinction (van der Schaaf et al., 2022). However, another study found faster extinction of appetitive conditioned responses compared with aversive ones, suggesting evolutionary conservatism since threat signals are harder to forget (Andreatta and Pauli, 2015). Studies on different aversive stimuli are limited and suggest contradictory results. Comparisons between pain- and tone-predicting cues indicate stronger and faster acquisition for pain-predicting cues (Forkmann et al., 2023) although extinction seems less affected by threat value. Interoceptive threats such as internal pain cause stronger, longer-lasting negative expectations and are harder to extinguish (Koenen et al., 2021). Evidence from human and animal studies shows a shared core brain network that processes aversive stimuli, whether painful or non-painful, involving cortical and subcortical regions. However, the degree and timing of activation differ depending on the stimulus nature (Hayes and Northoff, 2012). Our study provides a novel contribution by suggesting that although acquisition speed and learning saturation are nearly identical for two aversive stimuli, extinction reveals distinct differences in memory resilience, which could indicate fundamentally different memory types rather than only variations in memory strength.

The consolidation of the extinction memory depends on the number of conditioning trials and the time interval between acquisition and extinction (Sandoz and Pham-Delegue, 2004; Stollhoff et al., 2005; Stollhoff and Eisenhardt, 2009; Felsenberg et al., 2018; Piqueret et al., 2019; de Bruijn et al., 2021). Studies across various taxa, from humans to insects (McComb et al., 2002; Berman et al., 2003; Lovibond, 2004; Phelps et al., 2004; Sandoz and Pham-Delegue, 2004; Stollhoff et al., 2005; Kalisch et al., 2006; Kindt et al., 2009; Stollhoff and Eisenhardt, 2009; Hepp et al., 2010; Burns et al., 2011; Plath et al., 2012; Alvarez et al., 2014; Eisenhardt, 2014; Gramsch et al., 2014; de Carvalho Myskiw et al., 2015; Haubrich et al., 2015; Felsenberg et al., 2017; Kenney et al., 2017; Gao et al., 2019; Piqueret et al., 2019; de Bruijn et al., 2021), suggest that instead of being erased, existing memory is modified and updated through the formation of an additional memory that suppresses the recall of the original associations (Bouton, 2002; Myers and Davis, 2002; Bouton and Moody, 2004; Felsenberg et al., 2018). In vertebrates, brain regions, including the amygdala, prefrontal cortex, hippocampus, thalamus, periaqueductal gray and hypothalamus, process aversive learning and fear by encoding emotional responses to negative experiences (Gross and Canteras, 2012; Herry and Johansen, 2014;

Ozawa and Johansen, 2018). In various insect species such as bees (Nunez et al., 1997; Balderrama et al., 2002; Avargues-Weber et al., 2010; Tedjakumala et al., 2013; Junca and Sandoz, 2015; Kirkerud et al., 2017; Nouvian and Galizia, 2019; Black et al., 2021), wasps (Dyer and Howard, 2023), flies (Yarali et al., 2008; Schnaitmann et al., 2010; Zhao et al., 2019; Wu et al., 2023), ants (Desmedt et al., 2017; Iakovlev and Reznikova, 2019; Wystrach et al., 2020) and butterflies (Blackiston et al., 2008; Rodrigues et al., 2010; Salloum et al., 2011), the ability to learn and avoid negative stimuli is well documented. This learning is supported by neural mechanisms centred in the mushroom bodies, key integrative brain centres responsible for processing multisensory integration and associative memories (Owald et al., 2015). During the extinction phase, even after experiencing that previously punished flowers were safe, bees that had experienced electric shock made significantly fewer visits to these flowers in subsequent bouts. This response can be an adaptive and protective strategy, as severely risky stimuli, which might be associated with pain and potential injury, should promote long-term caution (Fraser and Duncan, 1998; Elwood, 2011; Nishi et al., 2019; Gatzounis and Meulders, 2020; Gibbons et al., 2022a; Forkmann et al., 2023). Our study revealed that bees learned to avoid flowers with salt solution and began to revisit those flowers more often after discovering that the aversive stimulus had been removed. In insects, the process of extinction involves altered activity in the mushroom body output neurons (MBONs), regulated by specific dopaminergic neurons. Interestingly, these dopaminergic neurons not only suppress aversive memories but also encode the extinction process as a positive experience (Felsenberg et al., 2018).

Differences in extinction could in principle arise from differences in the perceived intensity of the aversive unconditioned stimuli rather than from their sensory nature per se. In insects, aversive reinforcement signals are known to converge onto dopaminergic neurons, and stronger aversive stimuli typically produce stronger conditioned stimulus–unconditioned stimulus (CS–US) associations and more persistent memories (Wright et al., 2010). However, if electric shock simply acted as a stronger unconditioned stimulus, this should have been apparent during learning as faster acquisition. Instead, bees learned both associations at similar speeds and to similar levels, with the clear difference appearing only during extinction, where avoidance of the shock-predicting cue was far more persistent. This pattern is not easily explained by stimulus strength alone and suggests that the two aversive stimuli give rise to memories that could differ in their resistance to extinction.

We observed a gradual weakening of avoidance behaviour over time. However, in the first visits of each new bout, especially on days 5 and 7, bees again started with avoidance, suggesting that the original memory re-emerged after a break. This pattern raises the question of whether bees were learning a new association (that the flowers were now safe) or if the original memory was being erased. Although our experiment cannot clearly separate these two possibilities, the spontaneous recovery seen at the beginning of bouts (day 7 and 14) (Fig. S4; data were insufficient for formal statistical analysis) supports the idea that extinction does not erase the original aversive memory. Instead, it suggests that the memory is still present but temporarily suppressed and can return after a delay.

Aversive stimuli that are not directly related to the animal's survival, such as unpleasant food sources, are associated with greater behavioural flexibility (Devineni and Scaplen, 2021). Although high concentrations of salt are aversive to bees, they are generally attracted to low concentrations (Bestea et al., 2021). This is consistent with findings in *Drosophila*, where salt aversion is modulated by nutritional state (Jaeger et al., 2018). In contrast, nociceptive experiences such as electric shock are probably experienced as more serious threats, resulting in long-lasting avoidance even when the danger is absent. Behavioural flexibility is critical in environments with variable resource availability. When access to resources is limited, animals must balance the costs and benefits of what they learned. Foraging bees are known to adjust their strategies to prioritize efficient food sources (Greggers and Mauelshagen, 1997) and they often avoid flowers associated with predation risk, even when equally rewarding (Dukas, 2001; Dukas and Morse, 2003; Heiling and Herberstein, 2004; Abbott, 2006; Ings and Chittka, 2008; Jones and Dornhaus, 2011). This is especially concerning in the context of climate change and habitat loss, where behavioural flexibility is crucial for pollinators such as bees to enhance their optimal foraging and population stability.

Despite similar learning speed and final performance for two aversive stimuli, the extinction process was significantly different. These results suggest that although fast learning to avoid danger is critical for survival, how long the memory lasts might depend on how strongly the experience affects the animal. Our findings suggest that exposure to an unfavourable food source still allows for behavioural flexibility and enables bees to adapt to changing conditions. In comparison, a nociceptive stimulus might reinforce avoidance behaviour such that behavioural flexibility is impaired. While this heightened avoidance may reduce foraging efficiency, it probably serves as a survival mechanism in high-risk environments.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.S., H.M., C.K., L.C.; Data curation: S.S.; Formal analysis: S.S., H.M., C.K.; Funding acquisition: S.S., A.A.; Investigation: S.S.; Methodology: S.S., H.M., L.C.; Project administration: S.S., L.C.; Resources: A.A., S.H.G.; Software: S.S., C.K.; Supervision: A.A., S.H.G., C.K., L.C.; Validation: S.S.; Visualization: S.S., H.M., C.K.; Writing – original draft: S.S., H.M., C.K., L.C.; Writing – review & editing: S.S., A.A., S.H.G., H.M., C.K., L.C.

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Data and resource availability

All relevant data and details of resources can be found within the article and its supplementary information. The datasets generated and analysed during this study are available in Figshare at https://doi.org/10.6084/m9.figshare.31129489.

ECR Spotlight

This article has an associated ECR Spotlight interview with Sajedeh Sarlak.

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