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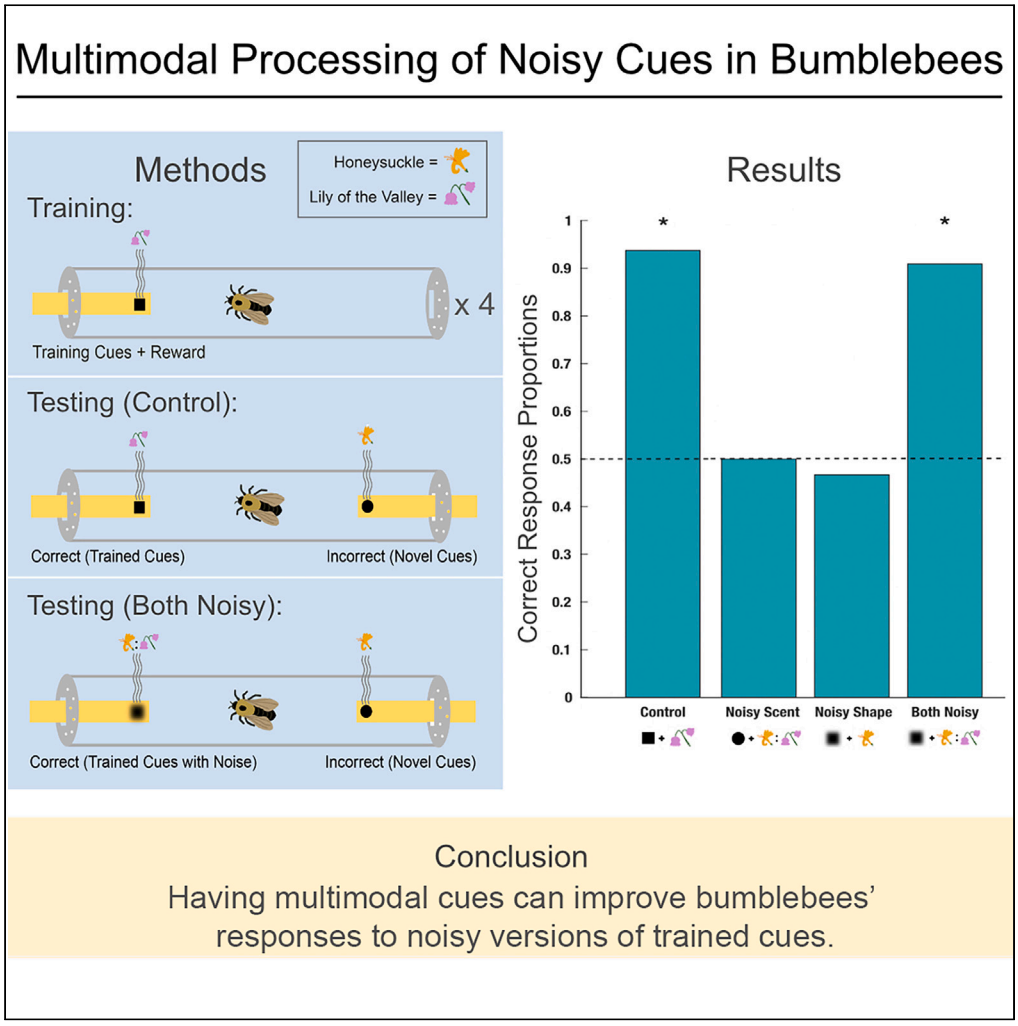

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Highlights

Bumblebees don't respond correctly to learned visual or olfactory cues with noise

Bumblebees can respond correctly to multimodal cues when both cues are noisy

Jordan et al., iScience 27, 108587
January 19, 2024 © 2023 The Authors.
https://doi.org/10.1016/j.isci.2023.108587

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Multimodal processing of noisy cues in bumblebees

Katherine A. Jordan,[1,*] Jordanna D.H. Sprayberry,[2] Wilsaan M. Joiner,[1] and Stacey A. Combes[1,3,*]

SUMMARY

Multimodal cues can improve behavioral responses by enhancing the detection and localization of sensory cues and reducing response times. Across species, studies have shown that multisensory integration of visual and olfactory cues can improve response accuracy. However, in real-world settings, sensory cues are often noisy; visual and olfactory cues can be deteriorated, masked, or mixed, making the target cue less clear to the receiver. In this study, we use an associative learning paradigm (Free Moving Proboscis Extension Reflex, FMPER) to show that having multimodal cues may improve the accuracy of bees' responses to noisy cues. Adding a noisy visual cue improves the accuracy of response to a noisy olfactory cue, despite neither the clear nor noisy visual cue being sufficient when paired with a novel olfactory cue. This may provide insight into the neural mechanisms underlying multimodal processing and the effects of environmental change on pollination services.

INTRODUCTION

Multimodal processing is an important aspect of brain function underlying many behaviors. Multisensory integration has been shown to improve behavioral responses including detection of sensory cues, localization of sensory cues, and response time.[1] Many studies investigating multimodal processing use clear, unambiguous sensory cues, varying only the intensity of the cue. However, in real-world settings, sensory cues are often noisy. Visual, auditory, and olfactory cues can be deteriorated, masked, or mixed with other cues in ways that make the target cue less clear to the receiver.

In humans, studies have shown that the level of uncertainty of sensory cues influences how cues from multiple modalities are processed.[2] The presence of a clear cue in one modality can help receivers interpret noisy or ambiguous cues in another modality. For example, a clear visual cue improves the auditory processing of speech in noisy auditory conditions.[3] Similarly, as visual uncertainty increases, auditory cues have more influence over visual perception.[4] Far fewer studies on multimodal processing in humans have included olfactory cues, but, Kuang[5] recently found that olfactory cues can influence visual perception when the visual cue is ambiguous.

Despite widespread interest in multimodal processing and perception of noisy cues in both human and animal studies, nearly all studies to date have involved combining a noisy cue in one modality with a clear cue in a different modality. Thus, the question of how multimodal processing affects response accuracy when both cues are noisy, as is likely the case in many natural settings, remains largely unresolved. Using bumblebees to address this fundamental question provides several advantages. Bees are highly motivated foragers, learn new cues quickly, and rely on both olfaction and vision. Additionally, both sensory modalities have been studied extensively in bees, both separately and in multimodal contexts.[6]

As in human studies, multisensory integration of visual and olfactory cues in bees appears to improve response accuracy when stimulus intensity is low.[7] Several studies have also shown that bees are better at discriminating between flowers that differ in both their olfactory and visual cues, as reviewed by Leonard and Masek.[6] The majority of investigations into visual learning in bees focus on color as the visual cue.[6] However, bees also use the shape of flowers as a visual cue when foraging and decision-making is improved when flowers differ in both shape and scent.[8]

In addition to bumblebees being a useful model system for investigating multimodal learning, the question of how noisy sensory cues impact bees' foraging behavior has ecological importance. Insect pollinators such as bees play a key role in our environment, pollinating roughly 35% of the global food supply and numerous native plants. Thus, disruptions to their foraging behavior could have significant environmental and economic consequences.[9] Several recent studies show that air pollutants react with floral odorants to degrade or alter their scent.[9] In honeybees, the addition of diesel exhaust causes 2 of 8 floral odorants emitted by oilseed rape to become undetectable and reduces honeybees' ability to recognize the blended odor as oilseed rape.[10] Modeling studies of foraging behavior suggest that the addition of air pollutants to floral odorants lowers the foraging success rate of honeybees,[9] potentially affecting both pollination services and colony health and growth. Bumblebee foraging is also known to be affected by scent pollution. Fungicides that are widely used to treat crops have been shown to affect bumblebee learning and recognition of floral scents,[11] and agrochemicals including fungicides and fertilizers affect bumblebees' foraging preferences and their ability to locate flowers.[12]

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https://doi.org/10.1016/j.isci.2023.108587

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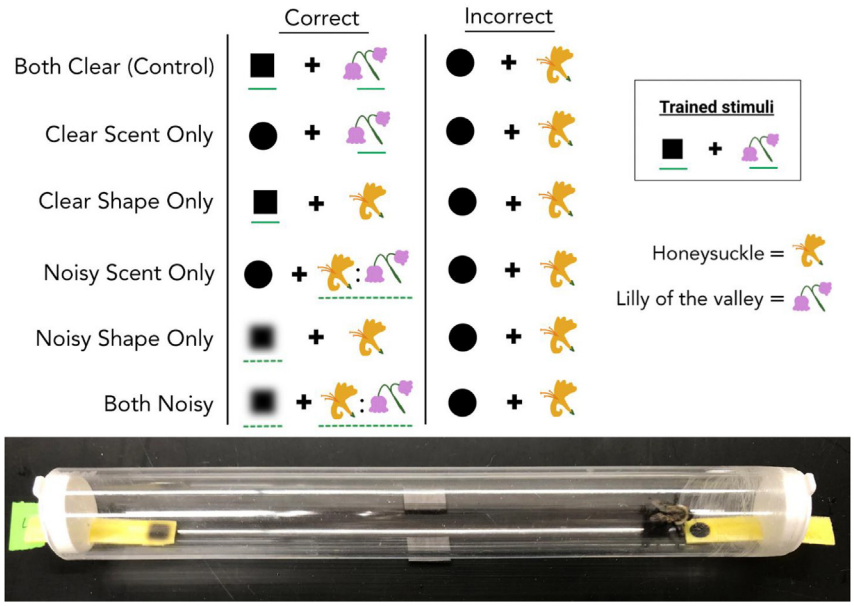

Figure 1. Experimental paradigm

Freely moving bees were trained to associate a reward of sucrose solution with a multimodal cue (a square paired with Lily of the Valley scent, on a yellow strip). They were then presented with a choice between a strip containing two novel cues (the "incorrect" choice) vs. a strip containing one or both of the trained cues, with noise added to the cues in some tests (the "correct" choice). Solid green lines indicate trained cues; dotted green lines indicate trained cues with noise, no line indicates a novel cue.

In addition to noisy olfactory cues, bees are likely to encounter noisy or ambiguous visual cues. From a color perspective, floral pigments are influenced by genetic and environmental factors, making them variable and therefore potentially ambiguous.[13] Far fewer studies have explored shape as a visual cue. The distinctive flower shapes that bees use as visual cues can become distorted by insect predation and other physical damage, adding varying levels of "noise" to the edge features that bees use to distinguish between shapes. One study showed that flowers damaged by beetles (such as those with chew marks on their petals) receive fewer visits from bumblebees than undamaged flowers,[14] though this study did not determine whether impaired visual recognition or other factors were responsible for the shift in bumblebee visitation.

Both visual and olfactory noise often occur in the environment, which can impact foraging behavior in bees. To understand the extent to which multimodal processing can be beneficial even if both sensory cues are noisy, we first trained bumblebees on clear multimodal cues. We then added noise to one or both cues and tested bees' accuracy using an adapted version of the Free Moving Proboscis Extension Response method originally developed by Muth et al.[15]

RESULTS

Experimental paradigm

Bees were placed in a transparent tube in which they could move freely, and were trained to associate a paired visual cue (a black square) and olfactory cue (Lily of the Valley extract, LoV) with a 50% sucrose reward ("trained stimuli" shown in box in Figure 1). After training, each bee was presented with two unrewarding (sucrose-free) test strips to choose between, according to one of the six testing conditions shown in Figure 1.

In each test, the "incorrect" strip contained two novel cues (circle and honeysuckle scent), while the "correct" strip contained one or both of the trained cues, with the addition of noise in some tests. In cases where only one of the trained cues was present on the "correct" test strip, a novel cue (circle or honeysuckle) was included in the other sensory modality, so that all training and testing cues were multimodal. We chose squares and circles for our visual cues because these shapes are less likely to be encountered in natural settings, so the likelihood of bees displaying an innate preference for one or the other is reduced, and previous studies have shown that bees are capable of learning to discriminate between squares and circles.[16] The visual difference between the circle and the square is approximately 13° which is larger than the bees' visual discrimination threshold of about 5°.[17] In addition, the key feature distinguishing the two (the corners of the square) could be blurred to create a more ambiguous visual cue. To add noise to the trained visual cue of a square, the edges of a 63x63 pixel square were blurred in Photoshop using a Gaussian blur with a radius of 5 pixels. The blur kernel had a size of 5 pixels, which equates to a 5° blur at a distance of 1 cm. However, the bee in Figure 1 is less than 0.5 cm from the shape, which would equate to a 10° angle. Thus, as bees get within 'proboscis' distance before making their choice, it is highly likely that they will be able to perceive a difference between the contrast gradient of the blurred 'noisy' edge and the high contrast edge of the clear shape. To add noise to the trained olfactory cue of Lily of the Valley scent,

LoV extract was blended 1:1 with honeysuckle extract, resulting in a chemical angle difference of 28.8° from pure LoV, making this a "noisy" scent that should lie just within bumblebees' generalization range for LoV.[18] The novel cues were a circle for shape and honeysuckle for scent.

The test strips were introduced simultaneously on opposite sides of the tube, with the side of the correct strip randomized. Following the Free Moving Proboscis Extension Response method,[15] if the bee extended its proboscis while touching one of the strips (indicating that it associated that strip with the sucrose reward), that strip would be marked as the bee's choice. If, however, the bee investigated each strip 3 times and at least 15 min had passed without it making a choice, the bee was marked as exhibiting "no choice" during the test.

Data processing and analysis

A total of 232 bees completed training, but only 83 of those made a definitive choice during testing by extending their proboscis on one of the strips. Although a sizable portion of "no choice" results have been found in previous studies (e.g., ~30% for high-contrast scent discrimination and up to ~60% for more difficult olfactory choices).[18] This was an unexpectedly high number of "no choice" results; even in the control condition, where the two trained cues were presented during the test, approximately 50% of bees failed to make a choice. The proportion of bees that did not make a choice varied between tests (see Figure S1 and Table S1). We compared the proportion of no choice responses to that of the control using a one-sided binomial test (using the control proportion as our expected value) and found that all were significantly different from the control ($p < 0.05$) except for pure scent ($p = 0.359$) and noisy scent ($p = 0.065$).

For the bees that did make a choice during testing, we calculated the proportion of correct responses for each testing condition (number of correct choices divided by the number of correct + incorrect choices). If the bees do not associate the cues presented during testing with the reward, the expected probability of success (probability of the bee choosing correctly) is 50%, the proportion expected for a random choice. To determine whether bees were able to recognize and associate the cues with a reward, we tested whether the proportion of correct responses was significantly greater than that expected by chance (50%), using a one-sided binomial test. This test compares the observed proportion of successes to the expected proportion of successes (50%), to determine whether the observed proportion of successes is significantly different from and higher than 50%.

The tests that were significantly different from chance were then compared to the control condition (and to each other) using the Fisher's exact test, which is used to evaluate categorical variables when sample sizes are small. This test analyzes a contingency table to determine if there are non-random associations between categories (in this case test type and response type). We found no non-random associations, meaning that none of the three tests in which bees performed significantly better than chance were significantly different from each other.

Response accuracy across conditions

For the control test (clear shape and scent), 93.75% of the bees that made a choice chose correctly, meaning that they extended their proboscis while in contact with the strip that contained the cues that they had been trained to associate with a reward (Binomial test, $p = 0.0003$; Figure 2), indicating that the training protocol was successful and that bees learned the association between the multimodal, clear cues and the reward.

The goal of the "clear shape only" and "clear scent only" tests was to determine how bees respond to only one of the trained cues when paired with a novel cue in the other modality. Of the bees that made a choice in the clear scent only (in which LoV, the trained scent, was paired with a novel shape contrasted against novel shape and scent), 91.675% chose the trained scent, a proportion that was higher than chance (binomial test, $p = 0.003$); the correct-choice frequency in this test was also not significantly different from the control condition (Fisher's exact test, adj. $p = 1$; Figure 2). In contrast, bees responding to the "clear shape only" tests (clear square presented with a novel scent) did not perform as well. Of the bees that made a choice, only 53.33% chose correctly, making the proportion of correct responses not significantly greater than chance (binomial test, $p = 0.5$; Figure 2).

The goal of the "noisy shape only" and "noisy scent only" tests was to determine how bees respond to only one of the trained cues when this cue is noisy (and paired with a novel cue in the other modality), to determine whether presenting "both noisy" cues together improves performance. While bees were able to choose correctly in the "clear scent only" condition, when presented with the "noisy scent only" (LoV:honeysuckle mixture, paired with a novel shape), the proportion of bees responding correctly declined to 50%, which was not significantly greater than chance (binomial test, $p = 0.605$; Figure 2). Likewise, bees did not choose the noisy shape at a frequency higher than chance (46.67% of choosing-bees, binomial test, $p = 0.696$; Figure 2).

However, among bees that made a choice in the "both noisy" test, 90.91% chose correctly, which was a significantly greater proportion than expected by chance (binomial test, $p = 0.00586$; Figure 2). The proportion of correct responses to the "both noisy" test was not significantly different from either the control condition (Fisher's exact test, adj. $p = 1$) or the "clear scent only" condition (adj. $p = 1$).

DISCUSSION

The relatively high proportion of bees that did not make a choice (>60%, averaged across treatments) despite completing the four training sessions was unexpected. Many of these bees repeatedly attempted to escape the tubes and appeared less interested in the strips. It is possible that after receiving up to 12 μL of sucrose solution during training these bees were less food motivated by the time of the testing. Another possible explanation is that after inspection of the strips some bees could tell that no sucrose solution was present. The control and clear scent conditions had the lowest proportion of no choice responses (see Figure S1) and both of the conditions also had high proportions

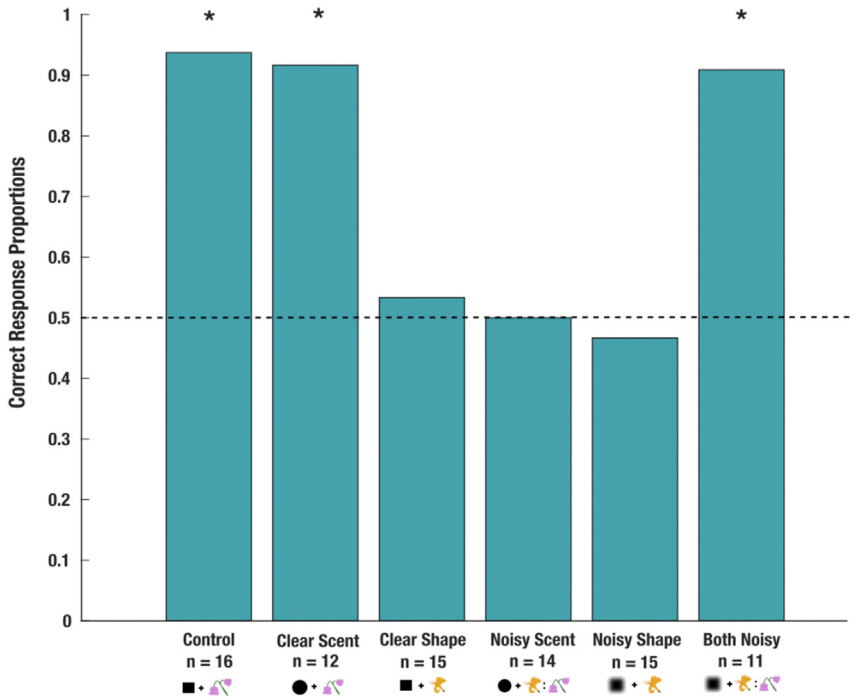

Figure 2. Response accuracy across conditions

Proportions of correct responses for each testing condition, with the total number of bees responding (excluding "no choice" trials) shown below. After being trained with a multimodal cue, the proportion of bees responding correctly to the control treatment (clear versions of both trained cues), a clear scent paired with a novel shape, and noisy versions of both trained cues was significantly higher than chance (50% accuracy, shown with a black dashed line). Treatments that had a correct response proportion significantly greater than chance are marked with a black star (binomial test, $p < 0.01$).

of correct choice responses. Interestingly, however, the noisy both condition had a high proportion of no choice responses despite also having a high proportion of correct choices. This could possibly indicate bees were more likely to make a choice when the cues were clearer. Future research is needed to explore whether or not no choice responses could be an indication of level of uncertainty or task difficulty.

As expected, nearly all of the bees chose correctly in the control test condition in which the cues on the correct strip were identical to the training strip. This indicates that the training protocol was successful and that bees learned the association between the multimodal, clear cues and the reward.

When presented with the clear version of the olfactory cue ("clear scent only") paired with a novel shape, nearly all bees responded correctly. However, when presented with the "noisy scent only" (LoV:honeysuckle mixture, paired with a novel shape), the proportion of bees responding correctly declined to 50% (Figure 2). This indicates that, despite recognizing the trained cue when presented with the clear scent plus a novel visual cue, bees did not associate the noisy olfactory cue plus a novel visual cue with the reward. Thus, a certain amount of information overlap between the trained cue and the test cue appears to be necessary for bees to make this association, and a noisy scent cue alone appears to be insufficient.

These results are in line with previous research showing that bees have difficulty discriminating "noisy" olfactory cues, particularly those contaminated by anthropogenic chemicals. Several studies have shown that air pollutants react with floral odorants to degrade or alter their scent.[9,10] In addition, fungicides that are widely used to treat crops have been shown to affect bumblebee learning and recognition of floral scents.[11] Finally, agrochemicals including fungicides and fertilizers affect bumblebees' foraging preferences and their ability to locate flowers.[12] Thus, while our results show that bumblebees will respond correctly when presented with only the clear, olfactory component of a learned, multimodal cue, these results along with previous studies suggest that "noisy" scents, particularly those contaminated by air pollutants or agrochemicals, may severely impair bees' ability to locate flowers that they have previously learned to associate with a reward.

Although the "clear scent only" tests showed that bees can respond correctly to just one component of a learned, multimodal cue, we found that bees responding to the "clear shape only" tests (clear square presented with a novel scent) did not perform as well. Of the bees that responded to the "clear shape only" test, only about half chose correctly (Figure 2); a frequency that was similar to "noisy shape only" tests. Unlike the "scent only" tests, where noise significantly affected the proportion of bees responding correctly, bees failed to perform better than chance in either the clear shape or noisy shape tests.

This could suggest that shape alone does not provide sufficient information for bees to recognize the cue as being associated with a reward in the absence of the trained olfactory component; or alternatively, that the trained shape presented with a dissonant (i.e., anomalous) olfactory cue inhibits association with the reward. Indeed, previous research on honeybees indicates that odor may be a dominant sensory

cue.[19,20] Honeybees form stronger associations between rewards and olfactory cues, and they form these associations more quickly than with visual cues.[20] The dominance of olfactory cues over visual cues in social bees could have an evolutionary basis. Bumblebees can learn scents brought back by successful foragers[21] and will preferentially forage on flowers with the same scent as that brought back to the hive by other foragers.[22] Visual cues cannot be shared with other potential foragers in this way, so relying strongly on olfactory cues could be more advantageous to the colony. Additionally, modeling has shown that odor is the sensory cue most frequently encountered by foraging bumblebees; finding that odor cues are present in 90% of successful floral encounters, while visual cues are present in 49%.[23] While odor cues are often paired with visual cues when bees are searching for larger flowers, bees must rely mostly on odor cues when searching for small flowers. This difference could potentially explain why the scent cues appeared to be more dominant than the visual cues.

Our results concerning bees' ability to associate the clear scent but not the clear shape with a reward may suggest that the impact (and possibly the mechanism) of multimodal learning may be different for olfactory vs. shape cues. Visual (shape) cues that were learned as part of a multimodal cue were severely affected by being paired with a novel odor, whereas odor cues that were learned as part of a multimodal cue were easily recognized by bees when paired with a novel shape, as long as the odor was not contaminated. Although these results might suggest that bees are only paying attention to the odor cue, the results from our final test condition ("both noisy") show that bees do in fact learn the shape component of the cue.

Results from the "both noisy" condition, in which bees were presented with the noisy versions of trained visual and olfactory cues, provide support for our hypothesis that multimodal processing improves decision-making when both cues are noisy. Among bees that responded to the "both noisy" test, approximately 90% chose correctly (Figure 2), a proportion that is significantly better than chance ($p = 0.00586$), unlike bees presented with the "noisy shape" ($p = 0.696$) or the "noisy scent" ($p = 0.605$). This indicates that multimodal processing provides an important advantage when cues in more than one modality are noisy. The most striking aspect of this result is that adding the noisy, trained shape cue to the noisy, trained scent cue (neither of which is recognized on its own) allowed bees to associate the reward with the clear, multimodal cues they had been trained with, despite the fact that the clear, trained shape paired with a novel olfactory cue was not associated with the reward. This reinforces the idea that shape alone may not be a sufficient cue for foraging bees, but shape can play a crucial role as part of a multimodal cue, especially when both cues are noisy.

These results enhance our understanding of the conditions in which multimodal processing can be advantageous, showing that this process may be particularly critical in environments where sensory cues are noisy. Most natural sensory cues are inherently variable (i.e., noisy), and many anthropogenic changes in the environment increase stimulus noise, which may reduce bees' learning and foraging performance. Future research could explore how color, texture, and other visual cues interact with shape and scent as bees learn multimodal cues, document the levels of noise at which bees become unable to identify learned cues, and determine how this "noise tolerance" varies across different sensory modalities, to provide a complete picture of the effects of noisy sensory cues on bumblebee learning and foraging.

Limitations of the study

One limitation of our study concerns our finding that bees did not associate the clear shape cue (combined with a novel olfactory cue) with the reward. With our study design, we cannot determine whether the explanation for this is that shape alone does not provide sufficient information for bees to form this association or whether the trained shape presented with a dissonant (i.e., anomalous) olfactory cue inhibits association with the reward. Additionally, future work might want to investigate the roles of noisy visual and olfactory cues in a free foraging assay. While the FMPER data presented here showed significant results, the high number of no-choice responses and low number of choosing bees results in a low-resolution assay. Thus, more subtle differences in behavior are not captured, but provide fertile ground for future investigation.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108587.

ACKNOWLEDGMENTS

This work was supported by the University of California, Davis Dean's Distinguished Graduate Fellowship.

We would like to acknowledge Nick Burnett and the members of the Combes Lab for their feedback on the manuscript.

AUTHOR CONTRIBUTIONS

Conceptualization, K.A.J., S.A.C., and W.M.J.; Methodology, K.A.J., S.A.C., and J.D.H.S.; Investigation, K.A.J.; Writing – Original Draft, K.A.J.; Writing – Review and Editing, K.A.J., S.A.C., W.M.J., and J.D.H.S.; Formal Analysis, K.A.J., S.A.C., W.M.J., and J.D.H.S.; Resources, S.A.C. and J.D.H.S; Supervision, W.M.J., and S.A.C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: July 3, 2023
Revised: October 20, 2023
Accepted: November 24, 2023
Published: November 25, 2023

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Bombus impatiens	Koppert	https://www.koppertus.com/natupol-excel/
Software and algorithms		
R version 4.2.2	The R Foundation for Statistical Computing	https://www.r-project.org/
Other		
Lily of the Valley	Piping rock fragrance oil	https://pipingrock.com/products/lily-of-the-valley-premium-fragrance-oil-1-fl-oz-30-ml-dropper-bottle
Honeysuckle	Piping rock fragrance oil	https://pipingrock.com/products/honeysuckle-premium-fragrance-oil-1-fl-oz-30ml-dropper-bottle

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Stacey Combes (sacombes@ucdavis.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This experiment was conducted on three hives of bumblebees (*Bombus impatiens*) which were purchased from Koppert. The bees were kept in their original hive box with a few minor alterations. A small hole was cut into the top of the box and covered with dark red, semi-transparent plastic so that bees and pollen could be placed into the hive through the opening but only minimal light would enter the hive. A rubber tube was also placed at the built-in opening to the box which allowed bees to crawl through to access a mesh foraging chamber where they could fly and forage. In the mesh foraging chamber was a container of 50% sucrose solution with a wick which was refilled regularly, and served as the bees' only source of sucrose (none was provided directly in the hive). Three times per week pollen mixed with sucrose solution was dropped through the opening created at the top of the hive box directly into the hive. Only female bees that were actively foraging (in the mesh foraging chamber) were used in experiments.

METHOD DETAILS

Our methods are adapted from the Free-Moving Proboscis Extension Response (FMPER) method originally conceived by Muth et al.[15] and modified by Sprayberry[18] for olfactory cues. Actively foraging, unmarked bumblebees (*Bombus impatiens*) were collected from a foraging cage with a bee vacuum and placed into ventilated tubes for $\geq$ 2 hours to acclimate and become hungry. Just prior to training, the bees were fed $\leq$ 3 μL of 50% sucrose solution to encourage foraging behavior. Bees were then placed in a clear plastic tube (~20 cm long, 2.5 cm diameter) with ventilation holes and a small slit cut out on either end. Visual and olfactory cues were introduced on plastic yellow strips (1 x 6 cm) that were inserted through the slits on the ends of the tube.

For visual cues, a shape was printed on a clear transparency sheet using black ink, trimmed, and glued (ink side down) to the top of each yellow testing strip. Both shapes were approximately 5.6 mm wide and bees should not have had any difficulty distinguishing between them. Assuming bees viewed shapes from an average distance of 1 cm (or less), the angular size of the shape subtended on the bees' eyes would be greater than 30 deg (31.3 deg), well above their visual discrimination threshold of ~5 deg.[17] For olfactory cues, a square of medical gauze tape was fixed to the bottom of each strip and 1μL of essential oil was pipetted onto the gauze prior to training; because sucrose rewards were

placed on the top of the strip the bees never came in direct contact with the oils via gustation. The essential oils were added to the strips just prior to each training/testing session and the strips were discarded at the end of each session or if soiled by a bee.

Approximately 5 bees were tested per session on average. During training, a reward of 3 μL of 50% sucrose solution was pipetted onto the top of the plastic strip in the middle of the shape. Once the bee appeared calm, the strip was introduced through the slit on one side. Bees were trained to associate the shape of a square and Lily of the Valley scent with the sucrose reward 4 times, with each training initiated 5 minutes after the bee completed the previous training (meaning they drank from the sucrose solution on the strip). During each training, the side of the tube through which the strip was introduced was randomized (unless the previous three trainings were all on the same side, in which case the final training occurred on the opposite side). Only bees that successfully completed all four trainings progressed to testing. All bees that began training (whether or not they completed training) were marked with a paint pen before being returned to the hive so that they would not be reused in the experiment.

Five minutes after a bee completed its fourth training, it was tested according to one of the six testing conditions shown in Figure 1. In each test, one strip (the incorrect choice) contained two novel cues (circle and honeysuckle scent), while the other strip contained one or both of the trained cues, which were either clear (as in the training) or noisy (see Figure 1). Once the bee was approximately in the middle of the tube and appeared calm, the plastic strips were introduced simultaneously, with the side of the "correct" strip randomized. If the bee extended its proboscis while touching one of the strips (indicating it associated that strip with the sugar water reward), that strip would be marked as the bee's choice. If, however, the bee investigated each strip 3 times and at least 15 min had passed without it making a choice, the bee was marked as having made "no choice" (as in Sprayberry, 2020)[18] and excluded from further analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was carried out in R[24]. A one-sided binomial test was used to determine whether the proportion of correct responses for each condition was greater than chance (0.5) using the binom.test function.[24] The three test conditions that produced a proportion of correct responses significantly greater than chance (control, clear scent only, and both noisy) were compared to determine whether the proportion of correct responses differed significantly between these conditions using a pairwise fisher test (with the pairwise_fisher_test function[25]), corrected for multiple comparisons using the Benjamini–Hochberg method.[25]