Behavioral state influences encoding mechanisms of color and odor integration in a model insect, the bumblebee Bombus impatiens.

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Abstract

Bumblebees rely on diverse sensory information to locate flowers while foraging. The majority of research exploring the relationship between visual and olfactory floral cues is performed at local spatial scales and applicable to understanding floral selection. Floral cue-use during search remains underexplored. This study investigates how the bumblebee *Bombus impatiens* uses visual versus olfactory information from flowers across behavioral states and spatial scales. At local spatial scales, non-flying animals in an associative learning paradigm will access learned multimodal cues in an elemental fashion - where a single component of the cue is capable of eliciting responses. However, bumblebees flying in a windtunnel shift cue-use strategy depending on the spatiotemporal scale of cue encounter. When both color and odor cues mimic local/ within patch spatial scale, bumblebees exhibit a gradient of elemental responses - with highest responses to the complete multimodal color+odor cue, followed by intact color, then intact odor. When cues mimic an intermediate/ between patch spatial scale, bumblebees exhibit configural responses to the learned cue - with high responses to only the learned color+odor cue. Thus the underlying physiological state, sensitive to both activity level and spatiotemporal scale of sensory information, is modulating encoding and utilization of multimodal floral cues.

Introduction

Bumblebees are essential pollinators in multiple ecosystems[1–4]. However, human activity has negatively impacted bumblebee populations through multiple pathways, including but not limited to habitat loss, disease, and pesticide exposure (as reviewed by Goulson et al 2015 [5]). In addition, anthropogenic activity can indirectly impact sensory signals that are utilized by bumblebees while foraging for floral resources. For example, negative effects of human activity on floral-scent signaling and reception include a reduction of distance traveled by floral scent [6,7], increased foraging times [8], decreases in floral scent recognition [9–12], and alterations of flower visitation rates [13,14]. These negative effects are potentially exacerbated by asymmetric impacts of sublethal pesticide exposure with odor-learning,but not color-learning, being impaired [15].

However, bumblebees do not exclusively rely on olfactory cues while foraging. Flowers provide a diversity of sensory information to pollinators (e.g. color, shape, patterning, electrostatic fields) (reviewed by Sommer et al. 2022 [16], Rands et al 2023 [17]). These signals are information-rich; improving detection, discrimination, and learning [18–21]. For example, bumblebees have been shown to recognize paired visual and olfactory patterns more efficiently than either cue on its own [20,22]. Multimodal signaling can also be used in a redundant manner by experienced foragers who must recognize rewarding cues across sensory modalities in a range of environmental conditions [22]. Learning multimodal floral signals likely improves foraging success for bumblebees, as access to richer floral information reduces uncertainty [20] and improves detection in variable sensory environments [23,24]. Understanding the ‘rules’ of multimodal integration of floral-information is critical to determining the potential impacts of

odor pollution, as these could be amplified or attenuated depending on how scent is integrated with other floral signals. The existing body of work strongly indicates that bumblebees utilize odor information in conjunction with visual cues such as color, but the rules of integration are less concrete. For example, work by Lawson et al shows that olfactory pattern learning can be transferred to visual patterns - implying that neural encoding of floral 'objects' flexibly integrates multiple modalities - while other studies have implied that color information dominates over scent [20,25]. To some extent varying conclusions across these studies can be explained by different methodologies - but what about methodological difference modulates sensory integration by bumblebees? It is possible that the 'rules' of sensory integration are dynamic and dependent on the behavioral-mode of a foraging bumblebee. Recent work proposed a framework for identifying and understanding foraging phases in terms of state and state-transitions (*search (S), acquisition (A), navigation (N), S→A, N→A, etc.*), forager-background (*naive (n), experienced (e), primed (p)*), and spatial scale (*local (l), intermediate (i), distant (d)*), with the understanding that different types of sensory information are relevant to different foraging-phases [16]. To start, floral-information is variable depending on distance to a flower/ patch, with olfactory information typically resolvable at greater distances from flowers than their floral displays [26]. Therefore a bumblebee searching for a novel floral resource might utilize floral-information differently than a bumblebee selecting a resource within a patch. The majority of multimodal-integration research has been performed at local spatial scales, typically less than or equal to 3.6 meters (reviewed by Sommer et al 2022 [16]). These studies provide valuable data about patch exploitation (*S→A*), but their findings may not directly translate to bumblebees in the search (S) or navigation (N) phases. Given that bumblebees forage large distances, up to 1 kilometer [27–29], an understanding of how spatial scale and behavioral state influence multimodal integration is crucial to furthering our understanding of foraging behavior. This phenomenon is not isolated to bumblebees, but applies to many species of central place foragers {c Sommer}. Our study investigates how bumblebees integrate color and odor information across behavioral modes via associative learning and wind-tunnel paradigms (Tables 1, 2). Specifically we use the free moving proboscis extension reflex paradigm [30,31]to ask if bumblebees trained to a multimodal color+odor cue will generalize to the learned color paired with a dissonant odor and vice versa. We use a wind tunnel assay to ask if foraging bumblebees trained to a multimodal color+odor cue will land on flowers with the learned color paired with a dissonant odor and vice versa. In addition, we manipulate access to visual and olfactory cues to simulate changes in spatial scale: tests where bees only have access to odor cues at the launch point are intended to mimic a farther distance from a flower, while those where both color and odor cues are available represent local spatial scales [26].

Methods

Animals

This study used 13 colonies of *Bombus impatiens* (Koppert Biological Systems or BioBest) between 2018 and 2023. The temperature of the colonies was maintained at 75–85◦ F by wrapping a seedling heat map with an attached thermostat around each. All colonies had continued access to ad-libitum pollen throughout the studies. Colonies used for FMPER experiments had ad-libitum access to 30% sucrose solution, while those used in wind-tunnel experiments only had access to sucrose during feeding windows of 2-3 hours to increase experimenter access to foraging bees for experimental-trials.

Stimulus Modalities

All experiments tested color and scent sensory modalities. The colors used were yellow and blue [32]; scents were lily of the valley (LoV) and juniper berry (JB) [31]. Conditioning for FMPER experiments is rapid enough that multiple associative color+scent conditions could be tested, while all wind tunnel experiments were based on association to blue+LoV-scented flowers (diameter 3.5”). Responses to combinations of novel and congruent combinations of trained modalities were then tested with different experimental paradigms to determine if behavioral state impacts the generalization of learned multimodal information (FMPER, Table 1; wind tunnel, Table 2).

Localized Associative Reward Learning: FMPER

Associative learning was assessed using Free Moving Proboscis Extension Reflex (FMPER) [30,31]. Active individual *B. impatiens* were selected from lab colonies and placed into screen backed vials to acclimate for 2 hours. During experiments the vials were placed into a ventilating FMPER apparatus (Fig 1). The ventilation system drew air in through two small holes in the vial lid and out of the back screens, with flow rates ranging between 0.1 to 0.3 m/s (VWR-21800-024 hot wire anemometer). During testing bees were able to walk back and forth in the vials, but could not fly and had a relatively small range of motion.

During conditioning, individual bees were offered a single drop of 50% sucrose on either a blue or yellow plastic strip that was inserted through one of the holes in the vial lid (hole-selection was randomized). Absorbent adhesive bandage tape (Cover Roll) was placed on the back of the strips in order to hold an odor-stimulus - 1 uL of either Lily of the Valley (LoV) or Juniper Berry (JB), depending on the condition being tested. Bumblebees would undergo four association trials, during which they were presented with these rewarding color+scent strips at 5 minute intertrial intervals. Individuals that actively participated in the four association trials would then undergo a test trial after an additional 5 minute wait. During tests bees were presented with two unrewarding color+scent strips; the sensory values were variable based on trial condition. Trials were designed to answer four different questions: 1) is a multimodal cue learned in this paradigm?; 2) does color or odor exhibit dominance in this paradigm?; 3) is accurate odor with dissonant color generalized?; 4) is accurate color with dissonant odor generalized? (Table 1). Proboscis extension onto a strip was considered a choice and individuals that approached the strips three times or went 45 seconds without exhibiting PER were

considered no-choice. All tested bees were tagged following experiments to prevent re-testing and ensure statistical independence of data points.

Question Asked	Associative Condition		Test Stimulus Conditions			
			Choice 1		Choice 2	
	color	odor	color	odor	color	odor
Is a multimodal cue learned?	blue	LoV	blue(✓)	LoV(✓)	yellow(X)	JB(X)
	yellow	LoV	yellow(✓)	LoV(✓)	blue(X)	JB(X)
Does color or odor exhibit dominance in multimodal learning?	blue	LoV	blue(✓)	JB(X)	yellow(X)	LoV(✓)
	yellow	LoV	yellow(✓)	JB(X)	blue(X)	LoV(✓)
Is accurate odor with dissonant color generalized?	blue	LoV	yellow(X)	LoV(✓)	yellow(X)	JB(X)
	yellow	LoV	blue(X)	LoV(✓)	blue(X)	JB(X)
Is accurate color with dissonant odor generalized?	blue	LoV	blue(✓)	JB(X)	yellow(X)	JB(X)
	blue	JB	blue(✓)	LoV(X)	yellow(X)	LoV(X)
	yellow	LoV	yellow(✓)	JB(X)	blue(X)	JB(X)
	yellow	JB	yellow(✓)	LoV(X)	blue(X)	LoV(X)

Table 1. Experimental conditions for FMPER experiments. Testing conditions that are congruent with training are highlighted green and marked with a check. Testing conditions that are novel are highlighted grey and marked with an X.

Wind Tunnel Tests

Association: During daily timed feeding sessions, wind-tunnel testing colonies were given access to a glass feeder with an LoV-scented blue flower/s (one or two) containing 30% sucrose. Flowers were 3-D printed with non-toxic PLA filament (eSun blue). Odor stimuli were added by pipetting 5 uL of the LoV essential oil onto a 2 cm x 2 cm piece of absorbent tape (Cover Bandage) on the back of each flower. After three consecutive days of flower color + scent exposure, colonies were eligible for wind tunnel trials. Occasionally throughout a colony's lifespan, the foraging activity within the timed feeding window was not robust enough to maintain adequate levels of honey for the hive. In these cases ad libitum feeding was offered without floral stimuli for two - three days. Following this, colonies would have an additional two days to re-associate with the color and scent stimuli. Following timed feeding sessions, 3D-printed flowers were washed and aired out overnight.

Wind tunnel structure: The wind tunnel dimensions was 5.625 ft x 1.625 ft x 1.541 ft; the walls and floor were white corrugated plastic and the ceiling was transparent plexi-glass. Green tape ran lengthwise on the walls, floor, and ceiling to provide visual cues of the wall locations without providing widefield field motion cues for distance measurement by flying bees [33]. Air flow through the tunnel is provided by a box fan, with insulation and grid-sheets to even out flow. Panels were placed at ⅓ and ⅔ of the way through the tunnel on opposite sides, making a serpentine-maze (Figure 2). Air flow through the tunnel is not laminar, but glycerine smoke-plumes were visualized to confirm that scent plumes are carried all the way through the tunnel. Average air flow sampled in the center of the tunnel in each section was 0.37 +- 0.11 m/s. Test bees were introduced into the tunnel at a consistent location, via a launch platform that

accepts bee-vials. Test flowers were placed at the opposite end of the tunnel, 1.5m (59in) from the launch platform.

Testing: Bees that were seen actively feeding from the training flowers in the colony were removed and placed in plastic vials for wind tunnel experimentation, waiting on a heating mat (80.6-82.4 F) for 15-40 minutes before being placed on the launch platform of the wind tunnel. The cap to the bee's vial was removed so that the bee could exit the vial, and this cap removal officially began a trial. Bees that did not exit their vial within five minutes were excluded from analysis. Bees that exited the vial were given 10 minutes to locate and/or land on the test flower. Test flowers were unrewarded 3D-printed copies of the training flowers (either blue or yellow), scented with 10 uL of odor (1:100 LoV or 1:100 JB; diluted in unscented carrier), depending on the experimental condition being tested (Table 2). Bees that landed on the test flower were given a "land" designation and the time in which they landed on the flower was recorded. Bees that did not choose to land after 10 minutes were removed from the tunnel and marked as "no-land." In addition, we recorded if bees navigated upwind to the third section of the wind tunnel. All tested bees were tagged and returned to their colony following experimentation to prevent re-selection and testing, ensuring statistical independence of data points.

Testing effect of cue encounter: Previous work indicates that searching bumblebees most likely encounter floral cues as either odor first, or odor and visual cues simultaneously (Sprayberry, 2018). In an effort to push bumblebees into a searching behavioral state (Sommer 2022), we placed panels at ⅓ and ⅔ of the way through the wind tunnel on opposite sides to create a serpentine-maze path. These tests endeavor to investigate search behavior in bumblebees at both a local spatial-scale, with clear panels allowing access to both visual and odor cues from the launch point, and an intermediate spatial-scale, with opaque panels obscuring visual cues (Figure 3). We used the number of tested individuals that traveled to the third section to calculate the percentage of 'searching' bees. We then used the number of individuals who landed divided by the number of searching bees to calculate percent landing.

Wind Tunnel Set-up	Test Flower Condition	
	color	odor
opaque panel	blue (✓)	LoV(✓)
	blue(✓)	JB(X)
	yellow(X)	LoV(✓)
	yellow(X)	JB(X)
clear panel	blue(✓)	LoV(✓)
	blue(✓)	JB(X)
	yellow(X)	LoV(✓)
	yellow(X)	JB(X)

Table 2. Wind tunnel experimental conditions. All bees were trained to a blue + lily of the valley (LoV) scented flower. Congruent testing conditions are green highlighted and marked with a check. Incongruent conditions are grey highlighted and marked with an x.

Statistical Analysis

FMPER Analysis: For each experimental question analysis of trial data proceeded as follows: 1) Full response distributions (including no choice) were tested against random (33%) with a fisher's exact test. 2) Choice data were tested against random (50%) with a binomial distribution

test. 3) All no choice data across trial conditions within an experimental question were tested with pairwise Fisher comparisons (using the pairwise.fisher.test function in R). Alpha values for the first two tests were Holm-Bonferroni corrected. P values for the third test were corrected with the Benjamani-Hockberg method. All analyses are summarized in Table 3.

Wind Tunnel Analysis: Both search and land data were analyzed as no-choice data above. For each, data for all stimulus conditions within a set of wind tunnel trials (opaque or clear maze-dividers) were tested with pairwise Fisher comparisons (using the pairwise.fisher.test function in R). P values were corrected with the Benjamani-Hockberg method. All analyses are summarized in Table 4.

Results

Non-foraging bumblebees will utilize components of a learned multimodal cue elementally.

To test bumblebees' ability to learn a color+odor cue, we trained bees to associate a scented-color strip with a sucrose reward using the free-moving proboscis extension reflex paradigm (FMPER), which tests for proboscis extension reflex (PER) after conditioning using two unrewarding strips: one matching their training condition, and one with dissonant color+odor (Table 1, Fig 1). In both training conditions (blue+lily of the valley (LoV) and yellow+LoV) the trained cue was selected more frequently than the dissonant cue ($p<0.01$, Table 3). We then tested if either the color or odor component of a learned color+odor cue was dominant by testing trained bees with a choice of learned-color + novel-odor versus novel-color + learned-odor stimuli. In these tests bumblebees were equally likely to select the learned-color or learned-odor (Fig 1d, Table 3). We also tested if bumblebees would generalize to individual learned components of a color+odor cue when compared against an entirely novel cue. This was performed for each sensory modality, where bumblebees trained to a color+odor cue were tested against the learned-color+novel-odor in one set of experiments, and against the learned-odor+novel-color in another set (Figure 2). Bumblebees did generalize to a learned cue-component for at least one associative condition in each modality: bees trained to blue+LoV selected yellow+LoV at a higher frequency than yellow+juniper berry (JB); and bees trained to yellow-LoV selected yellow+JB at a higher frequency than blue+JB (Figure 2, Table 3).

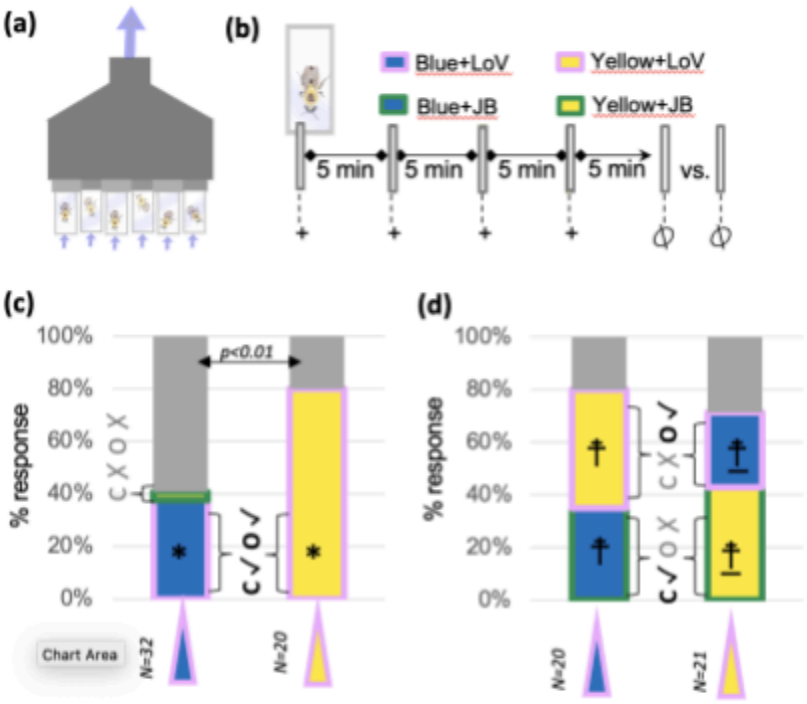

Figure 1. Free-moving proboscis extension reflex (PER) experiments use absolute conditioning to explore multimodal (color+odor) cue learning. A) A ventilating array holds six bees in screen backed tubes, allowing for multiple bees to be tested. B) Tube-lids have two holes, each of which allow a scented+ colored strip to be inserted. The odor was lily of the valley (LoV) or juniper berry (JB) and the color was blue or yellow; the rectangles in the legend show the color condition as their fill color, and the scent condition as their outline color (LoV = pink and JB = green). This color schema is consistent throughout all figures. For the four training trials the strips contain a drop of 50% sucrose. For test trials two unrewarding strips were inserted. If bumblebees extended their proboscis onto a strip that choice was recorded and both strips were removed. If bees did not make a choice, a "no choice" response was recorded. All trials (training and testing) were separated by approximately 5 minutes. C&D) training conditions are indicated by coloring of triangles below response distributions, while colors within the response distribution indicate choice frequencies for test conditions. No choice responses are plotted in grey , with arrows indicated p values of no-choice comparisons across treatments.. Congruous testing cue components are market with a ✓, while dissonant components are marked with an ✗. Symbols on response-choices denote p values for binomial comparisons of choices: *p<0.001, † p>0.2. An underscore (_) indicates a confidence interval > 0.5, due to a small number of bees making a choice. C) Bumblebees will choose a learned multimodal cue more frequently than a completely dissonant cue. D) Both odor and color are generalized by bumblebees, with neither cue showing dominance.

Question Asked	Associative Condition	test against random distribution			test for correct responses > incorrect responses				test for impact of associative condition in no-choice responses	
		p value	Holm Thresh.	n	p value	Holm Thresh.	delta CI	n	comparison	p value
Is a multimodal cue learned?	blue-LoV	0.00006	0.025	32	0.002	0.05	0.32	13		
	yellow-LoV	0.00001	0.025	20	0.00003	0.05	0.21	16	B-LoV vs Y-LoV	0.009
Does color or odor exhibit dominance in multimodal	blue-LoV	0.43	0.025	20	0.44	0.05	0.5	16		
	yellow-LoV	0.77	0.05	21	0.6	0.025	*0.52*	15	B-LoV vs Y-LoV	0.72
Is accurate odor with dissonant color generalized?	blue-LoV	0.02	0.025	19	0.02	0.05	0.44	10		
	yellow-LoV	0.01	0.05	21	0.09	0.025	*0.55*	*9*	B-LoV vs Y-LoV	0.54
Is accurate color with dissonant odor generalized?	blue-LoV	0.002	0.025	20	0.11	0.05	*0.58*	6	B-LoV vs. B-JB	0.28
									B-LoV vs Y-LoV	0.002
									B-LoV vs Y-JB	0.75
	blue-JB	0.0001	n/a	26	1	0.025	0.24	13	B-JB vs Y-LoV	0.004
									B-JB vs Y-JB	<0.001
	yellow-LoV	0.00001	0.025	23	0.0001	0.05	0.17	19		
	yellow-JB	0.00000006	0.025	32	0.004	0.05	0.31	8	Y-LoV vs Y-JB	0.000008

Table 3. Statistics for FMPER experiments

Color and odor identity of learned multimodal cues influences the likelihood that non-foraging bumblebees influence will access sensory information in an elemental fashion

The responsiveness of bumblebees within this FMPER paradigm was variable based on the color and odor values of multimodal cues (Table 3). Bumblebees associated to blue-strips demonstrated a higher percentage of no-choice responses than those associated to yellow-strips in tests of multimodal learning ability (p=0.009, Table 3). Experiments on bumblebee likelihood of generalizing to a learned color + novel odor show similar trends; all tests utilizing blue associative strips have a higher percentage of no-choice responses (Table 3). These tests also show an increase in no-choice when the associative odor is juniper berry. Indeed, color generalization trials where bumblebees were associated to blue+JB not only showed a high percentage of no-choice responses, bees did not generalize to the learned color. Rather they chose yellow+LoV in their test phase. Likewise,bees trained on yellow+LoV had the highest percentage of generalization-responses; and bees trained to yellow+JB showed a significant increase in no-choice responses (Figure 2).

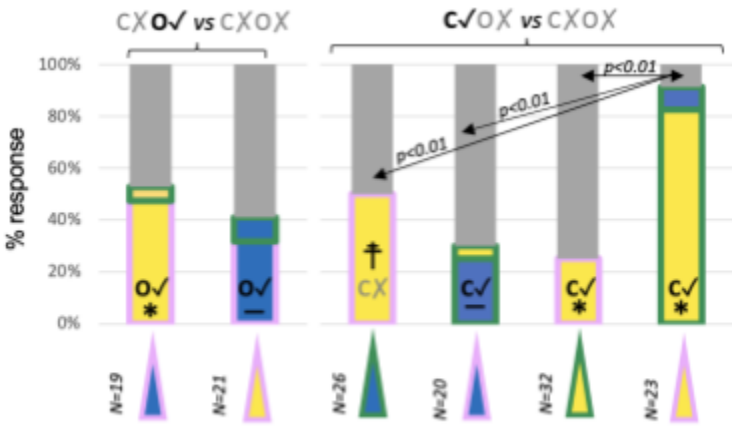

Figure 2. In the majority of experimental conditions bumblebees will generalize a unimodal component of a learned multimodal cue when paired with a novel cross sensory cue. Training conditions are indicated by coloring of triangles below response distributions, while colors within the response distribution indicate choice frequencies for test conditions. No choice responses are plotted in grey, with arrows indicated p values of no-choice comparisons across treatments. Congruous testing cue components are market with a ✓, while dissonant components are marked with an ✗. Symbols on response-choices denote p values for choice comparisons with a binomial comparison asking if responses to the learned cue are *greater* than those for the novel cue: *p<0.001, † p>0.2. An underscore (_) indicates a confidence interval > 0.5, due to a small number of bees making a choice.

Foraging bumblebees access multimodal information configurally

To test how actively foraging bumblebees respond to components of learned multimodal cues, we trained colonies to forage on blue-LoV scented flower feeders. Foraging bees were then collected from feeders and flown in a wind tunnel divided into a three section maze with offset panels; the final section held an unrewarding flower. We used the number of tested individuals that traveled to the third section to calculate the percentage of navigating bees. We then used the number of individuals who landed on the test flower divided by the number of navigating bees to calculate percent landing (Figure 3). Wind tunnel experiments measured responses of actively

foraging bees to various novel or congruent combinations of color and odor (Table 2). The percentage of navigating bees was consistent across all treatments; unaffected by panel-status, color, or scent (p>0.3, Figure 4, Table 4). However, cue-value had a substantial effect on landing frequency, with the blue-LoV flowers having the highest landing frequency, and flowers with any dissonant modalities showing reduced frequency in most conditions (Figure 4, Table 4).

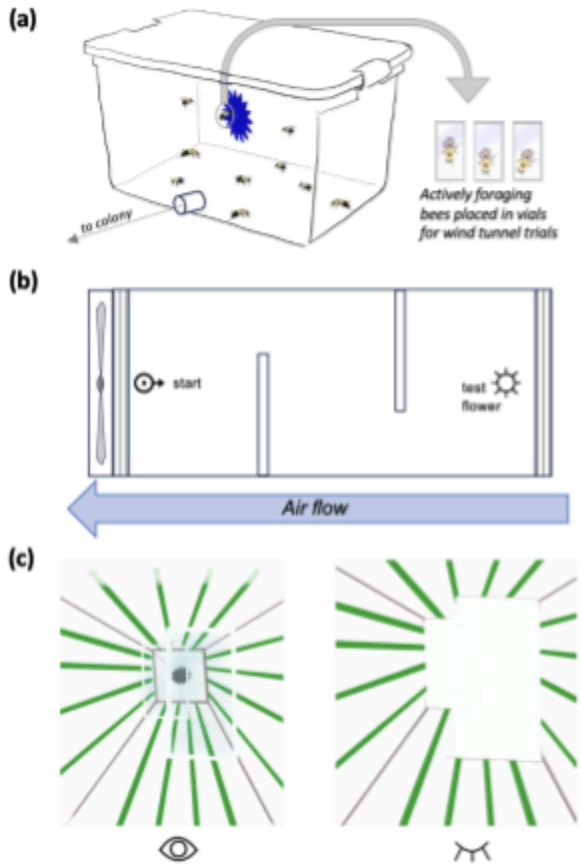

Figure 3. (a) Wind tunnel experiments select actively foraging bumblebees from the colonly's training flower and set them in vials for wind tunnel trials. (b) The wind tunnel has two panels that break it into a serpentine path over three sections between the start and an unrewarding test flower. Bumblebee vials are placed at the 'start' of the wind tunnel. After vials are uncapped bees have 10 minutes to complete the wind tunnel maze. Data on which bees navigate to the third section, and which land on the test flower are recorded. (c) The panels that divide the wind tunnel are either clear, allowing a starting bee to both see and smell the test flower, or opaque, resulting in the starting bee only having access to scent cues.

Spatiotemporal scale of sensory encounter can partially recover elemental cue use in foraging bumblebees

Wind tunnel tests endeavor to investigate behavior in bumblebees across spatial scales; with clear panels allowing access to both visual and odor cues from the launch point simulating a local-spatial scale; and opaque panels obscuring visual cues but allowing access to odor cues simulating an intermediate spatial scale (Figure 3) (Sommer et al). Bees tested at 'intermediate' scales, where odor encounter precedes visual, showed bimodal landing responses, responding robustly to only the learned multimodal cue combination blue+LoV (Figure 4). Bees tested at 'local' scales, where visual cues are encountered in synchrony with or ahead of odor, show a gradient of landing frequencies; with the highest response to learned-color + learned-odor cues, followed by learned-color + novel-odor, then novel-color + learned-odor, and no response to a fully novel cue (Figure 4).

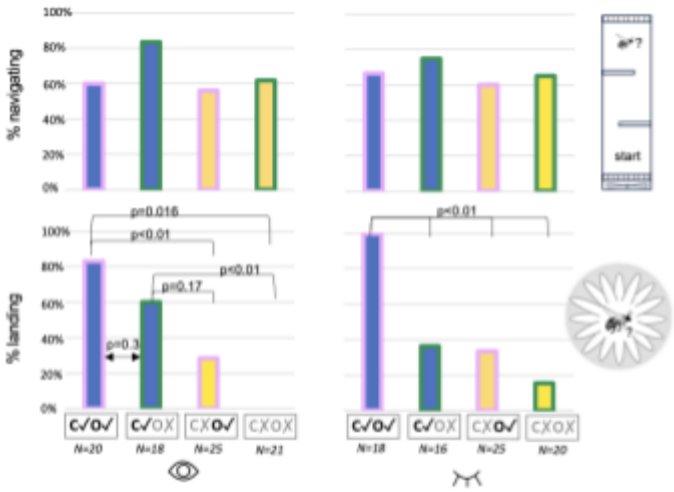

Figure 4. Foraging bumblebees flown in the wind tunnel utilize multimodal cue information differently depending on availability of cues at takeoff. All bees in these experiments were associated to a blue-LoV scented flower. The top row of graphs show the percentage of tested bees that flew upwind to the third wind tunnel section (% navigating). The bottom row of graphs shows the percentage of navigating bees that landed on an unrewarding test flower (% landing). Test flower conditions varied; congruous testing cue components are marked with a ✓, while dissonant components are marked with an ✗. The left-hand column of plots show data for wind tunnel tests with clear panels, so that both visual and olfactory information was available at launch. The right-hand column of plots show data for tests with opaque panels, so that only odor information is available at launch. All p-values shown are from pairwise Fisher comparison tests and corrected with the Benjamani-Hockberg method.

Panel Condition	Test Flower	Sample Size	Searching: comparison p-values			Landing: comparison p-values		
			yellow-**LoV**	**blue**-JB	yellow-JB	yellow-**LoV**	**blue**-JB	yellow-JB
clear	**blue-LoV**	20	1	0.34	1	0.02	0.24	<0.001
	blue-JB	18	0.34	-	0.34	0.17	-	0.002
	yellow-**LoV**	25	-	0.34	1	-	0.17	0.15
	yellow-JB	21	1	0.34	-	0.15	0.002	-
opaque	**blue-LoV**	18	0.92	0.92	1	0.001	0.003	<0.001
	blue-JB	16	0.92	-	0.92	1	-	0.5
	yellow-**LoV**	25	-	0.92	0.92	-	1	0.5
	yellow-JB	20	0.92	0.92	-	0.5	0.5	-

Table 4 Wind Tunnel experiment statistics

Discussion

The spatiotemporal scale of cue encounter modulates encoding of multimodal information

Previous multimodal studies on bumblebees have shown support for the 'efficacy backup hypothesis', where access to information across multiple sensory modalities improves learning and recall in noisy environments [20,22,23,34]. The data presented here also support this hypothesis; with our findings that unimodal components from a learned multimodal cue are capable of influencing behavioral choices in our associative learning FMPER paradigm (figs 1,2). In addition a substantial percentage of bees flown in the clear paneled wind tunnel were responsive to the learned color component of trained floral cues (fig 4). From a neural circuitry perspective this implies elemental access to the unimodal components of learned multimodal cues during activation of behavioral responses. However, bumblebee responses to unimodal cue-components paired with dissonant values are not uniform across experimental paradigms.

Bumblebees flown in an opaque paneled wind-tunnel, intended to simulate an experienced forager navigating at an intermediate spatial scale (foraging state *N(i,e)*), showed highest landing frequencies on flowers with intact multimodal cues. From a neural circuitry perspective this implies that behavioral responses are activated in response to a synthesized color+odor percept (i.e. configurally). The principle difference between wind tunnel paradigms is the temporal access to unimodal cue components; where opaque panels allow access to odor cues *before* color. Indeed this phased cue encounter is how 'intermediate' spatial scales in central place foragers has been defined [16]. Clear panels facilitate either simultaneous color+odor, or color cue encounter first. The neural mechanisms behind how timing of multimodal cues can modulate the relationship between unimodal cues has received little attention. A recent study utilizing conditioning of the proboscis extension reflex in harnessed bumblebees investigated the impact of color and odor synchronicity on bumblebee learning [25]. In their experimental context they also found evidence of elemental cue access, with readily learned conditions showing stronger responses to odor alone. Interestingly bees trained with odor preceding color showed the lowest responses to unimodal cues, providing some support for hypothesizing that the temporal structure of odor versus color encounter could shift bumble bees from elemental towards configural responses. However, that treatment was also poorly learned in their experimental paradigm; thus the mechanisms behind spatiotemporal scales of multimodal cues and behavioral activation remain an intriguing site for future study.

The idea that 'state' influences sensory processing in insects is not novel. Studies on fruit flies, hawk moths, and mosquitoes have explored how shifts in state can underlie behavioral flexibility [35–38]. Flight activity is known to modulate sensory processing - enhancing visual processing of landing in fruit flies [37] and olfactory processing in hawk moths [38]. Hunger can modulate responses to odor stimuli - turning aversion to attraction - in fruit flies [36]. Similarly, in mosquitoes one sensory cue (CO_2) can influence responses to an additional cue (host odors) [35]. In the current body of literature, the presence of one sensory cue (internal or external) is modulating the responses to an additional cue. In the results presented here, internal activity state could certainly be influencing some of the differences we see between FMPER and wind tunnel experiments. However, the shift from configural to elemental cue integration within wind tunnel treatments represents a more complicated instance of state modulation of sensory processing.

Not all stimuli are created equal: color and odor values influence how bumblebees respond to learned multimodal cue information

As reviewed by Leonard and Masek [21], the results of multimodal experiments can be shaped by the salience of chosen stimuli. While existing literature supports that bumblebees can learn all of the stimulus values used in these experiments [31,32,39,40], our results indicate that in our FMPER experimental context, yellow and lily of the valley were more appetitive than their counterparts (blue and juniper berry) (figures 1,2). The FMPER experiments, while excellent for probing cognitive capability, are far from a free foraging environment; thus direct translation of

this work into field preferences is premature. However, these experiments do highlight that bumblebee responses to multimodal stimuli are driven by neural circuitry that is performing more complex operations than simply matching potential resources to a learned search image. Learning clearly has a strong influence on responses, but is operating in conversation with additional factors such as salience and/ or valence (how appetitive or aversive a stimulus is). This hints at the kind of cognitive (and thus behavioral) flexibility that makes bumblebees effective generalist pollinators.

Ecological implications and limitations

Understanding how the multimodal information flowers provide is utilized by bumblebees in different foraging phases and across gradients of sensory disruption is critical to conservation efforts. For example, if a learned-color paired with a novel-odor is generalized to the learned multimodal-cue by a substantial portion of within-patch foragers - as indicated by our clear-panel wind tunnel tests - then local disruption of floral odors via anthropogenic activities may be ameliorated. However, generalization tendencies are likely impacted by the valence and foraging state of any odor-disruptions. For example Ryalls et al found that diesel pollution on a local level substantially reduced visitation of a broad spectrum of pollinators, including bumblebees [13]. A subsequent study found variable effects of diesel-driven odor pollution dependent on floral color [14]. Interestingly diesel exhaust contains sulfurous components - an attribute shared by several fungicides that have also been shown to disrupt bumblebee behavior on a local scale [11,12]. Thus our ability to fully interpret these findings is limited by our incomplete understanding of an odor-valence spectrum for bumblebees.

When considering bumblebees searching for resources at a larger spatial scale, the impacts of anthropogenic disruption are potentially more dire. Bumblebees travel large distances from their nest during foraging bouts [27–29], presumably searching for resources if they are not navigating directly to a previously learned patch. Recent work showed that bumblebees prefer to fly upwind [41], which primes them to utilize olfactory navigation if they encounter an ‘acceptable’ odor-plume [42,43]. If, as our opaque-panel tests indicate, experienced foraging bees are less flexible in terms of accepting disrupted color+odor combinations they are more likely to pass over discovered patches whose odors have been modulated by human activity. Given that habitat loss and fragmentation both reduce total resources available to bumblebees and increase distance between viable resource-patches (reviewed by Goulson [5]), anthropogenic-odor disruption could result in consequential reductions in foraging efficiency. Indeed, understanding the impact of spatial scale on odor generalization within multimodal cues is a critical next step in determining the conservation impacts of odor pollution.

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Data Availability

Data will be made available for download after peer review and publication.

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