Active sensing: Different plume structures affect movements of antennae in honey bees (*Apis mellifera*)

Christopher M Jernigan[1], Erin Connor[2], Hong Lei[1], Jonathan Victor[3], John Crimaldi[2], Brian H Smith[1,*]

[1]School of Life Sciences, Arizona State University
[2]Civil, Environmental & Architectural Engineering, University of Colorado Boulder
[3]Feil Family Brain and Mind Institute, Weill Cornell Medical College

*Corresponding author: brian.h.smith@asu.edu

Abstract

Insects move their antennae to actively sense their environment. Regarding olfaction, it is not clear how these movements might be optimized for sampling the odor environment. Honey bees have movable rod-like antennae the last of segment of which contains several thousand pore plate sensillae that contain dendrites of olfactory sensory neurons. Walking honey bees typically move antennae in an almost constant manner. These movements can be impacted by odor valence, either innate or learned, suggesting that these movements are under sensory control. However, it is unclear if these movements are under active control or are simply fixed responses to stimulation. Here we evaluated antennal movements of stationary bees when placed in odor plumes with different structures. Antennae took up on average two different positions both in the absence and presence of odor in the plume. One corresponded to upwind and toward the odor source. The other position was across the plume. Bees rapidly switched between positions both in the presence and absence of odor. The frequency of forward and lateral positioning depended on the presence/absence of odor and on the structure of the plume, which suggests that movement is involved in sensing of odor filaments. We conclude that these movements represent active sensing, analogous to sniffing in mammals. Future investigations need to focus on the connection between antennal movements and physiological sensing as well as on analyses of odor-driven antennal movements in freely moving bees. Our results also suggest that active sensing may differ across insects with different antennal morphologies.

Summary statement

Honey bee antennal movements are correlated to differences in structured odor plumes, representing active sensing analogous to sniffing in mammals.

Introduction

Insect antennae are multisensory organs designed to detect information in an insect's environment (Elgar et al., 2018). An array of sensory receptors from several different modalities, including hygroreceptors, mechanoreceptors, gustatory and olfactory sensory receptors, are distributed along antennae in different species. Many species move or position their antennae specifically to seek out and monitor static and dynamic information in these sensory modalities, which is called 'active sensing' (Crimaldi et al., 2022; Dürr et al., 2022). For example, the long filamentous antennae of stick insects and of orthopteroid insects such as cockroaches, crickets and locusts are actively used in tactile sensing (Mérida-Calvo et al., 2021; Prescott et al., 2011; Staudacher et al., 2005). Prior to contact with an object, stick insects move antennae in cyclical patterns consistent with exploratory behavior. After contact with an object, movement patterns change in ways specific to the type of contact – e.g. wall vs edges. Similarly, orthopteroid insects use tactile sensation to discriminate objects and determine whether to approach or avoid an object. These movements can also be guided by vision as antennae are moved to investigate objects in the peripheral visual field (Willis et al., 2011). Male cockroaches orienting to sex pheromone under different plume conditions hold antennae at a relatively fixed position so as to monitor plume position and boundaries (Willis & Avondet, 2005). Finally, in locusts contact with an odor filament in a plume triggers targeted antennal movements that increase certainty about the location of the filament (Huston et al., 2015). More focused studies evaluating how antennae move with respect to different stimuli have the potential for revealing more detailed aspects of strategies for active sensing.

Honey bees have geniculate antennae with three segments - the scape, pedicel and flagellum (Schneider, 1964). This type of rodlike, segmented antenna can be moved in three dimensions around the base as well as bent at the 'elbow' joint formed between the pedicel and flagellum. Thus the antennae can be moved using different degrees of freedom in order to actively investigate the surroundings. Flying honey bees hold the antennae in a relatively fixed position, like in walking cockroaches, but the position and movement depends on and tracks information from the visual flow field and windspeed (Roy Khurana & Sane, 2016). In this situation back-and-forth casting alternating with forward surging body movements in an odor plume can enhance active sensing (Carde & Willis, 2008; Demir et al., 2020).

In contrast, walking honey bees actively move their antennae. Introduction of an odor into an air stream moving over the antennae induces rapid movement (Lambin et al., 2005) and tracking the position of the odor stream relative to the head (Suzuki, 1975). Like in other insects, mechanoreceptors on the antennae are used in tactile sensation to discriminate textures on different objects (Erber et al., 1998). Moreover, when honey bees learn about the association of odor with reinforcement, the movements induced by odor reveal the valence – appetitive/aversive - of the reinforcement (Cholé et al., 2015; Cholé et al., 2022; Gascue et al., 2022). More recently, Claverie et al. (Claverie et al., 2023) have shown that naive bumble bees increase both vertical and lateral antennal movement when presented with an odor, the former of which might enhance flow speed around the antenna and hence odor capture (Claverie et al., 2022), and the latter movement might enhance determination of odor plume boundaries or odor gradients, as in cockroaches (Willis & Avondet, 2005). Accordingly, increased flow over crustacean antennules increases electrophysiological responses, which suggests an increase in odor molecules transferred to receptors per time as a result of thinning of the boundary (Moore et al., 1991).

Thus, there is now considerable evidence that antennal movements of stationary or walking honey bees are used to actively investigate odors. We therefore hypothesize that, if antennal movements represent information exploration or exploitation, the movements should depend on the structure of the odor environment. Air streams flowing over and around objects and through thermal gradients may become turbulent. When this happens, the turbulence breaks up odor packets into thin filaments, and the encounter with a filament might be as short as a few ms depending on flow conditions, the relative movement of the insect, and its antennal movement. Here we quantifiably manipulate odor flow conditions (Connor et al., 2018) while monitoring antennal movements of fixed honey bees. We show that movements depend on flow conditions, and we discuss how these movements might be extracting information from the flow.

Materials and methods

Odor mixture

For all odor presentations we used a mixture of 11 components loosely based on the natural mixture of the honey bee pollinated plant *Brassica rapa* (Knauer & Schiestl, 2015). Those components were (pg/L): α-Farnesene (10^{14}); acetophenone (10^{12}); benzyl nitrile (10^{14}); decanal (10^{12}); indole (10^{14}); methyl benzoate (10^{14}); methyl salicylate (10^{14}); nonanal (10^{12}); p-anisaldehyde (10^{13}); phenyl acetaldehyde (10^{13}); z-3-hexenyl acetate (10^{14}). We previously confirmed using electroantennograms that honey bees can detect each of these components.

Honey bee behavioral responses to odor

Twenty honey bee workers were collected at the hive entrance, placed briefly on ice, and harnessed in small plastic tubes using light colored masking tape (Birgiolas et al., 2017; Smith & Burden, 2014). Tape was placed under the back of the head such that the head, antenna, and proboscis were free to move. The back of the head of each bee was fixed in place with a small amount of wax so that a bee was unable to turn its head; this provided a fixed reference in video frames. Harnessed honey bees were placed 20 cm from the odor output port in a wind tunnel previously used to characterize odor plumes (Connor et al., 2018). Antennal responses were recorded from above using a Flea 3 camera at 30 frames per second. Each bee was recorded for 90 seconds, and at the 20 second mark the odor port was turned on, allowing 20 seconds for recording of antennal movements without odor stimulation and 70 seconds of recording of odorant plume structure responses. Odor plume structures were presented to each bee twice in randomized order (two rounds for each odor plume structure). Bees were allowed to rest for 15 min between each odor presentation. Odor was replaced in the odor port after 30 presentations.

Antennae were tracked from video recordings using SwarmSight (Birgiolas et al., 2017). That program returns the angular position of each antenna at each video frame, which was then used to generate the kernel density plots below. We then calculated at each video frame for each bee and each presentation trial the following measures: (1) the fraction of the time that the antenna occupied either of two angular modes (forward vs. lateral) identified in the kernel density plots and (2) an antennal coordination score, which represents the sign of the antennal coordination, i.e., positive if tended to be positioned in the same angular mode, and negative if they tended to be positioned in opposite modes. An antennal coordination score of zero represents no coordination, i.e., independent positioning of the two antennae. The antennal coordination score is calculated as follows:

$$Antennal\ coordination\ score =$$

$$0.5 * \log_e\left(\frac{P_t\ both\ antenna\ are\ forward * P_t\ both\ antenna\ are\ lateral}{P_t\ left\ antenna\ is\ forward\ \&\ right\ is\ lateral * P_t\ right\ antenna\ is\ forward\ \&\ left\ is\ lateral}\right),$$ where

P_t is the proportion of time the two antenna are in a given mode.

Bees were presented with four pre-defined plume structure scenarios in a wind tunnel (Figure 1). These scenarios were presented in the same wind tunnel and under the same conditions used for quantification of acetone plume structures just before our data were collected (Connor et al., 2018). The chamber was carefully calibrated to achieve the same flow conditions. The wind tunnel dimensions were 30 cm wide x 30 cm high x 100 cm long. Air entered through a bell-shaped contraction and exited out the back through a contraction that tapered to a 5 cm x 5 cm cross section housing a 12 V fan to generate the flow.

Analyses using laser sheet imaging of acetone plumes released at the mid-line of the entrance to the tunnel described the statistical properties of each of the four dynamic plume conditions we use here (Connor et al., 2018). Supplemental videos S1-S4 show restrained bees with moving antennae placed into an acetone plume so that encounters with the plume could be visualized under different conditions. 'Bounded' refers to release of the plume 6 mm off of the bed of the tunnel, where it initially disperses within a viscous sublayer next to the bed. Bounded plumes rapidly spread laterally, maintain high concentrations across several measurement points, and have relatively small fluctuations across the field (Suppl video S1). 'Unbounded' refers to release of the odor plume centered in the tunnel entrance, well away from the viscous sublayer next to the bed. Unbounded plumes are more diffuse, showing more fluctuations and less lateral spreading (Suppl videos S2 and S3). Higher flow rates (e.g. 20 cm/s in S3 versus 10 cm/s in S2) in the unbounded condition resulted in more rapid, energetic fluctuations. Finally, 'obstacle' refers to a block (5 cm L x 5 cm W x 16 cm H) placed 5 cm from the source of the odor delivery to create more complex eddies in the plume (Suppl video S4).

Statistical analyses

We established independence of antennal measures to satisfy statistical tests via a crosscorrelation analysis and determined that ~1.7s (50 frames, at 30fps video) was sufficient to obtain independent antennal measures in our data set. We next established bimodality in our antennal angle data with the Hartigan's dip test (D statistic below) using the *diptest* package in R (Hartigan & Hartigan, 1985), again taking only 1 of every 50 data points and testing each antenna separately but pooling across all other factors in the data. We found a bimodal split point for both the right and left antenna (see below) and thus converted our continuous angular data to bimodal forward or lateral modal position data based on that split.

The total number of data points used for GLM derives from the following procedure. 20 bees were each shown 4 plume structures over in two replicate trials, for a total of 160=20*4*2 presentations. Each presentation was 89.9 s long. Although SwarmSight extracted antennal position data for each frame of a 30 fps video (2697 frames), but we only analyzed frames 10, 60, 110, 160, etc.; this 1:50 downsampling is based on the cross-correlation analysis above that showed that it would yield independent measures. Each trial began with 20 seconds of odor off (600 frames, 12 frames analyzed) and was followed by 70 seconds of odor (2100 frames, 42 frames analyzed). This yielded, for each antenna, 160*(12+42)=8640 values of antennal position for each antenna (8640*2=17280 data points), and 8,640 antennal coordination values.

We used generalized linear models (GLM) in R (www.R-project.org) with odor presence, antenna, plume structure, and Bee ID as fixed effects on antennal modal position. We used a generalized linear model (GLM) with odor presence and Bee as fixed effects on antennal coordination score. Odor on vs. off and flow scenario were independent factors. Conversely, odor on vs. off was pooled for analyzing the effects of the plume scenarios. We also evaluated interactions between these factors but did not find any that were significant. Additionally, when removing 'off' condition data and re-running GLM analyses, we find identical significant effects of plume condition on antennal coordination and antennal modal position.

The residual estimates in figures below are the change in log odds of the model measure (antennal modal position or coordination) associated with one unit increase in the fixed effect factor variable. Essentially, a larger residual estimate means a bigger impact that a fixed factor component has on the value the model is trying to predict; i.e. antennal modal position or antennal coordination. The residuals are converted into probabilities in figures below by the following equations:

Antennal position - $p = \frac{1}{1+e^{-x-x_0}}$, where *p* is the probability that the antenna is forward, x_0 is the sum of the residuals for all fixed effects in the model including the intercept, and x is the residual value for a specific factor.

Antennal coordination - The coordination score, defined above, is given by x, the value of the residuals plotted on the x-axis, plus x_0, where x_0 is the sum of the residuals for all fixed effects in the model including the intercept. More positive coordination scores mean that the antennae are more likely to be in the same state than would be expected if they moved independently; more negative scores mean the opposite. As an example, if the antennae are equally likely to be forward or back, a coordination score of 0.1 (or -0.1) means that the probability that they are in the same state is 0.525 (or .475). The index we use to measure correlation takes into account the biases that each antenna (forward vs. lateral) might have, so the behavior in a single antenna cannot account for the changes in correlation: the shift to lateral position and the change in correlation are independent observations.

Results

Antennal positioning in air flow is bimodal

We find a striking feature in every condition for both antennae is the presence of two bands that extend across the entire recording, from prior to odor onset until the termination of the trial (Fig.2). The top band indicates antennal positioning toward the source of the odor and more parallel to the direction of air flow. The bottom band indicates antennal positioning more lateral and across the air flow. These bands show that on average and across bees antennae were positioned in one or the other position, with rapid transitions between positions.

Fig. 3A & B shows that transitions for both antennae range from a low of zero (only a few cases limited to the no odor condition) to as high as four to six transitions per second with means and medians around two. Therefore, bees did not hold their antenna in fixed locations for prolonged periods over the course of any trial. We ran a generalized linear model (GLM) comparing the rate of bimodal position transitions antennae (antennal transition rate) made across the experiment with plume structure, odor presence/absence, trial, and antenna as fixed effects. Antennal transition rate was not significantly impacted by any of our measured fixed effects (GLM, $p>0.05$).

We then collapsed across time to evaluate the significance of the bimodality (Fig. 4). In each of the four test conditions, there was a clear and significant dip in position probability around 50 degrees from the direction of air flow (Right: $D=0.0113$, $p<0.0001$; Left: $D=0.01$, $p<0.001$). This dip represents the fast transition in position shown in Figure 2. It was present under all flow conditions for both antennae both before and after odor onset.

Positioning of antennae depends on odor and flow conditions

We next analyzed how the presence of odor in different flow conditions influenced the distribution of antennae into the two modal positions identified above (Fig. 5). We find a significant impact of odor presence as well as plume structure on the modal position of antennae, but no differential impact on left vs right antenna. Prior to odor onset both antennae tended to be equally distributed across forward and lateral positions. During odor bees are more likely to hold their antenna lateral (away from the odor source) relative to the pre-odor periods, likely maximizing odor capture along the antenna. When examining differences across plume structures we find bees were more likely to hold antenna in the more lateral position for plume structures with very broad distributions either very constant in the environment (bounded condition) or highly variable (obstacle condition). However, we observe the opposite pattern in the unbounded flow conditions with narrowly defined linear plumes, in which bees were more likely to have their antenna pointing towards the odor source.

Coordination across antennae depends on odor and flow

We also find that the coordination of antenna was significantly impacted by odors and plume structures (Fig. 6), indicating that antennae can be moved independently of each other. In general, antennae tended to be in anti-correlated positions (one forward and the other lateral/backwards). However, they became significantly more positively correlated in the presence of odor. Again, as with positioning, flow conditions had additional impacts on antennal coordination. Coordination was highest in the obstacle condition and least coordinated in the unbounded 10 cm/s flow condition. The remaining two conditions showed no differential effects on antennal coordination.

Discussion

Our objective was to investigate antennal movements in odor environments that have documented differences in temporally fluctuating characteristics of odor encounter at a fixed location in a plume. We examined four different plume structures, three of which have well characterized statistical properties (Connor et al., 2018). Antennal movements averaged across bees in each flow condition show broad similarities in how antennae are positioned and moved, suggesting common strategies for locating and identifying odors. Yet there were also significant differences in movement patterns across plume structure through time that may further reflect active sampling of plumes with different encounter rate and distribution probabilities.

The similarities in antennal movements across plume conditions arise from two observations. First, both the left and the right antennae tended to occupy one of two positions, one forward, pointing more upwind toward the source, and the other extended out more laterally across the air stream. These positions show up as two distinct bands across time in each of the flow conditions. The separation of the bands represents a rapid and frequent transition from one mode to the other with little hesitation in between. At present, we do not know whether these positions represent different ways that the antennae seek or capture information from the plume. For example, wider held antennae may enhance the chance of odor filament capture. Antennae (and sensors) pointed upwind might enhance information about odor identity. Second, the left and right antennae are not always symmetrically in the same corresponding position in all plume structures. The reason for this difference could reflect turbulence created by the specific flow conditions in our experimental apparatus. The ability to move antennae fairly independently into positions that extract different features of the plume could also enhance active sampling. Future investigations, now justified by our analyses, would need to couple more precise control of plume conditions – e.g. intermittency, fluctuation, mean concentration – with electrophysiological recordings or imaging in the antennal lobe to unravel these relationships.

One other possible reason for antennal movement relates to flow conditions over the antennae. In the honey bee, olfactory receptor neurons on the antennae are housed in a few thousand ‘pore plates’ (Lacher, 1964), which are oval depressions in the cuticle with small pores arranged around the inside edge of the pore plate. Each pore plate contains the dendrites of a few to a few dozen olfactory receptor neurons. The pores, where there is an interface between air and the aqueous hemolymph in the sensillum, allow for the hydrophobic ligands to be picked up by transport proteins that move the ligands to the dendrite surface where they release a ligand to bind to the receptor (Leal, 2013). For an odor ligand to arrive at the dendrites and bind to odor receptors it first must diffuse through a boundary layer of air that surrounds the outside of the antennae. Oscillatory movements of the antenna can enhance the capture rate of odor ligands by increasing encounter rates (Claverie et al., 2022). In a recent numerical modal, Goulet (Goulet et al., 2025) showed that moving air thins the boundary layer at the leading edge of the antennae, which allows for more efficient odor capture. Turbulence around the back edge of the antennae reduces capture efficiency. This model shows why pore plates are not evenly distributed around the antennae(Lacher, 1964), with more of them being concentrated on the side that would be the leading edge during flight, when the air flow would be generated by body movement. This model also suggests that one reason for movement when walking. This movement pattern, in addition to enhancing odor search, would generate flow over the antennae and increase odor ligand capture particularly when antennae transition from one position to the other. This explanation for antennal movements is analogous to sniffing in mammals (Wachowiak, 2011).

Differences in antennal movements across plume conditions require an understanding of how plumes differed as a result of speed, bounded conditions and obstacles. Our figures represent antennal positions over time and plume structure averaged across a number of bees. Interpretation of on-average similarities or differences across these scales therefore need to reference average values of the plume structures, since the likelihood of an odor value at any point in space or time will be somewhat stochastic between different replicates of any plume structure. Connor (Connor et al., 2018) analyzed plume statistics for bounded and both unbounded flow conditions in the same apparatus we used for our study. Whether or not the plume was bounded, by having been created within the relatively laminar flow in the viscous sublayer near the floor or in the (unbounded) free air flow above floor, and changes in wind speed in the unbounded condition, influence the plume dynamics. At a fixed distance from the source, bounded plumes are more diffusive. They spread laterally such that concentrations at any point, particularly in the middle of the plume, average above zero with fluctuations uniformly above and below that mean. In contrast, unbounded flows are more turbulent and patchy, and they thus are characterized by mean values at or near zero with intermittent fluctuations above zero. Increased wind speeds disperse unbounded plumes further downstream. Connor (Connor et al., 2018) did not analyze the effect of obstacles. However, it is clear that obstacles increase turbulence. All of these characteristics are clear in the supplemental videos.

It is worth noting that there is a diversity of antennal morphologies in insects, and that these morphologies might be correlated to different types of active sensing movements. Thus what is reported for one species may not generalize to other species with different types of antennae. For example, some moths have long filiform antennae that, unlike bee antennae, extend well above the head. During flight those long antennae can interact with vortices produced by wing beats to enhance odor capture (Lou et al., 2024). Male silk moths also beat their wings when walking and orienting to female sex pheromone (Loudon & Koehl, 2000), and the wing movements enhance odor detection by the feather-like plumose antennae that extend well above the head. In contrast, flying honey bees hold their relatively shorter antennae in a position (Roy Khurana & Sane, 2016) that may be less likely to be affected by wing vortices in the same way as in moths, and that may be reflected in the lack of receptors on the downwind side of the antennae. Walking honey bees, which was our motivating interest for the current study, do not move their wings, which could contribute to the need for antennal movements that we have described in order to increase odor capture. In contrast to both honey bees and moths, fruit flies have much shorter antennae, which actively move in response to deflections by wind but not in response to odor stimulation (Suver et al., 2019).

In conclusion, it seems clear that antennal movements are affected by odor encounters. There would be no reason to expect differences across plume structures if antennal movements were not actively driven by odor encounters, that is, if they were driven by reflex and/or mechanosensory information. However, it remains to be determined how these movements affect extraction of information from odor plumes. We predict, for example, that the frequency of odor encounters with an antenna will be influenced by switching from one position into another, or by more stable positioning either forward or lateral in the air flow. As noted above, testing this hypothesis will require coupled behavior and electrophysiological recording from the antennal lobe (Lei et al., 2021). Our current data were collected from bees restrained in such a way that will allow coupling recordings with behavior. However, we will also need to map these movements onto freely walking honey bees as they move in more natural situations in order to understand the adaptive nature of these movements.

Acknowledgements

We recognize feedback on development of this project from in particular Shawn Mahoney and other members of the NSF funded NeuroNex network Odor-2-Action.

Funding

This project is supported by the NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program (Award #2014217) to B.H.S, J.D.V. and J.C.; www.odor2action.org.

References

Birgiolas, J., Jernigan, C. M., Gerkin, R. C., Smith, B. H., & Crook, S. M. (2017). SwarmSight: Real-time Tracking of Insect Antenna Movements and Proboscis Extension Reflex Using a Common Preparation and Conventional Hardware. *J Vis Exp*(130). https://doi.org/10.3791/56803

Carde, R. T., & Willis, M. A. (2008). Navigational strategies used by insects to find distant, wind-borne sources of odor [Review]. *J Chem Ecol*, *34*(7), 854-866. https://doi.org/10.1007/s10886-008-9484-5

Cholé, H., Junca, P., & Sandoz, J. C. (2015). Appetitive but not aversive olfactory conditioning modifies antennal movements in honeybees. *Learn Mem*, *22*(12), 604-616. https://doi.org/10.1101/lm.038448.115

Cholé, H., Merlin, A., Henderson, N., Paupy, E., Mahé, P., Arnold, G., & Sandoz, J. C. (2022). Antenna movements as a function of odorants' biological value in honeybees (Apis mellifera L.). *Sci Rep*, *12*(1), 11674. https://doi.org/10.1038/s41598-022-14354-z

Claverie, N., Buvat, P., & Casas, J. (2023). Active Sensing in Bees Through Antennal Movements Is Independent of Odor Molecule. *Integr Comp Biol*, *63*(2), 315-331. https://doi.org/10.1093/icb/icad010

Claverie, N., Steinmann, T., Bandjee, M. J., Buvat, P., & Casas, J. (2022). Oscillations for active sensing in olfaction: bioinspiration from insect antennal movements. *Bioinspir Biomim*, *17*(5). https://doi.org/10.1088/1748-3190/ac877a

Connor, E. G., McHugh, M. K., & Crimaldi, J. P. (2018). Quantification of airborne odor plumes using planar laser-induced fluorescence. *Experiments in Fluids*, *59(9)*, 137.

Crimaldi, J., Lei, H., Schaefer, A., Schmuker, M., Smith, B. H., True, A. C.,…Victor, J. D. (2022). Active sensing in a dynamic olfactory world. *J Comput Neurosci*, *50*(1), 1-6. https://doi.org/10.1007/s10827-021-00798-1

Demir, M., Kadakia, N., Anderson, H. D., Clark, D. A., & Emonet, T. (2020). Walking Drosophila navigate complex plumes using stochastic decisions biased by the timing of odor encounters. In (Vol. 9:e57524
doi: 10.7554/eLife.57524). eLife.

Dürr, V., Berendes, V., & Strube-Bloss, M. (2022). Sensorimotor ecology of insect antenna: Active sampling by a multimodal sensory organ. *Advance in Insect Physiology*, *63*, 1-105.

Elgar, M. A., Zhang, D., Wang, Q., Wittwer, B., Thi Pham, H., Johnson, T. L.,…Coquilleau, M. (2018). Insect Antennal Morphology: The Evolution of Diverse Solutions to Odorant Perception. *Yale J Biol Med*, *91*(4), 457-469.

Erber, J., Kierzek, S., Sander, E., & Grandy, K. (1998). Tactile learning in the honeybee. *Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology*, *183*(6), 737-744.

Gascue, F., Marachlian, E., Azcueta, M., Locatelli, F. F., & Klappenbach, M. (2022). Antennal movements can be used as behavioral readout of odor valence in honey bees. *IBRO Neurosci Rep*, *12*, 323-332. https://doi.org/10.1016/j.ibneur.2022.04.005

Goulet, D., Smith, B., True, A., & Crimaldi, J. (2025). Pore plate sensilla scale and distribution modulate odor capture around honey bee antennae. *Sci Rep*, *15*(1), 41574. https://doi.org/10.1038/s41598-025-25426-1

Hartigan, J. A., & Hartigan, P. M. (1985). The dip test of unimodality. *Annals of Statistics*, *13*, 70-84.

Huston, S. J., Stopfer, M., Cassenaer, S., Aldworth, Z. N., & Laurent, G. (2015). Neural Encoding of Odors during Active Sampling and in Turbulent Plumes. *Neuron*, *88*(2), 403-418. https://doi.org/10.1016/j.neuron.2015.09.007

Knauer, A. C., & Schiestl, F. P. (2015). Bees use honest floral signals as indicators of reward when visiting flowers. *Ecol Lett*, *18*(2), 135-143. https://doi.org/10.1111/ele.12386

Lacher, V. (1964). ELEKTROPHYSIOLOGISCHE UNTERSUCHUNGEN AN EINZELNEN REZEPTOREN FUR GERUCH, KOHLENDIOXYD, LUFTFEUCHTIGKEIT UND TEMPERATUR AUF DEN ANTENNEN DER ARBEITSBIENE UND DER DROHNE (APIS MELLIFICA L.). *Z. vergl. Physiol.*, *48*, 587-623.

Lambin, M., Déglise, P., & Gauthier, M. (2005). Antennal movements as indicators of odor detection by worker honeybees. *Apidologie*, *36*(1), 119-126.

Leal, W. S. (2013). Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annu Rev Entomol*, *58*, 373-391. https://doi.org/10.1146/annurev-ento-120811-153635

Lei, H., Haney, S., Jernigan, C., Guo, X. J., Cook, C. N., Bazhenov, M., & Smith, B. H. (2021). Novelty detection in early olfactory processing of the honey bee, Apis mellifera. *bioRxiv*, 2021.2010.2006.463371. https://doi.org/10.1101/2021.10.06.463371

Lou, Z., Lei, M., Dong, H., & Li, C. (2024). Wing-antennae interaction reduces odour fatigue in butterfly odour-tracking flight. *Journal of Fluid Mechanics*, *998*. https://doi.org/10.1017/jfm.2024.644

Loudon, C., & Koehl, M. A. (2000). Sniffing by a silkworm moth: wing fanning enhances air penetration through and pheromone interception by antennae. *J Exp Biol*, *203*(Pt 19), 2977-2990. https://doi.org/10.1242/jeb.203.19.2977

Moore, P. A., Atema, J., & Gerhardt, G. A. (1991). Fluid dynamics and microscale chemical movement in the chemosensory appendages of the lobster, *Homarus americanus Chemical Senses*, *16*(6), 663-674. https://doi.org/10.1093/chemse/16.6.663

Mérida-Calvo, L., Feliu-Talegón, D., & Feliu-Batlle, V. (2021). Improving the Detection of the Contact Point in Active Sensing Antennae by Processing Combined Static and Dynamic Information. *Sensors (Basel)*, *21*(5). https://doi.org/10.3390/s21051808

Prescott, T. J., Diamond, M. E., & Wing, A. M. (2011). Active touch sensing. *Philos Trans R Soc Lond B Biol Sci*, *366*(1581), 2989-2995. https://doi.org/10.1098/rstb.2011.0167

Roy Khurana, T., & Sane, S. P. (2016). Airflow and optic flow mediate antennal positioning in flying honeybees. *Elife*, *5*. https://doi.org/10.7554/eLife.14449

Schneider, D. (1964). Insect antennae. *Annual Review of Entomology*, *9*, 103-122. .

Smith, B. H., & Burden, C. M. (2014). A proboscis extension response protocol for investigating behavioral plasticity in insects: application to basic, biomedical, and agricultural research. *J Vis Exp*(91), e51057. https://doi.org/10.3791/51057

Staudacher, E. M., Gebhardt, M., & Dürr, V. (2005). Antennal movements and mechanoreception: neurobiology of active tactile sensors. . *Advances in insect physiology*, *32*, 49-205.

Suver, M. P., Matheson, A. M. M., Sarkar, S., Damiata, M., Schoppik, D., & Nagel, K. I. (2019). Encoding of Wind Direction by Central Neurons in Drosophila. *Neuron*, *102*(4), 828-842.e827. https://doi.org/10.1016/j.neuron.2019.03.012

Suzuki, H. (1975). Antennal movements induced by odour and central projection of the antennal neurones in the honey-bee. *Journal of Insect Physiology*, *21*(4), 831-847.

Wachowiak, M. (2011). All in a sniff: olfaction as a model for active sensing. *Neuron*, *71*(6), 962-973. https://doi.org/10.1016/j.neuron.2011.08.030

Willis, M. A., & Avondet, J. L. (2005). Odor-modulated orientation in walking male cockroaches Periplaneta americana, and the effects of odor plumes of different structure. *J Exp Biol*, *208*(Pt 4), 721-735. https://doi.org/10.1242/jeb.01418

Willis, M. A., Avondet, J. L., & Zheng, E. (2011). The role of vision in odor-plume tracking by walking and flying insects. *J Exp Biol*, *214*(Pt 24), 4121-4132. https://doi.org/10.1242/jeb.036954

Figures

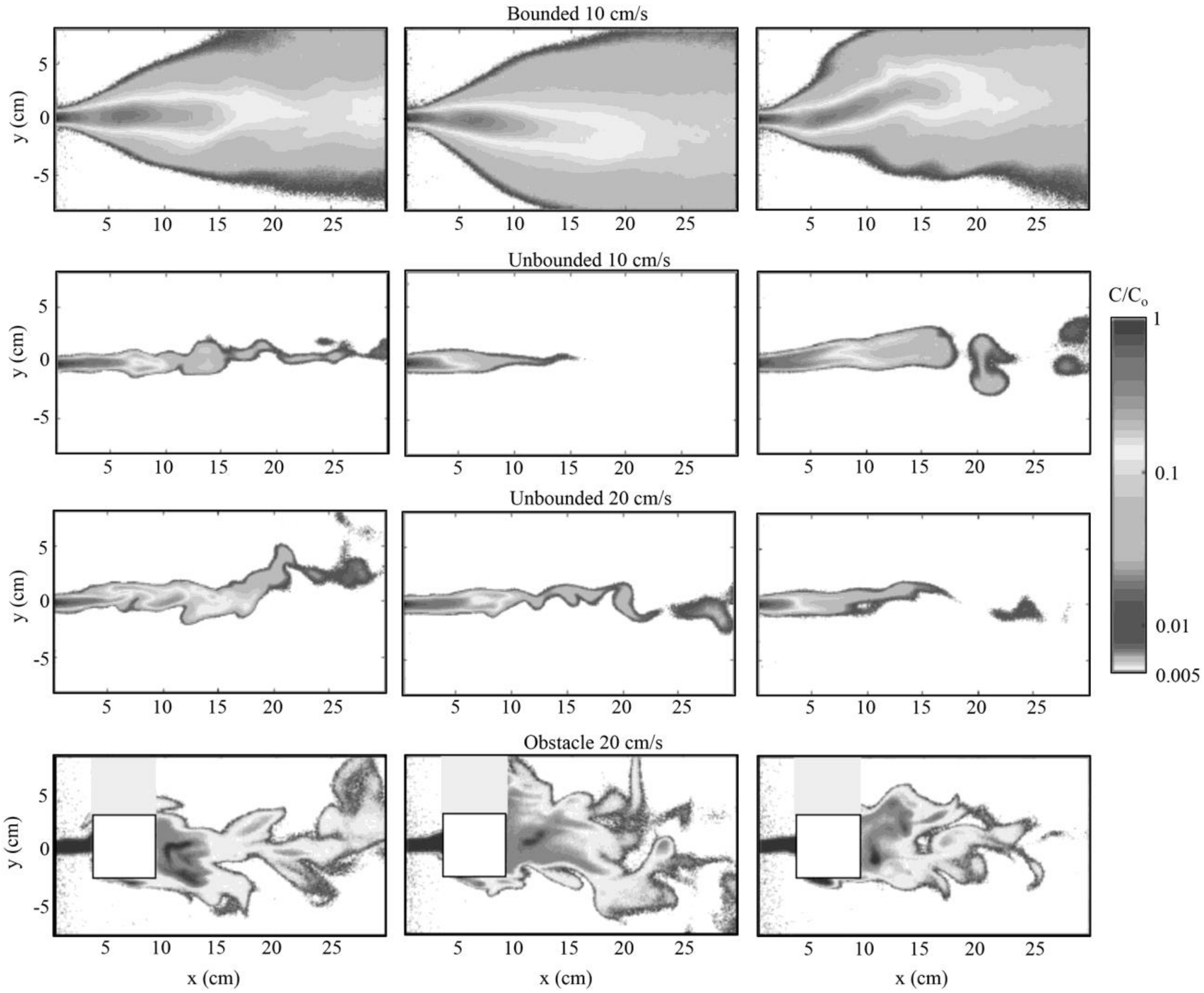


Fig. 1. Snapshots across three successive time points (left to right) of dispersion of acetone in the different plume conditions. Approximate ranges at 20 cm (the bee's position) for mean concentration (concentration relative to concentration at the source) and intermittency (maximum value 1.0 with values closer to zero indicating more intermittency) resp: Bounded 10 0.1-0.2 and 0.9-1.0; Unbounded 10 0-0.01 and 0.05-0.1; Unbounded 20 0-0.02 and 0.1-0.5; Obstacle 20 0.02-0.05 and 0.5-0.7.

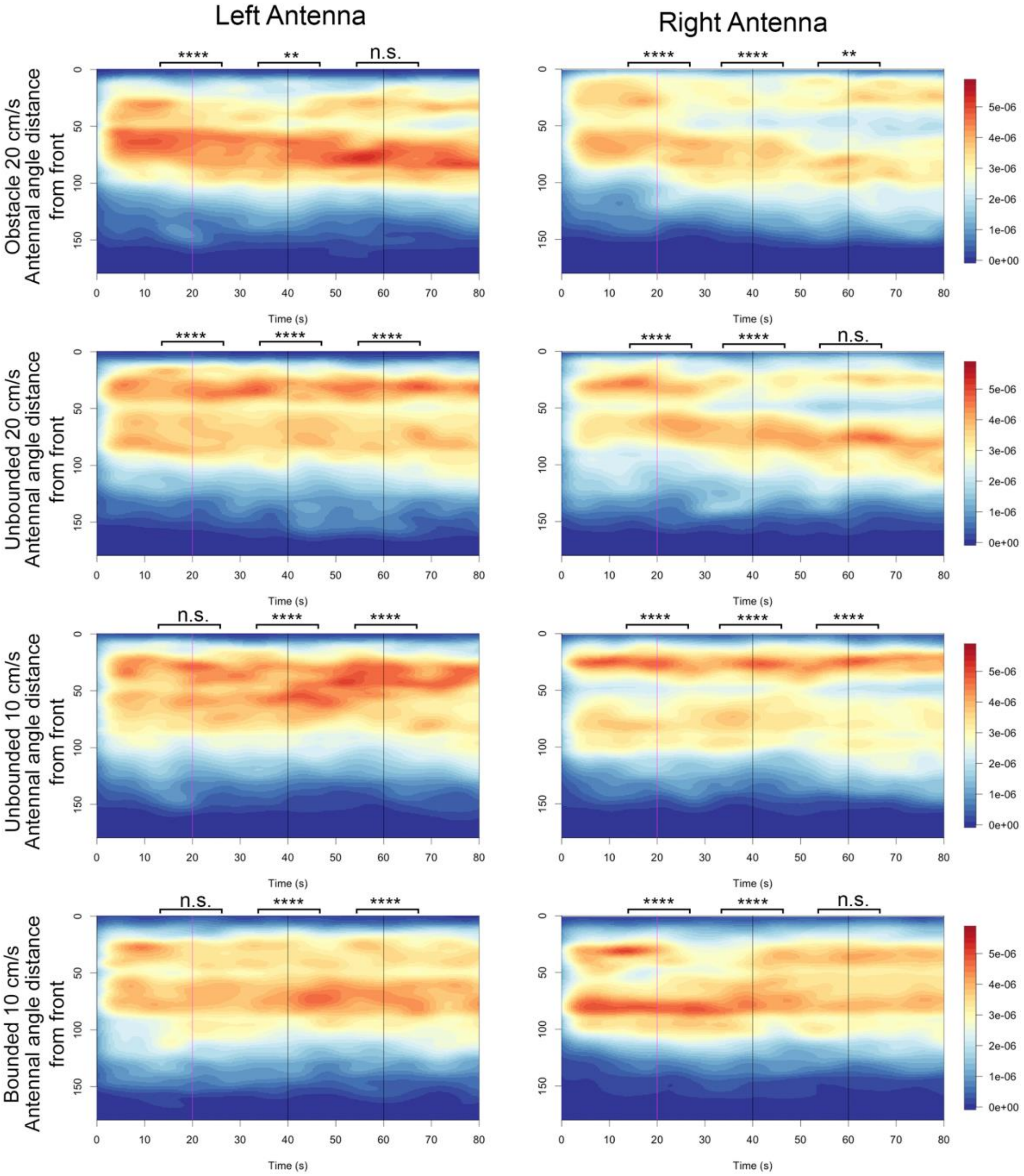


Fig. 2. The mean kernel density plots of antennal angle across time for left and right antenna for each of the 4 unique airflow conditions, pooling across replicate trials and bees. The x-axis displays time with the vertical black bar at 20s denoting odor onset. The y-axis displays antennal angle with 0 being pointing towards the mandibles and 180 representing antennal being held completely backwards (this is physically impossible for the bee giving rise to the zero-probability density). Color denotes density at a given angle. Scale is standardized across all plots and plotted to the right of each row.

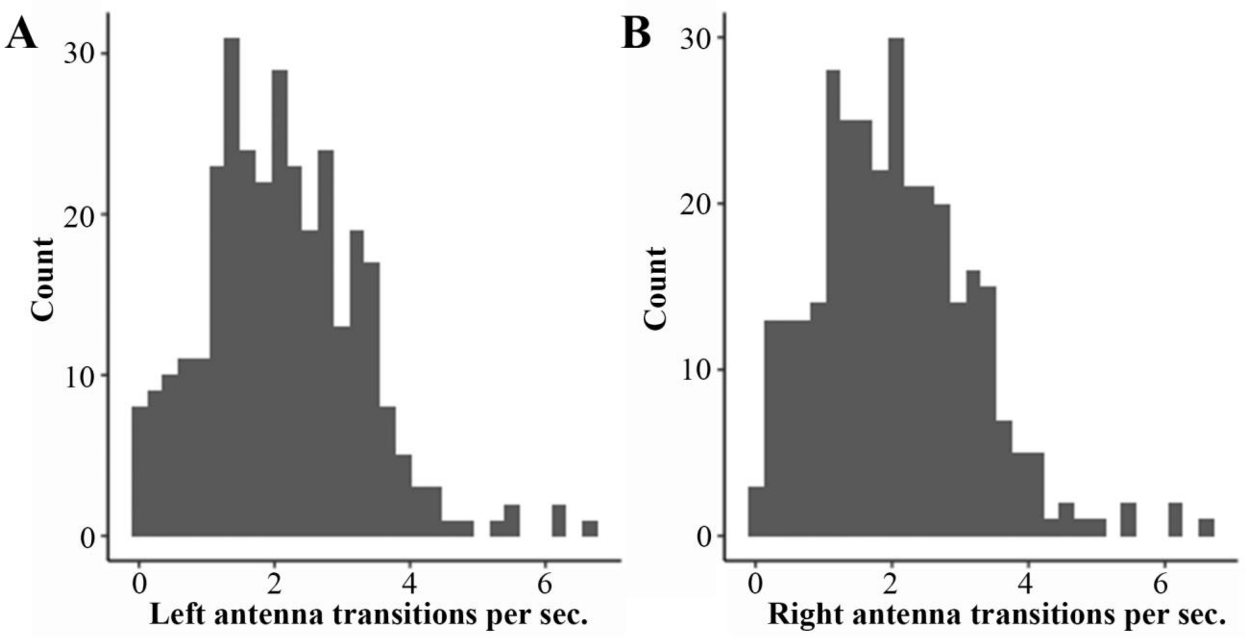


Fig. 3. Rate of transitions between forward and lateral positions for the left (A) and right (B) antennae. Data are summed across bees (n=20), plume structure (n=4), trial (n=2) and odor on/off (n=2). Means/medians: Left odor off 2.1/2.1; Left odor on 2.1/2.0; right odor off 2.1/1.9; right odor on 2.1/2.0.

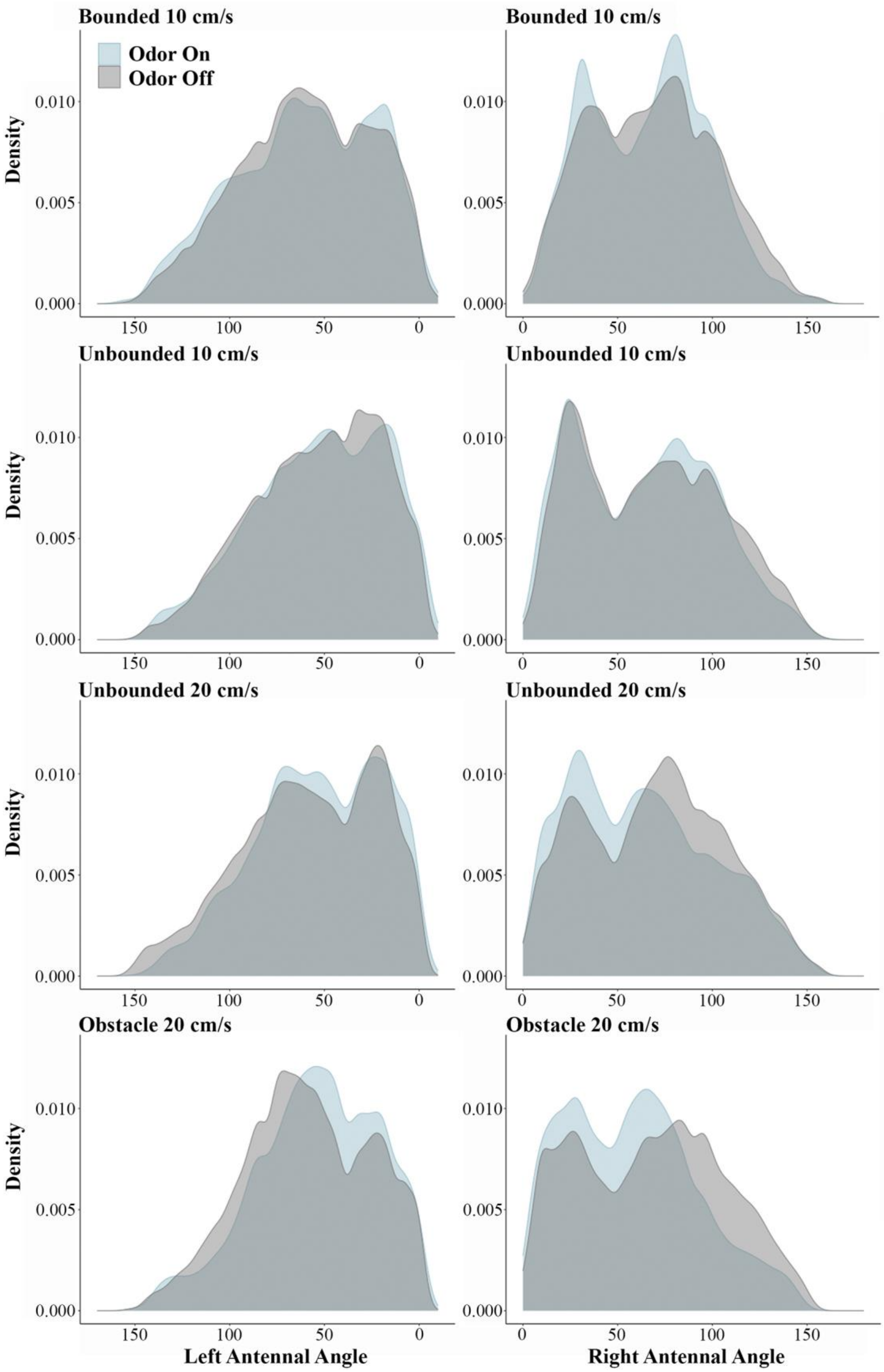

Bounded 10 cm/s
Odor On
Odor Off
Density
0.010
0.005
0.000
150
100
50
0
Bounded 10 cm/s
Unbounded 10 cm/s
Unbounded 10 cm/s
Unbounded 20 cm/s
Unbounded 20 cm/s
Obstacle 20 cm/s
Obstacle 20 cm/s
Left Antennal Angle
Right Antennal Angle

Fig. 4. Antennal angle density plots for left and right antenna across the 4 unique airflow conditions pooling across time, bees, and replicate trials. Note that across all conditions with and without odor present there is a bimodal distribution of antennal angles. Colors denote the presence of odor, with gray representing when odor is absent and blue representing when odor is present.

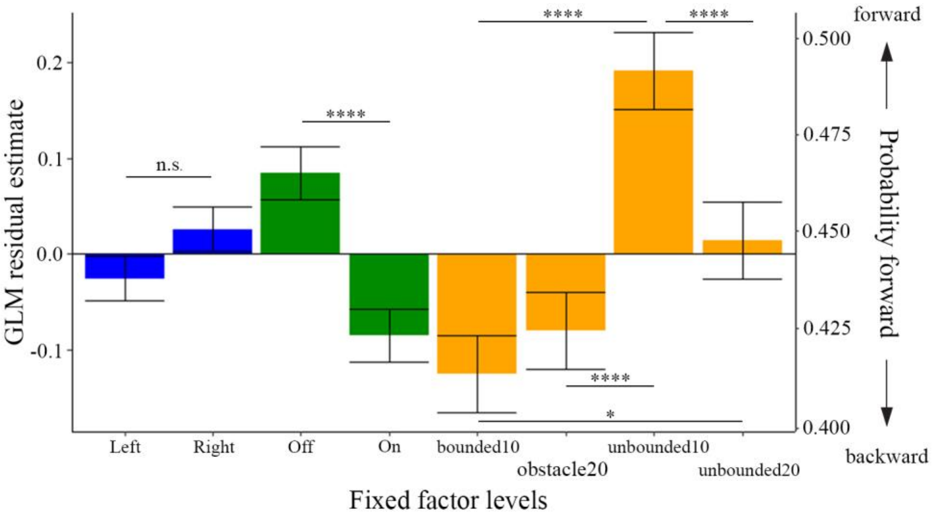


Fig. 5. The GLM residual estimates for the main fixed factors and their modeled distributions comparing the probability of being in the forward mode vs. the lateral mode with a bimodal distribution (GLM: Binary mode ~ antenna + flow condition + odor presence + bee ID). Plotted on the right is the conversion from residual to probability of the antenna being forward. Color denotes fixed factors. Statistical comparisons are only made within fixed factor. Symbols denote p-value: n.s.= non-significant, *= p<0.05, ****= p<0.0001

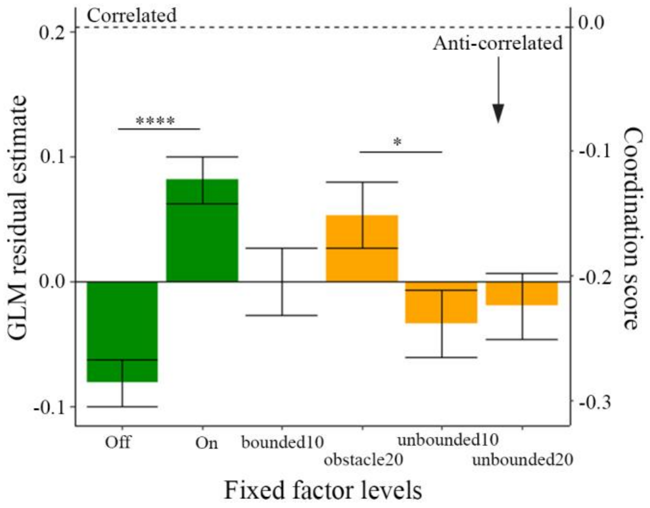
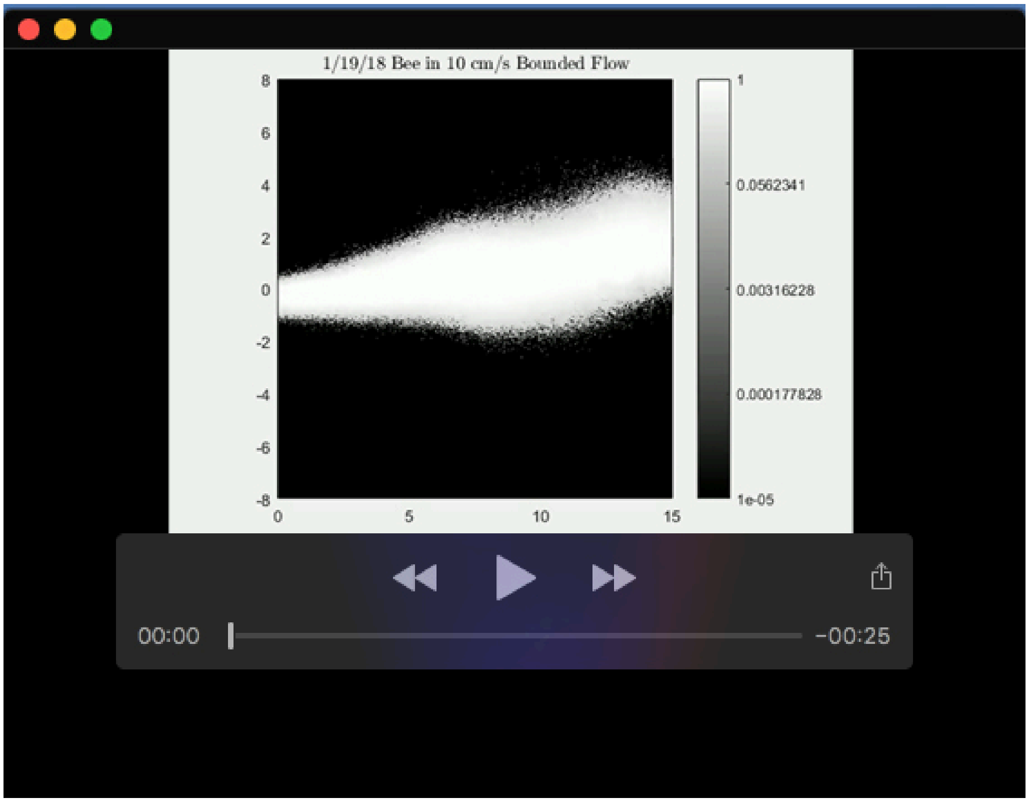
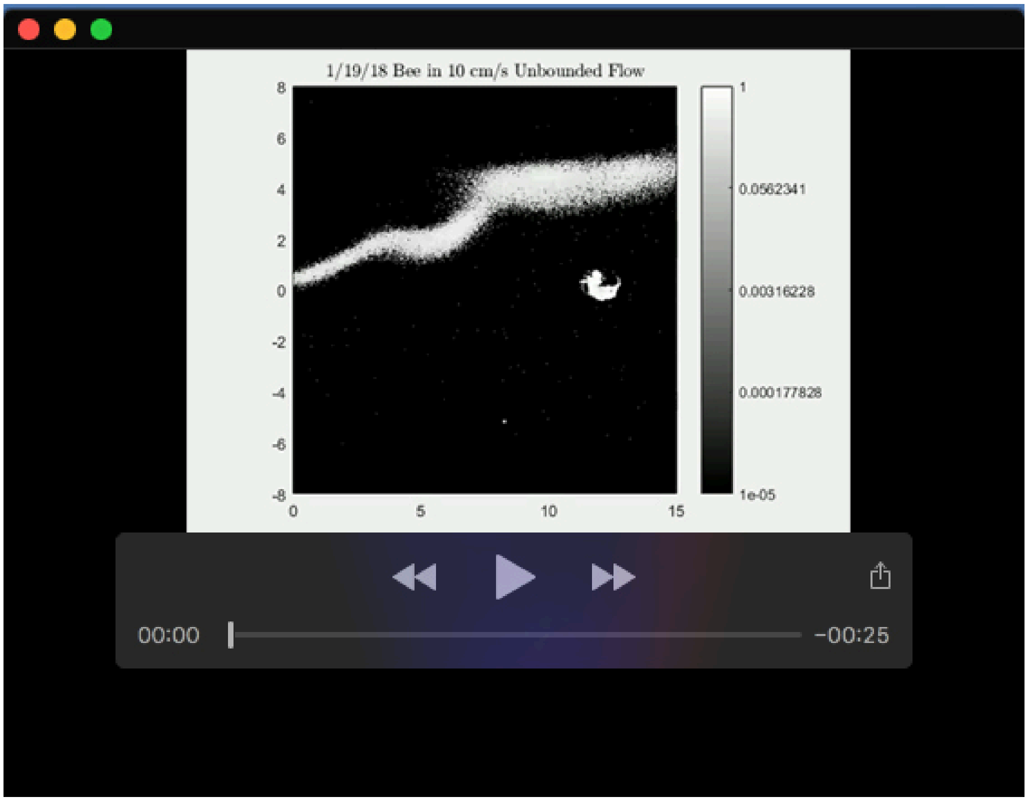


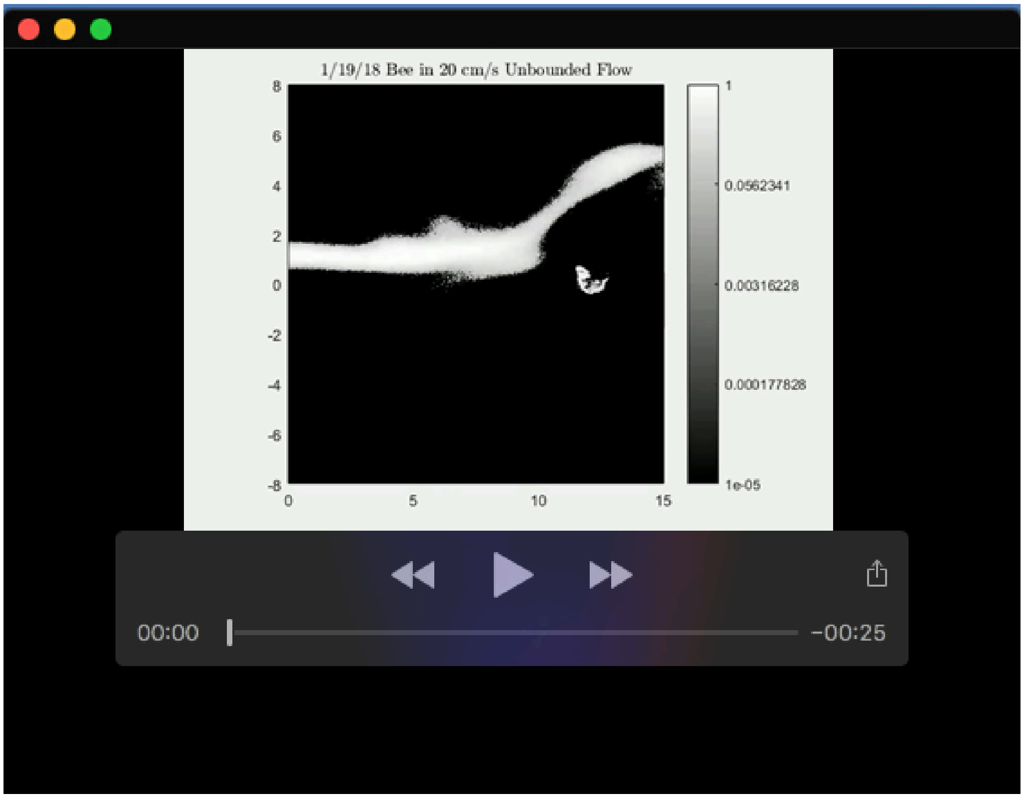
Fig. 6. The GLM residual estimates for the main fixed factors and their modeled antennal coordination (GLM: antennal coordination ~ presence of odor + airflow condition + bee). Plotted on the right is the relationship to antennal coordination. The horizontal dashed line represents 0 antennal coordination, all points below this line denote an anticorrelation. Color denotes fixed factors. Statistical comparisons are only made within fixed factor. Symbols denote p-value: n.s.= non-significant, *= p<0.05, ****= p<0.0001significant



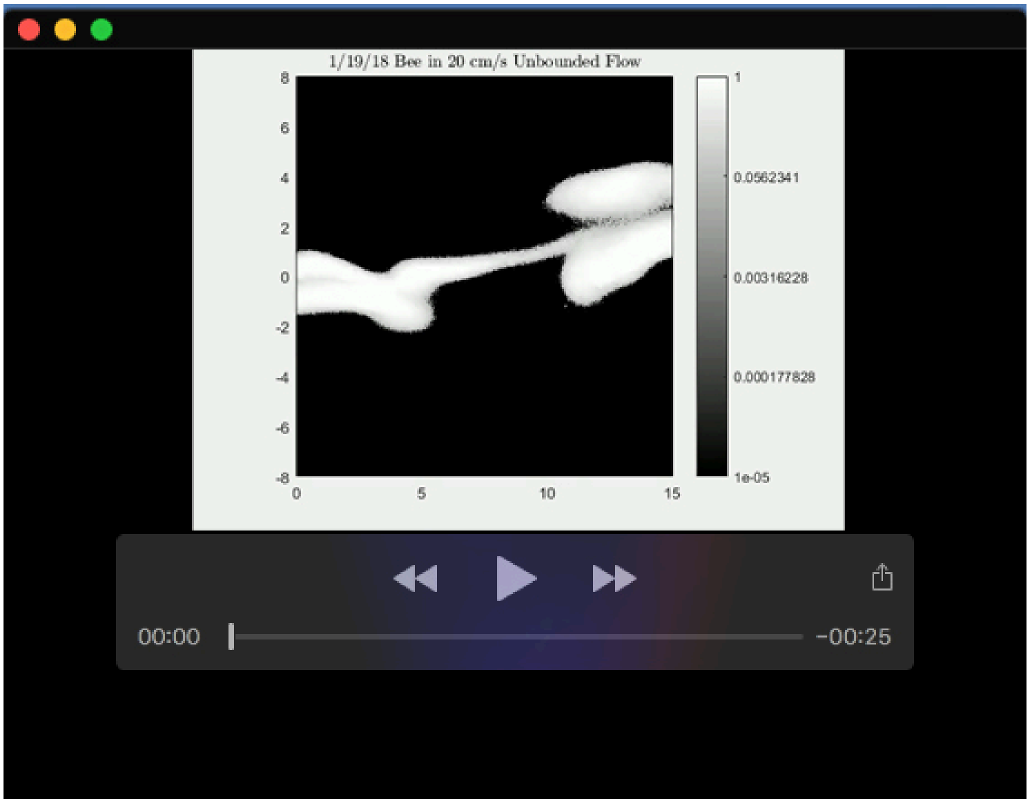
Movie 1. Honey bee in acetone plume bounded at 10 cm/s.



Movie 2. Honey bee in acetone plume unbounded at 10 cm/s.



Movie 3. Honey bee in acetone plume unbounded at 20 cm/s.



Movie 4. Honey bee in acetone plume unbounded at 20 cm/s with obstacle.