We have shown that members of the NM23/NDPK family interact directly and specifically with members of the dynamin superfamily and thus are positioned to maintain high local GTP concentrations and promote dynamin-dependent membrane remodeling. The role of NM23 is well supported by their high NDPK turnover number [k_{cat} 600 s^{-1} per active site (*21*)]. Furthermore, the localization of the NM23-H3 isoform at the mitochondrial outer membrane (fig. S11), where dynamin Drp1 is recruited to mediate mitochondrial fission, suggests that NM23-H3 could assist Drp1 in this process. These findings identify a general mechanism by which different NDPKs maintain high GTP concentration to high-turnover GTPase dynamins for efficient work in different membrane compartments.

REFERENCES AND NOTES

1. S. M. Ferguson, P. De Camilli, *Nat. Rev. Mol. Cell Biol.* **13**, 75–88 (2012).
2. S. L. Schmid, V. A. Frolov, *Annu. Rev. Cell Dev. Biol.* **27**, 79–105 (2011).
3. J. E. Hinshaw, S. L. Schmid, *Nature* **374**, 190–192 (1995).
4. K. Takei, P. S. McPherson, S. L. Schmid, P. De Camilli, *Nature* **374**, 186–190 (1995).
5. S. M. Sweitzer, J. E. Hinshaw, *Cell* **93**, 1021–1029 (1998).
6. A. Roux, K. Uyhazi, A. Frost, P. De Camilli, *Nature* **441**, 528–531 (2006).
7. S. Morlot *et al.*, *Cell* **151**, 619–629 (2012).
8. D. D. Binns *et al.*, *Biochemistry* **39**, 7188–7196 (2000).
9. B. Marks *et al.*, *Nature* **410**, 231–235 (2001).
10. K. S. Krishnan *et al.*, *Neuron* **30**, 197–210 (2001).
11. V. Dammai, B. Adryan, K. R. Lavenburg, T. Hsu, *Genes Dev.* **17**, 2812–2824 (2003).
12. G. Nallamothu, J. A. Woolworth, V. Dammai, T. Hsu, *Mol. Cell. Biol.* **28**, 1964–1973 (2008).
13. M. Boissan *et al.*, *Mol. Cell. Biochem.* **329**, 51–62 (2009).
14. L. Milon *et al.*, *J. Biol. Chem.* **275**, 14264–14272 (2000).
15. M. Tokarska-Schlattner *et al.*, *J. Biol. Chem.* **283**, 26198–26207 (2008).
16. A. M. van der Bliek, Q. Shen, S. Kawajiri, *Cold Spring Harb. Perspect. Biol.* **5**, a011072 (2013).
17. S. M. Ferguson *et al.*, *Dev. Cell* **17**, 811–822 (2009).
18. G. J. Praefcke, H. T. McMahon, *Nat. Rev. Mol. Cell Biol.* **5**, 133–147 (2004).
19. L. Griparic, N. N. van der Wel, I. J. Orozco, P. J. Peters, A. M. van der Bliek, *J. Biol. Chem.* **279**, 18792–18798 (2004).
20. U. Schlattner *et al.*, *J. Biol. Chem.* **288**, 111–121 (2013).
21. I. Lascu *et al.*, *J. Biol. Chem.* **272**, 15599–15602 (1997).

ACKNOWLEDGMENTS

We thank P. De Camilli, E. Smythe, and J. Bertoglio for reagents; S. L. Schmid and M. Mettlen for discussion; the Nikon Imaging Center@Institut Curie & Centre National de la Recherche Scientifique (CNRS) for image acquisition; and I. Hurbain for EM quantification. This work was supported by Institut Curie, CNRS, Fondation ARC pour la Recherche sur le Cancer, Groupement des Entreprises Françaises contre le Cancer, Fondation pour la Recherche Médicale, the Human Frontier Science Program, the Swiss National Fund for Research Grant, and the European Research Council.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/344/6191/1510/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S11
References (*22*–*33*)
Movies S1 to S3

24 March 2014; accepted 5 June 2014
10.1126/science.1253768

SENSORY BIOLOGY

Flower discrimination by pollinators in a dynamic chemical environment

Jeffrey A. Riffell,[1]* Eli Shlizerman,[2] Elischa Sanders,[1] Leif Abrell,[3] Billie Medina,[1] Armin J. Hinterwirth,[1] J. Nathan Kutz[2]

Pollinators use their sense of smell to locate flowers from long distances, but little is known about how they are able to discriminate their target odor from a mélange of other natural and anthropogenic odors. Here, we measured the plume from *Datura wrightii* flowers, a nectar resource for *Manduca sexta* moths, and show that the scent was dynamic and rapidly embedded among background odors. The moth's ability to track the odor was dependent on the background and odor frequency. By influencing the balance of excitation and inhibition in the antennal lobe, background odors altered the neuronal representation of the target odor and the ability of the moth to track the plume. These results show that the mix of odors present in the environment influences the pollinator's olfactory ability.

The olfactory environment is complex and rich, filled with natural, biogenically emitted volatile compounds (volatiles) and closely related volatiles from anthropogenic sources, such as those from combustion engines (*1*–*4*). Insects must successfully discriminate and locate biologically important scents, such as those emitted by food, mates, or hosts, from within this complex mixture (*5*–*8*). How does the insect olfactory system accomplish this task? Our understanding of these effects has been hampered by an inability to measure natural scents at time scales experienced by insects in nature and to link this information with an understanding of how the brain discriminates olfactory stimuli from the background odor landscape.

In the southwest USA, the *Manduca sexta* (hereafter, *Manduca*) moth navigates to, and pollinates, *Datura wrightii* flowers that are separated by hundreds of meters (*9*–*11*). *D. wrightii* (hereafter, *Datura*) often grow in dense stands of creosote bush (*Larrea tridentata*), which emit a high-intensity odor (>100 mg/h) that includes some of the same aromatic volatiles (e.g., benzaldehyde) as the scent of *Datura* (*9*, *12*).

A proton transfer–reaction mass spectrometer, which enables simultaneous measurement of multiple volatiles at subsecond time scales, allowed us to measure the scent plume from *Datura* flowers and characterize its dynamics (Fig. 1A). Measurement of ions from oxygenated aromatics (ARs, e.g., benzaldehyde) and monoterpenes (MOs, linalool and geraniol) showed that the floral plume increased in frequency and decreased in intermittency with increasing distance from the flower (Fig. 1, A to C). The ratio of volatiles in the plume also changed as the background volatiles from neighboring vegetation, including creosote bush plants, became intermixed with the plume (Fig. 1, D and E).

To determine how the changing frequency of the target odor influenced the moth's ability to locate the flower, we used a wind tunnel and a computer-controlled odor-stimulus system to test the moths' response to the *Datura* mixture at different frequencies (1 to 20 Hz) and embedded in different backgrounds [figs. S1 and S2; table S1; see supplementary materials (SM) for details]. Compared with the responses to a mineral oil (no-odor) control, moths exhibited the strongest response to odor pulses of 1 Hz (Fig. 2A_1) (2 × 2 χ^2 test, $P < 0.001$). However, frequencies higher than 1 to 2 Hz resulted in behavior similar to that displayed in response to the no-odor controls (Fig. 2, A_2 and B) (2 × 2 χ^2 test, $P > 0.33$).

We next tested the moths' ability to track the flower-odor plume at a frequency of 1 Hz among a background of different odors, ranging from volatiles that do not occur in the *Datura* mixture [nonoverlapping, ethyl sorbate] to those that do: for instance, (i) volatiles that occur in the flower odor and thereby change the constituent ratio (e.g., benzaldehyde) and (ii) the complex odor background of creosote bush (*L. tridentata*) that also shares volatiles with *Datura*. The volatile background significantly modified the moth's odor-tracking ability (Fig. 2, C and D). For example, when exposed to the *Datura* plume with a background of ethyl sorbate [a volatile that is not in the *Datura* floral odor and chemically dissimilar to constituents of the *Datura* mixture (*10*)], moths navigated to and located the odor source (Fig. 2, C_1 and D) (2 × 2 χ^2 test, $P < 0.001$). By contrast, when challenged with the *Datura* plume in a background of benzaldehyde (a volatile in creosote bush and *Datura* scents), the moth's ability to correctly navigate to the odor significantly decreased (Fig. 2C_2) (2 × 2 χ^2 test relative to *Datura* mixture, $P < 0.01$; χ^2 test relative to no odor control, $P = 0.44$). Similar results occurred

[1]Department of Biology, University of Washington, Seattle, WA 98195–1800, USA. [2]Department of Applied Mathematics, University of Washington, Seattle, WA 98195–3925, USA. [3]Department of Chemistry and Biochemistry and Department of Soil, Water, and Environmental Science, University of Arizona, AZ 85721–0077, USA
*Corresponding author. E-mail: jriffell@u.washington.edu

when the moth was exposed to the plume in the background of geraniol [a volatile that elicits strong antennal lobe (AL) responses (*13*)] or the complex creosote bush scent (Fig. 2D). Thus, altering the background can significantly modify the ability of the moth to discriminate and track the odor.

Neurons on the moth's antennae and in the AL, which is the site of combinatorial processing of olfactory information in the insect brain, are extremely sensitive to the *Datura* mixture (*13*, *14*). We investigated how peripheral and AL neurons processed the *Datura* mixture at different frequencies and how the volatile background modified the representation of the floral scent. Peripheral recordings from the moth's antennae showed that antennal responses were not modified when a background was presented simultaneously with the *Datura* mixture (fig. S3). We next examined whether the downstream AL neurons changed their activity in response to different odor frequencies and backgrounds. Inserting a 16-channel electrode into the moth's AL allowed recordings of neural ensemble activity in response to pulsed and background odor stimuli (Fig. 3A). Similar to the behavioral response, results at both the level of the single neuron and neuronal population showed that the greatest pulse-tracking occurred at a frequency of 1 Hz (fig. S4) (Kruskal-Wallis test, $P < 0.0001$), with higher frequencies not statistically different from the no-odor control (Fig. 3B and figs. S4 and S5) (Kruskal-Wallis test, $P > 0.05$). Similar results occurred when 1-Hz pulses of the *Datura* mixture were presented simultaneously with a background of benzaldehyde [Fig. 3B (middle trace) and fig. S6].

To gain further insight into how the neuronal population represented the odor, as well as how the frequency and background influenced perception, we used an odor-recognition classifier based on the *Datura* representation in the multivariate space (Fig. 3C and fig. S7; tables S2 and S3; see SM for details). In the multivariate space, when the dynamical trajectory representing the neural population responses reaches the prescribed neighborhood of the *Datura* representation, it is counted as evidence, or "recognition" of the given stimulus. We thus were able to compare the recognition scores between the *Datura* mixture at 1 Hz with the *Datura* mixture in different backgrounds or frequencies (Fig. 3C). This analysis showed similar results to those in the behavioral experiments: High odor frequencies (>5 Hz) and certain backgrounds (e.g., benzaldehyde) significantly modified the representation of the *Datura* mixture, which altered the perception of the flower (Fig. 3, D and E).

In addition to the interference caused by the plant volatile background, other volatiles could potentially modify the AL representation of the flower odor, including those that are commonly anthropogenic in origin such as toluene and *p*-xylene, which are chemically similar to benzaldehyde. We examined these effects by using volatile backgrounds at intensities equivalent to those found in urban environments (*1*, *2*) (fig. S2). Experiments showed that toluene and xylene elicited strong antennal responses and activated the same olfactory sensory neurons that responded to benzaldehyde and the *Datura* and creosote bush odors (fig. S8, A to F). Both volatiles influenced the AL representation of the *Datura* mixture (fig. S8, G to I) and significantly decreased the moth's ability to locate the *Datura* flower (fig. S8, J and K). When the moths were preexposed to the odor for 3 hours before testing, these results were exacerbated because of adaptation of these same olfactory "channels." Thus, biogenically or anthropogenically produced volatiles both play important roles in navigation by *Manduca* moths.

Inhibition in the AL is mediated by local interneurons that release γ-aminobutyric acid (GABA) onto projection neurons (PNs), and plays a profound role in odor representation and encoding odor pulses (*15*–*17*). To determine how altering the odor input into the AL (i.e., the composition and ratio of volatiles) also modified the balance of excitation and inhibition, we pharmacologically manipulated the inhibition by superfusing a GABA-receptor antagonist (50 μM CGP54626)

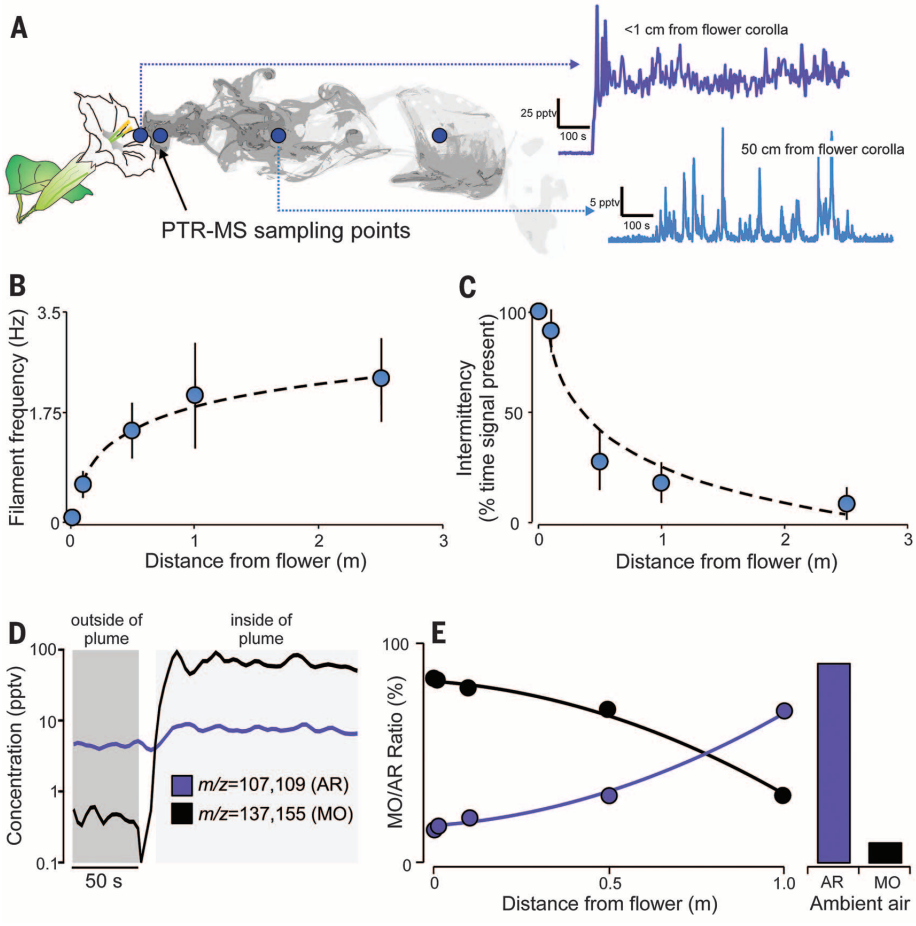

Fig. 1. The plume dynamics of a flower scent. (**A**) Single-ion monitoring (m/z = 137) from the *Datura* flower. (**B**) Odor frequency with increasing distance from the flower. (**C**) Intermittency (percent of time present) with increasing distance from the flower. Each symbol is the mean ± SEM; lines are the fitted regressions ($R^2 > 0.75$, $P < 0.001$). (**D**) Ions indicative of ARs (blue line) and monoterpenoids (black line) 1 cm from the flower. (**E**) (Left) Scatter plot showing the ratio of the percentage of monoterpene (black) and aromatic (blue) ions with increasing distance from the flower. Each symbol is the mean ± SEM; lines are the regressions (R^2 = 0.88, $P < 0.001$). (Right) The ions measured in the ambient environment outside of the plume.

onto the preparation during our experiments. When the vehicle control (saline) was superfused on the AL, neurons typically showed an excitatory response that was time-locked to the duration of the stimulus (Fig. 4A). By contrast, when the GABA-receptor antagonist was superfused, neurons showed a decreased ability to encode the pulses of odor, and the odor representation was significantly modified in a manner similar to that which occurred with the benzaldehyde background [Fig. 4, A (middle trace) and B]. These results were further validated by a computational model that allowed us to simulate different levels of inhibition in the AL (*18*): Decreasing inhibition by ~50% produced results similar to those of the *Datura* mixture embedded in benzaldehyde or those when the GABA antagonist was superfused on the preparation, which suggested a correlation between modifying the odor composition and modulation of the inhibitory circuitry (Fig. 4B and figs. S9 to S11).

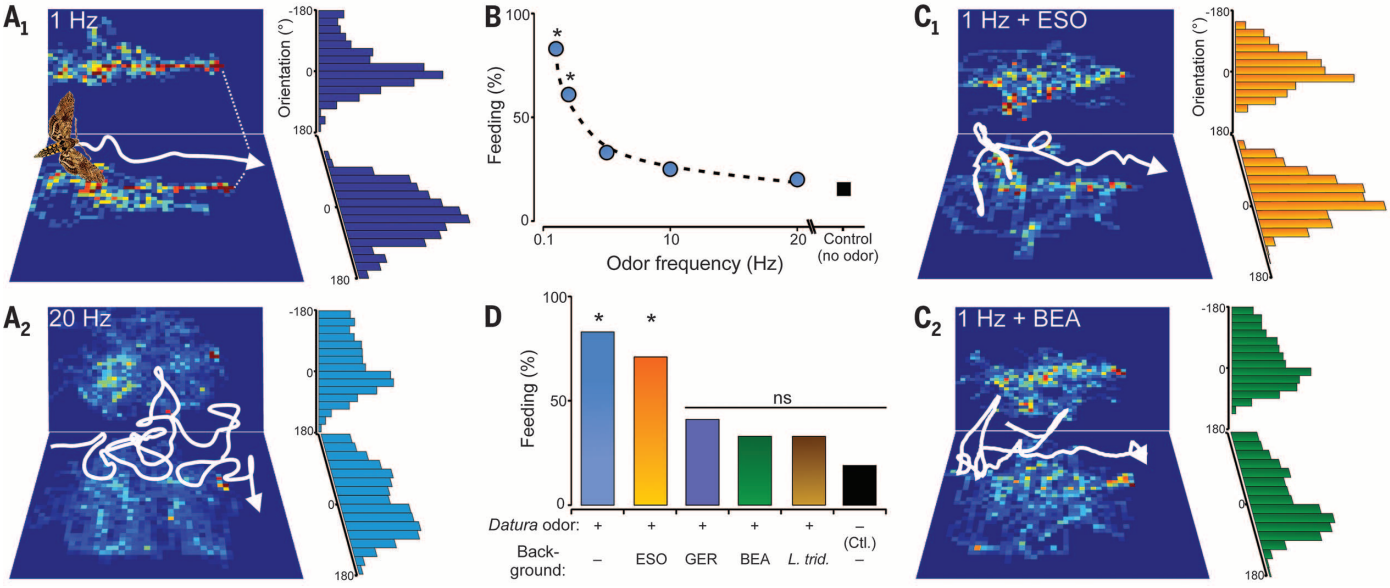

Fig. 2. Flower odor intermittency and background significantly modify moth behavior. (**A**) Flight tracks of moths navigating to an odor plume (white track) pulsed at 1 Hz (A_1) and 20 Hz (A_2). Moth trajectories were plotted as a transit probability plots in the *x*-*y* and *x*-*z* dimensions (left); orientations to the odor (0° origin) are shown as a track-angle distribution (right). (**B**) Increasing odor frequency caused a significant decrease in odor navigation and feeding (regression: $R^2 = 0.97$, $P < 0.01$) to levels not significantly different from control ($2 \times 2\ \chi^2$ test: $P > 0.48$). (**C**) Moth flight tracks to the *Datura* mixture embedded in different backgrounds [C_1: ethyl sorbate (ESO); C_2: benzaldehyde (BEA)]. (**D**) The percentage of moths that successfully navigated to the *Datura* mixture embedded in different backgrounds (N = 20 to 40 moths per treatment). ns indicates not significantly different from control ($P > 0.12$); *$P < 0.001$.

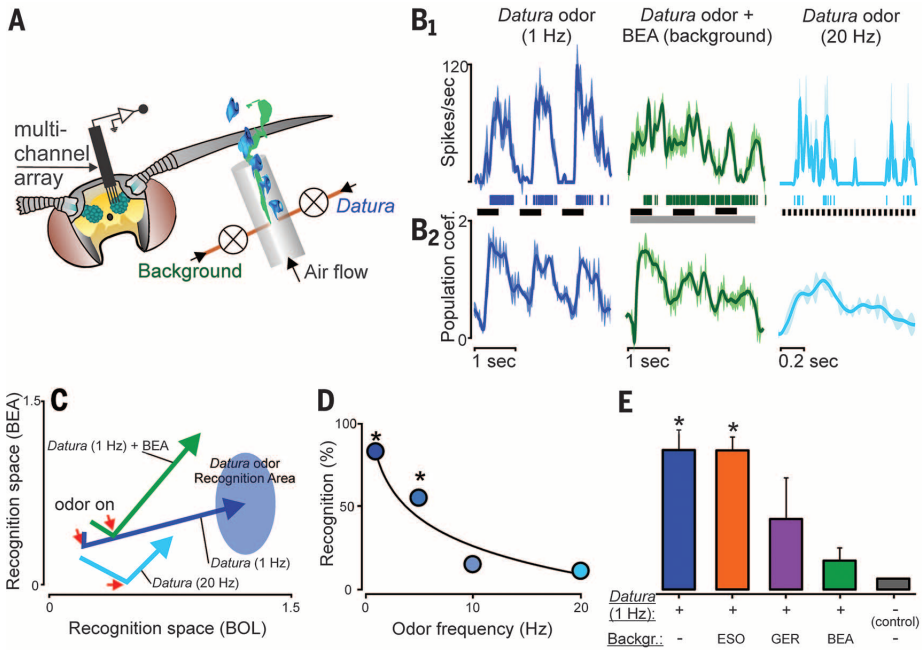

Fig. 3. Antennal-lobe neuronal response to *Datura* mixture at different frequencies and backgrounds. (**A**) Depiction of *Manduca* preparation and the odor delivery system (*Datura* mixture and background stimuli were presented by two solenoid valves). (**B**) Peristimulus time histogram (PSTH) of a single neuron response (B_1) to different stimuli (mean ± SEM from five stimulations). Rasters are plotted below the PSTHs. (B_2) Neural population response. Relative to the 1 Hz frequency, there was significant attenuation in response at the 20 Hz frequency and the BEA background (Kruskal-Wallis test: $P < 0.05$). (**C**) On the basis of the neural population response (10 preparations; N = 153 neurons) to the components in the *Datura* mixture [BEA and benzyl alcohol (BOL)] and 1-Hz pulses of the *Datura* mixture, a recognition region was calculated that describes the area that the trajectory returns to over the course of repeated stimulations. (**D** and **E**) Recognition scores computed as the percentage of time the trajectory spent in the *Datura* mixture recognition region. Increasing the odor frequency (D), or changing the background (E), caused a significant decrease in the recognition scores to levels approaching the control. Background volatiles included ESO, geraniol (GER), and BEA. Line is the regression fit ($R^2 = 0.91$, $P < 0.05$); asterisks denote responses significantly greater than control (Kruskal-Wallis test: $P < 0.05$).

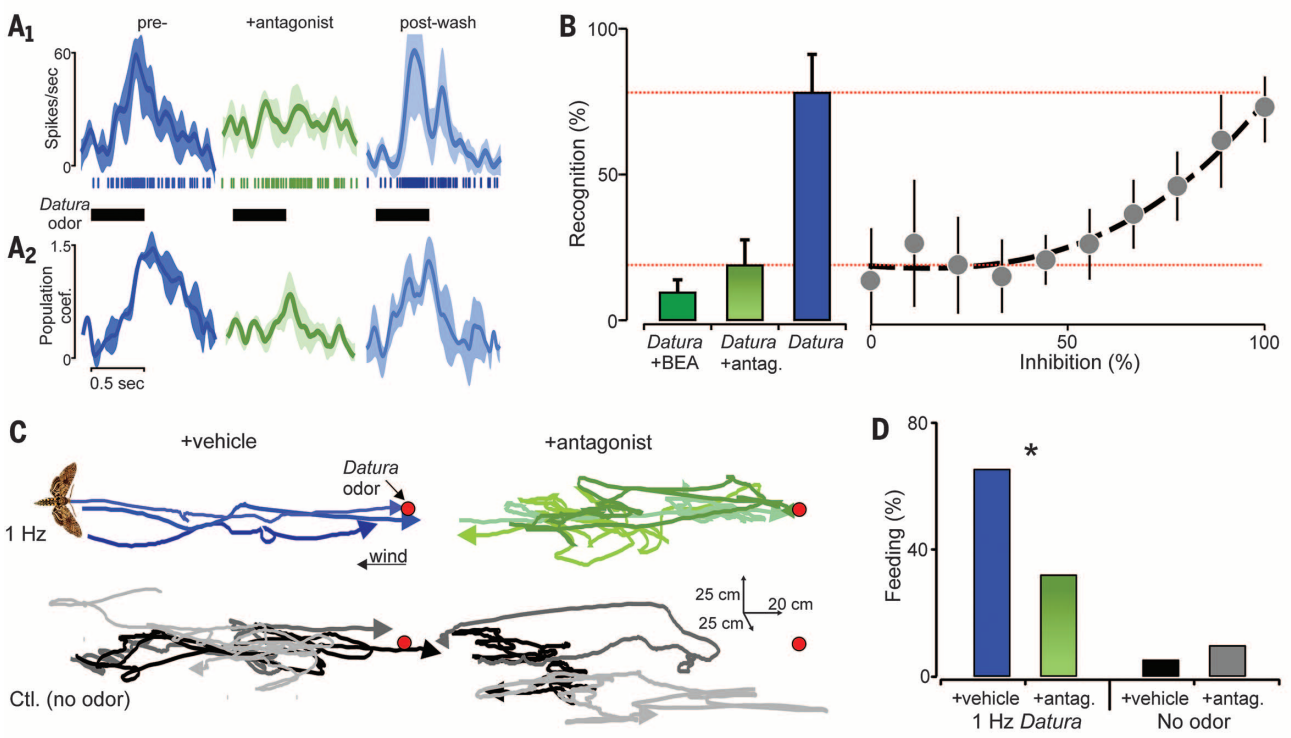

Fig. 4. The effects of inhibition in encoding the odor plume. (**A**) (A_1) PSTHs of a single neuron response and (A_2) population response (12 preparations, $N = 172$ neurons) to the *Datura* mixture (left, blue). Superfusion of the GABA receptor antagonist, (middle, green line), modifies the response to the *Datura* mixture relative to the pre- and postantagonist controls (left and right blue lines; paired *t* tests: $P < 0.05$). Rasters are plotted below the PSTHs; PSTHs are the average ± SEM of five stimulations. (**B**) The recognition scores for the recorded responses (left) in comparison with the model simulated under decreasing inhibition (right). Red lines denote the upper and lower bounds of the experimental manipulations of inhibition. (**C**) Behavioral flight tracks of saline- (left, blue lines) and antagonist-injected moths (right, green lines) to the *Datura* mixture or control (bottom, grey lines). (**D**) Percentage of moths that attempted to feed from the odor source. Asterisks denote a significant difference between saline- (blue bars) and antagonist-injected (green bars) moths (2×2 χ^2-test: $P < 0.05$). Each bar represents 20 to 30 moths.

Modification of the levels of excitation and inhibition in the AL need not indicate that odor navigation is altered or that moths are unable to locate flowers. Thus, we performed wind-tunnel experiments with moths that had either saline (vehicle control) or the GABA-receptor antagonist (CGP54626) microinjected into their ALs. Saline-injected moths navigated to the *Datura* mixture at a stimulus frequency of 1 Hz (Fig. 4, C and D). By contrast, antagonist-injected moths exhibited increased frequencies of cross-wind casting and a lower percentage of moths navigating to the odor (Figs. 4, C and D; fig. S11, C and D). Taken together, these results support the hypothesis that modifying AL inhibition severely alters the perception of the odor and disrupts the moth's ability to successfully navigate to the plume.

Together, our results show that the olfactory background can have important effects on the moth's ability to locate an odor source. At the neuronal level, odor backgrounds affect the ability of neurons to track the odor frequency and interfere with the odor representation through the alteration of the balance of excitation and inhibition. Our results have implications for ecological interactions among plants and their pollinators at the level of the individual flower, where the visual and morphological displays might offset the change of the odor (*19*). Additionally, these results have implications locally, where the plant community might have strong, indirect effects on the distances at which pollinators can recognize certain flowers (*6*, *20*), and, potentially, at larger scales because of the transport of volatiles from urban environments (*21*, *22*).

REFERENCES AND NOTES

1. A. Dorsey, P. McClure, A. McDonald, M. Singh, *Toxicological Profile for Toluene* (U.S. Department of Health and Human Services, Washington, DC, 2000).
2. D. Helmig, J. Arey, *Sci. Total Environ.* **112**, 233–250 (1992).
3. A. Herrmann, *The Chemistry and Biology of Volatiles* (Wiley Online Library, New York, 2010).
4. J. A. Riffell, L. Abrell, J. G. Hildebrand, *J. Chem. Ecol.* **34**, 837–853 (2008).
5. J. Murlis, J. S. Elkinton, R. T. Cardé, *Annu. Rev. Entomol.* **37**, 505–532 (1992).
6. R. Schröder, M. Hilker, *Bioscience* **58**, 308–316 (2008).
7. N. J. Vickers, T. A. Christensen, T. C. Baker, J. G. Hildebrand, *Nature* **410**, 466–470 (2001).
8. R. K. Zimmer, C. A. Zimmer, *J. Chem. Ecol.* **34**, 822–836 (2008).
9. R. A. Raguso, C. Henzel, S. L. Buchmann, G. P. Nabhan, *Int. J. Plant Sci.* **164**, 877–892 (2003).
10. J. A. Riffell *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 3404–3409 (2008).
11. R. E. Stockhouse II, *Am. Midl. Nat.* **96**, 241–245 (1976).
12. K. Jardine *et al.*, *Atmos. Chem. Phys.* **10**, 12191–12206 (2010).
13. J. A. Riffell, H. Lei, T. A. Christensen, J. G. Hildebrand, *Curr. Biol.* **19**, 335–340 (2009).
14. J. A. Riffell, H. Lei, L. Abrell, J. G. Hildebrand, *Science* **339**, 200–204 (2013).
15. T. A. Christensen, B. R. Waldrop, J. G. Hildebrand, *J. Neurosci.* **18**, 5999–6008 (1998).
16. T. Heinbockel, T. A. Christensen, J. G. Hildebrand, *J. Comp. Neurol.* **409**, 1–12 (1999).
17. S. R. Olsen, R. I. Wilson, *Nature* **452**, 956–960 (2008).
18. E. Shlizerman, J. A. Riffell, J. N. Kutz, *Front. Comput. Neurosci.*, in press; preprint available at http://arxiv.org/abs/1311.7450.
19. A. S. Leonard, A. Dornhaus, D. R. Papaj, *J. Exp. Biol.* **214**, 113–121 (2011).
20. D. Thiery, J. Visser, *Entomol. Exp. Appl.* **41**, 165–172 (1986).
21. R. D. Girling, I. Lusebrink, E. Farthing, T. A. Newman, G. M. Poppy, *Sci. Rep.* **3**, 2779 (2013).
22. R. A. Field, M. E. Goldstone, J. N. Lester, R. Perry, *Atmos. Environ. A-Gen.* **26**, 2983–2996 (1992).

ACKNOWLEDGMENTS

The authors thank B. Nguyen, W. Chan, K. Jardine, and J. Thornton for their assistance. This work was supported by funds from the University of Washington (J.A.R.). The electrophysiology data reported in this paper are tabulated in the supplementary materials (database S1).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/344/6191/1515/suppl/DC1
Materials and Methods
Figs. S1 to S12
Tables S1 to S3
References (*23*–*47*)
Database S1

20 January 2014; accepted 22 May 2014
10.1126/science.1251041

Science

Flower discrimination by pollinators in a dynamic chemical environment

Jeffrey A. Riffell, Eli Shlizerman, Elischa Sanders, Leif Abrell, Billie Medina, Armin J. Hinterwirth and J. Nathan Kutz

Science **344** (6191), 1515-1518.
DOI: 10.1126/science.1251041

How hawkmoths sniff out a flower

Pollinators such as butterflies and bees are the true targets of the flower odors we love so much. Though we might imagine insects "following their noses," the wealth of odors in the real world can drown out the smell of a flower, making it hard to find. Riffel *et al.* found that hawkmoths find angel's trumpets by creating a neuronal picture within their antennal lobe, the part of the moth brain that receives olfactory signals from the antennae (see the Perspective by Szyszka). The picture represents both the flower and the background odors. Finding a flower involves a complex reading of both background and target odors, and changes in the background odors—including human pollutants—can hinder the process.

Science, this issue p. 1515; see also p. 1454