Ca^{2+} because ATP potently induced Ca^{2+} increases (Fig. 4C).

The tripartite synapse forms a central element in work implicating astrocytes in long-term potentiation and complex neurocognitive functions such as sleep (*2*, *5*, *16*, *17*). Our analysis has broad implications because it shows that Gq-coupled group I mGluRs are not expressed by adult murine cortical and hippocampal astrocytes or by adult cortical human astrocytes. Astrocytic Ca^{2+} signaling evoked by synaptic release of glutamate may thus be confined to the young rodent pups, which most commonly are employed in studies of neuron-glia signaling (*3*). The observations reported here do not call into question that astrocytes can be indirectly activated by neural activity. A number of transmitters, including endocannabinoids, purines, norepinephrine, and acetylcholine, as well as changes in extracellular Ca^{2+}, can trigger astrocytic Ca^{2+} signaling (*14*, *18*–*21*). Yet, activation of these pathways is typically limited to episodes of intense glutamatergic transmission or to the global release of neuromodulators that occur in the setting of arousal or awakening.

Our analysis shows that adult astrocytes express mGluR3, which is a Gi/Go receptor negatively coupled to adenylate cyclase. Can activation of mGluR3 trigger gliotransmitter release? This seems unlikely, because mGluR3 agonist failed to trigger Ca^{2+} increases in astrocytes (Fig. 4, C and D), and regulated exocytosis generally is enhanced by cyclic adenosine monophosphate (cAMP)–dependent protein kinase (PKA). The slow time scale of Gi/Go-coupled processes likely excludes their involvement in rapid synaptic events. Thus, the existing literature plus the lack of mGluR5-induced Ca^{2+} increases suggest that mGluR3 receptors are not involved in gliotransmitter release.

In particular, our observations indicate that the Gi/Go-coupled suppression of cAMP signaling is the principal intracellular pathway by which astrocytes monitor local glutamatergic transmission in the adult CNS and further suggest that glutamatergic signaling per se is insufficient to trigger astrocytic Ca^{2+} signaling.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S7
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Neural Basis of a Pollinator's Buffet: Olfactory Specialization and Learning in *Manduca sexta*

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Pollinators exhibit a range of innate and learned behaviors that mediate interactions with flowers, but the olfactory bases of these responses in a naturalistic context remain poorly understood. The hawkmoth *Manduca sexta* is an important pollinator for many night-blooming flowers but can learn—through olfactory conditioning—to visit other nectar resources. Analysis of the flowers that are innately attractive to moths shows that the scents all have converged on a similar chemical profile that, in turn, is uniquely represented in the moth's antennal (olfactory) lobe. Flexibility in visitation to nonattractive flowers, however, is mediated by octopamine-associated modulation of antennal-lobe neurons during learning. Furthermore, this flexibility does not extinguish the innate preferences. Such processing of stimuli through two olfactory channels, one involving an innate bias and the other a learned association, allows the moths to exist within a dynamic floral environment while maintaining specialized associations.

Floral scent mediates a range of plant-pollinator associations, from specialized interactions (*1*–*3*) to those that are more generalized through associative learning of the scent and nectar reward by the pollinator (*1*, *4*–*7*). Although it is generally assumed that many flower species may have converged on a similar scent profile to attract a specific pollinator or pollinator class, identification of the compounds mediating the innate responses and how learning alters the behavior remain unclear. The hawkmoth *Manduca sexta* has a wide geographic distribution and is an important pollinator for many night-blooming plants, which make it an excellent system in which to explore the neural basis of olfactory specialization and learning. *M. sexta* has an innate olfactory preference for certain night-blooming plants that exhibit characteristics typical of moth-pollinated flowers (for example, *Datura wrightii* flowers). The moths, however, also have the ability to learn, through olfactory conditioning, to utilize other floral resources (*5*, *8*–*10*).

We characterized the floral scents of plant species that we and others have observed to be visited and pollinated by *M. sexta* moths (supplementary materials, table S1). To help control for phylogenetic differences between plant species, we also characterized the floral scents of related plant species that have more generalized pollinator associations. We collected floral volatile organic compounds (VOCs) using dynamic sorption methods and analyzed the trapped mixtures by gas chromatography with linked mass spectrometry (GCMS) (*11*, *12*). Although many of the hawkmoth-visited flowers differed qualitatively and quantitatively in their scent profiles, their scents were often dominated by oxygenated aromatic compounds (35 to 90% of the total floral headspace VOCs), especially methyl benzoate, benzyl alcohol, and benzaldhyde (Fig. 1A). By contrast, flowers that are visited by other pollinator taxa were dissimilar in their scents in comparison with the hawkmoth-visited flowers (Fig. 1). Moreover, similarity in scent composition between species

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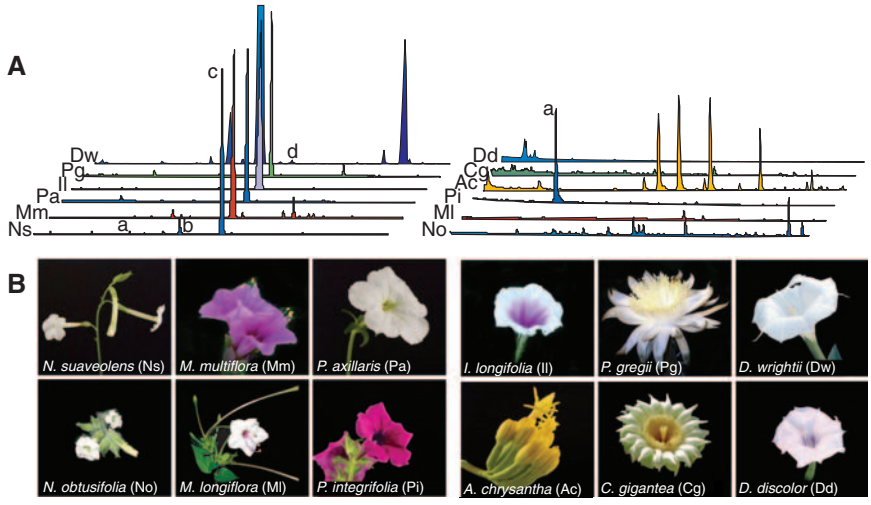

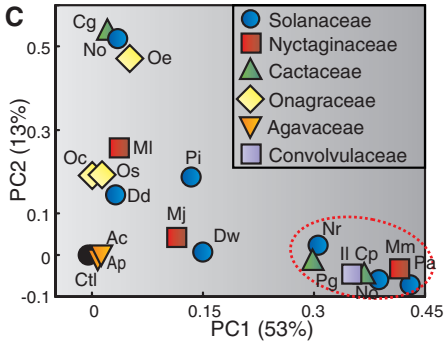

Fig. 1. Floral scent composition and similarity. (**A**) Total ion mass chromatograms of the scent from flowers visited by *M. sexta* (left) and species that have more generalized associations (right). Bioactive constituents are benzaldehyde (a), benzyl alcohol (b), methyl benzoate (c), and linalool (d). (**B**) The flowers analyzed in (**A**). (**C**) Principal components analysis (PCA) plot based on the composition of the floral scent identified by GCMS from 19 flower species. Flower species are abbreviated according to species name (table S1). Dotted red oval indicates those flowers with aromatic-dominant scents that are not significantly different from one another (analysis of variance: $P > 0.01$).

did not group according to phylogenetic relatedness (Fig. 1C), which suggested that the flowers may have converged on a similar sensory signal to attract hawkmoths.

To gain insight into the representation of floral scents in the olfactory system of the moth, we conducted electrophysiological recording experiments on the moth's antenna and in the antennal (olfactory) lobe (AL). When the ipsilateral antenna was stimulated with VOCs from the aromatic-rich flowers, projection (output) neurons in the anterior region of the AL responded strongly (Fig. 2A). Moreover, single-unit and neural-ensemble responses to the hawkmoth-preferred floral VOCs were not significantly different from one another (Fig. 2, B and C; Kruskal-Wallis test: $P = 0.69$), but were significantly different from ensemble responses to the VOCs of flowers visited by other pollinator taxa (Fig. 2, B to F; Kruskal-Wallis test: $P < 0.001$). Further recordings in the periphery and AL showed that oxygenated aromatics elicited the strongest responses and that the moth-visited flower species were processed similarly (figs. S1 and S2). These findings suggest a common olfactory percept of the aromatic-rich flower species even when the scents differed in composition and complexity.

But does olfactory response necessarily reflect behavioral preference by the moths? To answer this question, we performed behavioral experiments with naïve moths. Moths were exposed, one at a time, to an array of visually identical artificial

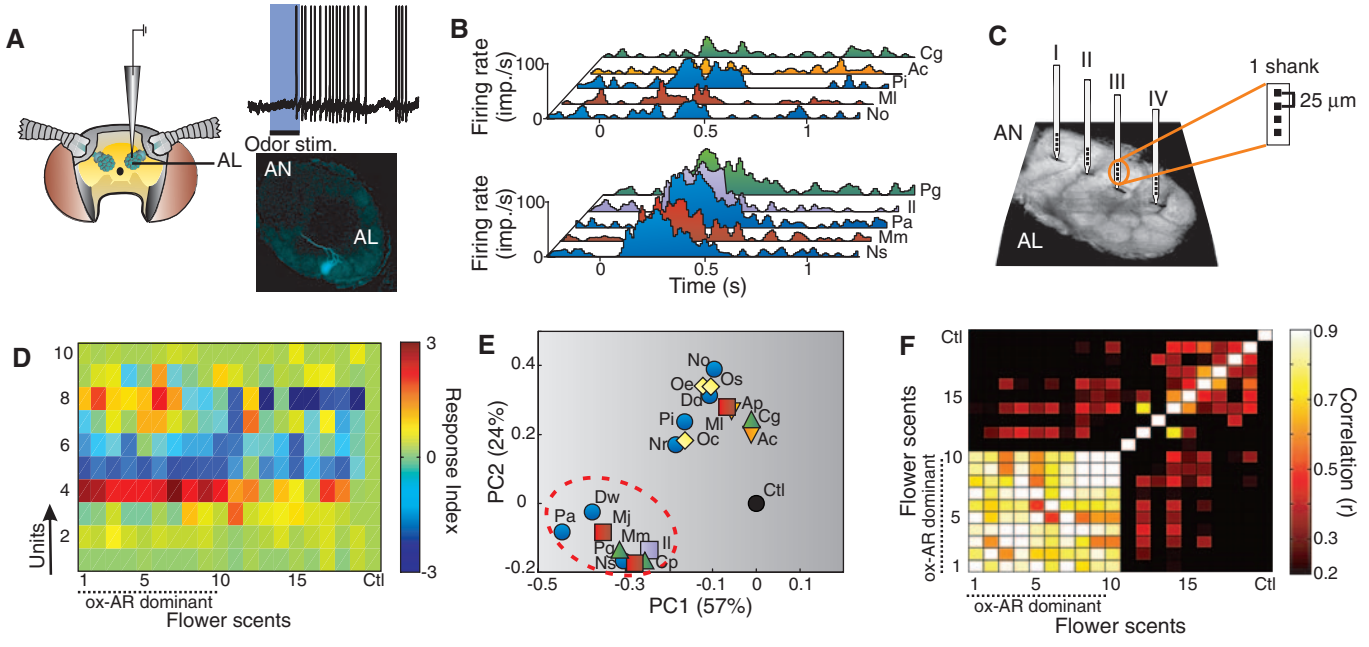

Fig. 2. Antennal-lobe neuronal response to floral scents. (**A**) Intracellular morphology and recordings of PN responses to scents from hawkmoth-visited flowers. AL, antennal lobe; AN, antennal nerve. (**B**) PN responses to scents from flowers not visited by *M. sexta* (top) and hawkmoth-visited flowers (bottom). (**C**) Confocal-microscopic image of AL showing the locations of the recording sites of the multichannel array used to record the neural ensemble responses. (**D**) Ensemble response to floral scents. (**E**) PCA of the firing-rate responses of the ensemble of neurons to the different floral scents. The red oval indicates not significantly different responses to hawkmoth-visited floral scents (Kruskal-Wallis test: $P > 0.10$). (**F**) Correlation coefficients between ensemble responses to the different flower scents.

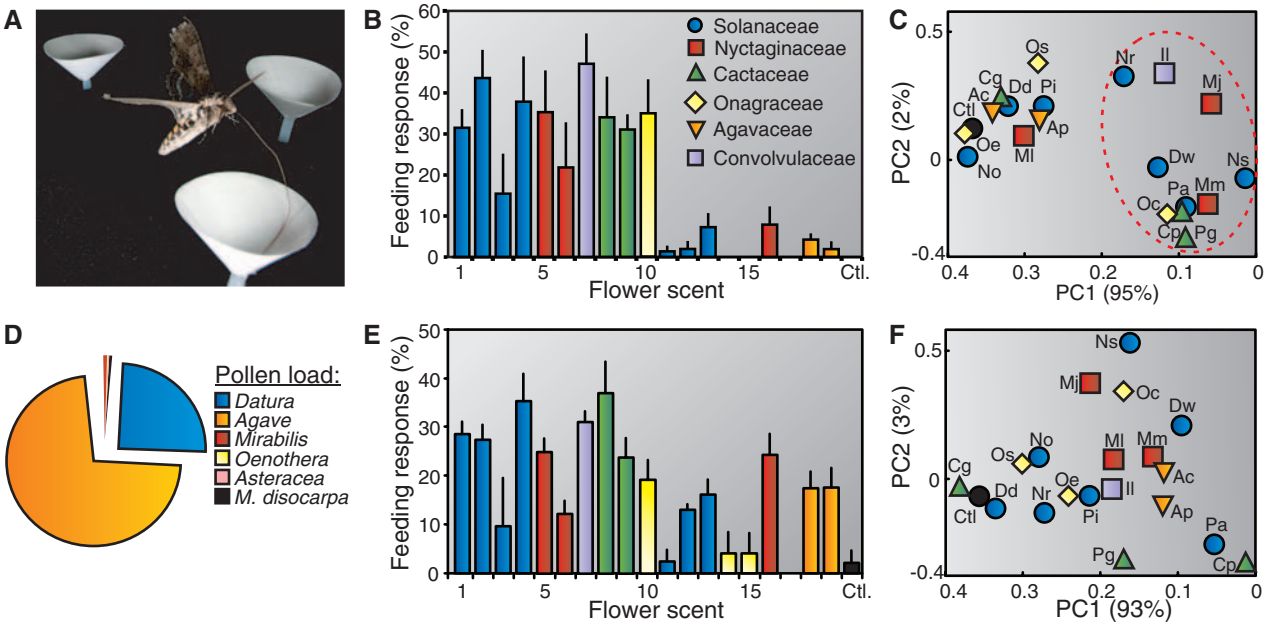

Fig. 3. (**A**) Image of a *M. sexta* moth attempting to feed from one in an array of 20 artificial flowers used to test the attractiveness of different floral scents. (**B**) Feeding responses by lab-reared *M. sexta* to the different floral scents. (**C**) PCA plot of the behavioral responses (table S3) to the different scents. Dashed red oval indicates those hawkmoth-visited floral scents that were not significantly different ($P > 0.05$). (**D**) Pollen species found on the proboscises of wild *M. sexta*. (**E** and **F**) Wild *M. sexta* feeding responses (E) and PCA plot of behavioral responses (F) to the different floral scents.

(paper) flowers, each emitting the VOC mixture from a different floral species (Fig. 3A). Results of these experiments showed that naïve moths attempted to feed from flowers emitting the aromatic-rich scents, and the naïve moths visited these flowers at approximately similar frequencies (Fig. 3B; two-by-two χ^2 test: $P > 0.05$). By contrast, scents of the majority of floral species that lacked the oxygenated aromatic compounds elicited significantly lower visitation and feeding rates (Fig. 3B; two-by-two χ^2 test: $P < 0.002$). Thus, the scents of hawkmoth-visited flowers were processed, and perceived, similarly by the moths to drive innate behavior.

A fundamental component of pollinator behavior is the flexibility to switch among floral resources on the basis of the pollinator's ability to learn (*7*, *13*), but how learning shapes the innate responses of pollinators remains unexplored. We therefore took advantage of the association between *M. sexta* moths and bat-adapted *Agave palmeri* flowers that occurs in the semi-arid Sonoran Desert of southern Arizona (*5*, *14*). *M. sexta* moths can learn to feed from the bat-adapted *A. palmeri* flowers by associating its abundant nectar reward with its floral scent, which is not innately attractive to moths (*5*). When wild moths that had previous experience with *Agave*, *Datura*, and *Mirabilis* spp. were exposed to the flower array, moths responded to those specific floral scents, but like naïve animals, they also responded to the other flowers with the aromatic-rich scents (Fig. 3, E and F). For more controlled examination of the effects of prior experience with flowers on moth preferences, we used laboratory-reared moths that had learned to feed from *A. palmeri* flowers (*5*). As with wild moths, *A. palmeri*–experienced moths also attempted to feed from flowers emitting *Agave* VOCs and from the hawkmoth flowers (table S5). Thus, olfactory conditioning provided moths with flexibility in their foraging behavior but did not extinguish their innate preferences for scents from the moth-pollinated flowers.

To determine how the olfactory system processes odor information as the moth learns, we performed experiments involving simultaneous multichannel AL recording and olfactory conditioning (Fig. 4A and fig. S3) (*15*). This approach allowed us to correlate the neural and behavioral responses directly as the animal learned the association between the *A. palmeri* scent and sugar reward (fig. S3) (*16*, *17*). After three training trials, the moths exhibited significantly increased proboscis-movement responses (PMRs) from 0 to 33% (χ^2 test: $P < 0.01$). The ability to learn the scent association was directly correlated with the change in AL neural responses evoked by the VOCs (Fig. 4B). As the moths learned, the firing rate of AL neurons significantly increased, response latency decreased, firing synchrony between neurons increased, and the pattern of neural activity by the ensemble changed significantly (Fig. 4 and fig. S4; Kruskal-Wallis test: $P < 0.02$). By contrast, moths that were not rewarded or for which the reward was paired randomly with VOC stimulation had significantly lower PMRs than the trained moths and did not learn the association (0% PMR for both treatments; χ^2 test: $P < 0.001$). Furthermore, AL neuronal activity in the control moths did not change significantly over the course of the experiment (fig. S4; Kruskal-Wallis test with multiple comparisons: $P \geq 0.10$). Last, differential conditioning experiments were conducted to determine how learning influences the coding of a scent paired with a reward (CS+; *A. palmeri*) versus an unpaired scent (CS–; *Carnegiea gigantea*). These experiments demonstrated that moths could readily learn to discriminate between the two complex scents and that modulation of the AL ensemble served to increase the difference in representation of the two scents (fig. S5; Kruskal-Wallis test: $P < 0.05$). Thus, learning the association between an ecologically important floral scent and sugar-water reward significantly modified AL neural responses and increased the contrast between scents.

The change in the AL associated with learning leads to the question of how this plasticity occurs. Biogenic amines—in particular, octopamine and dopamine—have been shown in other insects to participate in the acquisition of olfactory memory and modulation of AL neural circuitry (*18*–*21*). Because putative receptors for octopamine and dopamine are expressed in the *M. sexta* AL (*10*, *22*), we injected receptor antagonists or agonists into the AL before training the moths to associate the *A. palmeri* VOCs with a sugar reward. Results of these experiments demonstrated that microinjection of dopamine-receptor antagonists {fluphenazine [10^{-8} M], Le300 [10^{-6} M], or SCH23390 [10^{-6} M]} had no effect on the ability of moths to associate the reward with the *A. palmeri* odor and were not significantly different from the results obtained with control moths (two-by-two χ^2 test: $P \geq 0.55$). By contrast, injection of octopamine-receptor antagonists {epinastine or mianserine [10^{-6} M]} prevented the moths from learning the odor-reward association and elicited significantly lower PMRs

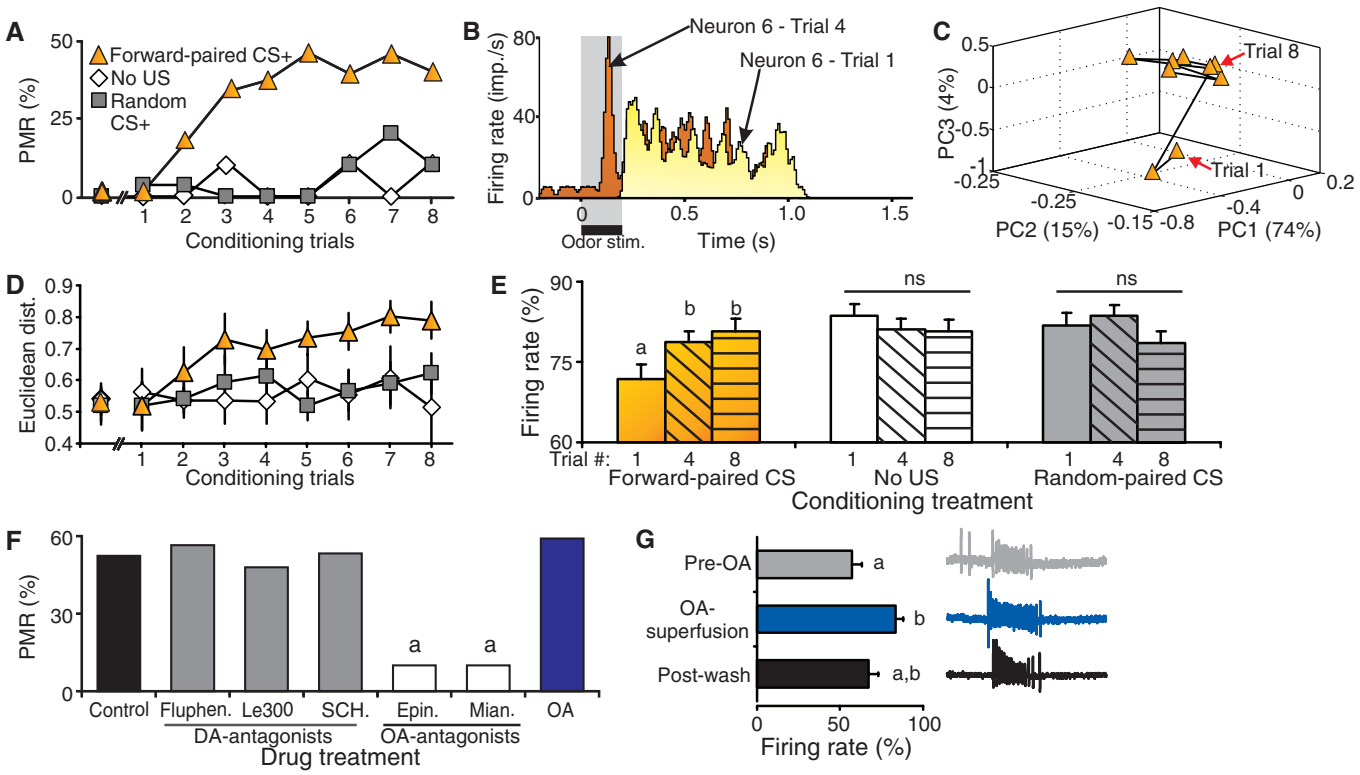

Fig. 4. Olfactory learning modifies the neural representation of the scent in the AL. (**A**) Olfactory learning during classical forward-paired conditioning experiments with *A. palmeri* scent and sucrose reward (orange triangles). Moths did not learn the association in the absence of the unconditioned stimulus (white diamonds), or the random paired conditioned stimulus (CS)–unconditioned stimulus (US) treatment (gray circles). (**B**) Response of an AL neuron to stimulation with *A. palmeri* scent at trial 1 (yellow) and trial 4 (orange). (**C**) PCA plot of the ensemble responses (e.g., PC1) to the *A. palmeri* scent through training. (**D**) Change in Euclidean distance of the ensemble representation of the *A. palmeri* scent during training. (**E**) Change in firing rate of AL neurons during trials 1, 4 (diagonal bars), and 8 (hashed bars) for moths in the different conditioning treatments: forward-paired (orange bars), no-US reward (white bars), and random-paired (gray bars). (**F**) In individual moths, focal microinjection of receptor antagonists [dopamine-receptor antagonists (DA) or octopamine-receptor antagonists (OA)], and agonist (OA) were conducted before conditioning. (**G**) Superfusion of octopamine significantly increased the firing rate of PN responses. Letters denote a significant difference between treatments ($P < 0.05$).

compared with the vehicle control (Fig. 4F; two-by-two χ^2 test: $P < 0.01$). Moreover, when octopamine (at 10^{-5} M) was injected into the AL and moths were stimulated with the VOCs without a paired sugar reward, these moths exhibited significantly higher PMRs in reaction to the VOC stimulus than did moths that were injected with the vehicle (Fig. 4F; two-by-two χ^2 test: $P < 0.001$). Finally, superfusion of octopamine (10^{-5} or 10^{-6} M) onto the AL during multichannel and juxtacellular neural recordings elicited effects similar to those observed when the moth learned. Neurons showed increased VOC-evoked firing rates, response latencies decreased, and firing synchrony between neurons increased (Fig. 4G and fig. S6). Thus, octopamine apparently acts as a key neuromodulator in the moth's olfactory pathway to mediate interactions with flowers in the field.

When considering the effects of learning on innate preferences, it is important to examine the strength or evolutionary importance of the association. Our results show that learning does not extinguish responses to the innately attractive floral scents and, thus, suggest that olfactory conditioning may operate in an olfactory "channel" separate from, but parallel to, that involved in the innate responses. In the case of *M. sexta*, many, but not all, of the flowers that elicit innate responses are also solanaceous plants that are host plants for ovipositing females. In such cases, the flower may signal an appropriate host or a site at which to locate a mate, and thus, the strength of the association may be a function of the important life-history events that are unrelated to the immediate foraging responses of the moths. However, some of the flowers that emit the aromatic-rich scents that elicit innate responses are not host plants, which raise the question of how these diverse species have evolved similar floral scents to attract hawkmoths. We here provide impetus for such future work by showing that moths perceive these diverse floral species similarly.

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Ezh2 Orchestrates Topographic Migration and Connectivity of Mouse Precerebellar Neurons

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We investigated the role of histone methyltransferase *Ezh2* in tangential migration of mouse precerebellar pontine nuclei, the main relay between neocortex and cerebellum. By counteracting the sonic hedgehog pathway, *Ezh2* represses *Netrin1* in dorsal hindbrain, which allows normal pontine neuron migration. In *Ezh2* mutants, ectopic *Netrin1* derepression results in abnormal migration and supernumerary nuclei integrating in brain circuitry. Moreover, intrinsic topographic organization of pontine nuclei according to rostrocaudal progenitor origin is maintained throughout migration and correlates with patterned cortical input. *Ezh2* maintains spatially restricted *Hox* expression, which, in turn, regulates differential expression of the repulsive receptor *Unc5b* in migrating neurons; together, they generate subsets with distinct responsiveness to environmental *Netrin1*. Thus, *Ezh2*-dependent epigenetic regulation of intrinsic and extrinsic transcriptional programs controls topographic neuronal guidance and connectivity in the cortico-ponto-cerebellar pathway.

In mammals, cortical motor and sensory information is mostly relayed to the cerebellum via the hindbrain precerebellar pontine nuclei (PNs), which include pontine gray and reticulotegmental nuclei. The developing hindbrain is rostrocaudally segregated into progenitor compartments, or rhombomeres (r1 to r8) (*1*), genetically defined by nested *Hox* gene expression (*2*). Mouse PN neurons are generated from r6 to r8 lower rhombic lip progenitors (*3*), undergo a long-distance caudorostral tangential migration via the anterior extramural stream (AES), and settle beside the ventral midline (Fig. 1, A and B) (*4, 5*). Intrinsic expression of transcription factors and guidance receptors and extrinsic distribution of ligands are important for AES migration (*6*-*8*). However, little is known about the epigenetic regulation of these transcriptional programs. Here, we addressed the role of *Ezh2*, which is member of the Polycomb repressive complex 2 and trimethylates histone H3 at lysine 27 (H3K27me3) (*9*).

Ezh2 transcripts are maintained through late stages in lower rhombic lip progenitors, migratory stream, and PN neurons (fig. S1), whereas H3K27m3 is detected throughout the hindbrain (fig. S2). To conditionally inactivate *Ezh2*, we generated transgenic lines in which *Cre* is driven by rhombomere-specific enhancers in spatially restricted regions tiling the caudal hindbrain (*10*) (figs. S3 and S4). To assess cell-autonomous and/or region-specific non–cell autonomous *Ezh2* function in pontine neuron migration, we first crossed *Krox20::Cre* (*10*) to an $Ezh2^{fl/fl}$ allele (*10*) (*Krox20::Cre;*$Ezh2^{fl/fl}$). Inactivation in r3

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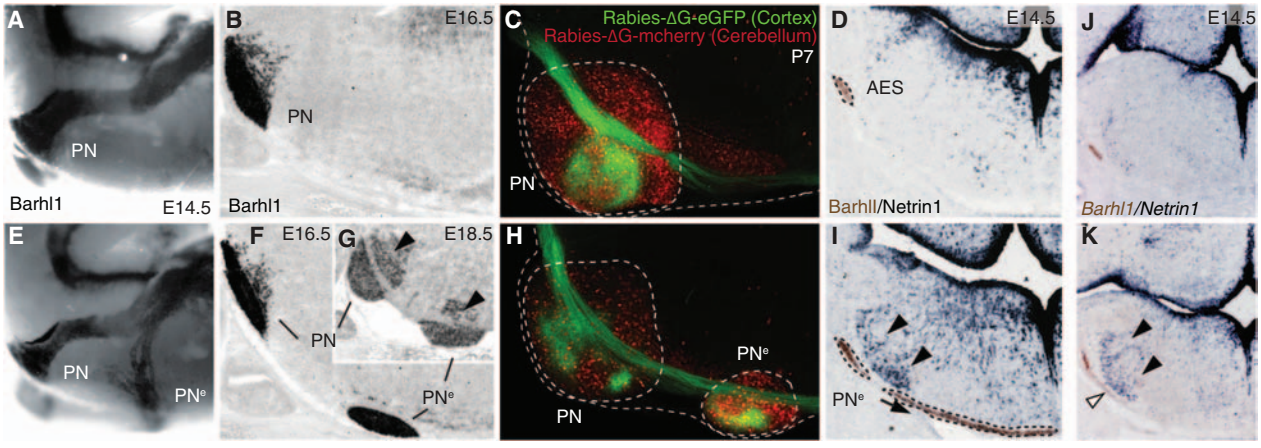

Fig. 1. *Ezh2* non–cell autonomous role in pontine neuron tangential migration. (**A**, **B**, **E**, **F**, and **G**) Migratory phenotypes in control (A) and (B) and *r5-6::Cre;*$Ezh2^{fl/fl}$ mutants (E) to (G). *Barhl1* in situ hybridization in E14.5 whole-mount (A) and (E), E16.5 (B) and (F), and E18.5 (G) sagittal sections. Pontine gray and reticulotegmental [arrowheads (G)] nuclei (PNs) are duplicated (PN^es). (**C** and **H**) Tracings from P7 cortex (rabies-ΔG-eGFP) and cerebellum (rabies-ΔG-mCherry) in controls (C) and *r5-6::Cre;*$Ezh2^{fl/fl}$ mutants (H). PNs and PN^es are connected to cortex and cerebellum. (**D**, **I**, **J**, and **K**) *Barlh1/Netrin1* expression in E14.5 control (D), *r5-6::Cre;*$Ezh2^{fl/fl}$ (I), *r5post::Cre;*$Ezh2^{fl/fl}$*;*$Shh^{fl/+}$ (K), and *r5post::Cre;*$Ezh2^{fl/fl}$*;*$Shh^{fl/fl}$ (J) coronal sections. Ectopic *Ntn1* [arrowheads: (I) and (K)] and PN^e ectopic migration [arrow and white arrowhead, respectively: (I) and (K)] are partially rescued (J).