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Peripheral and Central Olfactory Tuning in a Moth

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Abstract

Animals can be innately attracted to certain odorants. Because these attractants are particularly salient, they might be expected to induce relatively strong responses throughout the olfactory pathway, helping animals detect the most relevant odors but limiting flexibility to respond to other odors. Alternatively, specific neural wiring might link innately preferred odors to appropriate behaviors without a need for intensity biases. How nonpheromonal attractants are processed by the general olfactory system remains largely unknown. In the moth *Manduca sexta*, we studied this with a set of innately preferred host plant odors and other, neutral odors. Electroantennogram recordings showed that, as a population, olfactory receptor neurons (ORNs) did not respond with greater intensity to host plant odors, and further local field potential recordings showed that no specific amplification of signals induced by host plant odors occurred between the first olfactory center and the second. Moreover, when odorants were mutually diluted to elicit equally intense output from the ORNs, moths were able to learn to associate all tested odorants equally well with food reward. Together, these results suggest that, although nonpheromonal host plant odors activate broadly distributed responses, they may be linked to attractive behaviors mainly through specific wiring in the brain.

Key words: innate preference, insect, mushroom body, odor, olfactory learning, olfactory receptor neuron

Introduction

Olfaction provides critical cues animals need to locate food, oviposition sites, and mates (Karlson and Butenandt 1959; Renwick and Chew 1994; Raguso and Willis 2002). Many animals are, without prior learning experiences, attracted to species-specific subsets of volatiles. A well-studied example is the pheromone system in moths. In the antennae of male moths, a large fraction of olfactory receptor neurons (ORNs) is specifically tuned to respond to female sex pheromones (Kaissling 1996), providing the male moths great sensitivity to the presence of females. Information about sex pheromones gathered in the periphery is relayed to a dedicated neural circuit consisting of a small number of sex-specific glomeruli within the macroglomerular complex in the antennal lobe (AL, the first olfactory brain center) where subtle features in the composition and timing of sex pheromones are analyzed to identify those arising from conspecifics (Christensen and Hildebrand 1987).

In addition to pheromones, some floral volatiles that indicate appropriate food sources and mating sites are also innately attractive to nectarivorous animals including moths (Raguso and Willis 2002; Kessler and Baldwin 2007). Unlike pheromones, though, floral volatiles evoke activity in a broad assortment of ORNs, many of which respond to a range of different odorants (Hallem and Carlson 2006; Raman et al. 2010), and an assortment of distributed broadly responsive glomeruli (Galizia et al. 2000; Sandoz 2006; for review: Galizia and Menzel 2000) and populations of projection neurons (PNs), which respond to odorants with temporally complex firing patterns (Laurent and Davidowitz 1994; Laurent et al. 1996; Ito et al. 2008; for review: Kay and Stopfer 2006). It is not clear that circuit design features enabling pheromone detection also apply broadly for innately preferred general, nonpheromonal odors. How could this work? If host plant odors invoke relatively intense odor responses from the antenna, as do sex pheromones, detecting host plant odors could be a matter of detecting especially intense responses in a subset of PNs. Alternatively, specific wiring and network architecture might perform the task without needing such amplification.

Animals need to balance the benefits of circuitry specialized for detecting critical odorants with the flexibility of adapting to unpredictable and changing environmental conditions. What design strategies might animals use to cope with these 2 needs that compete for limited biological resources? We addressed this by testing potential sites of odor tuning in the olfactory pathway of the moth *Manduca sexta*. By delivering a set of specially diluted odorants (host and nonhost plant volatiles, and synthetic odorants not found in nature), we observed the following: 1) moth antennae provided responses of differing intensity to odorants that had been mutually diluted to equivalent vapor pressure, but, overall, the responses to volatiles released by host plants were not stronger than responses to other volatiles; 2) when odorants were mutually diluted to elicit equally intense output from the antenna, the moth AL faithfully transmitted those signals to the next target, the mushroom body (MB), without providing differential amplification; 3) moths were able, with similar efficacy, to learn to associate all tested odorants with food reward. Together, our results suggest that innate preferences for host plant odors in the moth are unlikely to arise from the differential strengths of signals originating in the antenna. At other locations, the general olfactory system in moth appears to process and associatively learn a broad range of odors similarly. This flexibility should improve the survival fitness of animals in case of unfavorable circumstances when primary food sources are not available.

Materials and methods

Experimental animals

Moths (*M. sexta*) were reared from eggs (NCSU Insectary) in our laboratory as described elsewhere (Ito et al. 2008). Only female *Manduca* at least 2 days posteclosion were used in this study.

Olfactory stimulation

Our odor stimulation method was modified from a previous study (Ito et al. 2008). Briefly, the odorized headspace in 60-mL glass bottles above mineral oil–diluted odorant solution was pushed by a controlled volume of clean air (0.75 L min^{-1}) into an activated carbon–filtered air stream (0.75 L min^{-1}) that flowed continuously across the antenna. Odorants were then continuously drawn away by a large vacuum funnel behind the preparation.

Odorants

All chemicals were purchased from Sigma-Aldrich unless otherwise noted. Although host plants typically release dozens of different volatiles, in *Manduca* it has been shown that delivery of only a few of these chemicals can suffice to trigger host-seeking behaviors (Riffell et al. 2009). In total, we included 8 host plant (methyl salicylate, benzylacetone, benzaldehyde, geraniol, decanal, *cis*-3-hexenyl proprionate, nonanal, and linalool) and 6 nonhost plant (β-Citronellene, cyclohexanone, ethyl isovalerate, β-pinene, 1-butanethiol, and 1,4-dichlorobenzene) odorants in our study (7 host plant and 5 nonhost plant odorants in Figure 1A and 3 host plant and 2 nonhost plant odorants in Figures 2–4). Methyl salicylate, benzylacetone, benzaldehyde, geraniol, and linalool are volatiles emitted by the *Manduca* host plant *Nicotiana attenuate* (Kessler and Baldwin 2007). Geraniol is also abundant in the *Manduca* host plant *Datura wrightii* (Riffell et al. 2008). Methyl salicylate and benzylacetone were both found to be attractive to *M. sexta* in behavioral assays (Kessler and Baldwin 2007). Both benzylacetone emission from *N. attenuata* (Baldwin et al. 1997) and benzaldehyde emission from the *Manduca* host plant *Petunia axillaris* (Hoballah et al. 2005) were found to be abundant and to follow a circadian rhythm of emission that peaks at night when *Manduca* is most active. Methyl salicylate, decanal, *cis*-3-hexenyl proprionate, and nonanal are all emitted by host plants *Lycopersicon esculentum*, *Capiscum annuum*, and *D. wrightii* and were components of a blend that was shown to attract female *Manduca* (Fraser et al. 2003). β-Citronellene (Reisenman et al. 2004) and cyclohexanone (Knudsen et al. 1993; Daly et al. 2001) are odorants not found in *Manduca* host plants. Ethyl isovalerate and β-pinene are bat-pollinated plant volatiles with structures and odors distinct from those released by moth-pollinated plants (Riffell et al. 2008). 1-Butanethiol has a skunk spray–like odor and is often added to natural gas. 1,4-Dichlorobenzene (Fisher Scientific) is a component of mothballs. Tomato plant vapor was prepared by placing about 10 g of fresh tomato stems into a clean glass bottle.

Electrophysiology

Moths were prepared and used for physiological recording as described previously (Ito et al. 2008).

Briefly, electroantennogram (EAG) recordings were made from isolated antennae or intact animals. In isolated antenna preparations, the moth antenna was sectioned at its base and tip, and Ag/AgCl wires were placed in both cut ends. In intact animals, only the tip of the antenna was cut, and Ag/AgCl wire was inserted there, and a reference Ag/AgCl wire was placed inside the head capsule. Local field potential (LFP) recordings were made using saline-filled blunt glass micropipettes (2–90 MΩ) inserted into the MB calyx. The indifferent electrode (Ag/AgCl pellet electrode) was placed in the bath.

Behavioral experiments

We used 116 moths for behavioral experiments. Each moth was classically conditioned as described previously (Ito et al. 2008) with 1 of the 5 odorants (Figure 4, 21–25 moths per odorant). Briefly, conditioning took place during the dark photoperiod and consisted of 5 training trials (5 min intertrial interval) and 1 test trial that followed 5 min after the last

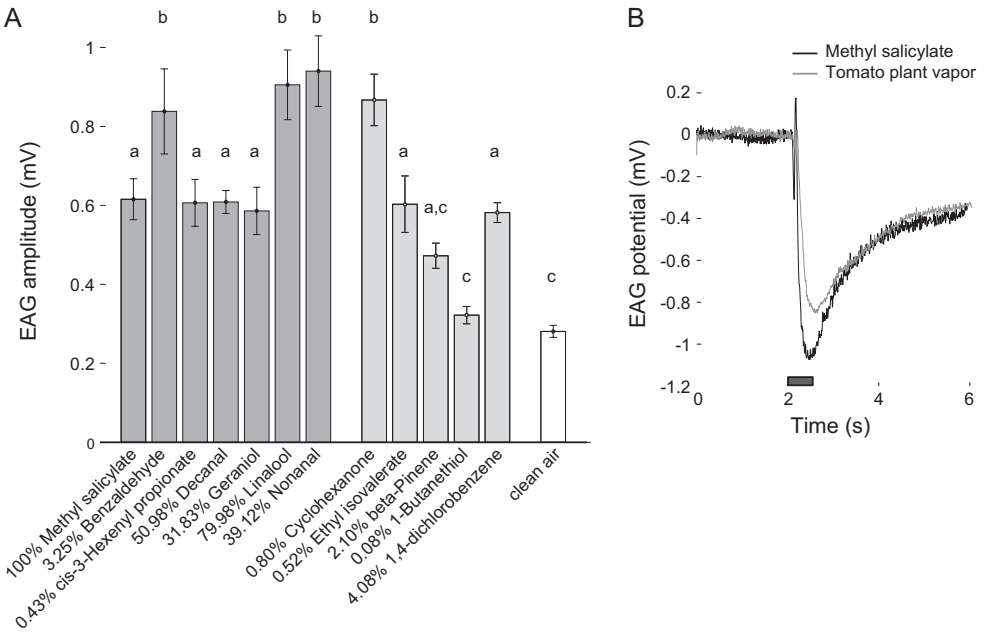

Figure 1 Moth antennae respond with differing intensities to odorants mutually diluted to equivalent vapor pressure. (**A**) Volatiles from host plants (dark bars) and nonhost plants (light bars), and clean air (open bar) evoked a range of EAG deflection amplitudes (2-way ANOVA, among all odors: $P < 0.0001$, among different groups (a, b, or c): $P < 0.005$, $n = 4–6$). Bars: mean ± standard error of the mean. (**B**) EAG responses elicited by vapor from a tomato stem and by methyl salicylate are similar in shape and amplitude.

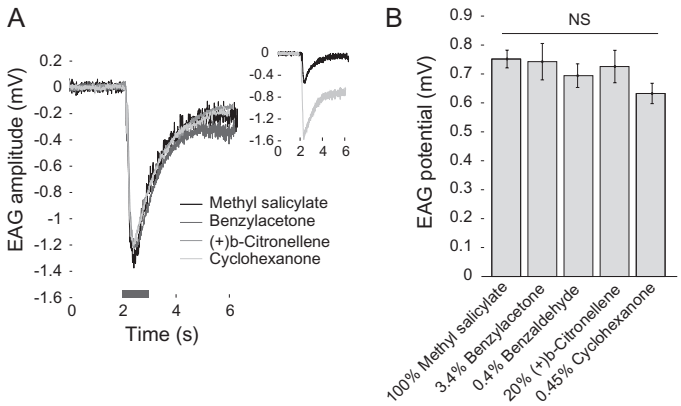

Figure 2 Normalizing activation of ORNs by mutually diluting odorants with mineral oil. (**A**) Normalized odor set elicits matched EAG deflections. Inset: EAG deflections elicited by methyl salicylate and cyclohexanone before dilution. (**B**) After dilution, odor-elicited EAG amplitudes are not different: 2-way ANOVA, among odors: $P > 0.05$, $n \geq 5$.

training trial. For each training trial, an odor was presented for 4 s to the moths' antennae paired with a measured amount (~0.01 g) of sucrose water (40% w/v) presented by hand to the tip of the proboscis using a metered micro-meter syringe (Gilmont, Cole-Parmer) 2 s after the odor's onset. Video analysis has shown ~98% of well-trained moths extend their proboscis within 1 s of odor onset (Ito et al. 2008). In training trials, proboscis movements with excursions exceeding 1 cm and occurring within 2 s after the odor onset were counted as responses to the odor. In nonrewarded test trials, a 1 min response window was allowed but nearly all responses occurred within 1 s of odor onset.

Data analysis

All analyses were carried out using custom programs written in MATLAB (MathWorks). Statistical tests were made using SAS version 9.0 (SAS Institute). All statistical tests were 2-tailed, and significance was judged at $P = 0.05$. We used logistic regression to model the relationship between a categorical response variable (in our case, whether moths responded or not to odor stimulus) and an explanatory variable (trial in this case). We used a repeated measures test because the same moths were tested repeatedly with the same odor throughout training and test trials (Agresti 2002; Wright and Smith 2004).

Results

Peripheral tuning to host plant and nonhost plant odors

We examined the overall sensitivity of the moth antenna to 2 categories of volatiles: *Manduca* host plant and nonhost plant volatiles. EAG recordings allowed us to assess the summed activity elicited by our odorant set within the antenna's population of ORNs. We tested 7 host plant volatiles and 5 nonhost plant volatiles (Figure 1A). Odorants used include aliphatics, aromatics, and terpenoids, with a variety of molecular sizes and functional groups.

Odorants in our set have different vapor pressures, so equivalent volumes of pure liquid odorant will release

different amounts of vapor. To allow us to deliver the same amount of each odorant to the moth antenna, we chose the least volatile odorant in our set, methyl salicylate, as a standard against which to normalize the vapor pressures of all other odorants by dilution with mineral oil (v/v) in accordance with Raoult's law. We expect the resulting concentrations of odorants fell into a physiologically meaningful range because EAGs elicited by undiluted methyl salicylate were comparable in amplitude to those evoked by the fragrant stems of fresh tomato plants; responses to our odor presentations corresponded to responses that likely occur in nature (Figure 1B, see Materials and methods).

Vapor pressure–normalized odorants evoked significantly different amounts of activity in the antennae (Figure 1A). We observed no clear categorical differences in responses evoked by host plant volatiles and nonhost plant volatiles. Several host plant volatiles (decanal, geraniol, and *cis*-3-hexenyl proprionate) evoked EAG responses similar in amplitude to that evoked by methyl salicylate, but benzaldehyde, linalool, and nonanal elicited responses that were significantly stronger ($P < 0.005$, 2-way analysis of variance [ANOVA], $n = 5$, Tukey–Kramer's test). Two of the 3 odorants in our host plant set eliciting the strongest responses, benzaldehyde and linalool, appear to hold particular relevance to moths: the release of benzaldehyde from the host plant *P. axillaris* peaks at night, overlapping with period the nocturnal *M. sexta* is most active (Hoballah et al. 2005); and the lateral large female glomerulus, a sexually dimorphic structure in the AL of female *Manduca*, responds to linalool preferentially (King et al. 2000) (although linalool did not attract flying female moths in a wind tunnel test, data not shown). Among all the nonhost plant odors, cyclohexanone, a synthetic chemical not encountered by moths in nature, evoked the greatest amount of receptor activation, a level exceeding that evoked by the host plant volatile methyl salicylate ($P < 0.001$, 2-way ANOVA, $n = 5$, Tukey–Kramer's test). β-Pinene and 1-butanethiol evoked EAG responses no stronger than those evoked by clean air ($P > 0.05$, 2-way ANOVA). Odorants that evoked relatively strong antennal activation greatly differ in molecular features.

Taken together, the population of *Manduca* ORNs was readily activated by all host plant volatiles and most nonhost plant volatiles in our set. Strong responses could be evoked by both host plant and nonhost plant odorants.

Central tuning to host plant and nonhost plant odors

Could some central mechanism further amplify or diminish more or less salient signals arising in the antenna? The odor-elicited spikes of ORNs travel through their axons to the AL. Our EAG recordings showed that, measured as a whole, the population of ORNs responded more intensely to some odors than to others, even when the odor solutions had been mutually diluted to deliver equal amounts of odorant to the antenna (Figure 1A). To explore the possibility of additional downstream variations, we sought a way to deliver our odorants such that each provided equally strong input to the central brain. Thus, we prepared a new series of dilutions to normalize our set of host plant and nonhost plant volatiles so that each odorant elicited equally strong EAG responses. The odor set included host plant volatiles methyl salicylate, benzaldehyde, and benzylacetone and nonhost plant volatiles (+)β-citronellene and cyclohexanone. After dilution, each of these odorants evoked the same EAG amplitude as undiluted methyl salicylate (Figure 2; 2-way ANOVA, among odors: $P > 0.05$, $n \geq 5$). The concentrations of EAG-equalized odorants spanned 4 orders of magnitude (Figure 2B).

In the AL, PNs receive excitatory input from ORNs and from other AL neurons. PNs typically respond to multiple odorants (Laurent and Davidowitz 1994; Ito et al. 2008). The PNs then send their output to the MBs and lateral horn (LH). LFP deflection amplitude varies with odor concentration (Stopfer et al. 2003). To test whether odor-specific amplification occurs in the AL, we measured the AL's output in the MBs. We stimulated animals with our set of odors that evoked the same EAG amplitudes and measured the amplitudes of the resulting LFP deflections in the MB calyx. Although it is known that some PNs project directly to the LH without sending collaterals to the MB, most PNs send axons to both MB and LH in *Manduca* (Homberg et al. 1988); in *Drosophila*, most PNs responding to food odors project to both the MB and the LH (Jefferis et al. 2007). Therefore, we expected recordings from the MB to detect the relative strengths of specific food (host plant)-elicited signals projecting from the AL. Differential amplification of some odor-elicited signals would be detected as differentially strong (or weak) responses in the MB.

We made LFP recordings from 12 locations in the MB. All tested odorants evoked responses in all electrode locations. We conducted 6 tests, each with a pair of LFP electrodes in different positions in the MB calyx (at least 100 μm apart), as a way to compare odor-driven output of PNs into different regions of the MB calyx. Among the 6 paired-LFP recordings, 3 pairs showed statistically significant correlations between deflections recorded at these sites across odors (Figure 3A, $P < 0.05$). The significant correlations suggest that individual PNs provide broadly distributed input to these regions of the MB, consistent with their diffuse anatomical projections patterns (Homberg et al. 1988). We also noted that often 1 LFP electrode produced a larger signal than its pair for every odor tested. This high correlation in absolute LFP amplitude was likely due to mundane nonbiological characteristics of each extracellular electrode's recording configuration rather than to biologically meaningful variations in the size of the signal reaching the MB. On the other hand, 3 paired recordings showed no significant correlations between the recording sites, indicating that some regions of the MB responded more vigorously to some odorants than to others, and suggesting that these regions may contain nonuniform densities of PN

arborization (Figure 3A,C). In 4 experiments in which we delivered puffs of clean air in addition to odors, all odors evoked stronger responses than did air in every recording position (data not shown), suggesting that PNs responsive to all the tested odors arborize throughout the MB. When averaged across individuals, no odor in our set evoked significantly stronger responses than any other (Figure 3D; 2-way ANOVA, among odors: $P > 0.05$, $n = 12$).

Our LFP recordings suggest PN outputs to the MB were diffuse and widespread but may also include odor-specific foci potentially carried by specific PN tracts. Overall, our results suggest that the flow of information from PNs to the MB proceeds without odor-specific modifications to the intensity of responses established by the ORNs.

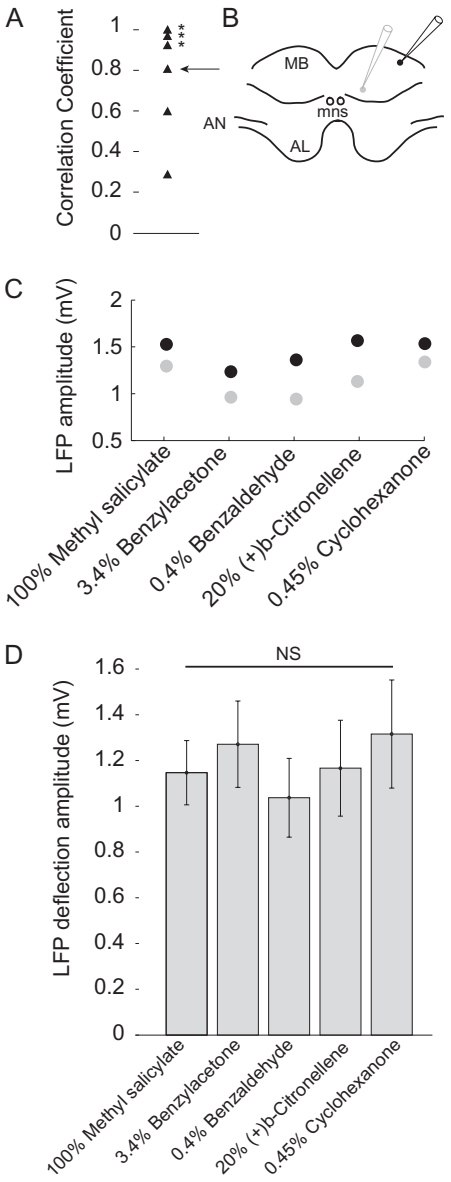

Figure 3 Paired recordings show EAG-normalized odorants evoked similar LFP deflections across the MB calyx. (**A**) Correlation coefficients (triangles) of all paired LFP recordings. *Significant correlations, $P < 0.05$. Arrow: LFP pair shown in **B** and **C**. (B) Example electrode positions in the MB calyx viewed from dorsal side; AN: Antennal nerve; AL: Antennal lobe; MB: Mushroom body; mnsc: medial neurosecretory neurons. (C) Amplitudes of LFP deflections elicited by different odors recorded simultaneously in 2 positions (one black, one gray as in B). (**D**) Overall, mean (±standard error of the mean) LFP deflections are not different from each other (2-way ANOVA, among odors: $P > 0.05$; $n = 12$ recording positions in 5 MBs).

Associative memories for host plant and nonhost plant odors

Finally, the processing of sensory information can culminate in the formation of new memories, the generation of new behaviors, or both. Therefore, using a conditioning paradigm, we tested whether, to a first approximation, some odorants are more easily associated with a food reward than others.

We trained individual moths, each with 1 of 5 odorants mutually diluted to evoke the same net output from ORNs. Using a classical conditioning proboscis extension paradigm in which a presentation of an odor predicts a sucrose reward, we trained a total of 116 moths and compared the learning curves of moths trained with different host plant and nonhost plant odors (Figure 4). We found that moths demonstrated statistically significant learning ($P < 0.0001$, repeated measures logistic regression; see Materials and methods) with no difference in learning efficacy among the odors ($P > 0.05$, repeated measures logistic regression). Our results suggest moths can learn to associate a sucrose reward with any type of odor equally well.

Discussion

Animals survive by maximizing predictable advantages within their ecological niches, but also by adapting to changes in the environment; thus, the behaviors of animals

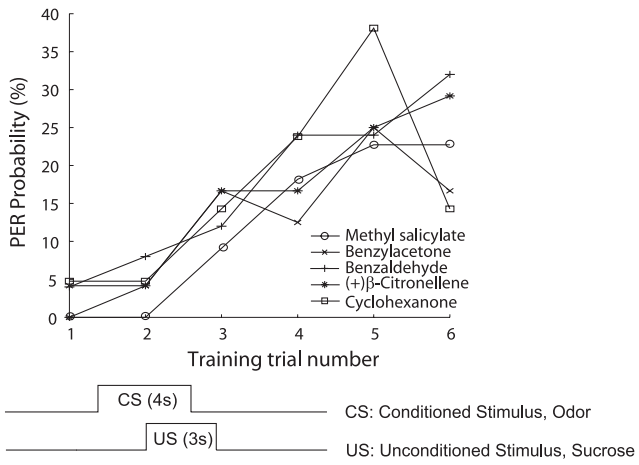

Figure 4 Increasing Proboscis Extension Reflex probability shows moths could learn to associate all EAG-normalized odors with reward ($P < 0.0001$, repeated measures logistic regression; n = 21–25 for each odor, see Materials and methods) and with similar efficacy ($P > 0.05$, repeated measures logistic regression; n = 21–25 for each odor).

reveal abilities to respond to sensory stimuli in ways determined by both innate and learned preferences (Kelber 2002; Cunningham et al. 2004; Riffell et al. 2008). Here, using an assortment of odorants, we investigated olfactory responses of the moth, focusing on 3 levels of odor processing: initial detection by populations of ORNs; early processing within the brain; and behavioral output during and after associative learning. Our stimulus set included nonpheromonal odorants that we expected to convey different degrees of innate meaning to moths. We found that the population of ORNs on the antenna responded with different intensities to different odorants even though these odorants had been normalized to exert equal vapor pressure (Figure 1A). However, our other measures of olfactory response offered no evidence of odor-specific sensitivity or amplification (Figures 3 and 4). Our findings suggest that the general olfactory system, unlike the moth pheromone system, does not employ a disproportionate fraction of receptor neurons for biologically relevant odors. The general system appears to maximize the ability to detect and learn about a number of odors regardless of their innate meanings.

Many types of insects have been shown to display innate olfactory preferences (e.g., moths: Cunningham et al. 2004; Hull et al. 2004; mosquitoes: Dekker et al. 2001; beetles: Dormont et al. 2010). Interactions with the environment can change the behavioral responses to innately preferred odors, though. For example, *Manduca* can learn to feed on the bat-adapted flower *Agave palmeri* when the primary host plant *D. wrightii* is not available but will readily return to *D. wrightii* when it is present (Riffell et al. 2008). *Drosophila* larvae readily learn to ignore the innate attractiveness of some odors (Saumweber et al. 2011). Consistent with this, our results show that moths can learn, with equal efficiency, to associate a food reward with host plant and nonhost plant odorants (Figure 4).

An earlier study in *Manduca* compared the acquisition rates of associative learning for 2 odors, the nonhost plant volatile cyclohexanone and the oviposition deterrent methyl jasmonate (Daly et al. 2001). This work concluded that *Manduca* could not associate methyl jasmonate with food reward likely because methyl jasmonate carries a different nonappetitive innate meaning. However, our EAG recordings showed that methyl jasmonate may not effectively activate *Manduca* ORNs: EAG responses evoked by undiluted methyl jasmonate were much smaller in amplitude than those evoked by other odors in our set and no larger than those evoked by odorless mineral oil (data not shown). The relatively weak excitation generated by methyl jasmonate may explain why *Manduca* were unable to associate the odor with food in the earlier work.

In this study, we used EAG and LFP recordings to compare the responses of olfactory neurons projecting from the antenna and projecting from the AL, respectively. Our results showed that different odors can elicit response of different intensities in the periphery. That these differences occurred even when the vapor pressures of the odorants were normalized to provide equivalent amounts of material (Figure 1) establishes that they are due to variations in receptor characteristics. In principle, such results could arise from a population of ORNs in which different types of receptors: 1) are expressed with a diversity of abundance; or 2) individually give rise to a diversity of response strengths; or 3) both. Furthermore, we observed more variability in responses across the nonhost plant odorants than across host plant odorants: the moth antenna responded to all the tested host plant odors, some of our tested nonhost plant odors failed to evoke EAG amplitudes stronger than those evoked by a puff of clean air (Figure 1A). This finding is consistent with work showing the antennae of 2 species of butterflies are particularly sensitive to volatiles from their preferred host plants (Mercader et al. 2008). In subsequent physiological and behavioral tests with odorants that had been mutually diluted to give rise to equivalent response intensities in the antenna (Figures 3 and 4), we found no evidence for systematic variability in response intensity downstream. These results suggest that much of odor selectivity may lie at the first stage of odor processing, in the receptors.

A simple way for an animal to link a small number of innately attractive odors to appetitive behaviors might be to overexpress ORNs that are highly sensitive to such odors and then orient toward odors that elicit strong responses. Because both host and nonhost plant odors could elicit strong EAG and LFP responses and could equally well support associative conditioning, our results do not support this idea and rather suggest specific wiring in the brain may be required to create this linkage. Host plant volatiles activate many types of ORNs and multiple generalist glomeruli (Shields and Hildebrand 2001; Hansson et al. 2003; Skiri et al. 2004; Hallem and Carlson 2006) in combinatorial patterns that vary not with the identities of their separate labeled lines but rather with the details of their spatiotemporal structures (Ito et al. 2008). As a general matter, how such activity patterns are transformed into appropriate actions is not well understood. In specialized instances including the coding of innate preferences, the responses of specific glomeruli may play critical roles in evoking behaviors (Semmelhack and Wang 2009); such glomeruli may function, as for pheromone detection, as parts of a labeled line system. Alternatively, all the glomeruli participating in the spatiotemporal activity patterns evoked by an odor may be important for reliably activating neurons in following pathways. It will be important to understand this balance.

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References

Agresti A. 2002. Categorical data analysis. Hoboken, NJ: Wiley-Interscience. p. 165–266.

Baldwin IT, Preston C, Euler M, Gorham D. 1997. Patterns and consequences of benzyl acetone floral emissions from Nicotiana attenuata plants. J Chem Ecol. 23:2327–2343.

Christensen TA, Hildebrand JG. 1987. Male-specific, sex pheromone-selective projection neurons in the antennal lobes of the moth Manduca sexta. J Comp Physiol A. 160:553–569.

Cunningham JP, Moore CJ, Zalucki MP, West SA. 2004. Learning, odour preference and flower foraging in moths. J Exp Biol. 207:87–94.

Daly KC, Durtschi ML, Smith BH. 2001. Olfactory-based discrimination learning in the moth, Manduca sexta. J Insect Physiol. 47:375–384.

Dekker T, Takken W, Braks MA. 2001. Innate preference for host-odor blends modulates degree of anthropophagy of Anopheles gambiae sensu lato (Diptera: Culicidae). J Med Entomol. 38:868–871.

Dormont L, Jay-Robert P, Bessière JM, Rapior S, Lumaret JP. 2010. Innate olfactory preferences in dung beetles. J Exp Biol. 213:3177–3186.

Fraser AM, Mechaber WL, Hildebrand JG. 2003. Electroantennographic and behavioral responses of the sphinx moth Manduca sexta to host plant headspace volatiles. J Chem Ecol. 29:1813–1833.

Galizia CG, Menzel R. 2000. Odour perception in honeybees: coding information in glomerular patterns. Curr Opin Neurobiol. 10:504–510.

Galizia CG, Sachse S, Mustaparta H. 2000. Calcium responses to pheromones and plant odours in the antennal lobe of the male and female moth Heliothis virescens. J Comp Physiol A. 186:1049–1063.

Hallem EA, Carlson JR. 2006. Coding of odors by a receptor repertoire. Cell. 125:143–160.

Hansson BS, Carlsson MA, Kalinova B. 2003. Olfactory activation patterns in the antennal lobe of the sphinx moth, Manduca sexta. J Comp Physiol A. 189:301–308.

Hoballah ME, Stuurman J, Turlings TCJ, Guerin PM, Connétable S, Kuhlemeier C. 2005. The composition and timing of flower odour emission by wild Petunia axillaris coincide with the antennal perception and nocturnal activity of the pollinator Manduca sexta. Planta. 222:141–150.

Homberg U, Montague RA, Hildebrand JG. 1988. Anatomy of antenno-cerebral pathways in the brain of the sphinx moth Manduca sexta. Cell Tissue Res. 254:255–281.

Hull CD, Cunningham JP, Moore CJ, Zalucki MP, Cribb BW. 2004. Discrepancy between antennal and behavioral responses for enantiomers of alpha-pinene: electrophysiology and behavior of Helicoverpa armigera (Lepidoptera). J Chem Ecol. 30:2071–2084.

Ito I, Ong RC, Raman B, Stopfer M. 2008. Sparse odor representation and olfactory learning. Nat Neurosci. 11:1177–1184.

Jefferis GS, Potter CJ, Chan AM, Marin EC, Rohlfing T, Maurer CR, Luo L. 2007. Comprehensive maps of Drosophila higher olfactory centers: spatially segregated fruit and pheromone representation. Cell. 128:1187–1203.

Kaissling KE. 1996. Peripheral mechanisms of pheromone reception in moths. Chem Senses. 21:257–268.

Karlson P, Butenandt A. 1959. Pheromones (ectohormones) in insects. Annu Rev Entomol. 4:39–58.

Kay LM, Stopfer M. 2006. Information processing in the olfactory systems of insects and vertebrates. Semin Cell Dev Biol. 17:433–442.

Kelber A. 2002. Pattern discrimination in a hawkmoth: innate preferences, learning performance and ecology. Proc Biol Sci. 269:2573–2577.

Kessler D, Baldwin IT. 2007. Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of Nicotiana attenuata. Plant J. 49:840–854.

King JR, Christensen TA, Hildebrand JG. 2000. Response characteristics of an identified, sexually dimorphic olfactory glomerulus. J Neurosci. 20:2391–2399.

Knudsen JT, Tollsten L, Bergstrom LG. 1993. Floral scents—a checklist of volatile compounds isolated by headspace techniques. Phytochemistry. 33:253–280.

Laurent G, Davidowitz H. 1994. Encoding of olfactory information with oscillating neural assemblies. Science. 265:1872–1875.

Laurent G, Wehr M, Davidowitz H. 1996. Temporal representations of odorants in an olfactory network. J Neurosci. 16(12):3837–3847.

Mercader RJ, Stelinski LL, Scriber JM. 2008. Differential antennal sensitivities of the generalist butterflies Papilio glaucus and P. canadensis to host plant and non-host plant extracts. J Lepid Soc. 62:84–88.

Raguso RA, Willis MA. 2002. Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, Manduca sexta. Anim Behav. 64:685.

Raman B, Joseph J, Tang J, Stopfer M. 2010. Temporally diverse firing patterns in olfactory receptor neurons underlie spatiotemporal neural codes for odors. J Neurosci. 30(6):1994–2006.

Reisenman CE, Christensen TA, Francke W, Hildebrand JG. 2004. Enantioselectivity of projection neurons innervating identified olfactory glomeruli. J Neurosci. 24:2602–2611.

Renwick JAA, Chew FS. 1994. Oviposition behavior in lepidoptera. Annu Rev Entomol. 39:377–400.

Riffell JA, Alarcón R, Abrell L, Davidowitz G, Bronstein JL, Hildebrand JG. 2008. Behavioral consequences of innate preferences and olfactory learning in hawkmoth-flower interactions. Proc Natl Acad Sci U S A. 105:3404–3409.

Riffell JA, Lei H, Hildebrand JG. 2009. Neural correlates of behavior in the moth Manduca sexta in response to complex odors. Proc Natl Acad Sci U S A. 106:19219–19226.

Sandoz JC. 2006. Odour-evoked responses to queen pheromone components and to plant odours using optical imaging in the antennal lobe of the honey bee drone Apis mellifera L. J Exp Biol. 209:3587–3598.

Saumweber T, Husse J, Gerber B. 2011. Innate attractiveness and associative learnability of odors can be dissociated in larval Drosophila. Chem Senses. 36:223–235.

Semmelhack JL, Wang JW. 2009. Select Drosophila glomeruli mediate innate olfactory attraction and aversion. Nature. 459:218–223.

Shields VD, Hildebrand JG. 2001. Recent advances in insect olfaction, specifically regarding the morphology and sensory physiology of antennal sensilla of the female sphinx moth Manduca sexta. Microsc Res Tech. 55:307–329.

Skiri HT, Galizia CG, Mustaparta H. 2004. Representation of primary plant odorants in the antennal lobe of the moth Heliothis virescens using calcium imaging. Chem Senses. 29:253–267.

Stopfer M, Jayaraman V, Laurent G. 2003. Intensity versus identity coding in an olfactory system. Neuron. 39:991–1004.

Wright G, Smith B. 2004. Variation in complex olfactory stimuli and its influence on odour recognition. Chem Senses. 29:127–135.