Innate recognition of pheromone and food odors in moths: a common mechanism in the antennal lobe?

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The survival of an animal often depends on an innate response to a particular sensory stimulus. For an adult male moth, two categories of odors are innately attractive: pheromone released by conspecific females, and the floral scents of certain, often co-evolved, plants. These odors consist of multiple volatiles in characteristic mixtures. Here, we review evidence that both categories of odors are processed as sensory objects, and we suggest a mechanism in the primary olfactory center, the antennal lobe (AL), that encodes the configuration of these mixtures and may underlie recognition of innately attractive odors. In the pheromone system, mixtures of two or three volatiles elicit upwind flight. Peripheral changes are associated with behavioral changes in speciation, and suggest the existence of a pattern recognition mechanism for pheromone mixtures in the AL. Moths are similarly innately attracted to certain floral scents. Though floral scents consist of multiple volatiles that activate a broad array of receptor neurons, only a smaller subset, numerically comparable to pheromone mixtures, is necessary and sufficient to elicit behavior. Both pheromone and floral scent mixtures that produce attraction to the odor source elicit synchronous action potentials in particular populations of output (projection) neurons (PNs) in the AL. We propose a model in which the synchronous output of a population of PNs encodes the configuration of an innately attractive mixture, and thus comprises an innate mechanism for releasing odor-tracking behavior. The particular example of olfaction in moths may inform the general question of how sensory objects trigger innate responses.

Keywords: floral scent, moths, neuroethology, olfaction, pheromone, sensory coding, sensory object, synchrony

INTRODUCTION

The olfaction-based behavior of an animal is governed by a trade-off: focusing on a narrow range of innately attractive odors efficiently guides the animal to mates and food sources that are most likely to be rewarding, while a flexible olfactory system that explores new odors and associates new rewards with them is resistant to changes in the availability of any one source (Waser et al., 1996; Chittka et al., 1999; Memmott et al., 2004). For a moth emerging from its pupal stage, with only a few days to a few weeks of adult life ahead of it to eat and mate, coming prepared with pre-programmed search images for the mates and night-blooming flowers they are likely to encounter, along with a capacity to learn new sources of food, could be highly valuable.

Most moths take flight at night, when the utility of visual signals is at a nadir. As nocturnal flyers, moths rely mainly on olfaction (Balkenius et al., 2006). Chemical cues released into the air can guide moths to sources of food or mates over long distances (Wall and Perry, 1987), and are behaviorally active at remarkably low concentrations. For example, only a few molecules of pheromone or plant odors can trigger increased heart rates in moths (Angioy et al., 2003).

Here, we review neuroethological findings about selected, innate mate-seeking and feeding behaviors of moths and ask if there is a common operating principle for a neural substrate underlying recognition of an innately attractive odor. We propose that for both sex pheromone and food odors, the circuitry of the antennal lobe (AL) produces a pattern of coordinated output for attractive odors

from either category. Our review is not exhaustive but intends to make a case for the existence of such a mechanism in many, if not all moths.

SPECIES RECOGNITION BY PHEROMONE BLENDS

Animals are, by necessity, specialists when searching for mates. The consequences of pursuing a mate from a related but reproductively isolated species are dire for animals that expend much energy in long-distance flights (Bartholomew and Casey, 1978). Moths have evolved a system of sex-pheromonal communication between calling females (senders) and conspecific males (receivers), incorporating the production of a suite of chemicals by females. These consist, for the most part, of aliphatic acetates, alcohols, and/or aldehydes with hydrocarbon chains 10–22 carbon atoms in length and often with one or more double bonds (Byers, 2005). This chemical alphabet allows for numerous unique molecules to be used as components of signals. Considering only the possible variations of the most commonly used molecules, Byers (2005) estimates there are over 100,000 possible pheromonal volatiles. Here, we review the evidence from the peripheral reception of pheromone odors in moths that suggests the existence of a template in the AL for the recognition of complex odors. We particularly emphasize how changes in behavioral selectivity that follow changes in the periphery can be understood as an alteration of the input to a pre-existing AL template. In the proceeding sections, we will describe the evidence for the nature of this template.

The use of fine variations on a single molecular theme requires a male receiver to have sufficiently specific receptors to accept certain variants and reject all others. The antennae of male moths carry olfactory organules – olfactory sensilla-containing olfactory receptor cells (ORCs) that respond with such specificity. For instance, ORCs of one phenotype present in a particular species respond specifically to a 14-carbon acetate with a double bond at carbon 11 in the *cis* configuration, and not to the *trans* isomer (Wanner et al., 2010). Pheromone-binding proteins in the lymph that bathes ORCs in antennal sensilla can increase the specificity of receptor responses further (Grosse-Wilde et al., 2006).

Most moth species employ multi-component pheromone mixtures instead of monomolecular signals (Byers, 2006). Two factors likely necessitate this level of complexity. First, genera with closely related, sympatric species often have sex pheromones with at least one component in common (Byers, 2006). Second, there are limits to the specificity of an ORC, and there are many examples of ORCs in one species that respond to pheromone components used by other species (Grant et al., 1989; Lofstedt, 1990; Berg et al., 1995; Domingue et al., 2007b, 2008). This may reflect an upper limit to the ability of a receptor to reject molecules that are highly similar to the preferred ligand and would lead to ambiguity between a non-preferred ligand at high concentration and a preferred ligand at low concentration.

Related moth species also may use pheromone mixtures with identical components, but in ratios specific to each species (Baker, 2008). A pattern is emerging that describes a large number of moth pheromone mixtures: a "major" component (providing a majority of molecules in the blend) and one or more "minor" components (often present at a much lower concentration but nevertheless required to elicit behavior in a recipient moth). Moreover, attraction to a pheromone blend may be inhibited by "antagonistic" compounds in the pheromone mixtures of other species (Baker and Heath, 2004). Species recognition thus requires a form of rudimentary pattern recognition, dependent on the arrangement of features in a complex stimulus, in the olfactory system of a male moth (Baker, 2008).

Clues about the nature of the pattern-recognition mechanism come from several observations of individual moths for which the parameters of an acceptable pattern have shifted. Accompanying these behavioral shifts are some revealing changes in the responses of the ORCs to pheromone blends typically rejected by "normal" moths. In a laboratory colony of *Trichoplusia ni*, a strain spontaneously arose in which females emit roughly equivalent amounts of the major and minor pheromone components instead of the typical 1:100 ratio (Haynes and Hunt, 1990). Over several generations, males emerged that were attracted to the mutant blend (Liu and Haynes, 1994). In normal males, the ratio between the major and minor components is reflected in the ratio between responses of the corresponding "major" and "minor" ORCs (Domingue et al., 2009). In the males evolved to accept the mutant blend, the response of the minor ORC was decreased, such that the nearly equal ratio between components still produced an unequal ratio of responses between the types of ORCs (Domingue et al., 2009). The ratio of ORC responses of evolved males to the mutant blend was thus similar to that of normal males to the normal blend.

Another case study provides an additional clue. In males of the species *Ostrinia nubilalis*, ORCs for the major and minor components of the conspecific sex-pheromone blend also respond weakly to the pheromone components of a related species, *O. furnacalis*, which differ only in the position of the double bond (Domingue et al., 2007a). This illustrates the utility of encoding schemes that depend on more than just the presence of a particular pheromone molecule (i.e. "labeled-line" coding), as a high concentration of *O. furnacalis* components would be indistinguishable from a low concentration of conspecific components. In contrast, the relative activity of neurons responding to an odor is typically consistent across concentrations, and is hypothesized to underlie "concentration invariant" encoding of odor identity (Cleland et al., 2007; Uchida and Mainen, 2007; Asahina et al., 2009).

Despite the incomplete specificity of their ORCs, only rare *O. nubilalis* males are attracted to the *O. furnacalis* pheromone, in which the major and minor components are present in an approximately 1:1 ratio (Linn et al., 2003). Major and minor ORCs in both normal and rare males have similar sensitivity to the major and minor components of the conspecific pheromone and thus produce a ratio of responses congruent with the 99:1 ratio of components in an attractive blend (Domingue et al., 2007a). Responses to the heterospecific *O. furnacalis* components in normal males also reflect the 1:1 ratio of components in the pheromone of that species, allowing the animal to discriminate between the blends. In the rare males, the response of the minor ORC to the heterospecific pheromone component is greatly diminished, producing a response closer to 99:1 when presented with a 1:1 stimulus and thus facilitating a behavioral response to the odor (Domingue et al., 2007a).

These studies and others on peripheral changes in pheromone processing suggest the existence of an internal template for the ratio of components in a conspecific pheromone blend (Baker, 2008). In both examples, sensitivity in the periphery changed, producing a response to a new mixture that had a ratio of major and minor ORC activation similar to that observed in response to the conspecific blend. Where might the template for such a pattern be located? The axons of pheromone-responsive ORCs terminate in the AL in a set of large, male-specific glomeruli called the macroglomerular complex (MGC) (Matsumoto and Hildebrand, 1981). An additional clue from the periphery suggests that the template exists there, at least in part.

The species *O. nubilalis* comprises two strains, differing only in the ratio of components produced by females and attractive to males: an "E-strain" and a "Z-strain," named for the isomer of the major pheromone component (Carde et al., 1978; Anglade and Stockel, 1984). The major component for one strain is the minor component for the other, and vice versa. In both strains, major ORCs terminate in the larger of two glomeruli in the MGC, and minor ORCs in the smaller (Karpati et al., 2008).

This arrangement is also found in male moths of the subfamily Heliothinae. Evidence from both the input ORCs (Berg et al., 1998, 2005; Galizia et al., 2000; Lee et al., 2006a,b) and output projection neurons (PNs) (Christensen et al., 1995b; Vickers and Christensen, 1998, 2003; Vickers et al., 1998) in the MGC demonstrates that the major component, shared across all four species studied in detail, is processed in the largest glomerulus, called the cumulus. One or more minor components, and in some species a component

that antagonizes behavior, are processed in smaller glomeruli surrounding the cumulus. The proximity of these glomeruli, and the conserved functional relationship across species, suggests that they are incorporated into a conserved network at the level of the AL that performs the initial processing necessary for species-specific pattern recognition.

The network architecture of the AL consists primarily of inhibitory, GABAergic neurons that have arborizations throughout the AL (Anton and Homberg, 1999). These local interneurons (LNs) connect the glomeruli of the MGC and facilitate reciprocal inhibition between them (Waldrop et al., 1987; Christensen et al., 1993; Christensen and Hildebrand, 1997; Lei et al., 2002). This is best established in *Manduca sexta*, a species for which two pheromone components in a 1:2 ratio are necessary and sufficient for attraction of male moths to the source of the stimulus (Tumlinson et al., 1989). Stimulation with one component activates ORCs projecting to one glomerulus (Kaissling et al., 1989; Christensen et al., 1995a), PNs arborizing in that glomerulus (Christensen and Hildebrand, 1987; Hansson et al., 1991), and LNs arborizing in both glomeruli (Christensen et al., 1993), and inhibits the background firing of PNs in the MGC glomerulus activated by the other component (Christensen and Hildebrand, 1997; Lei et al., 2002).

Information about the presence, and potentially the quantity, of each component is thus transmitted between glomeruli. The effect of these inhibitory inputs is not to reduce the output carried by PNs in response to a blend of both pheromone components, but rather to increase the coordination, or synchrony, of their action potentials (Lei et al., 2002). Synchrony between spikes produced by PNs arborizing in the same glomerulus, but not by PNs arborizing in neighboring glomeruli, increased in response to the blend. This result is similar to what is seen in the *Drosophila* AL, though in that system the effect does not rely on interglomerular inhibition (Kazama and Wilson, 2009). In contrast, the degree of synchrony between moth MGC PNs is correlated with the strength of inhibition they receive from the neighboring glomerulus (Lei et al., 2002). Synchrony provides an additional coding dimension (Singer, 1999; Biederlack et al., 2006) in the output of the MGC, such that the presence and intensity of each pheromone component can be encoded by the rate of firing of individual PNs, while the presence of both components together in a mixture is encoded in the coordination of firing of PNs. Conceptually, synchronous firing of two neurons can be thought of as a new, active, virtual neuron that is more effective in driving responses down-stream and more selective to behaviorally relevant mixtures than either of the neurons that produce it (Ghose et al., 1994).

Synchrony, typically shaped by inhibitory networks, has been investigated and debated for years as a possible mechanism for "binding" the features of a complex stimulus to produce a unitary representation (Engel et al., 1992; Engel and Singer, 2001; Lestienne, 2001; Robertson, 2003; Averbeck and Lee, 2004). We propose that the inhibitory network linking MGC glomeruli provides the mechanism by which the features of an encountered pheromone mixture are compared to an internal template for the conspecific mixture, and the output of synchronous spikes encodes a blend that fits this template.

INNATELY ATTRACTIVE FLORAL ODORS AND SYNCHRONOUS CODES

Beyond the enlarged glomeruli of the MGC in the AL of a male moth lie a larger number of "ordinary" glomeruli, in a region called the main AL (Anton and Homberg, 1999). These glomeruli process sensory input about volatiles from flowers (sources of nectar) and foliage of host plants (on which females lay eggs) (Galizia et al., 2000). Although more is known about the pheromone-processing pathways of the MGC, emerging evidence suggests that the processing of innately attractive, complex odors in both regions of the AL share fundamental traits.

While the MGC and main AL mediate different olfactory behaviors, there are many similarities between the two compartments (Christensen and Hildebrand, 2002). However, evidence from calcium-imaging (Galizia et al., 2000; Carlsson et al., 2002; Hansson et al., 2003) indicates that aggregate activity (calcium activity representing input, local circuitry, and output) in response to pheromone and plant odors is isolated to the MGC and main AL, respectively. Also, intracellular recordings (Reisenman et al., 2008) suggest that while the main AL receives significant inhibition originating from the MGC, the MGC receives no inhibition from some glomeruli, and only receives inhibition when the AL is activated by high concentrations of floral odors. Thus, the two are functionally separate compartments that may interact when the animal encounters both pheromone and floral odors. A similar arrangement is suggested by the anatomical arrangement of glomeruli in the antennal lobe of *Drosophila*, where classes of local neurons arborize in glomeruli across the AL, but avoid those involved in processing putative pheromone odors (Wilson and Laurent, 2005; Chou et al., 2010).

While pheromonal stimuli are the gold standard for innate, olfaction-based attraction and discrimination, naive moths are also innately attracted to the scent of certain flowers in preference to others (Plepys et al., 2002; Raguso and Willis, 2002; Riffell et al., 2008). The co-evolution of flowers and their moth pollinators is most remarkable in flowers pollinated by hawk moths (Grant and Grant, 1983). Those flowers have long, slender nectaries accessible to the moth's long proboscis (Darwin, 1862; Nilsson, 1988), large white, reflective surfaces (Raguso et al., 2003), and a strong, sweet fragrance. *M. sexta* moths exhibit innate attraction to the scent of the flowers of *Datura wrightii*, which is seasonally abundant in part of their range (Raguso and Willis, 2002; Riffell et al., 2008). Although this flower releases a mixture of volatiles consisting of more than 65 components (Raguso et al., 2003), neurons in the AL respond robustly to only nine of those compounds (Riffell et al., 2009a), and a mixture of just three of these volatiles is sufficient to attract naive moths (Riffell et al., 2009b). Thus the initially daunting complexity of a floral scent may be reduced in the olfactory processing of an animal to something more closely approximating that of a pheromone blend. A behavioral focus on a reduced subset of volatiles in a complex mixture also has been observed recently in honey bees (Reinhard et al., 2010).

Owing perhaps in part to the more broadly tuned ORCs that innervate the main AL (Wang et al., 2003; Hallem and Carlson, 2006), mixtures of plant volatiles activate a significantly larger number of glomeruli (Lei et al., 2004; Skiri et al., 2004; Pinero et al., 2008) than do pheromones. Nevertheless, simultaneous recordings from neurons across the AL show that in response to an innately attractive floral scent, a pattern of firing synchrony emerges (Riffell et al., 2009b). This

pattern is conserved in response to attractive blends with reduced numbers of components and is distinct from patterns generated by non-attractive mixtures and single components (Riffell et al., 2009b). Attractive odors also generate a distinctive pattern of firing rates across the ensemble of neurons. However, by using a shift-predictor measure of synchrony (Perkel et al., 1967), the authors ensure that the measures of synchrony and firing rate are independent. Thus both firing rate and synchrony may independently encode the presence of attractive floral scents (Riffell et al., 2009b). Synchrony, but not firing rate, is maintained across a range of concentrations that were detectable and attractive to the animal, providing a neural correlate of "concentration invariance" (Riffell et al., 2009a). An ensemble of synchronously responding neurons, innervating a larger number of glomeruli, thus is involved in encoding an innately attractive floral odor in a manner similar to that observed with sex pheromone in the MGC.

A fairly superficial analysis of the output of the AL thus has suggested that pheromonal and plant-odor processing share common mechanisms, wherein innately attractive mixtures of volatiles activate innate templates in the AL network, producing synchronous output among PNs. Particular patterns of synchrony are correlated with innate olfactory behaviors, and are absent in response to stimuli that are not attractive (Riffell et al., 2009b). Definitive proof of this hypothesis will require a pharmacological or genetic manipulation that disrupts synchrony in response to a normally attractive odor, and consequently behavior.

As local neurons arborize similarly among glomeruli in the main AL and in the MGC (Hoskins et al., 1986), it is likely that similar networks of reciprocal inhibition are involved in both regions of the AL. Indeed, data from studies of inhibition between glomeruli suggest a possible mechanism. For a small number of glomeruli that have been tested, interglomerular inhibition is not symmetrical (Reisenman et al., 2008). Data from calcium-imaging studies of honey bees and *Drosophila melanogaster* suggest that the strength of inhibitory connection is specific to each pair of glomeruli (Sachse and Galizia, 2002, 2003; Linster et al., 2005; Silbering and Galizia, 2007). A network of inhibitory connections, set according to some genetic program, could transform ORC inputs responding to a range of innately attractive odors into particular patterns of synchronized PN output, to be read at higher levels of processing.

Like honey bees, moths can learn readily to associate odors with rewards by both classical conditioning (Hartlieb, 1996; Fan et al., 1997; Daly and Smith, 2000) and in more naturalistic protocols related to foraging (Cunningham et al., 2004; Riffell et al., 2008). Evidence for learning in the wild also exists, as moths are found to feed from flowers to which they are not innately attracted when the preferred, innately attractive flower is scarce (Riffell et al., 2008). Simultaneous recordings from moths learning to associate an odor with a sucrose-solution reward reveal that neurons are recruited into the ensemble encoding the rewarded odor (Daly et al., 2004). Further research will clarify whether these changes make the representation of a learned odor more similar to an innately attractive odor.

CONCLUSIONS

We have reviewed evidence that innate odor attraction in moths is mediated by mechanisms in the AL that recognize and respond to the configuration of a complex odor. In both the specialized, pheromone-processing MGC and the more generalized, plant-

odor-processing main AL, innately attractive odors produce patterns of synchronous output. We have presented the available evidence that synchrony is the feature of AL output that encodes the innate salience of an odor. The mechanism underlying this firing synchrony is unknown, but future investigations can benefit from comparisons between pheromonal- and plant-odor-coding networks. It is important to note that this function of the AL does not preclude other functions, such as lateral inhibition, decorrelation, and gain control (Wilson and Mainen, 2006), which may occur in tandem. Nor does it invert the tendency to attribute too little to the AL by attributing too much, as there are certainly more processes in higher olfactory centers linking stimulus and behavior.

Our model of processing of innately attractive odors in the AL is depicted in **Figure 1**. Both sex pheromone and plant odors consist of mixtures of components present in various proportions (grayscale and colored dots in **Figures 1A,E**, respectively). A moth apparently requires only a subset of these volatiles to initiate innate behaviors. In the male-specific, pheromone-processing subsystem, each of multiple highly specific receptors (represented by various shades of gray in **Figure 1B**) responds to only one of the components of the mixture. In contrast, receptors in the plant-odor-processing subsystem are variously selective (indicated by the color of the ORN in **Figure 1F**) and sensitive (indicated by the saturation of the color) to one or more components of the plant odor. Plant odors thus are represented across a population of ORCs. A map of the connectivity of ORCs to their main glomerular targets in the AL is not yet available in detail. Pheromone-responsive ORCs provide synaptic input to the large glomeruli of the MGC (**Figure 1C**), where LNs (represented by blue arrows) mediate reciprocal inhibition between glomeruli. Although most moths have 3–4 MGC glomeruli, we currently have evidence for only the interaction of two MGC glomeruli in encoding a pheromone mixture.

In response to stimulation with the pheromonal mixture, PNs in each MGC glomerulus produce more synchronous spikes (red raster lines superimposed on gray and black arrows in 1C) with other PNs in the same glomerulus. Similarly, an innately attractive odor produces a pattern of synchrony in the main AL (indicated by black lines in **Figure 1G**). The output of each subsystem (green arrows in **Figures 1D,H**) that encodes the odor is thus a pattern of synchrony between PNs in the same glomerulus for pheromone odors (**Figure 1D**) and across multiple, heterogeneous PNs in the main AL (**Figure 1H**).

The particular features of this scheme, i.e. the importance of the configuration of a complex stimulus and encoding of higher dimensions of a stimulus via synchrony, parallel those uncovered in other work (Meister, 1996; Dan et al., 1998; Krahe et al., 2002; Cleland et al., 2007; Uchida and Mainen, 2007; Marsat et al., 2009; Avargues-Weber et al., 2010) and stems naturally from the observation that sensory systems are tuned, at various levels, to stimuli that are important for survival (Atick, 1992; Dusenberry, 1992). The fundamental similarities between olfactory information processing and storage in brains separated by hundreds of millions of years of evolution are becoming clearer and more numerous over time (Hildebrand and Shepherd, 1997; Davis, 2004; Ache and Young, 2005; Wilson and Mainen, 2006; Touhara and Vosshall, 2009). It seems likely that much of what is learned from moths will find parallels in other animals.
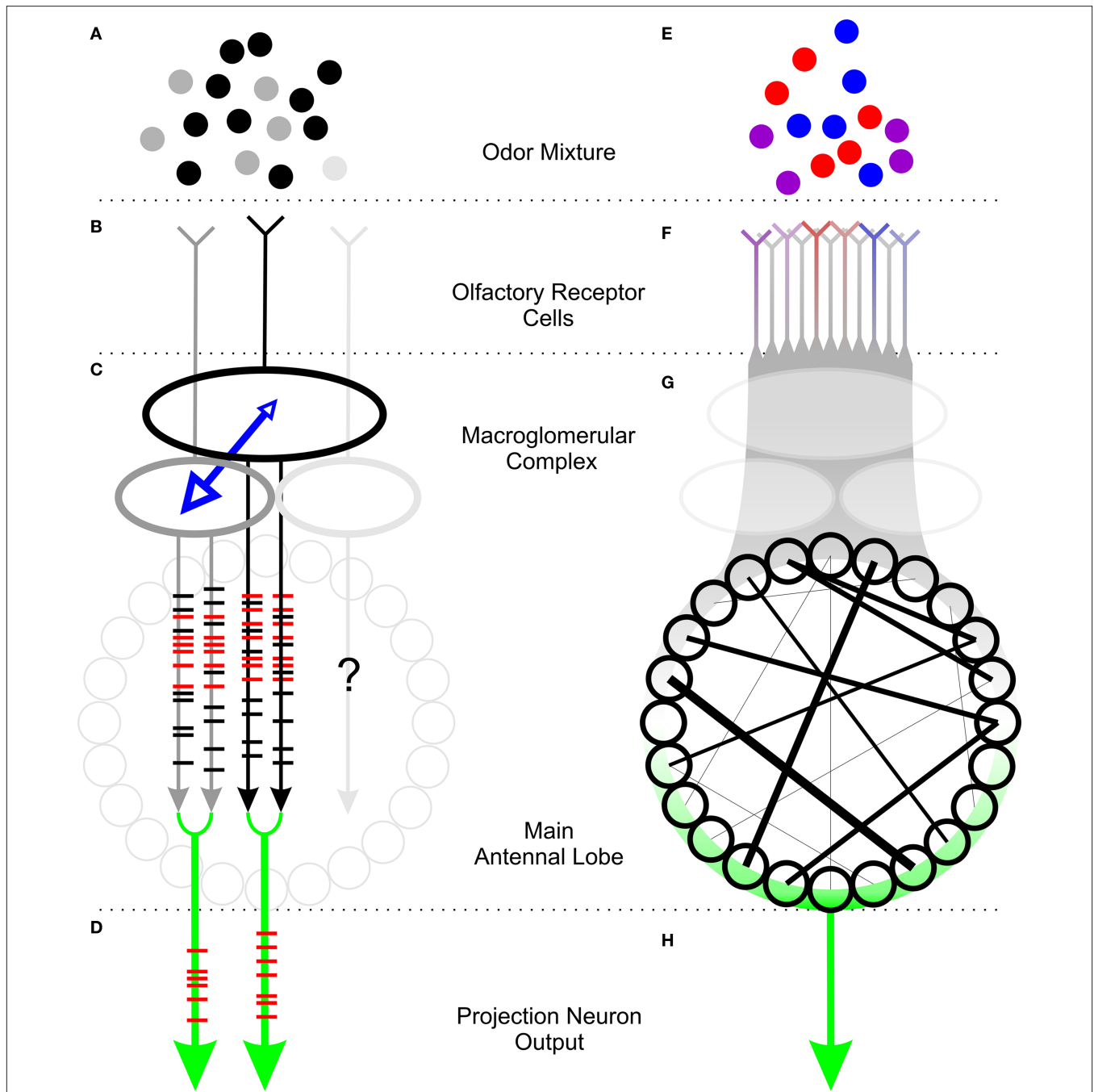
FIGURE 1 | Processing of innately attractive odor mixtures in the sex-pheromonal (A–D) and plant-odor (E–H) subsystems of the moth's antennal lobe. (A) Sex pheromones of moths typically comprise a "major" component (black circles) and one or more "minor" components (gray and light gray circles). **(B)** Pheromonal ORCs respond specifically to one of the components of the pheromone (colors corresponding to component colors in **A**). **(C)** Pheromonal ORCs synapse in glomeruli (large ovals). Glomeruli are connected by LNs (blue arrows) that mediate reciprocal inhibition. The output of PNs in each glomerulus (gray and black lines with superimposed spike rasters) in response to the pheromone includes a high proportion of synchronized spikes (red rasters). **(D)** Trains of synchronous spikes comprise the mixture-specific output of the MGC. **(E)** Plant odors typically include a large number of volatiles (colored circles), of which only a few may be necessary to elicit behavior. **(F)** Multiple ORCs respond to plant volatiles to varying degrees (colors represent specificity to correspondingly colored component from E, saturation of color represents sensitivity). **(G)** Stimulation with an innately attractive odor produces a pattern of synchrony (black lines) across the AL. **(H)** The mixture-specific output of the main AL is characterized by a pattern of synchronized firing of PNs from across the AL.

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