Trends in
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Spotlight

Cracking the encoding of human scent in the mosquito brain

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In a recent study, Zhao *et al.* decipher how the olfactory system encodes human versus animal odors in the mosquito *Aedes aegypti.* By combining genome engineering, *in vivo* calcium imaging, advanced chemistry, and behavioral analysis, the authors provide compelling evidence that the discriminatory coding of host odors is surprisingly simple – and bridges labeled line with combinatorial coding.

The sense of smell enables animals to recognize objects and organisms based on the volatile compounds they emit. This ability may perhaps seem trivial: with ease, on smelling a cup of coffee while lying sleepily in bed, we can visualize the cup on the kitchen countertop. This apparently simple task, however, involves solving a major challenge: each object produces hundreds of diverse odor molecules, many of which are shared with other very different objects, but we can often unambiguously associate a particular smell with a specific item.

To fulfill this task, the olfactory system relies on a limited set of olfactory receptors, most of which are broadly tuned to multiple compounds. The discrepancy between the number of receptors and the vast variety of odors recognized by animals has led to the formulation of the combinatorial coding model of odor recognition [1]: individual odors are identified by reading out the activity of all olfactory sensory neurons (OSNs) – each expressing a single (or very few) olfactory receptor(s) – giving olfactory systems the ability to encode many more odors than the number of olfactory receptors that are present. However, olfactory systems also encompass some narrowly tuned receptors that respond very specifically to select chemicals. A classic example is provided by specific pheromones that are detected by a single OSN population, and whose activation is sufficient to signal to an animal the presence of a suitable mating partner. This model of olfactory coding is referred to as the 'labeled line' model, and is also used to encode some non-pheromone odors that are of particular ecological importance [1] (Figure 1a).

Olfactory systems use both strategies to make sense of the chemical world around them, but how these are integrated, especially when processing the complex odor blends found in nature, remains the subject of active investigation.

In a recent article in *Nature,* Zhao and colleagues [2] revisit this question in blood-feeding female *Aedes aegypti* mosquitoes and their unique preference for human hosts. The choice made by a female mosquito to bite a human as opposed to a nearby animal – for example a sheep – perfectly exemplifies the challenge of olfactory discrimination. Both humans and sheep emanate complex odor blends that share many components, but – from the mosquito's point of view (or rather smell) – what is it that makes us human?

Building on previous work showing that *Ae. aegypti* mosquitoes lacking a functional odorant receptor (Or) coreceptor Orco (that is essential for Or function) fail to distinguish between human and animal hosts [3], Zhao *et al.* genetically engineered mosquitoes to express a calcium activity indicator specifically in Orco^{+} OSNs. This genetic reagent labels about 60% of all OSNs; the remaining OSNs express olfactory receptors belonging to two other families – ionotropic receptors (Irs) and gustatory receptors (Grs). The axons of OSNs expressing the same receptor project to a single glomerular structure within the antennal lobe in the central brain. The novel *orco* transgenic mosquitoes, together with a fast volumetric two-photon imaging set-up, enabled the authors to nearly simultaneously record the activity of all Orco^{+} glomeruli, thus visualizing their combinatorial activation in response to odor exposure. However, odor delivery required careful refinement because the different odorants in a blend have specific properties that affect the way in which they become airborne and reach OSNs. To overcome this limitation the authors developed thermal desorption-based odor delivery methods to precisely present natural blends at concentrations that could be matched across samples. The stage was now set to address the question: how do mosquitoes distinguish human from animal odor blends?

At low odor concentrations, likely relevant for behavioral attraction at a distance, the answer was surprisingly simple: although all odor samples strongly activated one broadly tuned (B) glomerulus, animal blends predominantly coactivated a second, animal-specific glomerulus (A). In turn, with human odor samples, a human-specific (H) glomerulus lit up together with the B glomerulus (Figure 1).

The authors then asked which components within the complex human odor bouquet activate the H glomerulus and are therefore crucial for host discrimination. Both animal and human blends share similar volatiles, but the blend ratios display a clear human-specific signature in which long-chain aldehydes are particularly abundant. Using these compounds alone or in combination, the authors observed strong activity in the H glomerulus, confirming the physiological relevance of the human-enriched odors.

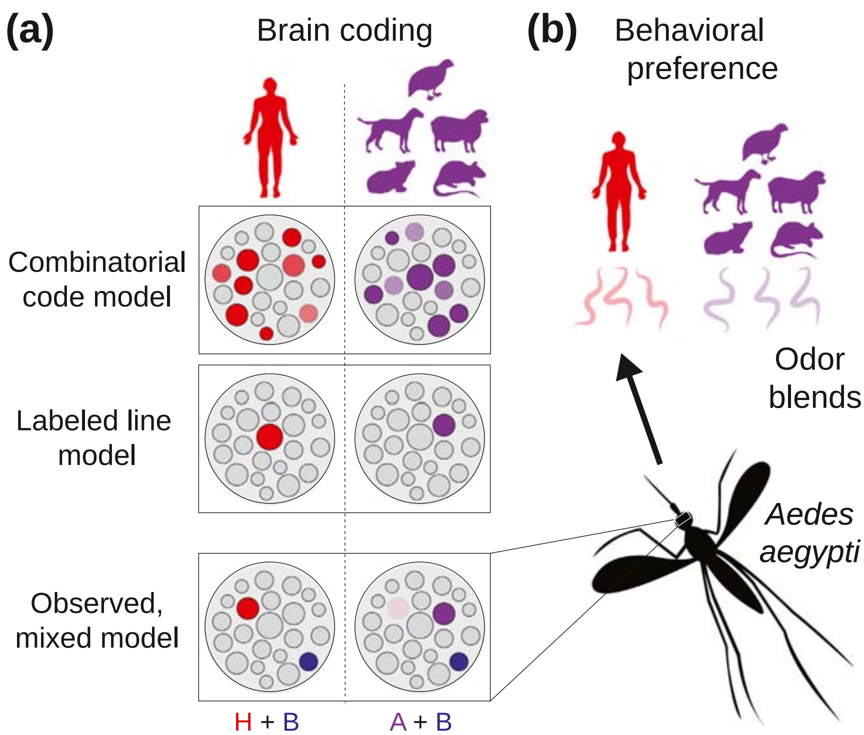

Figure 1. Odor coding in the mosquito brain. (a) A recent study by Zhao *et al.* [2] showed that, to distinguish a human from an animal host, mosquitoes use a simple olfactory code in their antennal lobe. The observed, mixed model (bottom) represents an intermediate scenario between a combinatorial code (top) and labeled line model (middle) of olfactory coding. Animal odors mainly activate a broadly tuned glomerulus (B, in blue) together with an animal-specific A glomerulus (A, in purple), whereas human scents coactivate the B glomerulus and a human-specific H glomerulus (H, in red). (b) *Aedes aegypti* show a clear behavioral preference for human odor blends.

The key question remained of whether H glomerulus activation is really the signal that the mosquitoes use to recognize human hosts. To address this point, the authors filmed mosquito flight trajectories in a wind tunnel while they were exposed to different stimuli. Delivered within a plume of CO_2 (that is necessary to induce a host-seeking state [4]) a binary blend of B and H glomerulus-activating odors was more potent in eliciting host-seeking behavior than either of the two components individually. This result underscores the importance of the binary code for long-range attractive behavior. However, these experiments do not resolve whether activation of the B+A glomeruli (signaling an animal host) induces weaker attraction than B+H activation, and whether silencing the H glomerulus abolishes human attraction.

Mosquito host-seeking behavior is a multistep process involving long-range olfactory signals, CO_2 detection, and visual stimuli to promote target approach, as well as short-range olfactory cues, thermosensation, and gustation to mediate landing and eventually biting [4,5]. In previous work the authors identified a receptor (Or4) sensitive to sulcatone – another human-enriched volatile – that is important for human preference [6]. However, the glomerulus receiving input from Or4 OSNs was not active at the low odor concentrations used in this study, prompting the hypothesis that Or4 is instead important for short-range attraction. Similarly, in addition to the role of Ors in human host selection, Irs are key for attraction to humans [7]. These receptors mainly sense carboxylic acids, which could not be reliably analyzed by Zhao *et al.*, and a recent preprint reports that the levels of these acids in human skin blends correlate with the differential attraction of mosquitoes towards particular individuals [8]. In the future, it will be important to identify the specific receptors that detect human attractive compounds. What receptor is expressed in the OSNs projecting to the H glomerulus? The presence of Orco in these OSNs suggests that it must be an Or. However, recently uncovered coexpression of Orco and the Ir subsystem [9] opens the possibility that H glomerulus activity is conferred by either of the two systems, or even by multiple receptors.

The study of Zhao *et al.* sets the stage for further work on the evolution of mosquito host-seeking behavior. The species used by the authors, *Aedes aegypti aegypti*, evolved preference for humans relatively recently, in evolutionary terms, whereas its close relative *Aedes aegypti formosus* feeds on animals [10]. What is the pattern of glomerular activation in *Aedes aegypti formosus* when it is exposed to human odors? Is the response of the H glomerulus absent, or does it respond to animal odors instead?

Finally, the study by Zhao *et al.* underscores the importance of sensory ecology to neuroscience research. Interrogating neural circuits with ecologically relevant stimuli is essential to truly grasp the computational problems that neural systems need to solve, leading to a more comprehensive understanding of brain function. Moreover, together with previous studies, it highlights the value of employing multiple complementary approaches when tackling the neuronal bases of behaviors. With their groundbreaking work, the authors have shed light on a long-standing question of olfactory coding and paved the way for a better understanding of the fascinating biology of a devastating disease vector.

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Declaration of interests

The authors declare no conflicts of interest.

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