Olfactory coding: sniffing out signals

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A report in *Nature* describes a physiological screen used to identify a previously unknown chemical signal in mouse urine. The chemical's selective response in the olfactory bulb raises interesting questions for how socially relevant odors are encoded.

Across the animal kingdom, chemical cues represent the most widespread means of communication. Chemical signaling allows for tremendous diversity and specificity, by virtue of the huge number of possible compounds and combinations. This diversity, however, makes it challenging for humans to decipher chemical signals: although our own senses often allow us to 'eavesdrop' on animals' visual or auditory communication, our own sense of smell rarely picks out the salient compounds in a chemical signal. Therefore, despite considerable progress, much remains undiscovered about the exact nature of chemical communication, even for relatively well-studied laboratory animals such as mice. Now, Lin *et al.*[1], in a recent issue of *Nature*, detect the salient compounds in mouse urine using an elegant method—by recording neural responses in the mouse olfactory system—and identify a new molecule, (methylthio)methanethiol (MTMT), in the urine of male mice that attracts females (**Fig. 1**). Their results also suggest that the neural representation of these natural stimuli is surprisingly sparse, indicating that our views of olfactory coding, at least for these social odors, merit closer examination.

Most land-dwelling vertebrates have two separate olfactory systems, main and accessory. The main olfactory system detects volatile odors in air, whereas the accessory olfactory system typically detects cues sampled through direct physical contact. Although the best-known class of social odors, pheromones, are most commonly detected by the accessory olfactory system, the main olfactory system is also important[2]. Lin *et al.* focused their efforts on the main olfactory bulb. They recorded electrically from the mitral cells, which receive direct excitatory inputs from olfactory receptor neurons, to test their responses to mouse urine (a stimulus of central importance to mice). In agreement with previous experiments using gene expression as an indicator of neural activity[3], they found responsive neurons in relatively restricted regions of the main olfactory bulb.

To isolate individual compounds, Lin *et al.* then turned to solid-phase microextraction of urinary volatiles and separated them by gas chromatography (GC). The effluent from the GC apparatus was split, with one stream going to an analytical detector and the other directed at the mouse's nose (**Fig. 2**). In this way, individual peaks from the GC were correlated with their ability to induce a neural response. These methods are built on decades of work on insect pheromones, which have identified hundreds of salient compounds using similar techniques[4].

When urine-responsive neurons were tested with the separated urine components, most neurons were stimulated by only a single peak. Moreover, a third of the responsive neurons were excited by one particular compound, present in male but not female mouse urine. Analysis of the fragmentation pattern of this compound by mass spectrometry identified it as MTMT. Synthetic MTMT behaved identically to the natural compound in terms of its retention time in GC and its fragmentation pattern. In neurons excited by the natural compound, synthetic MTMT was also able to induce a response. Together, these observations convincingly demonstrate that MTMT is one of the salient compounds in male mouse urine. MTMT is highly volatile, and the threshold for detection of this compound is very low, about 10 parts per billion in water.

This sulfur-containing molecule is able to activate mitral cells in defined regions of the mouse olfactory bulb, but what is its biological significance? Previous work[5] showed that female mice spend more time investigating urine from intact males than from castrated males. Because MTMT was not detected in the urine of castrated male mice, the authors tested whether its presence determined the interest of females. MTMT added to castrated male mouse urine induced substantially more investigation. Importantly, another compound (acetophenone) present in the urine of intact male mice did not have a similar effect. Thus, MTMT increases investigation of urine from castrated male mice. However, MTMT did not restore the behavior fully: females still spent more time investigating intact male urine than MTMT-doped urine from castrated males, and MTMT added to water induced only a small amount of exploration. Hence, the authors conclude that MTMT, although an important component, must act in concert with other compounds in urine to elicit an attraction response from female mice.

This study also raises intriguing questions about how social odors are represented in the main olfactory bulb. The GC analysis of urine volatiles, as captured by solid-phase microextraction, revealed more than 100 compounds. The authors identified at least 25 different GC elution time points (presumably corresponding to 25 different compounds) inducing responses in the recorded population of mitral cells. Most (80%) neurons responded to only a single component. For the compounds extracted from urine, mitral cells are highly selective, consistent with a 'labeled line' view of olfactory coding. Although direct comparisons are not entirely straightforward, previous physiological studies in mammals, fish and insects suggest a much lower degree of specificity of response by mitral cells (or their insect analogs, anten-

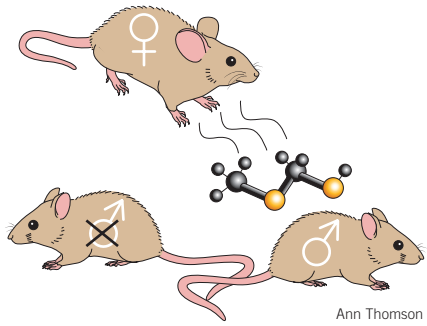

Figure 1 (Methylthio)methanethiol is secreted by male mice and is attractive to females. It is absent from the urine of females and castrated males.

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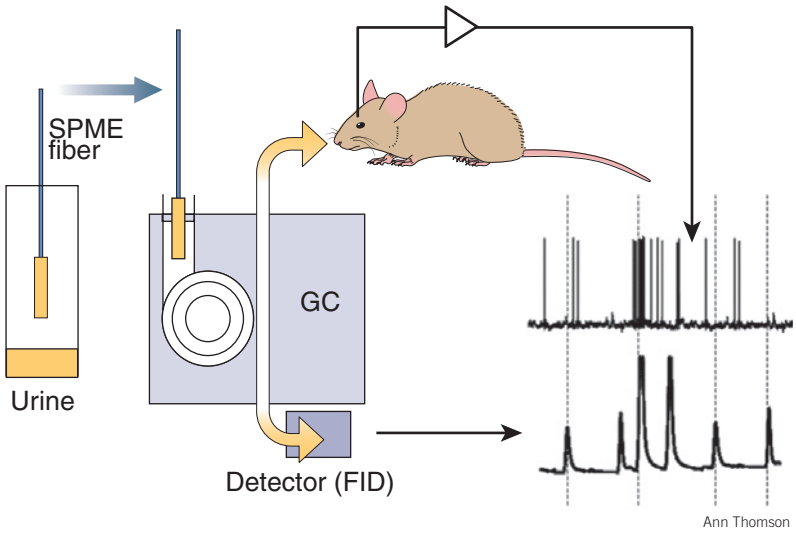

Figure 2 Setup of the electrophysiology–gas chromatography procedure. Urine volatiles were absorbed onto a solid-phase microextraction (SPME) fiber and introduced into a gas chromatograph (GC). The volatile constituents elute at different times, and are simultaneously detected by a flame-ionization detector (FID) and by neural recording from the olfactory bulb of an anesthetized mouse.

nal lobe projection neurons) to commonly tested odorants. In some of these studies, particular mitral cells responded to over half of the compounds presented, an observation that might favor a very different view of olfactory coding[6,7].

What might account for the apparent differences between these studies, and the corresponding views of olfactory coding they inspire? Most simply, perhaps urine volatiles are in a special category, because the mouse's olfactory system has, through millions of years of evolution, become exquisitely sensitive and selective for a stimulus of such importance. This notion has some merit, particularly because at least some of the cells recorded by Lin *et al.* might be associated with regions of the bulb previously identified by their unusual cholinergic innervation[8]. Therefore, with respect to just these stimuli, the main olfactory system might behave more like the accessory olfactory system, which seems to be highly selective[9]. However, Lin *et al.* mention unpublished observations indicating that other, non-social, natural odors behave similarly, suggesting that the discrepancy might not be so easily resolved.

In accounting for differences, one parameter worth noting is stimulus concentration. In both olfactory sensory neurons and mitral/projection neurons[10–12], manipulating concentration can have a large influence on the fraction of responsive cells. For example, in an optical physiology study of *Drosophila* olfactory receptor neurons, increasing the concentration of an odorant by six orders of magnitude increased the number of active glomeruli (regions that pool inputs from sensory neurons expressing the same receptor type) from a few percent to about 70 percent[11]. Concentration might therefore be a determining factor in assessing the specificity of these mitral neurons. The study of Lin *et al.* is appealing in that it uses a natural stimulus at behaviorally relevant concentrations. However, their procedure for extracting and separating the volatiles could either dilute or concentrate individual components, depending on each compound's affinity for the solid substrate. Ideally, each component should be presented at its natural concentration, as was done for MTMT, but to do this universally would seem a daunting task.

A final explanation stems from the possibility that natural odors, such as urine, contain volatiles with little similarity to each other. Because of this diversity, perhaps at most one of these activates an individual cell. However, the same cell, when tested with a well-chosen group of closely related compounds, might respond to several compounds. In such cases, any assessment of 'broad' or 'narrow' tuning is actually a product of the choice of stimuli rather than any property of the neuron itself. Until we have a useful quantitative metric on the space of chemical compounds, and means to sample this space in an unbiased way, assessments of the tuning widths of individual neurons are unavoidably subjective. However, it is less subjective to speak about the percentage of neurons activated by a particular stimulus, because this assessment is unaffected by choices of number or type of the remaining stimuli. In other words, it may be better to view the question of olfactory tuning from the side of the stimuli rather than the side of the neurons: rather than debating 'specialist' and 'generalist' neurons[13], we can more concretely speak of specialist and generalist compounds. Although Lin *et al.* present some data relevant to this issue (fewer than 10% of all mitral cells responded to whole-urine volatiles, and this is likely to represent only an upper bound), single-electrode studies only rarely sample the large number (2,733) of mitral cells reported by these authors. Fundamentally, single-electrode studies face considerable obstacles in answering such questions; fortunately, imaging[14] and multi-electrode recording[15] point to possible ways forward.

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