Social Signals: The Secret Language of Mice

Animals are known to produce substances that modulate social and sexual behavior of conspecifics, but the mechanistic details underlying these phenomena have been elusive. A recent paper identifies a male-specific compound in mouse urine that activates olfactory bulb neurons and mediates behavioral attraction.

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While we humans go to great effort and expense to mask our animal scent with perfume, deodorant and hygienic sprays, other animals use such odors to communicate precise information about themselves to other members of their species. For instance, domesticated dogs intently sample scent marks left by other dogs, allowing them to determine the age, gender, sexual receptivity, and exact identity of the animal that left the mark behind [1,2]. Social communication in rodents is equally robust (reviewed in [3–5]). Male hamsters efficiently choose new female sexual partners over old ones, a phenomenon known as the Coolidge Effect [6]. The onset of estrus and successful fetal implantation in female mice are both modulated by male odors [7]. Mice have the ability to discriminate conspecifics that differ in MHC odortype [8] and can determine whether others of their species are infected by viruses or parasites, presumably a skill of use in selecting a healthy mate [9,10].

Such social odors are typically produced in urine or secreted from scent glands distributed over the body. Both volatile and non-volatile cues are known to be produced [11–14]. The accessory olfactory system, comprising the vomeronasal organ and the accessory olfactory bulb, responds largely to non-volatile cues, while the main olfactory system receives volatile signals. Although mammalian pheromones are classically thought to activate the accessory olfactory system, several newly described pheromones are volatile and may act through the main olfactory system (for example [13]). Chemical signals have a number of advantages in social communication over signals that act on other sensory modalities: they are energetically cheap to produce, often being metabolic by-products; they are volatile and can therefore be broadcast within a large territory; and they can continue to emit signal after the animal has moved to a new location [15].

What are the specific, behaviorally active chemical signals present in urine? What sensory neurons respond to these cues? Can a single such compound be behaviorally active? A recent paper by Lin *et al*. [16] succeeds spectacularly in answering all three questions. The authors applied chemistry, electrophysiology and behavior to this problem, and identified biologically active volatiles in male urine that activate both male and female main olfactory bulb mitral cells. They have elucidated the chemical identity of a single such male-specific urine component that both activates olfactory bulb mitral cells and elicits behaviors in female mice.

The new study [16] builds on earlier work from Diego Restrepo's group that described regions in the olfactory bulb activated upon exposure to whole mouse urine [17,18]. Larry Katz's group [16] borrowed a technique from insect chemical ecology that has been classically used to identify insect pheromones (for example [19]) and used it to identify specific compounds in male urine that activate main olfactory bulb mitral cells. Solid phase microextraction (SPME; Figure 1A) coupled to gas chromatography and single-unit electrophysiology (GC-E) combines chromatographic separation of complex volatiles using gas chromatography with direct recording of biological activity in neurons (Figure 1B). Using this approach, Lin *et al*. [16] recorded from thousands of olfactory bulb mitral cells while fractions of male and female mouse urine volatiles wafted over the olfactory epithelium. By synchronizing the output of the gas chromatography with the electrophysiological traces, they linked specific mitral cell responses to identified peaks in the chromatogram (Figure 1B). Remarkably specific responses were obtained: male and female neurons selectively activated by male-specific urine components; strain-selective neurons; and very rarely, male neurons that preferred female urine.

Identifying one of these active components among hundreds of male-specific volatiles required a major feat of chemical detective work. Lin *et al.* [16] focused their attention on a biologically active peak in male urine, which was absent in urine from castrated males. Mitral cells in a restricted region of the main olfactory bulb fired robustly at precisely 508 seconds into the gas chromatography run, a few seconds after a large peak corresponding to a known mouse pheromone, 6-hydroxy-6-methyl-3-heptanone (HMH) [14]. Further inspection, however, showed a small peak that matched the onset of neuronal activity more precisely.

Separating the imposter HMH peak from the true stimulus required several additional rounds of analytical chemistry that definitively ruled HMH out as the biologically active signal and identified a sulfurous compound as the true signal. Mass spectroscopy narrowed down the possible list of suspects further and one of these, (methylthio)methanothiol (MTMT), was shown to be an exact match for the compound in urine. It elutes identically on gas chromatography, has the same biological activity in mouse olfactory bulb, and is perceived to have the same garlic odor by humans. Two aspects of MTMT-tuned mitral cell responses are of particular interest. First,

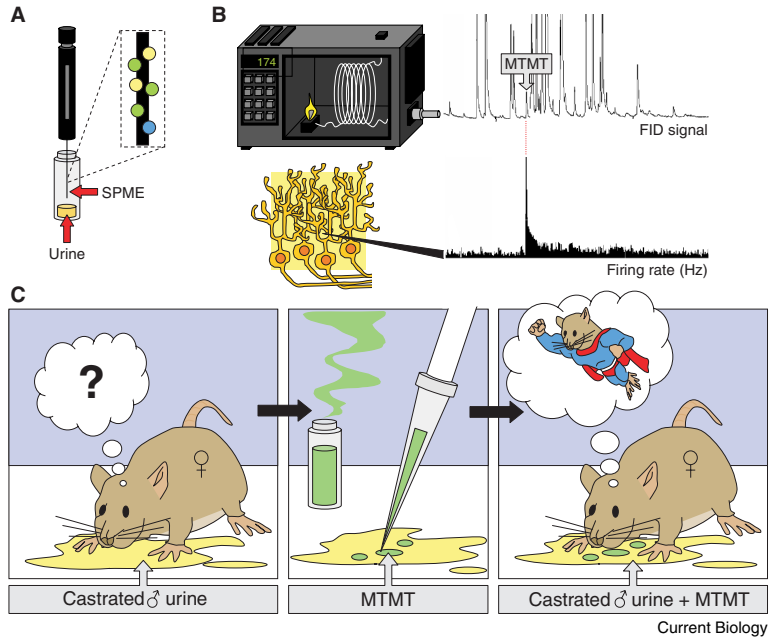

Figure 1. Tracking down the components of olfactory-guided social behavior in mice.

(A) Solid phase microextraction (SPME) is an efficient method for collecting volatiles released from complex odor sources, such as mouse urine. Specific compounds (colored dots) are absorbed by the SPME fiber. (B) Gas-chromatography linked with single unit electrophysiology permits the sequential screening of large numbers of different biologically relevant odorants present in urine. Relying on this technique, Lin *et al.* [16] performed extensive recordings from olfactory bulb mitral cells (lower trace, spikes/sec) which were stimulated with SPME-collected urine volatiles (upper trace, flame ionization detection (FID) signal). As in the schematic recording, many cells displayed a remarkable degree of selectivity in responding only to a single component. This compound, identified as methylthio(methanethiol) (MTMT) is a novel male-specific mouse social signal. (C) Female mice are uninterested in urine from castrated males (left panel), preferring the odor of intact male urine (not shown). The attractiveness of castrated male urine is enhanced by the addition of synthetic MTMT (center and right panels). (Illustration courtesy of Marcus C. Stensmyr.)

these neurons are incredibly sensitive to this compound, responding at a threshold of 10 parts per billion. Secondly, the neurons do not respond to any other of the hundred or so volatiles present in urine or other sulfurous compounds that are structurally similar to MTMT. In this regard, they resemble the classical 'specialist' neurons of the insect that respond only to a single pheromonal component. Such specific responses are particularly surprising given many previous reports that mitral cells are broadly tuned.

Finally, Lin *et al.* [16] showed that MTMT elicits specific attraction in female mice. They confirmed the well-known result that females are more interested in urine produced by intact males than castrated ones (Figure 1C, left). Adding synthetic MTMT to castrated male urine increased the attractiveness of the urine to female mice (Figure 1C, center and right). These results show that even a single component of male urine can be behaviorally active in female mice. This study succeeds in analyzing a social signal from the specific chemical to its effect on identified olfactory bulb neurons all the way to the production of a behavior.

A number of interesting questions remain for future studies. While female mice clearly prefer MTMT dissolved in urine over water, mitral cells do not discriminate between these stimuli. This suggests that integration of multiple urine-derived signals must be occurring higher up in the olfactory circuit. Lin *et al*. [16] identified 112 peaks in intact male urine, but only 57 in castrated male urine. It will be of interest to determine how many of the 55 additional peaks in intact males provoke specific responses in the female mouse. Finally, in this year of the olfaction Nobel Prize [20], it seems irresistible to ask which odorant receptor genes and which specific olfactory bulb glomeruli process urine odors. Are these odorant receptors a specific sub-class dedicated to perceiving social odors? What has happened to these odorant receptors in the course of vertebrate evolution and do we have traces of such putative social signal receptors in our own genomes? Perhaps through the cloud of perfume and deodorant, our own unique scents are still sending a message that others can receive.

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SUMO Modification: Wrestling with Protein Conformation

SUMO modification of human thymine-DNA glycosylase facilitates the processing of base excision repair substrates by an unusual mechanism: while leaving the catalytic center unaffected, it induces product release by eliciting a conformational change in the enzyme.

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Two common strategies to secure a victory are available to a traditional Sumo wrestler: his goal is achieved if he can force his opponent either to step out of the combat arena or to touch the ground with any body part other than his feet. In cell biology, the actions of the small ubiquitin-related modifier SUMO appear to be guided by a similar, but even more flexible set of rules: covalent attachment of SUMO to a protein usually forces the modified target to undergo a change in its localization, its interactions with other cellular components, its stability or its enzymatic activity [1,2]. By affecting the properties of its targets in such ways, SUMO contributes to the regulation of numerous biological processes, ranging from nuclear transport [3] to signal transduction [4], transcription [5] and genome integrity [6].

New SUMO targets are being identified almost by the day, though elucidation of the biological consequences of sumoylation lags far behind the discovery of target proteins. In fact, the mechanisms by which SUMO changes the properties of its targets are rarely well understood on a molecular basis. New work by Steinacher and Schär [7], reported in this issue of *Current Biology*, has now begun to shed light on the mechanism of SUMO function in one particular case.

Human thymine-DNA glycosylase (TDG) promotes DNA base excision repair by recognizing thymine (T) or uracil (U) when mispaired with guanine (G) in double-stranded DNA [8,9]. It cleaves the N-glycosidic bond between the base and the sugar backbone, thus releasing the mismatched base and creating an abasic (AP) site. This structure is then processed by downstream enzymes, which cleave the DNA backbone and initiate restoration of the nucleotide. The reaction intermediate, the AP site, is a

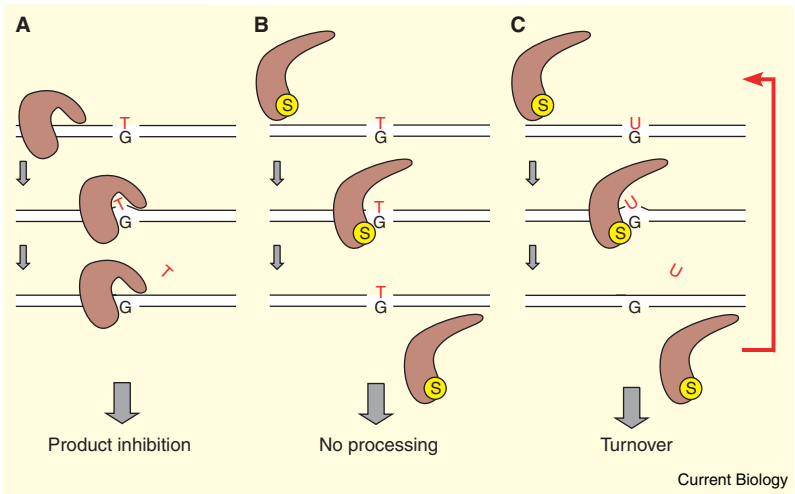

Figure 1. Influence of SUMO modification on the catalytic activity of human TDG *in vitro*.
(A) Unmodified TDG (brown) displays a high affinity for its substrates, including G•T and G•U mismatches in double-stranded DNA, but also for the reaction product, the AP site. Its high affinity is due to the contribution of the amino-terminal domain to non-specific DNA binding and allows the enzyme to process both G•U and G•T mismatches, but also results in a near complete product inhibition due to a failure to release the AP site after excision of the mismatched base. (B) SUMO modification of TDG induces a conformational change in the amino-terminal domain that reduces the overall affinity of the enzyme for DNA. As a consequence, the G•T mismatch, which requires strong DNA binding for recognition, is no longer processed. (C) In contrast to the G•T mismatch, the less demanding G•U mismatch is processed despite a reduced affinity of sumoylated TDG for DNA. Because of the reduction in affinity, however, product inhibition is abolished, and the enzyme is now competent for multiple catalytic turnovers.