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Pheromones Control Gender Recognition in Mice

Researchers have found the first molecular clues about how a group of poorly understood chemical signals, called pheromones, enable mice to distinguish male from female.

In knocking out a gene for a pheromone receptor in mice, the researchers discovered that pheromones appear important for gender recognition. Not only did the male knockout mice lack aggression toward other males — because they didn't recognize them as being male — they readily attempted to mate with both males and females, said senior author Catherine Dulac, a Howard Hughes Medical Institute investigator at Harvard University.

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— Catherine Dulac

The findings by Dulac and colleagues at Harvard were published online January 31, 2002, in *Science Express*, which provides rapid electronic publication of select articles that will appear in the journal *Science*.

Dulac and her colleagues have a longstanding interest in the vomeronasal organ (VNO), a chemical-sensing structure found in the nasal cavities of many animals that is anatomically and functionally distinct from the olfactory system. The VNO, which possesses receptors that respond to secreted pheromones, is wired to a different part of the brain than the olfactory system.

"It had been widely believed that the VNO controlled both mating and aggression, such that when the animal received one type of pheromone, it induced mating and another induced aggression," said Dulac. To better understand the VNO, the scientists produced knockout mice that lacked an important ion channel, called TRP2, which was thought to mediate pheromone signaling in the VNO. Previous studies revealed that TRP2 is found exclusively in the VNO.

"To our great surprise — and, at first, disappointment — we found that these knockout males were perfectly able to mate with females," said Dulac. To understand why this was happening, co-authors Markus Meister and Timothy Holy performed electrophysiological studies on *in vitro* preparations of VNO tissue from the knockout mice. Meister and Holy applied mouse urine — known to contain a mix of pheromones — to VNO tissue and used multi-electrode arrays to measure the electrical activity of the VNO tissue. The studies confirmed that the VNO from the knockout mice did not respond properly to pheromone signals. Additional physiological studies of the knockout mice revealed that the wiring of their VNOs appeared normal, ruling out a developmental defect as the reason why mating behavior persisted in the knockout mice.

After the scientists established that mice with otherwise normal VNOs could not respond to pheromones, lead author Lisa Stowers, an HHMI research associate, began to study the behavioral effects of knocking out *TRP2*. In one experiment, Stowers painted the backs of male mice with urine and introduced them into the cages of knockout mice. The knockout mice failed to show aggression toward their new cage mates.

"It is well known that if you put a male mouse in a cage for a while, it establishes the cage as its territory; and if you put another male in the cage, it will be attacked," said Dulac. "And this attack relies on detection of pheromones by the resident male.

"Besides this lack of aggression by the knockout mice, Lisa Stowers observed another very strange thing — the knockout males tried to mate with the intruder males," said Dulac. "It took us a while to realize what that might mean," she said. "We theorized that the knockout male could be hyper-sexed — willing to mate with any animal — or it might not be able to detect the difference."

The scientists solved the puzzle when they placed the knockout mice in cages with either males or females and found that the knockout mice attempted to mate with either sex. Additional studies showed that the knockout mice emitted the same mating-related ultrasound vocalizations with males and females, demonstrating that a full range of courtship behavior was affected by the loss of *TRP2*.

"Surprisingly, we found that by knocking out this receptor, we are in a sense uncoupling the mating behavior itself and the gender-specificity of the mating," said Dulac. The discoveries apply only to mice, said Dulac, since pheromone signaling may be different in other rodents and mammals and is thought to be absent in higher primates and humans.

Dulac emphasized that the *TRP2* -knockout mouse could have many more pheromone-controlled behavioral effects that the scientists have not yet observed. "We have probably seen only the tip of the iceberg," she said. "We still haven't studied the effects of the knockout in females, and we would like to find out when VNO function is required to establish normal behavior. One might imagine that there is a time period during development when the

mouse needs to have a functioning VNO, but after awhile, the VNO is no longer necessary because the animal can rely on other sensory information." Also, said Dulac, the *TRP2* -knockout mouse could enable the scientists to trace the neural circuitry by which pheromone signals enable the mouse to discriminate sex.