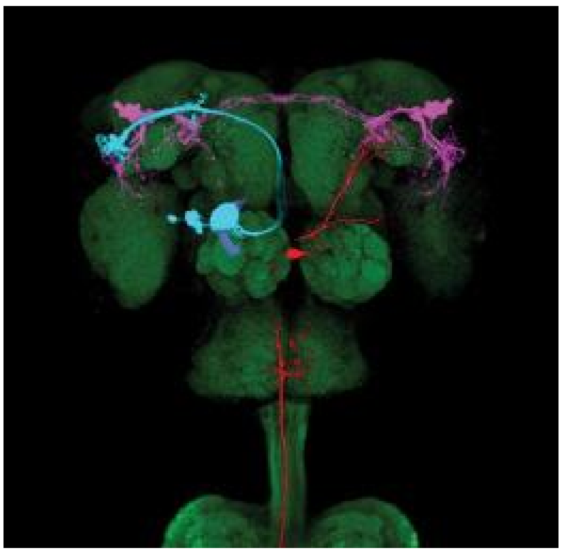
The taste of love: What turns male fruit flies on

June 5 2012, By Diana Lutz



A four-neuron circuit in the male fruit fly brain, called the sex circuit, transforms chemical scents into a physical response. A group of scientists at Washington University in St. Louis have found an ion channel they think activates neurons that feed into the sex circuit, releasing male courtship behavior. The image, which was published in Nature in 2010, was produced by Vanessa Ruta, PhD, a postdoctoral fellow at Columbia University in New York. Credit: VANESSA RUTA

(Phys.org) -- Fruit fly courtship is so highly stylized and repetitive, it is as instantly recognizable as the knee jerk or Achilles reflex.

A male lines himself up behind a female and then chases her, licking her and tapping her with his forelegs while vibrating his wings to sing to her. If she responds to these blandishments, the male attempts to mount her. (To hear the song, see below.)

Scientists at Washington University in St. Louis have found a gene that seems to unleash the courtship ritual. Males missing this gene are capable of courtship; they just have trouble getting started.

This lackadaisical behavior is remarkable because males are usually “highly sexed,” to the point that they will court and mount “perfumed dummies,” decapitated females coated in waxy pheromones.

The problem seems to be that the males with a mutation in this gene, which codes for an ion channel, can’t “taste” the waxy pheromone on the female’s cuticle and without that trigger, the courtship ritual isn’t released, says Yehuda Ben-Shahar, PhD, assistant professor of biology in Arts & Sciences, who led the team.

The study results appeared in *PLoS Genetics* in March.

A mysterious family of ion channels

Ben-Shahar’s team started out with the observation of a simple discrepancy. Fruit flies have 31 genes that code for a specific kind of ion channel, called a degenerin/epithelial sodium ion channel (DEG/ENaC). Mammals have eight or nine, depending on the species.

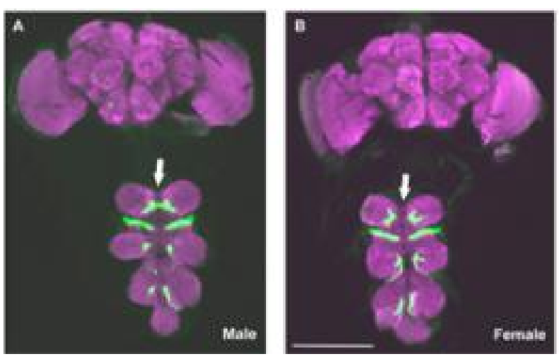
“I began wondering why this superfamily of channels is so diversified, especially in the fruit fly,” Ben-Shahar says.

Ion channels are pore-forming proteins that regulate the flow of ions (in this case sodium ions) across the membrane in cells. Many are involved in the transmission of nerve impulses, which is why most animal venoms disable them in various ways.

Very little is known about the DEG/ENaC superfamily of channels, but Ben-Shahar and his team suspected for various reasons that some of them might be involved in chemosensory-driven behaviors in the fly.

The bodies of insects are covered with hairs that are actually sensory bristles, Ben-Shahar says. Each of these hairs has several neurons in it that are tuned for very specific stimuli. Some are tuned to mechanical stimulation, and others to chemosensory (olfactory or gustatory) cues.

“The term gustatory is actually a bit of a misnomer,” he says. “These bristles aren’t necessarily used to sense food but rather chemicals in a liquid or solid form —such as perfumed waxes that serve as sexual attractants.”



Neurons expressing ppk23, here revealed by a protein that fluoresces green, branch differently in the male (left) than in the female (right). In the male, they cross the midline of the thoracic ganglion, considered part of the fruit fly brain. Credit: BEN-SHAHAR ET AL.

Channel gene Pickpocket 23

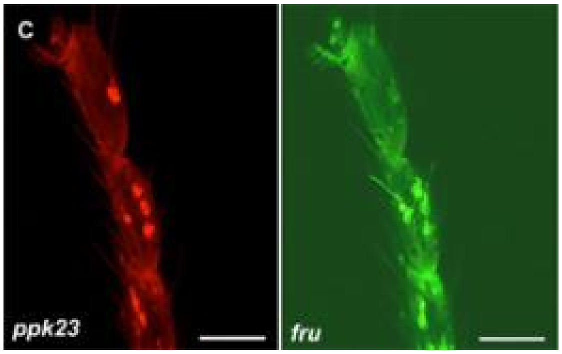
Because the scientists knew some DEG/ENaC channels are expressed in fruit fly chemosensory organs, they reasoned they might be able to find genes in this superfamily by comparing wild-type flies (flies as they exist in nature) with flies with a mutation that causes them to have only mechanosensory bristles.

When they looked at the genes the two types of flies expressed, they found that one ion channel, called pickpocket 23, or ppk23, was markedly reduced in that mutant flies, suggesting this ion channel involved in chemosensation.

By linking the ppk23 gene to one that makes cells glow green, postdoctoral fellow Beika Lu, PhD, was able to see where it was expressed. It turned out to be abundant in the forelegs of male flies, which sported twice as many ppk23-expressing neurons on their legs as did females.

“We knew that the male uses his forelegs in the courtship ritual,” Ben-Shahar says. “The male hugs the female, rubbing his legs and proboscis on her cuticle to test how she tastes. So it made sense that they have something in their legs that could detect the waxy pheromones she secretes.”

When they looked closely at the neurons expressing ppk23, it turned out that they also branch differently in male flies than they do in female flies.



Intriguingly, most ppk23-expressing cells in the male fruit fly's forelegs also express a gene called fruitless. Discovered in 1996, this gene seems to govern almost the entire courtship repertoire. Mutations in fruitless prevent courting entirely or disrupt parts of the repertoire. The ppk23 neurons probably play a role in stimulating the fruitless-dependent circuit to produce sex-appropriate behaviors in males. Credit: BEN-SHAHAR ET AL

They may be feeding into a male-specific neural circuit, called the sex circuit, which determines sexual behaviors and whether an individual fly behaves as a male or a female.

"Courtship is like a reflex with these males," Ben-Shahar says. "You put a wild-type male in with a virgin female and it takes two seconds and he's courting and trying to mate with her."

A courtship assay

At this point, Ben-Shahar and his team had lots of circumstantial evidence that ppk23 was somehow involved in fruit fly courtship, but they didn't really know for sure how it affected fly behavior. To find out, they would need to disable ppk23 and compare the behavior of the flies without functioning ppk23 to that of the wild type.

They designed several different behavioral assays for this purpose, including assays for feeding, tasting and courtship. The feeding and tasting assays showed that the ppk23-disabled flies behaved like wild-type flies, so clearly ppk23 didn't have to do with feeding behavior.

The courtship assay, however, clearly separated the ppk23-deficient flies from the wild-type flies.

The assay was carried out under red light, which left the flies essentially blind. The targets were decapitated females that had been carefully washed in a solvent and then coated with the pheromone 7,11 HD.

"In the assays, the ppk23-deficient males run and run and run, and bump into the female, but never get interested," Ben-Shahar says. "Wild-type flies, the moment they bump into a female, they start courting."

The ppk23-less males were capable of courtship, Ben-Shahar says, they just weren't very good at it. "If you put them in a vial with lots of females, they will mate and you'll get living offspring, so they're not sterile in any way."

"But if you put them in competition with wild-type flies, they wouldn't stand a chance. By the time they decided to court a female, she will already have mated with somebody else.

"We don't know what these ion channels do in females, Ben-Shahar says, but at least in males, the ppk23 neurons are critical in the decision to chase and court a female." "

The inevitable question

What does it mean for people and human sexual behavior? "I'd be shocked," says Ben-Shahar, "if humans were such an anomaly in nature that sensory cues didn't affect sexual behavior."

On the other hand, he says, “Human sexual behavior is very plastic and not at all categorical. It’s never all or nothing, probably extremely complex genetically and strongly affected by the environment.”

Still, if you’re looking for an entree to human behavior, you could do worse than start with ion channels.

“A lot of people argue that if we just understand ion channels, we might be able to understand human behavior without deciphering all 2 million proteins our bodies make, because a lot of the receptors for neurotransmitters are ion channels.”

Provided by Washington University in St. Louis