Why do flies fall in love? Researchers tease out the signals behind fruit fly courtship songs

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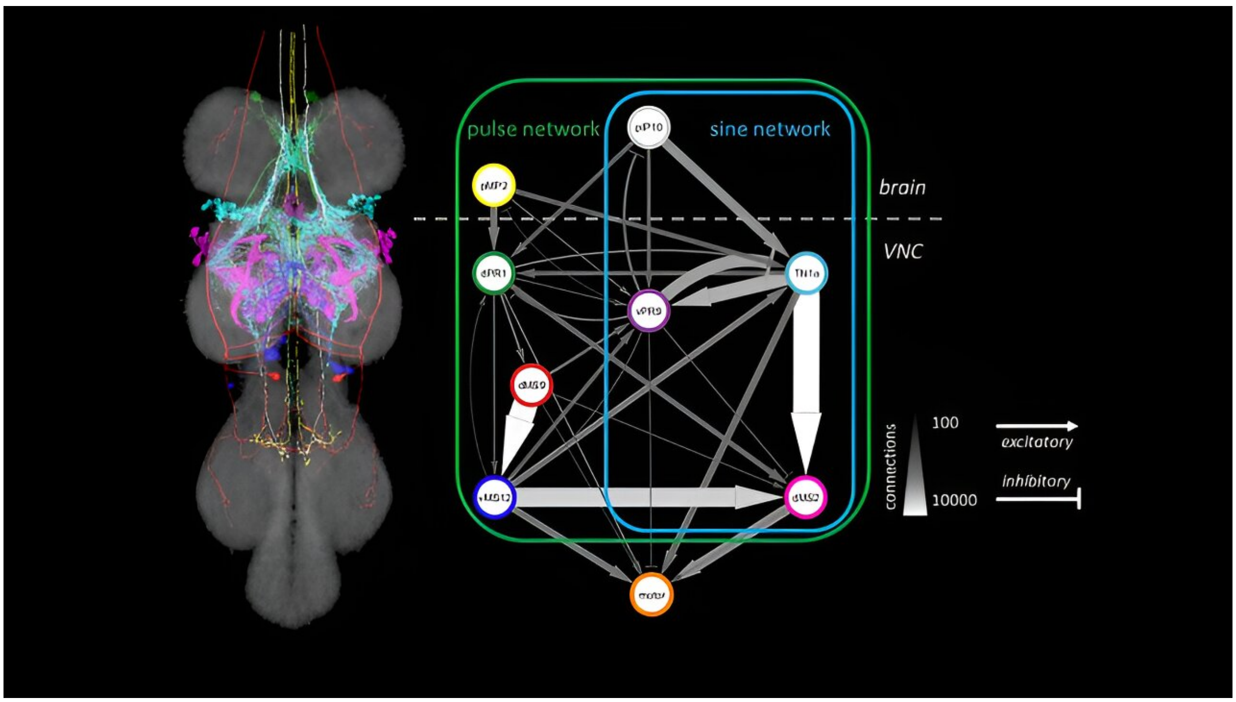

The fly song circuit neuron anatomy (left, from electron microscopy) and the song circuit wiring diagram (right) with the two song networks—pulse and sine—indicated. Credit: Joshua Lillvis / HHMI Janelia Research Campus

Like a Valentine's Day dinner or a box of chocolates, male fruit flies have their own rituals for wooing a potential mate.

As part of a complex courtship behavior, male flies vibrate their wings to produce a distinctive song that conveys a message to nearby females. Using internal information and cues from females and the environment, males decide from moment to moment whether to sing and how.

Although scientists now know a lot about how fly movements produce songs, it is still not clear which cells and circuits in the fly's nervous system enable the behavior.

Now, using a suite of novel tools, including a custom-built fly recording studio, researchers at HHMI's Janelia Research Campus have pinpointed the group of neurons in the nerve cord—a structure analogous to our spinal cord—that produce and pattern the fly's two major courtship songs. They also measured neuronal activity in these cells while flies were singing to understand how these neurons control each type of song.

The result is an in-depth view of how the fly nervous system coordinates a complex social behavior and generates multiple movements using a common set of muscles—information that could help researchers better understand how other animals, including humans, implement sophisticated actions.

The work also provides a new map of the neurons in the nerve cord required for fly courtship song, enabling researchers worldwide to probe further how the behavior evolved and how signals are produced.

"Combining several experimental approaches enabled us to examine the structural, physiological, and functional properties of the song circuit in order to learn how these behaviors are produced," says Joshua Lillvis, a research scientist who led one of two projects to characterize the neural circuits. "But on top of that, I think this will be a big resource for the community that people will be mining for many years."

Modeling behavior

The well-studied fruit fly is a key tool for neuroscientists investigating the neural underpinnings of behavior.

"It is a great model for complex motor programs and communication between the sexes and how those communications evolve," says Janelia Senior Group Leader David Stern, a senior author on the research. "Those questions are hard to answer in any other system."

Because flies are so actively studied, there are now many new tools available to probe these questions, including genetic tools to target specific cells and connectomes that map out the fly's neurons and their connections.

"All the pieces are coming together now to enable a really deep understanding of how these behaviors are constructed and interpreted by the female and then how these behaviors are evolving," Stern says.

One question, two approaches

By taking advantage of many of these new tools, researchers at Janelia set out to investigate the neurons and circuits underlying the movements that produce fly courtship songs in two different but complementary ways.

One project, led by Lillvis, used a collection of genetically engineered flies developed at Janelia that target more than 40 different types of cells that connect to the fly's wings and nerve cord. These fly strains allowed for systematic testing of the roles different neurons play in generating courtship songs.

The team used a custom-built fly recording studio to record the songs generated by 96 flies simultaneously. As they activated or silenced each cell type while the flies sang, the researchers could tease out the role of different neurons in generating the signal. They analyzed more than 1,800 hours of song from more than 5,000 male flies to quantify how changes in neuronal activity affected different characteristics of the two songs.

Once they identified the neurons involved, the team then used the fly ventral nerve cord connectome, completed by Janelia researchers and collaborators last year, to trace how the neurons were connected to each other.

The team found that a small number of neurons are critical for producing fly songs and that these cells form a highly connected, overlapping circuit that generates the two main types of songs. The full circuit of neurons produces one song—the more ancestral of the two songs—while a subset of neurons in the circuit produces the second, more recently evolved, song.

"We think that this might be a common mechanism: as an animal evolves new behaviors, it takes a portion of the circuits that already exist and modifies what they do," Lillvis says.

In a complementary project led by Janelia Research Scientist Hiroshi Shiozaki, the researchers examined the neural activity of singing flies to understand how the neurons produce the song.

To do this, the researchers used a novel instrument Shiozaki developed in Japan and brought to Janelia in his suitcase. Using the combination recording device and microscope, the team was able to image the neural activity in the fly's nerve cord while the insect sang—something that had not been done before. This allowed the team to home in on which neurons contributed to different aspects of behavior.

Remarkably, these two approaches converged on the same conclusion: one nested circuit controls both song types. The results also suggest there is one pathway in the brain that defines when to sing and another pathway that specifies what type of song to sing. These "when" and "what" pathways provide input to nerve cord neurons, activating different cells in the nested circuit that enable the production of the different songs.

For Shiozaki, seeing the project come to fruition is a long-term dream, one that he and Lillvis hope to build on as they further probe how fly song evolved, including in different species of fruit flies.

"It is exciting because it opens up lots of new directions to study the long-term evolution of behavior and more detailed analysis of how complex motor behaviors are produced," Shiozaki says.

The paper is published in the journal *Current Biology*.

More information: Joshua L. Lillvis et al, Nested neural circuits generate distinct acoustic signals during Drosophila courtship, *Current Biology* (2024). DOI: 10.1016/j.cub.2024.01.015

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