How female fruit flies know when to say 'yes'

October 7 2014

Drosophila sp fly. Credit: Muhammad Mahdi Karim / Wikipedia. GNU Free Documentation License, Version 1.2

A fundamental question in neurobiology is how animals, including humans, make decisions. A new study publishing in the open access journal *PLOS Biology* on October 7 reveals how fruit fly females make a very important decision: to either accept or reject male courtship. This decision appears to be generated by a very small number of excitatory neurons that use acetylcholine as their neurotransmitter located in three brain regions. This study provides the framework to understand how decisions are generated and suggests that a decision is reached because that option is literally the most exciting.

In choosing mates, females select traits that they like, which will be inherited by their offspring. Because the chosen traits will likely be displayed by following generations, mate choice is central to both the conservation and diversification of species, and, thus, partially responsible for the great variety of organisms on earth. However, despite how important mate choice is, the genes, cells, and circuits that are required to make such a decision remain largely unknown. To investigate this problem, the authors turned to the brain of fruit flies. Flies are posed, as every other animal on Earth, with daily situations in which they have to make a decision (Should I approach that ripe banana?). In addition, as with the human brain, the fly brain is compartmentalized into regions that process different sensory information (visual, acoustic, olfactory), and it uses the same types of neurotransmitters as humans. Therefore, a fly brain is a good model for studying decision-making.

To tease apart how neurons make a decision, the authors took a genetic approach: they searched for genes that control mate choice. They found one gene that, when mutated, rendered the female flies incapable of deciding whether to mate or not. "This gene, named dati, was hiding in one the most genetically inaccessible regions of the genome" says Dr. Rui Sousa-Neves from the Department of Genetics and Genome Sciences of Case Western Reserve University, the senior author of the publication. "What is so remarkable about dati is that females that are mutant for just this single gene can never decide to accept males, no matter how hard the males try to impress them" says Joseph Schinaman, a PhD student and first author of the publication. Normally, fruit fly females decide whether or not to mate within 15 minutes of a male courting them by dancing, singing (with their wings), and releasing pheromones. If the male has not succeeded after 30 minutes of courtship, it is certain to fail.

The authors went on to determine where, spatially, this gene acted to influence courtship acceptance of the female fly. To test that, the authors reduced dati expression in different tissues using a technology called RNA interference (RNAi) and found it to be required in the nervous system, in cholinergic neurons.

"This was a very exciting moment and we wanted to know where these neurons were located in the brain" says Dr. Sousa-Neves.

To solve this problem, the authors developed a genetic system to generate small patches of neurons lacking dati in an otherwise normal brain. To visualize the brain locations of these patches, they engineered the dati lacking mutant cells to express a green fluorescent protein. The authors systematically analyzed a large number of flies in which different patches of neurons lacked dati, and were able to map the rejection behavior to three distinct regions in the brain, two of which had never been previously associated with mating behavior. They narrowed the circuit down even further by determining which neurons in these brain regions both expressed dati and were cholinergic. To their great surprise they found that a mere 15 cells in two of the regions, and as few as 4 in the third region, were capable of producing mating rejection behavior.

"It is remarkable that such a complex behavior could be generated from such a small number of neurons" says Dr. Sousa-Neves.

"Our results provided a molecular and cellular basis of a hypothesis elaborated more than 50 years ago on how fruit fly females accept males", says Dr. Claudia M. Mizutani, one of the collaborators in this study, from the Biology Department of Case Western Reserve University. Dr. Sousa-Neves explains that this hypothesis, known as "summation hypothesis", predicts that females integrate, or summate, all types of sensory information given by male courtship in order to reach a state of excitement that, according to this study, seems to be provided by a small number of excitatory neurons arranged in a circuit that projects to main sensory centers in the brain.

"This gives us quite an exciting springboard to fully map out this decision-making circuit from all the sensory inputs leading into the brain and how the brain parses and compares these signals, and comes to a decision," states Schinaman.

More information: Schinaman JM, Giesey RL, Mizutani CM, Lukacsovich T, Sousa-Neves R (2014) The KRÜPPEL-Like Transcription Factor DATILÓGRAFO Is Required in Specific Cholinergic Neurons for Sexual Receptivity in Drosophila Females. *PLoS Biol* 12(10): e1001964. DOI: 10.1371/journal.pbio.1001964

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