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Keep Going or Give Up?

The brain of the fruit fly Drosophila contains around 100,000 nerve cells, about one million times less than that of humans.

Picture credit: Nicolas Gompel; Drosophila connectomics group; Graphics: ediundsepp

In day-to-day life, fruit flies are a nuisance we’re keen to get rid of. Ilona Grunwald Kadow, by contrast, cares for almost a million of them and pays close attention to their every move. For the neurobiologist, these tiny insects represent animal models to tackle the fascinating question of how the brain makes decisions.

Kurzfassung

Aufgeben oder Weitermachen?

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Entscheidungen, wie beispielsweise über die Futtersuche, beruhen auf sensorischen Eindrücken, aber auch auf früheren Erfahrungen und dem inneren Zustand eines Tieres oder Menschen. Wo im Gehirn werden solche Entscheidungen getroffen? Welche Nervenzellen sind daran beteiligt und wie bestimmen sie das Verhalten? Diesen Fragen gehen die Neurobiologin Ilona Grunwald Kadow und die Mathematikerin Julijana Gjorgjieva an der TUM School of Life Sciences in Weihenstephan auf den Grund. Als Modell dienen Fruchtfliegen. Mithilfe von Experimenten und Computermodellen haben die beiden Professorinnen einen neuronalen Schaltkreis im Fliegengehirn identifiziert, der Motivation und Durchhaltevermögen bei der Futtersuche steuert.

Julijana Gjorgjieva entwickelte mit ihrem Team ein mathematisches Modell, das dieses Verhalten simulieren kann. Mittels hochauflösender Elektronenmikroskopie konnte Ilona Grunwald Kadow zusammen mit Kollegen in den USA und in England dann die entsprechenden anatomischen Strukturen im Fliegengehirn identifizieren. Experimente mit gentechnisch veränderten Fliegen erlaubten es schließlich, die beteiligten Nervenzellen gezielt an- und auszuschalten.

Der Schaltkreis liegt im Lern- und Erinnerungszentrum des Fliegengehirns. Er wird von zwei gegenläufig wirkenden Botenstoffen – Dopamin und Octopamin – angetrieben bzw. unterbrochen. Dopamin existiert auch im menschlichen Gehirn, und Octopamin hat seine Entsprechung in Noradrenalin. Die Verhaltensprogramme zur Nahrungssuche haben sich vor vielen Jahrmillionen entwickelt. Deshalb vermuten die Forscherinnen, dass sie beim Menschen einer ähnlichen neuronalen Kontrolle unterliegen wie bei den Fliegen. Die neuen Erkenntnisse könnten zu einem besseren Verständnis von Essstörungen beitragen. □

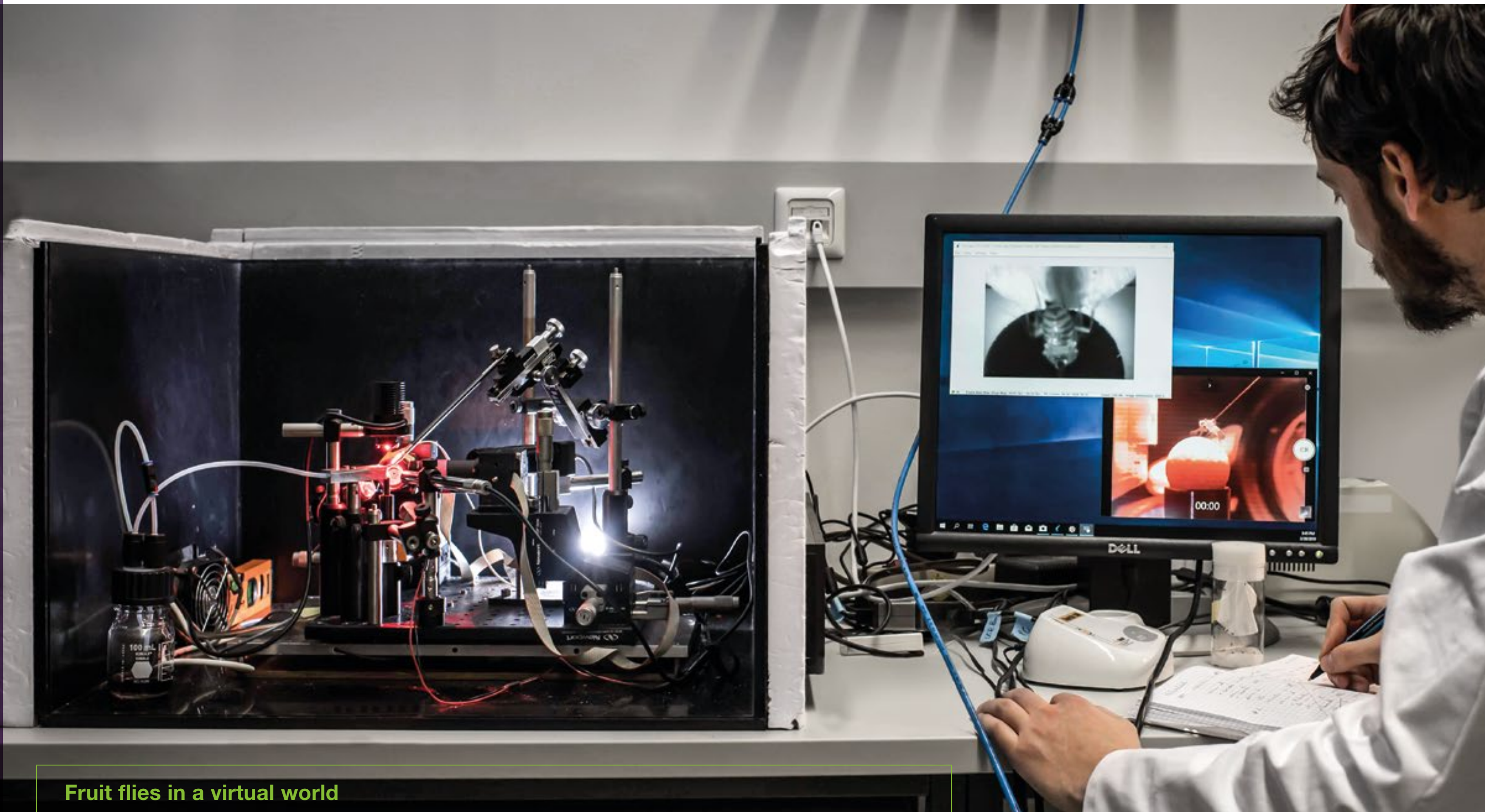
For her complex experiments, Ilona Grunwald Kadow needs a large number of transgenic fly strains that differ in important properties. To keep them apart, the flies are kept at a controlled temperature and humidity in carefully labeled plastic tubes with nutrient medium.

The fly is fixed to a holding bar and runs on the spot. Like Sisyphus, it struggles to get to the source of the scent without ever reaching its destination.

Picture credits: Astrid Eckert/TUM; Sercan Sayin

As lunchtime approaches, everyone agrees: it's time to eat. Work is put on the backburner as everyone moves – as one – to the canteen for the far more important matter of lunch. "In this respect, people and flies are pretty similar," reveals Prof. Ilona Grunwald Kadow. And the neurobiologist should certainly know, since her lab at the TUM School of Life Sciences in Weihenstephan is home to roughly one million flies of the *Drosophila* genus. Kept at an ideal temperature and provided with plenty of food, each fly does exactly as it pleases: flying or eating; crawling or fighting; grooming or mating. "Behavior differs greatly between individuals when they have no common objective," reports Grunwald Kadow. "But that changes when everyone is hungry. Then they suddenly have a shared motivation and pursue the same goal: finding food."

Looking beyond hunger and thirst, triggers that guide the behavior of flies and humans alike include sensory impressions, emotions, experiences and internal state. Where in the brain does this information converge? Which nerve cells (neurons) are involved and how do they determine behavior? Grunwald Kadow is searching for answers in her fruit flies. "A fly's brain has almost a million times fewer nerve cells than our own, so it's easier to find out what a single neuron does," the biologist explains. And the tiny insects are very suitable for her experiments in other ways, too. Similar to us humans, a hungry fly will drop everything to look for something to eat. As soon as it smells the scent of food, it heads straight for the source. And on reaching its destination, it stops and eats. ▷



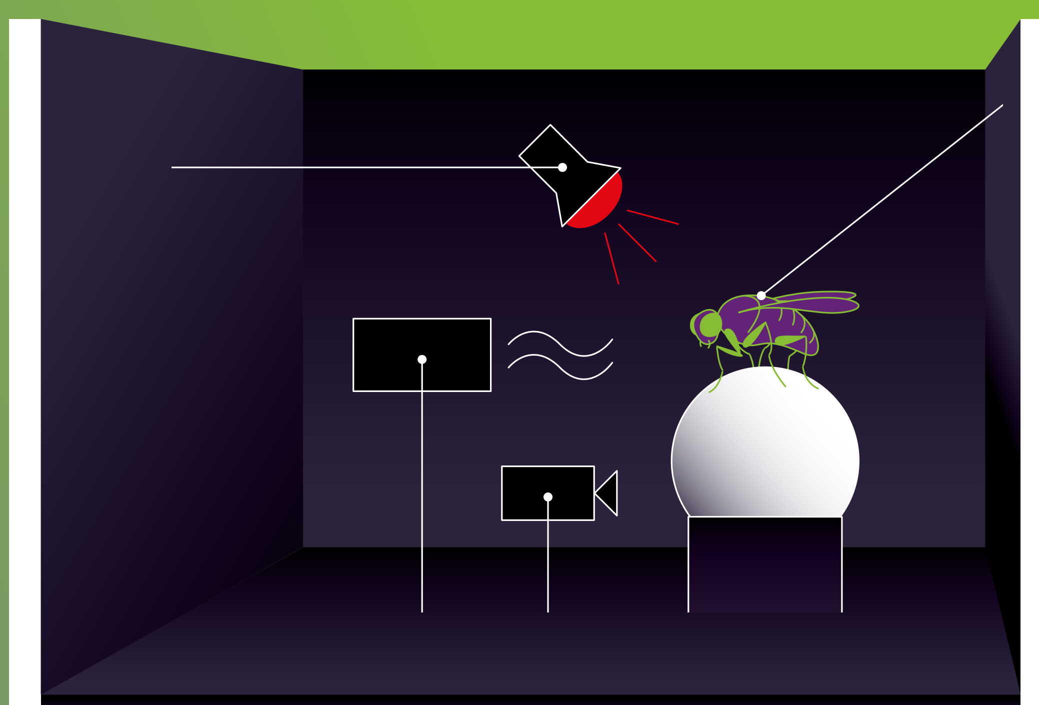
Fruit flies in a virtual world

The experimental setup, shown in real on the left and schematically on the right: In order to decipher how the brain controls the motivation of a fruit fly, the animals are placed in a darkened box in a kind of “virtual reality”. There, they are allowed to run on a moving ball (here in the center, illuminated in red) and a delicious scent is blown in front of their noses. So they imagine that they are moving towards food. In fact, however, they are glued to a holder on their back and are running on the spot. This trick allows the experimenter to observe individual flies from the workstation on the right, either on the monitor or under the microscope.

In the process of searching for food, it displays perseverance, even if it doesn’t immediately find anything.
“Using this behavior as a model, we are trying to gain an understanding of basic processes in the brain. We ask ourselves questions such as: How does the nervous system keep the fly on task even if it doesn’t immediately achieve its goal? And how can it suddenly abandon this goal and engage in a new, contrasting behavior as soon as it finds food – even though the scent of food, which previously triggered the food search, is still there?” explains Grunwald Kadow. This requires a flexible yet reliable neural mechanism capable of triggering the appropriate behavior and suppressing other behaviors in every situation. Hence, there is a kind of hierarchy within the nervous system – and the prevailing response depends on the situation, objective and internal state.
To find out how this works, the flies in Weihenstephan are placed into a type of virtual reality situation. An appetizing scent is wafted in front of their noses as they run on a moving ball in the belief that they are on course to find food. In fact, their backs are glued so that they are running on the spot. This trick enables the researcher to observe individual flies running at full speed on a monitor or under the microscope. “This shows us that hungry insects run after a food scent for longer and in a faster and more targeted way than ones that are already full,” explains ▷

“We suspect that behavioral programs are subject to a fundamentally similar neural control process in flies and humans.”

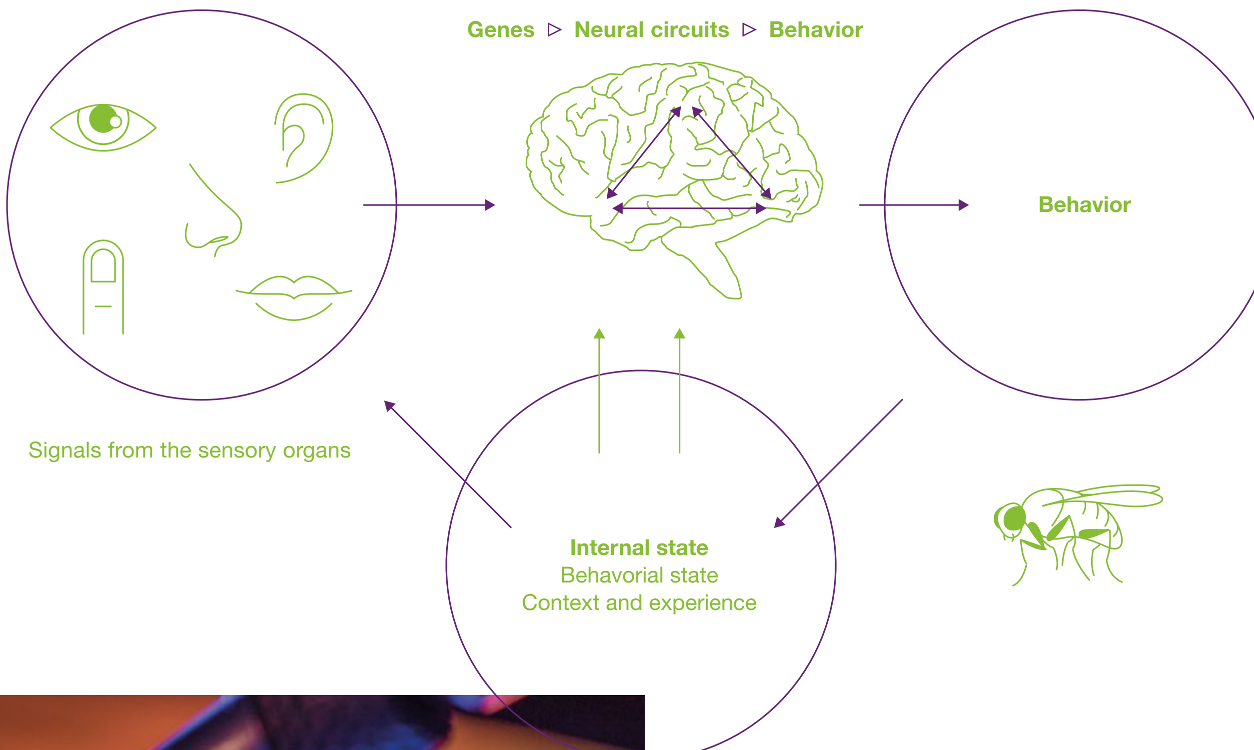
Ilona Grunwald Kadow



The schematic experimental setup: A fruit fly, stuck to a holder, runs on a moving ball. From the left, food scent flows towards it, while at the same time it is illuminated with red light so that its food neurons are activated. A camera transmits its behavior to the monitor.

Picture credit: Astrid Eckert/TUM; Graphics: ediundsepp

lead investigator Grunwald Kadow. That is hardly unexpected. But the more surprising finding is that, even after ten unsuccessful attempts, hungry flies do not give up. While full insects slacken quickly, the hungry ones try harder and harder each time. The "run and find" program seems to intensify each time as a result of their internal motivation.
"The easiest way to explain this is that there's a kind of feedback loop in the brain. So, we looked for corresponding brain structures in the flies," the neurobiologist continues. It was in the brain's learning and memory center – known as the "mushroom body" on account of its shape – that the TUM team made its finding: "Thanks to electron microscope images from our colleagues in the US and UK, we can see which neurons "talk" to each other. Thus, we were able to identify a two-way connection between neurons that communicate with the help of dopamine – a neurotransmitter or chemical messenger also found in humans," she reports. These "dopamine cells" not only communicate among themselves, but also with input and output cells, which respond to incoming scent signals and make the flies run. Cells sensitive to sugar and other taste stimuli also play an important role here. These "food neurons" communicate with the output cells via the messenger substance octopamine, whose function is similar to noradrenaline in humans.
At this point, Prof. Julijana Gjorgjieva enters the picture. The computational neuroscience expert has developed a mathematical model to replicate the flies' behavior based on interactions between the three types of neurons.



The team anesthetizes the flies with cold or CO_2 and uses tweezers and micromanipulators to position them under the microscope. They are glued into place with UV-cured dental adhesive, which hardens extremely fast.

“We were able to identify a two-way connection between neurons that communicate with the help of dopamine.”

Ilona Grunwald Kadow

By contact with red light, certain neurons of the genetically modified flies can be switched on and off. These flies are kept in blue light so that this only happens during the experiments.

Picture credit: Astrid Eckert/TUM; Graphics: ediundsepp

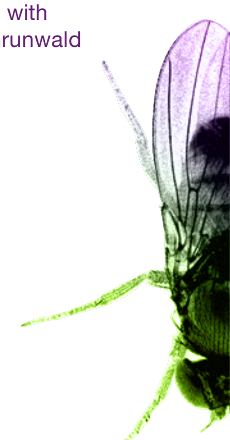
The model is able to simulate how hungry insects ramp up their efforts to reach food after each unsuccessful attempt. And it explains how food stimuli cause the flies to switch from “run and find food” to “stop and eat” mode. The two messenger substances play a decisive role here, exerting an opposite effect on the output cells: dopamine fuels the feedback loop, while octopamine inhibits it. “Mathematical models help us to unravel the complexity of neural circuits. Ultimately, though, they have to stand up to real-world behavioral responses,” emphasizes Juliana Gjorgjieva.

The reality check here takes place using genetically modified flies, where very specific types of nerve cells have been selectively manipulated. This is done by inserting genetic elements into the flies’ DNA that respond to temperature increases or light stimuli, either switching off or activating the relevant neurons. “We generate some of these transgenic *Drosophila* strains ourselves and obtain others from colleagues,” reveals Grunwald Kadow. Her team uses these specially adapted flies to turn on and off all of the elements in the neural circuit of interest, one step at a time – in the living insect. In some flies, the researchers block the suspected feedback mechanism within the dopamine or output cells. To achieve this, they need only increase the temperature in the room by a few degrees Celsius. The transgenic flies are equipped ▷

with a heat-sensitive protein that prevents the release of dopamine in the targeted nerve cells, and therefore avoids activating the recipient neurons – but only at temperatures of 30 degrees or above. Therefore, flies that receive this heat-sensitive protein then lack the dopamine-driven feedback loop and thus receive no feedback about previous unsuccessful attempts. Instead of stepping up their efforts each time like regular flies, they always run at the same speed and give up faster.

Other flies are genetically engineered so that their food neurons contain a light-sensitive protein channel. When exposed to red light, which penetrates from the outside through the fly's body tissue, the channel opens. This allows sodium and potassium ions to pass through the membrane of the nerve cell, triggering an excited state that leads to the release of octopamine. Since this messenger substance inhibits the output cells, these insects stop running – even if they are hungry and can smell food.

Julijana Gjorgjieva (on screen) develops mathematical models to decipher the complexity of neural circuits. Via Skype she discusses the results of her computer simulations with her colleague Ilona Grunwald Kadow.



Prof. Ilona Grunwald Kadow

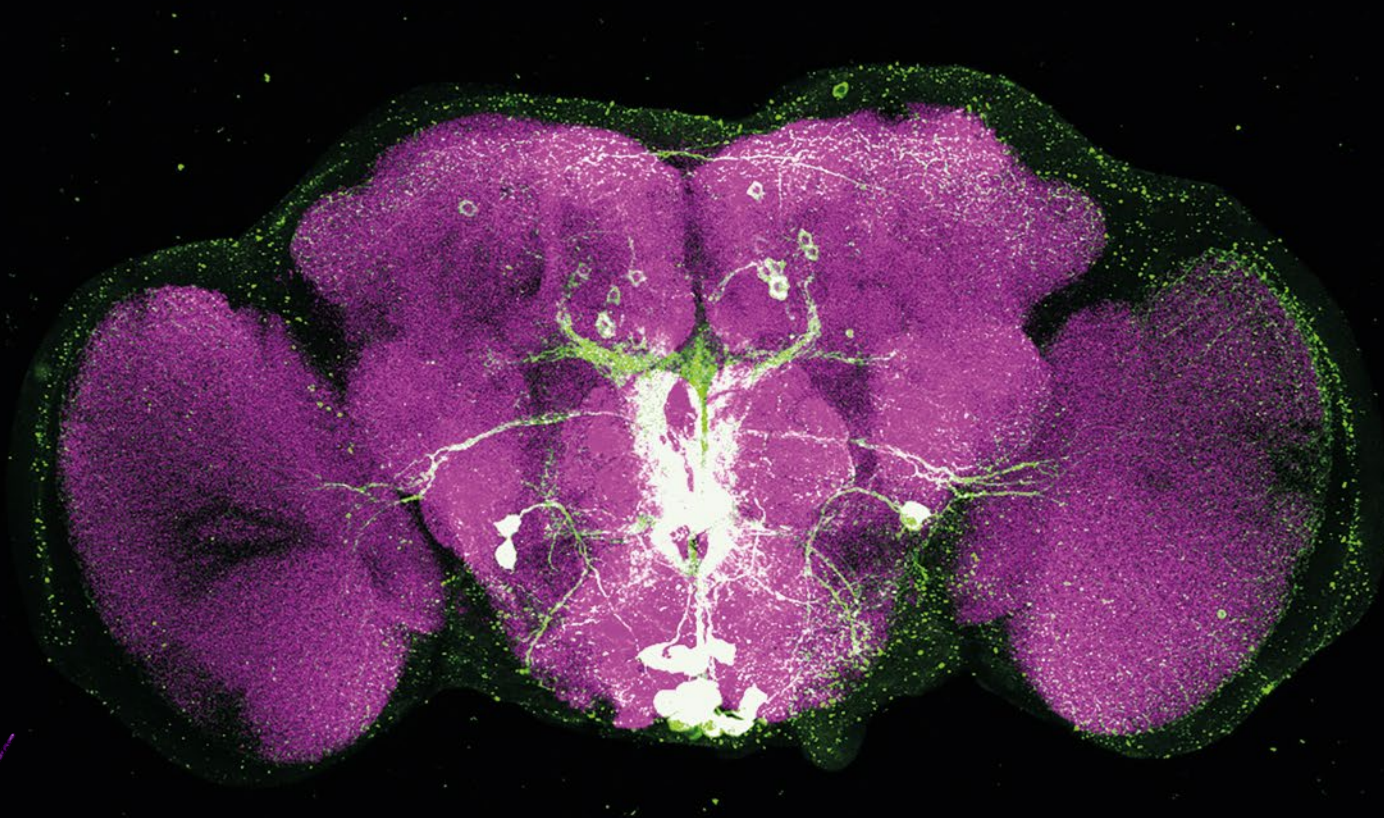
Geneticist at the forefront of brain research

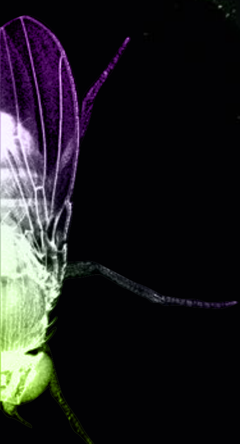
After studying biology in Göttingen (Germany) and San Diego (US), Ilona Grunwald Kadow completed her doctorate in neuroscience at the European Molecular Biology Laboratory and the University of Heidelberg (Germany). Following a postdoc at the University of California in Los Angeles (US) and at the Max Planck Institute (MPI) for Neurobiology in Martinsried, Munich (Germany), she spent eight years as an Emmy Noether and then a Max Planck research group leader at the Martinsried MPI. She took up the newly created professorship for Neural Circuits and Metabolism at the TUM School of Life Sciences in Weihenstephan at the start of 2017.

Prof. Julijana Gjorgjieva

Young leader with interdisciplinary focus

Born in Macedonia, Julijana Gjorgjieva completed her Master's in mathematics and her doctorate at the University of Cambridge (UK). Two summer schools sparked her interest in systems biology and neuroscience, initially taking her to the Center for Brain Science at Harvard and then to Brandeis University (US) as a postdoc. She has been leading a research group at the Max Planck Institute for Brain Research in Frankfurt (Germany) since 2016 and also holds a professorship in Computational Neuroscience at the TUM School of Life Sciences in Weihenstephan.





The green highlighted nerve cells use the messenger substance octopamine to transmit signals from the brain stem of the fly to the rest of the brain. The magenta coloration is produced by antibodies and identifies synapses that are everywhere in the brain. Thus, the green and white coloration indicates signaling pathways in the brain.

Picture credits: Astrid Eckert; Nicolas Gompel; Anja Friedrich

"Thus, the flies behave exactly as our model predicts. That is a very strong indication of the existence of this circuit," Gjorgjieva sums up.

What, then, can we learn from *Drosophila* flies? "Hunger is a major area of similarity between flies and humans. The associated behavioral programs evolved millions of years ago. Therefore, we suspect that they are subject to a fundamentally similar neural control process in both species," explains Grunwald Kadow. The fact that this control does not always work properly can be seen in patients with eating disorders: Some are unable to stop eating long after they are actually full, while people with anorexia are no longer able to respond to their hunger signals and, in extreme cases, can even starve themselves to death. "Something is way off track there. A program that is definitely flawed has managed to suppress a program vital to survival, which is actually supposed to be dominant. We want to understand how and where in the brain that happens and how we could potentially intervene," the TUM researcher concludes. And now that she knows how the brain circuit works in the fly, she is another step closer to that aim. ■ *Monika Offenberger*