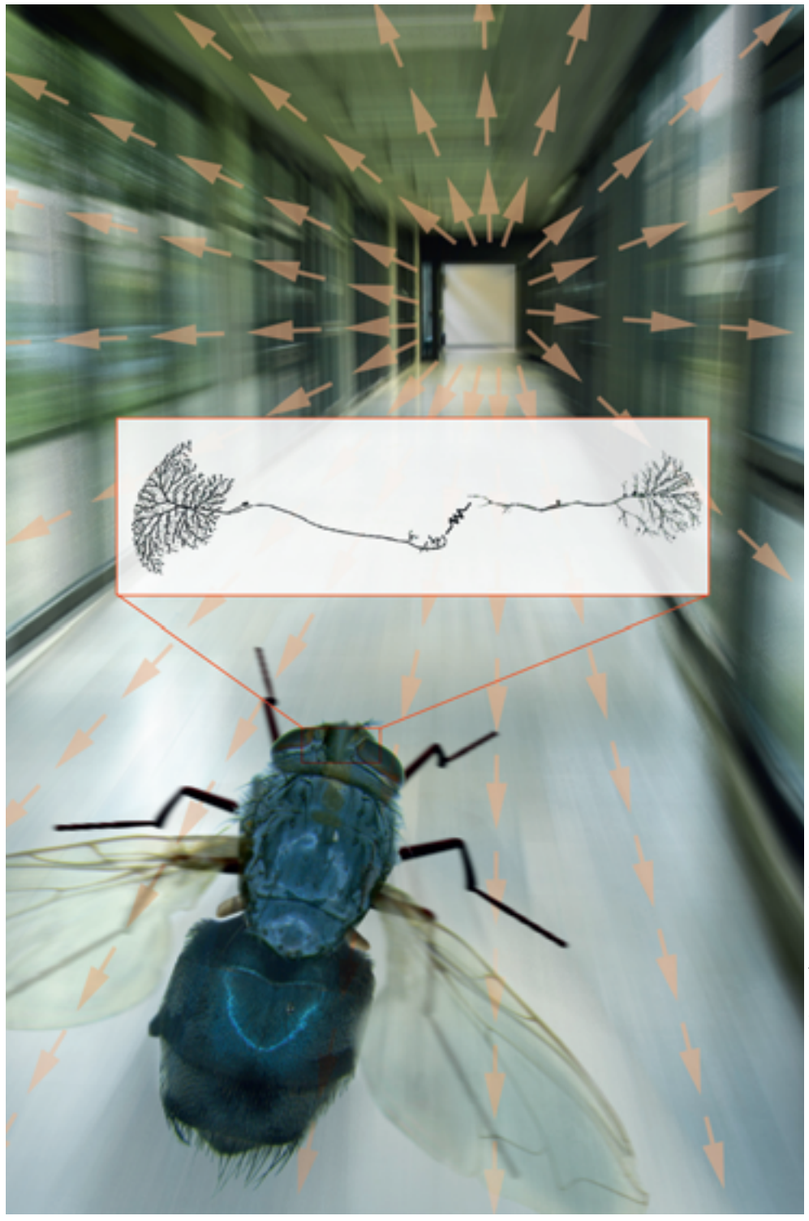
Inside a Fly's Head

A thousandth of a gram of nerve cells packed into a cubic millimeter of space: that pretty much sums up the brain of a fly – on the surface, anyway. Yet it's an amazing organ. In fractions of a second, it translates optical information into steering commands, enabling flies to perform aerial acrobatics.

Alexander Borst, *Director at the* **Max Planck Institute of Neurobiology** *in Martinsried, is studying the circuitry and components of this incredibly high-performance onboard computer.*



When it is flying along a corridor, the images a fly sees of its surroundings change constantly (illustrated by arrows). To control and correct the flight path, this "flow field" has to be analyzed at a higher level of the visual center, the lobula plate. To control rotations, two nerve cells must be directly linked, the HSE cells (right) and the H2 cells (left).

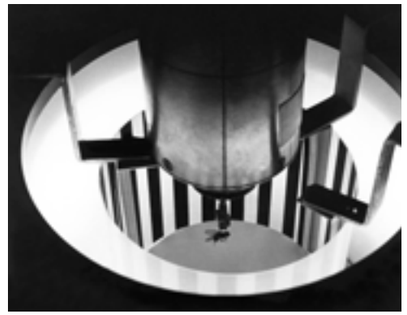
Photos: MPI of Neurobiology – Robert Schörner / Blachian – MPS

Borst came across the objects of his interest – flies – when he was a student under Martin Heisenberg at the University of Würzburg in the late 1970s. After finishing his degree, he joined the Max Planck Institute for Biological Cybernetics in Tübingen to work with Karl-Georg Goetz and Werner Reichardt, two researchers who had perfected some ingenious methods for observing flies in flight in such detail that conclusions could be drawn about their brain activity.

This involved "integrated analysis behavioral research," conducting experiments in which a fly is exposed to a defined optical environment and its flight reaction measured. The objective was to discover the relationship between stimulus and flight reaction and to use this to work out how the nerve cells behind the retina of the fly's eye were interconnected and how they worked.

Musca domestica, the common housefly, served as the lab animal, and a sophisticated flight simulator was used for the experiments. It comprised a revolving hollow cylinder in the middle of which the fly was tethered with its back to the axis of a torque compensator, its vertical body axis coinciding with the compensator axis and the centerline of the cylinder.

This meant that, while the fly was not able to turn at all, its flight forces around the vertical body axis could be measured. The fly was then presented with simple optical environments, such as a single vertical black bar on the otherwise uniform white walls of the cylinder, or a periodic grating, again consisting of vertical bars on a white background. The cylinder could either be turned from the outside and the reaction of the fly registered, or the fly could be "coupled" to its optical environment by using the output signal of the compensator to steer the cylinder, allowing the fly to move its environment itself and thus simulating free-flight conditions.



In a revolving hollow cylinder, a fly is tethered with its back to the axis of a torque compensator. By allowing the fly to determine the rotation of the cylinder, free-flight conditions on a horizontal plane can be simulated.

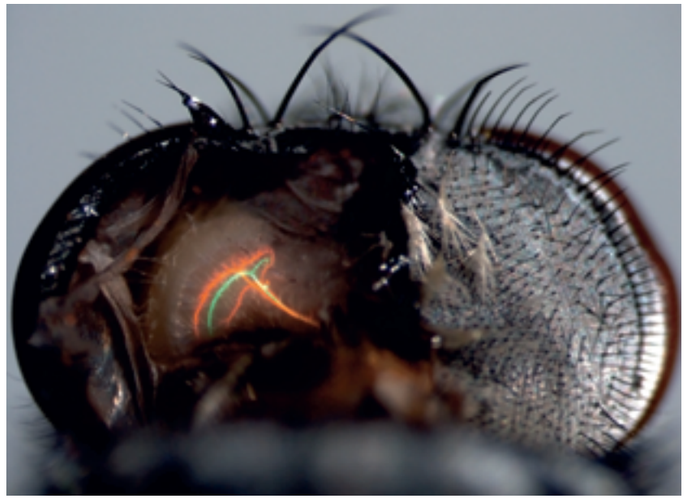
Such experiments allowed the Tübingen-based researchers to deduce the basic logical principles according to which the housefly's onboard computer analyzes its optical environment and passes on information about its position and movements, and about moving objects in its environment. These experiments, which revealed the software used in the fly's brain, as it were, provided the basis for the subsequent analysis of the hardware – in other words, the nerve cells and their function and circuitry. This is the project that Alexander Borst, now head of the Department of Systems and Computational Neurobiology at the Max Planck Institute of Neurobiology in Martinsried, is still working on.

Rather than the *Musca domestica*, the researchers are now studying the larger and more robust *Calliphora vicina*, the blowfly, whose brain works and is constructed according to the same principles as that of the housefly. Blowflies also glean information about their environment by evaluating and processing the signals they receive from receptors in their visual system. In principle, it makes no difference whether an object moves past or toward the fly while the fly is stationary, or whether the fly itself moves around. What is important are the changes in the light signals picked up by the receptors, such as the difference in the light from a lamp or light that is reflected by an object.

The Moving Environment Is a Vector Field

In order to perceive its environment and adapt its flight path to it, the fly must interpret what is known as a vector field: each individual image point and its current speed are registered. This allows the fly to derive information on how far away objects are and how the environment is structured spatially. This is because close objects pass through the field of vision more quickly than those that are farther away – like when we look out of the window of an express train: trees that are close to the track flash past while more distant features move more slowly and mountains in the background hardly change position at all.

A simple vector field can represent two basic types of movement: upward/downward movement and ro-



Nerve cells can be "loaded" with a fluorescent dye that glows when stimulated by a laser. By simultaneously injecting red and green fluorescent dyes, the researchers were able to demonstrate that all the VS cells in the fly are linked in a series circuit.

tation. The current velocity is represented by arrows, which are known as vectors. The tip of the arrow indicates the direction of movement, while its length is a measure of the speed, so the longer the arrow, the faster the movement.

However, to avoid misinterpretation, the whole picture must be taken into account, since a single arrow could belong to a vector field that describes the upward movement of the fly (from the point of view of the fly, the environment is moving away from it, or downwards), or to one that represents a rotation. "That is why neurons in visual steering systems cover such large receptive fields, whether in a fly or in the cortex of an ape. These cells are constantly analyzing nearly half the field of vision," says Alexander Borst.

The receptors that transmit optical information to the fly are located in the retina, which is effectively the outermost outpost of the visual system. In flies, a layer of nerve cells called the lamina is attached to the retina, and this is where light-dark adaptation takes place. Just as humans who move from the shade into dazzling sunlight have to squint until their eyes have adjusted to the light, the fly's visual system also has to adapt to light-dark changes.

Moreover, it can detect very small changes in light extremely well because of the way it processes the changes in the signals provided by the individual receptors in the lamina. To understand this, it helps to imagine a lamp that is continuously on. If this lamp is alternately switched to somewhat brighter and then returned to its original brightness, it is very difficult to distinguish the change between bright and very bright. So the signal is processed in such a way that the constant portion of light is excluded. Then the change is no longer between bright and very bright, but between dark and light, and the change is much easier to detect. This explains how flies are able to detect extremely fast movements so well and to easily dodge the hands of someone who wants to swat them.

Detecting the Direction of Movements

At this point (once the visual stimuli have passed the retina and lamina), the fly is still receiving local information that originates from an individual receptor. In the fly's visual system, the retinotopic neurons are responsible for detecting movement. This means that information from adjacent receptors – so also from neighboring image points in the surrounding environment – is processed in adjacent nerve cells. The human visual system functions the same way. The nerves that process the optical stimuli create a neuronal map of our surroundings, so to speak.

This retinotopic construction is by no means a given, as investigation of a different part of the fly's visual system reveals. The ocelli – round, light-sensing organs – measure only overall brightness. The information on the distribution of light is discarded, as if an image were of a black-and-white striped or checked pattern.

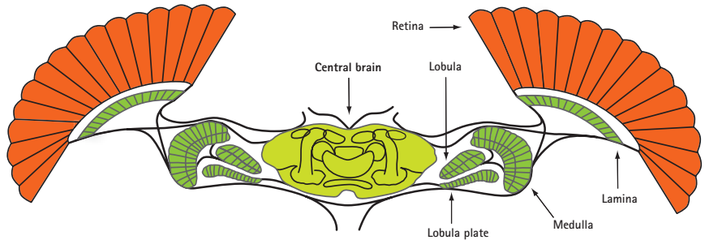
To receive movement information, neighboring receptors must be interconnected. They then form, for example, what is known as a Reichardt detector, which can make what is in fact a very complex decision: for them, a movement from right to left is very different than one from left to right. An individual receptor can distinguish only between light and dark, but not detect the direction of a movement, similar to a light barrier in a vault, which can detect only that someone has crossed the threshold of the vault, but can't determine whether that person has entered or left the vault. To make that decision, at least two interconnected light barriers are required.

Back to the fly: when it sees a moving pattern, the signals that reach the detector output depend on the direction in which the pattern is moving. The downstream nerve cells are stimulated accordingly – specifically in the layers of the nerve cells that connect from "outside to inside," from the retina to the center of the brain: in the medulla, the lobula and the underlying lobula plate.

Nerve Cells with Distinct Morphologies

In the lobula plate, distinctively formed neurons called LPTCs pick up the signals that are passed on by the Reichardt detectors. LPTC stands for lobula plate tangential cells. There are three types of LPTCs, and each type is sensitive to a specific movement direction: the HS and CH cells react primarily to horizontal movements – forward and backward movements and movements from left to right or vice versa – while the eleven vertically arranged VS cells register vertical movements, or upward and downward movements. Each individual VS cell is also specialized in a different type of movement. For example, the VS1 cell reacts best to vertical movements in front of the fly, while VS7 is specialized in vertical movements to the sides of the fly.

Jürgen Haag, who has been working with Alexander Borst for many years, found an interesting circuit layout in VS cells. They are connected by a series circuit, so that a stimulus

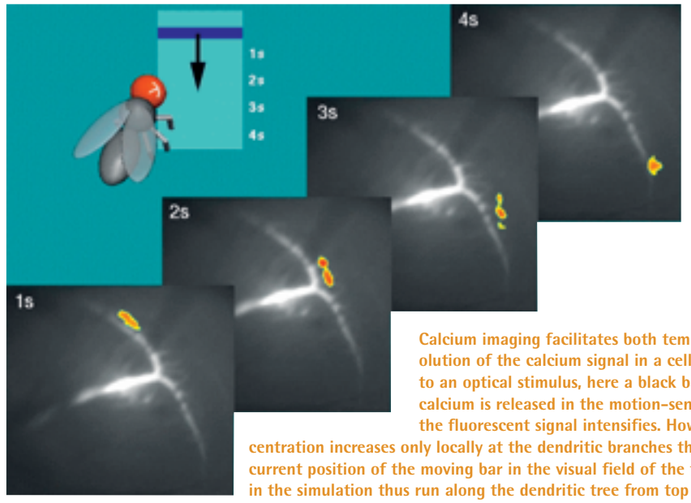


Schematic illustration of a horizontal section through the fly brain: On the outside is the retina with the light-sensitive receptors, then comes the lamina (where light-dark adaptation takes place); the medulla, lobula and lobula plate are connected to this, and in the middle is the central brain.

Illustration: Christoph Schneider, based on a template from the MPI of Neurobiology

Photos: MPI of Neurobiology

The morphology of the large tangential cells in the lobula plate of the fly brain also makes it easy to tell them apart (from left to right): the CH (centrifugal and horizontal system) cells and HS (horizontal system) cells process horizontal movement information, while the VS (vertical system) cells process vertical movements.



Calcium imaging facilitates both temporal and spatial resolution of the calcium signal in a cell. If the fly is exposed to an optical stimulus, here a black bar moving downward, calcium is released in the motion-sensitive nerve cell and the fluorescent signal intensifies. However, the calcium concentration increases only locally at the dendritic branches that correspond with the current position of the moving bar in the visual field of the fly. The color changes in the simulation thus run along the dendritic tree from top left to bottom right.

detected by VS1 is passed on to VS2, from there to VS3, and so on. This has two advantages for the fly. First, it expands the receptive field of each individual nerve cell. For example, if VS5 is stimulated, then VS2 automatically finds out. In other words, VS2 receives the optical information from VS5's receptive field without the need for any complex links of its own. What this does, in essence, is help flies expand their horizon.

Second, arranging its neurons in a series circuit helps the fly orient itself when rotating. It makes it easier for the insects to determine what point they are rotating around, which they need to know in order to adapt their movements to the rotation and, if necessary, correct their flight path. "This series circuit really stumped us," says Alexander Borst. "Initially we thought that it further impaired the spatial resolution, which wasn't that good to begin with. It wasn't until we carried out complex computer simulations with sequences of natural images that we understood that the series circuit greatly improves the representation of the rotational axis, even when the contrasts are distributed extremely unevenly, which is very typical of natural images."

To study how the LPTCs are linked, and thus to demonstrate, for example, that the VS cells are linked via a series circuit, Borst used imaging techniques. Now he is investigating another type of link: he wants to identify the neuronal components of the Reichardt detector. This involves its own unique experimental challenges. While LPTCs are relatively large and easily accessible neurons, physiological studies of the medulla neurons, where the cellular correlate of the Reichardt detector is thought to be located, have thus far been impossible, as the dye could not be injected into such small cells.

To get around this, Borst is now studying yet another species of fly: having already studied the blowfly, *Calliphora vicina*, he has now turned his attention to the fruit fly, *Drosophila melanogaster*. The idea is to insert into its genetic makeup a gene that produces the necessary dye in situ in the nerve cells. "It may seem absurd to look at an even smaller insect when the cells into which we want to inject the dye are already only a few micrometers in size," Borst explains, "but with Drosophila, we no longer need to inject the cells. Thanks to genetics, biology does that all on its own."

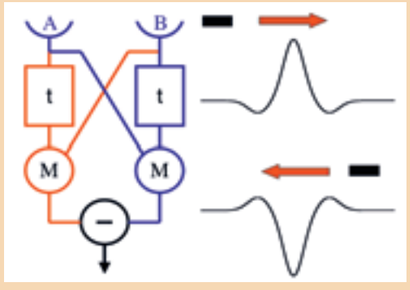
Using Genes to Color the Brain

It may seem surprising that it takes an entire spectrum of biological research techniques, from behavioral research and neurophysiology to genetic engineering – not to mention many years of work – simply to find out exactly how such a tiny brain as that of the fly functions. Ultimately, however, in its complexity, the fly brain, a conglomeration of nerve cells perfectly designed for their function and specialized in interpreting optical information, is a model for larger brains that are organized according to the same principles.

And Alexander Borst has yet another argument for the deeper significance of his research: "As yet, there is no technical system that would be able to solve the tasks involved in flight control as quickly as the fly's nervous system does. If we work out how a fly's brain does this, then we could learn a lesson from nature in how to create technical systems that perform equally well. Stefanie Hense

Key to the Circuit Layout of the Nerve Cells

The Reichardt detector consists of two mirror-symmetrical subunits, shown here in red and blue. To understand how it functions, let us first consider just one half of the detector:



If a signal moves from left to right, it first passes receptor A and then, a little later, receptor B. The signal received by A, however, is delayed, so ultimately, both signals – the one from A and the one from B – reach the next transmitter simultaneously. There, they are multiplied together, and the result is the curve shown above right. By contrast, if the signal moves from right to left, it reaches receptor B first, which immediately passes it on, and only then does it reach receptor A, where the signal – which is already delayed in arriving – is delayed even further. These two signals, in turn, are multiplied together and produce a curve with positive values. However, in order for the curves to be compared, one must take on positive values and the other negative values. And that is precisely what can be achieved by a detector comprising two mirror-symmetrical subunits whose output signals are subtracted from one another. Then a movement from left to right corresponds to the top curve, and one from right to left, the bottom curve. In the one case, the downstream nerve cell is depolarized and fires, releasing action potentials, and in the other, the nerve cell is hyperpolarized and does not fire.

The Reichardt detector is a true "Max Planck discovery," just as its name suggests – which stems from Werner E. Reichardt (1924 to 1992), Founding Director of the Max Planck Institute for Biological Cybernetics in Tübingen. Reichardt developed the concept in the 1960s, and Alexander Borst, who worked at the Max Planck Institute for Biological Cybernetics for many years, was one of the scientists involved in his research.