Guided by Light

A zebrafish larva that is only a few days old isn't yet very mobile: at this age, it is capable of a few vigorous tail movements and not much else. For **Herwig Baier** at the **Max Planck Institute of Neurobiology** in Martinsried, however, that's enough. For him, a simple and, above all, transparent brain is much more important. His particular aim is to switch individual neurons on and off using light and thus discover how the brain controls movement and behavior.

TEXT **HARALD RÖSCH**

Herwig Baier only needs to switch on one laser for the little zebrafish to beat its tail. A glass fiber just a few thousandths of a millimeter thick directs the laser light to the larva's head. To keep it from swimming away, its body is embedded in a gel-like agar, while its tail remains free to move. The tiny creature reacts to the push of a button: light on – movement; light off – pause.

Baier can use flashes of light to dictate to the animal what it should do. Optogenetics makes this possible: the gene for light-sensitive channel proteins, which comes from the single-celled alga *Chlamydomonas reinhardtii*, is inserted into the fish's genetic material in such a way that it is active in certain neurons. These proteins, known as channelrhodopsins, were first described a little more than ten years ago by Ernst Bamberg, Georg Nagel and Peter Hegemann. They allow ions to flow into the interior of the cell, where they no longer control the algal cell, but instead control the neurons in the fish's brain. Using this method, Herwig Baier wants to decode the networks that are important for the behavior of zebrafish.

Given that these little fish measure just a few millimeters in length, it may come as a surprise that the larvae even have a brain, let alone that we can learn something about the human brain from them. So, of all organisms, why are Baier and his research colleagues studying the nervous system of fish larvae?

First, because they are among the simplest vertebrates in existence: each zebrafish larva contains about a hundred thousand neurons; the human brain, in comparison, has around one hundred billion. Despite this, the basic architectures are comparable; the fish's brain just consists of fewer components. Moreover, the larva and its brain are transparent – for the scientists who use light stimuli for their research, this is an unbeatable argument.

PIONEERS OF OPTOGENETICS

When Baier first experimented with channelrhodopsins, he was still working as a researcher at the University of California in San Francisco. Optogenetics was still in its infancy at that time. In 2005, Karl Deisseroth and Ed Boyden at Stanford University were the first to transfer the channelrhodopsin proteins from the alga into neurons in cell culture and thus control their activity. Baier immediately recognized the huge potential of this new method. He obtained the coveted rhodopsins from Deisseroth and began to investigate the fish brain with them.

Together with his colleagues at the time in Berkeley and San Francisco, he achieved his first success with optogenetics. He did so initially with a different light-sensitive molecule, a light-controlled glutamate receptor. Glutamate receptors are located on synapses and respond to the neurotransmitter glutamate. This is one way that nerve signals travel from one cell to the next. However, the ion channel developed by Ehud Isacoff, Dirk Trauner and others in 2006 is not activated by glutamate, but by light. It has a small, light-sensitive molecule that changes its spatial structure and, as a result, slips into the receptor's binding pocket. Ions flow through the open channel pores and trigger an electrical signal in the neuron.

But that alone wasn't enough. In order for the researchers to be able to systematically switch individual neurons on and off, the light-sensitive proteins should be present only in these cells. >

Photo: Aristides Arrenberg

Lights on for light-sensitive proteins in the fish brain: The photo montage shows how a genetically modified zebrafish larva responds to light by vigorously beating its tail.

Hundreds of larvae in a petri dish: Only 3 to 4 mm long, the fish larvae have just been hatched. At this stage, they can only move with jerky tail movements.

Modern molecular biological methods that facilitate the genetic modification of fish helped Baier and his colleagues in this regard. It meant that they could place new genes in the genome in such a way that they become active only in a certain tissue or cell type – though which one wouldn't be known beforehand.

Thus, from a plethora of different genetic lines, they needed to identify the fish in which the channels were formed in nerve cells that are important for the behavior they wanted to study. In this way, the researchers came across a cell type whose activity can trigger swimming movements: "Each time the laser beam activated these cells in the spinal cord, the larvae began to beat their tails as if they were swimming normally," says Baier.

Further analysis revealed that he and his colleagues had stumbled across an old acquaintance: Kolmer-Agduhr cells. Instead of dendrites – the signal-receiving compartments of most neurons – these cells have a tuft of small finger-like appendages that extend into the central canal containing the spinal cord fluid. These "feelers" presumably measure chemical or mechanical changes there and forward these changes to the neural circuits in the spinal cord. Neurons that are in contact with the spinal cord fluid were observed for the first time almost 80 years ago and have since been discovered in all vertebrates studied, including humans. However, their function had remained a mystery over all those years.

A glass fiber 0.05 mm thick guides blue laser light to the larva's brain (left, center) and activates neurons in a small part of the brain with the light-sensitive channelrhodopsin (right). The researchers can deduce the function of the neurons from the tail movements. To keep the animal from swimming away, it is embedded in an agar gel, leaving its tail free to move (center, right: gray section of the image).

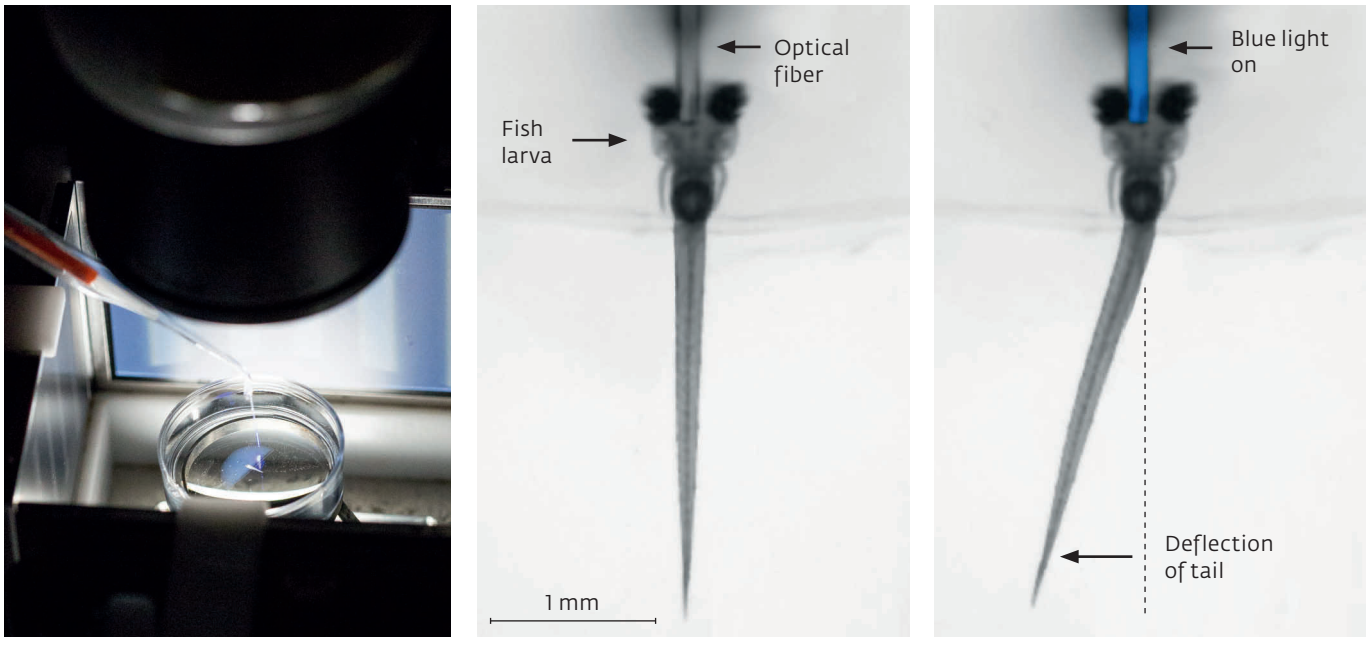
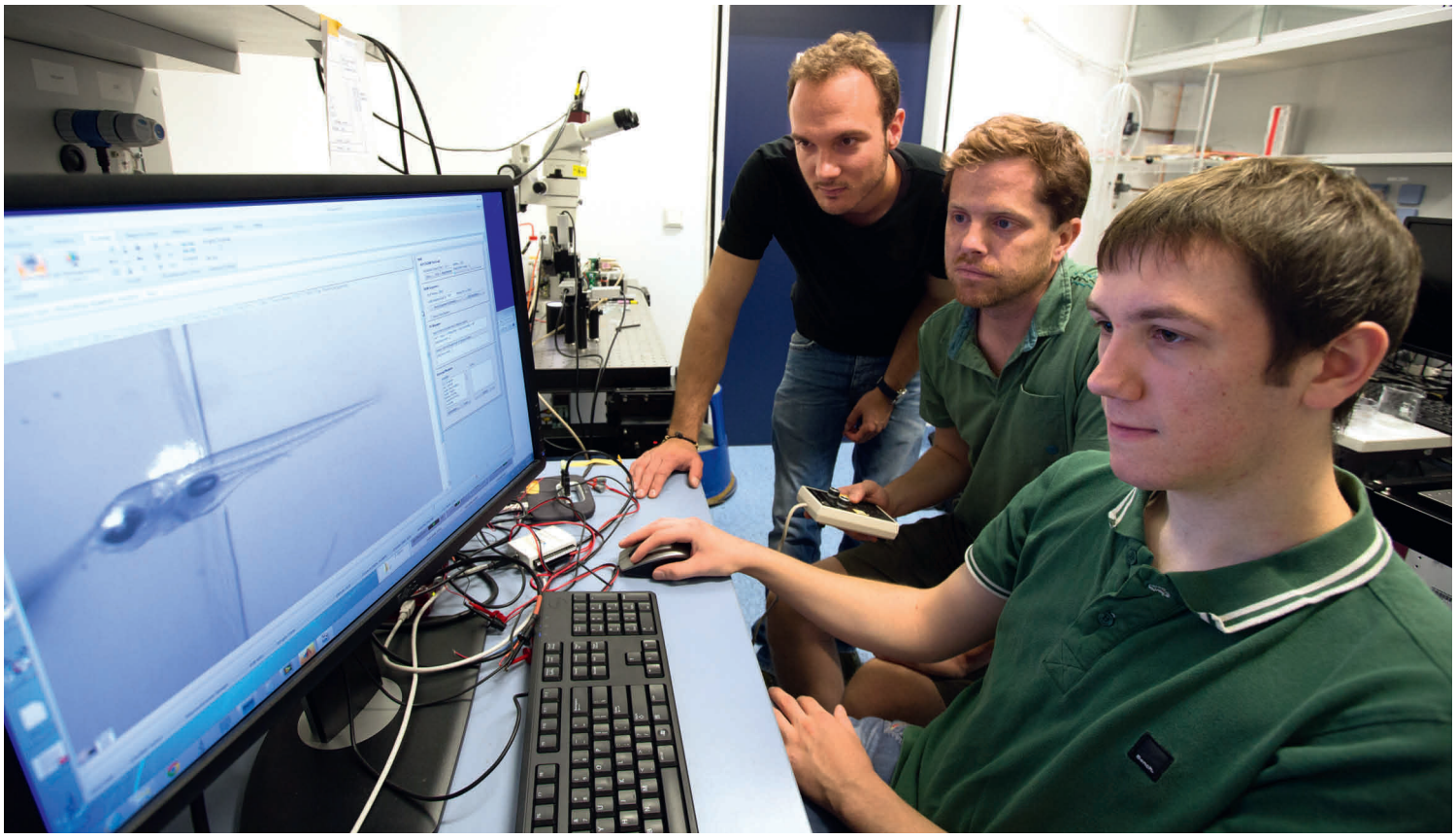


Photo: MPI for Developmental Biology (top), Denise Vernillo (bottom left), MPI of Neurobiology/Thiele (bottom right, 2)



Using a microscope and joystick, Duncan Mearns (front), Tod Thiele (center) and Thomas Helmbrecht (back) can place the optical fiber anywhere over the head of the fish larva. The laser light appears on the screen as a white mark on the animal's head.

Photo: Denise Vernillo

Now it was clear: the Kolmer-Agduhr cells are involved in stimulating movement, at least in fish. With their inhibitory neurotransmitter gamma-aminobutyric acid, or GABA, they influence other neurons, which are in contact with muscles, and regulate tail beating.

Even in such a simple organism as the zebrafish larva, swimming isn't just swimming. The animals can already master different behaviors such as orientation, approach and escape. The Kolmer-Agduhr cells seem to be involved in only forward swimming.

Escape movements, for example, can also be performed without these cells, even though the animal also flips its tail to make these movements. Another type of neuron in the spinal cord is involved in this: Rohon Beard cells. If these cells are equipped with light-sensitive ion channels, a light impulse triggers a C-shaped curvature of the body, as if the animal were trying to escape.

And the scientists noticed something else: the larvae don't need a brain for swimming! Even when the connection between the brain and the spinal cord is severed, the Kolmer-Agduhr cells still allow the animal to beat its tail. The neurons in the spinal cord seem to be connected in such a way that they can control the muscles as required all on their own.

The spinal cord is thus not just a connecting cable to the muscles, it also acts as a type of control center with its own networks. Some of these networks form central pattern generators. These are groups of neurons that are connected to one another in such a way that they are active in certain rhythms. Once they have been stimulated – by neurons such as Kolmer-Agduhr cells – they generate a rhythmic pattern of activity with which they control sequences of movements.

CIRCUITS FOR AUTOMATED MOVEMENTS

Such pattern generators govern motor processes across the entire animal kingdom: insects use them to coordinate the movement of their six legs, fish to coordinate their tail beating, and humans, their legs. These circuits are the reason we don't have to think about every step as we put one foot in front of the other. Instead, the brain has to intervene only occasionally, for example when it wants to start, modify or stop the movement. The advantage of this principle is that the complex signals for controlling movement are outsourced to the spinal cord, saving the brain space and energy.

Thanks to optogenetics, Baier and his colleagues were able to reveal the role of individual neuron types in the spinal cord. Since then, the technology has developed further. Instead of the light-sensitive glutamate receptor, Baier now relies almost exclusively on the channelrhodopsins obtained from *Chlamydomonas*. In the meantime, a light-controlled ion pump has also been added to the collection: halorhodopsin from the bacterium *Natronomonas pharaonis*. This can be used to switch neurons off rather than on. Several genetically modified zebrafish lines now exist that produce these proteins in almost all possible cell types.

Equipped with these tools, Baier set out to examine the motor circuits in the brain. Neurobiologists have long disagreed about whether complex behaviors are based on decentralized or modular networks in the brain. With a decentralized architecture, behavior arises from the activity of widely distributed circuits from many neurons in

different parts of the brain. The cells involved have no specific function. They often contribute in varying combinations to different movement patterns.

Circuits with a modular organization, on the other hand, are composed of smaller subunits. Different groups of neurons control different aspects of behavior. Cells have a specific task to perform within the modules. Complicated behaviors therefore arise out of the interaction of a small number of comparatively simple circuits.

The latter scenario actually appears to be the case for networks controlling movements in zebrafish larvae. Baier and his colleagues have proven that specific populations of neurons in the zebrafish brain control specific aspects of swimming. "It works similarly to how a boat works. A network produces the signals for swimming – that's the engine. Another steers – the tiller. And there's even a gearbox," explains Baier.

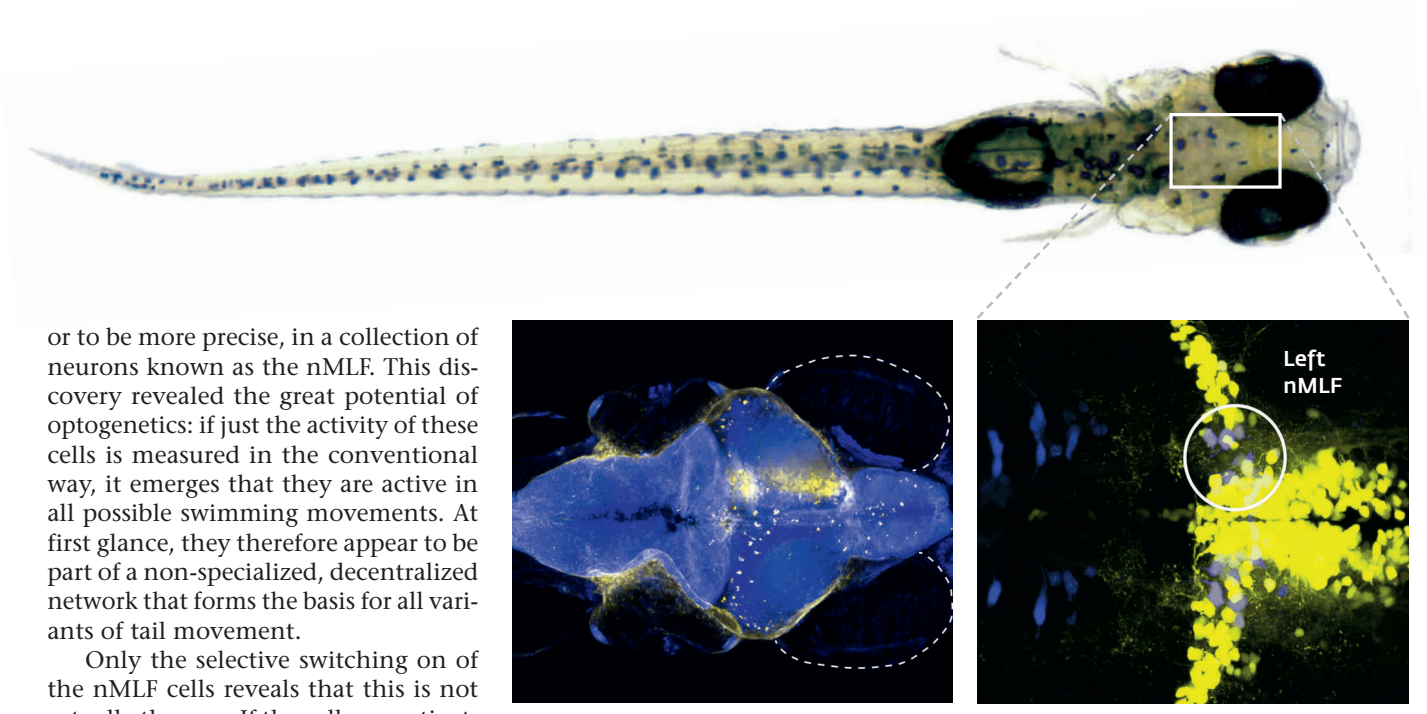
ENGINE AND TILLER IN THE FISH BRAIN

Baier discovered the engine first: neurons in the reticular formation near the spinal cord give the order to swim. "When we activate these cells with channelrhodopsin, the larvae start to beat their tails. If we inhibit them with halorhodopsin, they stop. In the brain, these neurons are the only ones that need to be active for the larva to be able to swim," says Baier.

Baier and his colleagues – at the Max Planck Institute in Martinsried since 2011 – recently also tracked down the rudder. They found it in the reticular formation in the brain of the fish,

Thomas Helmbrecht, Irene Arnold, Enrico Kühn and Anna Kramer (from front to back) in the Institute's aquarium. Genetic fish lines equipped with light-sensitive proteins swim in countless enclosed basins. Each line forms these proteins in a different neuron type.

Photo: Denise Vernillo



Upper image: Zebrafish are almost completely transparent at the larval stage. In addition to the two oversized eyes, the swim bladder in the stomach area is also dark. Different types of neurons can be activated in the fish brain using channelrhodopsins.

Image left: Neurons in the pretectum coordinate eye and swimming movements (yellow cells in the center; dashed line: implied eyes). Image right: The nMLF region contains other neurons, which control the position of the tail.

or to be more precise, in a collection of neurons known as the nMLF. This discovery revealed the great potential of optogenetics: if just the activity of these cells is measured in the conventional way, it emerges that they are active in all possible swimming movements. At first glance, they therefore appear to be part of a non-specialized, decentralized network that forms the basis for all variants of tail movement.

Only the selective switching on of the nMLF cells reveals that this is not actually the case. If the cells are activated on the left side of the brain, the tail bends to the left. If the nMLF cells on the right are switched on, the tail points to the right. In the experiment, it looks as if the tail follows the beam of light as the laser wanders from the left to the right nMLF. "The fish controls the swimming direction by bending its tail. The nMLF cells are therefore less responsible for the swimming as such, and more responsible for the steering," says Baier. This is also demonstrated when the scientists systematically switch off individual cells on one side of the brain. The larvae can still perform a wide variety of movements, such as beat their tails, but they can no longer swim straight ahead. The tail is deflected in one direction, causing the fish to turn in a circle.

Ruben Portugues, a Max Planck Research Group Leader at Baier's institute, and his colleagues have also identified the gear mechanism for tail beating in the reticular formation. Individual nMLF cells regulate the swimming speed by controlling the duration of tail beating. Others control the tail beat frequency. In this way, the animal can switch to a higher or lower gear and swim faster or slower.

The neurons in the fish brain thus perform a variety of tasks when swimming. Depending on whether the larva is swimming around looking for food, for example, or bolting from a predator, the different networks work together to generate the optimum behavior for the respective situation.

Swimming is therefore an example of a behavior that is based on a modular network. The individual modules involved may be relatively small: "A few dozen neurons in the brain are probably sufficient to position the tail of the fish," says Baier. When it comes to changing gears while swimming, less than a handful are needed: only four nMLF cells are required.

LARGE NETWORKS FOR MEMORY

In contrast, other brain functions may use decentralized networks. We know from the marine snail *Aplysia californica* that extensive networks are active when they withdraw their gills. In the human brain, as well, stored memory traces seem to be based on large networks. In these cases, the network's state of activity is crucial; the individual cell is presumably insignificant.

It's not yet completely clear which behaviors are based on decentralized networks and which are based on modular networks. In principle, both seem possible. The specific requirements of the respective brain function could play a role: memory traces, for example, must presumably be stored on a decentralized basis, as they comprise learned movements or specific recollections. Moreover, they are formed and retrieved before, during or after a behavior. Information that is learned can also influence very different patterns of behavior. Distributed networks, in which individual cells have no specific function and may be part of several circuits, are therefore probably better suited to this. Behaviors with a manageable number of variants, such as walking, running, jumping, turning, etc., differ fundamentally. In such cases, a modular organization presumably suffices.

Baier and his colleagues are interested in yet another aspect, namely how information from the sensory organs is incorporated into, and influences, behavior. For example, if a shadow falls across the eye of the zebrafish, its escape program is triggered. This process is known as sensorimotor transformation. One of the places this process takes

Photos: Thomas Helmbrecht (top), MPI of Neurobiology/Kubo (bottom left) and Thiele (bottom right)

From fish to human: Thomas Helmbrecht and Herwig Baier (right) are sure that the fish brain and human brain operate on similar principles.

place is the tectum, a region in the midbrain of fish and other vertebrates. Information from the sensory organs is processed here and forwarded to the motor system.

Thomas Helmbrecht, a doctoral student in Baier's department, discovered that escape movements can be induced by optogenetically activating cells at the back of the tectum. This can be explained by the architecture of this area: the tectum contains a map of the entire visual field. "Neurons in the eye that process, for example, stimuli from the back of the larva's visual field are also linked to the back of the tectum. An object that approaches from behind represents a potential danger. This could explain why a flight response is triggered primarily there," says Helmbrecht.

Baier is convinced that many of the findings from the brain and spinal cord of the fish can be applied generally: "In the development of the nervous system, evolution changes, first and foremost, what already exists. Whatever has proven its worth is retained or developed further. Since the gait of terrestrial vertebrates evolved from the fish's trunk and fin movements, the underlying circuits are probably based on the same principles."

From the light-sensitive proteins of an alga known only to botanists, to the nervous system of fish larvae, to the human brain, the paths along which science makes new discoveries are often tangled and unpredictable. The success story of optogenetics is a prime example of this. ◀

TO THE POINT

- **The swimming behavior of fish is based on neural networks in the brain and spinal cord that are comprised of small, local modules. Different modules can be combined with one another depending on the requirement at hand.**
- **Each neuron performs a specific task. Even switching a few cells on and off can change an entire behavior.**

GLOSSARY

Sensorimotor transformation: Movements are rarely performed completely independently of external influences. They are often triggered by environmental stimuli, which are perceived via the sensory organs. At the same time, sensory organs need to determine deviations from a desired movement so that the motor system can correct them via the relevant signals. Sensory and motor networks are therefore closely connected and exchange information.

Central pattern generators: Networks of neurons that are independently rhythmically active. Even three neurons, of which two mutually activate each other and the third inhibits the other two from a specific activity threshold, can form such a rhythmically active network. Pattern generators are present in invertebrates and vertebrates, and control involuntary standardized movements such as the beating of an insect's wings or the human gait. When walking, the pattern generators in the spinal cord are linked together for the two legs so that different gaits (walking, trotting or running) are generated.

Photo: Denise Vernillo