Memories Leave **Their Traces**

In the early days, only a small path connected the **Max Planck Institute of Neurobiology** in Martinsried with the outskirts of Munich. Now a huge biocampus is located on the periphery of Munich, and the path has been transformed into a wide road. According to **Tobias Bonhoeffer**, learning and memory function in a very similar way: intensively used pathways are expanded, while unimportant routes and dead ends are eliminated.

TEXT **HARALD RÖSCH**

The German city of Friedrichshafen had a Brazilian feel about it on April 1, 1984. Two days later, the German national newspaper Bild ran the headline "Rio at Lake Constance!" The reason for the euphoria: the city's volleyball team had emerged victorious over the frontrunners, Sindelfingen, securing its promotion into Germany's first volleyball division, the Bundesliga. The two teams battled it out for almost four hours before the home team wrapped up the fifth set and, with it, the longest volleyball match in the history of the Bundesliga. The losing players were bitterly disappointed. Among them was 24-year-old Tobias Bonhoeffer, at the time playing for the VfL Sindelfingen as well as member of the junior national team of Germany, and now Director at the Max Planck Institute of Neurobiology in Martinsried.

The opportunity for revenge came ten years later. The opponents from the 1984 game met once again for a friendly match. "After the game, my muscles ached more than ever before – probably because I wanted to play and move the same way I did when I was an active player, but my muscles were no longer up for it," says Bonhoeffer in retrospect. So the skills from his earlier years were still stored in his brain, only the rest of his body couldn't perform as it used to. This experience sums up what has been driving Bonhoeffer since the beginning of his scientific career: how does the brain store what it has learned?

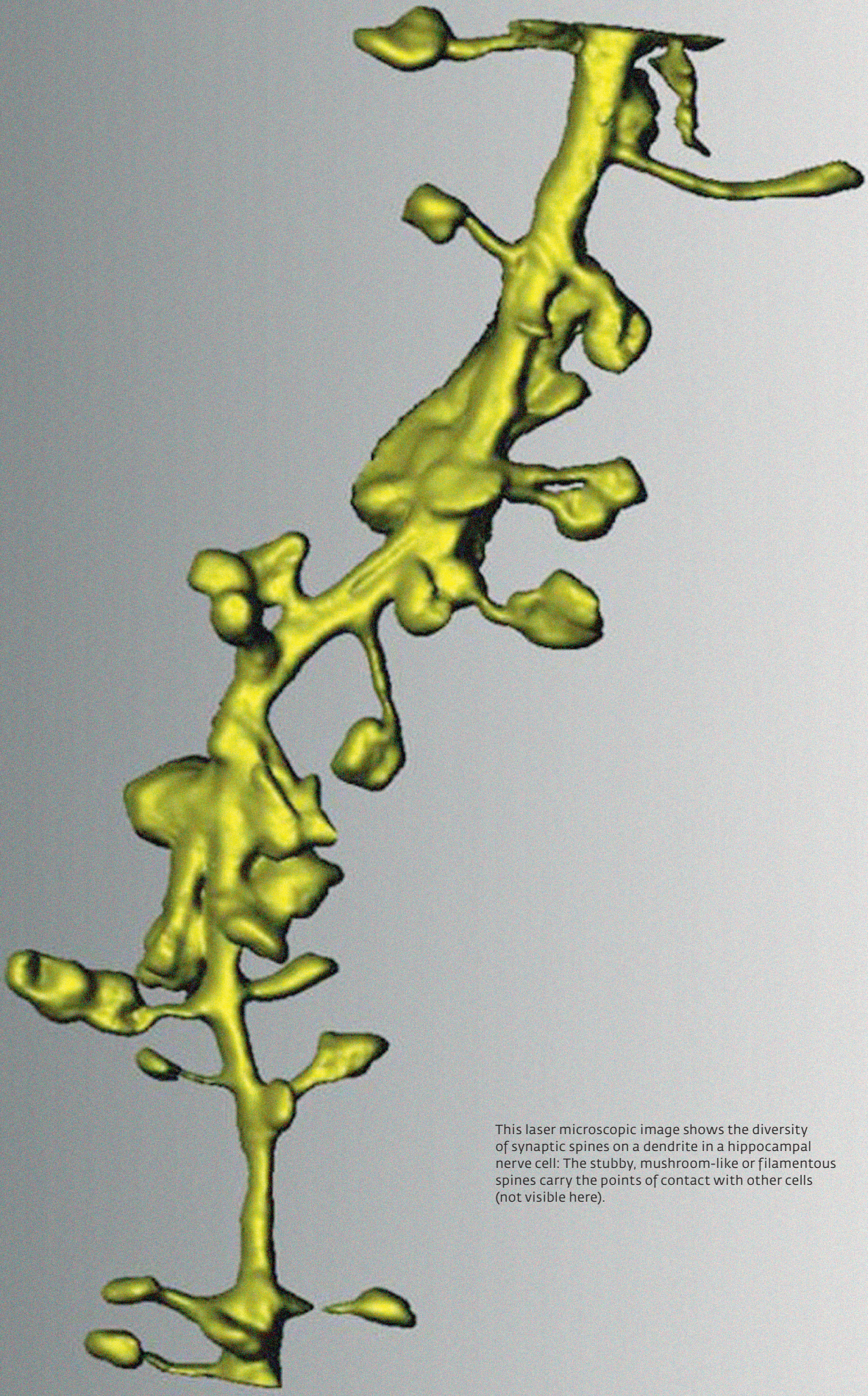
SYNAPSES AS DATA STORAGE UNITS

Unlike artificial storage media such as hard disks or DVDs, the brain doesn't use magnetism or lasers to hold information. Instead, the brain consists of countless nerve cells, which send electrical impulses from one cell to the next by way of so-called synapses. At these points, the nerve fiber or "axon," which conducts information from a nerve cell, meets the branched, tree-like input 'antennae' of another cell – the dendrites. At the synapse itself, the electrical signal is transmitted with a chemical neurotransmitter from one cell to the next.

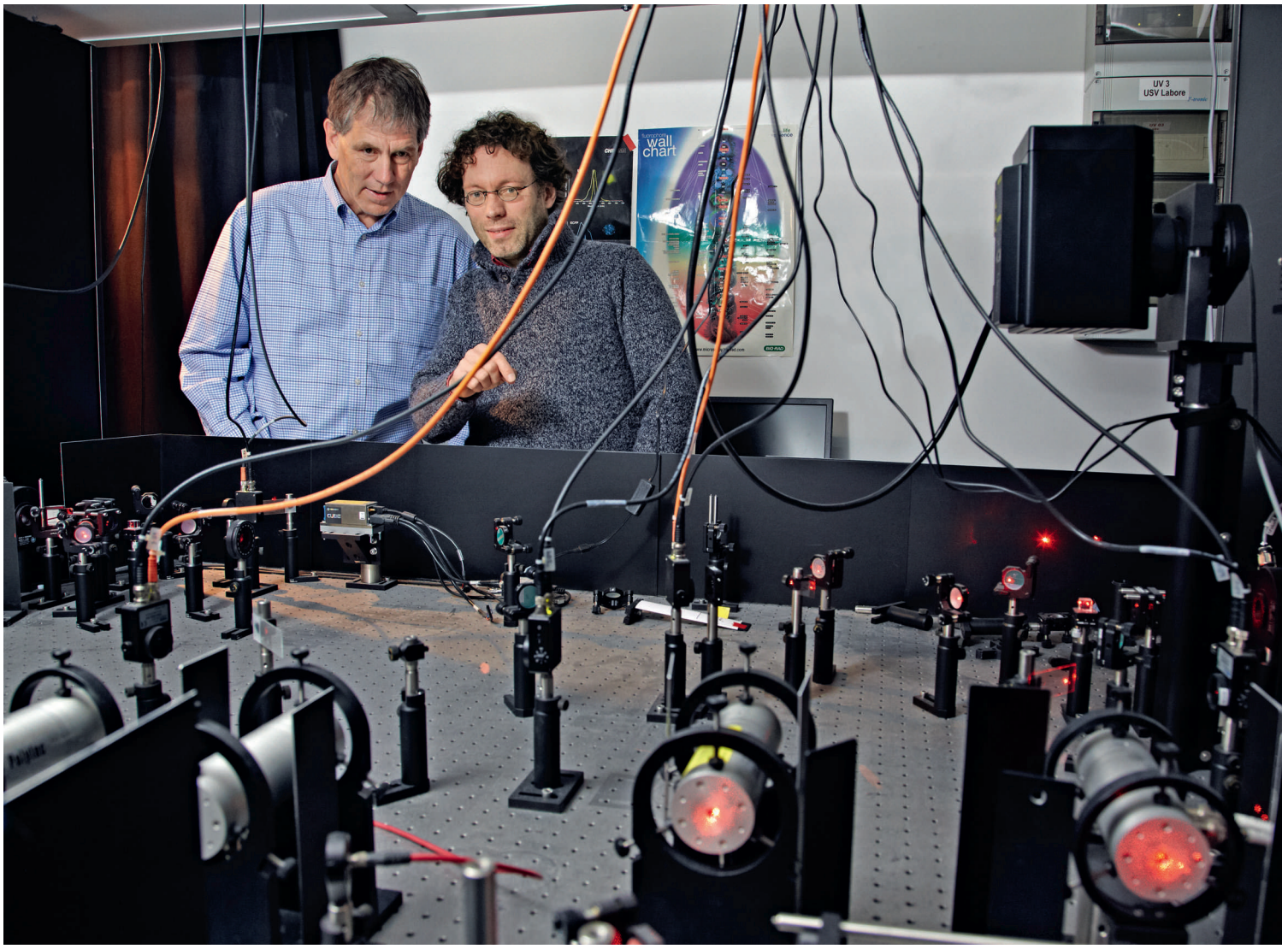
Synapses do more than transfer impulses: they are also responsible for storing information in the brain. They can conduct the electrical signals from one cell to the next with varying intensity, in other words, they can enhance or reduce the signals. In this way, the brain strengthens frequently used connections between cells and weakens those that are used less frequently. Known as synaptic plasticity – experts also refer to it as synaptic long-term potentiation (LTP) and long-term depression (LTD) – this is the mechanism that enables the brain to learn.

The effects of learning and memory can therefore be measured electrically at the synapses. But not only that: they also leave anatomical traces in the brain. Together with his fellow scientist

Photo: MPI of Neurobiology



This laser microscopic image shows the diversity of synaptic spines on a dendrite in a hippocampal nerve cell: The stubby, mushroom-like or filamentous spines carry the points of contact with other cells (not visible here).



Tobias Bonhoeffer (left) and Volker Scheuss plan a new beam path for a two-photon laser microscope. The laser must, with submillimeter-precision, hit mirrors and lenses before it reaches the microscope.

Florian Engert, Bonhoeffer was one of the first to see such traces with his own eyes at the end of the 1990s. They observed how new connections between nerve cells were made when information is stored in the brain.

This was made possible by a novel microscopy technology that had just emerged at that time – two-photon microscopy. With this technology, a laser beam scans an object layer by layer and excites a fluorescent dye. A computer reassembles the fluorescent light from every layer to form a three-dimensional image. Unlike previous microscopes, the laser beam of the two-photon microscope doesn't damage nerve cells, which means that scientists can observe living cells much more effectively.

Bonhoeffer's team initially used the technology on neurons from cell cultures. The scientists were interested mainly in the effect of synaptic long-term potentiation on "dendritic spines," small protrusions on the branched dendrites of the cells and their cell bodies. They contain the receptor part of the synapses. The transmitter part is located on the axon of the preceding cell, separated by a tiny gap. When strengthening or weakening of synapses modifies the connections between nerve cells, this should be visible on the spines.

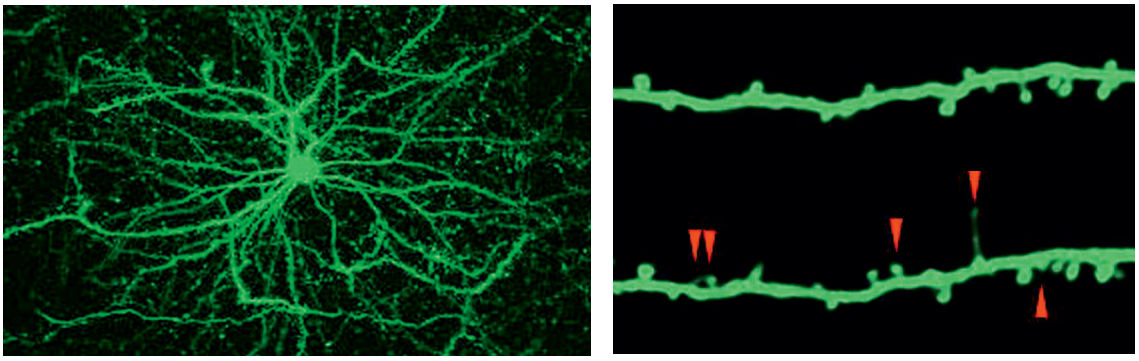
CELLS WITH MYRIADS OF CONTACTS

A dendritic spine never exists on its own, however: one nerve cell can have tens of thousands of spines. This enormous number is one of the reasons why it is so difficult to track anatomical changes at the synapses. After all, nobody knows which synapses connect certain nerve cells in the brain and which spines would then change as a result.

Nevertheless, in a groundbreaking experiment, the scientists in Martinsried succeeded in tracking what happened at the spines in real time. Their strategy was to first suppress the electrical activity of the nerve cells using a chemical inhibitor. Next, they washed the inhibitor out of a tiny area of the tissue using a flushing system and induced synaptic long-term potentiation. This enabled them to significantly limit the number of relevant synapses and observe that strengthening the connections causes new spines to grow.

In subsequent studies, the neurobiologists proved that the newly formed spines actually carry synapses, and that the transmitting terminals belonging to the spines on the axons are similarly modified. It was thus clear that particularly active nerve cells not only change the strength of their synapses, but can even form completely new

Photo: Felix Brandl



Luminous nerve cells: Genetically modified mice with a gene for a fluorescent protein in their genetic makeup make the researchers' work easier. They allow them to observe the nerve cells with all their dendritic branches and spines (left). Right: Learning processes result in the formation of new dendritic spines. Four spines are added shortly afterward (red arrows). One spine formed a filamentous extension, known as a filopodium (second arrow from right).

connections. The scientists also observed the reverse phenomenon: long-term depression of synapses causes spines to shrink or disappear altogether. Rarely used connections can therefore be abandoned.

But what do these results have to do with learning and memory? After all, the cells the scientists studied were nerve cells that had been stored in an incubator for several days. In addition, synaptic plasticity was triggered using artificial electrical stimuli. Therefore, the test conditions didn't exactly correspond to the natural learning processes in the brain.

Mark Hübener thus studied the processes at the synapses in the intact brain. Together with his colleagues, Bonhoeffer's long-time collaborator observed the nerve cells in the uppermost layers of the cerebral cortex of a mouse using a two-photon microscope. Hübener is not only an expert in such in-vivo studies, he is also very familiar with the visual system in the brain, particularly with the primary visual cortex. He measures the activity of nerve cells and analyzes how visual information from the eyes is processed. "That's how we know that this region of the brain can adapt if the incoming information from the eyes changes. The neurons learn to process new stimuli," explains Hübener.

The primary visual cortex is reorganized, for example, when nerve cells in the visual cortex don't receive any signals from a closed eye for some time. "Neurons that previously received impulses equally from both eyes react more strongly to the open eye after a few days. The input of the closed eye, in contrast, is reduced."

SWITCHING TO THE OTHER EYE

But what is the synaptic basis of these changes? Hübener discovered that, within a few days of closing one eye, the number of new spines doubled: the nerve cells subsequently had almost 10 percent more spines on their dendrites than before. "The new spines presumably carry synapses that connect the cells with the open eye," says Hübener.

During their experiments, the scientists operate like neurosurgeons in a hospital, using heart rate monitors and other devices to monitor the anesthesia. Through a small window in the skull, they can look up to a half millimeter deep into the brain and observe individual nerve cells and their spines – an amazing feat when we consider that even tiny vibrations originating from heartbeat and breathing are much larger than the spines and can therefore completely obliterate the images of the neurons. In the past, this work involved the laborious task of filling the nerve cells with a fluorescent dye using a micro-electrode to make them visible in the two-photon microscope. Now, genetically modified mice automatically produce the fluorescent light in individual nerve cells. In other words, they light up all by themselves.

The experiments therefore show that the brain modifies its wiring when it has to process new stimuli. According to Bonhoeffer and Hübener, learning and memory are also visible under the microscope: as newly emerging or disappearing dendritic spines. Critics maintain that typical learning processes are different from the adaptation to changed signals from the sensory organs and may function in another way.

Bonhoeffer doesn't agree with this argument: "These forms of plasticity don't differ fundamentally, only gradually. Whether our brain needs to respond to changed stimuli from the eyes, or whether we are learning a language or recalling sporting skills – it probably all happens in a very similar way at the cellular level."

Neuroscientists do, however, agree on one thing: learning takes place at the synapses. So does forgetting. But a rule of thumb stating that "new spines = learning, and destruction of spines = forgetting" would definitely be too simplistic. If spines are added during

Photos: MPI of Neurobiology (2)

» Learning and forgetting take place at the synapses. During these processes, new dendritic spines are formed and others are pruned.

Nerve cells are nature's works of art: Tobias Bonhoeffer and Mark Hübener (right) chat in front of an image of two nerve cells drawn, magnified several thousand times, onto the wall of the coffee break area.

Photo: Felix Brandl

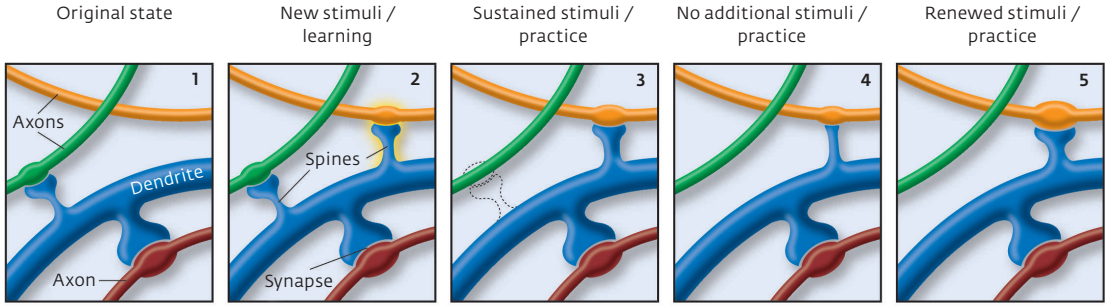
the learning process, others are eliminated at the same time, and vice versa, so the network of the brain remains in equilibrium.

CIRCUITS FOR MEMORIES

The paths between nerve cells become roads. Memory makes its way through the brain, connecting many – often widely scattered – nerve cells. Each item of memory makes its own way. "That's why playing football is of little use if you want to learn how to play volleyball. Both skills leave behind different traces in the brain."

Tobias Bonhoeffer also follows traces – those of his family. After all, the name Bonhoeffer has a long scientific tradition. Tobias Bonhoeffer's great-grandfather, Karl Bonhoeffer, was a famous psychiatrist at Berlin's Charité hospital. His grandfather, Karl Friedrich, was such an important figure at the Max Planck Institute for Physical Chemistry in Göttingen that the current institute bears his name. And his father, Friedrich, was a Director at the Max Planck Institute for Developmental Biology in Tübingen.

Three generations of science, two of them within the Max Planck Society. Yet Tobias Bonhoeffer felt no pressure to follow a similar path. "My father even advised me to study law." But perhaps a career as a scientist was



How learning modifies the synapses: 1: A dendrite belonging to a nerve cell (blue) bears synapses with axons of other cells (green, red) on its spines. 2: Learning or a new stimulus causes a dendritic spine to "sprout," connecting the dendrites with a new cell (yellow). 3: If the learned information is supplemented or if the stimulus is sustained for a longer period of time, the synapse on the new spine is expanded and the old spine is no longer needed, and disappears. 4: If there is no additional learning or training, or if the stimulus is removed, the spine shrinks and the associated synapse is weakened or inactivated. 5: If the same skill is used again, the existing contact can be expanded. As a consequence, learning occurs faster.

predestined by his family history: the young Tobias spent many Sundays with his father at the institute in Tübingen, where he marveled at the structure of spiders' legs and nettle leaves through the microscope.

Faced with the decision of whether to study biology or rather physics, he simply visited lectures at the university in his home town of Tübingen. A lecture about thermodynamics fascinated him much more than one on the tissue structure of deep-sea sponges, and that tipped the scales in favor of physics. Yet he studied a biological topic for his Ph.D. and, following research residencies in New York and Frankfurt, he came to Martinsried, where he initially headed up a research group and, later, the department "Synapses – Circuits – Plasticity."

CONSISTENCY DESPITE CONSTANT REORGANIZATION

So synapses can adapt to new requirements throughout life. Even the brain of an adult is far from inflexible and irrevocably hardwired, as was long assumed. On the contrary, it is so flexible that scientists are puzzled as to how it can even store anything over the long term. "Our measurements show that approximately one percent of the dendritic spines in the visual cortex are newly formed or disappear every day. If we extrapolate this, it means that all spines are replaced once every three months. It's a complete mystery how the brain can continue to work consistently under these conditions," says Bonhoeffer.

However, despite this constant reorganization, some spines remain stable for life. American scientists observed this in the mouse brain. Hübener's team also found in their experiment that some spines are more permanent than others. "The spines that were formed when the eye is closed endure. They just shrink a little when the eye reopens. If the eye closes once more, they grow again. Apparently they are then reactivated – which is, of course, a simpler and faster process than constantly forming completely new spines." This also explains why something that has been learned once is more easily remembered the second time around: the synaptic connections have already been predefined, the necessary spines are already there.

Thus, new spines don't necessarily always have to be formed, nor do existing spines have to disappear. Chang-

WHY DO NERVE CELLS HAVE DENDRITIC SPINES?

Immediately after birth, most of the brain's synapses are located directly on the dendrites. Later in life, almost all excitatory synapses sit on the dendritic spines. What advantages does this offer?

- A nerve cell can use its spines to more easily contact other cells in its environment: the axon's search for a postsynaptic partner is facilitated by the spines' ability to rapidly extend from the parent dendrite. In addition, the spines can be arranged in a spiral shape around the dendrites to maximize the probability of contact with an axon.
- Due to their small size and narrow diameter, the spines insulate their synapses from the dendrites electrically. This allows incoming electrical signals to be processed separately between synapses.
- Dendritic spines prevent signal molecules from spreading to other synapses. In this way, individual synapses can be strengthened or weakened.

Graphic: MPI of Neurobiology

es in size could be at least as important. Indeed, the findings of various research groups show that spines increase in size as a result of long-term potentiation, and shrink as a result of long-term depression.

Larger spines apparently carry larger and stronger synapses. Volker Scheuss, a researcher in Bonhoeffer's team, discovered that the number of important synapse proteins increases in growing spines. "Large spines are permanent only if they also have large synapses. Larger synapses can then transmit signals better," explains Scheuss (see box on p. 25).

FEWER SYNAPSES AS WE AGE

The human brain thus remains capable of learning over a lifetime. However, as we age, the synapses between nerve cells become fewer and the ability to make new connections diminishes. While particularly large numbers of new spines are formed in the first few weeks after birth, this is soon outweighed by their loss: the human primary visual cortex never again contains as many synapses as it does at the age of three months; in the prefrontal cortex – an area of the brain associated with higher cognitive functions – the maximum is reached after three to five years. The subsequent pruning is necessary for the brain to select the connections it needs and discard all the the others.

The brain learns particularly easily during childhood. There are also periods in which nerve cells are extremely receptive to very specific stimuli. Such sensitive phases begin suddenly but have no fixed end. Rather, they abate gradually. Language is one such example: the sensitive phase for language learning begins at around one year of age and continues until puberty. After that, the ability to learn languages slowly declines, but never disappears altogether.

Networks of inhibitory nerve cells – that is, cells that can diminish the activity of other cells – play a crucial role at the start of sensitive phases. Sensitive phases in development don't begin until these inhibitory networks have reached maturity.

Mark Hübener and his colleagues also studied such inhibitory nerve cells. If the researchers deactivated a small area in the retina of the eye using a laser flash, the inhibitory nerve cells in the visual cortex that normally receive signals from this area lose some of their spines. The inhibitory neurons reacted considerably faster than the previously observed excitatory nerve cells. Their synapses are already stable again when the reorganization of the excitatory cells is just beginning.

Hübener therefore suspects that the plasticity of inhibitory synapses is the first step in reorganizing the cerebral cortex. The pruning of spines on inhibitory neurons increases the activity of the excitatory nerve cells, allowing them to make new spines and remove existing ones. The affected region is then modified in such a way that it will be able to react to future visual input from adjacent, still functioning regions in the retina.

LEARNING WITH EMOTION

In addition to inhibitory nerve cells, there are also other factors that affect the ability to learn and remember: emotions, for example, or attention. But instead of affecting individual synapses, as is the case with inhibitory cells, emotions and attention regulate entire cells and brain regions. "That's why it's easier for us to learn if we're focused and if learning is fun," explains Bonhoeffer. Negative feelings, on the other hand, can be so strong that a single bad experience can last a lifetime.

The ultimate proof that changes in spines and synapses are a prerequisite for learning still eludes the scientists. Bonhoeffer has already thought of a suitable experiment to prove this: he would like to tag the new spines with a chemical marker that enables him to get rid of them once they are formed – in other words, a sort of suicide switch for dendritic spines. If the new connections were indeed essential, learned information should then be forgotten again.

"Unfortunately, no such switch exists yet. Also, you would probably have to mark thousands of spines individually," says Bonhoeffer. However, given the enormous progress neuroscientists have made in recent years, this thought experiment may not be so far-fetched after all. Maybe one day it will be possible to systematically delete memories from the brain by deactivating the associated spines, much like what we are already familiar with from science fiction movies.

So Tobias Bonhoeffer wants to learn a lot more about learning. That doesn't leave a lot of time for his previous activities. He rarely plays volleyball now. When he does, though, he's prepared: the dendritic spines he needs for it are already there. ◂

Film on the topic:

www.mpg.de/7331016/synapse-long-term-potentiation

TO THE POINT

- **Learning leaves anatomically visible traces in the brain: Some dendritic spines and their synapses increase in size or are formed completely anew. Others shrink or disappear entirely.**
- **The changed connections link new nerve cells with one another. Information that is learned is thus stored as a circuit of many nerve cells. In this way, an individual cell can be involved in many memory items simultaneously.**
- **The brain goes through sensitive phases in which it is particularly receptive to learning. Inhibitory nerve cells play an important role in the beginning of these phases: their synapses are the first to be reorganized.**