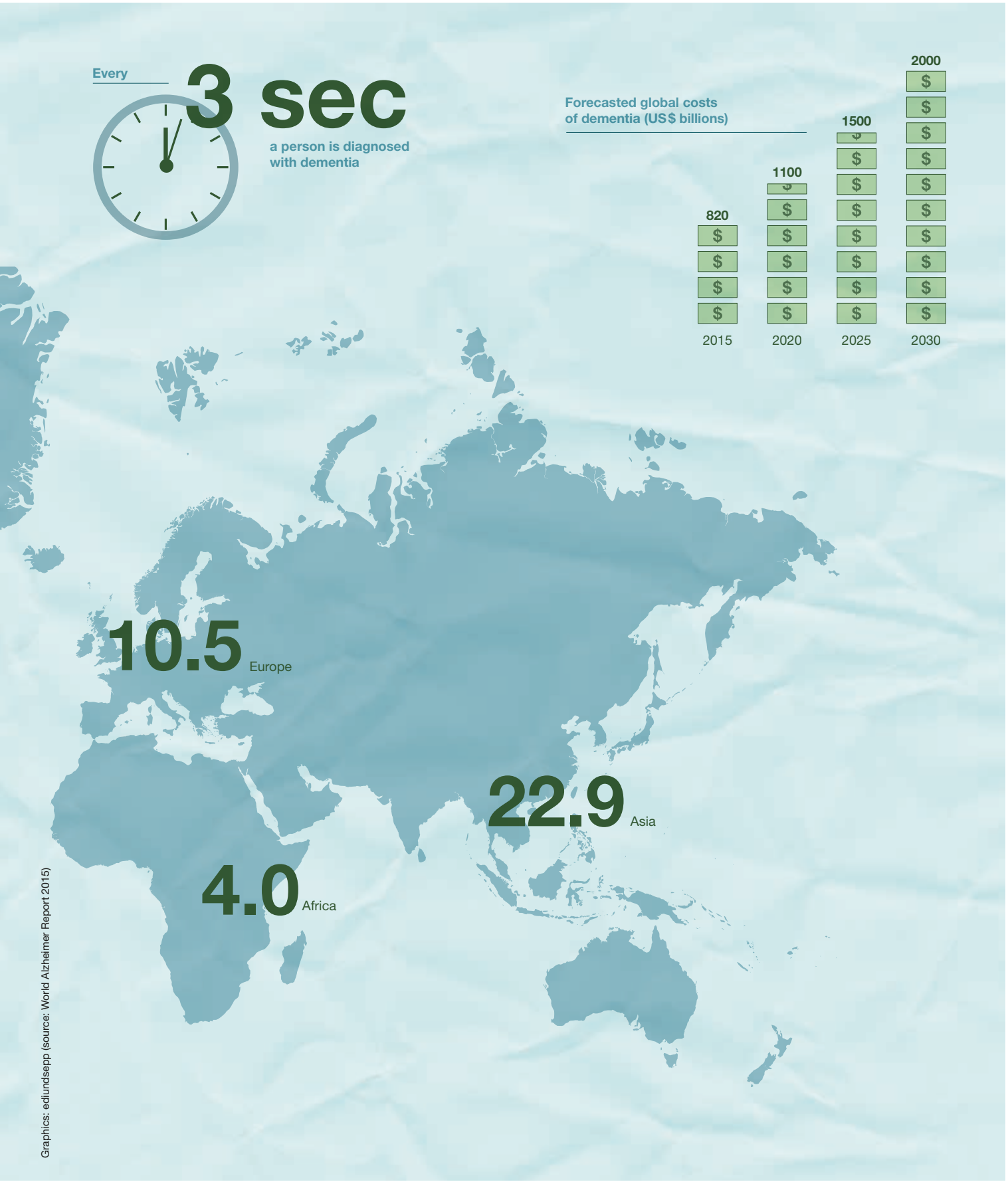
On the Trail of **Alzheimer's**

Watching the brain's neurons directly as they fire is a neuroscientist's dream. And for several years now, TUM researchers have been able to do just that. What's more, they are incorporating their methods into work relevant for patients, for instance in the field of Alzheimer's research – with findings that may hold the key to improving treatment.



Graphics: ediundsepp (source: World Alzheimer Report 2015)

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www.ifn.me.tum.de
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Brigitte Röthlein

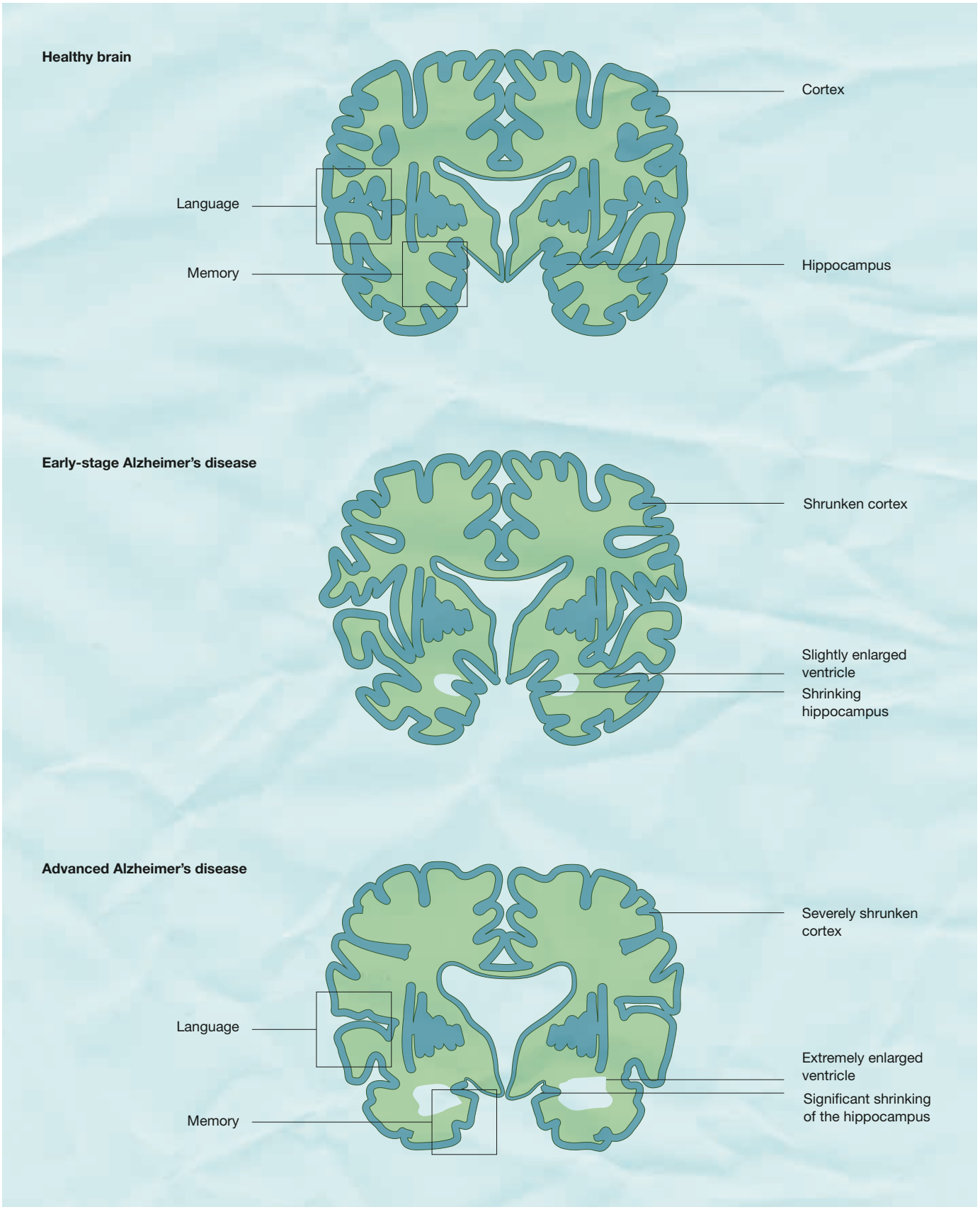
Fortschritte in der Alzheimer-Forschung

Neue Verfahren zur Beobachtung einzelner Nervenzellen im lebenden Tier, die Prof. Arthur Konnerth und seine Mitarbeiter am Institut für Neurowissenschaften der TUM entwickelt haben, ermöglichten es in den vergangenen Jahren, zusätzliche Erkenntnisse über die Arbeit des Gehirns zu gewinnen. Im Vordergrund steht dabei die Zwei-Photonen-Mikroskopie in Kombination mit der sogenannten Patch-Clamp-Technik. Erstere erlaubt es, mit roten und infraroten Laserimpulsen bis zu einem Millimeter tief ins Gewebe hineinzuschauen und dort dreidimensionale mikroskopische Aufnahmen zu machen, ohne dass die Zellen geschädigt werden. Man setzt dabei unterschiedliche Fluoreszenzfarbstoffe ein. Beim zweiten Verfahren benutzt man eine nur wenige Mikrometer dünne Glaspipette, die sich an einzelnen Zellen festsaugen kann. So kann man die Ionenkanäle untersuchen, die sich in der Membran der Zelle befinden und dafür sorgen, dass Ionen hinein- und herausfließen. Sticht man die Zelle mit der Pipette an, kann man auch gezielt Farbstoffe oder Pharmaka in sie einbringen.
Die Verfahren werden unter anderem bei der Alzheimer-Forschung angewandt und haben bereits zu unerwarteten Einblicken geführt. So gelang es beispielsweise Dr. Marc Aurel Busche, mit der Zwei-Photonen-Mikroskopie zu zeigen, dass es bei Alzheimer-Mäusen einen großen Anteil von Nervenzellen gibt, die besonders aktiv sind. Diese Hyperaktivität findet man zunächst im Hippocampus, also der Region tief im Gehirn, die für Lernen und Gedächtnis zuständig ist, und zwar in einem ganz frühen Stadium der Erkrankung, wenn sich die Alzheimer-Plaques noch gar nicht nachweisen lassen.
Ein weiteres wichtiges Ergebnis war die Erkenntnis, dass sich die krankmachenden Veränderungen im Gehirn auch auf die Vorgänge der Informationsspeicherung im Schlaf auswirken. Vor allem die langsamen Schlafwellen, die unser Gehirn nachts erzeugt, dienen dazu, Gelerntes zu verfestigen und Erinnerungen in den Langzeitspeicher zu verschieben. Die Wellen werden über ein Netzwerk an Nervenzellen in der Hirnrinde gebildet und breiten sich dann in andere Hirnareale wie den Hippocampus aus. Dieser Vorgang ist bei Alzheimer-Mäusen gestört. Derartige Befunde könnten dazu führen, dass man in der klinischen Forschung neue Therapien entwickeln kann. □

What's going on inside your head? Something we often wonder as we struggle to understand each other. And all the more so when we encounter someone with a mental illness such as Alzheimer's, causing them to gradually lose their memory, become disoriented and no longer recognize their own family. Wouldn't it be great if we could take a look at the brain's inner workings and see where the problem lies? Needless to say, researchers would also jump at the opportunity, since you can only cure a disease once you understand its cause. In the past few decades, they have certainly made significant progress, developing imaging techniques such as MRI (magnetic resonance imaging) and PET (positron emission tomography), which enable us to monitor brain activity to varying degrees of clarity. These have taken us a very long way – yet still leave the question open of what is actually happening in the individual brain cells. ▷

"The major problem with treating psychiatric disorders, in particular, is that we don't have a detailed picture of the way the brain works under normal conditions."

Arthur Konnerth



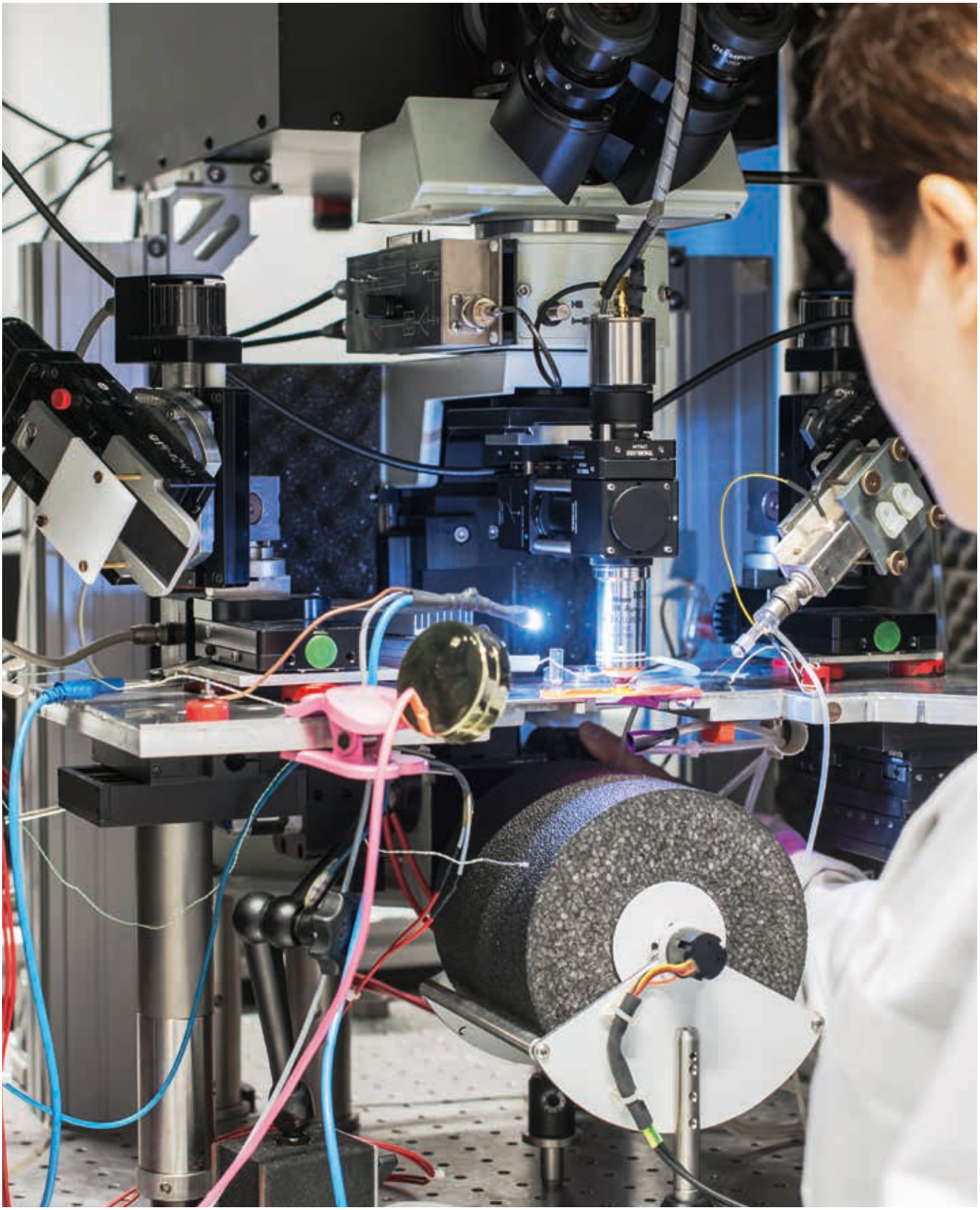
Graphics: ediundsepp (source: Alzheimer Forschung Initiative e.V.)

Alzheimer's is an incurable brain disease and the main cause of dementia – around two thirds of all dementia patients have Alzheimer's disease. It is characterized by the cells in certain brain regions ceasing to function and eventually dying.

Konnerth's working group combines patch clamp measurements, which involve attaching a pipette onto the nerve cell by suction, with two-photon microscopy. The micropipettes are fabricated internally by the group.

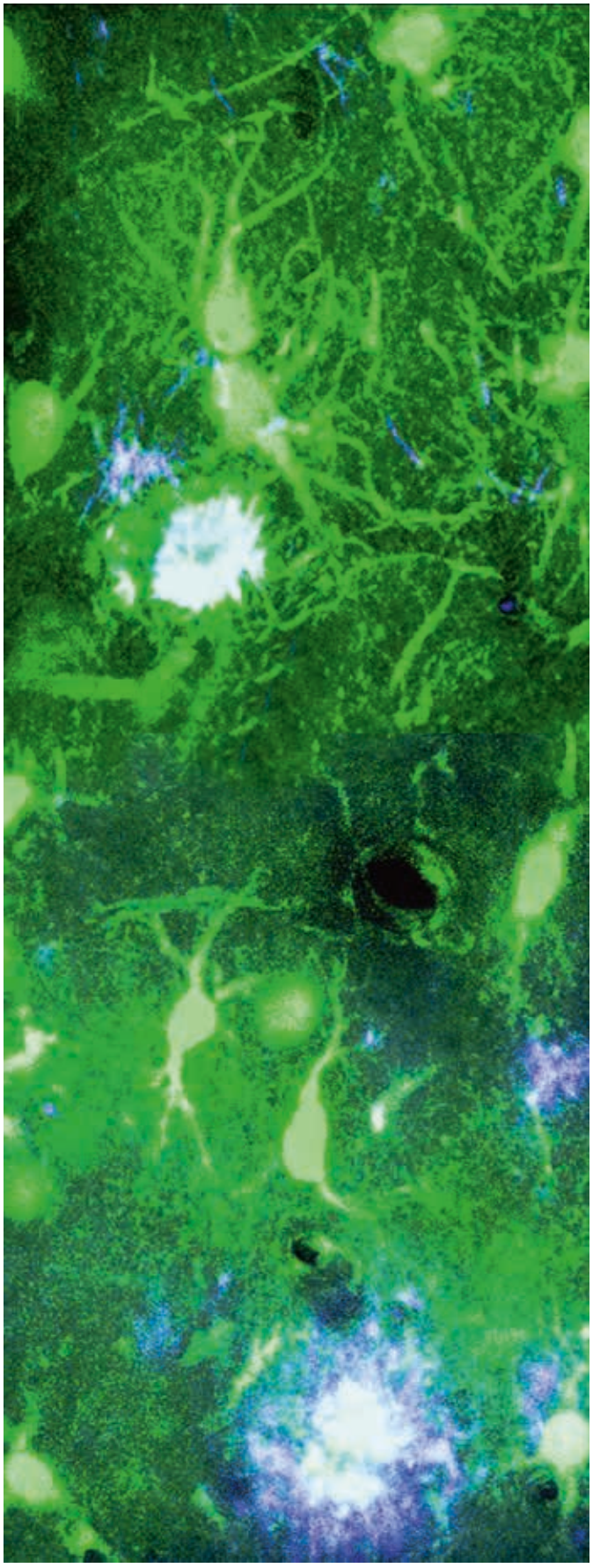
Scientists are however on the case, following every lead like detectives. Their most important equipment was previously the light microscope, enabling them to examine and analyze thin sections of the brain. They also inserted fine electrodes into the intact brains of living organisms to measure electrical activity. But it was in 2003 that research took a major leap forward, when Prof. Arthur Konnerth and his team at TUM's Institute of Neuroscience succeeded in developing a method to monitor the activity of individual nerve cells in a living brain. Today, the team's methods are used in many laboratories worldwide, for instance to improve our understanding of how the brain controls behavior and how disorders arise. "An effective treatment of diseases benefits enormously from a good understanding of the basic mechanisms of the normal function," declares the award-winning professor, now aged 63. "The major problem with treating psychiatric disorders, in particular, is that we don't have a detailed picture of the way the brain works under normal conditions. So we are trying to repair a system we don't fully understand in the first place. That is why therapies to date are often not sufficiently effective and have too many side effects."

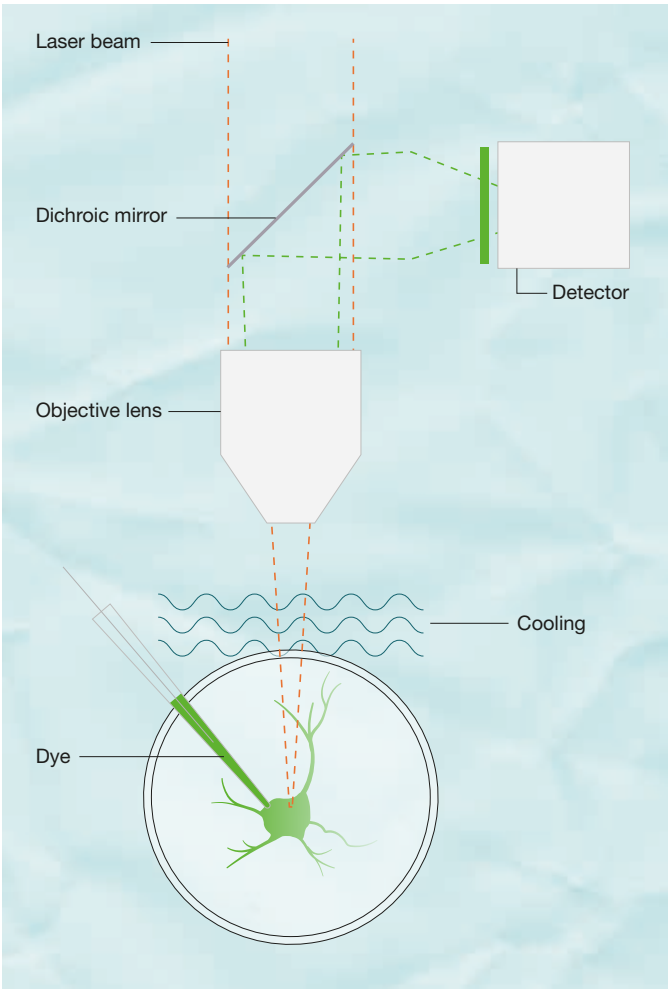
This also applies to Alzheimer's disease. As far back as the nineteenth century, specific deposits known as plaques were found in the brains of dead dementia patients. The condition was named after Alois Alzheimer who had first observed these plaques in the brain of a presenile patient in 1906. Many studies since have shown that the plaques consist of beta-amyloid clumps and are typical of the disease. But whether they are its cause or simply a side effect is something researchers still cannot say for certain. Alongside plaques, scientists have identified other changes in the brain that are also highly likely to play a role. These include smaller, still flexible clusters of beta-amyloid, the presence of neurofibrillary tangles (NFTs) formed by tau protein inside the nerve cells, and signs of inflammation. In the end, though, reliably identifying the brain processes associated with Alzheimer's disease means watching the cells in action. ▷



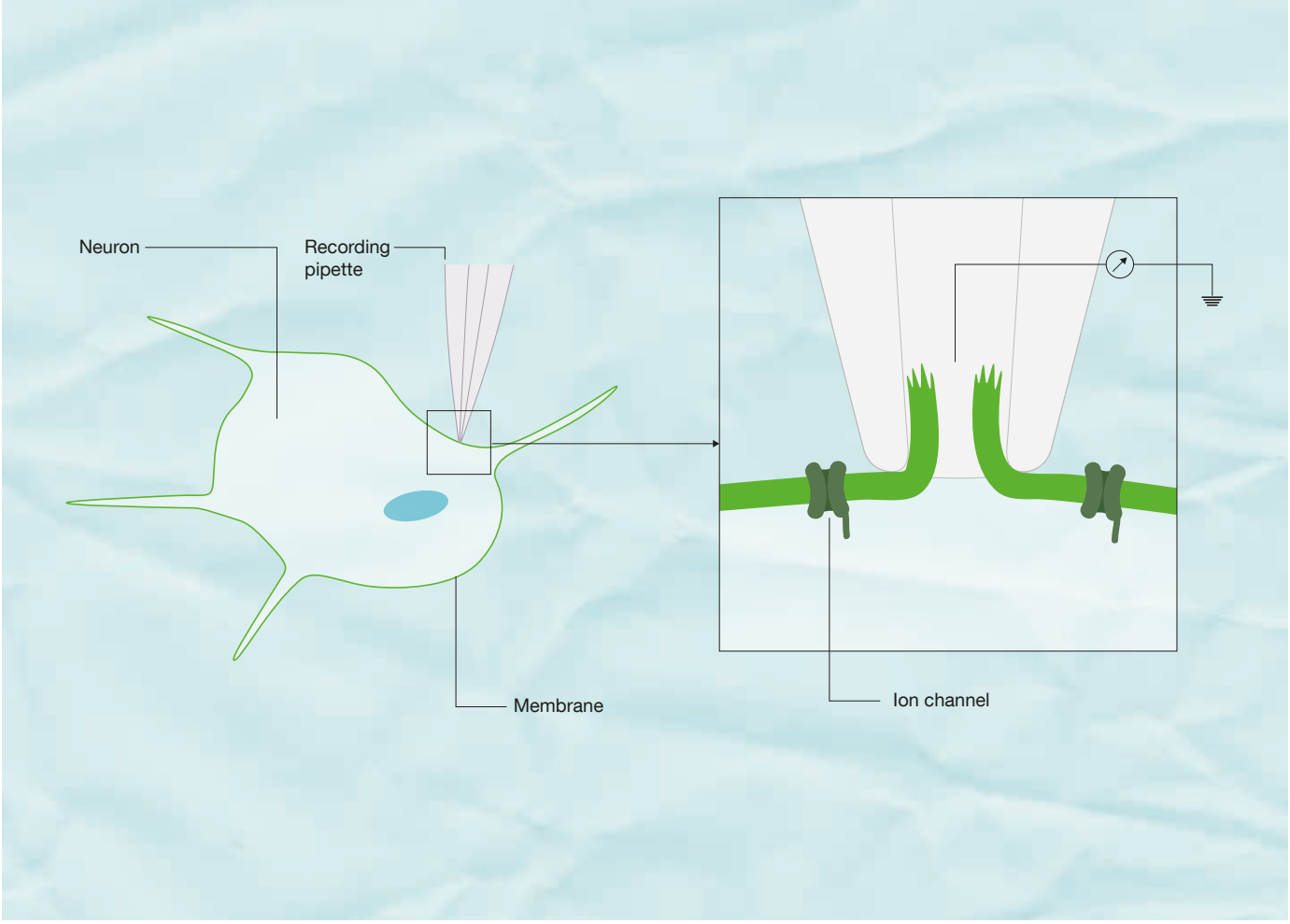
Picture credits: Bauer

A two-photon microscope allows the researchers to observe the nerve cells in the brain of live animals. The pipette for the patch clamp measurement can be seen on the right.





Left: Two-photon microscopy image of nerve cells (green) and beta-amyloid plaques (blue) in the brain of a mouse with Alzheimer's.
Above: In two-photon microscopy, a red laser beam penetrates the brain. A glass micropipette is used to inject dye into the nerve cells at the same time. The red laser beam causes the dye to emit a fluorescent green light under very specific circumstances, and this is registered by the detector. Since the dye binds to calcium, a molecule that always flows if the nerve cell is active, neuronal activity will be indicated by an increased brightness of that cell.



The patch clamp technique is used to measure the ion currents in the nerve cell or the cell membrane. It involves attaching a pipette to the cell membrane by suction. In order to measure the current throughout the cell, the cell membrane is perforated by strong negative pressure. Konnerth's group combines this technique with two-photon measurements, whereby fluorescent calcium indicator dyes are injected into the cells to allow the monitoring of neuronal activity.

Graphics: ediundsepp, Picture credit: TUM/Marc Aurel Busche

Shedding light on the living brain

Inside the labs at Konnerth's Friedrich Schiedel Chair of Neuroscience, researchers can look directly into the brains of living mice. Here, they have a raft of highly refined methods at their disposal, which they can also combine – for instance two-photon microscopy and the patch clamp technique. Two-photon microscopy uses red and infrared laser beams to penetrate the living brain tissue at depths of up to one millimeter and obtain highly detailed microscope images without damage to the cells. To achieve this, various flourescent indicator dyes are injected into the cells. For the precise recording of electrical activity they use the patch clamp technique which involves a glass pipette of just a few micrometers in diameter that can attach to individual cells. This enables scientists to examine ion channels in the cell membrane through which ions flow in and out of the cell. If the membrane under the tip of the micropipette is perforated, dye or pharmaceuticals can also be introduced into the cell.

In addition to these fundamental procedures, the researchers have also honed several other methods of investigating and influencing live neurons, right down to observing single synapses. These are the junctions between cells that enable the transmission of impulses. "We have a whole arsenal of tools at our disposal," confirms Konnerth, who was awarded the Brain Prize in 2015 for his pioneering work. "It's actually more physics than medicine." To further advance interdisciplinary competence, he and his colleagues at the TUM Medical School established a new study program for medical students to learn and apply these advanced methods. ▷

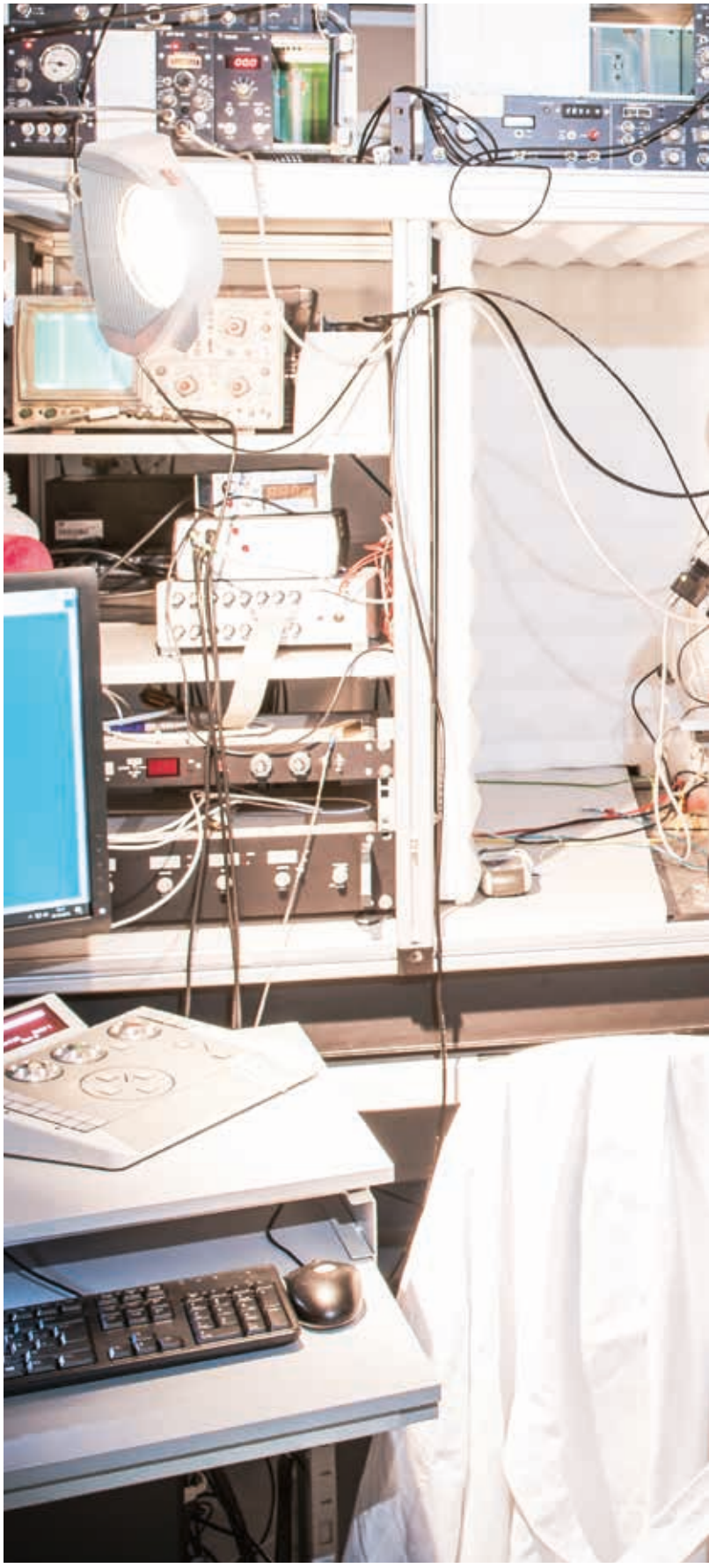
Prof. Arthur Konnerth

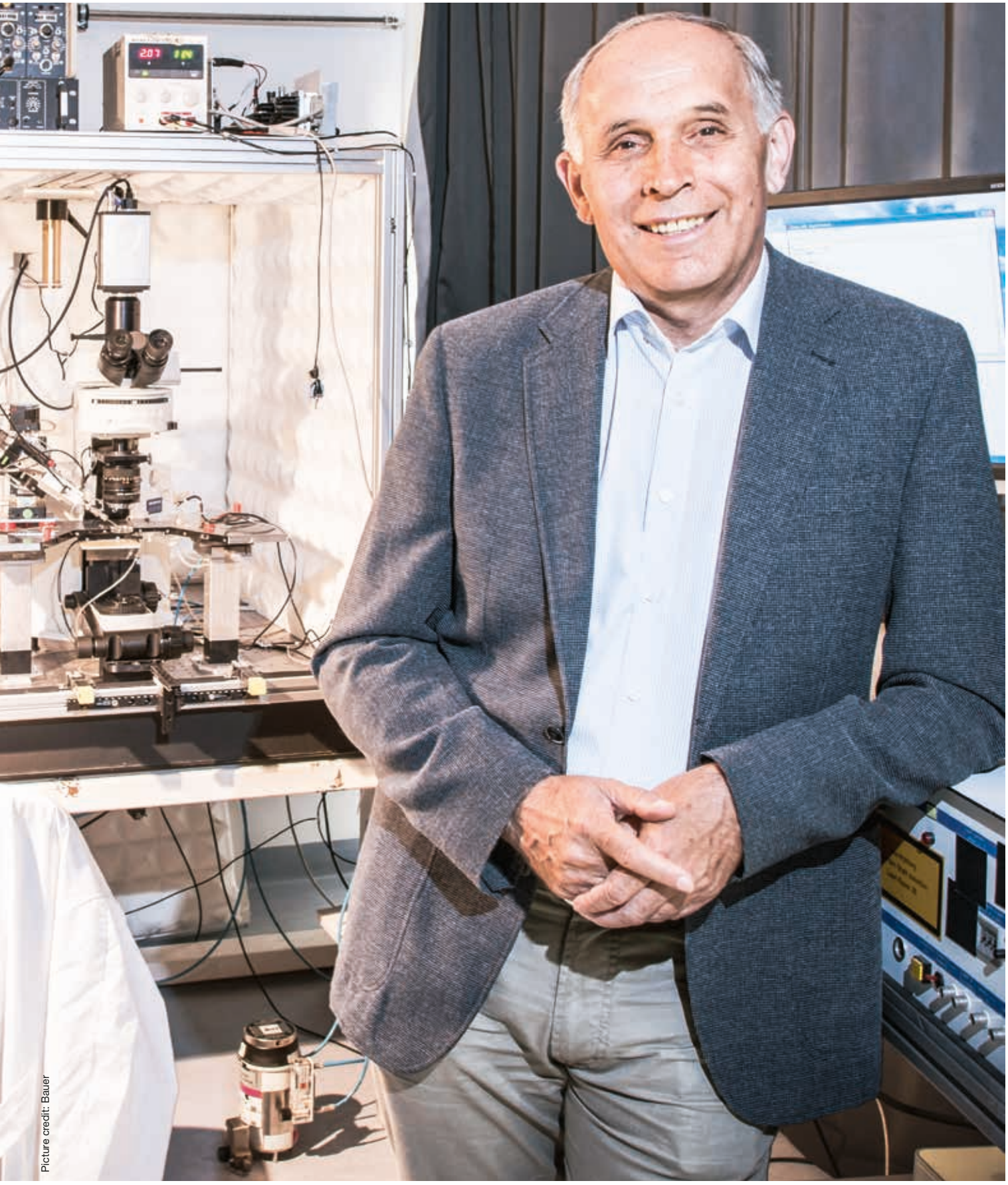
An international family

The latest breakthroughs in brain research were made possible in particular by interdisciplinary collaboration. Prof. Arthur Konnerth himself unites several of the disciplines in question, which is probably the secret of his phenomenal success – combining indepth physics and engineering knowledge with a thorough grounding in medicine. And he is quite determined not to limit himself to a single specialty, preferring to see himself as a "basic researcher and academic mentor."

Born in a German speaking region of Romania, Konnerth immigrated to Germany with his parents in 1974 and started his medical studies soon after his arrival. Even back then, he was determined to pursue basic research, hoping to be able to combine research with clinical practice. However, when he came to the end of his medical education, "Germany just didn't have the necessary structures in place," he recalls with regret. "It wasn't possible to combine the research and clinical work in an effective way at the time." Undaunted, he began his lab work already during his undergraduate studies, being especially interested in the development of new methods for his research. This was made possible in part because he was "lucky enough to be working alongside outstanding academic teachers from early on." He studied medicine in Munich and attended the city's Max Planck Institute of Psychiatry. After a period in the US, Konnerth moved to Göttingen in 1985 to work with Bert Sakmann, who received the Nobel Prize together with Erwin Neher in 1991 for development of the patch clamp technique. Konnerth later went on to lead the Cellular Neurophysiology working group in Neher's lab, before taking up appointments at various German universities.

The versatile researcher rapidly rose to success in all his various endeavors. As a result, he has already been distinguished with almost every award in the field. The latest in a long list is the million euro Brain Prize, which he received alongside three other researchers in 2015. Looking back over his career, Konnerth now says: "It wasn't always a dream come true, but things have certainly gone well. This field offers plenty of freedom to go after the things you think are important and I really appreciated that. But of course good health and good fortune play an important role too."





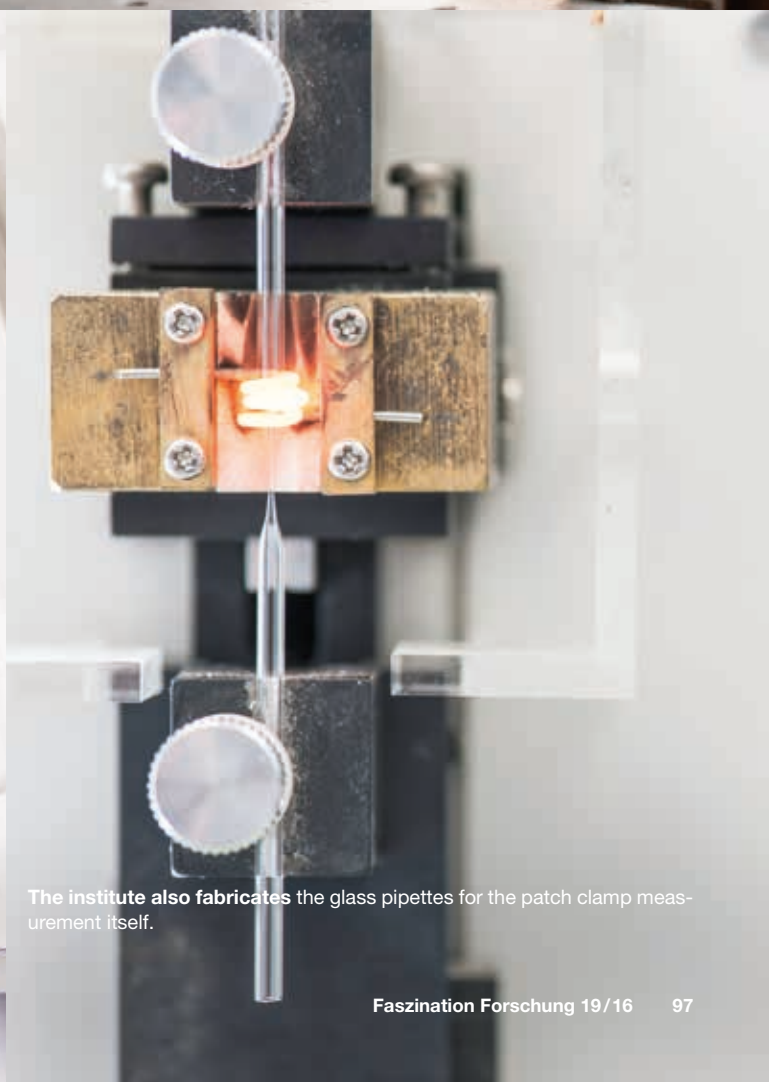
Picture credit: Bauer

Konnerth's institute produces two-photon microscopes itself and uses its own workshop for this purpose. Shown here is the construction of one component of the microscope, the so-called scan box. This is where the team makes fine opto-mechanical components for a two-photon microscope.

Construction of a tissue holding device.

Alignment of the two-photon microscope's red laser beam.

Embedded brain tissue is finely sliced and then stained to make even the tiniest protrusions from the nerve cells visible.



The institute also fabricates the glass pipettes for the patch clamp measurement itself.

Picture credits: Bauer

"I was especially drawn to Alzheimer's as a research topic. Psychiatric conditions are particularly fascinating, since they have an impact on us as people. And yet we know so little about them."

Marc Aurel Busche

Dr. Marc Aurel Busche, 33, took advantage of this opportunity and went on to complete his Ph.D. in parallel to his medical studies. He is one of the scientists bridging the gap to clinical research. In his case, this entailed specializing in psychiatry and psychotherapy at TUM's university hospital, Klinikum rechts der Isar, with a particular interest in Alzheimer's disease. "I'm basically a hybrid," he grins. "I was especially drawn to Alzheimer's as a research topic. Psychiatric conditions are particularly fascinating, since they have an impact on us as people. And yet we know so little about them. Now that we have these methods, there's a huge opportunity to take our research to a whole new playing field – both with animal models and in our work with humans."

Challenging and developing established concepts

Busche and his colleagues have indeed been successful in gaining new insights into Alzheimer's disease over the past few years. "For instance, we were the first group able to use two-photon microscopy on a living brain to reveal that mice with Alzheimer's have large numbers of particularly active neurons. This hyperactivity initially occurs in the hippocampus – a region deep in the brain responsible for learning and memory – and at a very early stage of the disease, before the Alzheimer's plaques are even present."

These findings came as a surprise to his doctoral advisor, Arthur Konnerth: "It had long been suspected that neurons surrounding the plaques were less functional than elsewhere – because of earlier evidence that beta-amyloid from plaques interferes with synaptic transmission. At the outset, we were actually looking to interrupt this slowdown. And then came the major surprise that, contrary to our expectations, some of the cells were in fact hyperactive." This outcome fits in well with independent findings of US researcher Lennart Mucke in San Francisco. Through studies both in mice and Alzheimer's patients, he was able to observe brain impairments that were similar to those occurring in epilepsy. "This overexcitation is what Marc Aurel Busche can see through the two-photon microscope," as Professor Hans Förstl puts it. The Director of the Department of Psychiatry and Psychotherapy at Klinikum rechts der Isar goes on to explain: "The body is not equipped to downregulate that level of hyperactivity. You could say the brain is overheating." This finding thus suggests the use of antiepileptic drugs to treat some aspects of Alzheimer's, although additional treatment is still needed. Various promising studies have now been conducted on mice, and clinical trials have recently gone ahead with patients in Munich and the US.

Marc Aurel Busche is a physician at TUM's university hospital and also conducts research into Alzheimer's at Konnerth's laboratory. With the assistance of two-photon microscopy, he discovered an unusually high number of very active nerve cells in the brains of mice with Alzheimer's. This is consistent with the hyperactive behavior which many physicians observe in Alzheimer's patients.

Busche and his team gained another fundamental insight into brain abnormalities accompanying Alzheimer's disease by monitoring neuronal activity during sleep. Sleep plays an important role in memory formation. In particular, the slow oscillations our brains generate at night are key to consolidating what we have learned and to shifting memories into long-term storage. These waves of activity are formed throughout a network of nerve cells in the brain's cortex and then spread out into other parts of the brain, such as the hippocampus. "There is a high degree of coherence between distant neural networks while we are asleep. But this is disrupted in mice with Alzheimer's," explains the researcher. "You could liken it to a heart flutter, where the chambers beat out of sync. In our case, wave activity still occurs locally in various regions of the brain's cortex, but the waves are no longer able to spread and synchronize properly." The original observation of neuronal hyperactivity may offer an explanation here too, particularly since Alzheimer's patients often have difficulties sleeping – and usually long before they become forgetful.

Busche and Konnerth published their findings in the October and November 2015 editions of "Nature Neuroscience". "The concept offers a completely new approach to understanding this condition – the great thing being that clinical interests align here with the cellular techniques that Marc Aurel Busche masters so well," concludes Hans Förstl.

Arthur Konnerth shares this opinion and has great expectations that the latest methods will take us further still: "Our hope is that we will one day gain a full understanding of the way cells actually work. What we need to accomplish now towards this goal with the highest priority is to gain an understanding of how single cells deal with incoming signals." This is still a source of major controversy among scientists. But if we could achieve clarity here, it would be a fundamental advance in understanding how the brain works. *Brigitte Röthlein*

Picture credits: Bauer