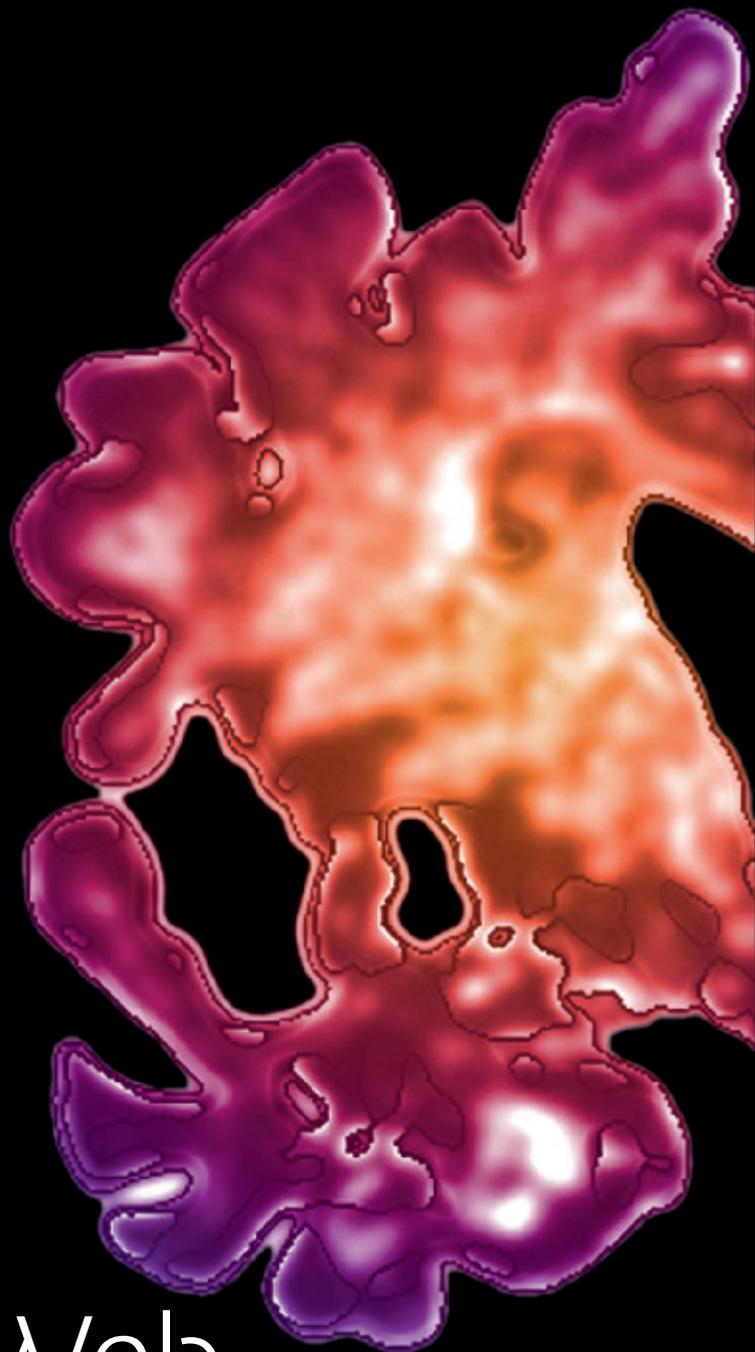


Diminishing brain: Computer graphic of a vertical section of a brain affected by Alzheimer's disease (left) and a healthy brain (right). The loss of brain cells causes the diseased brain to shrink.



Unraveling the Tangled Tau Web

Particle accelerators do not automatically spring to mind as a tool for use in Alzheimer's research. However, the German Synchrotron Research Center (DESY) in Hamburg has provided invaluable service to Alzheimer's researchers **Eva-Maria and Eckhard Mandelkow** from the **Max Planck Research Unit for Structural Molecular Biology**. With DESY's help, the Mandelkows have succeeded in illuminating the processes that lead to the memory loss associated with Alzheimer's disease. Their research findings raise great hopes for the development of effective treatments.

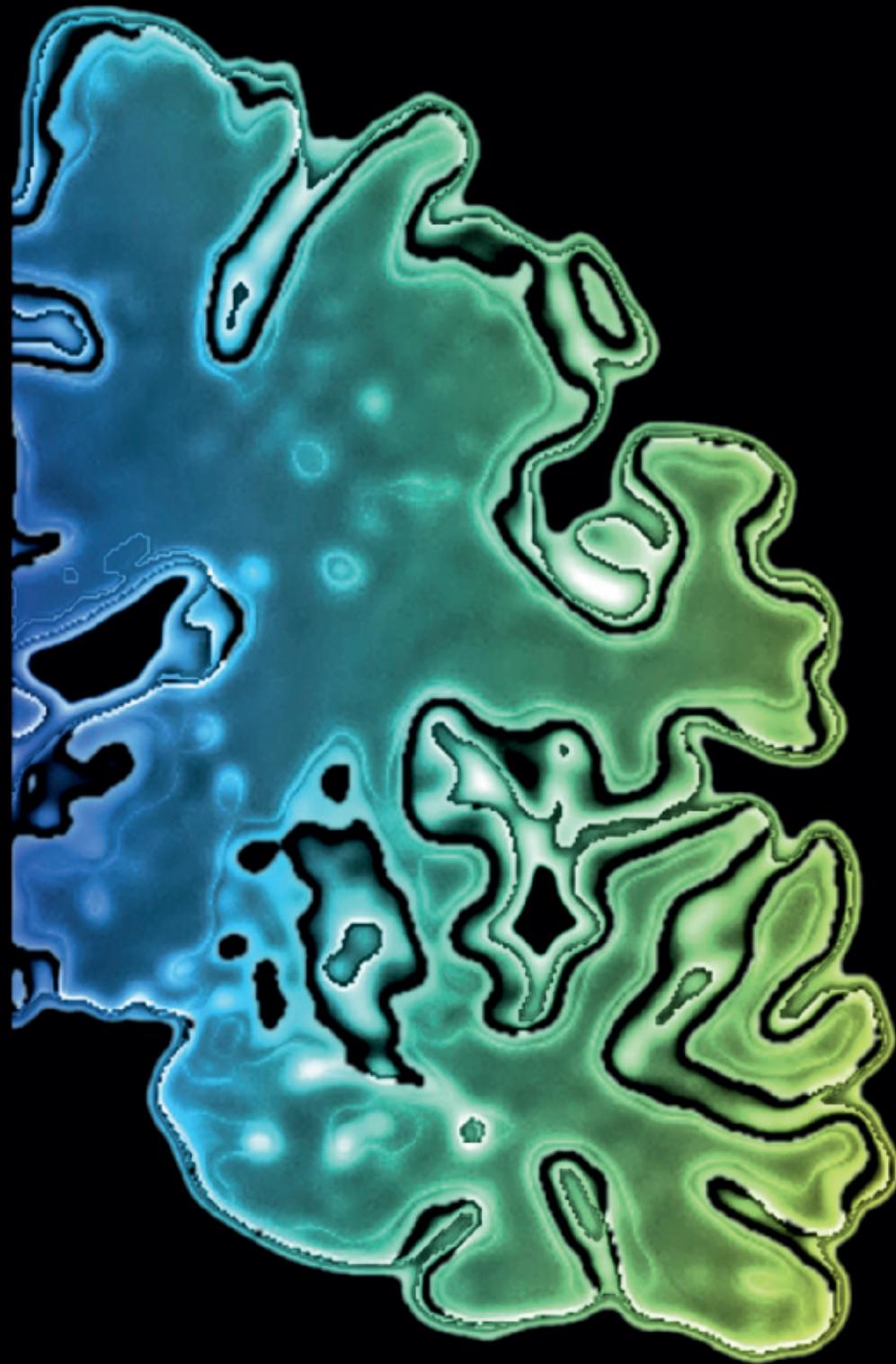


Photo: SPL - Agentur Focus

TEXT CATARINA PIETSCHMANN

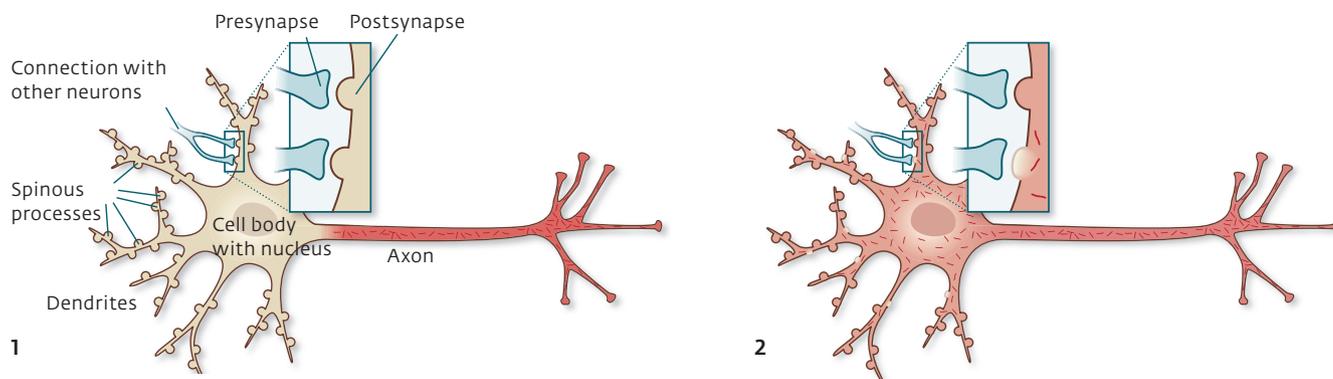
A constant humming can be heard throughout the complex. Metallic, not very deep, distinct – the noise is generated by the power supply to giant magnets. These are required to deflect the electrons propelled at an unimaginable speed through the storage rings of the DESY, DORIS and PETRA 3 particle accelerators, a process that can tease out vast numbers of protons from matter. When focused, these protons generate a very bright, energy-rich beam that can be used, for instance, to examine proteins in minute detail. Every year, 3,000 guest researchers from all over the world come to DESY in Hamburg for this very purpose.

Unlike their international colleagues, Eckhard and Eva-Maria Mandelkow are permanently based at the source. They work with DORIS, which the two scientists, who have made an enormous contribution to shining light on the darkness surrounding the cause of Alzheimer's disease, describe as the "ultimate torch."

First things first: Where can I find the Mandelkows? Without saying a word, the porter picks up a large map of the complex and, using a thick felt pen, traces the path to the Max Planck Research Unit for Structural Molecular Biology. "A good ten minutes on foot – walking at a brisk pace," he adds with a grin. The site, which is the size of a small city neighborhood, comprises 80 buildings. Even a young and fit person could easily lose their way here. For someone suffering from dementia, even the best map in the world would be of no use.

Alzheimer's – the diagnosis always comes as a shock. For the patients themselves, but also for their families. It means a process has begun that can't be halted: the loss of memory – experience, acquired and everyday knowledge, information about people and things loved and, ultimately, even awareness of the self.

The causes of the disease remain largely a mystery. The consequences are clearly recognizable while the pa-



tient is still alive, but up to now, they really become clear only after death: protein deposits and a conspicuous loss of nerve cells, primarily in the hippocampus, the part of the brain where the memory resides.

Our memory is like a personal library. We can access it whenever we like, select individual books and retreat to particular places and situations, even if they occurred many decades ago. All it takes is a photograph, a smell, a noise, or sometimes just a particular incidence of light, and images that were long believed forgotten trigger chains of thought. *Light like that time in Siena, in the early afternoon. It was September '94 ... I had just bought those tan shoes and we passed this small pasticceria. It smelled amazing! Afterwards, we had those warm almond cookies ... what were they called? ... that's right, ricciarelli ... and the café...? A day later, the name of the café comes back again: "Café Nannini"*

ALZHEIMER'S CAN AFFECT ANYONE

But what happens when one book is missing? And then several books? Later, entire years, and even entire epochs, have vanished into thin air. And at some point, all that is left on the empty shelves are dog-eared volumes from childhood and youth. So we read them again and again, almost compulsively. This is more or less how someone who has Alzheimer's must feel.

An estimated 24 million people throughout the world currently suffer from this most common form of dementia. Fewer than 2 percent of them suffer from familial Alzheimer's disease. In those patients, certain mutations arise on a chromosome and can be identified

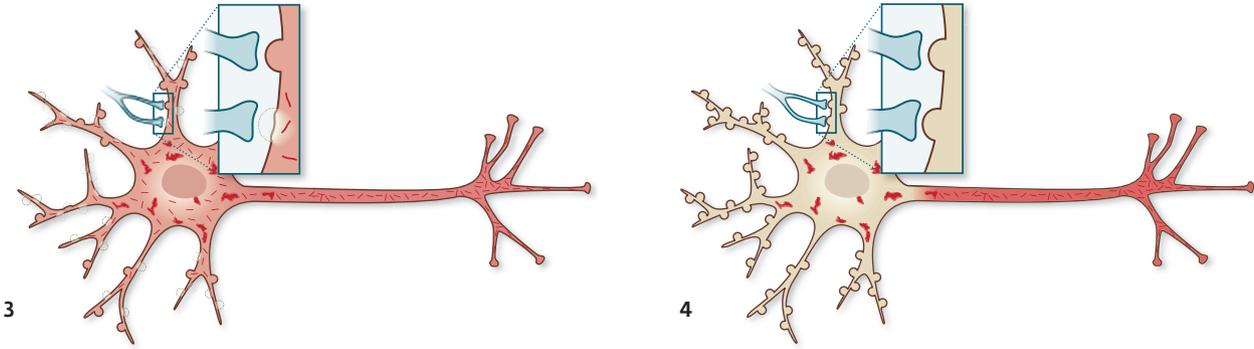
In a healthy neuron, the tau protein (red) stabilizes the cell skeleton in the cell axon (1). In a cell with an additional tau gene, which rapidly forms aggregating tau protein, tau also arises in the body of the cell and in the dendrites of the neuron, where the protein causes the dismantling of the synaptic thorns (2). The tau then forms clumps and the neuron loses more and more synapses (3). If the additional gene is switched off, the tau is found only in the axon again and new synapses form (4).

using a genetic test. The vast majority of sufferers have sporadic Alzheimer's disease. The good news is that anyone whose relatives did not display typical Alzheimer's symptoms from around the age of 50 can feel relatively safe until they are 80. Only three out of one hundred 75-year-olds contract the disease; in 80-year-olds, the corresponding figure is around 20 percent. After 85, it rises to between 30 and 50 percent – depending on the statistics used. "The

main risk factor for Alzheimer's is simply age," says Eva-Maria Mandelkow. "Just as we develop arthritis in the knee or hip joint, the nervous system is also subject to wear and tear."

"The astonishing thing is that the majority of us do not get Alzheimer's in old age," stresses Eckhard Mandelkow. "All elderly people have protein deposits in their brains. Do they have a protective gene? Good metabolism? We simply don't know."





The disease is highly specific. In addition to the loss of neurons, the pathologist will find two kinds of deposits in the brain tissue of Alzheimer's patients: the protein amyloid-beta ($A\beta$), also referred to as "plaque," will be observed between the neurons, and tau protein will be found inside the neurons. Only when both occur – and predominantly in the hippocampus – does the patient develop Alzheimer's. On their own, tau deposits, which are known as tau pathology, are indicative of various other forms of dementia. For example, the presence of tau in combination with the protein alpha-synuclein is a typical feature of Parkinson's disease. It is also possible to develop both Alzheimer's and Parkinson's.

The Mandelkows and their interdisciplinary team have long had tau in their sights. The protein stabilizes the microtubules, the tubular protein fibers that constitute a key component of the basic structure of all cells. They literally play a "supporting" role because they provide mechanical support to the cell. Moreover, during cell division, they form the spindle apparatus, to which the chromosomes in the emerging daughter cells migrate. They also facilitate the cell's "freight transport" system: valuable supplies access the cell through the cell projections, the axons and dendrites, and are carried "on piggyback" by motor proteins like kinesin, the cell's very own "freight carrier." The supplies in question range from pro-

teins and nutrients to entire cell organelles, for example mitochondria and peroxisomes – cone-shaped mini-containers for enzymes.

The entire process can be observed live and in color under the fluorescence microscope, as tau and other cell components can be marked using fluorescent proteins. The image under the microscope resembles slow-moving traffic at the entrance to a tunnel.

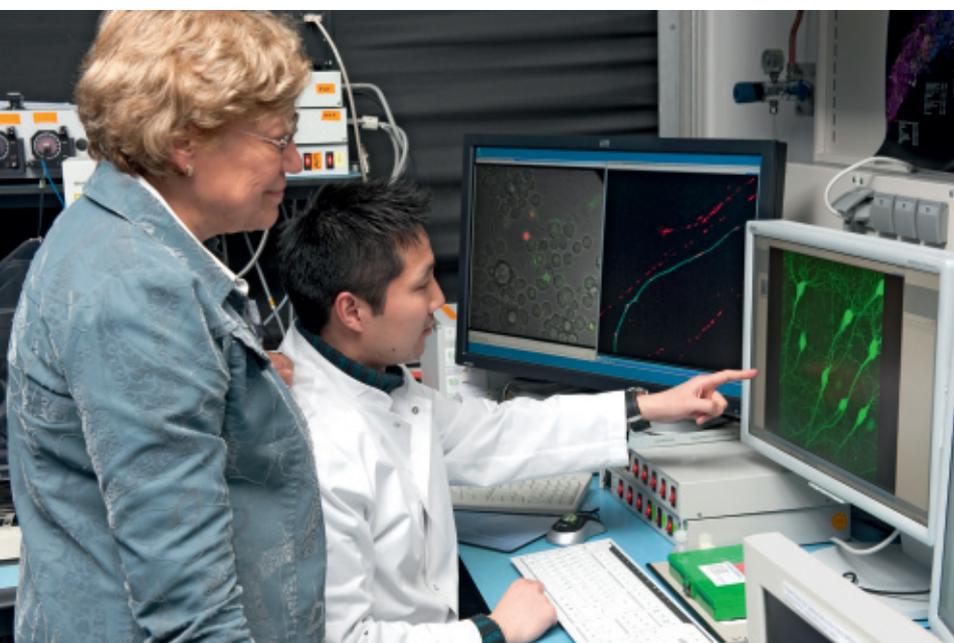
PART OF THE CELL SKELETON

Tau binds loosely to the microtubules and stabilizes them, helping to ensure that the cellular transport process runs smoothly. This protein is something of an exotic species, as it is almost entirely unfolded. As a result, it is very flexible and can't be impaired by either heat or acid. "We had already been working on tau and other microtubule proteins for some time when the link between tau and Alzheimer's was discovered more than 20 years ago. This prompted us to concentrate on the role of tau in this disease," explains Eva-Maria Mandelkow. In diseased neurons, the protein behaves "improperly" and no longer functions correctly. "In Alzheimer's, tau is over-phosphorylated by hyperactive enzymes, the protein kinases, such as MARK kinase. This means that phosphate groups suddenly arise at many locations in the tau protein. As a result, it

Doctoral student Lars Messing prepares brain tissue for testing. In order for the scientists to be able to identify changes in the neurons under the microscope, they must use a microcutting device to create tissue sections just a few thousandths of a millimeter in thickness.

Photos: Andreas Garreils (2); diagram: Emde Graflik (4)





Laser scanning microscopes provide particularly detailed images of the brain: Eva-Maria Mandelkow analyzes microscope images of neurons with her husband (top) and Xiaoyu Li (bottom).

is no longer able to fulfill its functions. It detaches itself from the microtubules and clumps together.”

Tangled protein fibrils that are unable to function are effectively waste, and this process marks the beginning of the end for the affected neurons. It is also the point at which memory loss starts, as too little tau on the tracks inhibits transport operations: the microtubules break up and the motor proteins can no longer hitch up to them correctly. Then, due to lack of supplies, the synapses, the minute protuberances of the axons and dendrites through

which the neurons communicate with each other both chemically and electrically, disappear. This is followed by the death of the axons and dendrites and, eventually, the entire neuron. “It doesn’t matter where you examine it – in the test tube, in the cell or in the mouse model – it’s the same picture everywhere,” says Eva-Maria Mandelkow.

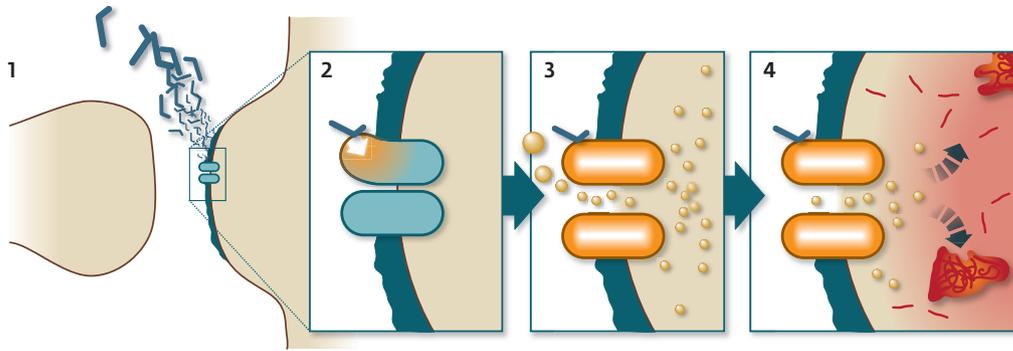
This is what happens in the neurons. But what does this have to do with the much-debated extracellular amyloid plaques? “That’s one of the key questions that we and other laboratories are investigating,” says Eckhard

Mandelkow. The researchers applied the loosely clumped “sick” A β protein to rat cells from the hippocampus and observed what happened in the neurons: the first pathological signs of Alzheimer’s emerged with bewildering speed – just two hours later. Tau, which is normally found in the axon, was also found in the dendrites. And wherever the tau arose in the wrong place, the parts of the synapses where signal transmission takes place – the postsynapses or spinous processes – disappeared. This is fatal, as the communication between the nerve cells takes place through the contact of the presynapse on the axon and the postsynapse on the dendrite of the downstream neuron. One neuron has around 10,000 synapses. Even if these points of contact are only partially destroyed, the “radio silence” between the neurons begins.

TAU AND A β – THE SEARCH FOR CAUSE AND EFFECT

Eckhard Mandelkow takes a piece of paper and starts to draw. “When A β forms clumps, it settles on the postsynapses like a thin film. It is assumed that it binds to a receptor – the NMDA receptor, for example. When it is activated, calcium flows into the cell. That is its job. In this case, however, the NMDA receptor is over-activated, and too much calcium flows into the cell interior. This triggers, among other things, tau dysregulation.”

Does this mean that A β is ultimately the trigger of the disease? Mandelkow shakes his head and continues drawing.



Aβ binds in the synapses to receptor molecules for the neurotransmitter glutamate (1, 2). Large volumes of calcium flow into the synapse through the open receptor channels (3) and trigger the formation of tau clumps (4).

“That’s not quite the case. It’s always said that Aβ dysregulation leads to tau dysregulation and this, in turn, causes synapse loss and neuronal cell death. But what actually causes the Aβ to clump? Mutations could be one possible cause, but these arise only in the rare cases of familial Alzheimer’s.” Many scientists believe that the precursor of Aβ, the cell surface protein APP, is split too often and in the wrong place. And the short Aβ protein that arises as a result has the tendency to form clumps. The gene for APP is on chromosome 21. This is why, for example, people with trisomy 21 (Down Syndrome) have a higher risk of developing dementia early on. An increased amount of Aβ is formed in their brains, as they have three copies of the APP gene.

“By the way, you’ve just drawn the postsynapse incorrectly,” notes Eva-Maria Mandelkow. He smiles. “I don’t like it at all when my wife corrects me ...” She laughs and continues. “Well, you did. You drew it as a presynapse. Doesn’t matter, forget it!”

Is it always easy to work together? They glance at each other. “Together we’re actually very creative,” she says. “I do everything by gut feeling.” Eckhard adds: “And I don’t let anyone walk all over me!” They both laugh. They don’t always agree when it comes to the interpretation of their research findings. This is hardly surprising, considering that he is a physicist, and she, a medical doctor.

And, as a doctor, Eva-Maria Mandelkow is interested not only in finding out how Alzheimer’s arises, but also

in developing a treatment. The path to this goal leads from cell tests to transgenic mice. These are animals into which a manipulated form of the human tau gene has been transplanted. Some of the rodents received a gene with an anti-aggregation mutation – tau is simply unable to form clumps in them. Others were given the pro-aggregation variant, in which the tau aggregates particularly quickly. These mice develop Alzheimer’s-like symptoms in a matter of a few months. The clever thing is that the gene can be switched on and off again.

MEMORY TEST FOR MICE

The scientists have recently been carrying out behavioral tests on the mice. The scenario: The rodents must swim to find their way to a platform in a two-meter-wide water basin. The platform is concealed beneath the surface of a milky fluid. They have already undergone training in finding the platform, four sessions per day. A camera films the mice from above and shows their swimming track as a red line.

And now for the test: First, the healthy mouse, the wild type. Ready, set, go! Within 15 seconds, it paddles to the platform and climbs onto it. Then it’s the turn of the “Alzheimer’s” mice. Their behavior is eerily reminiscent of a person with dementia who is unable to find the way home. One mouse moves through the basin in endless confused circles and even swims right by the platform at one point and then finds it – by accident, because it

brushes up against it. The time: 1 minute, 7 seconds – four times longer than the healthy mouse. “This film tells us so much more about the disease than all of the graphs and diagrams,” says Eva Mandelkow, pensively.

Finally, the same mouse, the one that already had “Alzheimer’s” and in which the toxic human pro-aggregation tau had been switched off for 4 weeks, does the test again. The mouse sets off purposefully, turns two quick pirouettes, as if to say, “I’ll be right there!” and, presto, reaches “dry land.” In a mere 10 seconds! “If you examine the neurons in the brains of these mice in detail, you’ll see that they still have tau aggregates and neuronal loss, but the synapses have re-formed!” Eva-Maria Mandelkow’s eyes shine with euphoria.

The brains of these “Alzheimer’s” mice contain clumps of mouse tau and human tau. To the scientist’s amazement, in the animals in which the mutated gene was later switched off, the human tau dissolved again, but the mouse tau remained entangled. Mouse tau doesn’t usually clump together. Strange. It appears it was prompted to do so by the toxic human protein. “That means that mutated – that is, pathogenic – tau can alter healthy tau! Like prions in Creutzfeldt-Jakob disease, but not infectious.” She grins. “My husband doesn’t like it when I say that.” He shakes his head. He sees it differently, from a physical perspective: mutated tau acts more like a crystallization germ that can also cause normal tau to form clumps. The real sensation, how-

ever, is that the memory loss is reversible – at least if intervention comes before too many neurons die off.

A SOLVENT FOR TAU CLUMPS

Switching off a gene – something that works well in mice – is not possible in humans. However, it should be possible to dissolve the tau aggregates using active substances. The Mandelkows' group has already tested 200,000 substances, identified promising lead structures and chemically refined them with the help of colleagues from other Max Planck institutes and universities. Two of the active substance classes examined have what it takes to dissolve the tau tangles and thus allow the synapses to grow again: derivatives of rhodanine and phenylthiazolyl-hydrazide.

Eckhard Mandelkow pops next door for a minute to consult his computer. His wife explains how they met. "As scholarship recipients of the Evangelisches Studienwerk, a scholarship orga-

nization for gifted students funded by the Protestant church of Germany, we were both involved in a performance of the opera *Dido and Aeneas*." A voice from next door calls out: "She as a flutist and I as a cembalist." When Eckhard went to New Orleans on a Fulbright Scholarship, she followed and completed a clinical internship. They both then wrote their doctoral theses at the Max Planck Institute for Medical Research in Heidelberg. This was followed by a period working as postdocs in the US, and the birth of their two children. "My dream was actually to work as a doctor in Africa. But I fell in love with this physicist!" Their daughter, a future surgeon, is now doing just that. She has already spent a year working for Médecins Sans Frontières in the Congo and during the cholera epidemic in Zimbabwe. Their son followed in his father's footsteps, studying physics and completing his doctorate on magnetic resonance tomography of the brain. He now works in the field of brain research.

The Mandelkows came to Hamburg to the newly established Max Planck laboratory in DESY in 1986 and have worked together ever since. "I focus on the biochemical dimension and the electron microscopy, and my husband does the rest," says Eva-Maria Mandelkow. As a doctor, structural biology was not quite her cup of tea. But when the tau protein came into play, and with it, Alzheimer's disease, she was hooked.

Together with colleagues in Munich, Freiburg and Cologne, they are now working on developing other transgenic animal models of Alzheimer's disease, such as fruit flies, zebra fish and worms. "When the 'tau worm' develops the clumping form of tau, it is no longer able to move," says Mrs. Mandelkow. They would now like to treat the worm using the drugs they recently tested on the mice. "The worm is ideal, of course, as it takes only a few days to go from one generation to the next, and the symptoms of a disease become apparent in a matter of hours.

Photos: Andreas Garrels (2)

Eva-Maria Mandelkow and Stefanie Könen train a mouse in the swimming pool (right). The creature must remember the figures on the edge of the basin to be able to find a platform concealed just below the surface of the water (left).





View from above of the swimming paths of a healthy mouse (1), a genetically modified mouse with rapidly aggregating tau protein (2) and a mouse in which the inserted tau protein was switched off again (3). Even after intensive training, the animal with dementia has to search for a long time before it finds the platform (light gray). When its tau gene is switched off, just a few weeks later, the mouse can remember the platform more or less as well as a healthy animal.

With the mice, we have to wait several months until we can observe symptoms." For the time being, it's all about providing the "proof of concept." As soon as the substances are available, the intention is to use them in patients.

ALZHEIMER'S RESEARCH IN BONN AND HAMBURG

Before things get that far, however, the two researchers will be moving to a new city. Next year they are set to transfer to a laboratory in the German Center for Neurodegenerative Diseases (DZNE), which was established in 2009. The DZNE is located on the premises of the "caesar" research institute, an institute of the Max Planck Society. At the DZNE, the Mandelkows intend to research other diseases in which tau plays a role. In addition to an 11.5 Tesla MRI scanner for mice, the scientists will have a host of new possibilities for observing the inside of the brains of live mice in Bonn. "We are really well resourced here in Hamburg – but we don't have anything like that in our current institute." However, they would like to keep one part of the laboratory in Hamburg for the time being. "Because of the mice, if nothing else. They can't simply be moved in the middle of experi-

ments. We work in close cooperation with the University Medical Center Hamburg-Eppendorf and keep more than 1,000 mice there at different stages of aging and treatment."

Eva-Maria and Eckhard Mandelkow spend between 10 and 12 hours a day at work. This doesn't leave them much free time. She has to laugh. "The children always used to say 'our parents have premature Alzheimer's. They always forget what they promised us.' That's what happens when your work and hobby overlap." Apropos free time: Do you both have other hobbies? "We used to," he says drily. "My husband plays the piano very well and we are interested in political developments," she adds. "But our research is so fascinating and we regularly meet our friends at conferences. We occasionally find time to go to the opera. And for relaxation, we go for walks along the banks of the Elbe River."

As experts, would they want to know whether they had Alzheimer's – assuming that a test were available with which the first signs of Alzheimer's-specific protein deposits could be observed at an early stage? She answers without hesitation. "Definitely!"

Is there anything you can do to prevent these protein clumps from form-

ing? "Of course there is. What's good for your heart is also good for your brain! As long as the brain is well supplied with blood, the supply is right, you've done just about everything that can be done at present," he says. "Lots of exercise, low cholesterol – that's very important. And then the usual things: vitamins, fruit, fish, reduced calorie intake," she adds.

Those who have a tendency to develop diabetes also have a much higher risk of developing Alzheimer's. "The tsunami is on its way, as many children are already too fat! There will be a huge increase in the incidence of diabetes and, for that reason alone, Alzheimer's as well," stresses Eckhard Mandelkow. So what about ginkgo or curcumin? Eva-Maria Mandelkow laughs again. "We don't believe in that." Indian and Chinese medicine? "Most of it could probably be thrown out," he growls.

Despite this worrying prospect, however, the Mandelkows are in good spirits. It's quite likely that drugs that are able to resolve the A β -tau problem will be available on the market in the foreseeable future. And the two scientists will have a well-stocked "library" to consult in their old age, containing shelves filled with memories of their long and shared path through scientific research. ◀

GLOSSARY

Presynapse/postsynapse

The presynapse is the transmitting section of a synapse. This is a specialized region of the axon in which the incoming electrical signal triggers the release of a neurotransmitter. This neurotransmitter binds to receptors in the neighboring postsynapse on a dendrite of the recipient cell and activates further electrical and chemical signal chains there.

Hippocampus

A region of the brain that plays an important role in learning and memory. Its name derives from its curved shape (Latin: sea-horse). It is part of the two hemispheres of the cerebrum, so there is also a right and a left hippocampus. Information from various regions of the brain enter the hippocampus and are connected with each other there.

Protein deposits

The proteins consisting of one or more amino acid chains fold during formation into complicated spatial arrangements. Incorrectly folded proteins can combine to form long and short fibers. They not only lose their functionality as a result, but also become insoluble and toxic and can no longer be dismantled by cells.