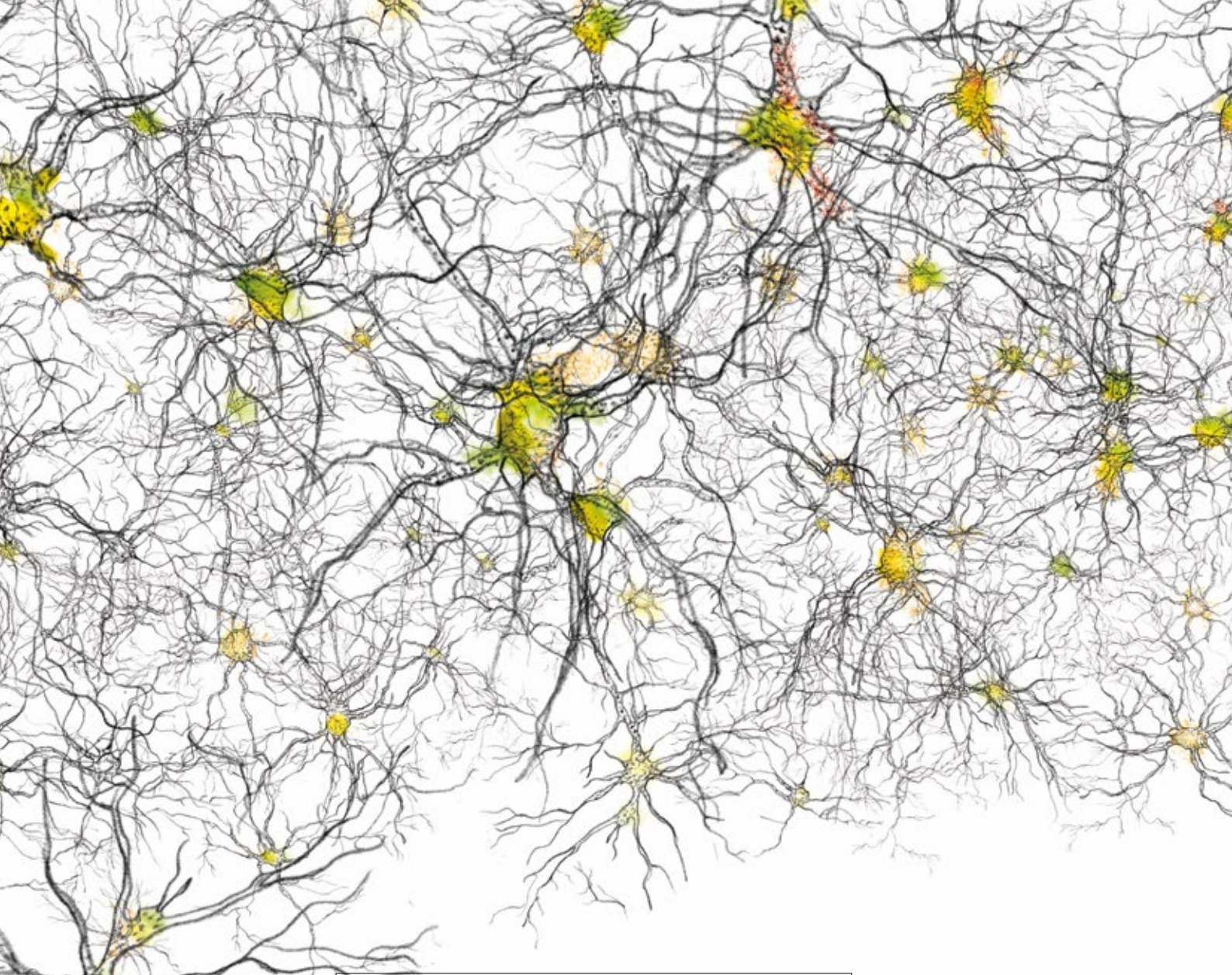


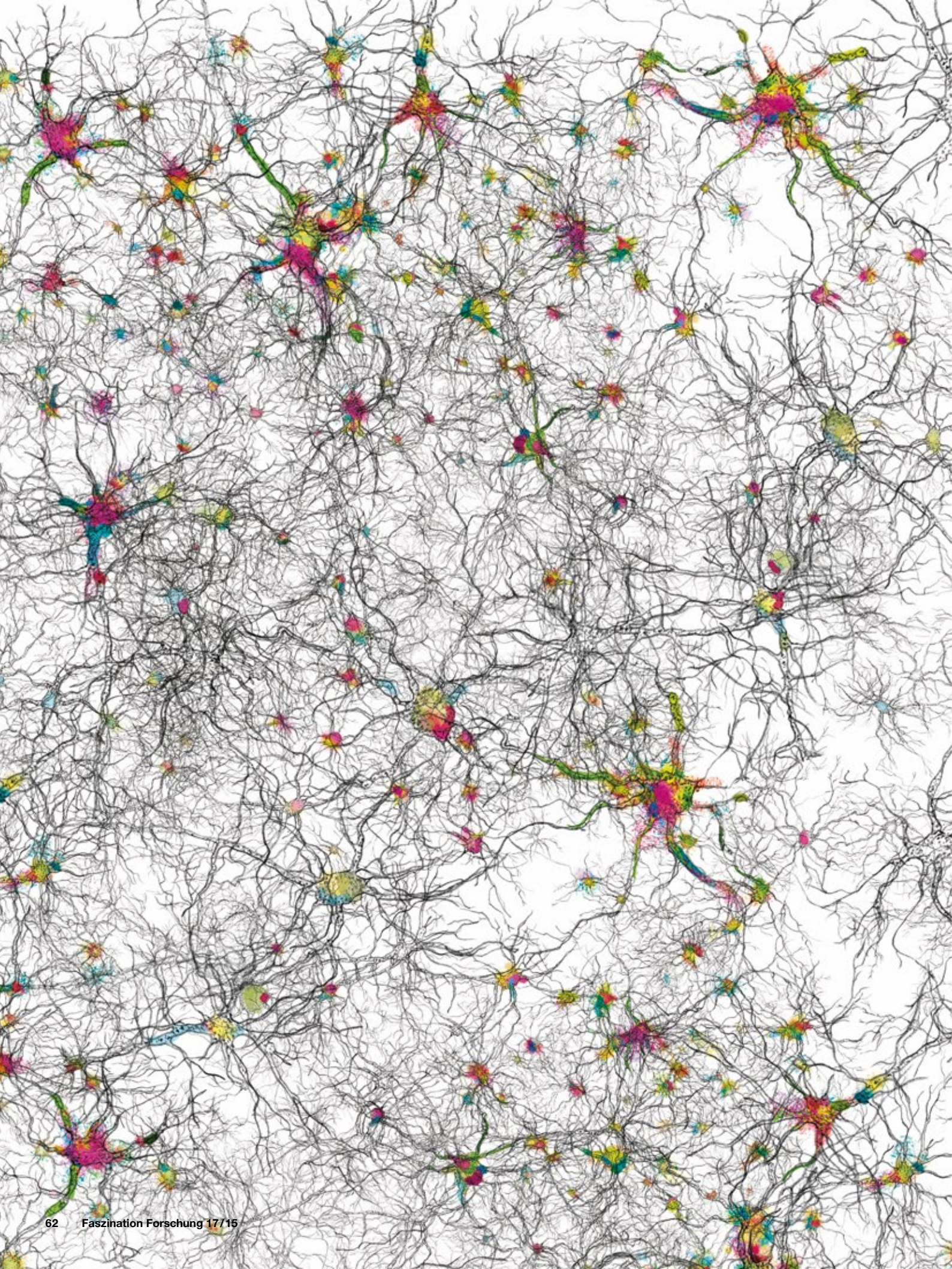
Link
www.misgeld-lab.me.tum.de

A Double-Edged Axe **in the Axon Forest**

There's rich variety of form and function among the hundred billion neurons that make up the human nervous system, yet almost every neuron has an axon. This extension from the cell body, typically resembling a trunk with many branches, carries electrical and chemical signals outward to other cells. During early development, the brain's axon "forest" is shaped by a pruning process that selectively cuts branches and clears the way for optimal neural networking. The same process shows a destructive aspect in old age, however, playing a role in neurodegenerative diseases. Prof. Thomas Misgeld is investigating the dual nature of axon loss.

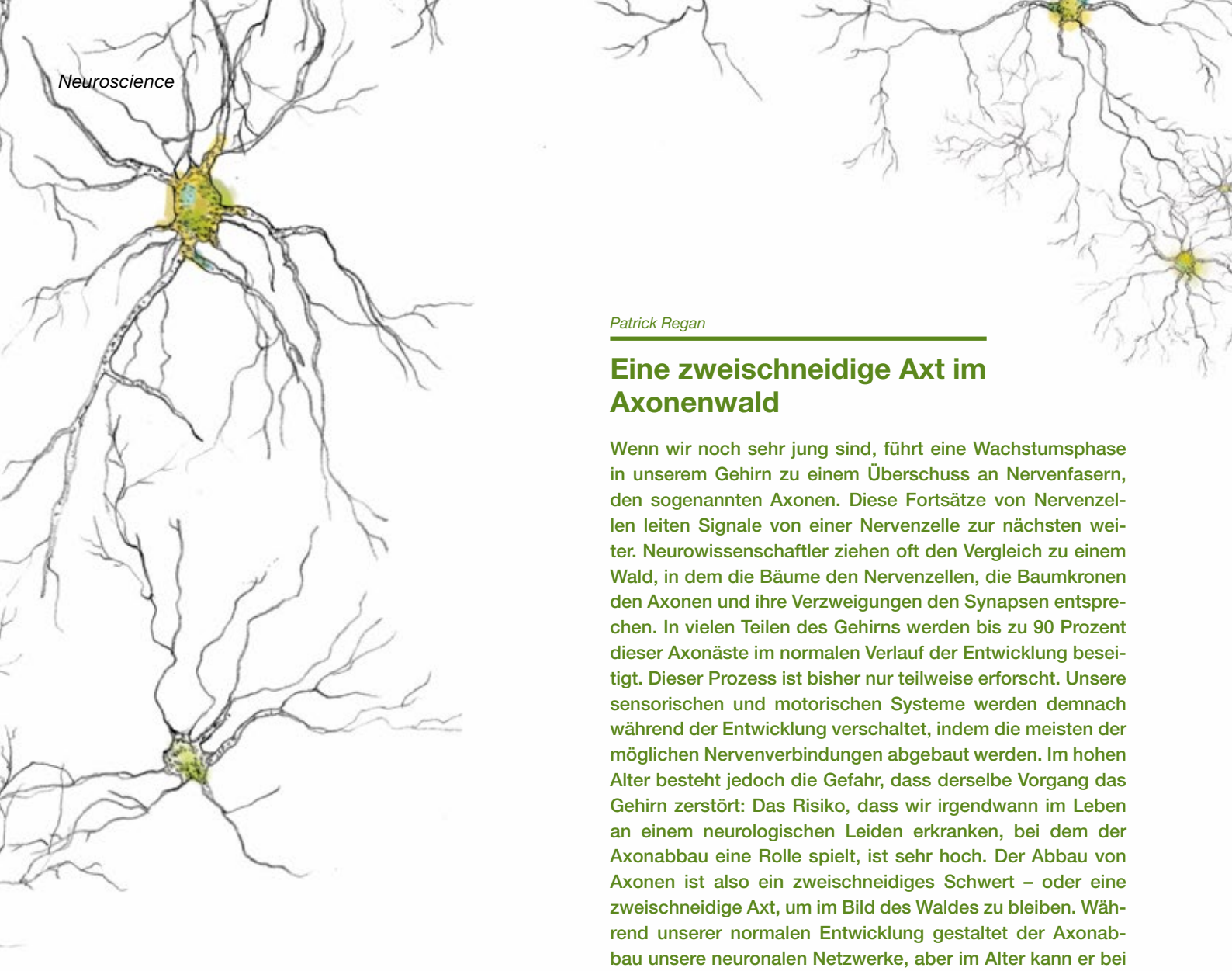
When we are very young, as young as Thomas Misgeld is in this picture, our brain builds up a rich abundance of nerve fibers called axons, which transmit signals between nerve cells.

Graphics: ediundsepp



During childhood, our brains exhibit the highest number of synaptic connections and axons. Thereafter, the pruning of the axon forest sets in and excess connections are cut.

Graphics: ediundsepp

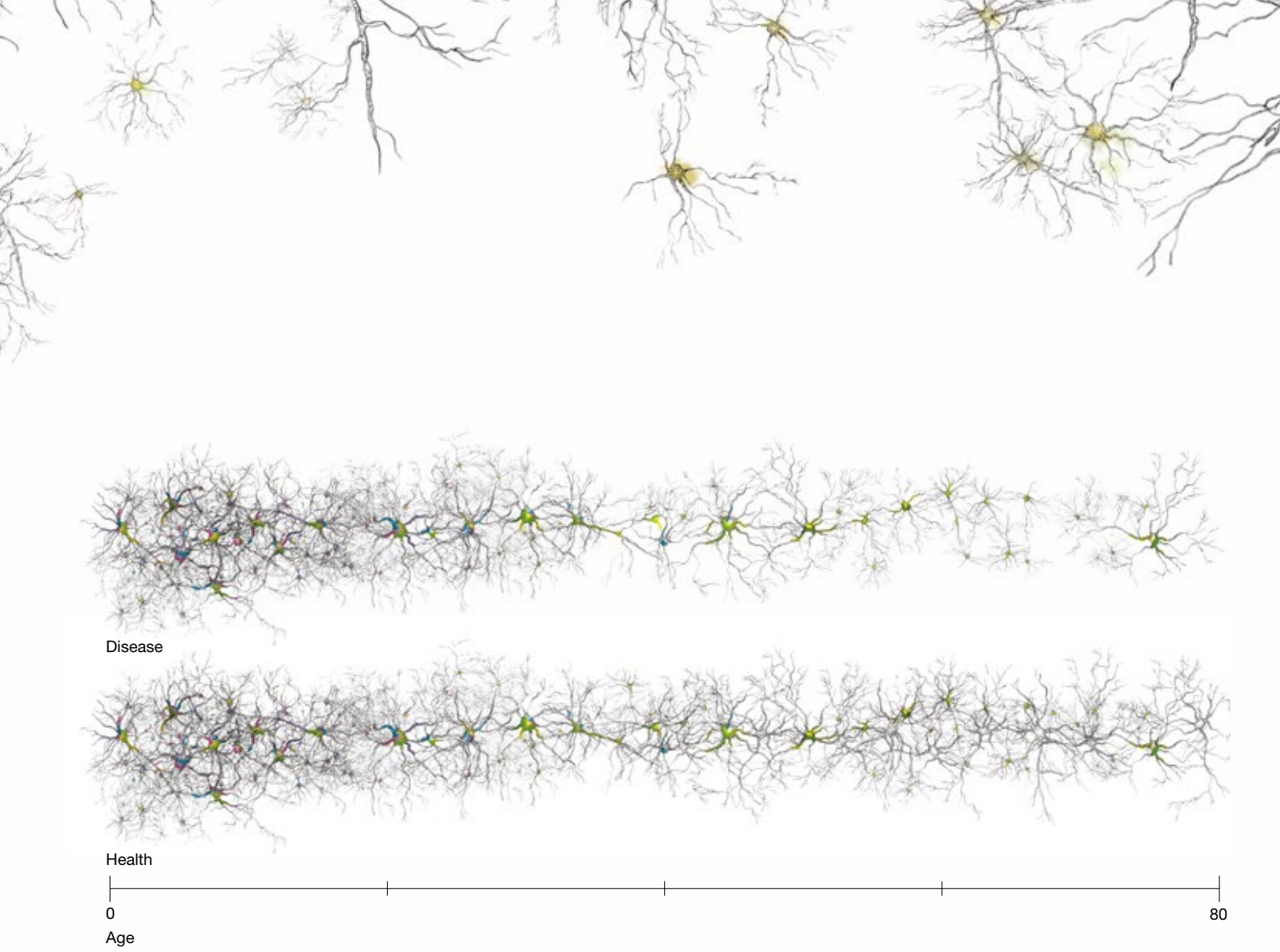


Patrick Regan

Eine zweischneidige Axt im Axonenwald

Wenn wir noch sehr jung sind, führt eine Wachstumsphase in unserem Gehirn zu einem Überschuss an Nervenfasern, den sogenannten Axonen. Diese Fortsätze von Nervenzellen leiten Signale von einer Nervenzelle zur nächsten weiter. Neurowissenschaftler ziehen oft den Vergleich zu einem Wald, in dem die Bäume den Nervenzellen, die Baumkronen den Axonen und ihre Verzweigungen den Synapsen entsprechen. In vielen Teilen des Gehirns werden bis zu 90 Prozent dieser Axonäste im normalen Verlauf der Entwicklung beseitigt. Dieser Prozess ist bisher nur teilweise erforscht. Unsere sensorischen und motorischen Systeme werden demnach während der Entwicklung verschaltet, indem die meisten der möglichen Nervenverbindungen abgebaut werden. Im hohen Alter besteht jedoch die Gefahr, dass derselbe Vorgang das Gehirn zerstört: Das Risiko, dass wir irgendwann im Leben an einem neurologischen Leiden erkranken, bei dem der Axonabbau eine Rolle spielt, ist sehr hoch. Der Abbau von Axonen ist also ein zweischneidiges Schwert – oder eine zweischneidige Axt, um im Bild des Waldes zu bleiben. Während unserer normalen Entwicklung gestaltet der Axonabbau unsere neuronalen Netzwerke, aber im Alter kann er bei verschiedenen Erkrankungen zur Zerstörung von kognitiven, sensorischen oder motorischen Funktionen beitragen.

Prof. Thomas Misgeld, Direktor des Instituts für Zellbiologie des Nervensystems an der TUM, will diesen Vorgang besser verstehen. Dafür hat er nun einen mit zwei Millionen Euro dotierten ERC Consolidator Grant eingeworben. Misgeld ist einer der Pioniere im Hinblick auf den Einsatz von Bildgebungstechniken, die moderne Mikroskopie und genetisch kodierte Biomarker kombinieren, um Veränderungen in der Struktur und der Funktion von Axonen *in vivo* beobachten zu können. In dem neuen Projekt werden die Mechanismen des Axonabbaus sowohl in motorischen Neuronen als auch im Zentralnervensystem während der normalen Entwicklung und der Krankheitsentstehung erforscht. Dabei handelt es sich primär um Grundlagenforschung, die Erkenntnisse über die Entwicklung und Anpassungsfähigkeit unseres Gehirns liefern soll. Obwohl die Forschung nicht primär darauf ausgerichtet ist, Therapien gegen neurodegenerative Erkrankungen zu entwickeln, so sind grundsätzliche Erkenntnisse dieser Art unerlässlich, um das Verständnis dieser häufigen Erkrankungen zu verbessern und damit mögliche therapeutische Ansatzpunkte zu identifizieren. □

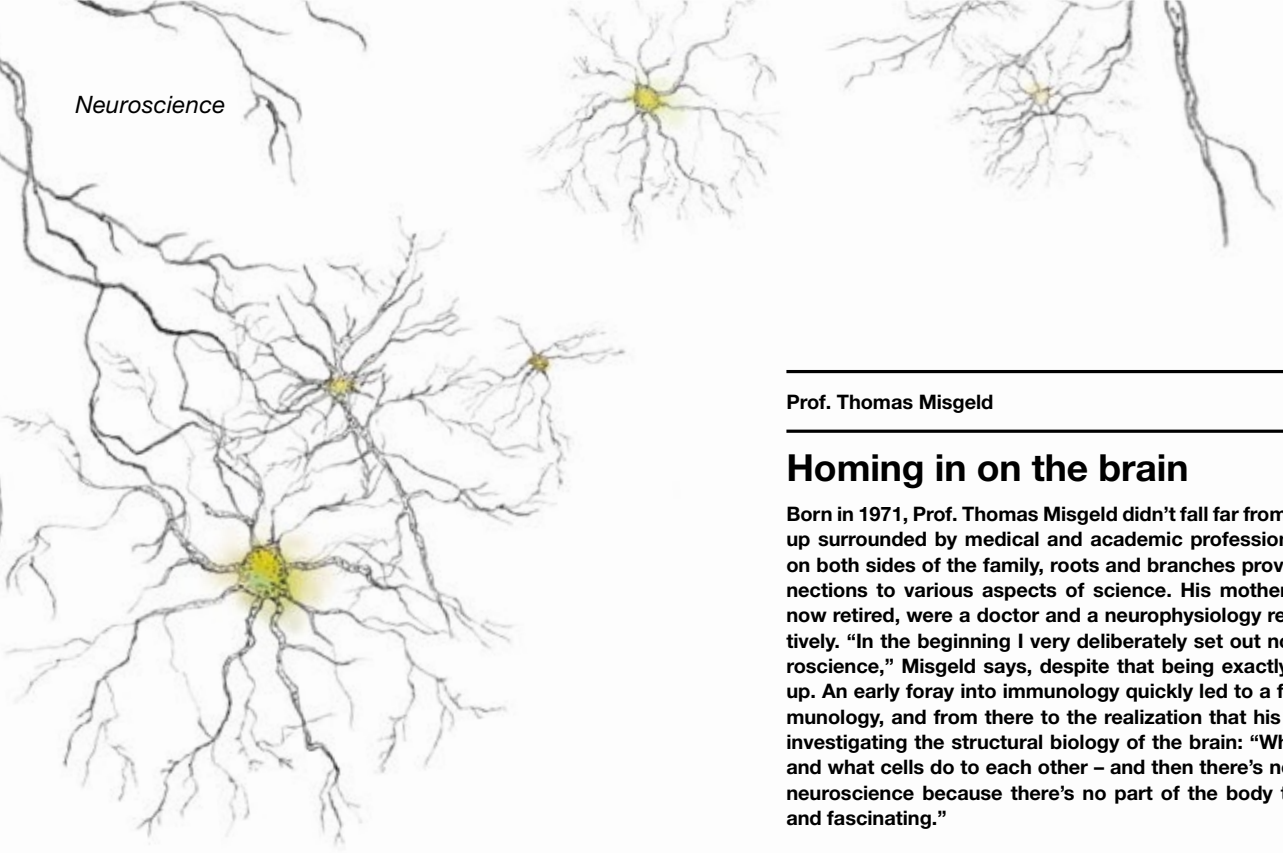


When your brain was very young, a period of exuberant growth produced a rich abundance of nerve fibers called axons – extensions of nerve cells that transmit impulses from one neuron to another – far more of them than would ultimately be put to use. Neuroscientists often liken this growth to a forest, with the trees being neurons and the canopy overhead consisting of axons and their synapse-bearing branches. Some of your young neurons, like trees growing in unsuitable places, were eliminated by apoptosis, a virtually universal mechanism for cell death. In addition, many of the remaining axon branches, up to 90 percent, were cleared away during the normal course of development through a "pruning" process that is only partly understood.

Largely in response to your specific environment and experience, your sensory and motor systems were wired up for life by taking down many of the possible connections. With old age, however, comes the danger that the same process will be the brain's undoing: If you grow very old, your lifetime risk of developing a disease in which axon loss plays a role – such as multiple sclerosis, amyotrophic lateral sclerosis, or Alzheimer's disease – could be very high.

Thus the mechanism is like a double-edged sword – or a double-edged axe, in keeping with the forest analogy – that both shapes and destroys. Axon loss can be observed sculpting neuronal networks during development and then, in a number of different diseases, contributing to the disruption of cognitive and neuromuscular functions. Gaining a more detailed understanding would be a big step in neuroscience and might also open new avenues for medical interventions against the looming epidemic of neurodegenerative disease. ▷

Graphics: ediundsepp



Ripe for discovery

There's no shortage of questions: How exactly does activity translate to morphology, leaving the imprint of experience on the circuitry of the nervous system? What kinds of signals set axon loss in motion? What mechanism allows a single branch to be selectively dismantled while leaving surrounding branches unharmed or even strengthened? What rules determine which branches will flourish and which will die off? If, as it appears, there is some kind of competition at play, what are the axons competing for? If competition alone can't explain the observations, what else is going on? Does the initial reversibility sometimes observed point toward a therapeutic window for disease-related axon loss? Will it be possible to identify pathways in axon loss that could be targeted for treatment?

"I don't understand it, and no one does" is a recurring refrain in conversations with Prof. Thomas Misgeld, director of the Institute of Cell Biology at TUM. He says this with excitement, never a hint of frustration. Every unknown could be an opportunity, and some of the most intriguing gaps in the neurobiological understanding of development and disease appear ripe for investigation using his techniques.

Misgeld's group and associated researchers have developed imaging techniques that make changes in the structure and function of axons observable *in vivo*. Two main areas of innovation come together here: novel approaches to microscopy and genetically encoded biosensors. Key examples would be two-photon microscopy and genetically encoded calcium indicators. Together, these lines of research have produced a versatile toolkit for making selected parts of individual ▷

Prof. Thomas Misgeld

Homing in on the brain

Born in 1971, Prof. Thomas Misgeld didn't fall far from the tree. He grew up surrounded by medical and academic professionals, with doctors on both sides of the family, roots and branches providing natural connections to various aspects of science. His mother and father, both now retired, were a doctor and a neurophysiology researcher, respectively. "In the beginning I very deliberately set out not to work in neuroscience," Misgeld says, despite that being exactly where he ended up. An early foray into immunology quickly led to a focus on neuroimmunology, and from there to the realization that his true passion was investigating the structural biology of the brain: "What cells look like, and what cells do to each other – and then there's nothing better than neuroscience because there's no part of the body that's as complex and fascinating."

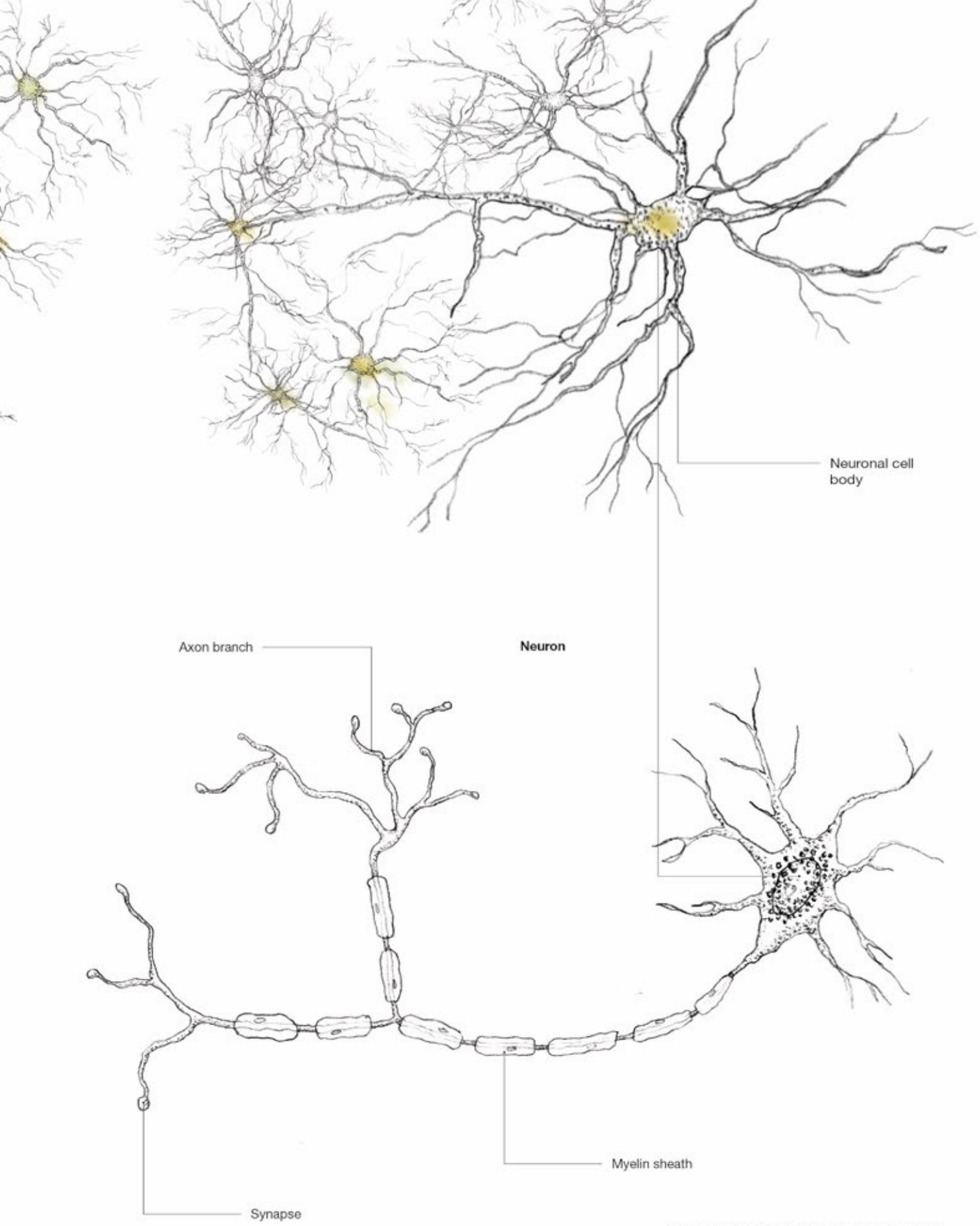
Following medical training in Munich, at TUM and the Max Planck Institute for Neurobiology, Thomas Misgeld did postdoctoral research in the United States. At Washington University in St. Louis and at Harvard, he acquired expertise in cutting-edge microscopy techniques for *in vivo* imaging that continue to shed new light on the life of individual nerve cells. Returning to TUM in 2006 as a Kovalevskaja group leader within the Institute of Neuroscience, he became a fellow of the TUM Institute for Advanced Study and one of the university's first tenure track professors, as well as a principal investigator in the Excellence Cluster CIPS-M (Center for Integrated Protein Science Munich). Now a full professor, he is director of the TUM Institute of Cell Biology and an associate member of the German Center for Neurodegenerative Diseases (DZNE). In 2012, Misgeld and a number of collaborators at TUM, DZNE and LMU established the Munich Cluster for Systems Neurology (SyNergy), an Excellence Cluster dedicated to investigating the mechanistic basis of neurological diseases. Misgeld and Prof. Christian Haass, a renowned Alzheimer's researcher at DZNE and LMU, are co-coordinators of SyNergy.

While in America, Misgeld made a new family connection that would prove vital for his research as well. It was in St. Louis that he met his wife, Dr. Leanne Godinho. An accomplished scientist in her own right, Godinho is the anchor for much of the lab's work on developmental neurobiology, and particularly for research employing the zebrafish model. "Developing a dual career path in Germany is hard, as it is everywhere," Misgeld says, "but the environment here really allows us to do the kind of research we want, and to build up capabilities that will become increasingly important in the future."

Asked why he applied for an ERC Consolidator Grant, Misgeld first cites the obvious advantages: It brings in a substantial amount of funding (roughly two million euros in this case), and it's good for the prestige of the university. Beyond that, he stresses that the ERC offers one of the few "bottom-up" funding schemes in Europe. "That means you can do what you want rather than what some advisory committee – whether wisely or not so wisely – has recommended be investigated now." And the Consolidator Grant in particular fills an important gap, he says: Young scientists in Europe, especially in Germany, have a number of different ways to raise money for the first critical years of independent research, but very few third-party funding options that would enable them to continue at the same level. For Misgeld, these funds allow him to expand the more basic, biological side of his lab's research – while the more disease-oriented work continues – and to upgrade the lab's already formidable experimental infrastructure.

As an adult, our brains have substantially fewer axon branches and synapses than in our youth. Now, our neurons form a network of reliable and specific connections.

Picture credit: edlundsepp



Schematic representation of a neuron and its processes. The axon shown is not to scale; in reality it would be much longer.

In the healthy brain, the number of axons and synapses remains fairly stable, even in the adulthood. However, there is a risk that the process of axon degeneration may resume and contribute to diseases such as Alzheimer's disease or multiple sclerosis.

neurons – in animal models such as mice and zebrafish – reveal their location, status and function. Misgeld and colleagues pioneered *in vivo* single-axon microscopy and have, over the past few years, applied their methods to questions regarding axon degeneration.

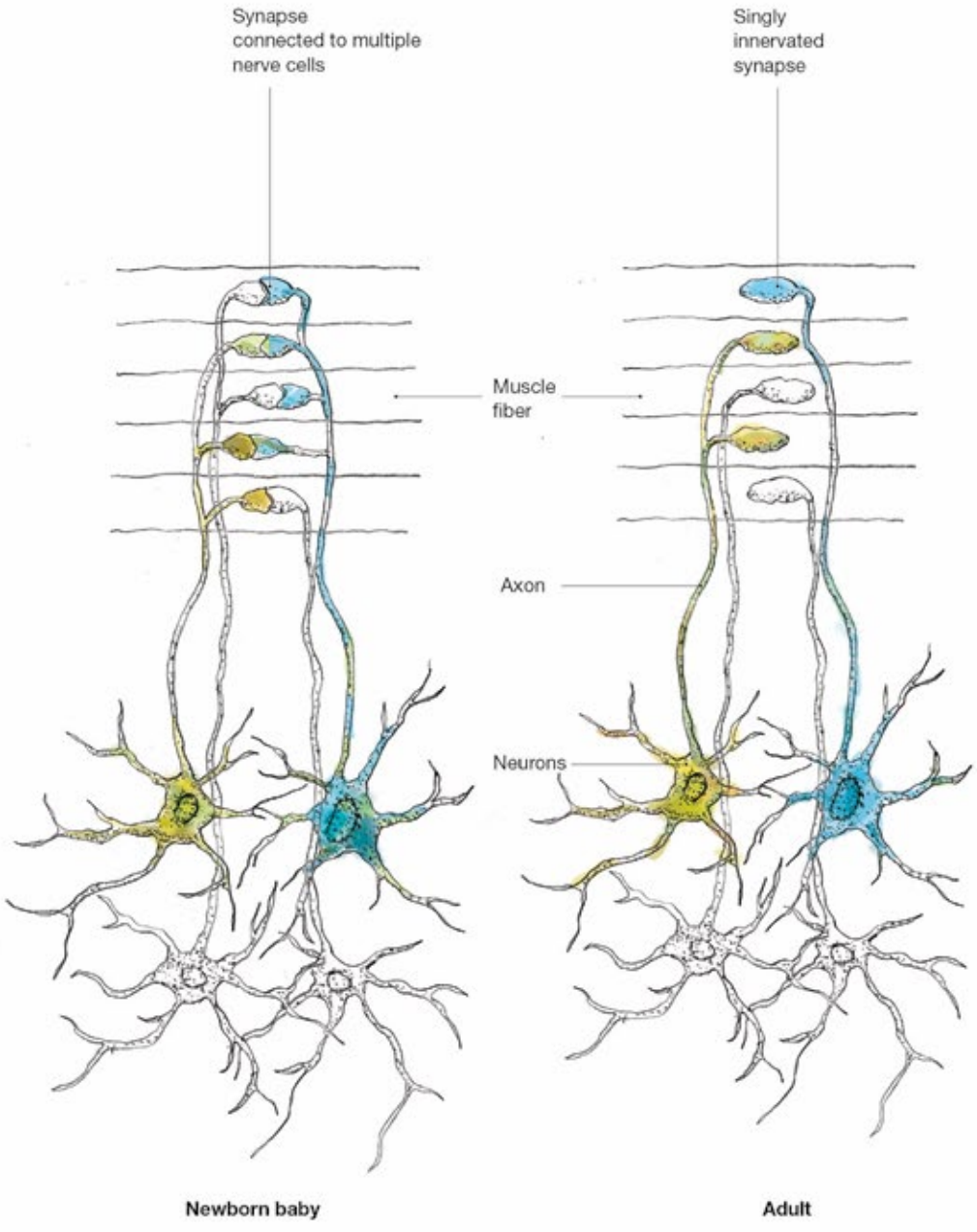
Models closer to nature

This effort is now being expanded and sharpened with support from the European Research Council, which awarded Misgeld an ERC Consolidator Grant to study "Mechanisms of Developmental and Injury-related Axon Branch Loss", a project nicknamed DIABLo. The mission is the further exploration of axon loss in the mammalian nervous system, developing and diseased, at the level of cellular and molecular biology.

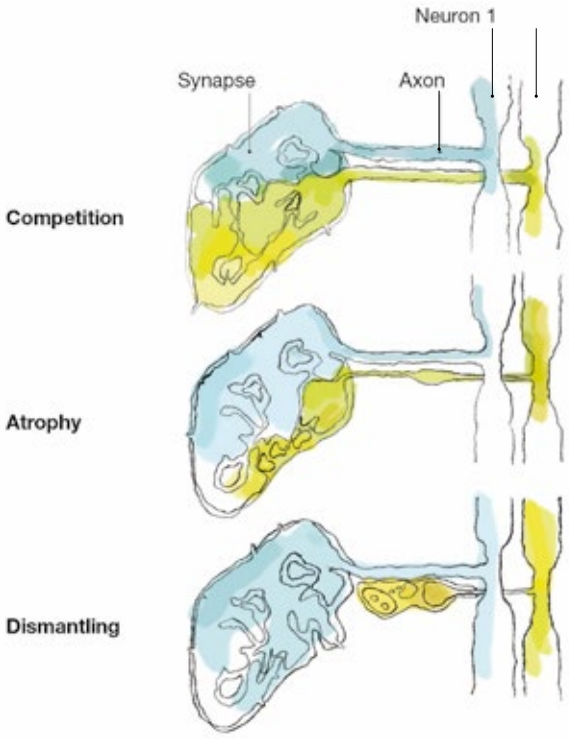
One distinctive feature of the project is a shift away from the standard way to trigger axon loss for study in the lab – an injury that completely cuts the nerves. "Except for a few specific cases, the approach of cutting something and then looking at what happens to the disconnected part doesn't seem so relevant to most neurological diseases," Misgeld says, "where that's not the initial mechanism. In most of the more common neurological diseases, something happens in the beginning, something that we don't even understand, to cause the axons to go away."

Not by chance, his group and collaborators already have experience with diverse animal models that share this common trait: Axons disappear without initially being cut. And they find more similarity between the responses to such ▷

Picture credits: ediundsepp



Innervation – meaning the "wiring up" of synapses – changes over a person's lifetime. For example, around the time of birth, each muscle fiber receives several weak inputs, which later remodel into a single, but strong input.



Axon branch loss occurs in distinct stages. In the normal "pruning" process, scientists assume that axons are competing. In disease as well as development, axons to be eliminated undergo thinning (atrophy) followed by localized swellings (dystrophy). In the final, dismantling stage, the axon is completely disassembled and cleared away.

"non-transecting" axon injuries in disease and development models than between either and the kind of axon loss triggered by a cut. One example is a process they have observed at developing neuromuscular synapses, which Misgeld suspects may be found to act elsewhere. Branches singled out to be disassembled go through a series of changes – overall thinning (atrophy), localized swellings (dystrophy), and local fragmentation at the tip repeated until completed – resembling the way an icicle melts. These individual steps also occur in diseases involving axon loss. Another characteristic that appears to be the same in development and disease is the role of glial cells, which surround and sheath neurons. Glial cells definitely do clean-up work by scavenging fragments of axon branches and might even – it remains to be seen – actively drive axon loss.

Four paths forward

Therefore, in DIABLo, Misgeld will concentrate on non-transecting models of axon loss, despite the increased difficulty of defining when exactly the process of axon dismantling begins. There are four main components of the project. The first, using developing motor axons as a model, will explore intrinsic mechanisms of axon loss. One set of experiments will focus on subcellular events that precede atrophy, particularly changes in axonal transport thought to destabilize selected, resource-starved branches while strengthening others.

The second component of the project, again using a motor axon model, will closely examine the role of glial cells in the death, breakup, and "self-digestion" of individual axon branches. During the initial atrophy phase, glial cells – which normally provide protective sheathing – "turn from friends to foes," Misgeld says. They first loosen their tight association with axons and synapses before engulfing fragments and finally disappearing – perhaps being digested by other members of the body's clean-up crew. The details of this sequence are unknown, and should be revealing.

The third and fourth experimental programs turn to the central nervous system in development and disease. The basic question is whether or not the mechanisms that operate in the brain and spinal cord are the same as those observed in the motor axon models. Misgeld suspects that they are. If so, the proof could expedite future research, since experiments with motor neuron models are technically less challenging than imaging individual cells in the central nervous system of living animals. In the development model, all of the group's prior experience will be brought to bear as they attempt to label corticospinal axons destined to undergo pruning and then to image the entire process *in vivo*. The fourth component of DIABLo will use a minimum-impact contusion technique to cause a non-transecting spinal injury and trigger axon loss, thus modeling disease in the central nervous system. This final element forms the most direct link between the ERC-funded project and other ongoing work in Misgeld's institute, including experiments involving models of multiple sclerosis, motor neuron disease and neuromyelitis optica. Such research is not aimed at developing cures, but it might yield knowledge that could lead in that direction.

"My group is not translational," Misgeld explains. "What we are doing is a step removed even from what most people call preclinical translation. My lab doesn't do quasiclinical studies, for example, where you look to see whether the mice in cohort A or cohort B are doing better. But we are always on the lookout for reasonable targets that other labs might want to study in that way."

Patrick Regan

"There's no part of the body that's more fascinating than the nervous system." *Thomas Misgeld*

Picture credits: ediundsepp (Source TUM)