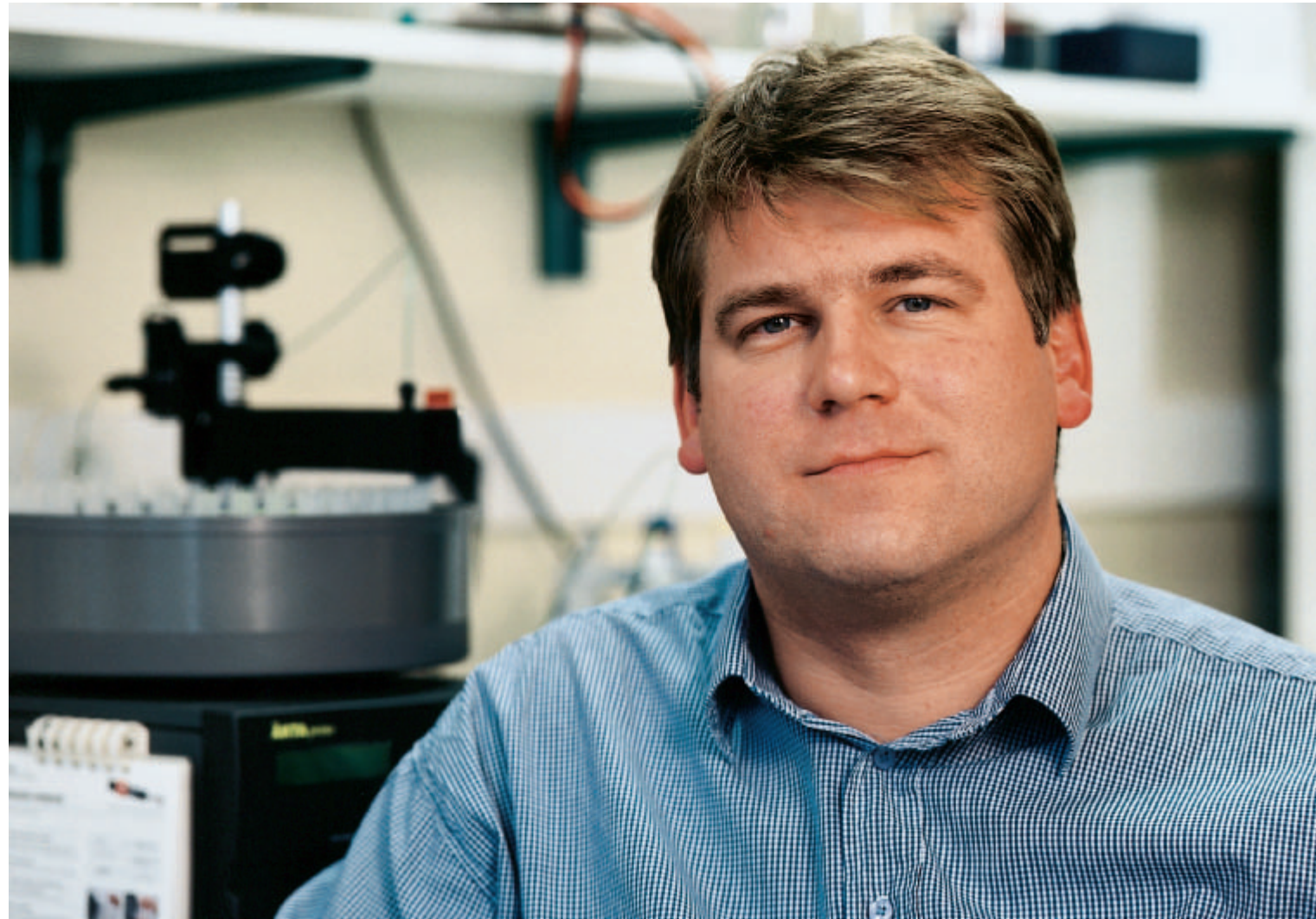


# Frank Pfrieder



*The Max Planck Society has set up an **INDEPENDENT JUNIOR RESEARCH** group named **"DEVELOPMENT OF SYNAPTIC CONNECTIONS"** at the **NEUROCHEMISTRY CENTRE** of the **CNRS** (French National Centre for Scientific Research) in Strasbourg. The main aim of this research group is to investigate the formation of contacts between nerve cells. The leader of this group, **DR. FRANK PFRIEGER**, and his colleagues recently found themselves on "somewhat sticky ground": Pfrieder and his research team have discovered that cholesterol – hitherto associated with a reputation as a "bad" blood fat – makes an important contribution to the formation of stable contacts between nerve cells, and thus to the brain's function.*

**N**ow is not exactly the ideal time for Frank Pfrieder to be looking forward to a break from work. However, it's the end of the year and Pfrieder's four-strong team at the Neurochemistry Centre in Strasbourg is taking a well-earned holiday. Meanwhile, the 36-year-old biologist is itching to get on and make progress with his experiments. The reason for this is quite understandable. As leader of a junior research group at the Max Planck Institute, a scientist may just be entering the most crucial phase of his/her career. To Pfrieder's own surprise and to the surprise of many of his colleagues, his team has identified the very blood fat with the "bad" reputation – namely cholesterol – as the crucial ingredient without which the nerve cells cannot form stable connections with one another. No one had reckoned with that: without cholesterol, the brain would not function.

"Our discovery has pushed open a door. There is now a string of new questions, and I am keen to set about finding answers to them", says Pfrieder, adding: "This is the best thing that can happen to a scientist: hitting on a discovery which leads to completely new hypotheses and shakes up current informed opinion."

This, of course, could not have been predicted back in 1994, when the young biologist who had studied in Constance and Munich took a three-year postdoctoral post with Ben Barres at Stanford University in California. Pfrieder wanted to carry out research there into the way in which nerve cells establish contact with one another. These connection points are called synapses, which are "buds" with a characteristic shape and which always release chemical messenger substances when a signal is to be transmitted from one nerve cell to the

next. Some nerve cells form over 10,000 such contacts with their neighbours – and their characteristics determine whether and how a stimulus spreads out in the brain, and which responses it triggers. The human brain contains 10 thousand million nerve cells. But it is only with the help of synapses that this huge collection of cells is made into an organ capable of thinking and feeling; synapses thus represent the key to memory and intelligence. It is therefore all the more astounding that scientists have known the significance of the synapses for almost a hundred years, yet they have so far barely come to understand the biological rules by which the nerve cells create and dismantle the connections.

The explanation for this gap in knowledge lies in the complexity of the brain. "It is virtually impossible to study the formation of synapses in the living organ because there are too many influences all taking effect simultaneously", says Pfrieder. His idea, therefore, was to observe a small number of living nerve cells outside the brain "in a test-tube", where conditions can be regulated and altered at will.

When Pfrieder started working in Barres' laboratory in 1994, Barres' team had already laid important foundations for the project. At the end of the eighties this research team had developed a method of obtaining nerve cells extremely cleanly from the retinas of young rats – uncontaminated by any other types of cell. The team was exploiting the fact that certain nerve cells display a characteristic protein on their surface. Barres used antibodies in order to detect this protein and to fish out exactly the cells he required: from 200 cells that have been cleaned in this way there will be no more than one which is not a nerve cell. But

this was only the first step: "At first, the isolated nerve cells were dying within a few days of being introduced to the culture", says Pfrieder – and that wasn't long enough to allow the researchers to study the formation of synapses.

The second important preliminary task for the Californian team was to keep the retinal cells alive for longer: it was another seven years before Barres' team had identified the substances which needed to be added to the culture to enable these cells to survive. By 1995, the American team had discovered the culture conditions in which the nerve cells could be kept alive for three weeks.

#### WHAT HELPS NERVE CELLS TO FORM NETWORKS?

Using this system, Pfrieder could now try to find answers to his questions such as whether nerve cells form synapses amongst themselves even in the absence of other types of cells. The purified nerve cells formed synapses in the culture dish, but these were neither especially numerous nor especially good contact points. "At this point in time we already suspected that nerve cells could be dependent on support from other cells", says Frank Pfrieder. The scientists guessed that these were cells known as neuroglia (or glial cells). There are three different types of these cells in the brain: oligodendrocytes form insulation for the nerve fibres that stretch right through the brain; astrocytes make sure that the environmental and metabolic conditions are stable, and microglia operate as specialised defence cells. These three types of cells are found throughout the brain as close companions of the nerve cells (see box on page 83). The hypothesis that nerve cells could be dependent on support from glial cells rests first-

ly on the spatial relationship of the cells: astrocytes are closely linked with the majority of synapses. Another factor is a conspicuous time correlation during brain development: although at birth the mammalian brain already contains most of the cells that it will require in the course of its life, those cells are linked only by a relatively small number of synapses. Most of these contacts develop only after birth. Glial cells are also still extremely rare at birth – is this coincidence or is there some kind of underlying principle? The glial cells multiply in parallel with the formation of the synapses.

Barres methods now led to the test system for investigating influences on the formation of synapses: the researchers could introduce additional substances at their own discretion and observe their effect on the formation of synapses. Pfrieger's idea was now to add glial cells to the nerve cells from rats' retinas. The results were dramatic: the level of electrical activity generated by synapses increased by a factor of 70. Further experiments demonstrated that this effect was due not to direct contact between glia and nerve cells, but to some substance which the glial cells were secreting into the nutrient solution: simply "feeding" nerve cells with the nutrient solution in which glial cells had been growing for a few days was sufficient to significantly stimulate the formation of synapses.

Thus, by the summer of 1997 the main players involved in the process had been identified. Frank Pfrieger then moved back to Germany, to the Max-Delbrück-Center for Molecular Medicine in Berlin, where he set up his own junior research group. "I had agreed with Barres that I would carry on working in the same field", says Pfrieger. The two colleagues found themselves in friendly competition with one another to find an answer to the all-important question of what the glial cells provide the nerve cells with. Identification of this factor could be expected to provide impor-



His idea that the infamous cholesterol molecule could be a kind of "brain lard" did not initially earn Frank Pfrieger a great deal of acclaim – even within his own team: "At the time we were pretty sceptical", admits his colleague Daniela Mauch (right).

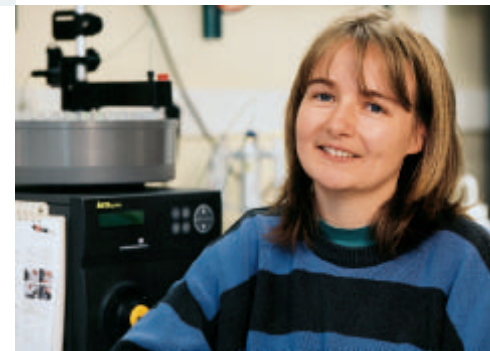
tant insights into the mechanisms of synapse formation. "We started off with a conventional approach", explains Pfrieger. This meant doing a large amount of spadework: the team performed long test series using biochemical methods to sort the substances affecting cell survival according to specific properties such as "size". It eventually became clear that the "added ingredient" in the glial cells appeared to be an unusually large molecule. No further progress was made until the protein components of the nerve cells were analysed: apolipoprotein E (ApoE) appeared to be the crucial substance.

#### "BULL'S EYE" – FATTY GLOBULES

It had been known for many years that astrocytes are the only cells in the brain which synthesise this protein. The cells produce droplets containing fat and cholesterol which they secrete into the brain's fluid. To each of these fatty droplets they then attach a number of ApoE molecules, which help other cells – including nerve cells – to detect and absorb the droplets. Further experiments showed, however, that ApoE in its pure form did not improve synapse formation: so was it one of the substances in the fatty droplets that

contained ApoE? "I can still remember the moment during a meeting when I suggested that cholesterol could be the substance we were looking for", says Pfrieger. At first, the other team members were not particularly impressed with this brainwave. "We were pretty sceptical at the time", says Daniela Mauch, a member of Pfrieger's team. Mauch was given responsibility for the task of testing the idea. The hunch turned out to be a "bull's eye". The addition of cholesterol improved the formation of synapses just as well as the presence of glial cells. And when the researchers used drugs to suppress the production of cholesterol in glial cells, then the cells lost their ability to stimulate synapse formation. "At first, the rediscovery of cholesterol was the worst-case scenario: I suppose there's nothing worse really than spending years looking for a molecule only to discover that it's been right under your nose all along", reflects Pfrieger. In the meantime, the sense of frustration had given way to a "feeling of mild euphoria", he says, as the discovery threw new light on cholesterol metabolism in the brain, a subject that has received little attention in the past. Until then researchers had assumed that nerve cells produce suffi-

cient cholesterol themselves: "It hadn't occurred to anyone that nerve cells might rely on cholesterol being imported", says Pfrieger. But now his results were pointing towards that very conclusion. Neurons themselves appear to produce just enough cholesterol in order to survive and to manage to produce axons (cell extensions) and a small number of synapses. Large-scale increase in the number of contact points, however, seems to require such large quantities of cholesterol that if the nerve cells had to produce the substance themselves, then they would no longer have sufficient energy for



their actual task. Furthermore, nerve cells cannot gain access to the cholesterol circulating in the bloodstream: they are screened off from this reservoir by what is known as the blood-brain barrier, a layer of cells which surrounds all blood vessels, and which protects the brain from toxins amongst other things. "This might finally explain why glial cells produce ApoE at all, and why they release particles containing cholesterol", muses Pfrieger. This scenario throws up a string of new questions: until now Pfrieger's team has been conducting experiments only on cells outside the brain, in other words "in test tubes". But what goes on in the brain? Do the glial cells and the nerve cells cooperate in the same way there? The questions continue: do all nerve cells depend on an external supply of cholesterol, or do some cells have their own sources? There are certain clues suggesting that cholesterol is, as a rule, imported: "In most areas of the

brain, the majority of the synapses are not formed until after the macroglia", says Pfrieger. Last but not least comes the interesting question from the cell-biological point of view: how does cholesterol stimulate synapse formation?

This discovery could also be important in medical terms – with Alzheimer's disease clearly at the top of the list. Several years ago, research scientists in the USA discovered that hereditary differences in the structure of the ApoE molecule increase the risk of suffering from this form of senile dementia. Pfrieger's discovery supports the supposition that changes in the transportation or absorption of cholesterol are responsible for the characteristic loss of synapses associated with Alzheimer's disease. Pfrieger will now be collaborating with other colleagues in his quest for the answers to these questions. The search for the factor with which glial cells feed nerve cells occupied his small team so fully during the time he was working in Berlin, that the scientist only managed to publish very few papers. Needless to say, Pfrieger is hereby out of step with the prevailing system for evaluating the achievements of research scientists, which is based on the motto "Publish or Perish".

#### "A COMPLETELY DIFFERENT TYPE OF ADVENTURE"

There was little chance that Pfrieger's post at the MDC in Berlin would be extended, so by the end of 1999 he accepted an opportunity to set up a junior research group under the auspices of an exchange programme between the Max Planck Society and the Centre National de la Recherche Scientifique (CNRS), the largest national research organisation in France. This took him to Strasbourg: "I now find myself in the midst of a completely different type of adventure. Science is organised in a totally different way in France, and first we need to find our feet here." The scientist is clearly surprised by the degree of difference

#### IT TAKES MORE THAN JUST NERVE CELLS TO FORM A BRAIN

It is no longer possible to understand how the brain works without considering the role of the glial cells: rapidly developing methods in the fields of cellular and molecular biology in the early nineteen-eighties focused the spotlight firmly on this "silent majority" of cells in the brain. Oligodendrocytes, astrocytes, and microglia represent around two-thirds of the cells in the central nervous system. What's more, as has become clear over recent years, the three types of glial cells by no means merely form simple supporting and nourishing tissue, but are equal partners to the ten thousand million or so nerve cells. The three types of glial cells perform different functions: microglia are the brain's defence cells; in a healthy, uninjured brain these relatively small cells lie between the neurons or along the nerve fibres. Without touching one another, they branch out to form a close-meshed network. The comparatively large oligodendrocytes and astrocytes are both classified as "macroglia". Oligodendrocytes act above all as insulators, for example "winding round" the long axons of the nerve cells, thus improving the transfer of signals from cell to cell. The third type of cell – the astrocyte – has different functions according to the phase of the brain's development: in a developing brain, the astrocytes not only form the "guide rails" for selective growth- and migratory movements of the nerve cells, but they also produce some of the growth factors required by the neurons. Once these growth processes have been completed, the astrocytes build a cohesive canal system through contacts with one another which is also closely linked with other cell types and the blood vessels. In conjunction with the nerve cells, astrocytes make a crucial contribution to the maintenance of ambient conditions in the brain. K.K.

between the scientific cultures of Germany and France.

One example is the way in which grants for doctorate research are awarded: in France the best students in each year are awarded a state grant, their ranking order being determined by grades from written and oral examinations. Another example is that the opportunities in France to apply for outside funding are very limited – at least in the case of neurobiologists – because there is no institution equivalent to the German Research Foundation. Pfrieger is nonetheless quite happy, and he certainly values the exchange programme: "It is good to broaden your horizons and to gain an insight into other research systems. Only by doing so can you gain a full understanding of what your own country has to offer." The young scientist can hardly wait for his team to come back from holiday, roll up their sleeves and get down to work ...

KLAUS KOCH