A conversation with Gero Miesenböck, Oxford, UK

"It's Like Solving a Sudoku Puzzle"

At the moment, optogenetics is on every neuroscientist's lips. Klaus Taschwer spoke to the man, who was first to come up with the idea of using light to specifically switch on neurons in the brain first.

Lab Times: You are considered the actual founder of 'optogenetics', a new approach in neurobiology, which was elected method of the year by 'Nature Methods' *at the end of 2010. What is so special about this?*

Miesenböck: Optogenetics is an experimental tool allowing us to understand certain mysteries of the brain. I like to compare the resulting possibilities with different ways of solving a Sudoku puzzle. If you only stare at the Sudoku, it is difficult to find the solution – but brain researchers did exactly that during the last 100 years: they tried to draw conclusions about the working mechanisms of the brain from detailed observations. Solving a Sudoku, however, is much easier, if you can play with the various little boxes and try how it might function. And exactly that became possible with the brain through optogenetics.

How can that be envisaged?

Miesenböck: Through various methods influencing neurons with light pulses it is, for the first time, possible to interfere precisely and selectively with the communication and signal transduction between neurons – and therefore to test, which interference induces what effect. This way one gets, in the end, a much better understanding of the connection of single neurons and of the role the circuits play in behaviour control.

You work with one of those method to gain insights into the brain of a fruitfly. Why of all organisms always Drosophila*?*

Miesenböck: Well, flies show a lot of practical advantages: they are small, cheap and breed quickly.

Is their brain not a bit small and too simply built to draw comparisons to higher animals or even humans?

Miesenböck: I would say the brain is complicated enough to allow flies to exhibit intelligent behaviour and simple enough to allow researchers to gain intelligent insights in its function. This topic was a long debate between Francis Crick, one of the discoverers of the DNA structure, and Seymour Benzer, who established the fruitfly as a neurobiological model organism. These two argued whether flies have consciousness. Crick always denied it, while Benzer said polemically, "Francis, the flies are able to do everything you can. And even more: Can you fly to the ceiling and stay there?" I believe there is, at least, a little bit of truth in this polemic.

"If we can understand how Drosophila *decides and how they learn from their mistakes, then there is every chance that this is similar in higher living organisms."*

In what sense?

Miesenböck: Nature very rarely develops two completely different solutions for the same problem. For me the philosophy therefore always was: look for the simplest system in which you can study a biological process. And for many questions that is the fly. If we can understand how *Drosophila* decides and how they learn from their mistakes, then there is every chance that this is similar in higher living organisms – including humans.

How did you discover that fruitflies learn from their mistakes?

Miesenböck: Using optogenetics, we discovered a group of neurons, which, if they are activated, convey to the flies that they made a mistake. I call these neurons casually "critic-cells" in the fly brain. They virtually say, "Don't do that again, try something different." With a light pulse we were able to switch on specifically these cells and thus reprogramme the behaviour of the fly – in other words, by an artificial intervention from the outside, we wrote a completely new content into the memory of the fly.

You think that these mechanisms are similar in higher animals?

Miesenböck: All findings that we have, so far, indicate exactly that. In addition, there are astonishing details. We could, for example, show that flies require more time for difficult decisions and that they are able to trade off the evidence for or against a certain action. I.e. this small brain really has a short term memory for information as well as other brain areas, in which information is accumulated and compared. All these are fundamental processes.

Do you also investigate these in higher animals?

Gero Miesenböck

(45), is neurobiologist and professor for Physiology at the University of Oxford. Born in Upper Austria, he received his medical degree from the University of Innsbruck in 1993, worked then at the Memorial Sloan-Kettering Cancer Center in New York and at Yale University, before moving on to Oxford in 2007.

Miesenböck: We published a study recently, where we also looked into the brain of mice. The main question there was why the different areas in the cerebral cortex processing visual, acoustic, motoric or completely different stimuli are nevertheless surprisingly similar. We excited different brain cells with light and found that there really are canonical calculations or something like a canonical circuit within the cerebral cortex.

How detailed can these circuits be described already?

Miesenböck: The wiring pattern, as we know it today, is still rather coarse. The cerebral cortex has six layers and we know that the signals arrive in the fourth layer and move on to the second or third layer – and from there to the fifth. But what the single connections look like in detail has still to be discovered.

Do you also compare notes about that with cognitive psychologists?

Miesenböck: Within my newly founded research centre in Oxford we are trying exactly that. I believe that the collaboration between cognitive neurobiology and mechanistic neurobiology will be very fruitful because it helps to reduce the mutual frustration. Many cognitive psychologists – at least the better ones – are frustrated because they don't understand what really happens in the brain. It will always remain a black box to a certain extent. We mechanistic neurobiologists, however, are frustrated because we are missing the big theoretical hit. And that does matter to us.

Is our brain maybe too complex for such theories?

Miesenböck: I don't think so. I can't imagine that our brain is constructed of an inscrutable collection of very different wiring patterns. I much rather believe that there are 50, 100, maybe 200 single building blocks constructed similarly in different brains and conducting arithmetic operations in a similar way, e.g. an oscillator measuring time, circuits comparing information or integrating it over certain time intervals or addressable information memory. If we were able to find and understand these building blocks then that would be a big advance.

Do your studies have practical implications?

Miesenböck: For rational medicine, building on an understanding of biological mechanisms, this kind of basic research is certainly essential.

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Optogenetic methods are already being tested in animal experiments to cure illnesses like epilepsy. How much further is it until the application in humans?

Miesenböck: I think there won't be an application in the next few years. The biggest hurdle thereby is not the influence on the brain. A glass of wine in the evening is also a kind of influence on the consciousness. The problem is to get genetic material into humans – but this would be a prerequisite to apply optogenetics in this way. That is a form of gene therapy giving rise to manifold problems.

Also in optogenetics, it's the fruit fly's head that is on the block

Can they be solved?

Miesenböck: At the moment there are too many reasons speaking against it. The technology to replace a defect gene exactly through a healthy one is, up to now, not far enough developed for it to function safely and without risks. Even if we only insert genes into the genome of fruit flies, it sometimes happens that an essential gene gets destroyed.

What is the reason for this?

Miesenböck: You have to imagine somebody firing a shotgun at the genome. Most "hits" won't have dramatic consequences but the side effects, to a small percentage, are detrimental. However, there is every indication that the replacement of a defect gene would, in principle, be the ideal therapy.

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Only in the past months, a number of leading British neuroscientists relocated to North America. What are your working conditions like in Oxford?

Miesenböck: My situation as researcher in Great Britain is very good. I managed to get a large amount of funding from the Wellcome Trust and I get ten million pounds for my new institute from the Trust of Lord Sainsbury, a former science minister and owner of a chain of supermarkets, whose main interest is in basic neurobiological research.

What are you planning to do with all that money?

Miesenböck: We are about to found a research institute, called the 'Centre for Neural Circuits and Behaviour', which will be directed by me. The University of Oxford also supports the institute generously with infrastructure: we got a building, which is just about to be renovated and in which we would then like to conduct a small social experiment.

Namely?

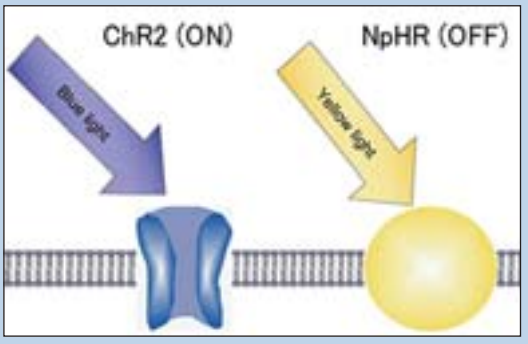
Miesenböck: I would like to arrange it in a way that the groups from different areas collaborate in a very small area. I believe that a high density of personnel is important for science to function well. You really have to peck at each other and then it works best. In addition, there won't be any borders between the laboratories any more. Everything within this institute shall be transparent and open.

Before Oxford you used to work at the US-elite Yale University. Where do you see the largest differences between the Anglo-American and the continental European research system?

Miesenböck: The biggest difference for me is that the research groups at Anglo-American universities are not endowed with internal money from the university. I just got paid my own salary. And that is still the same today. I haven't got 15 university positions for postdocs, which I can assign but I have to look for third-party funds for each single position. I think this is a good system because it keeps you awake. Our institute is organised in a way that established researchers support young researchers with their third-party funds during their first steps to independence. And when the young scientists have established themselves successfully the

Optogenetics: The Lowdown

It all began in 2002 at the Memorial Sloan-Kettering Cancer Center in New York, when Gero Miesenböck and his colleagues came up with a method for "stimulating groups of genetically designated neurons optically"– making it possible to manipulate transduced neurons by giving them a visible light stimulus and, on top of it all, in a temporally precise manner (viz. millisecond-scale). At the centre of their multi-component approach stood the co-expression of several Drosophila photoreceptor genes (Neuron; 33(1):15-22). But there has to be a simpler solution, thought Karl Deisseroth from Stanford University and, in 2005, developed a one-component system that brought genetic and optical elements even closer together. Now, more than 800 labs around the world use optogenetic techniques to answer their (neuro) biological questions.



Stimulated by light: Channelrhodopsin-2 (ChR2) and Halorhodopsin (HR)

At the very heart of all the approaches are opsins, which, together with retinal, form the well-known "visual purple" rhodopsin. For optogenetic studies, opsins from microbial sources (bacteria, algae fungi) are most commonly employed. They belong to two types: light-gated ion pumps and light-gated ion channels. One famous example of the ion pump-type opsins is Halorhodopsin, isolated from the Archaeon *Natronomonas pharaonis* (NpHR). This pump works by transporting negatively-charged chloride ions from the outside to the inside of the cell – generating a hyperpolarising membrane potential. Channelrhodopsin-2 (ChR2) is an example of an ion channel-type opsin. It was isolated from the unicellular alga *Chlamydomonas reinhardtii* and conducts protons as well as the cations Na^+ and K^+, creating a depolarisation along the way. According to *www.openoptogenetics.org* "within the past 5 years more than 12 channelrhodopsin variants and 4 light-driven hyperpolarizing pumps have been either cloned or engineered". And work to enhance the performance (on-off kinetics) of both will certainly go on.

What comes after finding the right opsin for your experimental needs? A *Nature* News Feature (456:26-8) nicely sums the whole procedure up in six easy steps: 1. Piece together genetic construct [including cell-type specific promoters – e.g. the CaMKIIα promoter for excitatory glutamatergic neurons or the GFAP promotor for astrocytes – and your opsin gene] 2. Insert construct into virus [lentivirus or adeno-associated virus will do] 3. Inject virus into animal brain, opsin is expressed in targeted neurons 4. Insert fibre-optic cable plus electrode 5. Laser light of specific wavelength opens ion channels in neurons 6. Record electrophysiological and behavioural results. Aaaand you're done. You have now taken control of the activity of a specific cell population "with temporal precision". For more detailed information on how to "deliver microbial opsin genes to deep mammalian brain structures in vivo" Zhang *et al.* have published a step-by-step manual in *Nature Protocols*, 5:439-56. According to the authors, the procedure can be completed in four to five weeks.

How it works in practice is illustrated in the following experiment. When still working at Yale, Gero Miesenböck and Susana Lima manipulated neurons of the *Drosophila* giant fibre (GF) system (consisting of a pair of interneurons in the brain and their synaptic targets in the thorax, a motor neuron and another interneuron) to become light-sensitive. In an "extreme demonstration" the two authors decapitated the flies and lo and behold "the headless bodies stood characteristically motionless in the open arena until illuminated and then took flight on circuitous, collision-prone trajectories" (*Cell*, 121:141-52). Thus, it was possible to simulate a signal even though the actual signal giver, the GF neurons along with the rest of the brain, was completely missing.

There's another new way to remotely control the firing of specific neurons on the horizon, called magnetogenetics. In contrast to optogenetics, this approach commands engineered neurons – not by using light but with iron nanoparticles and magnetic fields (*Nature Nanotechnology*, 5:602-6). -KG-

wheel turns again. This way the system gets younger all the time. But it is not a turning door which puts successful young researchers automatically on the road after a certain time.

Are there also disadvantages?

Miesenböck: In the Anglo-American area there is a tendency for research support to be more and more orientated towards applications. Meanwhile one of the criteria for public funding in Great Britain is that the research serves the economic wealth of the country. The aims are then really patents and licences.

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But that is not the case with the Wellcome Trust?

Miesenböck: No, but they are just about to change their funding principles. While in the past also small applications were supported, they are now going to try to give less people more money. That certainly is good for the institutions, which are excellent. But a lot of universities and institutes are now naturally trembling enormously. In addition, the risk is growing that solid and important, but maybe not spectacular, research is now going to go by the board. This would undermine the whole of research.

That would be the science-political translation of the Matthew effect: "For to all those who have, more will be given."

Miesenböck: Exactly. Or in Upper Austrian: „Der Teufel scheißt zum größten Haufen." – "The devil shits on the biggest pile."

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