

Healthcare industry BW

Calcium and memory

Calcium in the nuclei of neurons controls the transcription of genes that play a role in the structural changes that are responsible for the formation of long-term memory. Neurobiologists at Heidelberg University have identified this cellular “switch” in *Drosophila melanogaster* and have used sensational experiments to analyse its function in the formation of the long-term memory of flies.

The human brain is perhaps the most complex structure in the known universe. The adult human brain is made up of around a trillion (10^{12}) neurons and the number of interconnections (synapses) is estimated to be a thousand times higher.



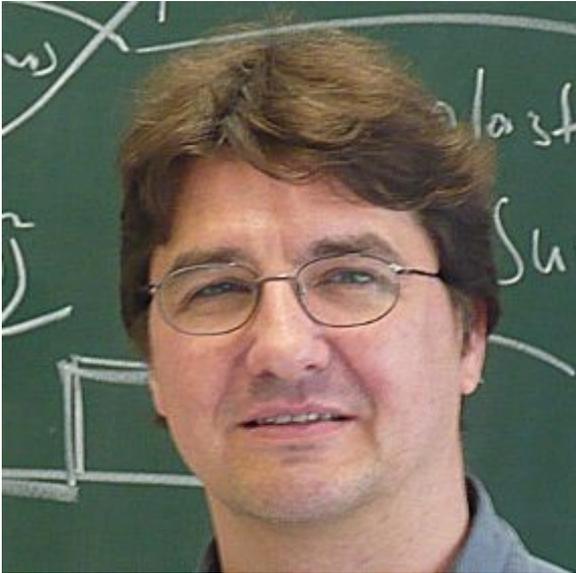
Prof. Dr. Christoph M. Schuster, Interdisciplinary Centre for Neurosciences
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Scientists need to study simpler model systems in order to understand the basic functions of such a complex organ. It may be difficult for some to grasp how studying the brain of the tiny fruit fly *Drosophila* can lead to insights into human learning and memory. Not only is the brain of the fruit fly a million times smaller than the human brain, the phylogenetic lineages of mammals and insects also took separate paths way back in the Precambrium around 600 million years ago when multicellular animals were just emerging. Despite this enormous evolutionary gap, the development of various forms of memory in humans and flies is remarkably similar. It can therefore be assumed that these vital processes have been conserved during evolution and use similar cellular mechanisms in humans, mice and flies.

“These commonalities suggest that the formation of long-term memory is an age-old phenomenon

that already existed in the common ancestor of insects and vertebrates. Both species probably use similar cellular mechanisms for forming long-term memory, including the nuclear calcium switch,” says Christoph Schuster, neurobiologist at Heidelberg University.

Short-term and long-term memory



Prof. Dr. Hilmar Bading, Interdisciplinary Centre for Neurosciences
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The researchers' finding that calcium in the nuclei (nuclear calcium) of certain *Drosophila* neurons triggers a genetic programme that controls long-term memory has made headlines. The study, which was published in the prestigious journal "Science Signaling", is the result of a cooperative project carried out by research teams led by Prof. Dr. Hilmar Bading and Prof. Schuster at the Interdisciplinary Centre for Neurosciences (IZN) at the University of Heidelberg.

The Heidelberg scientists were able to show with impressive experiments that nuclear calcium, and not cytoplasmic calcium, is responsible for the transition of unstable, short-term forms of memory into stable, long-lasting transcription-dependent forms. This process involves many neurons, including those known as Kenyon cells in the mushroom bodies, which serve as association and learning centres in the insect brain. The researchers' work revealed that when the nuclear calcium switch was blocked, the flies were unable to form long-term memory. Short-term memory was not affected.

What distinguishes short-term memory from long-term memory? Described in simplified form, short-term memory is based on alterations to existing neuronal connections caused by the post-translational modification of proteins that affects protein activity, frequency and distribution. In contrast, long-term memory depends on structural alterations to neuronal connections, the formation of stable synapses and new connections. This relies on the transcription of genes.

Bading has been working for many years on the big questions as to how electrically activated neurons can switch on genes and how these alterations are related to learning and memory. He is specifically interested in calcium ions that enter neurons upon activation at the synapses and are then guided onwards into the nucleus. Bading's team employs different model systems, including cell cultures, for studying the nuclear calcium-switch-dependent expression of genes. However, the molecular mechanisms related to learning and memory also need to be studied in living animals. But how can fruit fly memory be investigated?

Tiny winged Pavlov's dogs



Drosophila melanogaster as a tiny Pavlov's dog: antennae (olfactory organs) are shown on the right; two legs where the insects receive electric shocks to their feet are shown at the top.

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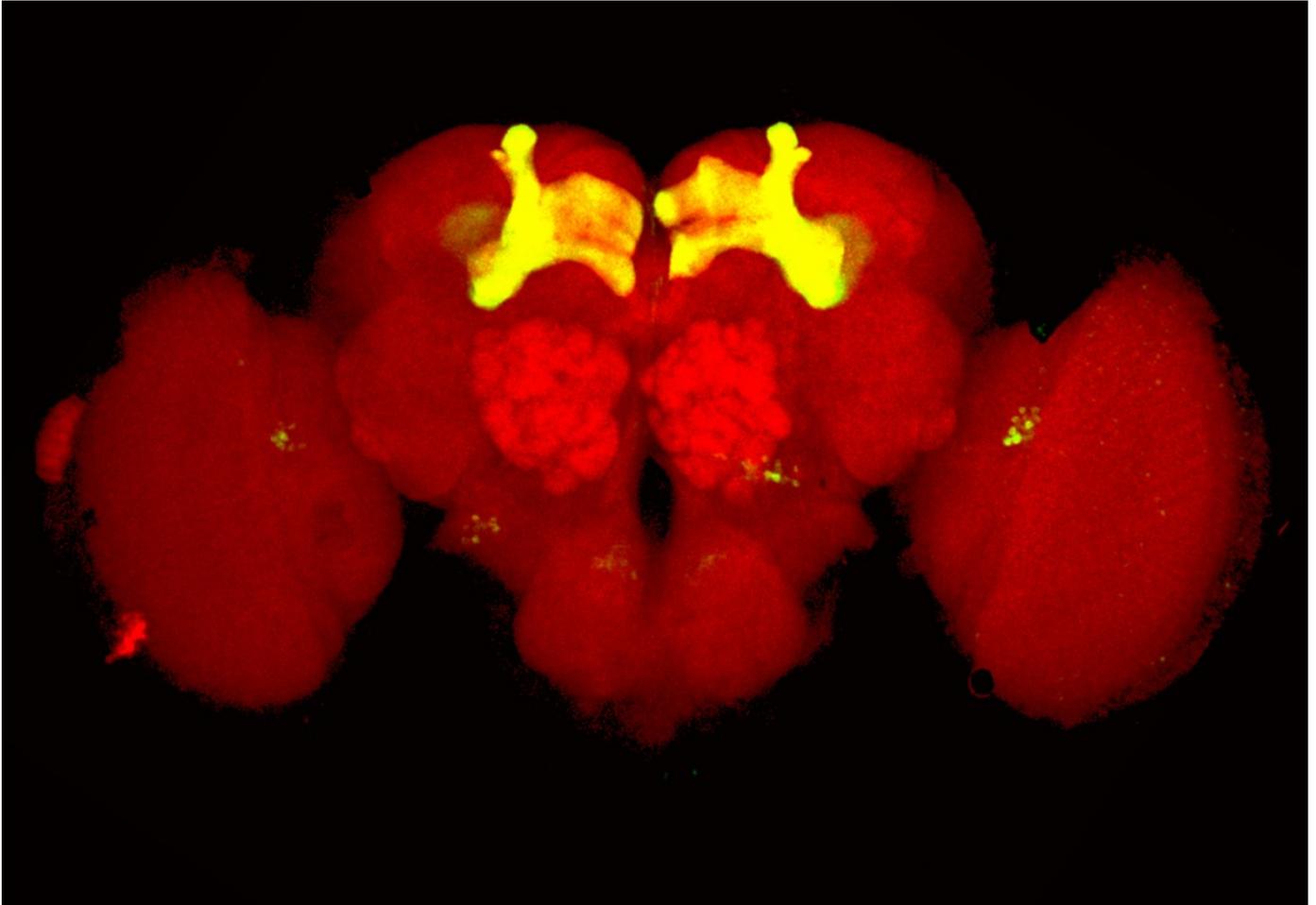
The neurobiologists from Heidelberg carried out conditioning experiments with fruit flies that were similar to the Russian physiologist Iwan Pavlov's experiments with dogs more than a hundred years ago. They temporarily exposed the flies to an odour which the flies perceive as nearly neutral. The odour of bananas (or a similar chemical odour) is detected in the antennae by olfactory receptor neurons. When the flies were exposed to banana odours, they received electric shocks to their feet. When they were exposed to orange odours, the flies received no electric shock to their feet.

The researchers tested the function of short-term memory after a single training session by giving the animals 60 seconds to choose between the odour of oranges or that of bananas without electric shocks. In order to test long-term function, the flies were given ten training sessions at intervals of 15 minutes ("spaced learning") during which they were exposed to odours (banana odour paired with electric shock or orange odour without electric shock). The flies' reactions were measured 24 hours after the sessions.

The researchers also manipulated neuronal calcium signalling. It is mainly known from experiments with mammals that calmodulin is the most important nuclear calcium sensor. This calcium-binding messenger protein is expressed in eukaryotic cells, and therefore also in flies. Nuclear calcium-dependent gene transcription in neuronal nuclei also involves calmodulin kinases and phosphatases as well as transcription factors like CREB.

Bading's and Schuster's team used transgenic flies in which it was possible to block components of the nuclear calcium signalling pathway. If the pathway was blocked during Pavlovian conditioning, the flies were unable to form long-term memory (e.g. flies avoiding banana odour); blockage was reversible and short-term memory was not affected. These results prove that the nuclear calcium switch is an evolutionarily conserved signalling pathway required by insects and vertebrates alike for the consolidation of transcription-dependent memory.

Looking into the fly's brain during learning



Drosophila brain with yellow mushroom bodies, which are the flies' association and learning centres.
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Using a smart experimental setup, the IZN researchers demonstrated the particular advantage of using *Drosophila* fruit flies for studying memory and learning. Using what is known as "in vivo imaging", they were able to observe the effect of calcium directly in the brain of living flies.

They used transgenic flies in which recombinant calmodulin was specifically fused with GFP (green fluorescent protein) so that it lit up under fluorescence light when calmodulin was bound to calcium. In vivo imaging was performed with a wide-field fluorescence microscope through a small opening in the flies' head cuticle (which did not measurably affect the insects); their heads were glued to a cover slip and their feet were in contact with a copper grid that delivered the electric shocks. The flies' antennae received a constant stream of air passed through a vial containing mineral oil and odour substances. The researchers then directly observed the points at which the calcium concentrations changed. The localisation of the calcium switch in the nucleus could also be established by immunohistochemical investigations using fly brain preparations and cell cultures with antibodies against GFP of a recombinant calcium indicator that is exclusively present in the nucleus.

Nuclear calcium also plays a role in learning and memory in organisms other than *Drosophila*. Bading explains that the memory of pain and specific protection and survival functions of neurons also involves the nuclear calcium switch. The decline in memory performance in the elderly might potentially be explained by the fact that this cellular switch is no longer fully functional. The studies carried out by Bading's and Schuster's research groups at the IZN on the long-term memory of fruit flies might open up new perspectives for the treatment of age- and disease-related brain function

alterations.

Publication:

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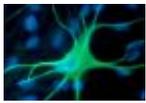
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