

Blind flies without recycling: How Drosophila recovers the neurotransmitter histamine

March 18 2013



Interaction of Tan, Black and Ebony: The enzymes Tan (blue), Ebony (magenta) and Black (green) in a brain section of Drosophila. The photoreceptor cells of



the retina produce the enzyme Tan (blue, top left). From the retina, the visual information finds its way to the so-called optical lobes of the brain, which consist, among other things, of lamina and medulla. Special glial cells of the lamina (light structure in the middle), and medulla (structure on the right) produce the two enzymes Ebony and Black. The researchers stained the enzymes with antibodies. © Anna Ziegler, RUB, Dissertation 2010

In the fruit fly *Drosophila*, the functions of the three enzymes Tan, Ebony and Black are closely intertwined - among other things they are involved in neurotransmitter recycling for the visual process. Ruhr-Universitaet-Bochum researchers from the Department of Biochemistry showed for the first time that flies cannot see without this recycling.

Their analysis of the <u>enzyme</u> Black also raises new questions as to its function. Anna Ziegler, Florian Brüsselbach and Bernhard Hovemann report in the *Journal of Comparative Neurology*, which chose this topic as cover story.

Tan, Ebony and Black are important for the visual process and the formation of the cuticle

The fruit fly's <u>genes</u> tan, ebony and black contain the construction plans for three enzymes with the same names that work together in hardening the <u>outer shell</u> of the body, the cuticle. The same enzymes also occur in the compound eye of the fly. Researchers therefore assume that Tan, Ebony and Black work together in vision - similar to the way they do in the formation of the cuticle. In fact, flies with <u>mutations</u> of the ebony and tan genes cannot see. A mutation of the black gene, however, has no such effect. Prof. Hovemann's team examined where the enzyme Black appears in the compound eye and the role it plays in vision.



Black and Ebony always occur together

First, the scientists tested where the genes ebony and black are active in the compound eye of the fruit fly and in its extra eyes on the head, the ocelli. They put different types of light-sensitive cells called photoreceptors, under the microscope. The result: both genes are always read together - just like in the cuticle. This suggests that the functions of the enzymes Ebony and Black are closely linked.

Vision requires a continuous flow of the neurotransmitter histamine

When light falls into the compound eye, the photoreceptors release the neurotransmitter histamine. In previous studies, Bochum's biochemists already demonstrated that histamine is recycled via the glial cells surrounding the photoreceptors. There, the enzyme Ebony inactivates the neurotransmitter histamine by binding it to the amino acid *B*-alanine, thus creating *B*-alanyl-histamine. This molecule is transported from the glial cells back into the photoreceptors. Here, *B*-alanine is split off again by the enzyme Tan, and histamine is produced. Previously, it was assumed that the enzyme Black is responsible for producing the ßalanine, which is required for the inactivation of histamine. However, if a fly's eye has no functional Black, the visual process still runs normally. Hovemann's team therefore looked into the question of whether there is another supply route for ß-alanine. They also tested whether the fly eye can get around the recycling of histamine; this would be possible if the photoreceptors could directly reabsorb the released neurotransmitter, without it being inactivated in the glial cells.

No functioning sense of sight without histamine recycling



The researchers examined flies that were neither able to produce histamine themselves nor recycle it, because they lacked the enzyme for histamine synthesis and the enzyme Ebony. The team measured the flies' vision using so-termed electroretinography, which not only shows the excitation of the photoreceptor cells, but also the transmission of the signal to the brain. Even when the researchers added histamine from outside, the flies were blind. With this test, they showed for the first time that, for vision, *Drosophila* is dependent on the histamine recycling in the glial cells. Without recycling the enzyme Ebony, the cells in the insect eye cannot make any use of the neurotransmitter.

Flies can also see with disturbed **B**-alanine production

Cells are not only able to produce β -alanine with the aid of the enzyme Black, but also by converting the molecule uracil into *B*-alanine using other enzymes. Hovemann's team inactivated both production pathways for B-alanine and tested the vision of the fruit fly again. According to the electroretinogram, the animals' sense of sight was not impaired by the double mutation. "The results seem to represent a contradiction", says Bernhard Hovemann. "Although the insect eyes with the double mutation cannot produce ß-alanine, the animals seem to have normal vision. At the same time, our data clearly shows that the <u>recycling</u> by attaching β alanine is necessary for the animals to see." The researchers suggest that B-alanine – like histamine – is recycled in a circuit between glial cells and photoreceptors. This would mean that the enzyme Black merely compensates for β-alanine losses. "That would explain why we do not immediately find visual defects in flies which cannot produce new ßalanine", says Hovemann. These puzzles can, however, only be solved by further studies.

More information: A.B. Ziegler, F. Brüsselbach, B.T. Hovemann (2013): Activity and coexpression of Drosophila black with ebony in fly optic lobes reveals putative cooperative tasks in vision that evade



electroretinographic detection, Journal of Comparative Neurology, <u>DOI:</u> <u>10.1002/cne.23247</u>

Provided by Ruhr-Universitaet-Bochum

Citation: Blind flies without recycling: How Drosophila recovers the neurotransmitter histamine (2013, March 18) retrieved 3 May 2024 from <u>https://phys.org/news/2013-03-flies-recycling-drosophila-recovers-neurotransmitter.html</u>

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