Focusing on fish. Biologist Nils Ohnesorge is investigating the nervous system of zebrafish at the German Centre for the Protection of Laboratory Animals.

When in doubt, for the fish

Research for animal welfare: biologist Dr. Nils Ohnesorge is investigating how zebrafish embryos react to unpleasant stimuli.

"What It Is Like To Be A Bat" – this is the famous essay by the American philosopher Thomas Nagel. In it, he explains why we humans will maybe never be able to put ourselves in the consciousness of a bat and experience the same sensations. The gulf between bat and human being is simply too wide. It is even greater with Dr. Nils Ohnesorge and his laboratory animals.

The biologist is investigating whether the zebrafish feels pain and if so, how exactly this happens. Ohnesorge wants to improve the conditions in which *Danio rerio* (the Latin name for the zebrafish) are kept. "I research the animal for the animal," says Ohnesorge. This is done at the German Centre for the Protection of Laboratory Animals in Berlin-Marienfelde, which is part of the BfR.

The zebrafish is slender and fast. The sociable animals, whose "zebra stripes" sparkle bright blue when exposed to oblique incident light, are the length of a match stick, reproduce rapidly (a female can lay 300 eggs per week) and are easy to breed. They are popular in science because they can be used to study hereditary factors, body processes, diseases or the effects of toxic substances. Around 70 percent of zebrafish genes are found in a similar form in humans.

Today, there is a real boom in research on zebrafish. After mice and rats, "zebras" are the most widely used laboratory animals. This also has to do with the fact that new procedures, such as the CRISPR/Cas9 DNA scissors, make it possible to breed fish with desired characteristics in no time.

> **It was assumed that fish were unable to feel pain.**

The fish: a strange creature

"Just a few years ago, the fish was a strange creature," explains Ohnesorge. "A creature that could not express itself, whose behaviour was difficult to interpret." That perception has changed and "fish welfare" has become more important. The German Animal Welfare Act also stipulates that suffering in experiments must be kept as low as possible. "It was thought that fish were unable to feel pain," says the researcher. "But a debate has now been sparked."

The prerequisites for pain perception are also present in fish. There are "pain sensors" (receptors) and corresponding nerve cells; the animals perceive unpleasant stimuli and evade them. "All the signals that report harm work," says Ohnesorge. But does that mean pain? "Pain is an individual, negative experience," explains the biologist. "And, therefore, a feeling that is incredibly difficult to detect – unlike physical stress, which can be measured."

The feeling of pain is probably different for fish than for humans, but if this feeling exists, it must be taken into account. For example, in animal experiments with fish, where painkillers should also be used.

Zebrafish bustle about in 60 aquariums

Ohnesorge suspects that fish perceive pain. But a conviction is not proof. It has to be tested in a laboratory. And on that note: welcome to zebrafish husbandry at the German Centre for the Protection of Laboratory Animals at the BfR, which the scientist has built over the four years that he has been at the Institute. Around 60 aquariums are connected to a pump system that automatically circulates the water, cleans it, heats it to 28 degrees Celsius and controls the pH value (acidity).

As schooling fish, zebrafish live together without problems in a comparatively small space. A pair is only transferred to the breeding aquarium to spawn. Zebrafish mature as though in a time lapse. Where a human embryo needs one month for one stage of development, the fish embryo needs just one day. The embryos still rolled up in the embryonic membrane and the freshly hatched larvae are hardly visible to the naked eye. This is not only because they are so tiny; they are also completely transparent. The transparency allows Ohnesorge to look inside the animal without hurting it. The microscope allows a view of its rapidly growing brain, in which every single cell is still visible.

A pain imprint in the brain

How do unpleasant stimuli, such as a certain chemical substance, affect the nervous system of fish larva? Do they perhaps leave their own pattern behind in the animals' brains, a kind of pain imprint? This would be a strong argument for Ohnesorge's assumption that zebrafish actually have this feeling. He is conducting research on genetically modified animals whose nerve cells (more precisely: the nuclei of these cells) light up under laser light if they are active. The special microscope is, therefore, able to register every single nerve cell at work in the brain and even record its progress. By doing so, a film can be recorded that shows the consequences of an unpleasant stimulus on the brain.

All of this is still a long way off for Ohnesorge. The technical possibilities are as immense as they are challenging; we are talking about tens of thousands of nerve cells and their behaviour. To begin with, the biologist wants to determine which regions of the brain are likely to be important to pain perception. He will then use them to determine the effects of pain stimuli and how they can be prevented in animal experiments, for example, with drugs.

A great deal of work lies ahead of Ohnesorge and his team. But the researcher is driven by the fact that his work directly benefits experimental animals. Just as with bats, perhaps we will never truly understand zebrafish. But there are remedies also for fish in animal experiments that can alleviate potential suffering. ◘