

How Light Gets on the Nerves

The ragworm is an unusual laboratory animal. However, for **Gáspár Jékely** of the **Max Planck Institute for Developmental Biology** in Tübingen, this marine inhabitant has all the qualities of a perfect model organism: the larvae possess the simplest eyes in the world and later develop a simple nervous system made up of just a few hundred cells. This means that the scientist can track how sensory stimuli trigger behavioral changes.

TEXT **STEFANIE REINBERGER**

In Tübingen, the sea is up a slope. To be more precise, it can be found in the basement of the Max Planck Institute for Developmental Biology, located on a hill in the north of the city. This basement houses a tank containing 1,000 liters of seawater. Every month, a tanker truck drives up to replenish supplies. The watery cargo is intended for Gáspár Jékely's lab animals, namely ragworms, known by the scientific name *Platynereis dumerilii*.

"These animals are really low-maintenance," says the biologist, who comes from Hungary. "All they really need to keep them going is plenty of fresh sea-

water." Plus a lamp that imitates the phases of the moon, which synchronizes the breeding of the animals. They can be fed with fish food and spinach leaves. These worms, which are found throughout the world's oceans, aren't particularly demanding.

A SIMPLE NERVOUS SYSTEM IS ENOUGH FOR SURVIVAL

But this easy-care aspect isn't the only reason Jékely chose these unusual study subjects. The head of the "Neurobiology of Marine Zooplankton" research group wants to understand how simple

nervous systems work and how they have developed over the course of evolution. It's no wonder he's diving into the sea to do this: "Life comes from the sea, and the first neurological systems also evolved there," says Jékely. In addition, the ragworm *Platynereis dumerilii* is regarded as a living fossil. For millions of years, it has existed virtually unchanged in the coastal waters of temperate and tropical oceans.

Intriguingly, the worm's larvae possess, during their first days of life, the simplest eyes in the world. Each eye consists of a single photoreceptor and a single pigment cell, which screens



Ventral view of a young ragworm (*Platynereis dumerilii*), about half a millimeter in length, under the scanning electron microscope. The mouth opening (top) is surrounded by spherical and thread-like appendages with which the animal can perceive chemical stimuli from the environment.

the sensory photoreceptor cell on one side. This exactly matches Darwin's idea of the prototype eye: as early as 1859, the father of the theory of evolution speculated that the visual organs of all living creatures might have evolved from a simple system – a light-sensitive sensory cell and a pigment cell. "When we study the larva's eyespots, we come as close as we can possibly get to the evolutionary origin of the eye," says Jékely.

Of course, with their simple eyes, the ragworm larvae can't see in the true

sense of the word. But they can tell light from dark, and will swim toward light sources. To prove this, the scientist opens the door of the lab's refrigerator and takes out one of the little date-labeled glass beakers. It contains two-day-old larvae. But the naked eye can make out only water and some fine, floating particles, as the larvae are still just 0.2 millimeters in size.

The light microscope, however, reveals the outlines of some tiny, transparent bubbles that have a little dent on one side, marking the head region.

This gives them the shape of a miniature hazelnut. "Look what happens next," says Gáspár Jékely. He holds the microscope lamp against the side of the beaker. And presto – the minuscule larvae move toward the light, head first.

This swimming toward the light, also referred to as phototaxis, is important for the annelid's spread and survival. The larvae use the surface water currents to travel long distances until they find a suitable environment in which to settle and grow into mature, bottom-dwelling worms.

- 1 To label individual cells in the ragworm's body, Gáspár Jékely stains them with pigments coupled to special antibodies.
- 2 Mature specimen of *Platynereis dumerilii*. Each of its body segments, all of them similar, carries a pair of legs that the worm uses to move along with a crawling motion. The animal can regenerate any damaged or severed segments.
- 3 On its head, the ragworm carries two pairs of eyes (dark brown), as well as four pairs of thread-like processes with sensory cells. Pigment cells on the body give the animal its coloring.



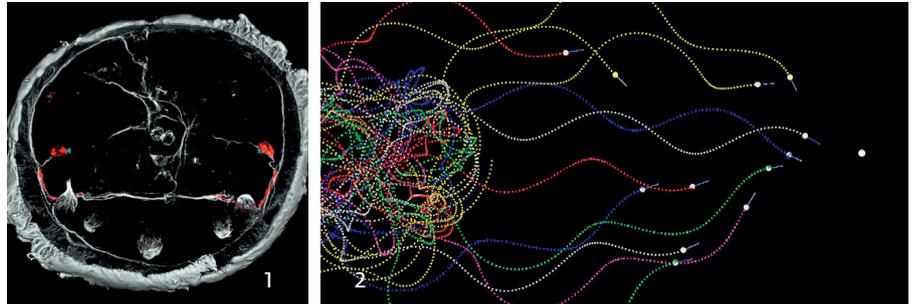
»» When we study the larva's eyespots, we come as close as we can possibly get to the evolutionary origin of the eye.

For a long time, it was a complete mystery to scientists how the larvae, with their simple two-cell eyes, could achieve this type of sensory skill and swim accurately toward the light. Until Gáspár Jékely, together with his colleagues at the European Molecular Biology Laboratory (EMBL) in Heidelberg, finally solved the puzzle: the photoreceptor is directly linked to the larvae's locomotion system. Below the head region, the larvae sport a dense wreath of cilia, rather like a collar. The beat of these tiny hairs propels the animal forward. A nerve fiber connects the cilia with the eyespot.

When light hits the sensory cell, it sends a signal to the cilia on that side of the body, which then beat more slowly. This makes the driving power one-sided, and the larvae go into something of a tailspin. They not only swim forward, but also rotate around their own axes – until the light hits the photoreceptor on the other side of the body. The ciliary beat on that side then slows down, and the larvae continue to rotate and are driven even further toward the light. In doing so, the tiny animals look as though they are climbing an invisible spiral staircase. They rotate themselves forward like a miniature propeller.

“From computer calculations, we know that only such spiraling locomotion can function in an organism with such basic equipment,” says Jékely. That is, as a result of the rotating movement, the light repeatedly hits the photoreceptor cell. The cell doesn't adapt to the stimulus; instead, it is repeatedly re-stimulated, thus constantly controlling the beat of the cilia.

“We are, in fact, working with the simplest neuronal system in the animal kingdom here: a sensory cell that directly controls an organ of locomotion as a single neuron,” says the Max



- 1 Front view of a two-day-old, spherical ragworm larva with eyes each consisting of one light-sensing cell. The two sensory cells and their processes leading to the cilia are colored red, while the larvae's ciliary bands and nerves are gray. The light-sensitive part of the sensory cells appears blue.
- 2 Computer model of the locomotion of *Platynereis* larvae. Spherical objects with ciliary bands that generate a constant, asymmetric force rotate around their own axes and are propelled along in a spiraling motion – very similar to the real larvae. If there are any localized changes in the ciliary beat, the model generates swim paths toward a virtual light source (white point on the right).

Planck researcher. “We suspect that this sort of direct link also existed in the first eyes that appeared early during evolution.”

A MODEL OF EVOLUTIONARY SUCCESS

Incidentally, the ragworm offspring aren't the only ones with this light-powered locomotion system: the larvae of mussels, sea cucumbers and flatworms also move in this propeller-like fashion. They also possess phototactic equipment of comparable simplicity; it would seem that the “direct-drive” eye has caught on.

But the scientists in Jékely's group have only a short time window in which to study these proto-eyes. They therefore need a constant supply of new larvae from their ragworm farm in the building's basement. The time for the eye prototype runs out in just three days. Interestingly, the larva's first simple pair of eyes degenerates as

it matures. In their place, new visual organs are formed a bit further back on the head. These replacements are now more advanced, and consist of an increased number of photosensor cells, a pigment cup and even a simple lens. They are the precursors of the adult animals' eyes. The fully grown ragworms possess two pairs of them, each pointing in a different direction to cover the maximum possible visual field.

It's as if several stages of evolution can be seen in a single animal. It may be that the genome of the ancestors of today's *Platynereis* representatives contained two copies of the genes that code for light perception. Evolution was thus at liberty to produce a new biological variant: a complex eye with which the animals could truly perceive their environment.

With its very simple eye, one pigment cup and one lens, *Platynereis dumerilii* makes an ideal study subject for researchers like Gáspár Jékely. Further-

»» Even the oldest eyes in evolution must have had a direct link between the sensory cell and the organ of locomotion.

more, as they develop, the larvae form more and more nerve cells – like a sort of primitive brain. These nerve cells include a great variety of different cell types, even ones that were long thought to exist only in vertebrates. Jékely points to a picture on his office wall. It shows a larva in cross section under the microscope. The animal is encircled by its ciliary band, with a network of various brightly colored nerve cells in the middle.

“This provides us with a model for a very simple nervous system that we can use to study the function and interaction of various cell types,” says the scientist. Colleagues who work with mice or rats, or even try to unlock the secrets of the human brain, have a much harder time. “When faced with brains made up of many millions or

even billions of neurons, it is incredibly difficult to stay on top of things,” explains Jékely.

So, anyone searching for basic connections at the cellular level would be well advised to get hold of a simple, manageable system – as manageable as that of the ragworm larvae. They even allow us to observe directly how single nerve cells control behavior.

The Hungarian scientist and his team are working on a sort of map of the larvae’s nervous system. In this map, the scientists aim to make a record of which nerve cells react to certain environmental signals, and what behavior results from this. Stimuli from the microscopic animals’ environment – such as light, water temperature or even chemicals – don’t just determine the direction in which they swim. Rath-

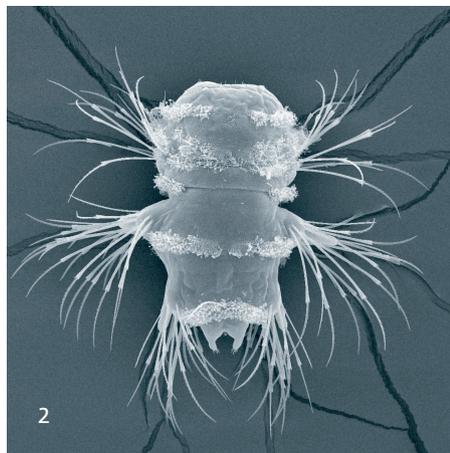
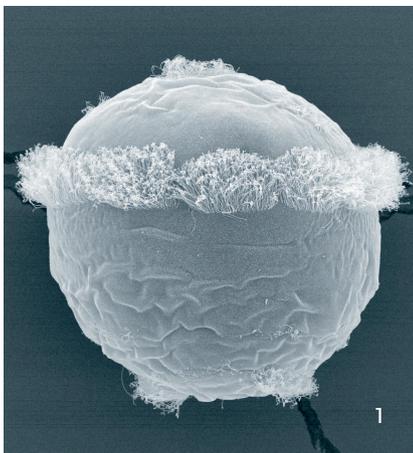
er, environmental signals appear to steer their entire development. The larvae thus bob around in the water until they find themselves in an environment that is suitable for them to settle on the ocean floor. It is only then that their body segments are formed and that they mature into full-grown ragworms. “How the larvae recognize this is not yet clear,” says Jékely. “It’s possible that it involves a chemical signal that originates from a food source.”

MESSENGER SUBSTANCES CONVEY ENVIRONMENTAL STIMULI

The number of potential external stimuli is enormous. To start from this end and feel one’s way toward the nervous system’s reactions and the resultant behaviors of the animals would be like looking for the proverbial needle in a haystack – particularly when one considers the great multitude of chemical stimuli emanating from the environment. The Tübingen-based Max Planck researchers are approaching the problem from the opposite end, by first putting the function of the neurons themselves under the microscope.

In this way, the scientists identified a series of neuropeptides that the worm larvae’s various nerve cells use as messenger substances. Among them, the researchers found representatives such as enkephalin and serotonin, which occur not only in the ragworm, but also in vertebrates. And, in 2011, they were able to prove that the animals also control their swimming depth with the aid of these signal molecules. After all, life is not all about swimming toward the light. During the dark nights, for instance, they would then simply sink to

- 1 | Electron micrograph of a two-day-old ragworm larva with its typical, wreath-like ciliary band. The larva is about 0.2 millimeters in size at this stage.
- 2 | A three-day-old larva possesses several ciliary bands for swimming, together with three pairs of bristles, with which it can attach itself to the ground (the front part of the animal is pointing upward).





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- 1 | A pipette ensures particularly gentle handling of the ragworm. This allows the researchers to transport the fragile animals without risk of injury.
- 2 | The worms build dwelling tubes (black) in their tank, and leave these tubes only when they are sexually mature. They are then collected by the researchers and paired off. The purple color is produced by the algae on which the worms feed.
- 3 | *Platynereis* thrives in shallow dishes filled with seawater. This allows a large number of animals to be kept and bred at the ragworm breeding station.



3



Twelve eyes are better than two: Members of Gáspár Jékely's research group analyze electron micrographs of *Platynereis*.

the bottom of the sea, where not a single ray from the sun would reach them the next morning.

The larvae maintain a fairly constant swimming depth. The researchers observed this by letting the tiny animals swim in transparent columns and following their movements with a camera. Normally, the larvae's ciliary beat ensures that they are propelled upward. If the cilia stop beating for a short time, the animals sink. Consequently, the larvae remain at the same water depth by keeping their upward and downward movement equal. The animals can tell whether they are too far from or too close to the surface through sensory stimuli such as light, temperature and pressure.

The neuropeptides act as messenger substances in this process. In a test, the scientists added several of these substances to the water. These signaling chemicals diffuse through tiny pores to reach the larvae's interior, where they

deliver their messages to the corresponding nerve cells or directly to the ciliary band. And, indeed, nine of the tested substances stimulated the ciliary band to increase the beat frequency of the delicate cilia hairs, making the larvae swim upward. Two other messenger substances caused the opposite to happen. The cilia stopped beating, and the microscopic animals began to sink. The neuropeptides not only guided the larvae up or down the column; they also controlled the extent to which they changed their position.

FROM A SINGLE CELL TO BEHAVIOR

"This means that we can observe individual cells translating a stimulus into a behavioral change," says Jékely, summarizing the significance of these experimental results. In mice and humans, this is much more complicated, due to the huge number of links in-

involved. There is, as it were, a black box between the incoming and outgoing signals.

In ragworm larvae, on the other hand, the researchers already have a fairly accurate idea of this black box. They therefore want to investigate the function of the individual nerve cell types step by step, so that they can work out the interrelationships of the worms' entire nervous system. And once the simple system of *Platynereis dumerilii* has been decoded, its basic principles can also be extrapolated to the complex networks of the higher animals.

However, the work of these scientists is not only of significance to neurobiology, developmental biology and evolution research. These findings also benefit zoologists, marine biologists and, in particular, the field of marine ecology. After all, a great variety of invertebrate organisms that live on the ocean floor go through a developmen-

tal stage as swimming larvae. Similar principles could well apply to all of them. Apart from this, the migrations of these marine plankton organisms also represent the biggest biomass transport on earth.

The Max Planck scientists are thus at the interface between different biological disciplines. They aim to develop the ragworm and its larvae into a model organism as powerful as mice, fruit flies and the roundworm *Caenorhabditis elegans*. "So far, there are only eight or ten groups in the world working with *Platynereis*," says Gáspár Jékely. Because of this, and unlike colleagues who work with standard experimental animals, they do not have

access to an exhaustive supply of molecular biology tools.

"We first have to develop methods for many of our experiments, but the range of options is constantly becoming

broader," says Jékely. And the biologist is convinced that, bit by bit, more and more scientists will recognize what a fascinating lab animal *Platynereis dumerilii* really is. ◀

TO THE POINT

- The ragworm *Platynereis dumerilii*, with its simple sensory and nervous system, can be used for research into how sensory stimuli induce behavioral reactions, and how eyes and nervous systems have evolved.
- With the aid of just one light-sensing cell on each side of its body, directly linked to a wreath-like ciliary band for locomotion, the ragworm larva can use its spiraling movements to swim accurately toward a light source.
- Environmental stimuli guide the worm larvae's behavior, with various neuro-peptides acting as messenger substances in this process.

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