Producing offspring is not always easy. This is especially true when your home is located underwater and the neighbors – a bizarre mix of phlegmatic relatives and agile, greedy finned creatures – are constantly passing through your living space. Invertebrate sea urchins are at a particular disadvantage. Sex is not a realistic option for a creature with a calcareous exoskeleton comprising hundreds of prickly needles. Fertilization? Yes, but only outside the body! Once a year sea urchins gather together on the sea floor, often in groups of up to 30 individuals, for the purpose of reproduction. They huddle together and release egg cells and sperm at depths of up to 40 meters, depending on the species. This cooperation increases their chances of success, as their reproduction efforts are frequently thwarted by the ocean current, fish and other marine creatures swimming by in their search for protein-rich snacks. There are no half measures here: a male sea urchin releases around 100 billion sperm – as compared with the paltry 200 million produced by the human male – and the female releases 50,000 eggs.

The really astonishing thing is that, despite the fact that different species of sea urchins, snails, fish and other cohabitants of the biotope also engage in extracorporeal reproduction, no accidental hybrid creatures ever arise. No “starfish urchins,” no “urchin snails,” no “fish urchins” – not even hybrids of the approximately 900 species of sea urchins. No, the sea urchin sperm find their way unerringly to the egg cells of their own species. It almost seems to happen by magic. But the magic here is chemotaxis.

Chemotaxis can be more or less defined as “movement in the direction of a chemical substance.” And that is precisely what happens: hungry bacteria and amoeba also use the technique, zigzagging toward their food sources like sniffing dogs. This process is referred to as a “random walk”. So what about the sea urchin sperm? They follow the trail of an attractant transmitted by the egg cell. The male gametes, which are 50 to 60 micrometers long, actively swim – in a pattern somewhat akin to a bent helix – in the direction of the increasing concentration of attractant. It all sounds fairly straightforward, just a question of following their noses. The reality, however, is not quite so simple.

Could You Point the Way to the Egg, Please?

Sea urchin sperm always follow their “noses” when swimming. Their olfactory organ is located in the tail and actually counts or calculates rather than smelling. The scientists working with Benjamin Kaupp, Scientific Director at the Center of Advanced European Studies and Research (caesar), have provided a molecular explanation for this peculiar process.

TEXT CATARINA PIETSCHMANN
Benjamin Kaupp and his team at the caesar research institute in Bonn are studying how this process works in detail. Molecular sensory systems is the general term used to describe the area in which Kaupp works, and it is also the title of his department. For more than 30 years, the biophysicist has been researching how sensory cells register and respond to stimuli in sensory processes, such as seeing, smelling and, in the case of sperm, chemotaxis. Despite being very different in many respects, these three processes have much in common. Each sensory cell transforms stimuli into electrical signals via a chain of biological reactions. Ion channels, which usually consist of several large proteins, play a key role in this process.

ATTRACTANT RECEPTORS ON THEIR TAILS

The scientists in Bonn are particularly interested in what are known as cyclic nucleotide-gated channels (CNG channels) and pacemaker channels (HCN channels). CNG channels are found, for example, in the membrane of the highly sensitive rod cells in the human retina. “Following the absorption of a single photon, hundreds of ion channels in the cell membrane close,” says Kaupp, who decoded the molecular structure of the CNG channels and discovered that these channels play a significant role in color-blindness. In the 1990s, similar ion channels were also found in the visual cells, known as cones, and in the fine hairs of the olfactory cells.

It was in 1997, while they were searching for channels in other forms of tissue, that Kaupp’s research group stumbled upon strange channels in the sperm of the sea urchin Strongylocentrotus purpuratus. These channels behaved just like those that control the heart beat and rhythmic activity of nerve cells in mammals – true pacemaker channels. The question was, what on earth were they doing in sperm? Are they a valuable tool that is used only for special purposes – possibly chemotaxis in this case?

This was reason enough to take a closer look at the sea urchin sperm and explore the question of which sensory organ they actually use to recognize their attractant. After all, these sperm are not intelligent creatures: they have no real nose, not to mention nerve cells that could transmit sensory stimuli to the brain, which, of course, they lack in the first place. Their heads contain only genetic information relating to their species. Theirraison d’êterelistsolely in conveying this information to the egg cell. Yet their tail, the flagellum, contains over a million receptors for the attractant – so their nose is actually located in their “feet.”
FOCUS: Olfaction

right UV flash as the starting signal: The sperm move aimlessly (top) until the flash releases the attractant within a circle. Sperm then swarm together to form a bright spot (bottom).

below The attractant can also be released under a Micky Mouse or striped mask (left). The sperm form the pattern shown on the mask (right).

The obvious question here is why such a crucial stage in the reproductive process is being examined using the sea urchin. “There are historical reasons for this,” explains Kaupp’s colleague Timo Strünker. “As it happens, the sea urchin is the species in which sperm chemotaxis was first discovered some 100 years ago.” Working at the Marine Biological Laboratory in Woods Hole on the east coast of the US, Frank R. Lillie observed something strange, which he published in the journal Science in October 1912: when he put a few drops of sea water containing unfertilized egg cells of the sea urchin Arbacia punctulata in with sperm of the same species, he noted that the sperm agglutinated so much that it was visible to the naked eye.

Lillie believed that this occurred because the egg cells had previously deposited something in the water that he called agglutinin. Decades later, it was discovered that the substance involved here was a peptide consisting of 14 amino acids. It is now known that all sea urchin species, all starfish and probably all species of marine animals that spawn have their very own agglutinin.

Of course human reproduction doesn’t involve this chaotic tumult of sperm originating from different species. Apart from the exceptional cases of in-vitro fertilization, the process takes place inside the body. So is chemotaxis required at all in this case? “It would appear that it is, but only in a narrow band around the egg,” says Strünker, “for precision control in the final few millimeters, so to speak.” But more on that later.

Back to the sea urchin. Tracking down the complete biochemical mechanism behind the chemotaxis of Arbacia called for a few tricks, ingenuity and the cooperation of physicists, chemists and biologists. The little peptide is now being produced artificially by chemist Michael Beyermann at the Leibniz Institute for Molecular Pharmacology (FMP) in Berlin. Once he has cleaned it up, his colleague Volker Hagen provides it with a “disguise” – a small protective group of chemicals that renders the peptide biologically inactive, but that can be split easily by a flash of UV light. The researchers call this a caged compound.

The peptide, now fixed in the chemical cage, is mixed with sea urchin sperm in a shallow measuring chamber and observed under a microscope at the caesar institute. The sperm – which can be identified by their light-colored heads – still swim more or less randomly around their swimming pool. Unlike in the sea, in the shallow cuvette, they simply paddle around in circles, which makes it easier to observe them. The chamber is then exposed to a UV flash through a striped, dotted or “Micky Mouse” mask – the researchers give their playful instincts free rein here. The “disguises” fall off and the attractant becomes active.

The sperm move fast as lightning toward the artificial attractant source and thus form the pattern shown on
The sea urchin sperm find their way unerringly to the egg cells of their own species. It almost seems to happen by magic. But the magic here is chemotaxis.

The attractant receptor in the sperm membrane, the enzyme guanylyl cyclase, was already known. The signaling pathway that causes the change in the swimming behavior was explained by Kaupp’s team: if an attractant molecule binds to the receptor, the aforementioned enzyme transforms GTP (guanosine triphosphate) into the intracellular messenger substance cGMP (cyclic guanosine monophosphate). According to Kaupp, cGMP is also the messenger in visual cells.

The cGMP opens potassium channels (CNGK), through which positively charged potassium ions flow from the cell. As a result, the cell interior becomes more negatively charged and the membrane potential decreases; this means that the membrane is hyperpolarized. This, in turn, results in the opening of the above-mentioned pacemaker channels. “These pacemaker channels are responsible for the rhythmic contractions in the heart muscle,” Kaupp mentions. Potassium then flows into the cell and the membrane potential increases again. The opening of the calcium channels associated with this depolarization ultimately allows the influx of calcium ions, as a result of which the beating pattern of the flagellum changes, and with it, the swimming trajectory of the sperm.

As the researchers established, sperm even react to single molecules of attractant. A single activated receptor is enough. Demonstrating this was no easy task, involving step-by-step experiments and control tests. “It would not have been possible at all without the optical switches, including the caged signaling substances,” stresses Timo Strünker.

So how does all of this work in humans? “We still don’t know very much about that. What we do know is that many elements of the process are different,” says Strünker. This starts with the fact that human sperm do not swim on a helix trajectory, but in a straight line. Their swimming also appears to involve more of a gliding movement along a surface – the epithelium of the fallopian tube – so they swim through a viscous medium, which is not comparable to swimming in sea water.

Moreover, in humans, only 10 to 20 percent of the ejaculated sperm are even in a position to reach the egg cell. This makes it difficult to observe them. But human sperm are also “attracted.” If follicular fluid is dropped into a solution containing human sperm, they swim toward it. “But no one has yet succeeded in identifying receptors, intracellular signaling substances or an attractant.” Some researchers believe that the process is more likely to involve thermotaxis than chemotaxis, as there is a temperature difference of between 1.6 and 1.8 degrees Celsius in the fallopian tube.

**STERIOD HORMONE AS A POTENTIAL ATTRACTANT**

But do human sperm not follow bourgeonal, the scent of Lily of the Valley, as claimed by the biologist Hanns Hatt from Bochum? Kaupp shakes his head: “From what we know of the sea urchin, we doubt it. What is certain is that, in both species, the motility of the sperm is controlled by a change in the calcium concentration.” In the experiments carried out using bourgeonal, the human sperm were exposed to a concentration of a few micromoles of the odorant per liter (1 micromole = 1 millionth of a mole). However, the sea urchin sperm react to concentrations in the femtomolar range (1 billionth of a mole per liter). Moreover, the team in Bochum didn’t test whether bourgeonal triggers the formation of the expected intracellular messenger substance. The researchers at caesar did test this and nothing happened.

A Japanese group also discovered that a different odorant is responsible for the reaction in mice. Kaupp has his doubts about both studies. “Because there are numerous substances that cause calcium changes in sperm, we sus-
CAESAR AND MAX PLANCK

cæsar stands for Center of Advanced European Studies and Research. The neuroscience research center in Bonn was established as a non-profit foundation in 1995. It is funded by the Federal Republic of Germany and the German federal state of North Rhine-Westphalia.

cæsar, which was previously located in temporary facilities in Bonn’s city center, moved to a newly constructed building in Bonn-Plittersdorf in 2003. The three-part complex can house not only three research departments, but also up to eight junior research groups and spin-offs.

In 2006, the Foundation Board passed a resolution in favor of focusing scientific research on neuroscience, and forging a link between cæsar and the Max Planck Society while retaining its mission and structure as a legally independent foundation.

The appointment of directors, the evaluation of research and the pursuit of scientific excellence are based on the criteria of the Max Planck Society.

Professor Benjamin Kaupp, Scientific Director of cæsar since 2008, is head of the department of Molecular Sensory Systems. He is also a Scientific Member of the Max Planck Institute for Neurological Research in Cologne. Departments of Neurodegeneration and Neurophotonics are currently being established at cæsar.

www.caesar.de

Promoting communication both among scientists and with the public was a key concern behind the architecture of the cæsar complex. The researchers’ “thinking cells” are located in the “wave” section – with a view of the Rheinaue leisure park. All of the laboratories are located in the central block. The cafeteria, seminar rooms, auditorium and administrative offices face the road, thus opening up the building to visitors.
above  A clear view of chaos: Benjamin Kaupp and Timo Strünker observe antibody colorings of pacemaker ion channels in brain sections. Such channels are also found in sperm.

below  Noses in their "feet": The receptors for the chemotactant in sea urchin sperm, here as seen under the optical microscope, are located on the flagellum.
pect that they are all lipophilic, or fat-soluble, compounds that can enter the membrane directly. The observations may well be experimental artifacts.”

So then what does attract human sperm? “There are a few possible candidates,” Kaupp remains rather circumspect. “It is thought that it might be progesterone.” It has been proven that the cumulus cells of the ovum release this steroid hormone. Progesterone normally regulates protein synthesis, and this takes anywhere from minutes to days. But mature sperm do not produce any proteins – why should they? Progesterone can also have a rapid effect: sperm react to it with an intracellular calcium signal within seconds. “We are currently carrying out tests with caged progesterone,” reveals Kaupp.

THE WEAKNESS IN THE FERTILITY TEST

Given that this is so complicated in humans, does it perhaps work on other mammalian models? “Yes, rats, mice and rabbits. We used to work with rabbits,” says Kaupp. Now, however, research is carried out on fresh human sperm at the caesar institute. The institute has built up a small network of volunteer donors for this purpose. Some scientists claim that the attractants in humans, rabbits and mice are identical.

“It is known that the human female’s follicular fluid also attracts rabbit sperm,” confirms Kaupp. “There is a certain logic to that: the sea urchin releases its gametes into the sea and abandons them to their fate. Chemotaxis plays a crucially important role there. In mammals, sexual intercourse precedes the release of sperm, so there seems to be no need for specificity of recognition via the attractant.” This phenomenon is exploited in the so-called hamster test, which checks the fertilization potential of human sperm: the ability of human sperm to penetrate into the egg cells of a hamster is seen as an indicator of healthy sperm function.

Timo Strünker has his doubts, though, as the test has a weak point. Sperm of knock-out mice lacking a calcium channel that occurs only in sperm are active but infertile. Their sperm cannot penetrate the egg membrane. “In the hamster test, however, the egg membrane is removed in advance. And today we know that there are people who have a defect in this channel.”

The potential application that could eventually arise from the basic research being carried out at caesar is clear. It could be used to help couples with fertility problems – on a diagnostic and perhaps even a therapeutic level. Certain ion channels, pumps and transporters are found only in sperm. These proteins are ideal targets for selective contraceptives. The “pill” for men, at last! Of course, there would also be a use for technical sensors that could identify individual molecules in a mixture containing trillions of other molecules. What excites Benjamin Kaupp about his work, however, is something entirely different: the evolutionary links. “Smelling, seeing, chemotaxis in sperm – for me, these are variations on a theme. How was that theme transformed to fulfill such different functions?”

DINING ON LOBSTER MAKES UP FOR WORKING IN THE DARK

The team is currently preparing for another trip to the east coast of the US. Measuring devices are being packed and shipped, the intention being – as every summer – to work with many other researchers from all over the world in the legendary Woods Hole Marine Biological Laboratory, which was established in 1888. Their trip takes them back to the roots of their subject, to Cape Cod, where chemotaxis was discovered in the sea urchin.

It must be wonderful to work in such a beautiful location. Timo Strünker dismisses this suggestion with a wave of his hand: “Well, we don’t see very much of that. Because the caged compounds are extremely sensitive to light, we spend hours working in the dark.” But there is no need to feel too sorry for the scientists. To compensate, they have freshly caught lobster for dinner, not to mention a magnificent view of the vast ocean, whose strange inhabitants still remain largely unexplored.

GLOSSARY

Extracorporeal
Located outside the body.

Chemotaxis
Refers to a movement within a chemical gradient toward a chemical stimulus. In the case of positive chemotaxis, the movement is toward the attractant; negative chemotaxis prompts a targeted withdrawal from the stimulus (alarm signal).

Helix
A screw that winds around the imaginary wall of a cylinder in a constant upward motion.

Ion channels
Pore-forming proteins that span the cell membrane and enable charged particles, known as ions, to permeate the membrane.

Flagellum
Long, threadlike structure that serves the function of movement.

Caged compound
A biologically relevant molecule that is inactive due to the presence of a protective chemical group (cage). The compound is photolabile and can be cleaved by a pulse of intense light of the appropriate wavelength in a photochemical reaction.

Membrane potential
The voltage difference between the interior and exterior of a biomembrane.

Follicular fluid
Fluid in the egg vesicles in which the egg matures.

Mole
A mole consists of $6.022 \times 10^{23}$ particles of a particular substance.