

HEARING COLORS, SEEKING GENES

TEXT: KLAUS WILHELM

- 54 Synesthesia is one of the most fascinating phenomena in psychology and the neurosciences. But only very slowly are its scientific mysteries being uncovered. Research in this field is gathering momentum, thanks to the studies being conducted by Simon Fisher from the Max Planck Institute for Psycholinguistics in the Dutch city of Nijmegen.

Even by non-scientific standards, synesthesia is baffling – at least for those who do not experience this perceptual phenomenon. Imagine you see or hear a letter, let's say the letter F, and immediately the color red lights up in your mind's eye. Or the letter Z – and the color that appears is green. Now imagine you are reading a book. You might find yourself seeing a continuous stream of colors at the same time. Other people hear certain words – and immediately experience a sweet, sour, or other kind of taste in their mouths – and so on.

Synesthesia literally means “perceiving together”. It is a neuropsychological phenomenon, in which the act of perception by one sense simultaneously and involuntarily stimulates another. It is “certainly not a disorder, much less a disease,” emphasizes Simon Fisher, Director at the Max Planck Institute for Psycholinguistics. It is estimated that there are 60 to 80 forms of synesthesia, which is a normal part of everyday life for around four percent of the human population. Lara Grabitz is one of them. The physics student has grapheme-visual synesthesia and sees the letters or numbers of a text in color directly on the paper or monitor. When she plays her cello, she also hears the tones in color. When she hears a year, she sees a kind of timeline in front of her with the years arranged along it. “This world feels completely normal to me,” says the young woman. “I’ve never known anything different, I would feel quite anxious if it suddenly went away.”

Simon Fisher belongs to the 96 percent of the population who have no direct, personal experience with synesthesia at first hand. He is a neuroscientist and geneticist, “a scientist with an interdisciplinary remit,” as he likes to say. He is interested in “looking at the DNA of genes and understanding the biological impact of genes on behavior, language, and other traits.” His team started by researching genes they’d discovered that influence specific speech disorders. At some point in his career, he met colleague Simon Baron-Cohen, Professor of Developmental Psychopathology at Cambridge University, who is known for his research on so-called autism spectrum disorders – as well as for his work on synesthesia. “I didn’t know much about the field at the time,” says Fisher, “but I found it incredibly exciting.” Back then, next to nothing was known about the genetic background of synesthesia.



KNOWLEDGE FROM

— BIOLOGY & MEDICINE

Painting for the ears: artist Wassily Kandinsky is one of the world's most famous synesthetes. Painted in 1926, this picture with the title "*Three Sounds*" embodies his idea that colors and shapes can convey music – and vice versa.



IMAGE: WASSILY KANDINSKY / HERITAGE IMAGES / FINE ART IMAGES / ARG-IMAGES

By the time he took up the post of Director at the Max Planck Institute for Psycholinguistics, research processes were being revolutionized by high-performance technology that could rapidly analyze entire genomes from thousands of people simultaneously. Ever more precise, ever more affordable, ever more sophisticated – a giant step forward. Yet “despite all the technology, it is still an intellectual challenge to analyze these raw DNA data files and to examine how genes ultimately influence a trait,” explains Fisher. “Because, even though genes provide instructions for human brain structure and behavior, we have to find out the ways in which these instructions are implemented. That’s the high art of DNA analysis.” And that’s the domain of bioinformatics. The experts investigate whether certain traits occur simultaneously with variants of certain DNA sequences or genes. “To ascertain this, you need skill, expertise, a lot of luck, and above all a large number of DNA samples from people with synesthesia,” the researcher says. This is because it is rare for a single gene to have a major effect on complex traits like synesthesia. As a rule, a combination of many gene sequences work together to make a trait more likely.

With this in mind, researchers have already spent many years seeking people with different forms of synesthesia for their studies. They are pursuing two different lines of research: firstly, the team needs as many unrelated test subjects with grapheme-color synesthesia as possible from among the general population. They are searching for so-called “polymorphisms” in their genes. These are “normal” variants of genes that often differ by just one building-

block. Although each polymorphism by itself has only a minor effect, the effects of multiple variants on various genes add up, and this is when people are more likely to develop synesthesia. However, the genomes of at least

1,000 synesthetes are required in order to make any statistically meaningful assessments. “We are almost there,” says Fisher. He and his team are now approaching their first goal: to examine the genomes of these

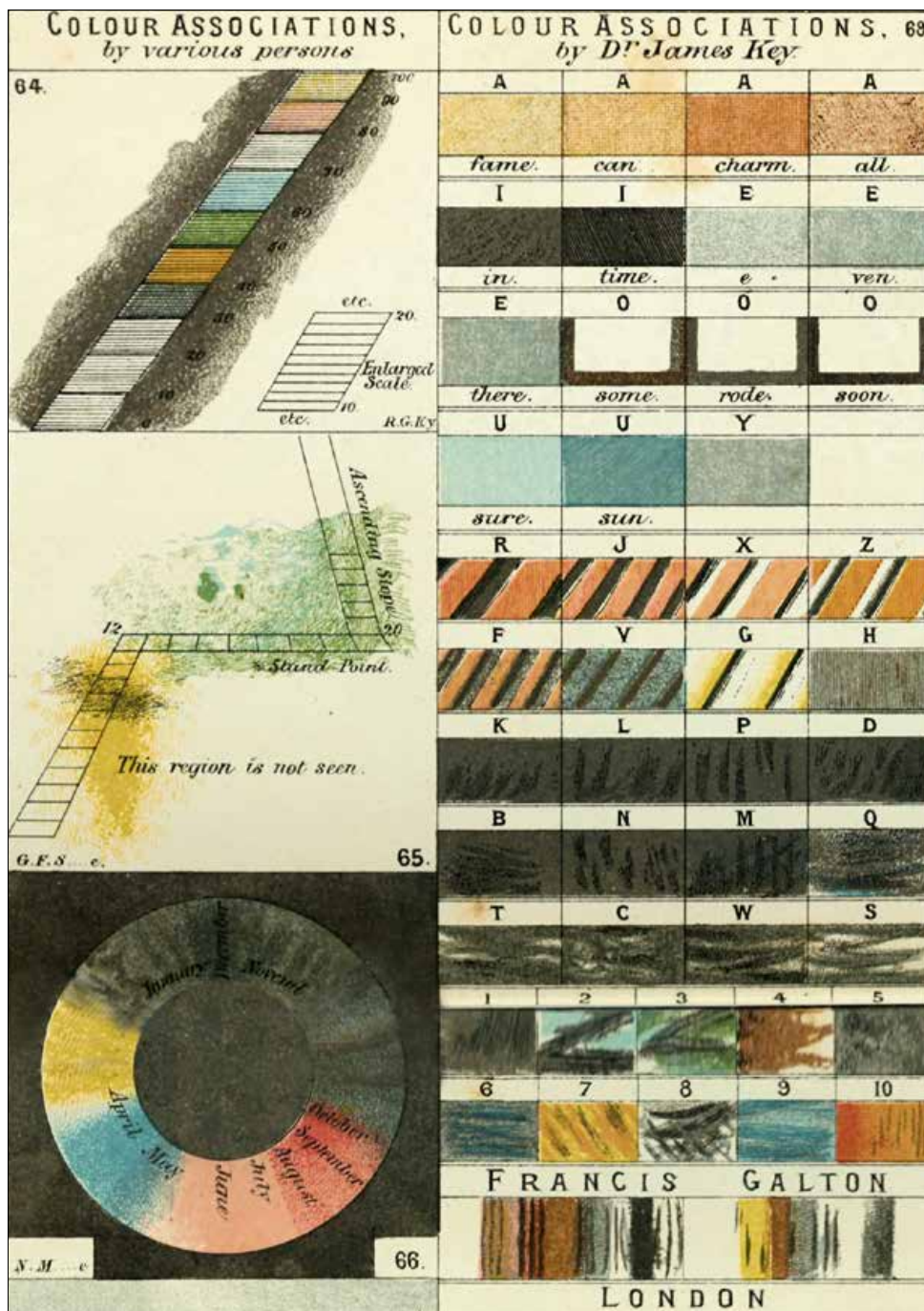


ILLUSTRATION: FRANCIS GALTON/INQUIRIES INTO HUMAN FACULTY (MACMILLAN 1883, SECOND EDITION, EVERYMAN, 1907)

Early categorization: published in 1883, Francis Galton’s book about psychology contains drawings by synesthetes.

Figs. 64 and 65 contain examples of how numbers are experienced in space, accompanied by their perceived colors.

In Fig. 66, the months of the year are arranged in a circle and associated with colors. Fig. 69 shows colors and patterns associated with various letters and numbers.

1,000 people and identify all “suspicious” polymorphisms. If specific DNA variants are found to be more common in people with synesthesia than in those without it, these variants could be associated with the trait.

Secondly, the team in Nijmegen is searching for families in which synesthesia occurs multiple times throughout several generations. The researchers are using their high-tech methods to comb the genomes of these people for genes that could help explain the unusually high incidence of synesthesia in these families. “We can only make statistically reliable statements if we can analyze large families,” Simon Fisher explains. “In similar studies on speech disorders which we conducted in the past, we investigated three generations of a family in which 15 out of 30 individuals were affected. The synesthesia families we have found so far are much smaller.”

Nevertheless, the first provisional results are already available. Through the collaboration with Simon Baron-Cohen, Fisher’s group got the chance to analyze the genomes of three families in which five or more members spanning at least three generations had sound-to-color syn-

esthesia. The team, led by Amanda Tilot, identified 37 candidate genes that might potentially be linked to synesthesia. Although different genes were altered in the three families, “we noticed that some of these genes function in a similar way,” explains Tilot. The researchers are interested in six of the genes in particular. These are genes that are thought to be important particular function in brain development during early childhood. They help to make sure that nerve cell extensions (known as “axons”) form and link up properly – a process known as “axonogenesis,” in which nerve cells form interconnections with neurons in other regions of the brain. The genes in question are particularly active in the visual and auditory centers. “There are many pathways that could lead to the enhancement of neuronal connectivity through slight variations in axonogenesis,” Tilot points out – for example, through the length and positioning of nerve fibers or through unusual branching and other changes in shape. The study shows how genetic differences can influence sensory experience – possibly by altering the connectivity of neurons in the brain. “Synesthesia is therefore a clear example of neurodiversity that we should respect and appreciate,” says Fisher.

“The possible role of genes involved in axonogenesis is really interesting, because it supports the hypothesis that people with synesthesia have increased connectivity between brain regions that are not usually linked.” However, there are other theories that could also explain synesthesia, such as a change in the balance between neuronal excitation and inhibition (the so-called E/I balance) in the brain. “Ultimately we need a lot more data,” says Fisher. This is why Fisher’s team are not letting up in their efforts to find synesthetes for their studies – through their cooperation with other research groups worldwide as well as through other channels. The researchers offer the “Synesthesia Battery” on the Max

Planck Institute’s website and as a smartphone app, for example; this self test was originally developed by Stanford University neuroscientist David Eagleman and can determine fairly reliably whether or not a person has synesthesia, at least for some of the most thoroughly-studied types like grapheme-color. Anyone who identifies as a synesthete on the test can take part in the Fisher team’s studies.

Genes and environment intertwined

Moreover, evidence exists that synesthesia-like perception can be learned. How? “Through intensive reading training using texts with colored letters,” explains Fisher. “This enables people to develop an artificial form of synesthesia even if they aren’t genetically predisposed to it. But it does not appear to be the same thing, because this ability disappears again.” People with true synesthesia experience it automatically (i.e. involuntarily, without having to focus on it) and they experience it consistently over a period of years – this is one of the most reliable characteristics of this phenomenon. As a rule, it begins to develop in childhood. “Synesthesia is a remarkably interesting example of how genes and environmental experiences interact,” says Fisher. “This genetic predisposition is present from birth.” However, the colors that people with synesthesia associate with specific sounds or letters, for example, are invariably the result of environmental experiences in childhood. This has been shown by studies conducted by synesthesia expert Julia Simner from the University of Sussex, for which she observed British elementary school children over a period of several years. In conclusion, synesthesia needs a long time to develop. Amanda Tilot adds, “The classroom may play a key part in the development of synesthesia since this is where the alphabet, numbers, and the calendar are connected with the child’s memory.”

57

SUMMARY

In children, synesthetic associations are initially chaotic and fluid but become more consistent over time.

People with autism spectrum disorders report synesthetic experiences much more frequently than other people.

The development of synesthesia could be influenced by several genes that help establish connectivity between nerve cells in different parts of the brain.



In the British study, the few children at each elementary school who became grapheme-color synesthetes gradually came to associate colors with each letter of the alphabet during their early years at school. These children selected specific colors for 34 percent of the alphabet at ages six to seven, 48 percent at ages seven to eight, and 71 percent at ages ten to eleven. “Initially, the synesthetic associations are chaotic and fluid,” says Fisher, “but in time they become more consistent.” Many of the children and adolescents are not even aware that their perception is different from other people’s.

Synesthesia can be useful

A study of 1,000 subjects recently found that men and women are equally represented among people who lead truly synesthetic lives. Simon Baron-Cohen’s team also found out that synesthesia is more common among people with autism spectrum disorders – up to 20 percent of this group report synesthetic experience. Tilot explains that “the findings bring to mind the remarkable case of the British author Daniel Tammet, who has autism and

a complex form of synesthesia.” Tammet’s synesthesia enables him to perform astonishing feats, such as memorizing and reciting the mathematical number pi to more than 20,000 decimal places. This shows that synesthesia can sometimes be useful. Grapheme-color synesthetes, for example, often say that the colors help them memorize telephone numbers or other numerical information. Studies have shown that people with synesthesia perform better in memory exercises than people without this ability, provided they are able to use their unusual perceptive experiences to boost their memory. In his book “Born on a Blue Day”, Daniel Tammet describes how his sense of the uniqueness of color, shape and location of each number helps him solve complex mathematical equations at lightning speed. In general, people with synesthesia are more creative than average.

An interdisciplinary approach: Simon Fisher’s work brings together knowledge in the fields of genetics and brain research in order to better understand the phenomenon of synesthesia.



PHOTO: INTERNATIONAL DYSLLEXIA ASSOCIATION, IDA CONFERENCE OCT. 2018

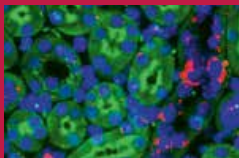
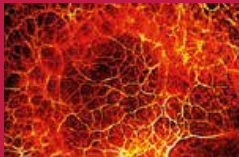
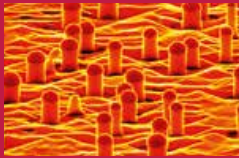
However, there is also a flipside to the coin, as Simon Fisher explains: “The many simultaneous perceptions can occasionally be overwhelming.” Sometimes, conflicts can also arise between concurrent perceptions. This can be disconcerting – for example when you are reading letters and perceive an odor of rotten eggs. “It gets difficult when systems collide, when my internal systems crash head on with the world’s systems,” says synesthete Lara Grabitz – for example, when the orchestra colleague with whom she shares a music stand writes numbers for fingerings on their shared sheets of music, but these mean nothing to her.

Synesthesia self-test (multi-lingual):
www.mpi.nl/page/join-our-synaesthesia-genetics-research





Connecting Science and Business.



Max Planck Innovation is responsible for the technology transfer of the Max Planck Society and, as such, the link between industry and basic research. With our interdisciplinary team we advise and support scientists in evaluating their inventions, filing patents and founding companies. We offer industry a unique access to the innovations of the Max Planck Institutes. Thus we perform an important task: the transfer of basic research results into products, which contribute to the economic and social progress.

Connecting Science and Business





Alpaca Britta after shearing. Alpacas originate from South America and are related to llamas. Their immune system produces antibodies with an especially simple structure. These have distinct advantages over conventional antibodies and can be developed for medical applications.

IMAGE: MPI FOR MULTIDISCIPLINARY SCIENCES/ CARMEN ROTTE

BOOSTER FROM ALPACAS

TEXT: KLAUS WILHELM

Basic research often takes a winding road and only indirectly leads to practical applications. The path of Dirk Görlich's research at the Max Planck Institute of Multidisciplinary Sciences in Goettingen began with defense proteins generated by the alpaca immune system. Görlich and his team have developed these proteins into mini-antibodies known as "nanobodies", which can block infection by SARS-CoV-2 and most of its variants. The efficacy and safety of these nanobodies will now have to be demonstrated in clinical trials.

When leaving their stable on the Fassberg hill in Goettingen, the alpacas Britta, Nora, and Xenia are met with a magnificent view of the university town at their feet. While it is admittedly not quite as breathtaking as their Andean homeland, these animals are participating in an extraordinary research project. The three alpacas are part of a herd of 22 animals at the Max Planck Institute of Multidisciplinary Sciences (until 2021 known as the Max

Planck Institute of Biophysical Chemistry).

In Germany, alpacas used to be common only in petting zoos but have recently become very popular with professional and amateur breeders. Several thousand of these camelids, which are native to South America are now at home in Germany. However, Dirk Görlich and his team are less interested in the alpacas' cute appearance or their wool, but rather in a peculiarity of their immune system: when camelids get infected, their immune systems produce not only standard antibodies but also simpler versions. While standard antibodies are composed of two heavy and two light chains, the simplified antibodies comprise just two heavy chains with a smaller antigen-binding module. This may allow them to target pathogens at buried sites that are inaccessible to standard antibodies. Nanobodies (or VHH antibodies) are the smallest antigen-binding fragments of such simpler alpaca/camel antibodies.

Antibodies are proteins that can bind very selectively to specific molecules, for example, a pathogen's surface, thereby, labeling the invaders for destruction by immune cells. However, they can also directly neutralize pathogenic molecules. Scientists use antibodies to label, visualize, or block molecules to study their function. The small quantities needed for this can be readily obtained from animals, such as rabbits or goats. This is done by immunizing the animal several times with the desired antigen. The animals then respond by making antibodies against the antigen. The antibodies can then be isolated from blood samples.

Given their ability to bind to specific molecules, antibodies can also be used as drugs, for example, to treat rheumatic diseases, cancer, or rabies. They are also being used to treat COVID-19. However, they cannot be obtained in the required quality and quantity from animal blood for such purposes. Instead, they are manufac-

tured in genetically modified hamster cells, in a procedure that is complex, time-consuming, and expensive. This is due to the complicated structure of the antibodies with light and heavy chains that are decorated with sugar chains. Nanobodies are different – thanks to their simpler structure, they are also easier to manufacture.

Approximately seven years ago, Dirk Görlich and his team came up with the idea of using nanobodies in their projects. At the time, the researchers were struggling to elucidate the three-dimensional structure of a protein complex from the cell's transport machinery by x-ray crystallography. But they just couldn't get the complex to crystallize – until they bound a nanobody from a Belgian company to the complex. "That solved the problem right away," Görlich remembers. This inspired the plan to generate nanobodies themselves and to establish a small herd of alpacas on the Institute's grounds. In the meantime, the Goettingen researchers have become experts in nanobodies. They use nanobodies as tools in fundamental research, for example, to block specific transport pathways in cells or as high-precision probes to localize proteins in fluorescence microscopy.

Then along came COVID-19. "It was clear that we could develop nanobodies that would stop infection with SARS-CoV-2," Görlich recounts. What followed was an emotional rollercoaster for the entire team, which pursued their project with immense dedication. "After all, there's a huge difference between producing nanobodies for basic research and developing them as drugs. In the laboratory, we use them just to bind specifically to their target structure, and a few milligrams are enough for thousands of experiments." For therapeutic purposes, however, they must not only bind to their target molecule but also neutralize it, and do so at very low concentrations to minimize side effects. In addition, very high stability is required to ensure that the nanobodies survive the production processes and years of storage without deteriorating. They also need to be produced in larger quantities – as a minimum on a kilogram scale.

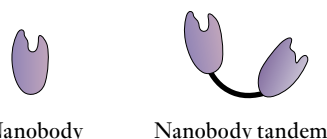
The researchers vaccinated Britta, Nora, and Xenia with a crucial part of the SARS-CoV-2 spike protein: the receptor-binding domain used by the coronavirus for invading its host cells. The immune system of the alpacas responded promptly and started producing antibodies against the virus. The team then collected blood samples and isolated the blueprints for more than a billion different nanobodies. Using the so-called phage display technique, they subsequently selected from this huge library the molecules that bind most strongly to the receptor-binding domain of the virus.

But which of these is most effective at neutralizing the virus and stopping an infection? The team of virologist Matthias Döbelstein, Director of the Institute of Molecular Oncology at the University Medical Center Goettingen, infected cell cultures with SARS-CoV-2 in the laboratory and tested how well the different nanobodies blocked virus replication. "The lower the neutralizing concentration of the nanobody is, the lower the risk of side effects will be,"

Görlich explains. The most effective nanobodies could block SARS-CoV-2 infection even at concentrations of less than one millionth of a gram per liter. The researchers then engineered these nanobodies to remain stable at temperatures of up to 95 °C without aggregation. Such a high level of stability makes the nanobodies easier to manufacture, process and store, as well as safer to use.

Tandems against variants

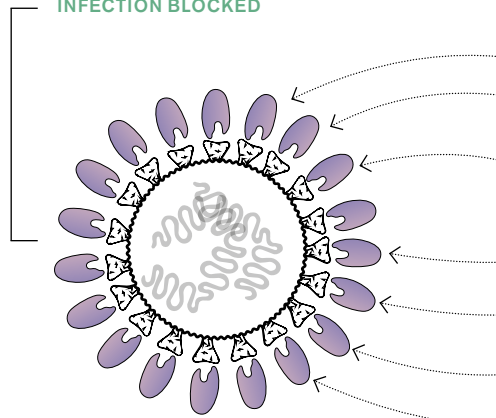
But then variants of the virus appeared that were even more infectious than the original strain. In addition, their spike protein had changed to make it



Nanobody

Nanobody tandem

NEUTRALIZED VIRUS:
INFECTION BLOCKED



SUMMARY

Compared to standard antibodies, nanobodies have a simpler structure and are easier to isolate and produce in large quantities for medical use.

The recently developed anti-SARS-CoV-2 nanobodies neutralize the virus even in minute amounts, are effective against new viral variants, and can be rapidly adapted to new SARS-CoV-2 strains.

Nanobodies could also be used for treating sepsis or snakebites.

harder for existing antibodies to neutralize the pathogen. Therefore, Dirk Görlich and his team fused two nanobodies that simultaneously recognize different regions of the receptor-binding domain. “These tandems bind so strongly that they can tolerate the new ‘immune escape’ mutations of the virus. This strategy worked perfectly with the alpha, beta, gamma, and delta variants of the virus,” explains Thomas Güttler, a scientist in Görlich’s team.

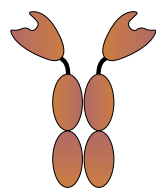
“By that time, we had re-immunized the alpacas with spikes from the viral variants and selected nanobodies that, already as monomers, can potentially neutralize not only the original Wuhan strain but also alpha to delta,” reports Metin Aksu, another scientist

in the team. The now dominant omicron variants are yet another challenge since they carry 15–17 mutations in their receptor-binding domains, ≥ 12 more than any previous variant of concern. It now seems that two distinct nanobodies are needed to potentially target all known variants. The Goettingen team has already isolated the first generation of anti-omicron nanobodies and is now optimizing a second set, which they obtained after boosting the alpacas twice with a specific omicron vaccine.

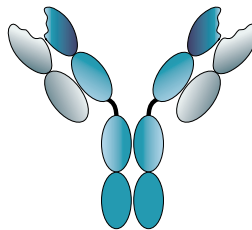
The only problem that remained was to produce these nanobodies at an industrial scale. A few milligrams of the mini-antibodies suffice for laboratory research, but kilogram amounts would be needed to make

a difference in the pandemic (and the foreseeable endemic) situation. Indeed, such a goal is feasible with the help of genetically re-programmed yeast cells and industrial-scale bioreactors.

But even if your laboratory team has succeeded in developing a potential drug to combat one of the worst epidemics in recent history, that doesn’t automatically pave the way to clinical trials. “I was a bit naïve” says Görlich. Initially, the intention was to start a spin-off company of the Max Planck Society to develop the nanobodies as drugs for patients and then test them on volunteers. However, it proved impossible to raise the necessary funding in a short period of time, even the major pharmaceutical



Alpaca antibody



Conventional antibody

Upper: Typical antibody proteins consist of two heavy and two light chains.

By contrast, the simpler antibodies of alpacas have only two heavy chains. Their antigen-binding domain can be separately produced and is then called “nanobody”.

Lower: The spike protein on the surface of the SARS-CoV-2 binds to the ACE2 receptors of the host cells and subsequently fuses the viral membrane with the plasma membrane, allowing entry of the viral RNA and thus infection. Nanobodies can block the spike protein, thereby neutralizing the virus and preventing infection.

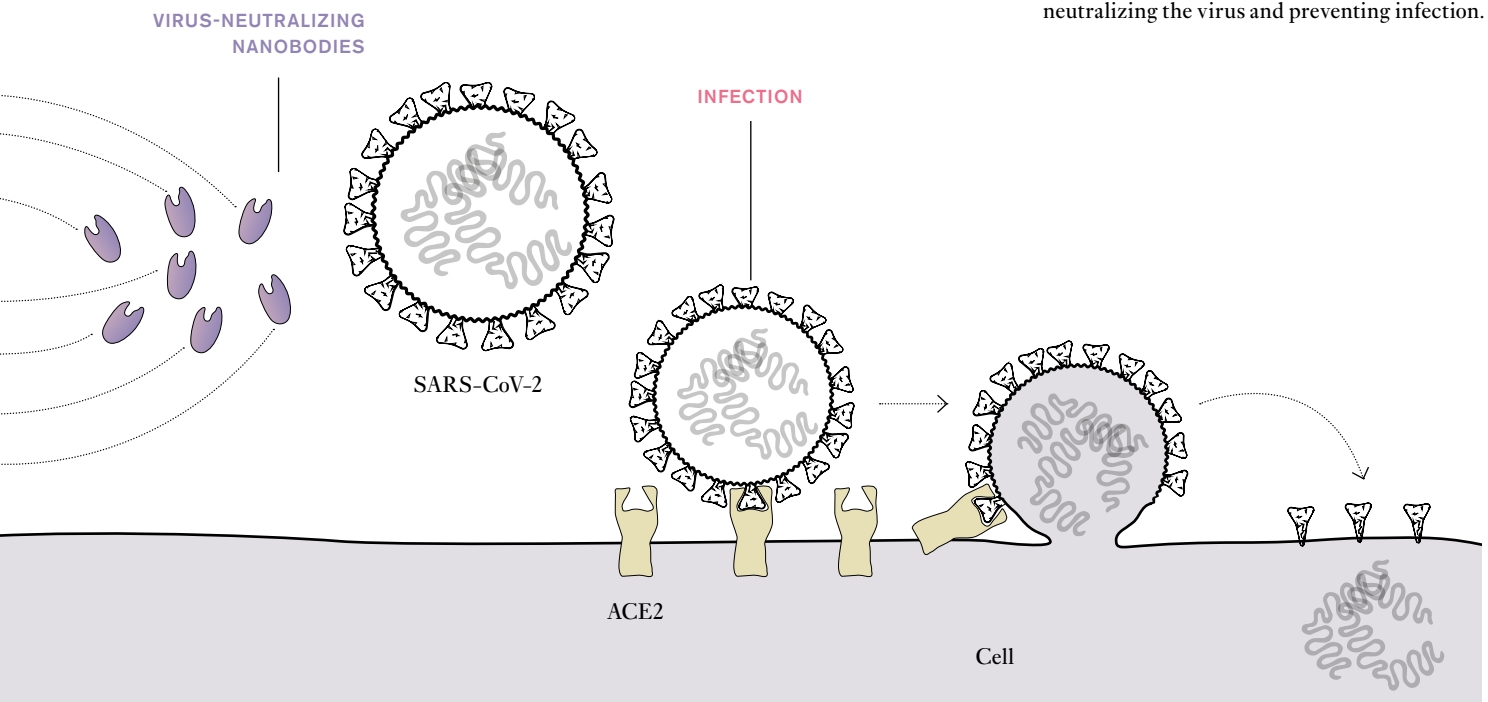




PHOTO: MICHAEL DANNENMANN THE MPG

In the Max Planck Institute in Goettingen the nanobodies are adapted to the spike protein of the coronavirus so that they can block different variants of the pathogen (left: Jürgen Schünemann and Kathrin Gregor, right: Waltraud Taxer (rear) and Renate Rees).

64

companies were very cautious. The industry's hesitancy was partly due to the fact that using nanobodies as drugs is still a very novel concept. Only one product has, as yet, made it to the market – a drug to treat a rare thrombotic disease.

Arduous path to drug development

It was only at the very last moment that the team was able to find a company willing to undertake the clinical development, an Israeli biotech venture. "The negotiations were rather tedious and complicated. We learned that the process of developing a drug follows its own set of rules," says Dirk Görlich. His team is being supported by technology transfer experts from Max Planck Innovation and the Lead Discovery Center. The Max Planck Foundation is providing financial support for the project.

Encouraged by the ultimately positive experience, the Goettingen researchers are now looking to develop nanobodies to treat sepsis. Commonly

referred to as blood poisoning, such systemic bacterial infection is often fatal, and, in fact, the most common cause of death amongst hospitalized patients. Widespread antibiotic resistances adds to the problem. And even if antibiotics are effective against the bacteria, they cannot eliminate the already secreted bacterial toxins. "We are now aiming to develop nanobody cocktails that target and block the toxins of the most dangerous bacteria," says Dirk Görlich. Nanobodies could also be used for treating venomous snakebites, which cause around 100,000 fatalities per year worldwide. Until now, anti-venoms are produced by injecting snake toxins into horses and then isolating antibodies from their blood. This is a mature (>100 years old) technology but comes with severe adverse effects in patients and with hardly acceptable animal welfare problems.

Nanobodies would provide an excellent alternative strategy. From an exotic antibody of an exotic laboratory animal to novel drugs – the development of this new antibody technology would never have been possible without basic research.

GLOSSARY

NANOBODIES

Fragments of antibodies with a particularly simple structure, which the immune system of camelids (for instance, alpacas, dromedaries, and llamas) produce against pathogens. Unlike typical antibodies, nanobodies are composed of only a single amino acid chain. This makes them more stable and easier to manufacture. They are also more effective at recognizing hidden binding sites of other proteins. Nanobodies are therefore being considered for a broader use in medicine.

PHAGE DISPLAY

A molecular biology technique to isolate nanobodies (or other "binders") from "immune libraries". Each nanobody of a library is directly coupled to its "own" DNA-encoded blueprint. This is achieved by packing the DNA blueprints into "phages" (viruses that infect bacteria) which then express and display DNA-encoded nanobodies on their surface. Such a library is then passed over an immobilized antigen surface. Phage nanobodies with a high affinity for the antigen bind to it and are isolated, while irrelevant binders are washed away.





Irresistible at heart

In 2020, Reinhard Genzel received the Nobel Prize in Physics for his research on the black hole at the heart of the Milky Way.

We support his project GRAVITY+ at the Max Planck Institute for Extraterrestrial Physics, because with the world's largest virtual optical telescope he wants to pin down the galactic gravity trap and write a new chapter in astronomy.

The Max Planck Foundation has supported the Max Planck Society for more than fifteen years by providing targeted funding for top-level innovative and cutting-edge research at the more than 80 institutes, enabling breakthroughs in frontier science. As a patron, you can make a crucial difference by creating additional scope to keep this research ahead of the curve in the international scientific competition. Join us!

**Max-Planck-Foundation
Deutsche Bank
IBAN DE46 7007 0010 0195 3306 00**

ADVERT

www.maxplanckfoundation.org



MAX PLANCK
Förderstiftung