

COULD THE BRAIN OF A TINY FISH HELP SPARK A BIG ADVANCE?

By Ty Burke
Photographs by Boaz Perlstein

NOVEL NEUROSCIENCE RESEARCH EXPLORES THE POTENTIAL OF PSILOCYBIN FOR TREATING DEPRESSION

For somebody with depression, everyday life can be a struggle. It is among the most common mental health disorders worldwide, affecting more than 280 million people. Depression can negatively impact personal relationships and work performance. In severe cases, it can be debilitating and contribute to suicide.

Depression is commonly treated with a class of antidepressants called selective serotonin reuptake inhibitors (SSRIs). These drugs have been in use since the 1980s and have helped countless patients cope, but their side effects can make them intolerable. And even though SSRIs have been around for decades, we still don't know much about how they actually work.

Weizmann Institute of Science neuroscientist Takashi Kawashima, who is based in the institute's Department of Brain Sciences and was an Azrieli Early Career Faculty Fellow until 2022, is exploring whether an entirely different kind of drug could yield similar benefits, without the unintended consequences of SSRIs. His research is probing the potential of a psychoactive chemical called psilocybin, which is better known for inducing hallucinations (see "A Brief History of Psilocybin," page 11). Kawashima is investigating whether psilocybin can mimic serotonin's effects in the brain and, if so, which mechanisms it uses. In the long run, this could help lead to the development of depression treatments that are more effective and faster acting than the drugs that doctors rely on now.

Serotonin is a chemical that functions as a neurotransmitter, a kind of messenger that transmits signals. A deficit of serotonin in the brain is thought to be a major underlying factor in depression. This is called the serotonin theory of depression, and it is widely accepted, although we don't know *how* serotonin deficiency actually leads to depressive symptoms.

Commonly prescribed SSRIs such as Prozac and Zoloft work by increasing the amount of serotonin in the brain. But these drugs have distinct drawbacks. In addition to mood regulation, serotonin is also involved in digestion, sleep, learning and sexual drive. When serotonin levels are increased, any of these functions can be affected. For some people, nausea and diarrhea make SSRIs intolerable.

Beyond that, SSRIs can take several weeks to have an impact. They work by blocking the brain's reabsorption of serotonin, which increases the amount of serotonin that's available. But this change takes time, so patients don't immediately know whether a prescription is working, and identifying the right regimen is a trial-and-error process. It often takes months for people to find a tolerable balance between recovery from depressive mood and the side effects of SSRIs. Some never do.



Zebrafish have few obvious similarities with humans, but their serotonergic system is one of them. All mammals are descended from fish, and this is one of the features we retained. And because zebrafish are transparent, this makes zebrafish an ideal research model for the *in vivo* imaging that Takashi Kawashima does to better understand brain activity in living animals.



Kawashima, a former Azrieli Early Career Faculty Fellow at the Weizmann Institute of Science, is analyzing a rich trove of data detailing the effects of psilocybin on zebrafish.

Psilocybin is different. It doesn't increase the amount of serotonin but appears to act as a serotonin mimic. Because psilocybin causes changes in the brain's function, treatments derived from it could work more quickly than SSRIs with fewer side effects. We don't know how psilocybin produces these effects either, but that's one of the things Kawashima is working on. And he is using an innovative new model to understand psilocybin: the tiny zebrafish (*Danio rerio*).

Kawashima's research sits at the intersection of neuroscience and computer science, and it builds on knowledge that he began developing while studying to become a medical doctor at the University of Tokyo. In Japan, his programming skills weren't useful at medical school, and although he enjoyed learning about psychiatry and neurosurgery, he was much less enthused about the prospect of working in a hierarchical hospital environment.

Instead, Kawashima pursued a PhD in neuroscience, and during his studies he began learning about *in vivo* imaging — using technology to study brain activity in living animals. He didn't start with psilocybin or zebrafish, though. Kawashima initially explored the neocortical (or outer brain layer) mechanisms of learning in rodents. Seeing limitations in rodents, he changed his model animal to zebrafish, focusing on the role of serotonin in learning. He

established his laboratory at Weizmann to continue basic research on serotonin, and it seemed as if this work would never cross paths with his medical past — until psilocybin began to re-emerge as a promising antidepressant.

Zebrafish have a highly unusual trait: they are transparent. And even though they have few obvious similarities with humans, our brains have a few things in common. The serotonergic system is one of them. All terrestrial vertebrates — including mammals — descended from fish. Over hundreds of millions of years of evolution, mammalian brains grew larger and more complex, but they retained some aspects of their distant ancestors' brains. One of these is the subcortical area — part of the central nervous system that encloses the brainstem and cerebellum, below the cerebral cortex — where serotonin is produced. Because of this, findings about how psilocybin affects fish can help us understand how it works in humans.

“The subcortical part of the brain is hidden beneath the neocortical area. That's where serotonin molecules controlled mood in our ancestors and still should in us,” says Kawashima. “This is a primitive and reactive area of the brain, but it is almost impossible to study it in mammals. That's why I chose to work with zebrafish. Because they are transparent, we can see the entire brain at once, including its serotonin system.”

‘In neuroscience, there are scientists who look at the molecular level, and there are scientists who think of the brain as a system with computational principles. Historically, there has not been very much research that bridges them, but Takashi does this. He makes observations about the molecular machinery and uses them to look at the whole network as a complete system, and then uses computational techniques to understand what’s happening.’

Neuroscience research often uses rodent models because mammal brains have anatomical similarities to humans. But in this case, that's exactly what gets in the way. Like human brains, mouse brains have outer layers — the neocortex — that are relatively easy to access with imaging technology. The majority of neuroscience research is focused on this area; it's where many of the human brain's high-level functions are performed, including language learning, sensory perception and cognition. Other important functions happen deeper in the brain, and they are much less studied.

To see what happens when zebrafish are given psilocybin, Kawashima puts them in a small water chamber and adds a pure form of psilocybin to the water. The fish ingest the psilocybin as they breathe; water is taken in through their mouths, and it passes it through their gills. And then they are put in a test arena under a camera.

This is not your average camera. Zebrafish swim fast; their acceleration rivals a Formula 1 racing car if scaled to that size. Interpreting every single aspect of their motions is necessary to decipher their emotional states. So even though Kawashima's experiments look simple, with tiny fish swimming around a palm-sized dish, his camera captures millions of pixels at the

A BRIEF HISTORY OF PSILOCYBIN

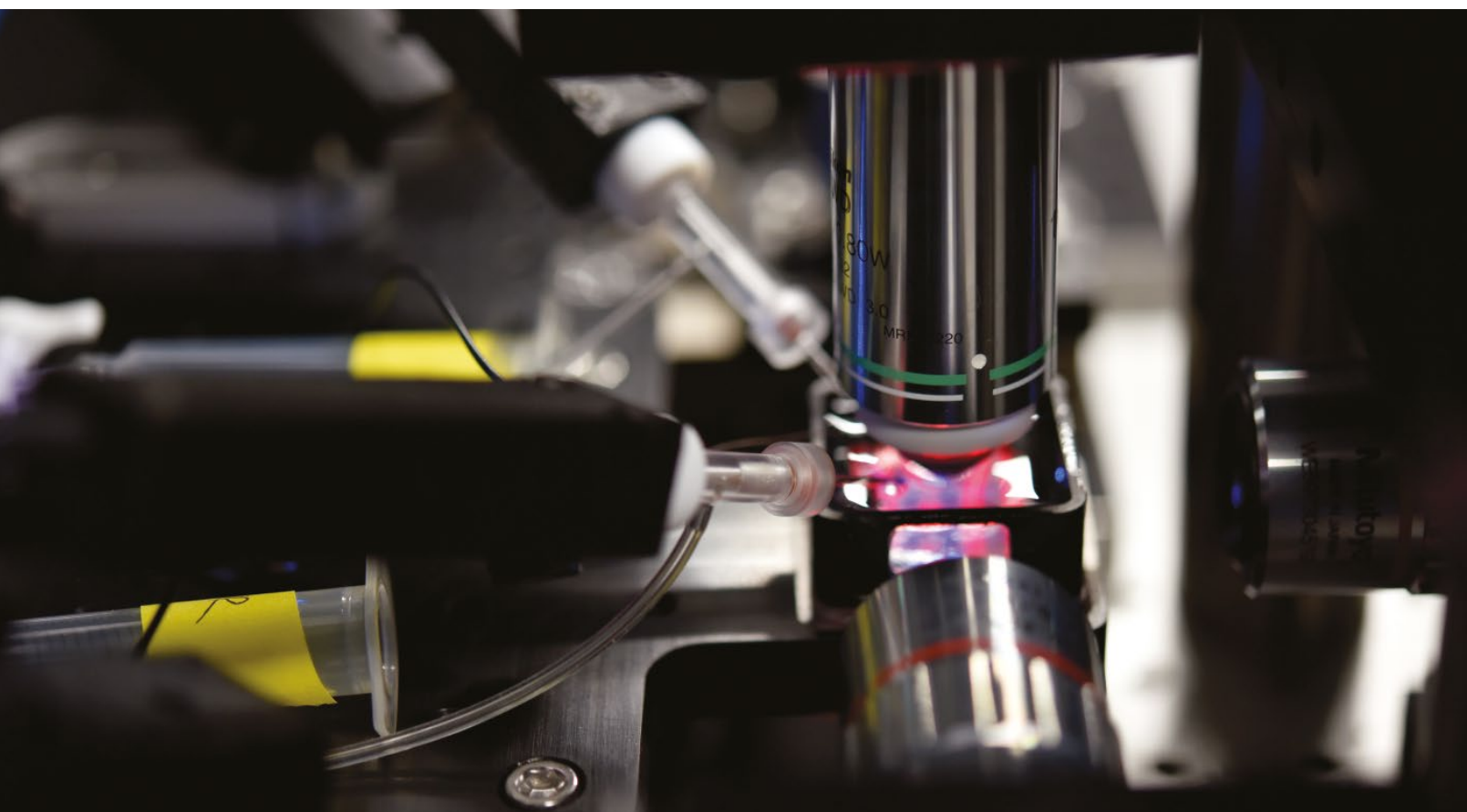
Psilocybin occurs naturally in more than 200 species of mushrooms. Their capacity to produce visual and auditory hallucination led to recreational use beginning in the 1950s. But they've been known for much longer. Indigenous peoples have used psilocybin-containing mushrooms for ceremonial and healing purposes for centuries.

These unusual fungi first piqued popular curiosity in 1957, when an American banker and amateur mycologist named Robert Gordon Wasson travelled to Mexico and consumed psilocybin mushrooms as part of a traditional Mazatec ceremony. They were not criminalized at the time, and the story about Wasson's experience made the cover of *Life* magazine, then one of the most widely read publications in the United States. *Life's* banner headline read “Seeking the Magic Mushroom,” and the name stuck. Today, many know psilocybin mushrooms by this moniker.

Psilocybin mushrooms went on to become part of the cultural zeitgeist of the psychedelic 1960s, with some scientists recognizing that their ability to shift perception could have therapeutic uses. But academic research was stunted in 1971 when the United Nations Convention on Psychotropic Substances was signed. Magic mushrooms were criminalized, and research paused for decades.

Today, psilocybin mushrooms are making a comeback. Academic research has resumed in several countries, and the rules are beginning to change. Recent studies have already yielded promising findings about psilocybin's ability to treat depression and alcohol addiction. It has also been used to reduce anxiety about death in the terminally ill. ▲●■





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The zebrafish larvae that Kawashima inspects under a microscope (above left) are roughly four millimetres long. Before Kawashima positions fish under his microscope (above), he attaches a pair of electrodes to the tail of each zebrafish to record its swim signals; these signals are used to infer zebrafish behaviour during brain scans. Kawashima uses a bespoke light-sheet microscope (left) to observe the activity of individual neurons across the brain in live zebrafish.

speed of several hundred frames per second, which amounts to a terabyte of data in half an hour. Kawashima analyzes these data with an artificial intelligence module that he programmed. And he has seen that the psilocybin appears to be increasing brain activity.

“When a fish is given psilocybin, it has a stimulatory effect, and its swimming patterns change,” explains Kawashima, whose AI algorithm identifies these changes. This appears to align with the stimulatory effect that psilocybin has in humans, but he doesn’t know yet exactly what is happening when this occurs.

Kawashima made another observation, one that aligns with serotonin’s affects in humans. Psilocybin seems to reduce zebrafish anxiety. “It is possible to induce anxiety in zebrafish by dropping the temperature of the water around them,” he says. “When this happens, the stressed fish typically begin swimming in a zigzag pattern. But fish that are given psilocybin are not affected by the stressors. This indicates that the psilocybin is potentially working in the fish’s brain in a way that is similar to its effect on the human brain.”

To further this research, Kawashima’s lab has developed a technology called whole brain neural activity imaging, or PyZebroscope. This is an open-source platform that can record the neural activity from every single neuron in a zebrafish’s brain. For this experiment, he uses fish that have been genetically modified so that they produce a protein when their neurons fire that allows for fluorescent capture. Kawashima uses the technology to determine the effects of psilocybin, but the approach could be used to image other aspects of subcortical function, such as locomotion and movement.

Kawashima’s work also helps bridge areas of neuroscience research that often functioned separately. “In neuroscience, there are scientists who look at the molecular level, and there are scientists who think of the brain as a system with computational principles,” says Rony Paz, one of Kawashima’s neuroscience colleagues at Weizmann. “Historically, there has not been very much research that bridges them, but Takashi’s research does this. He makes observations about the molecular machinery and uses them to look at the whole network as a complete system, and then uses computational techniques to understand what’s happening.

“Until recently, we didn’t have an animal model that allows these observations, but the fish model does. Moreover, the more models we have, the better. It is the only way to do neuroscience. You need to find the right model for the questions you have.”

But even though the zebrafish model reveals much more about the inner workings of the brain, it will take time to understand the results. Brain function is complex, and Kawashima has begun collaborating with researchers who use rodent models to build an understanding of how his findings connect with the data they have collected about the neocortex.

“We need to figure out the underlying neural mechanism, and that could take years,” he says. “Think of the brain’s serotonin system like a giant tree with many branches. There are so many possible pathways, and it’s very difficult to know which are most important. The brain is combinatorial, and the serotonin system doesn’t work alone. Finding the parts of the brain it works with is not a trivial task because there are thousands of possible combinations. But because we have the ability to scan the entire brain, maybe we can reveal the right ones.” ▲●■