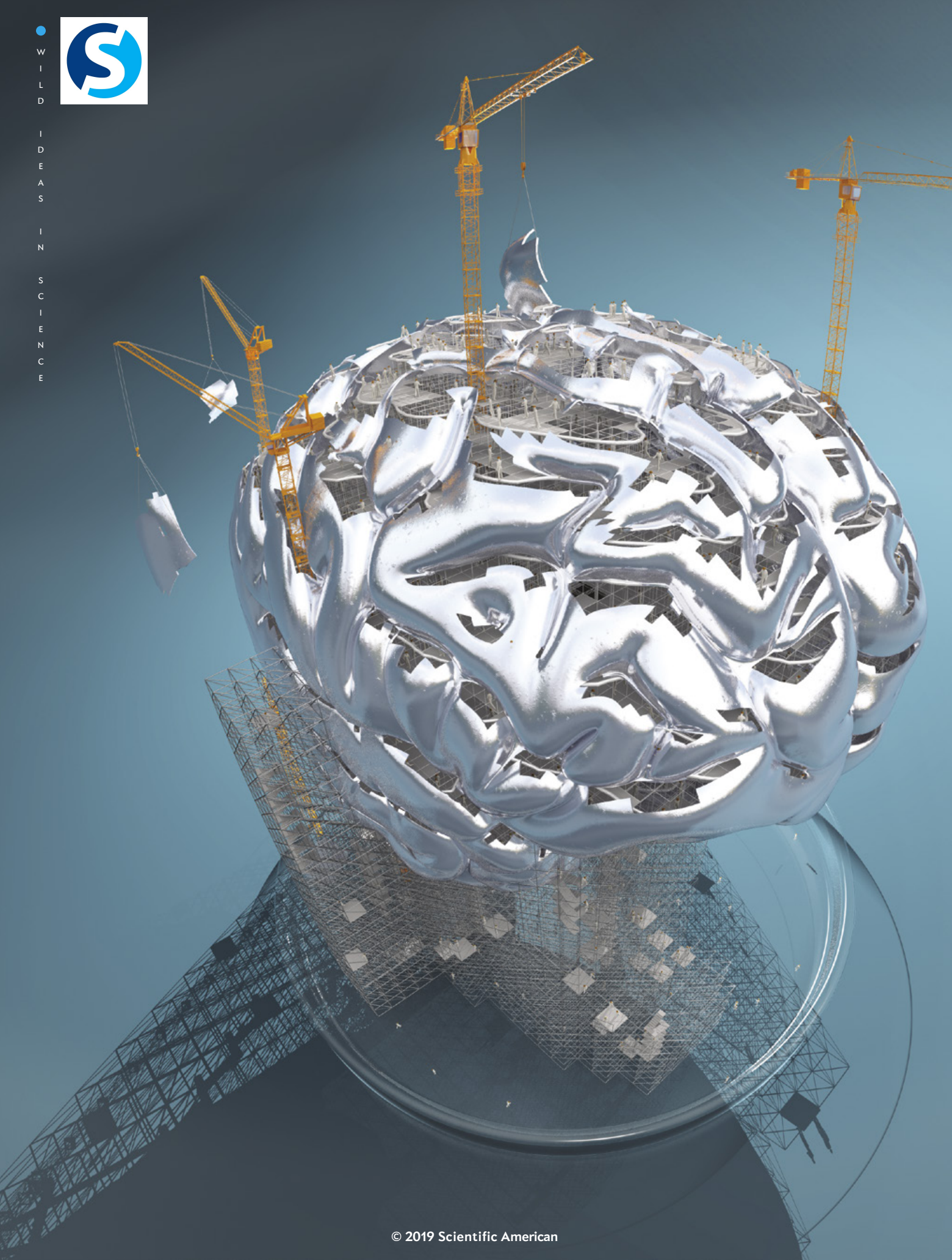
WILD IDEAS IN SCIENCE



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"What I cannot create, I do not understand."
—Richard Feynman, 1988

LAB-BUILT BRAINS

Scientists copy nature's most complex organ in the hope of solving the mysteries of brain disorders, from autism to Alzheimer's

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Illustration by Bryan Christie

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EVERYTHING THAT MAKES US HUMAN IS LOCATED WITHIN 1.4 KILOGRAMS of yellowish tissue composing the human brain. It is here that our thoughts develop, here that we feel love or hate, and where the most creative and most evil ideas of humankind arise. This walnut-shaped structure is also the most complex organ nature has generated. The brain harbors about 86 billion neurons, or nerve cells, that have to be born at the right time, migrate to the right place, and wire up in the right way if we are to survive and thrive.

Understanding exactly how the human brain develops and functions is the greatest challenge of modern biology. Most of what we have learned about the organ since the birth of neuroscience more than 100 years ago derives from experiments done on animals—frequently mice or rats. Scientists could justify this approach because mice and humans share a common brain architecture: they have many of the same types of nerve cells and rely on essentially the same parts of the brain to carry out shared mental processes. But humans and rodents differ in one key way. Whereas the mouse brain has a smooth surface, the human brain is highly folded.

To nonscientists, this difference might seem trivial. But neurobiologists believe that the folding makes a world of difference to human brain function. It allows for many more neurons to be placed within the same volume and is also a prominent feature of all "intelligent" animals, such as monkeys, cats, dogs and whales. Evolutionary biologists have shown that folding arose from another difference between mice and people: neurons in many parts of the brain arise from a specific set of precursor cells that exist only in minute numbers in mice.

Such differences may explain why many common genetic mutations responsible for severe neurological disorders in humans have little effect when bred into mice by researchers trying to study the mechanisms of human diseases. If the mutations affect the development or maintenance of proper human brain architecture or the functioning of cell types that are common only in humans, then the studies would be doomed to failure. In fact, the unique characteristics of the human brain may be one of the reasons that rodent studies have yielded no effective therapies for such brain disorders as schizophrenia, epilepsy and autism.

Recognition of the differences between mouse and human brains has spurred a hunt for more informative ways to conduct neuroscience experiments. Recently my laboratory has come up with an exciting approach: growing the largest part of the developing brain in miniature in a lab dish. These brain structures, called organoids, give neuroscientists a model of the human brain that should provide information they cannot obtain by running studies in mice. Researchers can observe what happens when the brain-in-a-dish, or mini brain, is exposed, for example, to the Zika virus, which can disrupt brain development in fetuses of infected women, or when an organoid is genetically engineered to mimic a brain afflicted with a neurological disease.

BRAIN-IN-A-DISH (SORT OF)

MY LAB BEGAN WORK on organoids in 2012, when Madeline A. Lancaster, then a postdoctoral scientist in the group, devised a way to replicate in a culture dish the essential processes that lead to brain formation in a human fetus during the first roughly 10 weeks of development [*see box on opposite page*]. Our procedure relies on human cells known as stem cells, which exhibit a remarkable feature called pluripotency. Pluripotent stem cells are the same type of cells found in the early embryo. When cultured under the right conditions, they can give rise to any kind of tissue, be it nerve, muscle, blood, bone or any other type. In the fetus, these new cells retain their pluripotency for only a few days. But using special lab cultures, researchers can preserve them in this state permanently and ultimately turn them into almost any desired cell type.

To start, we culture the cells in a liquid containing all the nutrients needed for growing the neuroectoderm, the part of a fetus that forms the nervous system. When the cells aggregate into a ball called an embryoid body, we embed the ball in an amazing substance called Matrigel. This gel, produced by cultured cells that were isolated from a mouse cartilage tumor,

IN BRIEF

Knowledge about the human brain often derives from experiments performed on mice, rats or other animals. Brains of these species share much in common with the human organ, but they lack a highly folded surface, a difference that affects neural functioning.

Unique qualities of the human brain may help explain why rodent studies have failed to yield new treatments for brain disorders ranging from schizophrenia to Alzheimer's disease. That has spurred a search for new ways to conduct neuroscience experiments.

One alternative entails growing the largest part of the developing brain in a laboratory dish. These "organoids" most likely will give brain scientists information that cannot be obtained from mouse studies; they are already being used in investigations of the Zika virus.

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Grow Your Own

The technology that coaxes stem cells to develop into different types of biological tissue has now been used to grow a part of the brain that contains the cortex and other structures and is responsible for such higher mental functions as processing information from the outside world, forming memories and making decisions. To create such a mini brain, researchers give a tiny ball of cells nutrients and a bed on which to grow; then the cells recapitulate much of the developmental process that occurs in the early embryo.

1 The procedure begins with embryonic stem cells or induced pluripotent stem cells capable of turning into any cell type in the body. The latter cells can be derived from adult skin or blood cells that have been genetically altered.

Embryonic stem cell

Reprogramming of adult cell

Adult skin cell

2 Days 0–5: The cells divide and aggregate into balls called embryoid bodies. Within three days, those cells start forming three distinct layers: ectoderm, mesoderm and endoderm.

Different cell types

Embryoid bodies

3 Days 6–10: Embryoid bodies, after being placed in a liquid containing the nutrients for the part of the fetus that forms the nervous system (the neuroectoderm), begin to cluster into layers that form the embryonic tissues that give rise to the human brain.

4 Days 11–15: Tiny balls of neuroectoderm are embedded in Matrigel—a medium rich in chemicals that stimulate cells to divide, prevent them from dying and provide an environment that supports growth of budlike appendages, a prelude to development of fully formed brain structures.

Matrigel droplet

Neuroectoderm tissue

Budlike appendage

Maturing cells

5 Days 15–30: Matrigel droplets are transferred to a spinning bioreactor or a device known as an orbital shaker. In the gel, the embryoid bodies grow into brain organoids—three-dimensional, white balls of tissue that resemble the forebrain of a growing human fetus. The organoids can be used to study brain development and disorders that occur early in life.

Spinning bioreactor

Brain organoid

Analogue

10-week-old embryo forebrain

Outcome: After a month of nurturing the stem cell concoction, the cultures are strikingly similar to the forebrain of a 10-week-old embryo. This brain region includes the cortex (the large, folded outer structure) and the choroid plexus (the region that generates cerebrospinal fluid).

Fully formed forebrain

SOURCE: "GENERATION OF CEREBRAL ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS," BY MADELINE A. LANCASTER AND JUERGEN A. KNOBLICH, IN *NATURE PROTOCOLS*, VOL. 9; OCTOBER 2014

Illustration by Tami Tolpa

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resembles the membrane on which cells sit in the fetus. Matrigel, which is rich in factors that both stimulate cells to divide and prevent them from dying, provides a scaffold that is stiff enough for cells to grasp but malleable enough to be modified by the cells, which in turn alter its shape.

The outcome of these experiments has been truly spectacular. Left to their own devices in the gel, the embryoid bodies grow into three-dimensional, white balls of tissue that resemble the embryonic human brain. Exposed to the proper chemical signals that trigger fetal brain development, stem cells grow into exact replicas of the human forebrain, the region responsible for higher mental functions. It includes such components as the cortex (the large, folded outer structure) and the choroid plexus (the region that generates cerebrospinal fluid). We also find other structures that guide cells to their proper place in the developing brain. The medial and lateral ganglionic eminences, which perform this function, assist in giving rise to cells that generally tamp down neural activity (interneurons) and the hippocampus, which is involved in memory formation.

WE HAVE DEMONSTRATED TECHNICAL FEATS WE COULD ONLY DREAM OF A FEW YEARS AGO.

Cells in a growing organoid arrange themselves identically to those in the brain of an eight- to 10-week-old human fetus. In rare cases, the organoids even grow small eyecups, indentations in the tissue that contain colored pigments, much as occurs when the human eye begins to form. Also, as happens in a developing brain, the cells divide and give rise to the kinds of nerve cells found in an embryo. And the nerve cells send out axons—long cables that make contact with other neurons to form an active signaling network. Before forming these networks, the neurons migrate from one area to another, much in the way they do in the fetus, potentially providing clues to what happens when neurons end up in the wrong place, as they often do in psychiatric disorders.

ON THE SHOULDERS OF GIANTS

THE IDEA OF BUILDING TISSUES in culture is not really new. As with most scientific discoveries, the current organoid boom relies on years of pathfinding research, some of it dating back more than a century. Already in 1907 zoologist Henry Wilson had demonstrated that certain lower animals, such as sponges, can put themselves back together after being broken up into single cells, an indication that the brain is endowed with a program for assembling its myriad parts.

In 1939 Johannes Holtfreter discovered that the various cells in a frog embryo will seek one another out and regenerate their shape even after they have been completely separated. During the 1980s this finding led to a huge boom in "reaggregation" studies, in which complex animal organs such as the retina and even the cortex were formed in the lab by bringing together their diverse cell types.

Building on early reaggregation experiments conducted from 2006 to 2010, the late Japanese scientist Yoshiki Sasai of the RIKEN Center for Developmental Biology pioneered the use of pluripotent stem cells for growing nervous system tissue, most notably the human retina. In fact, our brain organoid technology merged his techniques with groundbreaking work by Hans Clevers of Utrecht University in the Netherlands, who combined stem cells with Matrigel to establish a culture system that can be used for growing gut, stomach, and even liver and pancreatic tissue.

Beyond drawing lessons from these earlier studies, our work makes use of recently developed technologies that are dramatically turning the entire field of biomedical research upside down. One called reprogramming was developed by Japanese Nobel Prize laureate Shinya Yamanaka of Kyoto University. Through a simple set of genetic manipulations, reprogramming turns body cells that have already fully matured back into pluripotent stem cells—and it can do so for virtually any cell, from skin to blood cells. Stem cells from a sample of skin or blood can then be transformed into various types of brain cells, and those cells can then be grown into organoids. The approach can thereby avoid the need to use cells derived from embryos.

Reprogramming allows an organoid grown from the cells of a patient with a genetic disorder to be compared with ones from a healthy individual to ferret out underlying causes of a disease, because the genetic defect in the patient's cells should afflict the organoid much as it affects the developing fetus. In fact, we have already used the organoid technology to gain insight into microcephaly, in which patients are born with a brain of severely reduced size. We found that organoids grown from cells of a patient with microcephaly are much smaller than normal. Because we can grow the patient's cells in unlimited numbers, we can now undertake detailed analyses of the chain of molecular events that leads to microcephaly in a developing fetus. Much the same should be true for other brain disorders: using patients' cells to grow organoids may enable neuroscientists to better understand the defects in brain formation that underlie schizophrenia, epilepsy and other diseases that are difficult or impossible to study in animals.

Organoids derived from the reprogrammed cells of individuals who are not ill can also be useful. Indeed, they were put to good use during the Zika epidemic, which has been blamed for causing microcephaly in a number of babies born to women infected during pregnancy. Multiple labs working on organoids, first in Brazil and then in the U.S., have now established that the virus can lead to microcephaly—a link that would have remained hypothetical were it not for this new technology. When organoids are infected with the Zika virus, their nerve cells die and the resulting organoids are much smaller than their uninfected counterparts, much like the ones we have grown from our microcephaly patient.

Organoids most likely will help with research on other viruses as well. Multiple viruses, such as cytomegalovirus or herpes simplex virus, cause brain defects when infections occur during pregnancy. By growing organoids and infecting them with different viruses, we can try to understand why they cause damage, what they have in common and how the damage mechanisms differ from one another. Ultimately organoids may be used to identify the docking points, or receptors, used by the viruses to gain entry to cells—and they may be critical for test-

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ing potential antiviral drugs before moving them into clinical trials with patients.

A second technique propelling the use of organoids is genome engineering—a collection of methods that allows researchers to alter a cell's genetic code. Organoids engineered to incorporate mutations suspected of causing disease can enable researchers to determine whether the genetic defects actually do lead to illness. Investigators may someday be able to evaluate whether repairing those mutations would generate healthy organoids; if so, the work could lead to new treatments that counteract the mutations' effects.

Neuroscientists are eager to explore still other applications of mini-brain technology, such as drug development. The technology can assess whether new medications affect brain tissue in desired ways, obviating the need for animal testing and thus saving on the costs of drug development. The organoids can also let scientists identify unwanted effects on the developing human brain, thereby preventing drugs that would be harmful during gestation from ever reaching a pregnant woman. If the notorious drug thalidomide, which disrupts the developing brain early in pregnancy and causes other birth defects, had been tested in this way, it presumably would not have been prescribed for morning sickness in the late 1950s and 1960s.

Organoids are becoming an invaluable tool for evolutionary biologists. They can be used to identify genes responsible for the enormous size of the human brain compared with other primates. Contrasting human and primate genomes has already identified genes that might be responsible for cognitive functions, such as language, that are unique to humans. Understanding the workings of these genes has remained largely a matter of speculation. Now researchers have already generated organoids from chimpanzees and macaques and compared them with their human counterparts to identify key differences. Organoid technology should eventually allow us to test the role of those differences by replacing human genes with their monkey counterparts and studying the effects on organoids.

SHOULD WE BE AFRAID?

THE IDEA OF GROWING a human brain in a dish is sure to make some people squeamish. Movies such as *The Matrix* come to mind that evoke fantasies about lab-grown brains developing thoughts or even personalities. These are needless fears. The probability that a lab-grown brain will develop a mind of its own is nil. An organoid is not a "humanoid" in a jar and will not be one even in the far future. Any conscious being needs to be able to process information from the senses to develop an internal mental model of reality. Organoids are neither able to see nor hear and lack any sensory input. Even if we were to connect them to a camera and a microphone, the incoming visual and auditory information would still need to be translated into a form that could be understood by these brain cells in a dish—and, as things stand, providing that translation is an insurmountable technical challenge.

Organoids are not functional brains, only lumps of tissue that imitate the molecular and cellular functioning of the organ at spectacular levels of detail. They are similar to pieces of tissue removed during brain surgery, not conscious beings.

Still, growing an organoid does raise certain ethical and legal issues. All organoids derive from cells taken from individuals who have certain legal rights. As such, performing this work in the lab must conform to the same set of legal and ethical procedures used for samples taken from patients in any industrial country. Patients, of course, must give permission before their cells can be used for research. The same set of rules applies with organoids. But even when the benefits are clearly explained, donors may not at first feel comfortable with the idea of having their cells cultured into brainlike structures.

WHAT NEXT?

THE BENEFITS of this cellular technology outweigh any possible downside. Cerebral organoids have laid the foundation for performing realistic medical and toxicology experiments in human tissue, without the need for animal experiments. Even so, I and others would like to improve them. For instance, the current generation lacks blood vessels. That absence is not a problem during the early stages of organoid development, but over time cells start dying from lack of oxygen and nutrients. In theory, it should be possible to provide blood vessels, either through new 3-D-printing techniques or by growing them from stem cells. Blood vessels are known to grow into the brain, a process that could potentially be recapitulated with a 3-D culture.

In another challenge, we want to make organoids that, in common with an actual brain, have front-to-back, top-to-bottom and left-to-right axes. Unlike a real embryo that has clearly defined body axes, organoids lack a front-to-back and head-to-tail axis. As a result, they develop randomly, so that their individual parts have different orientations. In the developing brain, complex signaling systems give a brain its sense of up versus down—and these same chemicals may ultimately do so for organoids as well. Modern biotechnology methods can generate tissue cultures in which the chemicals needed to spur cell growth during development are present. These techniques may eventually result in the formation of organoids with a forebrain on one end and the hindbrain at the opposite end.

We have already begun to push forward to begin to look for ways to overcome these barriers. We have demonstrated technical feats that we could only dream of a few years ago. Organoids are already helping to achieve a better understanding of disease and are assisting in developing drug candidates. The ability to grow parts of a brain and work with the living sample has begun to open an entirely new chapter in biological research by providing vastly more realistic lab cultures—and at times even a reasonable alternative to using animals in doing research. SA

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MORE TO EXPLORE

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