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ARTICLE

Brain Model Technology and Its Implications

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Abstract

The complexity of the human brain creates a spectrum of sophisticated behavioral repertoires, such as language, tool use, self-awareness, symbolic thought, cultural learning, and consciousness. Understanding how the human brain achieves that has been a longstanding challenge for neuroscientists and may bring insights into the evolution of human cognition and disease states. Human pluripotent stem cells could differentiate into specialized cell types and tissues in vitro. From this pluripotent state, it is possible to generate models of the human brain, such as brain organoids. The recent observation that brain organoids can spontaneously develop complex neural network activity in a dish can help one understand how neural network oscillations evolve and vary between normal and disease states. Moreover, this finding can be leveraged to other applications outside medicine, including engineering and artificial intelligence. However, as the brain model technology becomes more complex, it raises a series of ethical and moral dilemmas. This article discusses the status of this technology, some of its current limitations, and a vision of the future.

Keywords: brain organoids; consciousness; stem cells; Neanderthal; evolution; microgravity

When we pause to think about the major problems that the world faces today or will face in the future, they include a discouragingly mounting list of such seemingly far-ranging issues as climate change, poverty, sustainable development, and mental health. What strikes us first is not only the enormity of the challenge but also how diverse they seem to be. Identifying problems is one thing, and finding solutions is another. However, upon closer inspection, it becomes clear that there is only one source for their solution—to use the human brain, especially the cortex, with its powers of cognition.

The problem is that we do not fully understand how the brain works and how it is formed. To do that would require examining the brain in utero. Unfortunately, that is an insurmountable barrier. We lack sufficient noninvasive tools to follow brain development with high definition in looking for the emergence of the first synapses, neurons to fire, or networks to form. All these changes take place within the womb, and the tools we do have, such as MRI and ultrasound, lack sufficient magnification or power to focus on these structures or even at the molecular level. Due to these and other limitations, the precise way in which the human brain forms during gestation is a black box. Bridging this gap in order to understand human brain development was the impetus to start my laboratory at the Departments of Pediatrics and Cellular & Molecular Medicine at UCSD School of Medicine in 2018.

At that time, most of the model systems to understand the human brain were based on animals and were far from perfect. Although mice were the preferred animal model to study the brain, they lack several of the very important stages of development present in humans. Whereas the mouse brain is fully formed in approximately 20–22 days, it takes at least 9 months for the human brain to be developed and several postnatal years to mature. Moreover, the mouse and human brains evolved differently and for different purposes at the genetic level, cellular composition, and neural networks. Therefore, the disconnection between the two models leads to an inappropriate comparison in terms of neuroscience.

For this reason, in my laboratory, we decided to create a human brain from scratch using human pluripotent stem cells, the type of stem cells that can create all tissues in our body, including the brain. Our research relies on brain organoids, structures created from stem cells that can be derived from human embryos themselves or reprogramed from somatic cells (skin, blood, hair, etc.) obtained from living persons. These brain organoids not only capture the development in vitro outside the womb but also more closely resemble human brain development than relying on a mouse model.

Developing this procedure became the focus of my work, and this is what we now call "disease modeling in a dish." In essence, the work of my laboratory is to create human model systems in vitro to mimic diseases. We focus on the cortex because it is one of the brain regions that is associated with human cognition and several psychiatric and neurological disorders. We are trying to understand the onset of these pathologies and how to intervene so that we can create better treatments that are more effective for neurological conditions. The process is simple and consists mainly of three steps: starting with single cells—human pluripotent stem cells—and treating them with different factors to become the desired types of neural progenitor cells. Next, we stimulate the progenitor cells to proliferate using growth factors. Finally, we remove the growth factors and let the cells in the organoids to self-organize and specialize in different brain cell types.

From the outset, it is important to emphasize that organoids are *not* "mini brains," a term sometimes used by researchers and the media to refer to this technology. A sensational image no doubt but one that conveys the erroneous idea that there is a miniaturized brain in a dish in the laboratory. This misnomer gives an idea of the hype that is an association with the field and incorrectly anthropomorphizes these structures. Despite advances, there are numerous limitations to this technology. The organoid is not vascularized, and that is why it only grows to about 0.5 cm. It does not represent the entire brain, we do not have all the components of the human brain, and it is not connected to other human tissues, such as the heart. In our laboratory, we grow many thousands of brain organoids using tissue from different people, primarily from those with neurological disorders. Although the brain organoid does not have the same number of neurons as the human brain, it does have about 2.5 million neurons, which is more than a bee brain, which has roughly 1 million. Although a very tiny structure, these organoids contain cell types that are major components of the brain necessary to form networks: neural progenitor cells, excitatory cells, inhibitory cells, and glial cells.

An example to illustrate the importance of using brain organoids to solve important medical questions is our work with the Zika virus. In 2016, several babies in the northeast of Brazil were born with microcephaly, a visible reduction in brain size with several malformations, leading to a severe syndromic condition. At the time doctors started to be aware of this epidemic, the cause of these malformations was not clear. Furthermore, it was also unclear whether the Zika virus was perhaps the culprit or the cause of these malformations. Although Zika was on the table as a feasible culprit, scientists were also considering possibilities such as pesticides and other chemicals that pregnant women were exposed to during that time. As a native of Brazil, I had access to a small sample of the virus that was isolated from one of the patients. When we exposed the brain organoids to the virus, we clearly noticed that the Zika virus was killing some of the intermediated neural progenitor cells, causing brain malformations similar to those that were manifested in babies. The use of brain organoids made it possible for us to show the mechanism of how the microcephalic brain was being affected in babies.

Underscoring the importance of the *human* organoids, in parallel, we initially failed to reproduce our findings in mice. Our failure was because the mouse brain develops so fast that the Zika virus did not have the opportunity to kill the progenitor cells, which led to the malformations. Although it would be possible to eventually show the connection in a mouse model, it would require a large amount of virus and other factors that make using a rodent to be a suboptimal model. But most importantly, 2 years after the successful demonstration of causation, we were actually able to find a drug that would protect the infected mother from having a baby also infected with the Zika virus. In the history of sciences, especially the biological sciences, moving from causation to treatment in a window of 2 years is an exceedingly short period of time. This rapid advance was made possible because we had the right tool in our hands: the brain organoids that mimicked human neurodevelopment so well.

The Zika example demonstrates that the brain organoid is proving itself to be a very valuable implement, not to completely replace existing models but to complement and provide new ways to gain insight into various conditions.

A subsequent challenge was generating brain organoids with functional networks that would "work" like the human brain and produce significant brain activity, or as colloquially referred to: "fire." By optimizing our protocol, we devised a method to capture and show complex electrical network activity that could be measured in human brain organoids for the first time. The beauty of this innovation allows us to see how human networks evolve over time. The emergence of complex network activity can be measured in a human brain using an electroencephalogram (EEG) to detect brain oscillations at different frequencies. Alterations in these frequencies are associated with different human behaviors (such as awake or sleep) and diseases (such as in epilepsy or autism). Thus, it would be quite a significant advancement to generate brain organoids with neural oscillations. But the creation of human brain tissue with this level of activity was never achieved by scientists before.

To put in perspective, the rodent brain fires at 18 Hz, and a primate brain at about 20 Hz. All in vitro work done with human brain cells could never pass the 5-Hz threshold. However, starting at about 25 weeks, our organoids grew exponentially in terms of activity to the point that in about 9 months, or 40 weeks, they reached 20 Hz. That was unprecedented in that never before in tissue culture had a human-made brain network reached that activity level in a petri dish.

We then decided to check whether we could generate neural oscillations like what could be captured by an EEG. This would be an important step because it would mean we could conceptually cross the bridge between basic biology and cognition. From our research, we were able to establish that neural oscillations from the brain organoids do exist. They start when the organoids are about 4 months of age and become highly synchronized at about 6 months. By 8 months or longer, they become exceedingly intricate. If the organoids continue long enough, they generate quite complex different frequencies of oscillations that match human neural development trajectories. This was confirmed using a machine learning algorithm to unbiasedly compare the electrical signal from an organoid with the EEG from preterm human babies' brains. Beginning at 25 weeks, there is a perfect correlation, suggesting that the same trajectory of a network being formed in the organoid is as formed in the human brain.

This was a significant step in technology. Earlier, we could use the morphology of the organoid, such as in the Zika case but not the functionality. Now we possess a tissue that behaves like the human brain in many ways. At present, we are using these organoids to test drugs or gene therapies, so we can generate proof-of-principle to enter faster into clinical trials. Currently, in my laboratory, we are involved in several exciting projects. Several continue to be on the disease modeling. For example, there are a number of monogenic conditions (caused by mutations in a single gene) that we can model very well in brain organoids and use them as a preclinical tool to demonstrate reversibility. We are employing strategies such as ASOs (allele-specific oligonucleotides), gene therapy approaches, or even the genome editing capabilities of CRISPR to correct the mutation in the genome of these organoids to see whether it is possible to reverse these conditions. Alterations in the brain organoid appear to go away if we fix or revert the mutation, indicating that many of these neurodevelopmental disorders might be reversible. We want to know whether we can unlock the potential of several of these very rare genetic conditions by using a gene therapy strategy.

We are also looking at ways to take this technology to other applications. One idea was to grow brain organoids at the International Space Station to understand the impact of the space environment, which includes radiation and microgravity, on human brain cells. This project was inspired by the NASA twins experiment in which scientists compared one of the genetically identical twins who spent approximately a year at the space station with his brother, who remained earth-bound. The twin who returned from space came back with systemic alternations in different tissues, including the brain. It became evident that microgravity affects different systems in the body, creating something similar to accelerated aging and associated comorbidities. We know that the nervous system was not evolved to cope with the very harsh environment of outer space environment; for that reason, finding ways to mitigate the environmental effects on cells will be important to help astronauts with future long-term interplanetary missions and space colonization. There are also important implications here on the Earth. Because organoids are

great models for neurodevelopment, they are not ideal for helping us find better treatments and cures for late-onset diseases. We are learning that we can leverage microgravity exposure to speed up the aging of brain cells so that we can model the adult-aged human brain, including conditions such as Alzheimer's, Parkinson's, and dementia, but without waiting 60–70 years. Without the valuable environment of the International Space Station and the use of organoids, currently, there would be no simple way to perform this type of research.

Brain organoids are also opening new possibilities with artificial intelligence (AI). Right now, AI uses very rigid artificial networks or machine learning to perform specific tasks. It requires an enormous amount of energy and storage to accommodate all the required data and extensive training systems. The human brain operates in a very different way. As soon as babies are born and open their eyes, they start exploring the environment and begin to learn. No one is teaching them; this activity is innate to the human brain, shaped by millions of years of evolution. We learn by sensing our environment and observing others in a social context. Exactly how the brain accomplishes this, we do not know. However, now with brain organoids, we can follow different stages of development in the brain and reconstruct their networks, exploring how the connections in the nervous system interact to produce behavior and cognition. Based on what we learn from organoids, we can propose innovative algorithms to explain how the brain works, which will be fundamental to creating a more human-like AI type. It will be like an organic way to perform AI, using biocomputers created by stem cells to have more humanized AI networks. Finally, the energy cost is so low that it is possible to perform several computational analyses simultaneously to a fraction of the cost that we currently have with AI.

We are also studying the impact of stimulation on human brain organoids. Different from the human brain, which is constantly stimulated in the body, brain organoids are grown in isolation inside an incubator. To add stimulation and embodiment to the brain organoids, we created in 2019 the first robotic interface, allowing the organoid to receive inputs from the external environment. This idea came to me from watching a Star Wars movie where a group of monks disconnected their brains from their bodies in a spider-like robotic machine, the so-called B'omarr monks. Separated from their corporal selves, they were free from the distractions of bodily sensations and pleasures and were able to reach an enlightenment higher state. I thought it was an interesting fictional system and wondered whether we might do something similar with a brain organoid.

There are several questions we want to answer using such brain organoids with a machine interface. For example, we can analyze the impact of chronic stimulation on brain organoids. Another opportunity is for understanding the principles and mechanisms of human learning and memory by creating a closed feedback loop. In this system, the organoid sends electrical signals to the robot that stimulates it back using a set of infrared sensors that inform the organoid when the robot is approaching an obstacle. By doing so, it creates a new response that drives the robot in another direction.

We are well aware of the increasingly complex ethical dilemmas generated by our organoid research. A primary ethical issue is the life of the organoid itself. The organoid is a piece of brain tissue that has derived from a person—it could be from any one of us or even from embryonic stem cells. Thus, informed consent should contain clear language about the potential uses of this technique. Another pressing concern is whether these organoids will reach a level of complexity that generates some consciousness level or self-aware. Current organoids are not highly super-complex samples of brain tissue. But at the pace that science is moving, this might be inevitable for organoids to reach a level that mimics what we believe to be evidence of consciousness in the human brain, independent of how one characterizes consciousness. If, or when, should organoids be treated with some moral status? Should science move forward after that boundary is crossed? I believe it is generally accepted that doing research on mice raises a lower bar of concern. Also, if we are working on developing human tissues to develop heart or liver organoids, there is likely no concern. But, if the aim involves a human brain, then we are in a different and much more complex area of ethical concern. At that point, we have entered the realm of an entity derived from a human with the potential of becoming self-aware and perhaps having conscious intent.

An experiment that we completed just recently was proposed by Nobel laureate physicist Roger Penrose and anesthesiologist Stuart Hameroff. They proposed that consciousness is coming from these

quantum vibrations in the cellular microtubules and that these vibrations can be blocked by an anesthetic. In our experiments, we treated the organoids with anesthetics: The answer to the question, "Do organoids stop vibrating when neurostimulation goes away?" is yes. Therefore, according to Penrose and Hameroff, the brain organoid has the essential components to become conscious. Italian neuroscientist Giulio Tononi believes that all we have are the cortical neurons and that consciousness is defined by one thing—experience. He uses the perturbation complexity index (PCI) as an objective measure for determining consciousness. Tononi provides a specific stimulus to the brain and then calculates how much reverberation is found. When this procedure is performed with people who are fully awake, a higher PCI is detected; in contrast, people in a coma show a lower PCI. When performed on those who are dead, the PCI is zero. What is the PCI of a brain organoid? We are working on it now.

It is important to note that none of these experiments we are doing now are actually providing final evidence for consciousness or lack of it. We are just building evidence that the necessary components of consciousness might be already preprogramed in cells. Perhaps a more important question should be: What does it change in our research? I believe that even if some level of consciousness is achieved, that should not mandate that research be abandoned. Research with mice is common, and I do not think people would argue that the mice are conscious. When it comes to ethics in research, the predominant issue is *how* that research will be conducted. For example, researchers using animal subjects cannot use as many or in whatever manner they wish. They must comply with a strict set of regulations that agree with the scientific community on how to perform their experiments in a humane way. I believe that if we reach the consciousness stage with organoids, the situation will be the same, with a system in place quantifying the number of organoids and rules for their use. Perhaps the rules will be the same, with a justification in place for the number of organoids for specific experiments, as well as how they are to be discarded. Would it be acceptable to trash the organoids, or must they be anesthetized before we dispose of them? These are examples of the kinds of ethical regulations currently governing research with animals and, which I believe, will require our developing similar rules for research with conscious organoids as well.

It is a challenge to control the ethical implications of our research, as well as the perception of the public, with this technology. In 1996, associates of the Roslin Institute in Scotland used nuclear transfer to create the first mammal cloned from an adult somatic cell. The arrival of "Dolly the Sheep" caused a media explosion and set off speculation untethered from reality (e.g., that cloning could end human reproduction.) Later, some involved with the creation of Dolly expressed regret that the public had not been better prepared for the event. Of the exciting projects that are currently underway in our laboratory, we are fully aware of the far-reaching and consequential aspects of the ethical issues they raise. Along with our team of scientists, we have philosophers-of-the-mind and ethicists who play an integral part. These projects are at a stage where I can see the potential and anticipate the fruits of this research. More to come next year.

Conflict of interest. A.R.M. is a cofounder and has equity interest in TISMOO, a company dedicated to genetic analysis and brain organoid modeling focusing on therapeutic applications customized for autism spectrum disorder and other neurological disorders with genetic origins. The terms of this arrangement have been reviewed and approved by the University of California San Diego in accordance with its conflict-of-interest policies.

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