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Out of the Cave, Into the Light? Modeling Mental Illness With Organoids

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> [T]hose who were chained would consider nothing besides the shadows of the artifacts.
>
> –Plato, The Allegory of the Cave

In *The Allegory of the Cave*, Plato envisions a dialogue between his mentor, Socrates, and his brother, Glaucon, that questions the nature of our reality. Imagine, Socrates suggests, a group of human beings who have lived in a cave for their entire lives (1). They are bound by chains such that they can only look toward the inside of the cave. However, they can see shadows from the outside world that are projected onto the wall in front of them. These shadows are devoid of color and physical form, distorted by the bending of light and their sense of perception. For the cave dwellers, this is the only truth they have ever known.

For Socrates (or, more accurately, Plato), the story was more than simply a thought experiment. When Glaucon expresses his amusement at the “unusual picture” of the “unusual prisoners,” the famous philosopher responds, “[t]hey are very much like us humans” (1). Plato suggests that humans are, largely, bound by fundamental limitations in the way we see the world. What we perceive as truth is merely a facsimile—not altogether false, but a clouded and incomplete representation of reality.

The concerns expressed by Plato have similarly shadowed the field of psychiatry. In other medical disciplines, physicians have a better ability to directly examine the cellular and molecular root cause of a clinical syndrome: a patient's hacking cough can be traced to pathogenic bacteria in sputum; a cancer diagnosis can be confirmed by genetic and molecular characterization of biopsy tissue. In psychiatry, however, a missing link persists between what mental illness *is* and how mental illness *looks*. Despite recent advances, a 2014 review described the state of the field rather bluntly: “There is not a single symptom of a single psychiatric disorder for which we fully understand its physiologic basis at a molecular, cellular, and microcircuit level” (2).

To address these gaps in knowledge, many researchers have focused on building better experimental models. However, developing these models has produced something of a paradox. Human data come with the advantage of studying the organism and disease of interest, but this same quality means that controlled biological intervention is both

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technically challenging and unethical. Conversely, animal models are more easily manipulated but fail to recapitulate many features of human illness, leading some to question their translatability. Until relatively recently, there has not been a viable middle path.

In 2006, Takahashi and Yamanaka (3) showed in a landmark study (that would later contribute to Yamanaka winning a Nobel Prize for Physiology or Medicine) that differentiated mouse cells could be reprogrammed back to induced pluripotent stem cells (iPSCs). With the right cocktail of transcription factors, cells are coaxed into losing their organ-specific function and regain the ability to take on a completely new identity. Another breakthrough occurred shortly thereafter when researchers used this technique to generate functional human neurons from a biopsy specimen obtained from human skin. In the summary of the article, the authors foresaw the promising applications of their findings: “Our approach enables large-scale studies of human neurons for questions such as analyses of human diseases, examination of human-specific genes, and drug screening” (4). Indeed, iPSCs have provided many novel insights into the human brain that were previously inaccessible. Starting from a biopsy specimen, researchers have been able to characterize differences in derived neurons between individuals with mental illness and healthy control subjects. In the context of autism spectrum disorders, studies of iPSCs demonstrated alterations in cellular proliferation, maturation, morphology, and electrophysiology (5). Growing human serotonergic neurons has advanced the study of depression, while culturing dopaminergic neurons has proved valuable insights for schizophrenia (6).

Despite the success of using iPSCs to study psychiatric disease, there are also notable limitations. First, homogenous iPSC cultures do not accurately depict the diverse cellular milieu in the brain. Second, and of equal importance, iPSCs exist only in a two-dimensional monolayer. This simple design does not accurately portray the brain on a molecular, architectural, or functional level. Growing neurons on a flat surface changes their genetic signature, and a two-dimensional framework lacks the multilayered organization that is the hallmark of evolved cortex (5,7). Moreover, many mental illnesses may be driven by defects in cellular organization and inter-connectivity, phenomena that cannot be fully described with a sheet of cells in a Petri dish (5). These shortcomings suggest that to more accurately study the function of the neuron it needs to be studied in a more natural environment.

Going from a homogenous, two-dimensional cell culture to a heterogeneous, three-dimensional tissue seems to present a substantial technical challenge: developing a brain would presumably require an intricate set of signals for growth and differentiation. Amazingly, however, much of this complexity may arise essentially spontaneously due to the self-organizing properties of human cells. In an extraordinary paper in 2009, Sato *et al.* (8) showed that intestinal stem cells gave rise to stable intestinal villi composed of all in vivo cell types (8). Incredibly, these three-dimensional in vitro assemblies of human cells, known as organoids, recapitulate many architectural and functional aspects of human tissues. Several years later, Lancaster *et al.* (9) showed that the same principle could be applied to build an approximation of perhaps the single most complex structure in the universe: the human brain. As stated by the authors, their protocol did not rely on a complex mix of growth factors, but instead the “environment necessary for intrinsic cues to influence development.” Neuroectoderm cells, derived from iPSCs, were cultured on an artificial

scaffolding meant to mimic an extracellular matrix and allowed to grow without instruction or restraint. This laissez-faire approach nonetheless developed brains with histologically distinguishable cortices, choroid plexuses, meninges, and retinas. Immunohistochemistry even identified markers of sub-specification within the cortex, suggesting the presence of similar cortical lobe divisions as found in humans. Further studies have corroborated these histologic data by showing that the genetic and epigenetic signatures of organoids are similar to those of the live human fetal brain (5). In addition, these organoids are remarkably stable in culture—the seminal paper on the topic reported that at 10 months the organoids were still viable in vitro (9). Suddenly, neuroscientists had a model that had elements of the best of both worlds: a version of a human brain that could be manipulated directly.

As organoids most closely resemble the fetal brain, the most logical application of this technology is in studying neurodevelopmental disease. A remarkable study generated iPSCs from patients with autism spectrum disorder and then used these cells to grow organoids (10). Genetic analysis of autism spectrum disorder organoids (compared with those from healthy control subjects) showed an increase in a transcription factor that led to increased cellular proliferation and overproduction of inhibitory neurons. Interestingly, this phenomenon was present in early neural development but not at later stages. This example shows the potential of organoids to push our boundaries even further: not only can they extend inquiry from a two-dimensional surface into a three-dimensional space, they also open a critical fourth dimension—“How did this brain grow over time?” Historically, psychiatric illness has been difficult to study in part because by the time we have identified clinical pathology we have already missed the initiation of disease. Organoids represent one way to partially address this problem, by looking at cells not just as they are, but also as they were. In this way, organoids represent a time capsule of sorts, providing insight as to how illness affects the beginning moments of the brain.

Many challenges still exist for this new and radically complex technology. From a technical and experimental standpoint, growing and studying a brain without a body presents several issues (to say nothing of the ethical questions). Oxygenating organoids without a cardiovascular system is challenging, leading to ischemia and necrosis as the tissue outstrips its supply of nutrients (9). In addition, without functional bone marrow, organoids lack cells involved in the immune system, which may be important in some neuropsychiatric illnesses. Another fundamental limitation is that it may not be possible to go much beyond midgestation-like maturity. Lastly, the sheer complexity of these models contributes to “batch syndrome,” wherein experiments lack reproducibility between trials.

Like the unchained cave dweller who sees daylight for the first time, scientists are using this nascent and promising technology to explore unchartered territory in a revolutionary way. One particularly promising approach is leveraging the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas gene editing technique to develop knock-in and knock-out models of specific genes. This approach would allow scientists to precisely delineate the role of individual genes on brain development and function by either selectively introducing mutations into “healthy” brain organoids, or, alternatively, using gene therapy on organoids derived from patients with neuropsychiatric disease to identify possible therapeutic targets. In addition, organoids have also been proposed as model systems for drug screening, drug

development, and ultimately an interface for calibrating personalized medicine (5). As we adjust to the novelty and complexity of organoids, time will tell whether these promising advances have moved us further away from the cave and into the light.

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