Epilepsy Research Now in 3D: Harnessing the Power of Brain Organoids in Epilepsy

Abstract
Brain organoids represent a powerful tool for studying human neurological diseases, particularly those that affect brain growth and structure. However, many diseases manifest with clear evidence of physiological and network abnormality in the absence of anatomical changes, raising the question of whether organoids possess sufficient neural network complexity to model these conditions. Here, we explore the network-level functions of brain organoids using calcium sensor imaging and extracellular recording approaches that together reveal the existence of complex network dynamics reminiscent of intact brain preparations. We demonstrate highly abnormal and epileptiform-like activity in organoids derived from induced pluripotent stem cells from individuals with Rett syndrome, accompanied by transcriptomic differences revealed by single-cell analyses. We also rescue key physiological activities with an unconventional neuroregulatory drug, pifithrin-α. Together, these findings provide an essential foundation for the utilization of brain organoids to study intact and disordered human brain network formation and illustrate their utility in therapeutic discovery.

Keywords
epilepsy, Rett syndrome, brain organoids, drug screening, epileptiform activity

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Commentary
Epilepsy is a complex brain disorder that can be caused by overt malformations of the brain but also simply manifest as subtle molecular and local circuit alterations. A challenge for epilepsy research, like brain research in general, is the limitation of available experimental models that reflect the complexity of neuronal circuits in the human brain while being accessible and easily manipulatable. Intact in vivo animal models, particular mouse models, provide a versatile tool to study gene defects and circuit abnormalities in epilepsy but are not easy to manipulate. Perhaps more importantly, murine brain development and the associated cellular and molecular composition are considerably different from human brains.

In 2013, scientists reported the generation of brain organoids, three-dimensional cultures of neurons derived from human embryonic stem cells or human-induced pluripotent stem cells that formed discrete brain regions and resembled the early development of a human brain. While these brain organoids are still far away from being fully functional human brains, this technological advance has opened new doors for brain research. Among many other diseases, they have been used to model and analyze several developmental epilepsy disorders. These studies recapitulated disease characteristics such as macrocephaly in epilepsy caused by mTORopathies and showed changes in cell differentiation, cell migration, and molecular signaling providing important insight into the underlying disease mechanisms and potential treatment strategies; however, an important unanswered question so far has been whether these organoids can truly mimic circuit-level phenotypes in epilepsy, such as defective network oscillations (rhythmic changes in electric activity of the brain) and epileptiform activity, including hypersynchrony and excitability. Altered brain networks are a hallmark of epilepsy, and therefore, for brain organoids to be a true model of epilepsy and an adequate testing platform to assess novel treatment strategies, they must recapitulate this defective neural circuit activity, which ultimately underlies the development of seizures.

Only recently, brain organoids were shown to form spontaneous functional neuronal networks and electrical oscillations similar to what occurs in the human brain, but whether disease-associated network defects are detectable in brain organoids was still unclear. A new study by Samarasinghe et al. took this and the previous work in brain organoids an important step further, and for the first time, reports abnormal electrical activity in brain organoids derived from patients with an epilepsy disorder, namely, Rett syndrome. Rett syndrome is a devastating, incurable X-linked neurodevelopmental disorder caused by mutations in MECP2 and is often accompanied by seizures. To generate brain organoids from these patients that were capable of circuit-level oscillations reminiscent of the intact brain, and to mimic the intricate network of excitatory and inhibitory neurons in the human cortex, the researchers generated cortical (excitatory) and ganglionic eminence (inhibitory) organoids that were then fused together. In these preparations, the inhibitory neurons started migrating into the cortical organoids and forming synaptic connections with excitatory neurons, mimicking processes occurring...
During development of the human brain, network function of these fusion organoids was then tested with intracellular calcium sensor imaging using two-photon microscopy and extracellular recordings of local field potentials. Indeed, brain organoids derived from healthy control cell lines produced rhythmic, synchronized waves of activity and oscillations at various frequencies when treated with subthreshold levels of kainic acid, suggesting that the organoids built functional networks resembling the intact brain.

When the scientists repeated these experiments with fusion organoids generated from two patients with Rett syndrome, they detected network oscillations that differed from the healthy control brain organoids and were characterized by hyperexcitability, hypersynchrony, epileptiform spikes, and high-frequency oscillations, reminiscent of epileptic brain networks. However, it is important to note that the brain organoids did not develop seizures, suggesting that they are still several steps away from truly modeling an epileptic brain.

Using an elegant approach of “mixed” fusion organoids generated with control cortical and mutant ganglionic eminence organoids, or vice versa, the authors showed that the network abnormalities were mostly mediated by the mutant inhibitory neurons. Taking these findings even further, the authors demonstrated that treatment with an experimental drug, pilocarpin-α, which targets a signaling pathway, TP53, previously shown to be altered in Rett syndrome, partially normalized these network abnormalities, while a common antiseizure drug, valproic acid, was less efficient. These results further support the prospect that brain organoids from patients with Rett syndrome and other epilepsy disorders may be used as screening platforms for drug testing and identification.9 Fluorescent methods like the calcium imaging employed here to detect network activity are suitable for automized drug screening, which could accelerate novel drug identification even further.

Remarkably, the authors found shared and distinct features in organoids derived from two different patients with Rett syndrome. This shows the power of the method to identify common disease pathologies in addition to individual phenotypes that could underly the spectrum of phenotypes often observed in brain disorders and be used to develop individualized and thus highly efficient precision-medicine therapies for patients.

It is important to note that brain organoids are far from being “mini-brains in a dish.” They do not form the exact structure of brain regions and, importantly, lack angiogenesis, leading to cell death in the center. Therefore, they, like all experimental model systems, do not fully reflect the human organism, and the clinical relevance of the results must not be overinterpreted. The most relevant phenotype in epilepsy are spontaneous seizures; however, as mentioned above, the brain organoids did not produce electrographic seizures. It remains to be seen if, in the future, the technology can be further advanced to generate brain organoids that seize.

As with every revolutionary new research method, brain organoids come with unique ethical challenges. While brain organoids are still far from producing any kind of “conscious awareness,” discussion about regulation of their use should be ongoing among scientists, patient advocacy groups, science policy makers as well as the general public and renewed with every new advance in brain organoid technology. As discussed previously,10 it would be unethical to not use this and similar new technologies, which hold great potential to help identify disease mechanisms and develop novel treatment strategies for currently untreatable human disorders, such as epilepsy; however, an open and inclusive debate is needed to regulate their use and ensure the publics’ acceptance.

Despite these limitations and challenges, the work by Samarasinghe and colleagues sparks hope for the future use of brain organoids to identify disease contributing molecular, cellular, and circuit-level defects in epilepsy. Most excitingly, this work advances the prospects of “personalized medicine,” which, with more research and further technical advances, may enable tailored and thus highly efficient therapies.

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