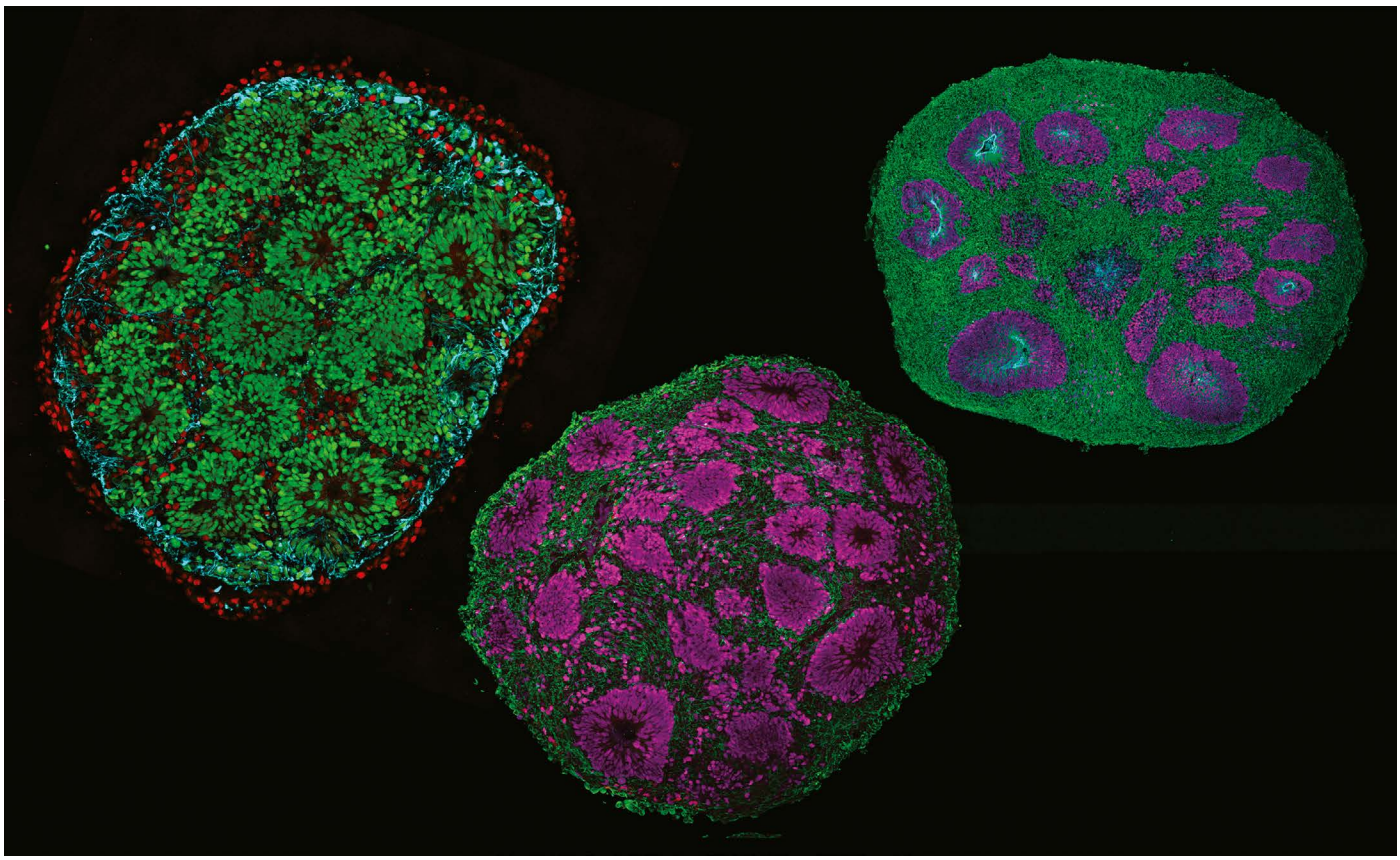


ARLOTTA LAB, NOELIA ANTÓN-BOLAÑOS, IRENE FARAVELLI, RAHEL KASTLI

Complex organoids, such as these cortical 'chimeroids', allow researchers to probe brain development and disease.

BUILDING A BRAIN, PIECE BY PIECE

With organoids, assembloids and a growing bioengineering toolkit, scientists are pushing the limits of human brain models. **By Elie Dolgin**

In the 2015 Pixar film *Inside Out*, a young girl's mind is brought vividly and hilariously to life through a cast of animated archetypes, each representing a distinct facet of emotion or thought. The brain, in this portrayal, is a modular structure, composed of interlocking yet discrete parts — a concept that mirrors one of the most advanced models in modern neuroscience.

Over the past decade, researchers have coaxed stem cells to form 3D clusters of neural tissue known as organoids, bathing the cells in carefully formulated chemical cocktails to generate brain regions such as the thalamus, where sensory information is processed, and the striatum, which integrates movement and reward signals.

Initially, efforts focused on isolated, region-specific models. But today, researchers routinely fuse organoids representing distinct brain areas to create unified 'assembloids', capable of reconstructing functional neural networks and offering insights into brain connectivity, development and disease. "It has opened a new direction in the field to really understand interactions between different parts of the brain," says In-Hyun Park, a stem-cell biologist at the Yale School of Medicine in New Haven, Connecticut.

Yet the brain is more than a patchwork of interconnected structures, stuck together like Lego bricks. It is a dynamic, self-organizing ecosystem in which neighbouring cells continuously shape one another's fate. Over time, an intricate web of synaptic pathways emerges through fine-tuned exchanges — both local and long-range — that help all the parts of the organ to mature in synchrony.

No one organoid model can capture this complexity. Assembloids provide a valuable window into regional interactions. But techniques are emerging to recapitulate further aspects of brain mechanics and circuit dynamics. From neurodevelopment to drug discovery, organoid models are opening up fresh investigative paths, each geared to different dimensions of brain biology — and with tools suited to a variety of lines of scientific inquiry.

"We cannot really talk about 'best' models, but rather models that are more adequate to answer specific questions," says Silvia Velasco, a developmental neurobiologist at the Murdoch Children's Research Institute in Parkville, Australia. And because our understanding of human brain development is "still largely fragmented", Velasco adds, a diverse modelling toolkit is not just helpful — it's essential.

Diversity, however, comes with trade-offs. Greater biological realism often means lower scalability and reproducibility, especially between research groups. This complicates efforts to draw broad conclusions

and translate discoveries reliably from the laboratory to the clinic. Still, researchers such as Paola Arlotta, a neuroscientist at Harvard University in Cambridge, Massachusetts, are working to close the gap. "We need models that have fidelity," she says.

Network effects

In Arlotta's lab, tiny white globs of brain cells swirl in a cerise-coloured bath of growth medium, like 'pearls' in a cup of hibiscus bubble tea. These are three-month-old organoids, made from human stem-cell-derived neural precursors that, together, form the cerebral cortex, the wrinkled outer layer of the brain. Each organoid comes from a population of carefully patterned stem cells, combined in just the right amounts and cultured over several months to create microcircuits. The result is a balanced mix of excitatory and inhibitory neurons that have developed in unison, forming intricate, functional networks.

"They are really growing together," exclaims Irene Faravelli, a neurologist in Arlotta's group. After years of tinkering to get the conditions just right, "now we are at the fun part of exploring what we can interrogate with this model", she says. "We can ask about the mechanisms controlling the formation of the human cerebral cortex, the site of highest cognitive function. Or dig deeper into how the unique features of the human brain are acquired and what makes our brain different."

Most importantly, she adds, the model lets researchers probe the way in which genes linked to conditions such as autism, schizophrenia and Alzheimer's disease shape brain development and function.

Last year, Arlotta, Faravelli and their colleagues described a reliable method for growing these composite organoids — dubbed chimeroids. But at the time, instead of mixing cells from different brain regions, the researchers pooled a single kind of neural precursor cell from donors[1].

Now, in unpublished follow-up work, they have adapted these cultures to start from a mix of progenitors corresponding to different brain regions. These are the spheres that Faravelli now gently coaxes along in the lab.

The goal, explains Arlotta, is to recapitulate cell–cell interactions that occur as the brain naturally develops — mimicking the choreography of cellular differentiation, migration and connectivity that unfolds as cells mature in a shared environment. "This is how the nervous system develops," she says.

"I like to think about the process of building a brain much like an orchestra producing a specific musical symphony," Arlotta continues. "Much like instruments playing at the wrong times and producing a different music, cells that do not interact with each other correctly during development will produce a different final tissue."

Assembloid models created by physically joining pre-formed brain organoids can also recapitulate some of the same circuit-level interactions — and Arlotta is quick to note that chimeroids and assembloids are complementary. But to investigate the earliest stages of brain development, she realized, a different approach was needed: hence, chimeroids.

"It's the only way you can watch these intricate processes of cell–cell interaction and communication that progressively build our beautiful brain," Arlotta says.

Model assembly

The careful calibration of timing and cell composition needed to engineer chimeroids stands in contrast to one of the field's most high-profile early successes.

In 2013, a team led by developmental neurobiologists Madeline Lancaster and Jürgen Knoblich at the Austrian Academy of Sciences' Institute of Molecular Biotechnology in Vienna showed that human stem cells, when embedded in a gelatinous protein matrix and cultured in spinning bioreactors, could spontaneously assemble into 3D, multilayered brain-like structures with discrete regions and architectural patterns[2].

"It's all about complex cell interactions that give rise to emergent properties."

A triumph of self-organization, these structures, with features akin to those found in the brain of a nine-week-old human fetus, provided an unprecedented window into neural development and disease — including microcephaly, a condition marked by severely impaired brain growth that is difficult to replicate in animal models. "It's not just one small part of the brain" in these organoids, says Lancaster, who is now at the MRC Laboratory of Molecular Biology in Cambridge, UK. "It's actually this whole complex make-up of different brain regions connected to each other."

Knoblich vividly recalls the reaction when he first presented the work at a scientific conference in 2012, ahead of publication. He was a last-minute replacement for another speaker and had no idea what to expect. Neither did the crowd — which erupted with excitement. "Never in my life have I given a talk that created this kind of attention," Knoblich says. "It was amazing."

Other researchers soon started adapting and refining the technique, showcasing its potential for studying complex neural circuits and disease mechanisms. But the self-organizing nature of these early models meant that variability was baked in. No two organoids were identical, which made reproducibility a persistent challenge. Researchers addressed this by introducing different growth factors and signalling molecules that control cell fate and tissue formation more tightly.

First came protocols for making just part of the cerebral cortex. Methods for generating midbrain and hypothalamic organoids soon followed, along with techniques for modelling the cerebellum, spinal cord and other structures — each requiring its own set of signalling cues and culture conditions, tailored to mimic region-specific development.

As the fidelity of region-specific brain organoids improved, researchers embraced a new frontier: modelling the connections between them. This led to the development of assembloids — hybrid constructs that recreate the functional wiring of the brain and offer a deeper look into its integrative architecture.

"It's all about complex cell interactions that give rise to emergent properties," says Sergiu Pașca, a neuroscientist at Stanford University in California who coined the term 'assembloid' and led one of three teams that independently introduced the concept in 2017 (refs 3–5; Knoblich and Park led the other two).

Neural knotwork

Assembloids allowed scientists, for the first time, to observe long-range neuronal crosstalk in a dish — such as the migration of inhibitory neurons from the ventral forebrain into the dorsal cortex — thereby recapitulating key events in fetal brain development. "No one knew how that migration was actually happening in humans," says Pașca. Assembloids also created a powerful platform for studying neuropsychiatric conditions such as Rett syndrome, epilepsy and schizophrenia, in which circuit-forming events go awry.

Knoblich credits the widespread adoption of assembloids to their mix-and-match nature. "There's not much to it," he says. "You take two organoids, you put them together, and they fuse — that's it."

"The challenge," he points out, "is to get the right interaction."

In 2020, Pașca's lab introduced three-part assembloids, fusing cortical, spinal-cord and muscle organoids to study how neural activity triggers muscle contractions[6]. Now, the team has pushed the approach even further. By stitching together organoids carrying neurons involved in each key stage of pain processing — from peripheral sensory neurons to the spinal cord, through to the thalamus and finally the cortex — the team was able to recapitulate the entire pathway and even watch it respond to a noxious stimulus such as capsaicin, the compound responsible for the spicy heat in chillies.

What's more, the Stanford researchers demonstrated last month how heritable mutations in a key signal-relaying protein can profoundly alter these pain-transmission dynamics[7]. This helps to explain why such

mutations can, depending on the nature of the genetic alteration, lead to either complete insensitivity to pain or a hypersensitivity disorder marked by sudden, intense burning or stabbing pain in the rectum, eyes and jaw.

Making the connection

A complementary approach to studying how brain regions interact comes from Yoshiho Ikeuchi, a bioengineer at the University of Tokyo. Instead of fusing organoids, Ikeuchi and his colleagues connect them using tiny plastic channels on a microfluidic device. This set-up keeps the organoids physically separated and encourages nerve fibres to close the gap, mimicking the long-distance communication patterns seen in the human brain[8].

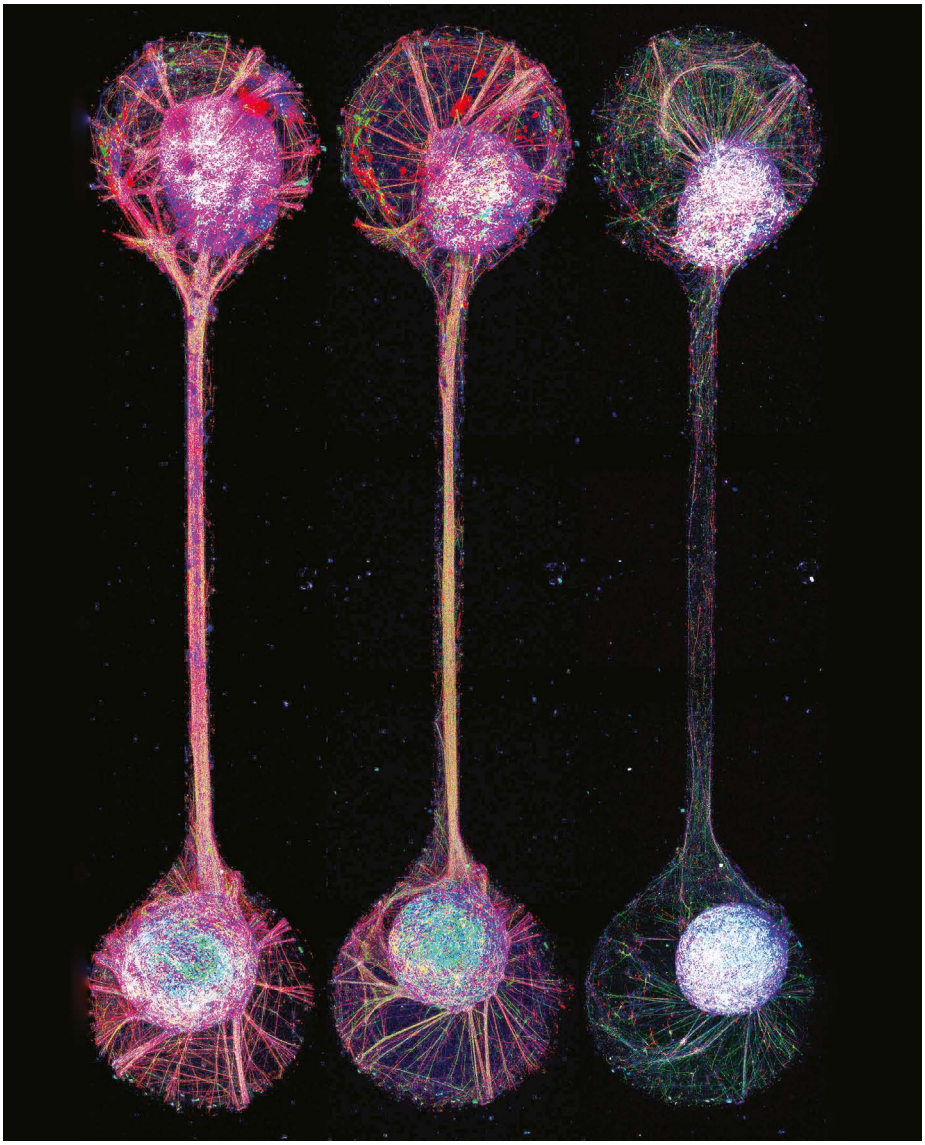
In unpublished work, Ikeuchi has connected as many as 18 organoids in a 6 × 3 grid — an effort he admits was driven more by curiosity than by a specific objective. "It's so fun to watch how they generate spontaneous activity and respond to our stimulation," he says. But, beyond the novelty, Ikeuchi sees real potential in using these 'connectoids' to study diseases such as schizophrenia, which involves disruptions in axonal function and synaptic integrity.

Connectoids also provide a way to study communication across the corpus callosum, the physical barrier that separates the two hemispheres of the brain. Last year, in collaboration with Knoblich's lab, Ikeuchi and his colleagues used paired organoids connected through microchannels to show how a gene mutation associated with intellectual disability disrupts the formation of long-range axonal projections, leading to connectivity defects[9].

Although physical fusion and engineered connections can help to simulate neural communication, a true understanding of how these interactions emerge during early brain development requires turning back the biological clock. That means guiding not just the final position of cells, but also the paths they take to get there — a process orchestrated by signalling molecules known as morphogens.

Increasingly, brain-organoid researchers are turning to microfluidics to harness these molecules with precision. By exposing developing tissues to carefully calibrated gradients of morphogens, researchers such as Flora Vaccarino, a developmental neurobiologist at Yale, have managed to steer neural precursor cells towards specific identities in a spatially organized way. "You kind of trigger what the organoids will be, and then it's a self-organizing system," says Vaccarino. "Everything else is like a chain of events."

A striking example, published last year, demonstrates how such gradients can shape complex tissue architecture. A team led by Jianping Fu, a mechanobiologist at the University of Michigan, Ann Arbor, designed a microfluidic device with reservoirs at either end of a central channel, allowing for stable and adjustable diffusion of key morphogens along two key developmental axes: dorsoventral (back-to-front) and rostral–caudal (head-to-tail).



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'Connectoids' use microfluid channels to recapitulate and study neural connections.

In collaboration with neuroscientists Guo-li Ming and Hongjun Song at the University of Pennsylvania Perelman School of Medicine in Philadelphia, the researchers then used this set-up to organize stem cells into neural tube-like structures that faithfully replicate many aspects of early brain and spinal-cord formation[10]. As Song points out, "All of the things that are naturally supposed to be next to each other are patterned that way" in this model.

Unlocking the next level

Instead of waiting for organoids to self-assemble, some labs are 3D printing brain tissue into specific layered arrangements, or putting down scaffolds that can support the patterning of organoids alongside other tissues designed to mimic the blood vessels that nourish and support the brain.

With these scaffolds in place, organoids can grow without the cell death and oxygen deprivation that often plague conventional culture systems — while also developing structures and cellular activities that more closely resemble those of the human brain. "This will unlock functionalization," says Steven Sloan, a biomedical engineer at Emory University School of Medicine in Atlanta, Georgia.

Still, 3D-printed constructs can only go so far. To sustain long-term growth and maturation, some researchers have turned to a more biological solution: transplantation.

By implanting organoids into the brains of newborn rodents, neuroscientist Fred Gage and his colleagues at the Salk Institute for Biological Studies in La Jolla, California, demonstrated in 2018 that they could harness the host's circulatory system to keep their 3D models alive[11]. This approach also supports the organoids' continued development and integration with the surrounding brain tissue — although it comes at the cost of losing some

experimental control over environmental cues and the timing of developmental processes.

Others have taken a scalpel to the problem of nutrient and oxygen deprivation — literally. In 2020, Ming, Song and their colleagues showed that slicing organoids into discs half a millimetre thick drastically improved nutrient diffusion. The result: healthier, longer-lived organoids that can develop more-mature cortical structures and better recapitulate the layered organization of the human brain[12].

Together, these strategies offer a growing toolkit for reconstructing the brain in a dish, each revealing a different facet of biology. No single method captures it all. So, increasingly, researchers are combining these systems in the same project, weaving together insights from a mosaic of models to get at the heart of how the brain develops, functions and breaks down.

Last year, for example, Pașca and his colleagues described how they had used a combination of standalone organoids, fused assembloids and a rat transplant model to identify and test a potential drug treatment for Timothy syndrome, a rare neurodevelopmental condition[13].

Using stem cells made from the skin of affected individuals, the team first generated cortical organoids — chosen for their scalability — to screen antisense oligonucleotide drugs targeting the faulty calcium channel at the root of the disease. Promising candidates then moved into assembloids, where neurons migrate between fused forebrain regions — a set-up that let the team assess the drugs' ability to restore proper circuit formation. Finally, the researchers transplanted cortical organoids into the brains of newborn rats. This gave the team a platform to test the leading drug candidate *in vivo* and confirm its molecular and functional impact. Clinical trials are expected to begin in the next year.

"It's the first therapeutic for a psychiatric disease that has been exclusively developed with human stem-cell models," notes Pașca, whose team is now applying the same integrated approach to conditions such as epilepsy, autism and schizophrenia. "It's the paradigm of how to do drug discovery at multiple levels," he says.

Finicky factors

Pașca's work highlights what's possible when organoid models are applied across multiple experimental systems — from dish to assembloid to animal. But it also points to the level of precision that such efforts demand. And even the most sophisticated models can be sensitive to subtle shifts in timing, media or handling, raising concerns about how reproducible these systems are between labs and, by extension, whether findings can reliably translate to clinical or physiological contexts.

"Everyone has their own protocol," says Jimena Andersen, a developmental neurobiologist at Emory. "So, it's really hard to compare things between labs."

Andersen and her colleagues have tried to address this variability through a centralized resource they call the brain organoid hub. Funded through institutional support and grant funding from member labs, the hub provides a way to benchmark commonly used methods side by side, starting with a systematic comparison of protocols for generating dorsal forebrain organoids. This effort revealed how the composition of the growth medium can drastically affect results and flagged several ways to reduce costs without sacrificing quality.

It also underscored the outsized impact that even minor procedural differences can have on organoid fate. "A lot of the variability comes from stupid things," notes Sloan. For instance, how someone closes an incubator door can affect the way in which bundles of cells settle in culture plates. "There's a difference between not slamming and being gentle," he says.

The solution, Sloan says, lies in automation — and at least one company is already moving in that direction. A:head Bio, a Vienna-based firm co-founded by Knoblich and Lancaster, has adapted its culture protocol and assay workflows to enable scalable, standardized cortical models for epilepsy. According to chief scientific officer Josh Bagley, the company is now seeking funding to build a fully automated platform, at which point it should be able to routinely produce tens of thousands of organoids under consistent conditions for high-throughput drug screening.

Organoids might never capture every nuance of the living brain — but they don't have to. As tools for asking targeted questions and generating testable answers, they've proved remarkably insightful, notes Knoblich. "Given the limitations, we are more surprised by what organoids can do than what organoids can't do," he says. Still, with continued innovation and a growing push for standardization, Knoblich and others expect these brain proxies to keep pushing the boundaries of neuroscience, one cell cluster at a time.

Elie Dolgin is a science journalist in Somerville, Massachusetts.

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