Organoids

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Eavesdropping on brain organoids

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A basket-like device records the electrical activity of neural organoids over months of maturation.

How the intricate neural circuitry of the human brain forms and matures during prenatal development is largely a mystery. Organoids — tiny clusters of cells that model tissue differentiation in vitro — might give us a unique window onto these questions, but only if we can listen to their electrical activity as they grow over weeks and months. In a new study, Yang et al.[1] describe a sophisticated electrode basket that supports long-term growth and electrical recording of brain organoids. Their kirigami-inspired system, reported in *Nature Biotechnology*, promises to allow rigorous analysis of both the formation of brain circuits and their dysregulation in neuropsychiatric disease.

Long before birth, during embryonic development, the human brain begins to assemble the neural circuitry that allows participation in the outside world. Even as these interwoven connections may dictate the floor and ceiling of cognitive ability, the biological processes that generate them cannot be studied directly. The recent emergence of organoid technology offers an exciting entryway into early development. Brain organoids are three-dimensional clusters of neural cells grown from pluripotent stem cells[2,3]. These minute tissues, on the scale of millimeters, recapitulate some key features of the brain, such as tissue morphology, cell type composition and certain aspects of function. They can be cultured for years, and over time they develop diverse cell types and projections and exhibit intrinsic activity. Human brain organoids have even been implanted into animal brains, where they integrate into circuits and alter behavior[4,5].

Brain regions are often anatomically defined by unique patterns of input–output connectivity. For example, the prefrontal cortex is defined as having reciprocal connections with the mediodorsal thalamus, whereas the motor cortex has unilateral projections to the striatum. The latter has been modeled in vitro by co-culturing organoids representing the cortex and the striatum[6]; eventually, the two organoids fuse and integrate to form functional cortico-striatal circuits. However, maturation of a neural circuit in vitro can take many months — similar to the developmental time frame in vivo, posing challenges for longitudinal study of neural activity during growth and differentiation.

Current technologies for measuring electrical activity in brain organoids include patch clamp, silicon probes and multi-electrode arrays[7,8]. While still considered state of the art, these techniques require special preparations, such as slicing of the organoid or plating onto a planar surface, making it difficult to study the same organoid longitudinally. Recently, more-compliant mesh electrode systems[9] have been developed to flexibly and stably adapt to the growing organoid and to enable chronic measurements. However, these approaches require organoids to be grown on a substrate, which may interfere with their intrinsic development or limit recordings to cells adherent to the bottom surface.

To enable chronic recording of organoids grown in suspension, Yang et al. searched for a structure that is flexible enough to cradle growing organoids while rigid enough to withstand months in solution. They turned to the Japanese art of kirigami, a practice of folding and cutting (*kiru*) paper (*kami*). To optimize a design that balances flexibility and mechanical robustness, they used a finite element simulation framework to quantify deformation under a load and to determine how stress accumulates at different locations. They eventually identified a spiral design that can accommodate the weight of an organoid under minimal strain. The resulting three-dimensional geometry is a basket with a diameter of 1 cm and bearing an array of 32 embedded microelectrodes of 25 µm diameter (Fig. 1).

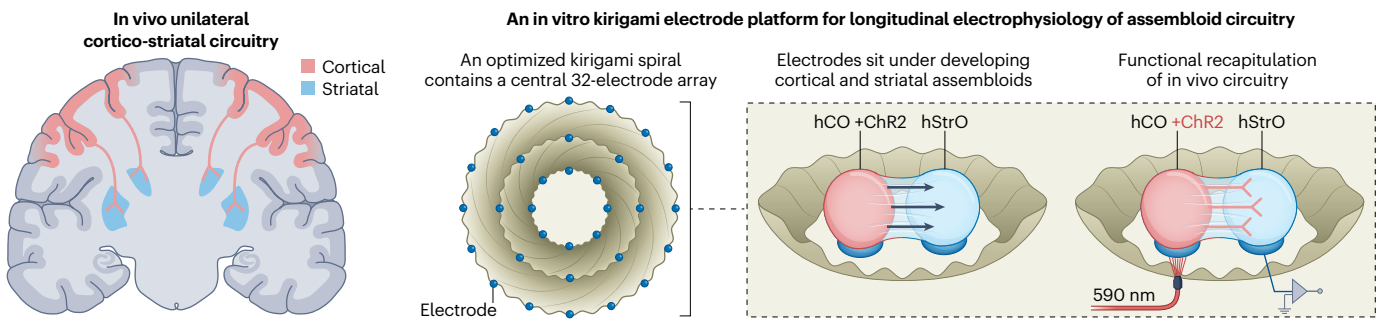

Fig. 1 | Kirigami electronics cradle and monitor the development of human assembloids. In vitro neural assembloids, formed from the fusion of organoids representing individual brain regions, recapitulate in vivo circuitry such as the unilateral projections from human cortex (red) to the striatum (light blue) (far left). To longitudinally monitor the emergence of electrical activity in assembloids, Yang et al. designed a kirigami-inspired spiral mesh that contains a 32-electrode array at its center (dark blue, center left). These extracellular electrodes sit under developing assembloids (dark blue, center right) while the mesh superstructure flexibly unfolds to allow simultaneous growth and recording over months. The recordings can be paired with pharmacologic or optogenetic modulation to probe circuit features at specific time points. The authors transduced a human cortical organoid (hCO, red) with channelrhodopsin (ChR2) and placed it adjacent to a human striatal organoid (hStrO, light blue) situated above the electrode array. As the assembloid develops, corticostriatal projections are observed (center right, arrows) and, once matured, can evoke neural activity responses in striatal cells (far right).

The authors then carried out experiments to define the capabilities of their kirigami electronics (Kiri-E). Through a series of live cell imaging, immunofluorescence and single-cell RNA sequencing studies, they demonstrated that the device can be embedded into cortical organoids as deep as 300–500 µm without substantially altering organoid morphology or cell composition. Next, they tested whether Kiri-E can reliably record spontaneous or evoked activity of human cortical organoids. They found that longitudinal recordings starting from 75 days post-induction (dpi) begin to show spontaneous electrical activity by 100 dpi. In the majority of cells, this activity remains stable over days, and activity persisted at least until the experiment was terminated at 179 dpi. In future studies, it would be interesting to assess whether organoids progressively develop giant depolarizing potentials, GABA polarity switch and oscillatory waves, thought to be characteristic of the developing fetal nervous system.

The authors also explored the compatibility of Kiri-E with pharmacological and optogenetic manipulations. They found, for instance, that potassium channel blockers lead to threefold increases in spontaneous neural activity and that optogenetic activation induces 20-fold increases in overall firing rates. Sodium channel blockers, conversely, fully abolish spontaneous activity, as expected. In a final set of experiments, the authors created cortico-striatal assembloids and recorded striatum while optogenetically activating cortex. These analyses demonstrate a framework for the longitudinal study of long-range neural circuits and their plasticity during development.

A key question in the study of assembloids is the limit of their complexity. Are neural cells in assembloids capable of complex activity patterns, such as state-dependent shifts in tonic versus phasic firing? Can they display emergent properties of complex networks, such as patterns of synchrony, oscillation and plasticity that are thought to be important for behavior? Recent claims to the establishment of emergent properties such as sentience in neural cell cultures have both captured the imagination and invited rational skepticism[10]. Improved longitudinal recording systems will surely shed light on these issues.

At least some of the current limitations on the complexity of assembloids spring from technical issues that are well understood and that may be addressable in the near future. For example, spherical organoids typically lack ventral–dorsal and rostral–caudal patterning and often have an avascular, necrotic core. In addition, there is incomplete representation of the cell types that are needed to produce emergent properties, such as inhibitory, neuromodulatory and glial cells[11]. Continued collaboration among developmental, stem-cell and neurobiologists promises to further characterize and expand the limits of brain assembloids.

We foresee applications of Kiri-E in diverse areas of neuroscience. In precision medicine, longitudinal monitoring of patient-derived assembloids may provide clues into both brain deficits and interventions in a personalized manner, including the possibility of organoid implants that ameliorate neural circuit deficits. In pharmacology, as assembloid cultures become more scalable and reproducible, they may be useful in drug screening to identify therapeutic compounds that are tailored to discrete developmental time periods. Finally, in basic research, we are fascinated by the promise of assembloids for understanding synaptic and microcircuit functions. Reductionist approaches have long provided elegant models to understand how networks of neurons generate complex physiology and behavior — for instance, using the *Aplysia* sensori-motor synapse or the lobster stomatogastric central pattern generator. By constructing neural circuits in vitro, one component at a time, can we define the minimal unit that governs specific synaptic computations and emergent network properties? Kiri-E could provide a crucial tool for such investigations.

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Competing interests

The authors declare no competing interests.