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Next-generation cortical organoid models: from self-organization to integrated systems

Abstract

Understanding human brain development remains challenging because animal models do not recapitulate many human-specific features, and access to developing human tissue is rare. Over the past decade, stem cell–derived 3D brain organoids have transformed this landscape by enabling controlled modeling of fate specification, cell diversification, migration, and emerging connectivity. Advances in patterning and culture now yield region-specific cortical organoids that recapitulate key human cortical cell types, trajectories, and disease-relevant phenotypes. Yet current models still struggle to capture the cortex's intricate architecture, long-range interactions, and extended maturation. In this review, we highlight emerging strategies to enhance cortical organoid complexity and the physiological relevance of their neural circuits.

Keywords: human brain development, cortical organoids, 3D brain organoids, stem cell

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Organoids as experimental models of the developing brain

Understanding human brain development is hampered by a paucity of experimental models capable of recapitulating its complexity and species-specific features. While traditional animal models are extremely useful to study brain characteristics shared across species, they cannot model human-specific traits such as extended neotenic periods, cell type-specific expansions, and human-specific cell proportions, in addition to the evolutionary expansion of brain areas and distinct functional properties of human circuits.[1,2] Moreover, animals cannot feasibly model complex human genetics, including polygenic disease states or the contribution of human genetic background. Experimental access to the human developing brain is limited, as most of its cell types and patterns of connections emerge during embryonic development and early postnatal life, and rare samples of *ex vivo* developing brain tissue are not amenable to expansion *in vitro* (Figure 1).

Over the past decade, stem cell-derived brain models, such as 3D brain organoids (also referred to as cerebral or neural organoids),[3] have become invaluable tools to explore and perturb human brain development. Organoid systems have undergone dramatic refinement and growth in complexity, fidelity, and reproducibility, emerging as powerful experimental models. These advances have been driven by organoid protocols that rely either on intrinsic self-organization or on external progenitor patterning (see reviews by Birtele et al.,[4] and Mayhew and Singhania,[5] for description of various organoid models). These approaches enable the generation of both unpatterned cerebral organoids, which arise through unguided differentiation and lack a defined regional identity,[6,7] and patterned, region-specific organoids, such as human cortical organoids (Figure 1).[8-11] The development of standardized differentiation and culture approaches has broadly increased reproducibility in organoid systems. Human organoids have been used to model key events of human brain development, including fate specification, cell diversification, and migration,[4,12-16] and are emerging as valuable models to study circuit organization, including long-distance connectivity.[12]

In this review, we focus on cortical organoid models, as the cerebral cortex represents a hallmark of human evolutionary expansion[2] and is prominently affected in neurodevelopmental disorders.[17-19] Cortical organoids can reliably recapitulate key features of human cortical development, including cellular composition, diversity, and developmental trajectories, with appropriate progenitor, neuronal, and glial populations emerging at defined stages.[4,5,20] These increasingly robust models are well-suited to capture disease-relevant characteristics and dissect human-specific mechanisms of cortical pathogenesis,[4,21] as their predictable developmental trajectories provide greater power to identify phenotypes linked to disorder-associated mutations and patient-specific genetic backgrounds (Figure 1).

However, even with these advantages, cortical organoids still encounter major obstacles when it comes to capturing the intricate circuitry of the human cortex. Much of this challenge arises from the difficulty of coordinating region-specific development, achieving formation of particular structural features (i.e., cortical layers), and sustaining growth over the long timescales required for the necessary degree of cell and circuit maturation.

The maturation of the cells and circuits of the brain involves multiple processes that can be defined across many axes. Organoid studies to date have generally assessed maturation through four broad categories: (i) cellular diversity, such as presence of distinct neural subtypes and glial classes, (ii) molecular maturation, as evidenced by transcriptional and epigenetic states associated with differentiated cell populations, (iii) cellular maturation, including the presence and density of dendritic spines and synapses, the complexity of dendritic and glial arborization, and myelin formation, and (iv) functional maturation, as assessed by electrophysiological properties such as network coordination and oscillatory dynamics. However, most studies address only a subset of these criteria, depending on the work's goals and guiding research questions. A comprehensive comparison assessing each of these features across the breadth of existing models has yet to be accomplished.

As researchers push forward, the next frontier lies in coaxing these miniature tissues toward more authentic circuit formation and specialization. Emerging efforts to enhance connectivity, improve cellular heterogeneity and maturation, and broaden regional diversity will help to advance this goal. Perhaps this is the start of a new chapter in organoid research, one that brings us closer to *in vitro* models that more faithfully mirror the brain's remarkable complexity.

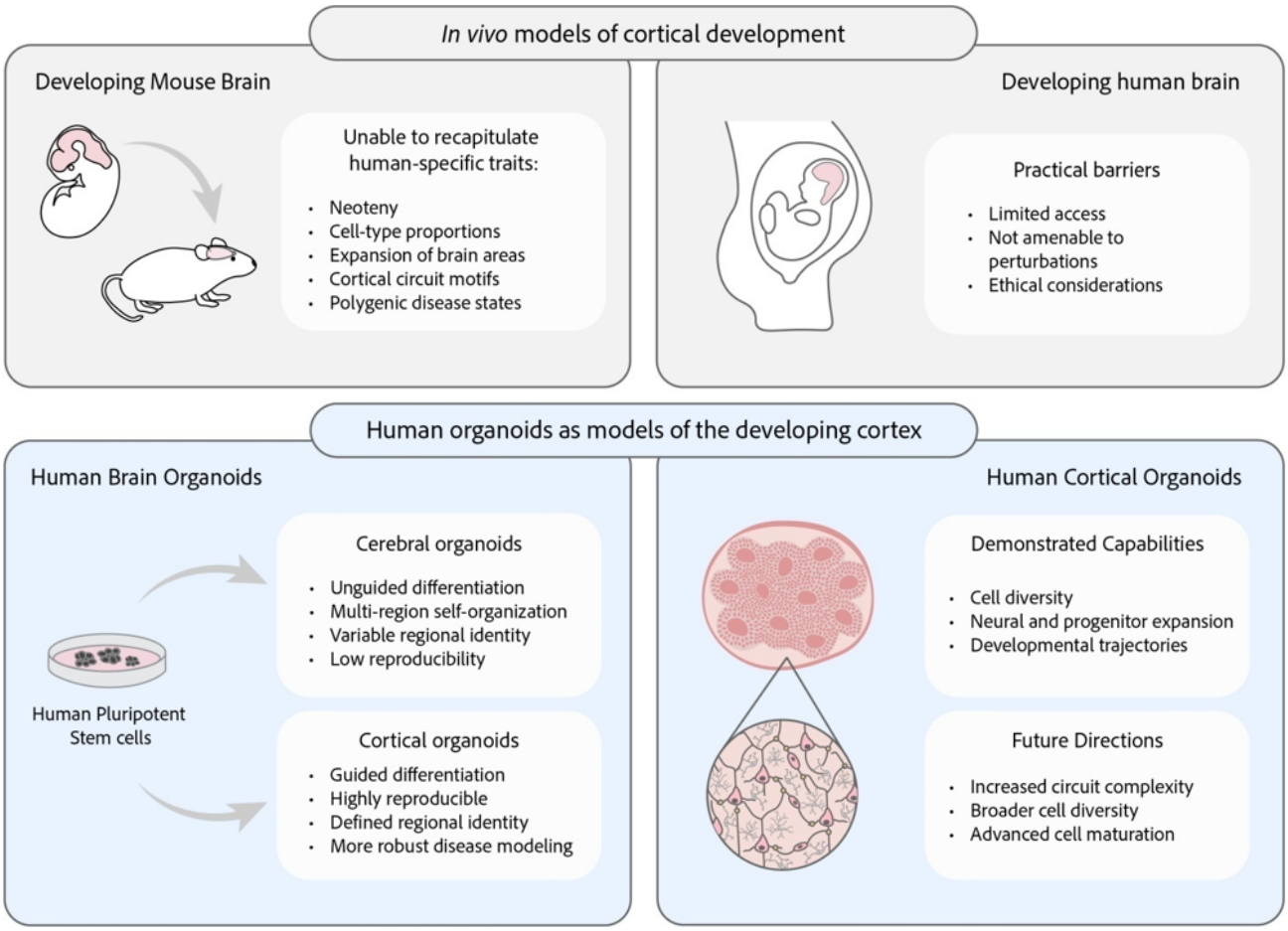

Figure 1 Human brain organoids as experimental models of cortical development. In vivo models are limited by species differences, restricted access to human tissue, and ethical and practical constraints. Human pluripotent stem cell–derived cortical organoids overcome some of these issues by offering reproducible, region-specific models that capture important features of human corticogenesis.

Toward greater complexity in cortical organoids

Intrinsic generation of cellular diversity and network complexity in cortical organoids

Cortical organoids have demonstrated a remarkable capacity to mature during long-term culture, recapitulating transcriptional trajectories that mirror the temporally coordinated stages of fetal brain development.[10,14,22-26] They undergo sequential neurogenesis, synaptogenesis, and gliogenesis, with astrocytes emerging first and oligodendrocyte precursor cells (OPCs) appearing later, alongside the generation of inhibitory neurons. Importantly, human cortical organoids also produce essential cell types, including outer radial glia and callosal projection neurons, populations that are highly expanded in humans.[2] This cellular diversity underscores the unprecedented potential of cortical organoids for studying human cortical development.

To date, organoids have largely been used to model early stages of prenatal brain development. Yet, many critical processes, such as synaptic refinement and circuit maturation, occur well into postnatal stages. Human brain maturation therefore spans a prolonged developmental window; for the cortex, this extends over tens of years postnatally.[27-29] The ability to model these events is essential not only for establishing human-specific features of cortical function but also for revealing mechanisms underlying neurodevelopmental and psychiatric disorders.[4] Understanding how far and how fast current cortical organoids can mature, and which developmental processes they can recapitulate when guided solely by intrinsic programs, is therefore crucial.

Only a limited number of studies have cultured cortical organoids beyond six months.[22,23,26,30] These studies report molecular markers of maturation, including time-dependent upregulation of synaptic, neurotransmitter-related, and ion channel genes,[22,23,26] as well as age-associated epigenetic signatures,[22,26] particularly when organoids are maintained in activity-permissive media.[22] Evidence of network maturation has also been described.[31] Network activity typically emerges around 3–6 months[22,24,26,30] and becomes more robust over time,[26,30] a pattern that has also been described in cerebral organoids.[32-34] Together, these findings demonstrate that cortical organoids can, through intrinsic programs alone, attain substantial molecular and functional complexity. However, achieving these maturation milestones requires long culture periods, which remain a major practical limitation.

Furthermore, cortical development is shaped by interactions with extra cortical regions, diverse non-neural cell types, and structured spontaneous and sensory-driven activity.[27,28] Key cell populations,

such as parvalbumin-expressing (PVALB+) interneurons[35] and oligodendrocytes (OLs),[36] rely heavily on these external cues and, therefore, show restricted maturation in standard organoid cultures. This raises the question of whether more advanced maturation could be achieved by increasing circuit complexity, integrating additional cell types or brain regions, or introducing patterned activity, features that cannot arise solely from intrinsic developmental programs. Accordingly, recent efforts have focused on incorporating these elements into organoid protocols to accelerate maturation and enhance the physiological relevance of these models (Figure 2).

Advancing circuit complexity through cross-regional interactions and external cues

Cortical organoids can achieve remarkable levels of maturation when cultured for extended periods, yet their progression remains constrained by slow developmental timelines, as well as the lack of interaction with other brain areas. To address these challenges, emerging strategies aim to enhance organoid maturation by introducing conditions that better recapitulate the interplay between the developing cortex and other brain structures that provide necessary cues, cell types, and input into cortical neurons (Figure 2).

Multi-region integration: The brain is not a uniform structure but a mosaic of distinctive, interconnected areas. Single-region organoids, by nature, capture only part of that landscape. The use of guided patterning in cortical organoid protocols boosts reproducibility[3,9-11] but limits the regional diversity generated in earlier, unguided cerebral organoid protocols.[6,7] This reduced interregional complexity constrains the physiological relevance of single-region organoids, since cortical circuits rely on the presence of cells generated in distinctly patterned germinal zones, local connections, and long-range interactions with other brain areas.[37] For example, interneurons generally act within the cortex, whereas extra-telencephalic projection neurons extend far beyond it, and many excitatory and inhibitory neurons depend on inputs from non-cortical sources. Without these partners, the full complexity of the human cortex cannot be achieved *in vitro*.

These insights have motivated multi-region organoid strategies that preserve patterned consistency while restoring richer cross-regional interactions (Figure 2). One such approach, "connectoids", promotes the development of long-range axon tracts between organoids to study projection architecture and connectivity.[38-40] These systems display short-term plasticity, complex network dynamics, and optogenetically-evoked responses,[40] and they provide a controlled setting to model disorders involving impaired long-range communication.[39] Thus, connectoids offer a versatile platform for exploring the principles of long-distance neuronal signaling in both health and disease.

In parallel, "assembloids" have gained prominence as a complementary strategy for restoring interregional interactions. By fusing single-region organoids patterned separately, assembloids enable integrated cellular and circuit-level dynamics.[12] These models can form long-range connections and exhibit synchronized activity across regions. The earliest cortical assembloids brought together dorsal and ventral forebrain regions, enabling direct observation of interneuron migration into the developing cortex.[41] Soon after, cortico-thalamic models[42-49] expanded the repertoire, opening windows into axon guidance, disease-related phenotypes, and long-range circuit assembly.[12] More elaborate combinations have since emerged, including cortico-striatal-midbrain assembloids,[50] cortico-spinal-muscle assembloids resembling the motor pathway,[51] somatosensory-spinal-thalamic-cortical assembloids modeling ascending sensory pathways,[52] and cortico-striatal-thalamic-cortical systems for the study of loop circuits.[53]

In cortico-striatal assembloids, for example, cortical input accelerates striatal projection neuron maturation, improving electrophysiological properties, promoting dendritic spine formation, and establishing functional excitatory connectivity.[53] Dorsoventral forebrain assembloids, similarly, promote the emergence of developmentally important interneuron subtypes such as PVALB+ cells[54,55] and exhibit inhibitory synapse formation by migrating interneurons.[4] Cortico-thalamic assembloids extend these insights by showing that thalamic input promotes cortical gene programs involved in axon growth and activity regulation, expands cortical progenitors, and increases extra-telencephalic neuron production. Thalamic signals also induce network dynamics absent in cortical organoids alone, including thalamus-initiated waves and widespread synchronous activity.[45] These models suggest enhanced synaptic plasticity at both thalamocortical and corticothalamic synapses, with electron microscopy evidence of reciprocal contacts,[47] as well as more elaborate neuronal architectures marked by increased branching, longer projections, and dendritic spines containing synapses.[43] They have also proven useful for studying phenotypes in neurodevelopmental diseases.[12,16] Finally, more complex assembloids further show the capacity of these systems to model physiological responses. In motor pathway models, cortical stimulation can drive muscle contraction,[51] whereas in sensory pathway models, peripheral sensory input can elicit coordinated activity across the connected tissues.[52]

However, even in these more intricate models, the regional components of assembloids are still generated separately and fused only after some maturation has occurred. This contrasts with the embryo, where the neural tube begins as a single structure and acquires regional identity through continuous exposure to morphogens, organizer centers, and direct cell–cell interactions.[56,57] These early cues shape regional identity, boundaries, and connectivity via a process of self-organization that can only be recreated if cells of different brain regions co-develop within a single organoid. Notably, *in vivo*, early cell–cell interactions establish transient tissue boundaries that regulate cell migration and connectivity. These boundaries also serve as progenitor sources for transient cell populations that can only form when cells from distinct brain regions interact at the right time and place.[56] These nuanced interactions are largely missing in organoid systems in which regions are developed in isolation and only later fused together.

To better capture these early processes, new approaches aim to recreate patterning within a unified tissue. Some methods introduce signaling sources such as morphogen-soaked beads or cell-based morphogen release centers activated by light or chemicals.[58] Other approaches use microfluidic platforms to establish stable gradients similar to those that guide regionalization in the embryo. Together, these strategies aim to restore aspects of the shared developmental environment that current models lack. In this context, the "chimeroid" approach introduced recently by Antón-Bolaños et al., [59] offers a possible foundation for future work achieving these goals. In this study, cortical organoids derived from different induced pluripotent stem cell (iPSC) lines were generated by patterning to the neural progenitor stage, then dissociated and reassembled into a single mixed organoid. This principle could be adapted to combine progenitors with different regional identities. Such adaptations might generate unified tissues in which early cell interactions support the self-emergence of processes like migration, neuronal maturation, and circuit formation. Chimeroid strategies could therefore complement efforts to model early brain patterning and increase the complexity of organoid systems.

Collectively, multi-region organoid systems have the potential to provide unprecedented access to the mechanisms by which brain regions communicate, mature, and assemble into functional circuits. These models show that interactions between regions can drive each other toward greater maturity, potentially yielding more advanced transcriptional states and richer neuronal morphology. Nevertheless, much work remains to characterize the cellular composition of each regional model and to define the specificity of the connections that different classes of neurons make within assembloids, compared to their *in vivo* counterparts. Thus, we are still in the early days of understanding how faithfully these systems recapitulate the human brain in terms of functional properties, cell diversity, and cellular maturation, beyond broad shared features. Although the degree to which multi-region organoid strategies capture the cellular complexity, connectivity specificity, and functional properties of *in vivo* circuits remains to be clarified, they represent very valuable tools for understanding the establishment and function of long-range connectivity.

Xenotransplantation: Another strategy to increase neuronal and circuit maturation is to engraft organoids into a host brain (Figure 2). Early studies showed that transplanted neurons could survive, mature, and functionally integrate within the rodent cortex,[60,61] hinting that an *in vivo* environment might provide crucial maturation cues absent *in vitro*. Building on this idea, multiple groups have transplanted both cerebral[62-65] and cortical organoids[24,66-70] into rodent brains. Once engrafted, these tissues integrated into the surrounding brain and established reciprocal synaptic connections with host neurons.[24,64] Several studies have shown that grafted organoids can also respond to sensory stimulation.[67-70] This enriched *in vivo* milieu often promoted deeper maturation of neurons and glia within the grafted organoids, revealing facets of development that are difficult to achieve *in vitro*. For instance, cells from transplanted cortical organoids show signatures of more advanced molecular and morphological maturation, including increased expression of genes associated with synaptic activity, greater dendritic complexity and spine density,[68] and enhanced astrocyte ramification.[69] A comparable enhancement of astrocyte complexity was reported in cerebral organoid grafts.[65]

Electrophysiological indicators of maturity have also been shown to be enhanced, including coordinated activity, some oscillatory patterns, and more stable firing properties.[68,70] Xenotransplantations have also proven useful for detecting and studying cell migration defects in neurodevelopmental disorders.[66] Despite these advances, several important aspects of these systems still need to be addressed. Species-specific interactions may influence how faithfully human cells integrate into a rodent brain and the degree to which organoid physiology is shaped by these manipulations requires careful evaluation. A further key challenge is the lengthy timeline for functional human maturation, which can take close to eight months in some cortical organoid grafts.[68] Thus, the developmental stage of transplanted human organoid cells can never be fully aligned with that of the host brain, limiting their capacity for functional integration. In addition, transplanted tissue generally persists as a discrete graft within the host brain rather than achieving widespread integration and cell-type-specific connectivity within defined endogenous networks. These limitations may hinder the faithful recapitulation of spontaneous waves of activity, as well as evoked activity patterns that shape cortical development *in vivo*.[27-29] Nonetheless; xenotransplantation approaches represent an important advance toward promoting higher levels of maturation and modeling cortical development in the context of a behaving organism rather than isolated cortical tissue.

Exogenous stimulation: External stimulation is being explored as a way to enhance cortical organoid maturation, aiming to mimic the activity that shapes cortical circuits *in vivo* (Figure 2). In the developing brain, spontaneous and sensory-evoked activity from other regions guides cortical growth, refines wiring, and establishes the rhythms that support long-term circuit formation.[27-29] Cortical neurons *in vitro* generate bursts of spontaneous activity that become increasingly complex,[34,71,72] but *in vivo* the corresponding endogenous circuits are further shaped by long-range waves of patterned activity and sensory experience.[27-29]

As an initial, rudimentary mimic of these additional external influences, direct stimulation has been used to provide structured cues that may promote more biologically-relevant maturation. Some studies have tested whether short-term electrical stimulation can rapidly modify cerebral organoid circuits.[40,73,74] Brief pulses of activity elicit acute synaptic plasticity, including short-term potentiation, showing that organoids can adjust their activity on fast timescales. These results provide initial evidence that activity can shape electrophysiological and synaptic behavior in organoids. While promising, more closely mimicking *in vivo* development requires sustained activation.

Longer-term stimulation protocols examine how prolonged activity influences maturation. In one study,[75] 45 days of activity-enhancing drug treatment increased synaptic gene expression and the number of synaptic puncta, reduced progenitor-state signatures, and produced more mature electrophysiological properties in cortical organoids. Electrical stimulation has shown similar benefits. In cortical organoids, an 8-day stimulation period during progenitor stages improved neurogenesis, synaptogenesis, and long-lasting plasticity, with effects that persisted for months, and promoted better integration after transplantation into the mouse brain.[76] Another recent work[77] using daily stimulation over two weeks in cerebral organoids revealed network-level reorganization, particularly when multiple organoids were interconnected, indicating that both chronic stimulation and multi-organoid architecture contribute to circuit maturation. Altogether, these findings suggest that sustained external stimulation can push organoids toward more advanced stages of cortical development.

Yet human cortical development unfolds over months and even years, suggesting that truly recapitulating this process may require much longer stimulation and continuous monitoring of network evolution, a technical challenge that is only beginning to be tackled.[52,78-81] To move toward this goal, emerging approaches include studies that add ions or neurotransmitters while recording activity in real time.[82] However, such exogenous stimulation can only approximate the richness and specificity of activity dynamics that shape cortical development *in vivo*.

Although external electrical or chemical stimulation can mimic certain activity patterns, *in vivo* cortical circuits receive highly specific inputs from subcortical and sensory regions that shape their development, first through waves of spontaneous patterned activity and later through sensory-driven signals.[27-29] Moreover, the absence of sensory input is known to hinder cortical maturation *in vivo*.[83] Providing these physiologically-relevant types of input would be an exciting next step. One potential way to achieve this would be to use multi-region organoid strategies, combining sensory (e.g., retinal) and cortical systems and stimulating them through the sensory component itself. Retino-cortical and retino-thalamo-cortical assemblies have been generated,[84,85] making this an appealing avenue for future exploration.

In summary, activity-based interventions show that patterned stimulation can promote more mature molecular, morphological and functional states in cortical organoids, but current paradigms

remain rudimentary and are still far from capturing the complex, temporally- and spatially-structured activity patterns that shape cortical development *in vivo*. Nonetheless, they provide crucial proof-of-principle that activity can be systematically manipulated to guide organoid development toward more complex circuit states.

Expanding cell diversity and neuromodulatory control

Cortical organoid models are increasingly being refined to maximize physiological relevance by more closely mimicking the brain's environment and multicellular complexity. Multi-region organoid integration, together with xenotransplantation and activity-based approaches, represent promising strategies to push cortical organoids toward more complex cellular and circuit states and to mitigate some intrinsic limitations of cortical organoids, such as the lack of interaction with other brain areas and a relatively simplified functional and molecular environment. However, other important constraints, including limited representation of extracortical cell types and the absence of neuromodulator-driven refinement of circuit states, are not fully addressed by these strategies.

Beyond neurons, non-neural cell types play essential roles in shaping cortical circuit development. Astrocytes are a notable example:[174] *in vivo*, they promote synapse formation, maturation, and activity-dependent synaptic engulfment. In cortical organoids, astrocytes spontaneously emerge[14,15] and mature over long-term culture.[14,21,69,86,87] Although evidence that mature astrocytes can promote cortical circuit development in organoids is limited, one study shows that astrocyte-derived secretomes can accelerate neuronal differentiation and enhance network activity.[88] By contrast, other key non-neural populations, such as microglia and endothelial cells, do not arise in cortical organoids, and others that do, such as OLs, show limited maturation. Neuromodulatory neurotransmitters that tune cortical states and circuit refinement are also missing. These gaps highlight the need to incorporate additional cell types and neuromodulatory inputs to achieve more physiologically complete cortical organoids (Figure 2).

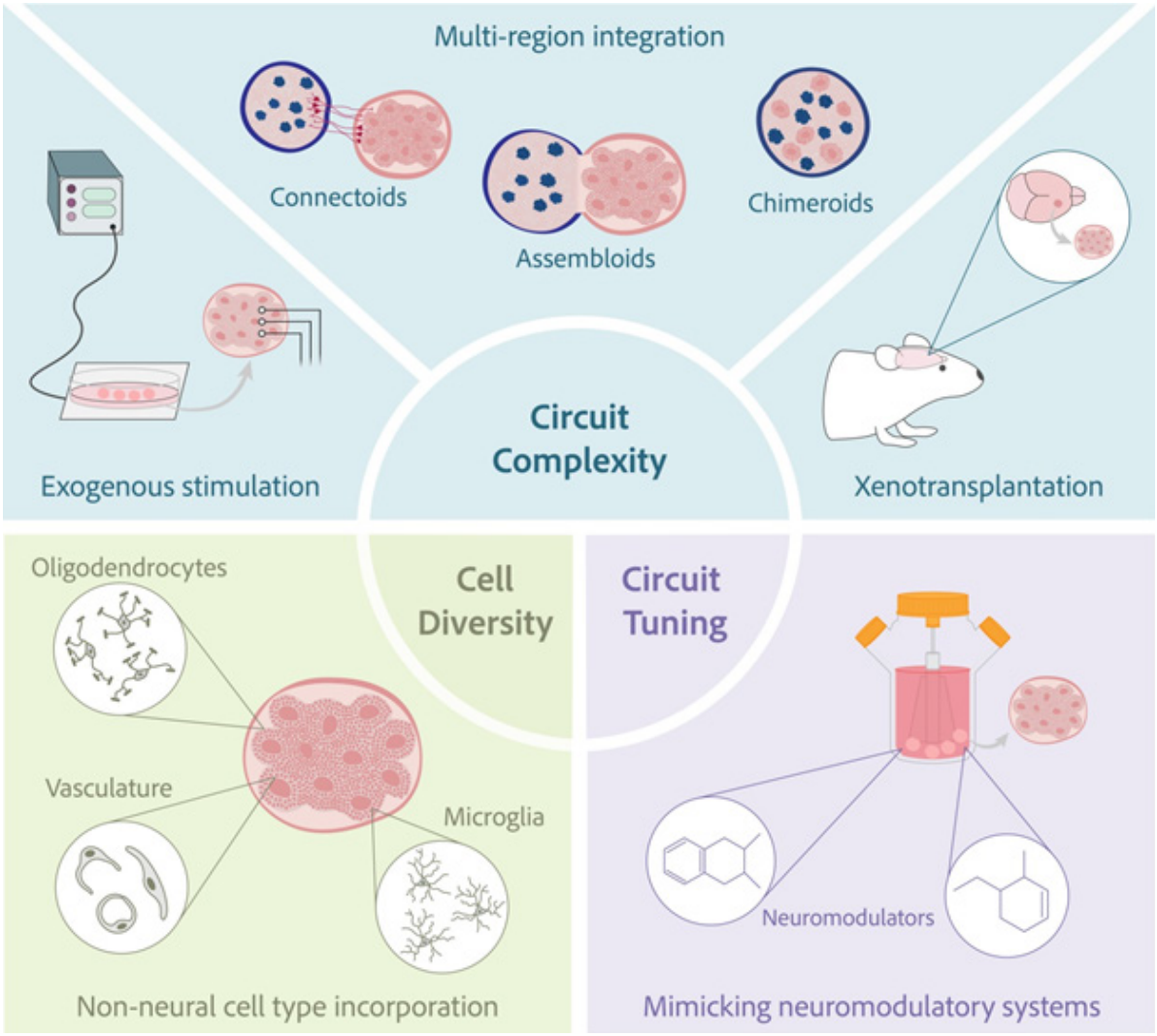

Figure 2 Strategies to enhance human cortical organoid maturation. Approaches include (i) modeling the interaction of diverse brain regions with the developing cortex in multi-region organoids; (ii) mimicking spontaneous and evoked activity during development through exogenous stimulation; (iii) providing in vivo-like endogenous cues to enhance maturation through transplantation into a host brain; (iv) expanding cell diversity by incorporating microglia and vasculature and promoting oligodendrocyte maturation; and, (v) controlling circuit state and tuning via added neuromodulation.

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Oligodendrocytes: OLs regulate cortical developmental timing, plasticity, and circuit function throughout life.[89,90] Beyond their well-characterized role in myelination, they act as active circuit-specific regulators by phagocytosing axons and synapses, integrating synaptic inputs, and releasing extracellular matrix-related factors.[89-93] Their dense innervation by cortical inhibitory neurons, which is especially pronounced during specific developmental windows, has linked them to key roles in activity-dependent circuit plasticity and refinement, particularly during critical periods.[92] They also contribute to synchrony across cortical regions,[94] and their dysfunction is associated with several pathological conditions, including neurodevelopmental disorders.[91]

During development, cortical OLs arise later than astrocytes, originating from OPCs that first appear in two early migrating ventral waves, followed by a third wave generated locally.[95] Accordingly, OPCs and OLs also emerge in both unpatterned and cortically-patterned organoids, but only after prolonged maturation and with limited progression, resulting in absent or very sparse and immature myelin formation.[7,14,15,96,97] Given this delayed and limited oligodendrogenesis, multiple approaches have been tested to accelerate OL appearance and maturation (reviewed in Huang et al.,[48] and extensively in Zeldich and Rajkumar[98]). In brief, adding OPC-promoting factors to dorsal forebrain organoids enriches late-wave OLs,[99] while ventral forebrain organoids supplemented with glia-promoting media produce early-wave OLs and can generate myelin by five months.[100] Fusing treated dorsal and ventral forebrain organoids enables OPC migration and produces more mature OL features within six weeks compared with single-region organoids.[101] However, myelination in these models remains limited, with few myelinated axons, poor compaction, and no nodes of Ranvier.[99-101] In contrast, these features do appear in midbrain organoids,[102] where myelination occurs earlier than in the cortex *in vivo*, suggesting that cortical models with more advanced myelination may also be possible. More recent strategies, including trophic factors, pro-OPC geneover expression, and transplantation into the mouse brain, can produce earlier myelin (as soon as 12 weeks)[103-106] and even nodes of Ranvier.[103] However, the effects of these developments on neurogenesis, cellular diversity, and astrogenesis remain untested.

Direct evidence that inclusion of mature OLs can enhance network dynamics in cortical organoids is currently absent. One recent study[30] detected network activity but did not assess whether the presence of OLs directly contributed to it. Overall, these strategies to increase myelination and OL maturation are encouraging, but further work is needed to clarify their potential impact on cortical circuit maturation in organoids.

Microglia: Microglia sculpts neuronal circuits by assisting with the elimination of weak synapses and releasing cytokines and growth factors that regulate neuronal excitability and plasticity.[107] They play a key role in refining cortical circuits, not only by shaping excitatory networks but also by regulating the development and output of cortical interneurons early in life.[108-111] Microglia also helps maintain neural circuitry during development by modifying the perisynaptic matrix, the diffuse extracellular material surrounding dendritic and axonal terminals.[112] Furthermore, alterations in microglial number or function have been linked to neurodevelopmental disorders.[113,114] Notably, microglia share a highly-conserved developmental origin across vertebrates: they largely arise from yolksac-derived mesodermal progenitors early in embryogenesis, enter the brain through the nascent vasculature, and gradually spread across the forming cortex.[115] In contrast, regionalized cortical organoids are patterned toward neuroectoderm and, thus, lack the mesodermal signals required to generate microglia, prompting the development of approaches to incorporate them into these models.

There are three main strategies to generate microglia-containing brain organoids:[116] (i) co-culture, in which independently-derived microglia are combined with mature neural organoids at defined ratios; (ii) addition of microglia or microglial progenitors into pre-generated organoids; and (iii) spontaneous generation, which allows mesodermal lineages to emerge and produce endogenous microglia. Across these approaches, microglia have been shown to accelerate intrinsic and network-level maturation in cerebral organoids,[117-119] midbrain organoids,[120] and cortical organoids, where microglia were generated through overexpression of a myeloid-specific factors.[121] Microglia has also been observed to support synaptic pruning in cerebral organoids[119,122] and in co-cultures with tripartite cortico-striatal-midbrain assembloids.[123] Interestingly, pathological overpruning and synaptic loss have been reported in cortical organoids containing microglia carrying genetic risk mutations associated with autism spectrum disorder.[123,124] Microglia may also play neuroprotective roles in organoids, as suggested by observations in long-term cerebral organoids containing integrated iPSC-derived microglia.[125] Although these findings are promising, further work will be needed to clearly elucidate how microglia contribute to the maturation and increased structural and functional complexity of cortical organoids.

Vasculature: Vascularization of the cortex occurs early in embryonic development, with cells from the vasculature originating extracortically and migrating into the developing tissue.[95] This process is tightly regulated by reciprocal communication between vascular and neural lineages. In the cortex, endothelial cells (the cells that line the interior surface of blood vessels) have been shown to influence circuit formation by regulating excitatory and inhibitory neuronal migration, synaptogenesis, and synaptic strength.[126-129] Disruption of these endothelial–neural interactions can alter migration patterns and may contribute to neurodevelopmental disorders.[130]

A major driving purpose behind vascularizing cerebral and cortical organoids has been to enhance their long-term survival and enable the modeling of neurovascular development, including the formation of the neurovascular unit (NVU), angiogenesis, and blood–brain barrier features. Therefore, multiple strategies have been developed for vascular development in organoids, including co-culture methods, factor-based differentiation, genetic engineering, *in vivo* engraftment, and microfluidic platforms. Across these methods, researchers consistently report the formation of mature and functional vascular structures and in some cases even NVUs.[131]

Beyond these motivations, accumulating evidence suggests that vascularization can improve neuronal circuit quality within cortical organoids, although this area of study is very limited. Adding endothelial cells through various strategies appears to enhance molecular signatures of neuronal maturation, although often without electrophysiological validation.[132-136] Complementary work using mouse endothelial–neuron co-culture models has shown pro-maturation effects, including enhanced neurite outgrowth, increased dendritic spine density, a higher number of excitatory synapses, and more advanced electrophysiological properties.[137] Notably, one study using human umbilical vein endothelial cells showed increased neuronal maturation, improved synaptic development, and more organized excitatory–inhibitory network activity in cortical organoids.[24] Together, these findings highlight that vascular components can actively contribute to the establishment of more mature and functionally organized cortical circuits. Future studies incorporating these cells into cortical organoids will be important to determine how they further improve cortical circuit development and function.

Neuromodulators: Five major neuromodulatory systems regulate cortical circuits: acetylcholine, histamine, dopamine, norepinephrine, and serotonin. They arise from subcortical nuclei in the basal forebrain, hypothalamus, midbrain, and hindbrain, which send long-range projections that broadly innervate the cortex.[138] Although traditionally considered slow and spatially-diffuse signals that act mainly through volume transmission, growing evidence shows that these neuromodulators can also exert rapid and spatially-precise effects.[138,139] Across systems, neuromodulators shape cortical physiology by regulating neuronal excitability, synaptic plasticity, and large-scale network states, including thalamocortical pathways.[140,141] They are essential for higher cognitive functions such as learning and memory[138,141] and have been implicated in cortical development and in vulnerability windows linked to neurodevelopmental and psychiatric disorders.[142-144] Despite their importance, their precise roles within cortical circuits remain understudied.

This knowledge gap is even greater in cortical organoids, where neuromodulatory signaling is largely absent. Although transcriptomic analyses indicate that many neuromodulatory neurotransmitters are present in cerebral organoids,[82] and region-specific models have been generated, including serotonergic hindbrain organoids,[145] locus coeruleus-like norepinephrine neurons,[146] and dopaminergic midbrain organoids,[147] few studies have integrated these populations with cortical organoids or assessed their influence on cortical network maturation.

A notable exception is a cortico-striatal-midbrain assembloid showing dopamine-dependent cortical activity,[50] though its effects on network maturation remain untested. Focusing on glia, another promising study[65] showed that astrocytes from cerebral organoids engrafted into the mouse cortex enhanced their calcium responses and morphological complexity through modulation by cholinergic and dopaminergic inputs. Given the essential role of neuromodulators in tuning cortical circuits, introducing neuromodulatory systems into cortical organoids may be particularly valuable. This could be done by applying exogenous neuromodulatory signals or, more physiologically, by adding subcortical regions to reconstitute long-range neuromodulatory pathways. Such approaches could provide a powerful platform to uncover human-specific mechanisms of cortical maturation and refinement and to clarify how their disruption contributes to disease.

Multi-lineage approaches: In the last few years, new strategies have begun to incorporate multiple non-neuronal cell types into cortical organoids, an important advance given the essential bidirectional crosstalk among microglia, OLs, and other non-neuronal lineages that supports cortical circuit maturation.[91-93] A recent model integrating both microglia and OLs into cerebral organoids[148] highlighted some microglia-mediated remyelination after injury, underscoring the importance of introducing multiple non-neuronal cell types to increase biological realism. Although such dual integration is promising, its direct impact on circuit-level enhancement within organoid systems remains unexplored.

Building on this concept, strategies combining vascular and microglial elements yield further biologically relevant outcomes: vascular–cerebral assembloids show that microglia remain immunocompetent within a vascularized microenvironment,[136] and an iPSC-derived hematopoietic/endothelial co-induction strategy promotes early astro cytogenesis, supports functional microglia, and achieves perfusion following *in vivo* transplantation.[149] Extending these multisystem approaches, a three-dimensional human co-culture incorporating iPSC-derived microglia, OLs, and vascular cells together with neurons and astrocytes reported enhanced neuronal synaptic protein expression, increased calcium transient frequency, elevated spike and burst rates, and reduced response latency to electrical stimulation relative to neuron-only monocultures. Consistently, transcriptomic signatures show heightened neuronal and synaptic maturation compared to neuron-astrocyte co-cultures, supporting the idea that diverse non-neuronal lineages may collaborate to promote cortical circuit maturation.[150]

Overall, the field has successfully incorporated non-neural cells into cortical and other organoids, with flexible strategies to either promote their endogenous development or add them exogenously. Across several studies, such multisystem models show more advanced maturation than neuron-only cultures. Yet *in vivo*, diverse cell classes interact in tightly-regulated spatial and temporal patterns, and species-specific differences in cell-type proportions critically shape brain properties, implying that these proportions may need to be controlled to achieve normal network function. Key open questions concern which developmental stages, timings, combinations, and relative ratios of cell types provide the greatest benefit, and whether these cells interact during appropriate developmental windows to shape circuit-level behavior. Together, converging evidence suggests that multisystem cortical organoids are a promising route to more physiologically-complete reconstructions of human cortical maturation, particularly as they begin to be applied to disease modeling.

Future directions: challenges, applications and opportunities

As cortical organoids develop more mature and diverse circuits, they are opening new avenues for exploring human cortical development and function. These advances enable a new range of biological questions to be addressed directly in a human context, expanding the frontiers of developmental neuroscience.

The increasing complexity of next-generation organoids will likely enable the investigation of human-specific aspects of cortical microcircuit development, moving the field beyond earlier developmental events, such as fate specification, cell diversification, and migration. Although human and mouse cortex differ in specific cell-type identities, such as expanded interneuron and glial diversity, these distinctions do not fully explain the major evolutionary changes of the human cortex. More fundamental shifts in cell properties are thought to be key, including the expansion of outer radial glia that drives cortical enlargement, increases in callosal projection neurons, altered interneuron proportions, and the larger, more complex morphology of human pyramidal neurons.[2,11] Building on this cellular foundation, major species differences also appear at the microcircuit level. While humans and rodents share the same basic cortical plan, human cortical microcircuits are sparser, more interneuron-centered, and more influenced by non-neuronal cells. Humans have fewer synapses overall, but these synapses (especially in upper layers and pyramidal-to-interneuron pathways) are stronger and more reliable.[151-153] Interneuron networks are expanded, with more inhibitory-to-inhibitory connections,[154] and primates show pronounced upper-layer expansion and cortico-cortical projection dominance.[152,155] Additional human-specific shifts include changes in neuromodulatory receptor expression[156,157] and more structured neuron–glial organization.[155,158] These differences likely contribute to the distinctive features of human brain function and cognition. Still, the scope of these human-specific microcircuit specializations, their origins, and their impacts on cortical development and disease risk remain unresolved, questions that advanced cortical organoids could help address.

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Beyond these microcircuit-level differences, the developmental tempo of human cortical networks is markedly prolonged, extending well into postnatal life and involving major shifts in circuit architecture, synaptic refinement, and myelination.[6,159-161] Consequently, humans maintain extended and gradually closing windows of plasticity, allowing experience to influence circuit formation for much longer than in other species.[159,160,162,163] Disruptions to this timing are often linked to neurodevelopmental disorders.[164] Next-generation organoids offer an experimentally accessible model for probing these slowly unfolding processes, perhaps highlighting how genetic or environmental factors shape the timing and flexibility of network formation and the mechanisms guiding human-specific cortical circuit maturation. Extending this framework, strategies linking cortical organoids to sensory structures or device-based interfaces could further clarify how experience molds developing circuits. Such systems would allow direct study of sensory processing, environmental responsiveness, and disorders affecting these pathways, providing a complementary platform for investigating experience-dependent refinement and critical period regulation.

Enhanced cortical organoids will also open the door to revealing mechanisms of neurodevelopmental disorders. Many of these conditions involve disruptions in synaptic function and circuit organization, to the point that they have been described as synaptopathies,165 or, more recently, as connectopathies.166 As organoids begin to capture more human-specific cellular and regional interactions and circuit motifs, they will enable deeper investigation of how genetic variants or environmental insults alter network-level function. These advances may also support more predictive therapeutic screening by allowing candidate compounds to be tested on human-like circuits that better reflect developing cortical dynamics. Emerging findings highlight this translational potential: a recent study66 showed that an antisense oligonucleotide therapy can rescue core cellular and circuit defects in Timothy syndrome across dorsoventral forebrain assembloids and transplantation models, and efforts are now underway to translate this approach clinically.

Together, these advances position next-generation cortical organoids as powerful platforms for uncovering the cellular and circuit-level principles that shape human cortical development and disease. However, as these models become increasingly complex and versatile, they raise some ethical considerations. As noted in recent articles,[167,168] the rapid advancement of neural organoid research is calling for discussions about higher order functions, the ethics of implanting organoids into other species, and the broader limits of the field.[169-170] Together, these challenges underscore the need for careful oversight and a clear ethical framework for this area of research at large.

By capturing increasingly human-like features of cortical organization, cortical organoids provide a framework for investigating the origins of unique human traits, clarifying mechanisms that underlie neurodevelopmental vulnerability, and guiding more precise strategies for intervention.[170-175] Organoids may never reproduce every nuance of the living human brain, but even without this, they hold promise to deliver powerful insights into human brain biology, development, and disease. With rapid methodological advances and growing efforts toward standardization, these models are becoming robust and flexible enough to drive new discoveries, opening avenues previously inaccessible in human neuroscience.

Author contributions

Marta Montero Crespo and Milagros Pereira Luppi contributed equally as co–first authors.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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