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Organoid intelligence (OI): the new frontier in biocomputing and intelligence-in-a-dish

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Abstract

Recent advances in human stem cell-derived brain organoids promise to replicate critical molecular and cellular aspects of learning and memory and possibly aspects of cognition *in vitro*. Coining the term "organoid intelligence" (OI) to encompass these developments, we present a collaborative program to implement the vision of a multidisciplinary field of OI. This aims to establish OI as a form of genuine biological computing that harnesses brain organoids using scientific and bioengineering advances in an ethically responsible manner. Standardized, 3D, myelinated brain organoids can now be produced with high cell density and enriched levels of glial cells and gene expression critical for learning. Integrated microfluidic perfusion systems can support scalable and durable

culturing, and spatiotemporal chemical signaling. Novel 3D microelectrode arrays permit high-resolution spatiotemporal electrophysiological signaling and recording to explore the capacity of brain organoids to recapitulate the molecular mechanisms of learning and memory formation and, ultimately, their computational potential. Technologies that could enable novel biocomputing models *via* stimulus-response training and organoid-computer interfaces are in development. We envisage complex, networked interfaces whereby brain organoids are connected with real-world sensors and output devices, and ultimately with each other and with sensory organ organoids (e.g. retinal organoids), and are trained using biofeedback, big-data warehousing, and machine learning methods. In parallel, we emphasize an embedded ethics approach to analyze the ethical aspects raised by OI research in an iterative, collaborative manner involving all relevant stakeholders. The many possible applications of this research urge the strategic development of OI as a scientific discipline. We anticipate OI-based biocomputing systems to allow faster decision-making, continuous learning during tasks, and greater energy and data efficiency. Furthermore, the development of "intelligence-in-a-dish" could help elucidate the pathophysiology of devastating developmental and degenerative diseases (such as dementia), potentially aiding the identification of novel therapeutic approaches to address major global unmet needs.

KEYWORDS

organoid intelligence, biocomputing, microphysiological systems, electrophysiology, cognition, artificial intelligence

Key points

- Biological computing (or biocomputing) could be faster, more efficient, and more powerful than silicon-based computing and AI, and only require a fraction of the energy.
- 'Organoid intelligence' (OI) describes an emerging multidisciplinary field working to develop biological computing using 3D cultures of human brain cells (brain organoids) and brain-machine interface technologies.
- OI requires scaling up current brain organoids into complex, durable 3D structures enriched with cells and genes associated with learning, and connecting these to next-generation input and output devices and AI/machine learning systems.
- OI requires new models, algorithms, and interface technologies to communicate with brain organoids, understand how they learn and compute, and process and store the massive amounts of data they will generate.
- OI research could also improve our understanding of brain development, learning, and memory, potentially helping to find treatments for neurological disorders such as dementia.
- Ensuring OI develops in an ethically and socially responsive manner requires an 'embedded ethics' approach where interdisciplinary and representative teams of ethicists, researchers, and members of the public identify, discuss, and analyze ethical issues and feed these back to inform future research and work.

Introduction

Human brains are slower than machines at processing simple information, such as arithmetic, but they far surpass machines in processing complex information as brains deal better with few and/or uncertain data. Brains can perform both sequential and parallel processing (whereas computers can do only the former), and they outperform computers in decision-making on large, highly heterogeneous, and incomplete datasets and other challenging forms of processing. The processing power of the brain is illustrated by the observation that in 2013, the world's fourth-largest computer took 40 minutes to model 1 second of 1% of a human's brain activity (1). Moreover, each brain has a storage capacity estimated at 2,500 TB, based on its 86–100 billion neurons having more than 10^{15} connections (2, 3). In this article, we describe the emerging field that we term "organoid intelligence" (OI), which aims to leverage the extraordinary biological processing power of the brain.

Superficially, both biological learning and machine learning/AI by an intelligent agent build internal representations of the world to improve their performance in conducting tasks. However, fundamental differences between biological and machine learning in the mechanisms of implementation and their goals result in two drastically different efficiencies. First, biological learning uses far less power to solve computational problems. For example, a larval zebrafish navigates the world to successfully hunt prey and avoid predators (4) using only 0.1 microwatts (5), while a human adult consumes 100 watts, of which brain consumption constitutes 20% (6, 7). In contrast, clusters used to master state-of-the-art machine learning models typically operate at around 10^6 watts. Since June 2022, the USA's Frontier has been the world's most powerful supercomputer, reaching 1102 petaFlops (1.102 exaFlops) on the LINPACK benchmarks. The power consumption of the new supercomputer is 21 megawatts, while the human brain operates at the estimated same 1 exaFlop and consumes only 20 watts (Table 1) (8–11). Thus, humans operate at a 10^6-fold better power efficiency relative to modern machines albeit while performing quite different tasks.

Second, biological learning uses fewer observations to learn how to solve problems. For example, humans learn a simple "same-versus-different" task using around 10 training samples (12); simpler organisms, such as honeybees, also need remarkably few samples ($\sim 10^2$) (13). In contrast, in 2011, machines could not learn these distinctions even with 10^6 samples (14) and in 2018, 10^7 samples remained insufficient (15). Thus, in this sense, at least, humans operate at a $>10^6$ times better data efficiency than modern machines. The AlphaGo system, which beat the world champion at the complex game Go, offers a concrete illustration (16, 17). AlphaGo was trained on data from 160,000 games (17); a human playing for five hours/day would have to play continuously for more than 175 years to experience the same number of training games – indicating the far higher efficiency of the brain in this complex learning activity. The implication is that AI and machine-learning approaches have limited usefulness for tasks requiring real-time learning and dynamic actions in a changing environment. The power and efficiency advantages of biological computing over machine learning are multiplicative. If it takes the same amount of time per sample in a human or machine, then the total energy spent to learn a new task requires 10^{10} times more energy for the machine. AlphaGo was trained for 4 weeks using 50 graphics processing units (GPUs) (17), requiring approximately 4×10^{10} J of energy – about the same amount of energy required to sustain the metabolism of an active adult human for a decade. This high energy consumption prevents AI from achieving many aspirational goals, for example matching or exceeding human capabilities for complex tasks such as driving (18). Even large multinational corporations are beginning to reach the limits of machine learning owing to its inefficiencies (19), and the associated exponential increase in energy consumption is unsustainable (20), especially if technology companies are to adhere to their commitments to become carbon negative by 2030 (21, 22). At a national level, already in 2016 it took the equivalent of 34 coal-powered plants, each generating 500 megawatts, to meet the power demands of US-based data centers (23). Being much more energy efficient than current computers, human brains could theoretically meet the same US data storage capacity using only 1,600 kilowatts of energy. Notably, the power demands of any current or future implementation of OI is very different from the energy consumption of the human body, especially considering the energy footprint of modern cell culture relative to small organoids today. These comparisons of brains and computers serve only as illustrations of the high efficiency of the human brain.

TABLE 1 Comparison of the latest supercomputer (June 2022) and a human brain.

	Frontier supercomputer (June 2020)	Human brain
Speed	1.102 exaFLOPS	~1 exaFLOPS (estimate)
Power requirements	21 MW	10–20 W
Dimensions	680 m^2 (7,300 sq ft)	1.3–1.4 kg (2.9–3.1 lb)
Cost	$600 million	Not applicable
Cabling	145 km (90 miles)	850,000 km (528,000 miles) of axons and dendrites
Memory	75 TB/s read; 35 TB/s write; 15 billion IOPS flash storage system, along with the 700 PB Orion site-wide Lustre file system	2.5 PB (petabyte)
Storage	58 billion transistors	125 trillion synapses, which can store 4.7 bits of information each

The Hewlett Packard Enterprise Frontier, or OLCF-5, is the world's first exascale supercomputer, hosted at the Oak Ridge Leadership Computing Facility (OLCF) in Tennessee. It is compared here with the human brain. For sources see (6–11).

Together, these observations have created high expectations for biological, brain-directed computing (24–26) as an alternative to silicon-based computing, with the potential for unprecedented advances in computing speed, processing power, data efficiency, and storage capabilities – all with lower energy needs. However, realizing the potential of biocomputing has proved challenging, and most research remains in its infancy. To date, the term "biological computing" has been used mainly to describe the use of DNA to store digital data (27, 28). An exception to this is the recent work by Kagan et al. (29), which uses the term "synthetic biological intelligence" (SBI) to describe the use of synthetic biology to generate intelligent systems through brain-directed computing, albeit using only simple 2D monolayer cell cultures (which poorly replicate the complexity of the *in vivo* brain) as a proof-of-concept.

We have coined the term "organoid intelligence" (OI) to describe an emerging field aiming to expand the definition of biocomputing toward brain-directed OI computing, i.e. to leverage the self-assembled machinery of 3D human brain cell cultures (brain organoids) to memorize and compute inputs. Brain organoids recapitulate organ histoarchitecture and functionality far more closely than traditional 2D cultures. They can contain myelinated axons (30–32) and not only show spontaneous electrophysiological activity (33) but also demonstrate complex oscillatory behavior (34), and exhibit high cell density and layering patterns, all of which make brain organoids superior to traditional monolayer cultures (34–36). The question is: can we learn from and harness the computing capacity of these

organoids? Achieving this will require major advances in the interfacing of brain cell cultures and computers. We envision using biofeedback to systematically train organoids with increasingly complex sensory inputs and output opportunities – interfacing the brain organoids with computers, sensors, and machine interfaces to facilitate supervised and unsupervised learning. We use the term "OI" for this approach to stress its complementarity to AI – where computers aim to perform tasks done by brains, often by modeling our understanding of learning. However, while AI aims to make computers more brain-like, OI research will explore how a 3D brain cell culture can be made more computer-like.

The many possible applications of this work include a new generation of biological and hybrid (biological-electronic) computing technologies, together with advances in our understanding of the physiology of cognition, learning, and memory, and the pathophysiological effects of developmental and degenerative diseases, intoxication, and infection – which in turn could stimulate drug development and other interventions. OI also has the potential to unlock new neuromimetic AI algorithms (with the potential to overcome current AI limitations) and aid the development of new brain-computer-interface technology.

The concept of brain-machine interfacing emerged around five decades ago. Before the advent of more complex human neuronal cultures and brain organoids, pioneering work on learning and memory was carried out using primitive animals such as the lamprey, showing long-term potentiation (37). This led to brain-machine interaction studies establishing bidirectional communications between the nervous system and external devices (38). Others used brain slices of different species to study the basic phenomena of learning on a cellular level (39). Neuron cultures were later shown to perform simple robotic tasks or demonstrate increased plasticity within a delayed closed-loop environment (40, 41). The combination of brain cell cultures and computers has also been attempted: 2D cultured rat neurons displayed evidence of self-organized activity in a computational task (blind source separation) when supplied with electrical information (42). A different study showed that these cultures learned to respond in the form of distinct electrophysiological patterns to low-frequency focal stimuli (43, 44).

To the best of our knowledge, however, no relevant approach using brain organoids as learning systems has been reported. Previous research has demonstrated spontaneous electrophysiological signals and synchronous neural network activity of dissociated organoids (45), sliced organoids (46), or developing full organoids (34, 47), and advanced manipulation of neural circuits within assembloids (organoids merging two distinct brain regions) was recently published (48). The only study resembling our vision is the recent work by Kagan et al. (29), which embedded monolayers of cortical neurons in a real-time closed-loop environment *via* electrophysiological stimulation and recording. These cultures self-organized to rapidly alter their activity to display goal-directed behavior in a simulated game environment. Similarly, the European Union-funded NEU-CHiP project aims to demonstrate the growth of layered networks of brain stem cells on microchips (49). In addition, the Human Brain Project models similar (but virtual) input-brain output machine models (50).

Obviously, terms such as "cognition," "intelligence," "sentience," and "consciousness," describing human capabilities, cannot be directly translated to simple cell culture models; they are used here to describe the realization of basic functions underlying these higher-order functionalities. A workshop to define adequate terminology for the field is in preparation. Please see the glossary included, which attempts to provide definitions in the context of this manuscript.

In this article, we present an architecture (Figure 1) and blueprint for an OI development and implementation program designed to:

- Determine the biofeedback characteristics of existing human brain organoids caged in microelectrode shells, potentially using AI to analyze recorded response patterns to electrical and chemical (neurotransmitters and their corresponding receptor agonists and antagonists) stimuli.
- Empirically test, refine, and, where needed, develop neurocomputational theories that elucidate the basis of *in vivo* biological intelligence and allow us to interact with and harness an OI system.
- Further scale up the brain organoid model to increase the quantity of biological matter, the complexity of brain organoids, the number of electrodes, algorithms for real-time interactions with brain organoids, and the connected input sources and output devices; and to develop big-data warehousing and machine learning methods to accommodate the resulting brain-directed computing capacity.
- Explore how this program could improve our understanding of the pathophysiology of neurodevelopmental and neurodegenerative disorders toward innovative approaches to treatment or prevention.
- Establish a community and a large-scale project to realize OI computing, taking full account of its ethical implications and developing a common ontology.

To the latter point, a community-forming workshop was held in February 2022 (51), which gave rise to the Baltimore Declaration Toward OI (52). It provides a statement of vision for an OI community that has led to the development of the program outlined here.

Prerequisites for biocomputing models

Culture and bioengineering technologies to advance 3D brain organoids

The advent of two biological technologies makes our OI approach possible: the groundbreaking work to reprogram human somatic cells back to stem cells [i.e. induced pluripotent stem cells (iPSC)] (53); and the more recent development of 3D brain organoids from iPSC.

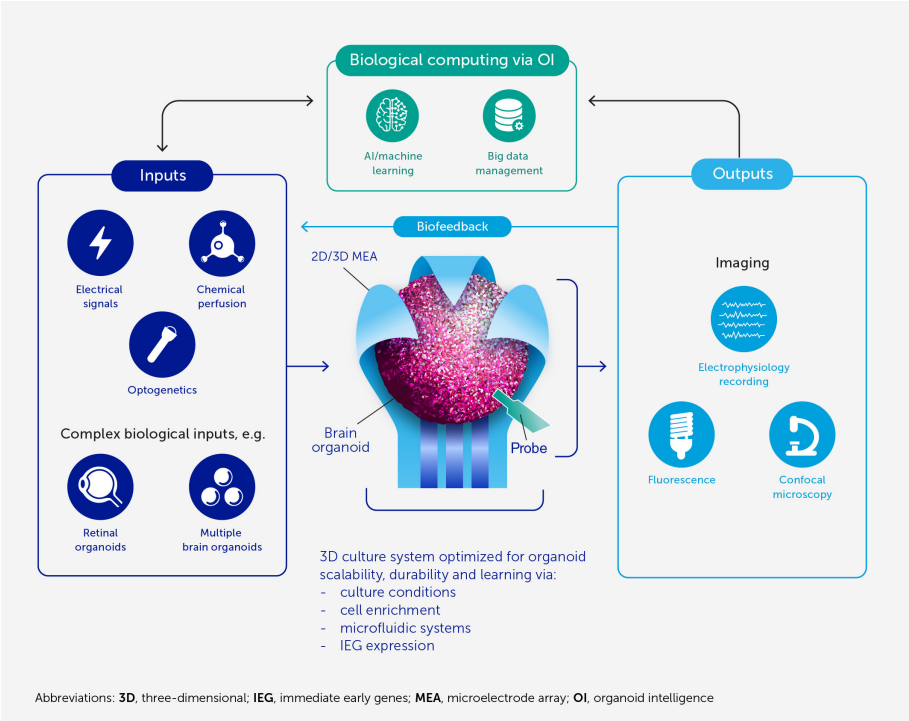


FIGURE 1
Architecture of an OI system for biological computing. At the core of OI is the 3D brain cell culture (organoid) that performs the computation. The learning potential of the organoid is optimized by culture conditions and enrichment by cells and genes critical for learning (including IEGs). The scalability, viability, and durability of the organoid are supported by integrated microfluidic systems. Various types of input can be provided to the organoid, including electrical and chemical signals, synthetic signals from machine sensors, and natural signals from connected sensory organoids (e.g. retinal). We anticipate high-resolution output measurement both by electrophysiological recordings obtained *via* specially designed 2D or 3D (shell) MEA, and potentially from implantable probes, and imaging of organoid structural and functional properties. These outputs can be used directly for computation purposes and as biofeedback to promote organoid learning. AI and machine learning are used throughout to encode and decode signals and to develop hybrid biocomputing solutions, in conjunction with a suitable big-data management system.

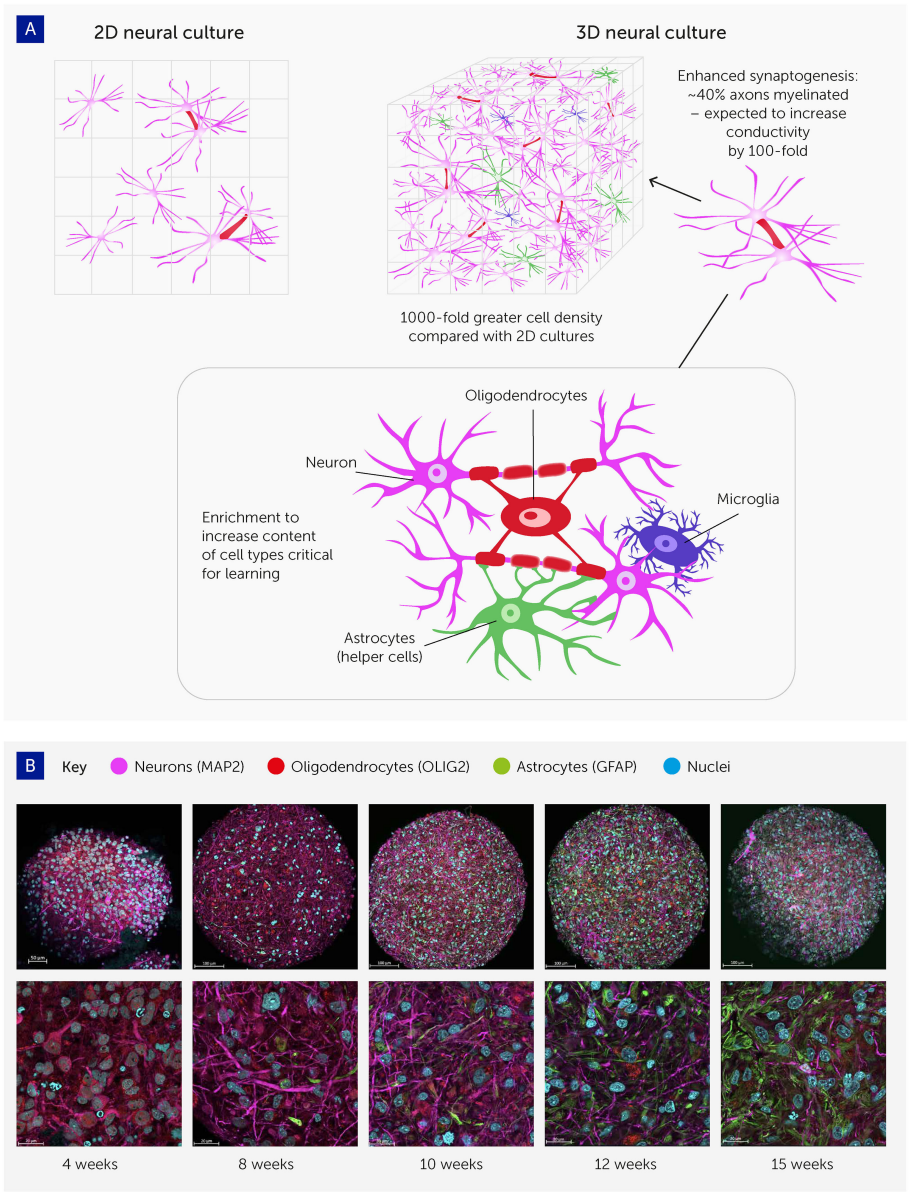
Advances in 3D organoid culture

The past decade has seen a revolution in brain cell cultures, moving from traditional monolayer cultures to more organ-like, organized 3D cultures – i.e. brain organoids (Figure 2A). These can be generated either from embryonic stem cells or from the less ethically problematic iPSC typically derived from skin samples (54). The Johns Hopkins Center for Alternatives to Animal Testing, among others, has produced such brain organoids with high levels of standardization and scalability (32) (Figure 2B). Having a diameter below 500 µm, and comprising fewer than 100,000 cells, each organoid is roughly one 3-millionth the size of the human brain (theoretically equating to 800 MB of memory storage). Other groups have reported brain organoids with average diameters of 3–5 mm and prolonged culture times exceeding 1 year (34–36, 55–59).

These organoids show various attributes that should improve their potential for biocomputing (Figure 2). First, cell density in these 3D models is similar to the *in vivo* cell density, and much higher than in monolayer cultures; the ratio of cells to media volume is also much higher compared to monolayers. Second, most of these brain organoids show spontaneous electrophysiological activity and reactivity to electrical stimulation (via evoked field potentials) (32), confirming the presence of active synapses. Trujilio et al. have shown patterning of cortex layers and oscillation waves comparable to electroencephalograms (EEGs) from human preterm babies' brains (34).

Third, axons in these organoids show extensive myelination. Pamies et al. were the first to develop a 3D human brain model showing significant myelination of axons (32). About 40% of axons in the brain organoids were myelinated (30, 31), which approaches the 50% found in the human brain (60, 61). Myelination has since been reproduced in other brain organoids (47, 62). Myelin reduces the capacitance of the axonal membrane and enables saltatory conduction from one node of Ranvier to the next. As myelination increases electrical conductivity approximately 100-fold, this promises to boost biological computing performance, though its functional impact in this model remains to be demonstrated.

Finally, these organoid cultures can be enriched with various cell types involved in biological learning, namely oligodendrocytes, microglia, and astrocytes. Glia cells are integrally important for the pruning of synapses in biological learning (63–65) but have not yet been reported at physiologically relevant levels in brain organoid models. Preliminary work in our organoid model has shown the potential for astroglia cell expansion to physiologically relevant levels (47). Furthermore, recent evidence that oligodendrocytes and astrocytes significantly contribute to learning plasticity and



Abbreviations: **2D/3D**, 2/3-dimensional; **MAP2**, microtubule associated protein 2; **OLIG2**, oligodendrocyte transcription factor; **GFAP**, glial fibrillary acidic protein

FIGURE 2
Advances in 3D cell culturing provide the foundation for systems to explore organoid intelligence. **(A)** 3D neural cell cultures have important advantages for biological learning, compared with conventional 2D monolayers – namely a far greater density of cells, enhanced synaptogenesis, high levels of myelination, and enrichment by cell types essential to learning. **(B)** Brain organoid differentiation over time from 4 to 15 weeks, showing neurons (microtubule associated protein 2 [MAP2]; pink), oligodendrocytes (oligodendrocyte transcription factor [OLIG2]; red), and astrocytes (glial fibrillary acidic protein [GFAP]; green). Nuclei are stained with Hoechst 33342 (blue). Images were taken with an LCM 880 confocal microscope with 20x and 63x magnification. Scale bars are 100 µm and 20 µm, respectively. The images show the presence of MAP2-positive neurons as early as 4 weeks, while glial cells emerge at 8 weeks and there is a continuous increase in the number of astrocytes over time.

memory suggests that these processes should be studied from a neuron-to-glia perspective, rather than the neuron-to-neuron paradigm generally used (63–65). In addition, optimizing the cell culture conditions to allow the expression of immediate early genes (IEGs) is expected to further boost the learning and memory capacities of brain organoids since these are key to learning processes and are expressed only in neurons involved in memory formation – as detailed below.

Scaling up these 3D organoids is a key early aim. We set out to produce brain organoids with about 10 million neural cells (66). Existing differentiation protocols for scaling up the cultures, and starting 3D differentiation directly from iPSC (bypassing the intermediate generation of neuroprogenitor cells), are very promising (67, 68). These developments benefit from general progress in microphysiological systems (MPS), which include organoids, and aim to establish organ architecture and functionality

such as OI as proposed here. Notably, some coauthors are involved in spearheading quality assurance guidance for Good Cell Culture Practice, expanding earlier guidance to stem cell-based models, MPS, organ-on-chip models (69), and establishing an annual MPS World Summit series (70) and international society.

Microfluidic perfusion systems

While brain organoids may recapitulate spatiotemporal molecular signatures, gene expression networks (71), certain histoarchitectures (e.g. cortex patterning), and neuron phenotypes within the human brain, they do not reflect its regional organization and the complexity of its neuronal circuitry to levels allowing for higher-order brain function (35, 72, 73). Part of the human brain's complexity stems from its size and the vasculature that supports its growth (74, 75). Although brain vasculature models are under development (76, 77), most brain organoid models so far are still avascular and rely on passive diffusion to deliver nutrients; the average scope of diffusion is approximately 300 µm before starving-derived necrosis occurs at the core (78) (Figure 3A). Thus, the lack of a perfusable vasculature is a major limitation for improving biological complexity and *in vivo*-like functionality (78).

Microfluidic systems that substitute for vasculature – allowing controlled perfusion of oxygen, nutrients, and growth factors and the removal of waste products – will be critical to the scalable and durable culturing of brain organoids (Figure 3B) (79, 80). These will support the homeostasis and viability of the organoids, allowing a more physiologic-like differentiation toward a more complex, sophisticated, and "*in vivo*-like" model. Flexible, self-folding microfluidics can already deliver chemicals with 3D spatiotemporal control (81), and recent advances in 3D printing with sacrificial materials offer the potential to create perfusable scaffolds for organoids (82, 83).

These microfluidic systems will also support chemical signaling to organoids. The importance of spatiotemporal chemical patterns to encode information is well established in neuroscience and behavioral science (84–86). Significant advances in micropatterning and microfluidics over the past two decades have already allowed 2D chips to offer significant tunability of the chemical microenvironment around neurons, and tree-like microfluidic gradient generators have been widely used to create chemical patterns (87). Importantly, 3D spatiotemporal microfluidic interfaces (88) now enable localized dosing and replication of chemical environments with neurotransmitters, neuropeptides, and other neurochemicals. A comprehensive list of the agonists and antagonists is available (89).

High-resolution recording of complex neuronal networks

3D microelectrode arrays for brain organoids

Robust and reproducible systems to record electrophysiological outputs from brain organoids are critical to developing OI systems and will need to address various challenges in reading and writing to complex neural assemblies. Brain-machine interface technologies have been in progress for at least two decades (90) but remain primitive. Microelectrode arrays (MEAs) form the heart of many such interfaces since they can be used to both stimulate and record,

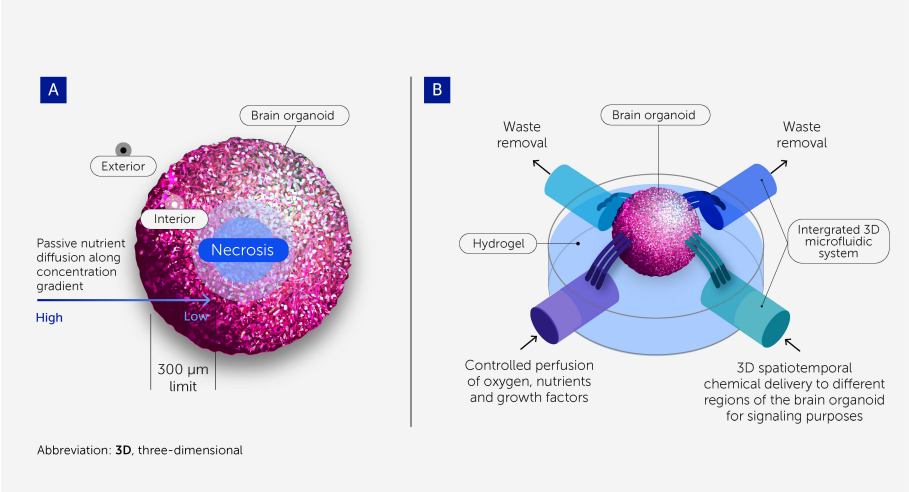


FIGURE 3
3D microfluidic devices to support scalability and long-term homeostasis of brain organoids. **(A)** Cells within brain organoids require perfusion with oxygen, nutrients, and growth factors, as well as the removal of waste products, to provide conditions approximating physiologic homeostasis. Passive diffusion penetrates to a depth of only around 300 µm, and so necrosis occurs at the core of larger organoids owing to starvation. This prevents brain organoids from being scaled up to the size and complexity required for OI research and limits their durability. **(B)** 3D microfluidic systems enable greater scalability and durability by providing controlled perfusion throughout larger organoids. They also enable 3D spatiotemporal dosing of chemicals for signaling purposes.

and offer unprecedented parallelism and individual addressability. However, most are predominantly in a 2D chip-based format, being designed for use with monolayer cell cultures (91). This represents a likely problem as brain organoids are spherical 3D structures that make limited contact with a 2D MEA chip. Furthermore, most 2D electrode chip interfaces are rigid, and a mismatch in the stiffness of the recording interface and cell system compromises performance (92, 93).

Therefore, we and others are developing novel 3D MEA interfaces specifically designed for organoids (93–96) and inspired by the EEG caps used to record brain electrical patterns from the scalp. Organoids are grown inside flexible, ultra-soft-coated, self-folding, and buckled shells, covered with patterned nanostructures and probes (92, 93, 97–99) (Figure 4). This model allows multichannel stimulation and recording spatiotemporally across the entire surface of the organoid with unprecedented resolution and high signal-to-noise ratio, resulting from the greatly enhanced recording surface areas (92, 93). After the spontaneous signal is stabilized and synchronized, the response to repeated chemical stimuli from neurotransmitter gradients [glutamate, γ-Aminobutyric acid (GABA), dopamine, serotonin, acetylcholine] and main receptors agonists/antagonists [e.g. kainic acid, kynurenic acid, γ-Amino-β-hydroxybutyric acid (GABOB), bicuculline, haloperidol, nicotine, methylbromide (89)] can be recorded to address and modulate the synaptic plasticity.

These shell MEA interfaces can be integrated with the aforementioned 3D microfluidic systems, supporting the scalability and durability of the system and chemical signaling *via* spatial patterning and gradients. Together, they create a robust platform to gain an iterative, in-depth understanding of organoid behavior and responses to a range of highly modifiable environmental and input stimuli, which in turn will allow us to explore their capacity to recapitulate the molecular mechanisms of learning and memory formation and ultimately their computational potential.

High-resolution implantable electrophysiology devices

We consider that the shell MEAs described above strike an appropriate balance by providing comprehensive, high-resolution electrophysiological recordings with minimal disruption to the organoid. However, future systems might permit organoids to be grown around implantable electrodes to further enhance signal resolution and to access the inside of the organoid. The efficiency of such systems must be balanced by their invasiveness since any damage to neuronal networks could alter the behavior of the organoid.

Neuropixels are silicon probes developed for extracellular recording in animals (mostly mice and rats) (100). They lend themselves to direct integration with brain organoids, though in principle, Neuropixels can also be integrated into shells. These large (10 mm), dense (100 sites/mm) implantable neural devices allow the recording of hundreds of well-resolved single neuron signal traces. They can be combined with light sources, electrical stimulation, and photometry, dramatically increasing the input and output opportunities and the mapping of activity in the learning organoid. These devices also appear uniquely capable of long-lived exposure to neural tissue. Chronic implants in rats and mice frequently last for 150 days or more with little degradation in recorded neural activity, indicating suitable compatibility and stability (100, 101). Recently, the Kosik laboratory (University of California Santa Barbara, CA, USA) used such CMOS shank probes in parallel with 2D high-density MEAs to record intrinsic network activity in brain organoids (46).

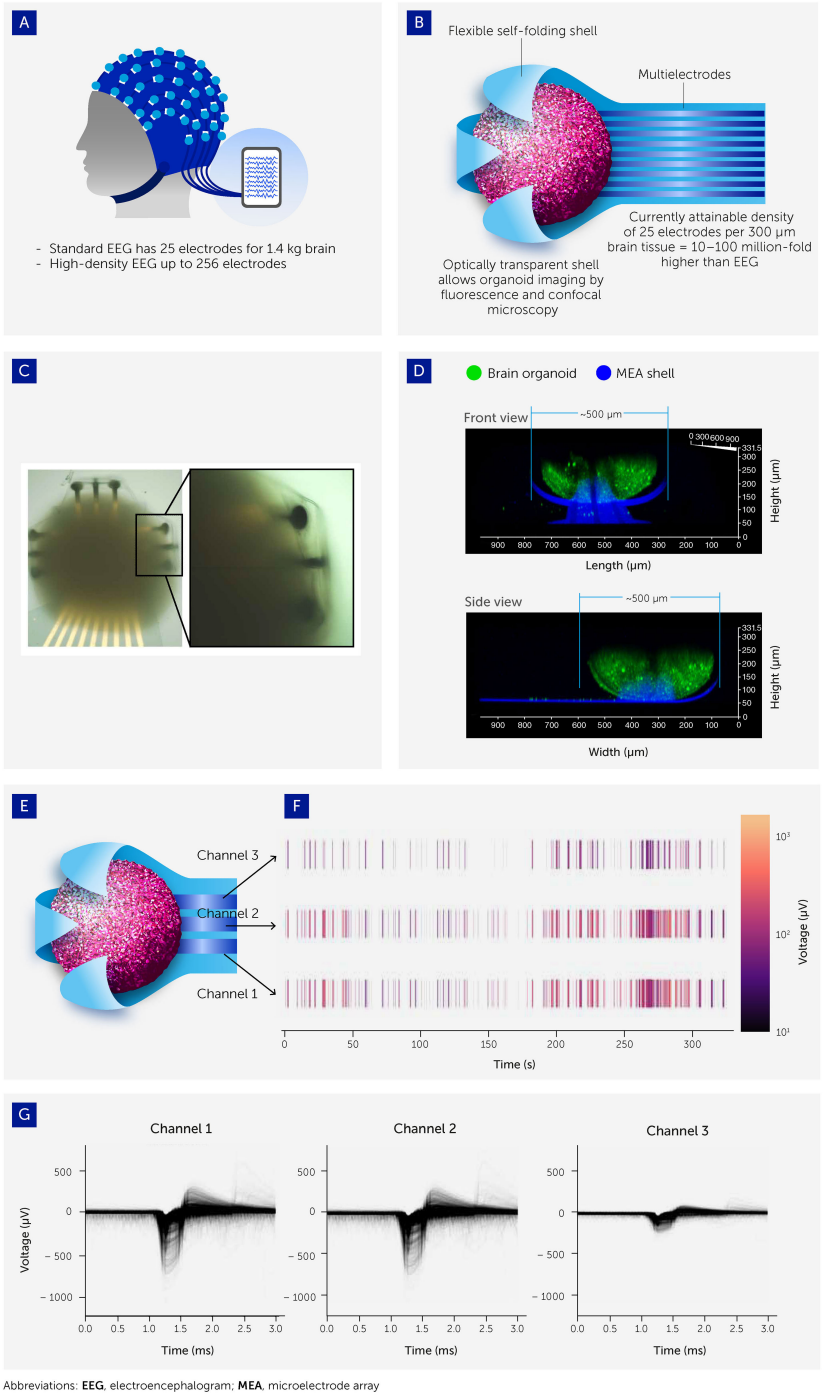
Mounting and inserting such probes into the organoid is a complex challenge, and work is ongoing to develop a new generation of suitable (more flexible) probes. If the organoid is grown in well plates, mounting of the probe would be like skull mounting in a rodent. If the growth medium needs to be exchanged periodically for organoid health without disturbing the probe-organoid interface, then an unconventional means for removing and adding media to the wells would need to be developed. The minimum size of the probe base (4.2 mm × 1 mm) allows sufficient "headroom" for this fluidic machinery. Once a microfluidic growth chamber is adopted, a membrane interface to the probe shank would be needed. Neuropixels are routinely used with a Kwik-Sil (WPI) silicon layer over the brain while recording and sealing chronic implants. Further investigation may be required to perfect this geometry, but interfacing with a low-pressure fluidic chamber does not present any fundamental problem. Finally, a Neuropixels 2.0 probe (101) has four shanks, each 10 mm long with a cross-section of 70 μm × 24 μm. For a 1 mm diameter organoid, approximately 500 electrodes would contact the cells, giving the capacity to record from 384 switch-selectable sites at a time. The probe electrodes would displace ~1.5% of the current organoid volume, likely an acceptable perturbation. Yields of 200–600 "units" are typical in recordings from rodents, limited by activity. The probe would be within detection distance of ~14% of the cells in a 1 mm diameter organoid.

Among the challenges facing brain-machine interface technologies is the scale of connectivity. Cortical neurons each have in the order of 10^4 input synapses and connect to in the order of 10^3 cells, some across many millimeters – even in small brains such as those of mice. It is not yet clear if organoids have similar or reduced synaptic counts. Current brain-machine interfaces have many unresolved cells per input (reading) for each electrode (all within 20–80 μm) and a largely unknown number of cells for output (writing or stimulation). Except for special cases in the visual cortex, the cellular understanding of writing remains difficult, while reading has enabled the control of prosthetic robots.

How might traction be achieved to begin harnessing organoid computing power? We propose that two paths be explored.

All-optical

An all-optical path for organoids would allow cell-by-cell excitation and whole organoid reading (again cell-resolved). While optical imaging is not likely a terminal technology for harnessing OI, it allows exploration of the system behavior, and the kinds of computation that could be initially (102) performed. There are rapidly developing technologies for large-volume imaging that make this attractive. Techniques such as Bessel



Abbreviations: **EEG**, electroencephalogram; **MEA**, microelectrode array

FIGURE 4
Interfacing organoids with 3D microelectrode arrays (MEAs) to allow electrophysiological output recording. **(A)** Organoid-MEA interfaces were inspired by the electroencephalograph (EEG) used to take electrophysiological recordings from the human brain. **(B)** Organoids are grown inside flexible, ultrasoft-coated, self-folding, and buckled shells covered with patterned multielectrode nanostructures and probes. These interfaces allow ultra-high-resolution 3D spatiotemporal stimulation and recording of electrophysiological patterns across the entire organoid surface (see also 93). **(C)** Brightfield image of brain organoid captured inside the shell. **(D)** Confocal image showing the side view (projected confocal stack) of a brain organoid (green; Fluo-4 calcium dye) with a diameter around 500 µm encapsulated in the electrodes of the 3D shell (blue). **(E, F)** Three channels of electrodes are distributed across the shell with representative raster plot showing the spontaneous electrical activity of the brain organoid. **(G)** Overlaid spike waveform from channels 1, 2, and 3.

holography can image volumes with hundreds of µm diameter at kHz rates, with an accurate cellular resolution if the activity is relatively sparse (102). Directly writing with opsins is also well established, and holographic methods are again exploited (103), but the cell write rate is significantly lower than the cell read rate. These methods are immediately available and would help inform the design of electrophysiological systems necessary to progress OI.

High-throughput electrophysiology

Electrophysiology recording capacity is impeded by the "dark matter" observation resulting from the fact that most neurons are not firing most of the time. As such, an electrophysiology channel sensitive to reading and writing individual local neurons can be inefficient: even the best-performing probes "see" <1% of the neurons within their detection range (104). It seems unnecessary to achieve a synapse level of interpretability of these systems if the base principles are elucidated, empirically tested, and used to control the system as a whole. Notably, bionic implants for humans have managed to convey significant information with relatively few input electrodes; e.g. the bionic eye (105) used 24 electrodes, and current cochlear implants use between 10 and 22 (106, 107). Therefore, different resolutions may be required for input vs. output.

Although organoid EEG and implantable neural devices are feasible to investigate the scale of required input/output contacts, both technologies need to be explored further to assess the recording patterns and learning potential of brain organoids.

Memory and learning in organoids: training using biofeedback, big data, and AI/machine learning

Understanding the capacity of brain organoids to learn is fundamental to determining whether they can be used computationally by harnessing the advantages of biological learning. At this stage, learning is identified as an increased frequency to show and memorize a response pattern to a stimulatory pattern. We aim to use the iterations of technologies described above to interface organoids and computers to initiate supervised learning simulations (i.e. trained stimulus response patterns). To achieve this goal, the brain organoids should be exposed to spatiotemporal patterns of electrical and chemical stimulation, with the associated recordings delineating relationships between inputs and outputs. A feedback loop is required to train a learning system. Changes in the brain organoid architecture and functionality (synaptic connections and electrophysiology) due to such training cycles can then be analyzed. These two factors affect synaptic plasticity – the main mechanism of memory formation and learning. Hence, the recorded responses to electrical or chemical stimuli should demonstrate whether and how learning may occur in the organoids.

Ultimately, robust, high-resolution encoding and decoding of signals going into and out of human cortical tissue is required. Recent achievements using intracortical microstimulation (ICMS) and decoding of motor and sensory cortices using MEA in human subjects offer promise (108, 109), though further advances in scale and resolution will be necessary.

AI analysis of responses

Organoid-MEAs will generate massive recording datasets that will themselves need to be analyzed by statistical and machine learning techniques. Given the recording density and volume, this will necessitate a novel big-data infrastructure and supercomputing capacity tailored to the sophisticated needs of this form of modern biological data.

Fundamentally, the two major challenges for AI analysis in this context are : (a) how to decode the input provided to an organoid (e.g. the game Pong) (29) to relate to changes with its architecture and/or functionality; and (b) how to relate these organoid changes to certain outputs (e.g. the improvement in playing Pong). In other words, biological computing includes OI as a mediating mechanistic process between the inputs and outputs. To answer these two challenges, we foresee the use of interdisciplinary tools integrating machine learning, statistics, signal processing, information theory, and optimization. We also believe that the questions raised will motivate new methodological developments in these fields.

Specifically, we believe the following three paths must be explored to relate OI inputs to outputs:

1. Machine learning and statistical algorithms are needed to quantify organoid function changes. This involves: (a) sensor integration to accelerate processing based on unsupervised learning and dimension reduction, such as principal component analysis (PCA), independent component analysis (ICA), and autoencoders including hierarchical versions (110–112); (b) signal detection using sequencing and time series (e.g. state-space) models (113–115), which are often used in brain imaging analysis; (c) pattern recognition to identify the real signal pattern and deconvolute it from the background noises (116).
2. Algorithms are also needed to quantify organoid architecture changes. Challenges include pinpointing the exact parts of the organoid that respond to the input [e.g. using mixture models (117)] and then quantifying these changes by monitoring their physical appearances. The application of multiscale unsupervised structure learning methods to the recorded responses can identify discrete, statistically distinguishable, observer-unbiased response phenotypes.
3. Models must then be trained to relate the quantified organoid changes to the output variables *via* multivariate causal models (118–120). OI will require novel developments integrating AI/machine learning and both space- and time-dependent causal modeling (121–123).

Clearly, inferring or estimating the connectivity of organoids will be a core endeavor. Connectivity, in neuronal circuits, is usually divided into structural, functional, and effective connectivity (124). The distinction between functional and effective connectivity is particularly prescient here: functional connectivity refers to the statistical correlations between neuronal fluctuations in different populations, while effective connectivity refers to the causal and directed connectivity between populations. The corresponding data analysis techniques can be parsed into frontal connectivity methods such as coherence analyses and Granger causality. These can be contrasted with inferences about directed (effective) connectivity, usually using some form of dynamic causal modeling (125).

Applying these statistical and computational methods and modern big and complex data tools, such as those from brain imaging and computational biology, will allow us to map input and outputs from organoids' neurological connections. As an example of

a relevant technique, a fundamentally different approach to neuron behavior mapping was developed in *Drosophila*: optogenetic activation of neurons and the application of multiscale unsupervised structure learning methods to the recorded responses to identify discrete, statistically distinguishable, observer-unbiased response phenotypes (126). This could be a starting point for connectivity- and activity-mapping studies to further investigate the mechanisms through which neurons mediate diverse behaviors. However, with respect to its application to brain organoid recordings, we are entering terra incognita.

Machine learning and other mathematical models are increasingly applied to certain parts of organoid research (127–129). However, machine learning, in the sense of deep learning and supervised learning, deserves further comment. This is because it presupposes that supervised learning is an appropriate theoretical formulation of self-organization in organoids, in the sense that the opportunities afforded by organoid research transcend questions about how to engineer a particular behavior through supervised learning (130). The opportunities probably require a more generic theoretical framework within which to formalize self-organization and active exchange between an organoid and its external milieu (131, 132). Practically, if one wanted to train an organoid to do this or that, it would be impossible to implement the procedures for supervised learning in machine learning (i.e. either backpropagation of errors or local energy-based schemes). Current developments favoring reinforcement learning, where self-organization is met by feedback on functionality, lend themselves to such problems. Strategically, if correct, this means that the direction of travel of organoid research may be either toward reinforcement machine learning or more aligned with some of the foundational questions posed in neurobiology (133) or, indeed, the physics of nonequilibrium self-organization (134, 135).

Big-data infrastructures

Providing suitable infrastructure for the storage, curation, and processing of OI big data is a scientific challenge of its own. Analytic datasets need to be stored in efficient shared memory structures; appropriate technology will be needed where manipulations, such as standard matrix or tensor calculations, can be obtained without partial or streaming access to the full dataset. Furthermore, a fast, robust, and scalable computational analytic and curation infrastructure for the resulting data will be needed. A likely requirement will be efficient dimension-reducing transforms of the 3D sensor arrays applicable for streaming data; e.g. by "scattering transform" (136), which has been particularly successful in analyzing audio streams (137).

Each deep biological network computes some function, transforming inputs to outputs depending on many variables, chief among them: (1) the weights and biases and (2) the activation functions. In both AI and OI, it is reasonably safe to assume that the activation functions are essentially static; i.e. any given node at any given time is activated only upon reaching a certain threshold. However, Sinapayen et al. (138) suggest that neural networks can autonomously change activity to avoid external stimulation. The key difference between AI and OI networks is that in OI networks, the weights and biases may dynamically change over time – for example, owing to growth and/or maintaining homeostatic equilibrium – however, with unclear changes in the network function. Indeed, vastly different neural networks can implement approximately the same function (139). A similar result is well established in biological networks (140). We, therefore, hypothesize that although the precise weights and architecture of the OI may be dynamic, the memories may be stable over time.

The amount of data and respective curation, compression, and processability will dramatically increase with boosted electrophysiological recordings from OI systems. Additional challenges come from spatiotemporal recording and the combination of electrophysiology and high-content imaging. In addition, the training and experimental data created will require efficient frameworks for storage and analyses. This could include a combination of *in vivo* proto-neural networks developed in brain organoids and *in silico* analog/digital hybrid, and/or neuromorphic computing (41, 90, 141, 142). Transmedia progressive learning will therefore exhibit the advantages of both biological and machine computing and learning, while mitigating the limitations of each. The main aspect of our strategy for storage is to develop a scheme resembling the Large Hadron Collider experiment at CERN, where sophisticated triggers are used to detect events in real time and only events with a potential discovery value are kept – greatly decimating the data rate. We envisage a similar event-driven way of analyzing the data: we will send discrete stimuli to the brain organoids, look for coordinated responses in many channels, and store only the discrete events related to these intervals. We will use insights gained through multiple versions of experiment reduction to develop a sensor correlation model and optimal event triggers that optimize the trade-offs between data reduction and discovery value.

Many large ongoing efforts aim to create data-processing tools, storage solutions, and standards to handle the scale of data generated by modern neuroscience experiments, e.g. the US BRAIN Initiative (143). Open-source community solutions, which the OI community could leverage, are being developed for terabyte- and even petabyte-scale data across multiple modalities. As high-throughput MEAs represent the most promising initial technology for interfacing with organoids, solutions from the invasive and *in vitro* electrophysiology communities can likely serve as the basis for the OI community. Standardized community-processing pipelines exist for this application, including machine learning tools such as DataJoint Elements (144). Many individual tools and techniques for electrode arrays have been developed for spike sorting (145) and analysis of local field potentials (146). Community cloud-based archives are available for publishing such electrophysiological data in an open and accessible manner, such as the OpenNeuro Archive (147) and DANDI (148). These archives are hosting an ever-increasing range of data from varied experimental paradigms. Several standards initiatives could be leveraged to facilitate reuse, reanalysis, and meta-analysis, such as the Brain Imaging Data Standard (BIDS) (149) or Neurodata Without Borders (NWB) (150). By leveraging the standards, processing pipelines, storage,

and dissemination techniques developed by the larger electrophysiology community, the OI community can rapidly establish a robust and reproducible big-data infrastructure.

Looking to the future, additional imaging modalities may become critical to providing further insights into organoid function and learning. Other processing tools and archives exist for relevant modalities such as fluorescence microscopy and also other forms of microscopy; for example, the CaImAn pipeline for calcium imaging data analysis (151) and the Brain Imaging Library (152) for archiving and storage. In the invasive *in vivo* neuroimaging community, functional and structural connectivity (or connectomics) is also being studied with improved resolution and larger volumes (153, 154). Cloud-based processing pipelines for deriving connectivity are under development (155), and the Brain Observatory Storage Service and Database (BossDB) ecosystem has been developed to host and archive large connectivity datasets at the neuron-synapse level (156). Many emerging graph analysis tools enable improved insight, such as statistical characterization of connectomics (157) and identification of repeated motifs (158). In time, multimodal datasets and infrastructure may play an important role in the development of the OI community.

Ultimately, the OI community should seek to build on these tools to establish standardized analysis and storage infrastructures. Open data sharing can be a powerful approach to grow the community and maximize the reuse of experimental data. Establishing a large-scale, standardized set of experimental data may rapidly improve processing tools, provide theoretical insight, and generate hypotheses for future experiments. An interesting model approach is the Human Connectome Project (159), which used standardized approaches to high-resolution magnetic resonance imaging to produce a gold-standard dataset for the emerging field of human connectomics. A similar data and infrastructure effort for the OI community could provide invaluable insights.

To summarize, a big-data ecosystem necessary to study OI will require:

- Standardization of experimental data and metadata, building on existing standards such as BIDS or NWB
- Robust, repeatable, and standardized processing pipelines that scale to large electrophysiological datasets
- Efficient, accessible, and open data storage, possibly leveraging existing cloud archives such as OpenNeuro or DANDI
- The potential development of multimodal OI datasets
- The establishment of standard, reference datasets for the community

In the foregoing discussion, we have anticipated a need for the collation, storage, and dissemination of big data, under the assumption that organoid research will recapitulate developments in neuroscience (and in particular imaging neuroscience). However, there is an alternative path, which reflects a move from "big data" to "smart data." In other words, we need only informative data that enables people to answer well-posed questions. In effect, this would represent a pushback against big data and a return to carefully designed experiments that elicit the right kind of data to make inferences about the dynamics, plasticity, and functional architectures in brain organoids. In short, the experiences of the neuroscience community may usefully inform the kind of resources needed to realize the full potential of organoid research over the ensuing years.

Advancing biocomputing complexity

Optimized algorithms for organoid-*in silico* interactions

Realizing the potential of OI requires more than interfacing a computer with an organoid. In OI, the organoid can take on the role of an embodied agent that interacts with an environment through the organoid-*in silico* interface. This will require optimized algorithms for organoid-*in silico* interactions as well as research into theoretical frameworks for learning and adaptations in organoids drawing from the theoretical neuroscience literature. The viability of OI is dependent on optimized algorithms for organoid-*in silico* interactions, in addition to the fast data storage and retrieval described above.

There are two broad environments in which OI may operate: open-loop or closed-loop:

- Open-loop involves feeding information into cells and measuring the response. Recent work leveraging this approach has found that neural systems can alter their activity to perform various tasks, including context-dependent encoding (160) and blind-source separation (42), and to display features such as adaption toward scale-free dynamics (161) or long-term activity-dependent plasticity (41).
- Closed-loop extends the open-loop environment to include feedback to the neural systems about the result of the system activity. Examples of this are limited owing to technical difficulties, but high-latency/low-temporal resolution analysis suggests that under these conditions, cultures can demonstrate changes in neural dynamics (41) and arbitrary learning (44). Moreover, real-time implementations have recently resulted in goal-directed learning displayed through a change in neural culture activity when applied in line with neurocomputational theories (29).

Exploring, applying, and refining empirically supported theories of what fundamentally drives learning intelligence is a critical factor. So far, numerous theories have been proposed to explain how, at the fundamental level, neural systems process and respond to information.

The first branch of theories focuses on how neural systems are organized, both structurally and functionally. Key notions include neural criticality (162), neural Darwinism (163), cell assembly and Hebbian plasticity (164), rule-based learning (165), and core ideas behind population coding approaches (166, 167). Generally, these

theories aim to explain how the incredibly complex organization within the brain results in its ultimate functioning. Thus, they offer generally compatible frameworks for analyzing and interacting with brain organoids, providing the opportunity for optimized input and decoding of output.

A second "optimization" category of theories generally focuses on how a system or agent works to maintain homeostasis in a dynamic environment. Broadly, this can be achieved either through maximizing a utility or reward or by minimizing surprise or uncertainty (165, 168). Key theories include the Bayesian brain hypothesis (169), efficient coding hypothesis (170), value-dependent learning (171), optimal control theory (172), and learning by stimulation avoidance (138). Attempts have been made to unify these theories, recently by exploring the underlying compatibilities, most notably through the proposal of the free energy principle (173), where the system or agent may engage in active inference to construct a generative model of the external environment and act in a manner to minimize the difference between the internal model and the perceived external world. At present, it is difficult to empirically test many of these theories in a controlled manner because *in vivo* organisms possess compensatory mechanisms that hamper the interpretation of results. OI offers a pathway for highly controlled experiments to empirically test these theories.

In addition to frameworks for learning in neural systems, the OI community will require methods to assess embodied intelligence; i.e. computational approaches to understand intelligent behavior in organoids for both open-loop and closed-loop environments. Previously, *in vitro* experiments have demonstrated the ability of cell cultures to control both physical robotic systems and simulated video games (29, 174). Experience within the AI community suggests that the OI community will likely benefit from standardized testing environments and conditions, accounting for variability and constraints in input/output interfacing in terms of channel count and bandwidth. The AI reinforcement learning community has produced a huge range of games, simulation environments, and physical systems that could be adapted to OI evaluation. Of particular interest to the OI community may be the field of continual or lifelong learning (175, 176), where embodied agents are assessed in learning that occurs over a sequence of experiences (often referred to as a "lifetime"). Such testing environments may serve the OI community by providing important benchmarks for understanding functional activity and learning in organoids.

Incorporating complex biological inputs in OI

Previous sections describe how we intend to interface organoids and computers. Combining organoids with various types of more complex inputs and output stimulation and recording interfaces (177, 178) will allow us to understand the potential for real-time controls. The consequences of such interconnections can be explored, starting with two brain organoids, one with complex input and one with complex output connections. A sensory organ, such as a retinal organoid, could then be connected with a brain organoid. Eventually, networks of organoids will be interconnected to implement more complex OI. The organoid will be interfaced with electrical and fluidic-sensing and simple outputs controlling machines through biofeedback on the cellular level; i.e. giving the brain organoid control by feeding back the results of its induced actions.

By connecting retinal and brain organoids we can determine whether signals can transfer between these different neuronal organoids and how this exogenous biological signaling will be interpreted by the brain organoid – establishing the initial baseline of organoid–organoid communication. Retinal organoids containing laminated retinas with outgrowth of outer segment-like structures and synaptic ribbons have been developed (179, 180). Synapse formation between two organoids is complex and was demonstrated recently in assembloids (48). Retinal and brain organoids can be connected either through electrodes or directly. Since methods to generate mature, endogenous, light-sensing retina are preliminary and very inefficient, engineered photosensitive ion channels, which are expressed under the control of a photoreceptor-specific promoter (181–183), can substitute as a proxy for light-reactive photoreceptors. Optimization of retinal organoid culture conditions to promote more robust signaling has recently been published (184).

Understanding and perhaps even influencing the connectivity of retinal and brain neurons would be extremely exciting. Although we and others are actively investigating ways to establish and modulate these connections, we are still quite far away from a system that can demonstrate robustly modifying neuronal connections in a directed manner. Ultimately, we aim to build on this simplified approximation or representation of visual input toward a system that more fully approximates vision.

Leveraging advances in the molecular basis of biological learning

Advances in the molecular biology of synaptic plasticity will be critical for optimizing the capacity of organoid systems for learning and OI. Growth conditions can now be optimized to allow organoid neurons to optimally express genes essential for learning in the human brain.

First, they need to express genes coding for the neurotransmitter receptors that mediate synaptic transmission. We have already characterized the expression dynamics of different subunits of the N-methyl-D-aspartate (NMDA) glutamate receptor during differentiation (unpublished observation). The long-term organoid maturation and age based on gene expression and switch of the main receptor subunits were recently extensively characterized (185). The machinery of the organoid's biochemistry, especially of neurotransmitters, will be an important part of understanding the signaling cascades and synaptic plasticity necessary for learning; to this end, a thorough characterization of protein expression is warranted.

Secondly, it will be important for organoids to express IEGs. IEGs mediate synaptic processes essential to memory consolidation and are rapidly transcribed in adult neurons as they process

information (186). Moreover, evidence indicates that IEGs are required for circuits capable of gamma frequency rhythmicity (187), which is associated with attention and information processing. Using RNA-sequencing to compare organoids (at different stages of maturation and grown under different culture conditions) with fetal, adolescent, and adult brains will allow the identification of growth conditions that optimize expression of these essential genes. It is critical to ensure IEG expression that is robust and dynamically responsive to activity evoked by a pharmacological block of $GABA_A$ inhibition (e.g. using bicuculline). Dynamic, activity-dependent IEG expression would assure the presence of molecular substrates necessary for organoids to establish circuits with balanced excitatory and inhibitory neurons and synapses. The cell culture conditions should be optimized so that 10–30% of neurons express IEGs in response to informational inputs. This level of sparsity is typical of brain regions involved in learning and memory (including the hippocampus and neocortex) and may ensure that multiple ensembles can be created to uniquely encode different streams of data. This contrasts with the global activation of IEGs that may occur with non-informational activity, such as a seizure. IEG expression and/or associated reporters can also provide a means to assess the formation of ensembles of neurons that are stably linked to specific inputs. In the brain, such ensembles are thought to represent memory engrams. EEG data can then be correlated with dynamic activity reporters (Ca^{2+} or voltage) and IEG expression data. Ultimately, neuronal activity can be stimulated and recorded optogenetically, as recently described (48). To our knowledge, IEG expression has not previously been utilized as an endpoint for optimizing organoid growth conditions. Successful use of this parameter would establish a biological basis for OI and address concerns regarding the uncertainty of the developmental state of organoids and their potential utility as information-processing memory units.

OI-led advances in medical research and innovation

In addition to pioneering the use of human brain organoids for computing and learning, OI research will also allow the exploration of inter-individual neurodevelopmental and neurodegenerative differences between stem cell donors. Alzheimer's disease and other dementias could represent one particularly important priority for research. Globally, more than 55 million people are living with dementia, and this number is projected to exceed 150 million by 2050 (188, 189). Dementia is among the top 10 leading causes of death (190) and globally costs at least $1 trillion annually (191, 192). Clinical trials of novel Alzheimer's disease therapies have shown very poor success rates, in part owing to premature translation of successful results in animal models that mirror only limited aspects of the pathology in humans (193, 194). The adaptation of OI research models to neurodegenerative diseases would offer the first human-based preclinical model to help us understand and develop effective treatments for these devastating diseases.

In addition to neurodegenerative diseases, neurodevelopmental disorders also lend themselves to OI, given that the different stages of brain development are reflected in the bioengineering of these models from stem cells. Conditions such as autism are major concerns owing to the enormous increase in prevalence. In the US, autism spectrum disorder (ASD) was diagnosed in the 1970s in 1 in 10,000 children, but according to the Centers for Disease Control (CDC), in 2021 it was 1 in 44 (195). While the disorder has heritable aspects, environmental influences are an increasing focus. The brain organoid model has shown promise for both developmental neurotoxicity (196, 197) and gene × environment (198) studies of autism. A varying combination of cognitive impairments characterizes this complex spectrum of diseases (199). Similarly, leukodystrophies are a diverse group of rare genetic disorders affecting white matter and linked to cognitive impairment (200). Using OI to explore the genetic basis of autism or leukodystrophies appears to represent an important path to understanding these disorders and to allowing screening of potential drugs that might boost underdeveloped cognitive functions.

Schizophrenia affects around 1% of the population worldwide and is one of the top 10 illnesses contributing to the global burden of disease (201). Schizophrenia has a prominent genetic basis, and it has been suggested that it may be neurodevelopmental in origin (202). Prenatal complications are an important contributor to the condition (203), while cognitive dysfunction is a hallmark (204), with a strong similarity to autism (205). Human iPSC lines, e.g. with genetic backgrounds associated with disorders, are available and continuously growing [e.g. SFARI base for autism (206)]. Organoids developed from iPSCs from individuals with various conditions could be compared against control samples to help identify differences that may elucidate the pathogenesis, risk factors, and treatments. The application of an OI approach using these cell lines would thus be very promising to aid further understanding and characterization of the etiology of the neurodegeneration, neurodevelopmental, and psychiatric disorders. This enables a multitude of applications, from de-risking (pediatric) drugs for adverse effects on cognitive development (207), the identification of toxicants and illicit drugs with long-term effects on cognitive capabilities, and the optimization of lead drug candidates acting on respective pharmacological targets.

The study of memory, learning, and cognition – and the impact of neurodegeneration on these functions – will require physiologically relevant neuron-to-glia ratios and high levels of biological complexity and interregional communication. Besides assembloids (48, 208, 209), aspects of ventral and dorsal regions in the absence of external morphogens or growth factors have been recapitulated (210–212), highlighting an innate ability of self-organization in both cases. Recently, Cederquist et al. (213) demonstrated that a chimeric assembloid of an early organoid and a cluster of sonic hedgehog (SHH)-secreting cells resulted in dorsal-ventral and anterior-posterior positional axes. However, organoids do not have predictable anatomy nor defined topography, and generally do not reflect the characteristic developmental asymmetry (208, 213). Nonetheless, brain organoids do show a remarkable self-organizational capacity. This might be further enhanced by functional demands, which may be

harnessed when scaling up the size and inducing regional polarization, thereby increasing the biological complexity of neural networks and interregional connections to better reflect brain architecture and function (48, 208–213). Bioprinting approaches might also help here.

Ethics of biological computing with organoids

Creating a human brain model with input and output as well as learning capabilities raises complex ethical questions. At 12 weeks of fetal development, a human brain has a weight of approximately 3 g, a volume of approximately 3.5 ml, and 3×10^9 cells in the neocortical part of the fetal telencephalon (214–216). An adult mouse brain weighs approximately 0.4 g. In comparison, current brain organoids have a diameter below 500 µm in culture, with less than 100,000 cells. The maturation of the brain organoid is accelerated by growth factors so that at 10 weeks of culture, organoids show some features (such as myelination) that start in the fetus after 20 weeks of gestation (217). Furthermore, the stimulation with information input might lead to very different organoid development, and much longer culture periods would be envisaged for training organoids, together possibly augmenting cognitive capabilities.

The ethical concerns raised by brain organoid research have mainly focused on questions about creating entities that could potentially exhibit consciousness (45). Could organoids experience pain and, if so, would they suffer – even in rudimentary ways? These concerns will mount during the development of OI, as the organoids become structurally more complex, receive inputs, generate outputs, and – at least theoretically – process information about their environment and build a primitive memory. This will require deeper analysis and research regarding the morally salient neurobiological features that contribute to human capacities, including consciousness, and the implications for OI research and implementation when some or all of these are met. Articulating the physiological conditions that are necessary and sufficient for consciousness is one of the most difficult puzzles of neuroscience (143, 218). To assess whether organoids exhibit the criteria for consciousness will require some consensus to be reached about what those criteria are (219). Work underway to uncover the neural basis of consciousness will inform the evaluation of the ethical issues raised by OI. However, it will also be important to distinguish "consciousness" from "sentience," formally considered as "awareness to stimuli," i.e. response to sensory input (220, 221). Such use of terminology can be debated and represents a critical challenge for the forming OI community. We use the term "sentience" in its most basic way, similar to the way in which many aspects of cognition have to be understood as very basic cellular mechanisms, not human-level brain functions. Even recent proposals (222) for the use of the perturbational complexity index (PCI) assume the more complex idea of phenomenological consciousness when the behavior could be explained by simpler sentience (174). Notably, the suggested OI program does not aim to recreate human consciousness, but rather functional aspects related to learning, cognition, and computing.

Nevertheless, as advances in the structural and functional complexity of OI systems begin to recapitulate aspects of human neurobiological (sub)-processes, such as learning and cognition, researchers will inevitably encounter the Greely Dilemma: a situation whereby incremental successes in modelling aspects of the human brain will raise the same kind of ethical concerns that originally motivated their development (223). Sufficient advances in OI will raise questions about the moral status of these entities and concerns for their welfare. Frameworks have been proposed to address these ethical concerns in research practices (224, 225) but it remains unknown whether these proposals adequately attend to moral concerns held by the public. For example, harm reduction policies are often unsuccessful in gaining public support when the underlying attitude is based on a moral conviction (226) with implications for public discourse (227). Comprehensive ethical analysis of OI will require input from diverse publics and relevant stakeholder groups (228), in order to (i) prevent misunderstandings from creating unintended moral appraisals, and (ii) and foster trust, confidence, and inclusion through responsible public engagement. Notably, moral attitudes toward OI may depend less on epistemological concerns mentioned above, such as the role of specific cognitive capacities in assessments of moral status, and more on ontological arguments of what constitutes a human being. Perceptions of (re)creating 'human-like' entities in the lab are likely to evoke concerns about infringing on human dignity that could reflect secular or theological beliefs about the 'essential' nature of the human being (229, 230). Our approach to embedded ethics in OI will seek to identify and attend to these ethical concerns by informing future public engagement and deliberation on OI.

Other issues anticipated to require attention include privacy concerns on the part of iPSC donors and aspects of intellectual property. What does the organoid exhibit about the cell donor? Is there a moral obligation to inform the donor if, for example, something relevant to their health is identified during research? Do donors have rights that extend beyond the donation?

In common with other scientific and bioengineering aspects of OI, this is truly uncharted territory. The ethical considerations and viewpoints can be expected to evolve with an increased understanding of organoid systems. It is therefore critical to frame the ethical considerations at the onset of this research in a manner that encompasses all anticipated issues, and which continually reflects on progress and new lessons. We propose to use an "embedded ethics" approach whereby an ethics team will identify, discuss, and analyze ethical issues as they arise in the course of this work. Embedded ethics is a standard approach in interdisciplinary ethics research, whereby expert ethicists join and collaborate integrally with research and development teams to consider and address ethical issues *via* an iterative and continuous process as the research evolves (231). Box 1 offers a preliminary framework of ethical considerations informed by discussions at the "First organoid intelligence (OI) workshop to form an OI community" workshop (22–24 February 2022) (51).

While the embedded ethics approach provides a mechanism for investigating the philosophical and scientific conditions relevant to the moral status of brain organoids, it has no inherent mechanisms for seeking, identifying, or incorporating public values in the development

BOX 1 Preliminary framework of ethical considerations in organoid intelligence research. Organoid intelligence research entails many important ethical aspects that warrant an iterative, collaborative ethical process as the field develops, involving all relevant stakeholders. Here we offer a preliminary framework of issues for consideration.

Domain	Potential issue
Cell donation	• Selection bias in samples from which organoids developed (risks of discrimination in standardized lines, limitations on neurodiversity, etc.,) • Donor informed consent • Donor rights beyond donation • Donor beliefs about ephemeral relationships and relational concerns
Bioculturing	• Cell culture practices • Limitations on embryo use • Gene editing • Disposal • Biosafety
Organoid-machine interface	• Effect on organoid via forms of suffering (however rudimentary) • Violation of foundational (cultural) distinctions (self vs. other, living vs. machine)
Learning and computation	• Artificial intelligence algorithms • Emergence of neurobiological features that contribute to human capacities • Data sharing (e.g., open access/data provisions) • Sharing of code and protocols
Applications	• Regulation/governance of use • Commercialization (e.g., intellectual property protection) • Equity in access to resulting scientific advances and technologies • Stakeholder input, scientism and trust

of OI. It is important to understand public perceptions of OI, and this cannot be delegated to ethicists alone. It needs to be embedded within the field as a three-way feedback loop involving researchers, ethicists, and members of the public, including stakeholders who could be especially impacted by advances in OI (e.g. neurodiversity advocates). This feedback loop will enable specific applications of OI to be articulated by researchers, analyzed by ethicists based on theoretical principles, and evaluated by members of the public with diverse moral perspectives. The views expressed by the public then inform the work of both scientists and ethicists seeking to advance OI in a socially responsive manner. This call for public dialogue has been echoed by a National Academy of Sciences report on human neural organoids (232), the recommendations on innovation in neurotechnology by the Organisation for Economic Co-operation and Development (233), and various neuroethics committees. Moreover, researchers in science communication and deliberative democracy have demonstrated that deliberative techniques are one of the most effective mechanisms for informing the public and mitigating the risk of polarization on contentious issues. Finally, public engagement on OI will not only be necessary to prevent adverse public reactions but will also maximize the future impact of the field and serve as an exemplar of how to embed society within science.

Of relevance in this context, coauthor JK co-chaired the neuroethics subcommittee for the new strategic plan for NIH BRAIN 2.0 (234). The only project we know of with relevant parallel aspects is the Brainstorm Project led by Insoo Hyun at Case Western University (235). We will be further actively promoting discussions between biologists, ethicists, and philosophers who are interested in brain organoids and navigating brain organoid ethics in research, as we did before (219). A recommended way forward would include: (1) an agreement on commonly used language, (2) the need for research on the neural basis for consciousness (as above); and (3) the development of best practice guidelines that consider the views of all relevant stakeholders.

Conclusion: an action plan for OI research

We present a collaborative, iterative multidisciplinary program aiming to establish OI as a form of genuine biological computing that harnesses brain organoids using the scientific and bioengineering approaches described here in an ethically responsible manner (Figure 5). Ultimately, we aim toward a revolution in biological

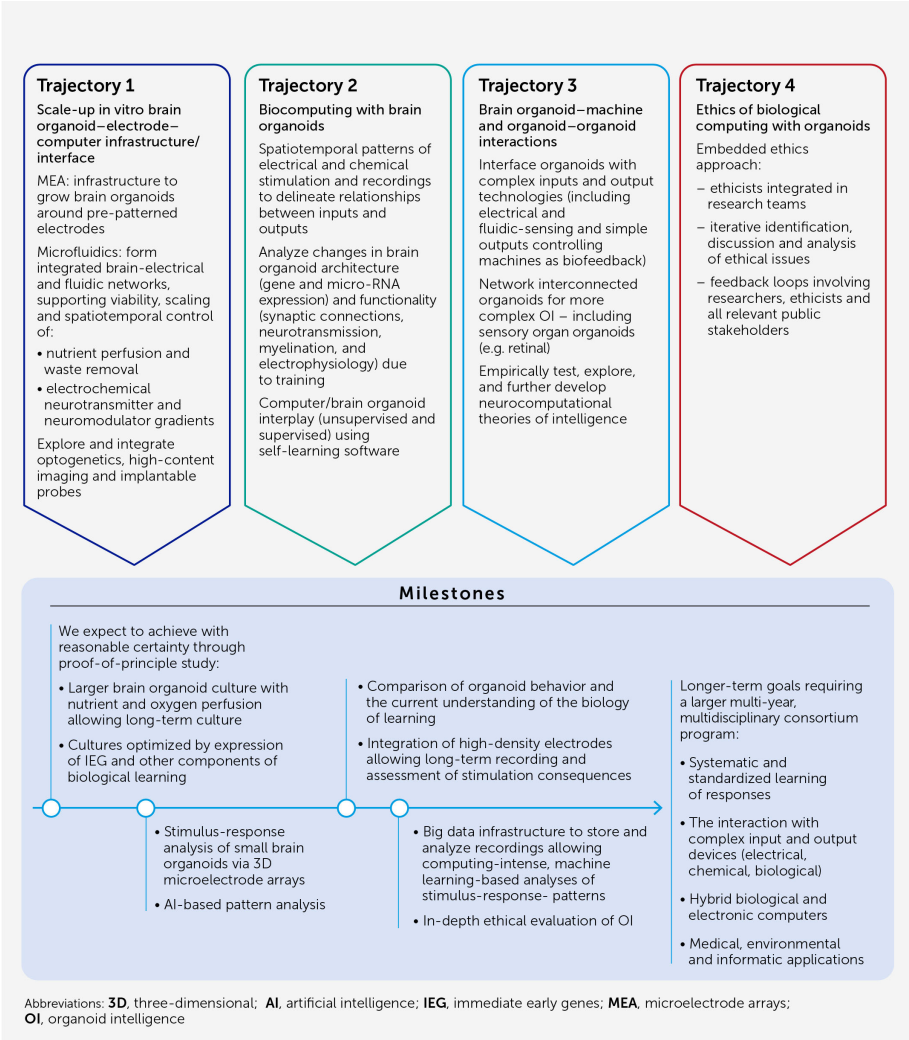


FIGURE 5
Organoid intelligence (OI) action plan and research trajectories. Our program foresees assembling multidisciplinary teams working in four research and development directions (or "trajectories") employing the scientific and bioengineering advances described to establish OI as a genuine biological computing field harnessing brain organoids in an iterative and ethically responsible manner.

computing that could overcome many of the limitations of silicon-based computing and AI and have significant implications worldwide. Specifically, we anticipate OI-based biocomputing systems to allow faster decision-making (including on massive, incomplete, and heterogenous datasets), continuous learning during tasks, and greater energy and data efficiency. Furthermore, the development of "intelligence-in-a-dish" offers unparalleled opportunities to elucidate the biological basis of human cognition, learning, and memory, together with various disorders associated with cognitive deficits – potentially aiding the identification of novel therapeutic approaches to address major global unmet needs.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

TH is named inventor on a patent by Johns Hopkins University on the production of brain organoids, which is licensed to AxoSim, New Orleans, LA, United States, and receives royalty shares. TH and LS consult AxoSim. JS is named as inventor on a patent by the University of Luxembourg on the production of midbrain organoids, which is licensed to OrganoTherapeutics SARL, Esch-sur-Alzette, Luxembourg. JS is also co-founder and shareholder of OrganoTherapeutics SARL. AM is a co-founder and has equity interest in TISMOO, a company dedicated to genetic analysis and human brain organogenesis, focusing on therapeutic applications customized for autism spectrum disorders and other neurological disorders with genetic origins. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict of interest policies. BK is an inventor on patents for technology related to this paper along with being employed at and holding shares in Cortical Labs Pty Ltd, Melbourne, Australia. No specific funding or other incentives were provided for involvement in this publication.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

Biological computation	calculation (not necessarily as mathematical operations) carried out by a biological system.
Biological computing	tasks typically done by computers carried out by biological systems.
Brain organoid	induced pluripotent stem cell (iPSC)-derived 3D neural cultures, recapitulating aspects of brain cellular composition, architecture, and functionality.
Cognition	the human mental action or process of acquiring knowledge and understanding through thought, experience, and the senses (236).
Cognition-in-a-dish	a basic ability to process an input and provide a measurably output; a learned adequate response to the stimuli which is enabled by the presence of the necessary molecular machinery and physiological features such as learning circuits of long-term memory.
Consciousness	the human state of being aware of and responsive to one's surroundings (236); a hypothetical organoid's state of being responsive to and "aware of" the environment.
Embodied intelligence	the computational approach to the design and understanding of intelligent behavior in embodied and situated agents through the consideration of the strict coupling between the agent and its environment (situatedness), mediated by the constraints of the agent's own body, perceptual and motor system, and brain (embodiment) (237).
Intelligence	the human ability to acquire and apply knowledge and skills (238).
Intelligence-in-a-dish	vision of OI-implementing cell models to perform computer functions (238) and to test substances (e.g. for toxicological or pharmacological purposes).
Learning and memory	in the context of OI, learning is identified as an increased frequency to show and memorize a response pattern to a stimulatory pattern.
Organoid intelligence (OI)	describes an emerging field aiming to expand the definition of biocomputing toward brain-directed OI computing, i.e. to leverage the self-assembled machinery of 3D human brain cell cultures (brain organoids) to memorize and compute inputs.
Sentience	in humans, the simplest or most primitive form of cognition, consisting of a conscious awareness of stimuli without association or interpretation (220); for OI, basic responsiveness to sensory input, e.g. light, heat, etc.