"The only way of discovering the limits of the possible is to venture a little way past them into the impossible."
Arthur C. Clarke (1917-2008) – one of Clarke's three laws

"It is now widely realized that nearly all the 'classical' problems of molecular biology have either been solved or will be solved in the next decade. The entry of large numbers of American and other biochemists into the field will ensure that all the chemical details of replication and transcription will be elucidated. Because of this, I have long felt that the future of molecular biology lies in the extension of research to other fields of biology, notably development and the nervous system."
Sydney Brenner (1927-2019, Nobel Prize in Physiology or Medicine 2002)[1]

Food for Thought ...

Organoid Intelligence (OI) – The Ultimate Functionality of a Brain Microphysiological System

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Abstract

Understanding brain function remains challenging as work with human and animal models is complicated by compensatory mechanisms, while *in vitro* models have been too simple until now. With the advent of human stem cells and the bioengineering of brain microphysiological systems (MPS), understanding how both cognition and long-term memory arise is now coming into reach. We suggest combining cutting-edge AI with MPS research to spearhead organoid intelligence (OI) as synthetic biological intelligence. The vision is to realize cognitive functions in brain MPS and scale them to achieve relevant short- and long-term memory capabilities and basic information processing as the ultimate functional experimental models for neurodevelopment and neurological function and as cell-based assays for drug and chemical testing. By advancing the frontiers of biological computing, we aim to (a) create models of intelligence-in-a-dish to study the basis of human cognitive functions, (b) provide models to advance the search for toxicants contributing to neurological diseases and identify remedies for neurological maladies, and (c) achieve relevant biological computational capacities to complement traditional computing. Increased understanding of brain functionality, in some respects still superior to today's supercomputers, may allow to imitate this in neuromorphic computer architectures or might even open up biological computing to complement silicon computers. At the same time, this raises ethical questions such as where sentience and consciousness start and what the relationship between a stem cell donor and the respective OI system is. Such ethical discussions will be critical for the socially acceptable advance of brain organoid models of cognition.

1 Introduction

Since the beginning of the computer era, engineering aimed to emulate brain-like functionality, most obviously by striving for artificial intelligence (AI). Alan Turing, a father of computing, asked in *Computing Machinery and Intelligence* in 1950, *"I propose to consider the question, 'Can machines think?' "* The influential mathematician John von Neumann wrote an unfinished book, begun shortly before his death and first published in 1958, *The Computer and the Brain*. He already discussed several important

[1] Letter to Max Perua, 5 June 1963. Quoted in William B. Wood (ed.), *The Nematode Caenorhabditis elegans* (1988), x-xi.

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Tab. 1: Comparison of the latest supercomputer (June 2022), a human brain, and a current laptop
Modified from Smirnova et al. (2023a) with additional information from Apple[a].

	Frontier supercomputer (June 2020)	Human brain	Current laptop, e.g., Apple MacPro M1max 14"
Speed	1.102 exaFLOPS	~1 exaFLOPS (estimate)	10 teraFLOPS
Power requirements	21 MW	10-20 W	10-100 W
Dimensions	680 m^2 (7,300 sq ft)	1.3-1.4 kg (2.9-3.1 lb)	1.5 kg
Cost	$600 million	Not applicable	~$3,000
Cabling	145 km (90 miles)	850,000 km (528,000 miles) of axons and dendrites	Not known
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)	32 GB Upgradable 64 GB
Storage	58 billion transistors	125 trillion synapses, which can store 4.7 bits of information each	1 TB RAM Upgradable 8 TB

[a] https://www.apple.com/macbook-pro-14-and-16/

differences between brains and computers of his day (such as processing speed and parallelism), and suggested directions for future research. Still, we are far away from achieving brain functionality *in silico* (Tab. 1). For example, the computational power of a human brain (estimated at 1 exaFlop) was exceeded for the first time by the fastest supercomputer in June 2022. At the same time, a current laptop with comparable weight and energy consumption as a human brain, has 100,000-fold less computational speed, 10,000-fold less memory, but you can buy 200,000 of them for the price of the Frontier computer. Sure, this is comparing Apples and orange brains…🙂. Put another way, according to Moore's law formulated in 1965 that computers double power every two years at half the price, it would take another 33 years until laptops reach the performance of a human brain. These comparisons can help us grasp the potential of biocomputing. With respect to functionality, the brain works differently to a computer: For example, human brains can work much better with incomplete information (intuitive thinking) and can add new information more easily.

Bioengineering of 3D organ equivalents (Alépée et al., 2014), based especially on induced pluripotent stem cells (iPSC), has revolutionized biomedical research (Marx et al., 2016, 2020; Roth et al., 2021), providing increasingly human-relevant model systems, also of the brain (Koo et al., 2019; Qian et al., 2019; Anderson et al., 2021). We contributed to this drive with the first mass-produced, standardized brain organoid model (Pamies et al., 2017). The different respective technologies including organoids and organ-on-chip models belong to microphysiological systems (MPS), which comprise a number of bioengineering breakthroughs that reproduce organ architecture and function *in vitro*. Fueled by stem cell technologies, a broad variety of especially human models and test systems have emerged, which make relevant experimental tools broadly available through international and multidisciplinary collaborations. MPS are increasingly considered also for use in regulatory applications (Andersen et al., 2014). The field is maturing, having established its own International MPS Society[2] and series of MPS World Summits[3]. We have discussed opportunities for MPS earlier in this series (Smirnova et al., 2018) and expanded Good Cell Culture Practice (GCCP, Coecke et al., 2005) recently to the MPS field (Pamies et al., 2022), following two workshops and a draft for stakeholder input (Pamies et al., 2017, 2018, 2020).

Cell culture aims to recreate human organ architecture and functionality; for the brain this ultimately means cognitive functions. With the advent of human stem cells and the bioengineering of MPS, this is now coming into reach with the machinery of learning and memory being realized in organoids and organ-on-chip systems. The prospect of experimental models of human cognitive functions ("cognition-in-a-dish") will allow their study for the first time with easy experimental interventions and immediate measurements. A proxy for short-term learning in neuronal cultures has been demonstrated recently (Kagan et al., 2022a): Human neuronal cultures can "learn" to play the computer game Pong; at least, they statistically improved keeping the ball in game in each training session but had to start from scratch the next day (Smirnova and Hartung, 2022). Brain organoids now bring glial cells (including added microglia) and self-organization to these models, promising further advances in synaptic plasticity and potentially long-term memory (Fig. 1).

[2] https://impss.org

[3] https://mpsworldsummit.com

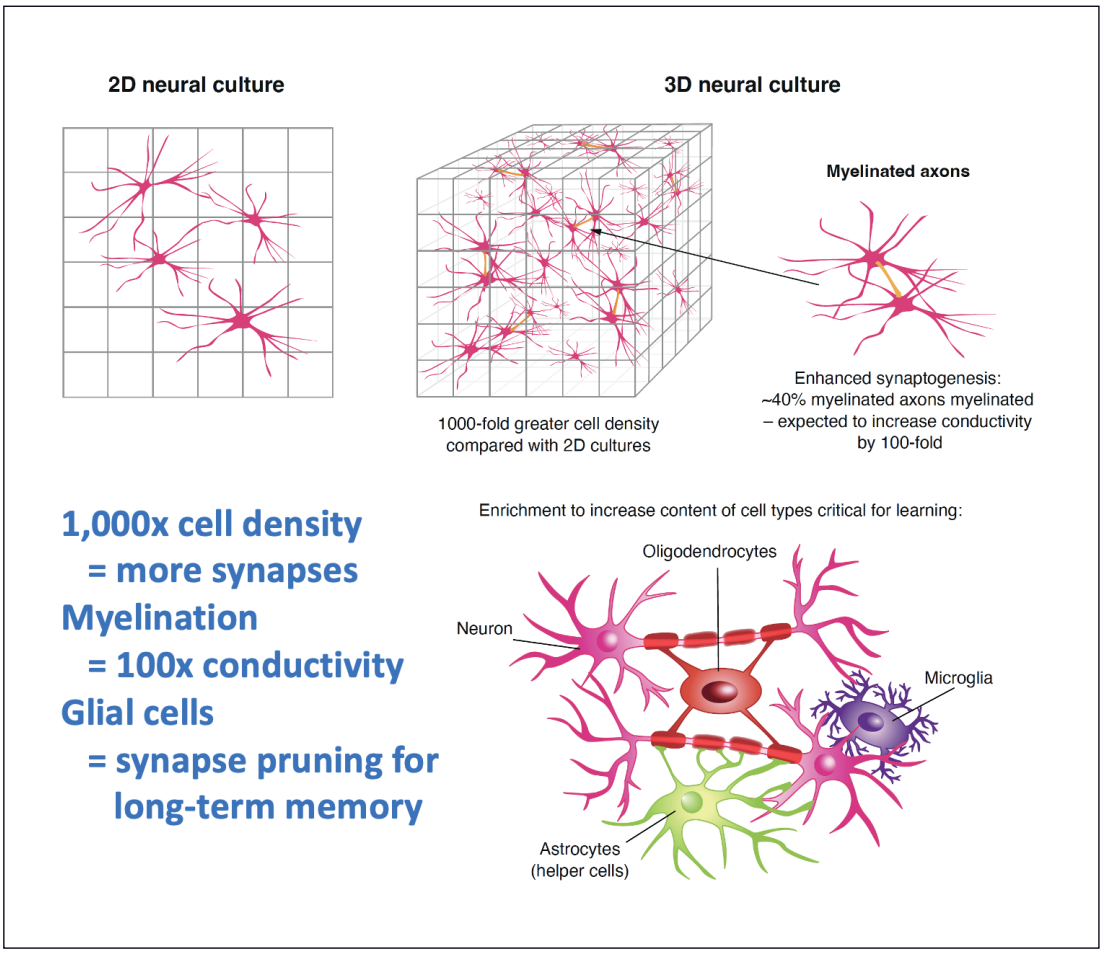

Fig. 1: The advance from 2D neuronal to 3D organoid brain culture
Modified from Smirnova et al. (2023a).

With the creation of disease models of neurodevelopmental disorders and neurodegeneration, some of our most costly and challenging diseases might benefit from novel tools for chemical safety and drug development. Imagine what experimental access to the most complex physiology of the human body enables for understanding, diagnosing, and treating the different states of the brain in health and disease. Questions including which exposures contribute to perturbation of brain development, impair brain function, or lead to earlier brain function decline, along with how to treat these conditions, can all be explored. These can be addressed with engineered MPS models, which promise cost- and time-efficient tests.

The vision is to realize cognitive functions in brain MPS and scale them to achieve relevant computational capabilities. By continuously advancing the frontiers of biological computing, we aim to (a) create models of intelligence-in-a-dish to study the basis of human cognitive functions, (b) provide models to advance the search for toxicants contributing to neurological diseases and identify remedies for neurological maladies, and (c) achieve relevant biological computational capacities to complement traditional computing. Is such novel biological AI needed? We will see, or to quote Steve Polyak, University of Washington, "*Before we work on artificial intelligence why don't we do something about natural stupidity?*" Perhaps we will bring natural stupidity to the computer…

At the same time, this field of research raises several ethical questions, which force us to consider where sentience and consciousness start and what the relation between a donor and the respective OI system is. Embedding such ethical discussions into the field will be critical for the socially acceptable advance of biological computing.

2 The history of OI

One of the authors (TH) started working on rat brain organoids (Honegger et al., 1979) in 2002 when joining ECVAM. After moving to Hopkins in 2009, his lab started humanizing this model in 2011. In 2016, they were the first to report the mass production of standardized brain organoids. At the time, a bit sloppily, he said, they are "thinking" because they are spontaneously electrophysiologically active and form neural circuits. When asked whether this means consciousness, he said, "*They have nothing to think about without input and output*". This statement prompt-

ed them four years ago to think about what would happen if they gave the organoids input and output? That was the starting point of organoid intelligence (OI), which so far has attracted more than 80 researchers. Bioengineering and stem cell technologies have since synergized to replicate organ architecture and function in the form of organoids and organ-on-chip systems. For the brain, the goal of these developments is ultimately to replicate cognitive functions and intelligence. A Hopkins-initiated movement has developed over the last years to create a new vision of OI, in which brain organoids are used to 1) understand and replicate learning (leveraged for, e.g., dementia research and drug development) and 2) develop actual biological computing that complements artificial intelligence (AI).

The idea to control simple robots by brain cell cultures or to measure simple learning tasks is more than 20 years old: For example, Shahaf and Marom (Shahaf and Marom, 2001; Marom and Shahaf, 2002) reported that cultures of rat primary cortical neurons could learn as they demonstrated a desired predefined response to low-frequency focal stimuli: after a learning curve, distinct electrophysiological patterns immediately followed the stimulus. The Potter group (Demarse et al., 2001; Bakkum et al., 2008) trained rat primary cultures to control a small moving device.

Last year, Kagan et al. (2022a) elegantly demonstrated aspects of learning by training human iPSC-derived neuronal cultures to play the simple computer game Pong. They used a feedback loop approach, where the culture was penalized with white noise (uninterpretable input) whenever the controlled paddle missed the ball. This was sufficient to improve game performance, and well-controlled follow-up experiments demonstrated that the type of feedback applied seemed related to the apparent learning effects. Likewise, numerous electrophysiological measurements, including nuanced metrics such as functional plasticity and information entropy, were found to accord with these findings. Beyond these thought-provoking findings, the paper suggests several directions for future research. The fascinating question is whether this represents learning, sentience or any form of intelligence. This shows on the one hand the challenge of applying terms of general use to these very different experimental settings but also the need to define the minimum characteristics to assign these capabilities to an experimental OI system. A white paper on terminology for OI and a workshop to develop definitions are underway. Smirnova et al. (2023a) use the following pragmatic definitions:

- *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.
- *Cognition:* the human mental action or process of acquiring knowledge and understanding through thought, experience, and the senses[4].
- *Cognition-in-a-dish:* a basic ability to process an input and provide a measurably [*sic*] 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[4]; 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).
- *Intelligence:* the human ability to acquire and apply knowledge and skills[5].
- *Intelligence-in-a-dish:* vision of OI-implementing cell models to perform computer functions[5] 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.
- *Sentience:* in humans, the simplest or most primitive form of cognition, consisting of a conscious awareness of stimuli without association or interpretation[6]; for OI, basic responsiveness to sensory input, e.g., light, heat, etc.

With the advent of brain organoids from iPSC as 3D neural cultures (Lancaster et al., 2013; Lancaster and Knoblich, 2014), models recapitulating aspects of brain cellular composition, architecture, and functionality became available. For the brain, functionality ultimately means cognition. We coined the term OI, combining organoids and AI, because these are the two key technologies – arguably we must add electrophysiology as part of sensor technologies – which promise implementation of cognitive functions. OI thus 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. We are at a stage where we can bioengineer cellular aspects of learning and memory. These models of "intelligence-in-a-dish" have immediate applications as research tools for neuroscience and for drug development. They can also help us identify toxicants that impair brain function. By exploring how the brain works, we can design better computer architectures as the brain is still unmatched in many aspects. Ultimately, if some of the advantages of the brain can be realized also in a bioengineered system, we might also exploit this as a biocomputer.

Over the last years, an OI community has formed, and in a community-forming workshop at Johns Hopkins on February 22-24, 2022 (Morales Pantoja et al., 2023), we developed a Baltimore Declaration toward OI (Box 1, Hartung et al., 2023).

[4] Oxford English Dictionary. https://www.oed.com

[5] Merriam-Webster Dictionary. https://www.merriam-webster.com

[6] American Psychological Association. https://dictionary.apa.org/

Box 1: The Baltimore declaration toward the exploration of organoid intelligence (Hartung et al., 2023)

We, the participants of the First Organoid Intelligence Workshop – "Forming an OI Community" (22-24 February 2022), call on the international scientific community to explore the potential of human brain-based organoid cell cultures to advance our understanding of the brain and unleash new forms of biocomputing while recognizing and addressing the associated ethical implications.

The term "organoid intelligence" (OI) has been coined to describe this research and development approach (1) in a manner consistent with the term "artificial intelligence" (AI) – used to describe the enablement of computers to perform tasks normally requiring human intelligence.

OI has the potential for diverse and far-reaching applications that could benefit humankind and our planet, and which urge the strategic development of OI as a collaborative scientific discipline. OI holds promise to elucidate the physiology of human cognitive functions such as memory and learning. It presents game-changing opportunities in biological and hybrid computing that could overcome significant limitations in silicon-based computing. It offers the prospect of unparalleled advances in interfaces between brains and machines. Finally, OI could allow breakthroughs in modeling and treating dementias and other neurogenerative disorders that cause an immense and growing disease burden globally.

Realizing the world-changing potential of OI will require scientific breakthroughs (1). We need advances in human stem cell technology and bioengineering to recreate brain architectures and to model their potential for pseudo-cognitive capabilities. We need interface breakthroughs to allow us to deliver input signals to organoids, measure output signals, and employ feedback mechanisms to model learning processes. We also need novel machine learning, big data, and AI technologies to allow us to understand brain organoids.

In addition to confronting these scientific and technical challenges, we also need to anticipate (as far as possible) and address the significant and largely unexplored ethical challenges associated with this research. We must be alert to any possibility that organoids could develop forms or aspects of consciousness and mitigate and safeguard against this. The cell donor's personal rights and interests are among other important considerations. These issues warrant stringent, ongoing discussions throughout the development of OI toward an accepted ethical framework. Such discussions should include all relevant stakeholders and take due account of public values.

We are only just beginning this multidisciplinary and multistakeholder endeavor. The potential benefits are world-changing, but the challenges are daunting. We call on the scientific community to join us on this journey. Only by collaborating will we be able to realize the full potential of OI to advance science, technology, and medicine.

The workshop was based on an early draft of the white paper (Smirnova et al., 2023a), was cosponsored by the Johns Hopkins Whiting School of Engineering and the publisher Frontiers, and had financial support from the Johns Hopkins University Center for Alternatives to Animal Testing through the transatlantic think tank for toxicology (t^4). It brought together partners who had recently published on human neurons playing the computer game Pong (Kagan et al., 2022a), several groups in the US and Europe who had started to combine 2D-neuron cultures with computer chips, and our partner Muotri, University of California San Diego, who controls robots with human brain organoids (unpublished[7]). The workshop report is the inaugural paper of the section Frontiers in Organoid Intelligence[8] of *Frontiers in Artificial Intelligence*.

The vision paper (Smirnova et al., 2023a) was published together with several editorials:

- Quirion (2023): *Brain organoids: are they for real?*
- Friston (2023): *The sentient organoid?*
- Miller (2023): *Organoid intelligence: smarter than the average cell culture.*
- Magliaro and Ahluwalia (2023): *To brain or not to brain organoids.*

In parallel, a policy laboratory article by Julian Kinderlerer "*Organoid intelligence: society must engage in the ethics*"[9], a version for kids (Smirnova et al., 2023b), a version for lay people, an author interview "*My dream is for AI and brain organoids to explore each other's capabilities*",[10] and a video[11] were published. A Frontiers Forum session on organoid intelligence with authors (TH and LS) and others is planned for June 21, 2023, 11:00 EDT[12].

The parallel press releases resulted in more than 500 press hits including the *Financial Times*, *Wallstreet Journal*, CNN, BBC, *Focus*, *La República*, *The Daily Beast, Science at Avenir*, *The Hindu, Psychology Today*, Deutschlandfunk, Radio New Zealand, DPA, and many others.

7 https://www.youtube.com/watch?v=nohbx-TXsyk

8 https://www.frontiersin.org/journals/artificial-intelligence/sections/organoid-intelligence

9 https://policylabs.frontiersin.org/content/policy-outlook-julian-kinderlerer-organoid-intelligence-society-must-engage-in-the-ethics

10 https://blog.frontiersin.org/2023/02/28/thomas-hartung-organoid-intelligence/

11 https://youtu.be/Dgihhl2SR20

12 https://events.blackthorn.io/en/58JetR6/frontiers-forum-deep-dive-or-organoid-intelligence-3a2q4KzPg/overview

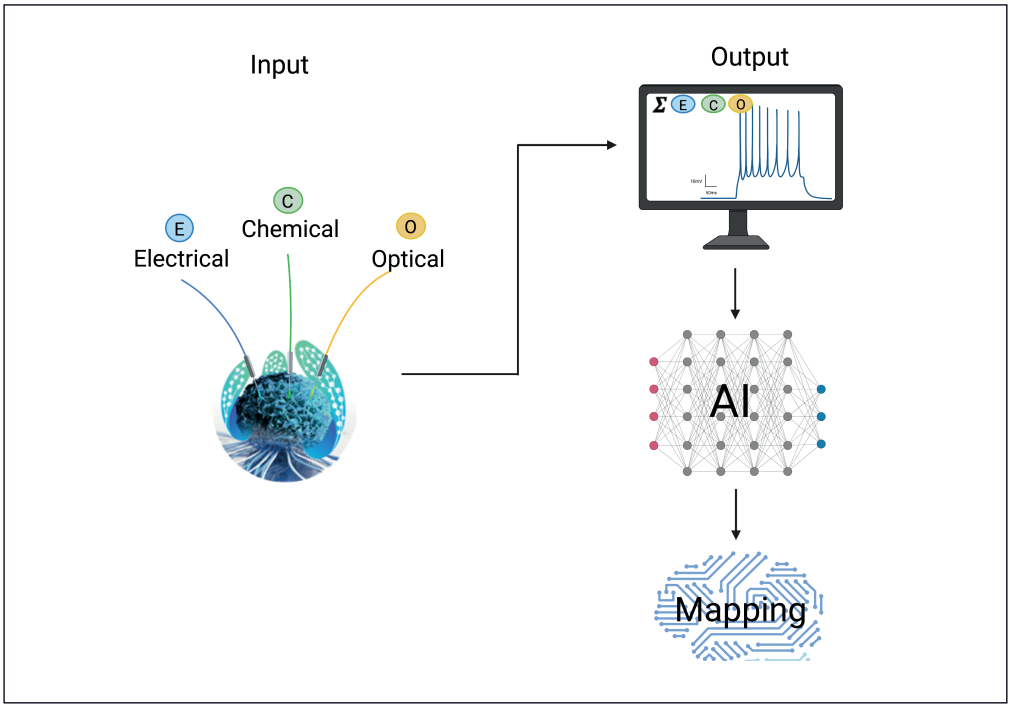

Fig. 2: Basic organoid intelligence (OI) concept
Beside electrodes, chemical (neurotransmitter) and optical stimuli (optogenetics) can serve to input information to a brain organoid. Through interfacing with AI and respective feedback, a mapping of responses is possible. Created with BioRender.com.

3 The challenges of OI

The OI initiative will establish and utilize OI as a platform to investigate the fundamental mechanisms of learning and development, giving unprecedented insight into human neuroscience without the need to access human or animal brains, which display overlapping compensatory mechanisms making interpretation difficult, and at a fraction of the cost for drug development. The ultimate goal is to revolutionize our understanding of neurological disease and information processing by neural systems. Progress in this grand challenge will require advances in several key research thrusts.

3.1 Growing large and diverse brain organoids

Actual biological AI that can compete with current silicon-based AI is certainly far away, if ever achievable. It is obvious that the current limitation of brain organoids to around 500 µm due to limited access to oxygen and nutrients in the core does not allow us to expect major cognitive capabilities.

We therefore have to learn to scale these organoids, and we must interface them with as many electrodes as possible. We will need to develop novel methods to grow brain organoids from 50,000 cells to at least 1 billion cells, which will be about 1 cm in diameter. This will require incorporated architecture and support structures including vasculature and a blood brain barrier. Perfusion of the model will allow continuous oxygen and nutrient access and overcome the drastic changes produced by changing cell culture media regarding oxygen, nutrient and waste levels as well as to some extent temperature and pH (Pamies and Hartung, 2017).

3.2 Communicating with the organoid – multi-scale data acquisition from organoids

The interface with electrodes, which provides input and output as well as feedback, is the prerequisite for training brain organoids. Building and training the intelligent brain organoid to expand cognitive functions allows to address whether our concepts of the physiology of human learning hold. This involves phenotypic characterization, imaging technologies, and connectome mapping, among others. Novel real-time and closed-loop electrophysiology systems and imaging experiments at multiple spatial and temporal scales are underway. Electrophysiology measurements through shell electrodes (Huang et al., 2022), microelectrode arrays (Kagan et al., 2022a), microelectrode meshes (McDonald et al., 2023), and neuropixels (Jun et al., 2017; Efros et al., 2018) are being explored.

With funding from the NIH BRAIN Initiative, our collaborator Tim Harris at Johns Hopkins is developing Neuropixels 2.0 probes (Steinmetz et al., 2021), which are small enough to accommodate more than 10,000 channels and can be inserted into grown organoids or used as scaffolding. Analysis of these recordings requires big data approaches and interfaces with AI. Noteworthy, Trujilio et al. (2019) have shown patterning of cortex layers and oscillation waves in brain organoids that were comparable to electroencephalograms (EEGs) from human preterm babies' brains.

3.3 Cognition experiments and functional analysis

Bioengineering allows to culture brain organoids with a complexity promising to realize basic cognitive functions. We are only beginning to find out how far this can allow the development of learning and memory, and how this changes the organoid.

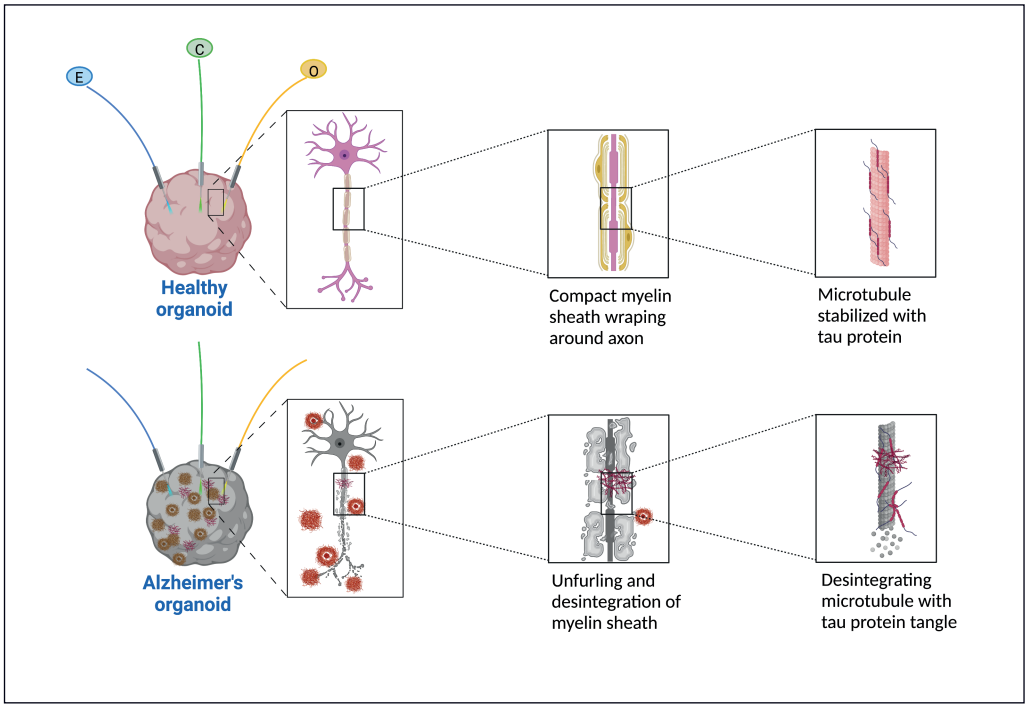

Fig. 3: Neurons and brain organoids from iPSC derived from Alzheimer patients show characteristics of Alzheimer pathology
Illustration of literature findings. Created with BioRender.com.

Experimental inputs will primarily be through electrical stimulation, but chemical signals (such as neurotransmitters or their pharmacological agonists) or optogenetics (Lee et al., 2020) can serve as well (Fig. 2). Organoid crosstalk – combining the brain organoid with sensory inputs such as assembloids, starting with the retina, and allowing complex inputs and outputs to control other organs – will be the next step.

Analysis techniques will be needed to investigate short-term and long-term learning, memory, and cell population dynamics in brain organoids. Establishing and executing theoretical frameworks and closed-loop experiments to investigate fundamental aspects of cognition such as memory formation and recall, plasticity, developmental trajectories, and predication will allow the study of the behavior of the brain organoid under variations of input.

3.4 Pharmacology and toxicology enabled by OI

The fact that the generation of brain organoids in many aspects reflects neurodevelopment promises especially relevant models. Such models can be used to investigate which exposures contribute to perturbation of brain development, impair brain function, or lead to earlier brain function decline and may generate hypotheses on how to improve such conditions.

For example, it has been shown that brain organoids derived from stem cells of patients with Alzheimer's disease show characteristics of the disease (Ochalek et al., 2017; Machairaki, 2020) (Fig. 3). Similarly, exploring the basis and drugability of developmental cognitive dysfunction, e.g., autism, by employing patient-derived brain organoids, is another important opportunity. Our recent work on combining an autism risk gene and a chemical risk factor resulting in synergistic toxicity in the brain organoid model (Modafferi et al., 2021) may serve as illustration. OI endpoints might further enhance the relevance of such model interactions.

Finding "dementiogens", i.e., chemicals that impair cognitive development and lead to neurodegeneration, through the brain exposome (Sillé et al., 2020) is an important perspective of OI's use. Figure 4 shows a workflow for how to assess neuroplasticity as an endpoint for such studies. Through the combination of genetics (through donor cells) and exposures, gene-environment interactions in these diseases can be studied.

Similarly, illicit drugs or tobacco products and their added flavors (Hartung, 2016) might be assessed. Another use scenario is the identification of threat agents and countermeasures against them (Hartung and Zurlo, 2012), as many of these are nerve agents targeting the CNS.

3.5 Ethics framework

Pioneering where the "birth of sentience and consciousness" starts requires embedded ethics (McLennan et al., 2022) in the form of ethical discussion in our team headed by the JH Berman Institute of Bioethics. An empirical ethics study of OI is aiming to identify factors that contribute to public support, trust, and perceived risk of the technology.

Could a biocomputer develop emotional intelligence? Sentience? Consciousness? Could it suffer? We are certainly far away from this, but it is difficult to exclude it, and the respective discussion has started (Lavazza, 2019; Boers et al., 2019; Reardon, 2020; NASEM, 2021; Sawai et al., 2022; Kagan et al., 2022b).

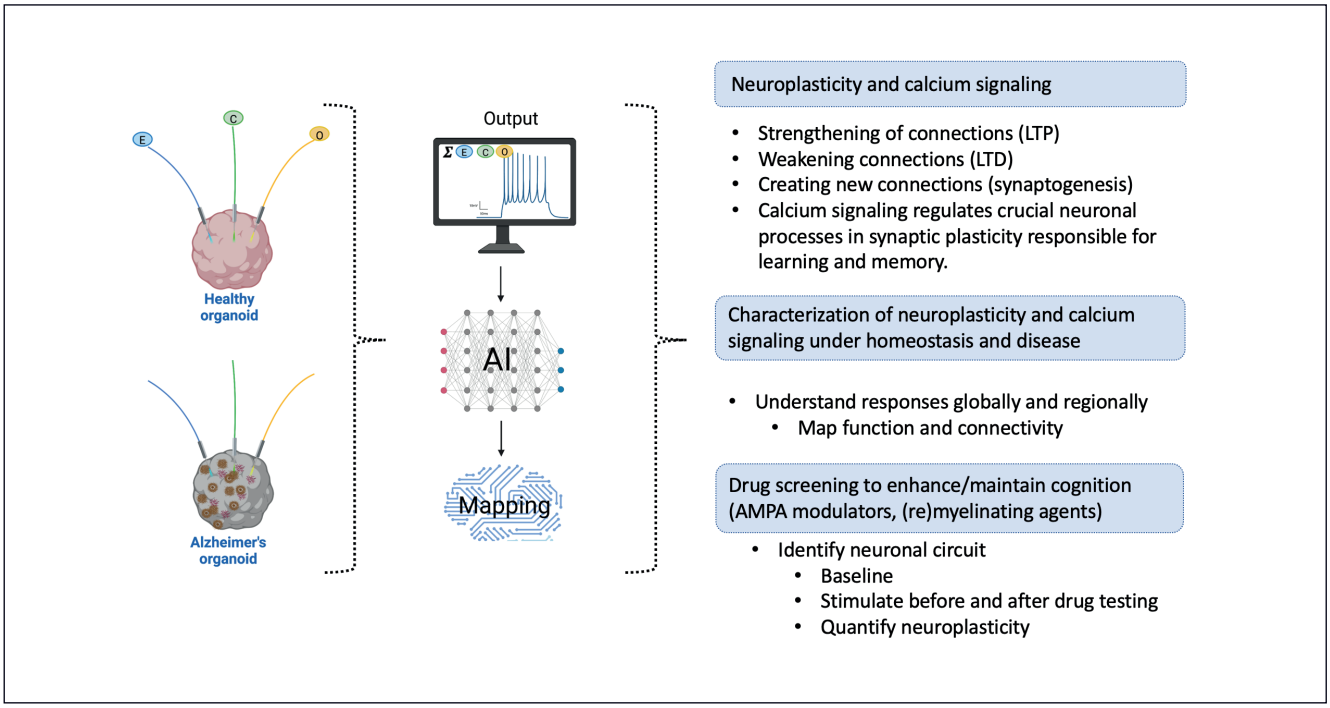

Fig. 4: Neuroplasticity underlying long-term learning as read-out for organoid intelligence (OI)
Long-term synaptic plasticity forms the model for memory storage as the connectome of neuronal circuits. Very active synapses are likely to become stronger (long-term potentiation, LTP), and those that are less active, or less effective at causing an action potential, tend to become weaker (long-term depression, LTD). AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) modulators of the AMPA receptor, a type of ionotropic glutamate receptor, which mediates most fast synaptic neurotransmission in the central nervous system. Created with BioRender.com.

The development of any cognition certainly depends on what input the system can be given. At this moment, the brain organoids are tiny, and the input and feedback on the organoid's output is very limited. No emotions without feeding in emotional content. No pain without pain receptors. However, we must anticipate such possibilities and want to proactively identify boundaries and set limits to such research. While it is fascinating to ask when sentience or consciousness start or when a model might be able to suffer, no such levels of cognition are to be expected in the foreseeable future. Further possible questions for debate could be whether organic matter could become uncontrollable or could be given any form of autonomy.

As the stem cells are derived from cell donors, questions must also be asked on how an organoid relates to the cell donor or what an informed consent needs to include to allow such research.

3.6 Biological computing

First, we should be clear that a biocomputer that can compete with a silicon computer is a vision, not a reality. While a superior biocomputer is certainly decades away, its uses for brain research and drug development are already starting. At this moment, we see foremost the opportunity to study cognition and find drugs or toxicants that influence cognition rather than for practical biocomputing. However, learning about the human machinery of cognition may suggest new computer designs. If we can realize some of the advantages a brain has over a computer (efficiency, intuition, progressive learning, creativity, emotional intelligence, etc.), there might be room for a biological component in our IT infrastructure, but this is currently science fiction.

More realistic, however, is that information gain on how the brain achieves its functionality can serve to further optimize computer architecture as neuromorphic computing (Schuhman et al., 2022). Neuromorphic computing is a method of computer engineering in which elements of a computer are modeled after systems in the human brain and nervous system. As OI combines three disruptive technologies (bioengineering based on stem cells, sensor technologies, and AI), we might see faster developments than we can currently imagine.

4 The prospect of replacing non-human primates in neuroscience

The possibility of replacing animals in neuroscience is particularly important, as non-human primates (NHP) are commonly used in this discipline. While in the US, there are relatively low

legal barriers, in the EU, Directive 2010/63/EU on the protection of animals used for scientific purposes[13] states in Article 8 that "*non-human primates shall not be used in procedures, with the exception of …* [basic research] *with view to the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions in human beings; or (b) there is scientific justification to the effect that the purpose of the procedure cannot be achieved by the use of species other than non-human primates*." This means that research can be done only for severe human conditions or when the research question can only be answered by using NHP.

Certain cognitive functions of NHP are closer to those of humans than those of other species. These experiments are often done on awake, fixated NHP over prolonged periods of time, often several hours per day for months to years, in many cases using fluid deprivation to force certain behaviors. Article 15 of Directive 2010/63/EU on the classification of severity of procedures requests that "*Member States shall ensure that a procedure is not performed if it involves severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated*".

Leading European organizations for laboratory animal welfare developed a working group guidance "*Classification and reporting of severity experienced by animals used in scientific procedures: FELASA*[14]*/ECLAM*[15]*/ESLAV*[16] *Working Group report*" (Smith et al., 2018). The majority of the criteria for severe experiments are met by the NHP experiments described above (e.g., separation from the social group, fixation of their head in a "primate chair" for many hours, transport from cage to laboratory, preparation of measurements, introduction of electrodes, limiting fluid access). It is remarkable that researchers in this field often argue that they need to work on NHP because of their similarity to humans, but on the other hand consider that these protocols, which would be perceived by humans as extremely severe, are of minor severity for the animals. The Pickard Report (2013)[17] (Report of the animal procedures primate subcommittee working group chaired by Prof. John Pickard) found that "*there were some nonhuman primates that could not cope and were removed from study. In a small minority of cases, premature euthanasia was performed as part of the terminal phase*." This suggests a high level of stress, similar to an evaluation by the Biotechnology and Biological Sciences Research Council (BBSRC), Medical Research Council (MRC) und Wellcome Trust (Review of Research Using Non-Human Primates - Report of a panel chaired by Professor Sir Patrick Bateson FRS)[18]: "*Electrophysiology studies in the awake, behaving state were generally assessed as imposing a high welfare impact due to the numerous procedures involved, their likely effects on the monkeys, and the lengthy duration of the experiments*."

The availability of human test models with even primitive forms of cognitive functions would create opportunities for some of this research to transition to brain MPS. This is enforced with Article 4 of the EU Directive 2010/63/EU "*Principle of replacement, reduction and refinement 1. Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure*." This means that with OI, alternatives to a particularly problematic form of animal research come into reach.

5 A vision for an interdisciplinary collaboration

The vision is to realize cognitive functions in brain MPS and scale them to achieve relevant computational capabilities. In how many years will organoid intelligence be reality? In its most primitive forms, such as the Pong-playing cultures, it is already a reality. We can already study the organoid's response to varying inputs, and we can already grow organoids from patients to compare them with those from healthy donors. Our ultimate goal is to culture mature organ models to observe, measure, and analyze human intelligence in action, including measuring human cognitive physiology in new ways that deepen our understanding of human cognitive health and disease.

Such organ models also open the prospect of a complement to AI. Synthetic biology and bioengineering will be explored to leverage advantages of human computing, such as progressive learning, decision-taking on limited datasets, and energy- and space-efficiency. To achieve targeted breakthroughs in OI, a multi-institute, multi-disciplinary team is required. There is no area of research and technology not affected by progress in AI, but the potential impact on basic neuroscience and the understanding of neurological diseases and the search for treatment regimens is evident. MPS research has been driven so far by the biomedical research community and bioengineering. The potential for computer science, i.e., leveraging the possibility to recreate cognitive capabilities in biological models at larger scale, requires crosstalk between engineering specialties and data science. To fully realize the potential of OI, contributions from many domains are required:

- *Human cellular biology:* Human stem cell technology and bioengineering are required to recreate brain architectures and to model their potential for cognitive capabilities.
- *Neuroscience:* Various subdisciplines of neuroscience, including developmental neuroscience, neuroanatomy, and theoretical neuroscience will be required to study brain organoid neurophysiology.

[13] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF

[14] Federation of European Laboratory Animal Science Associations, https://felasa.eu

[15] European College of Laboratory Animal Medicine, https://eclam.eu

[16] European Society of Laboratory Animal Veterinarians, https://www.eslav.org

[17] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/261687/cs_nhp_review_FINAL_2013_corrected.pdf

[18] https://wellcome.org/sites/default/files/wtvm052279_1.pdf

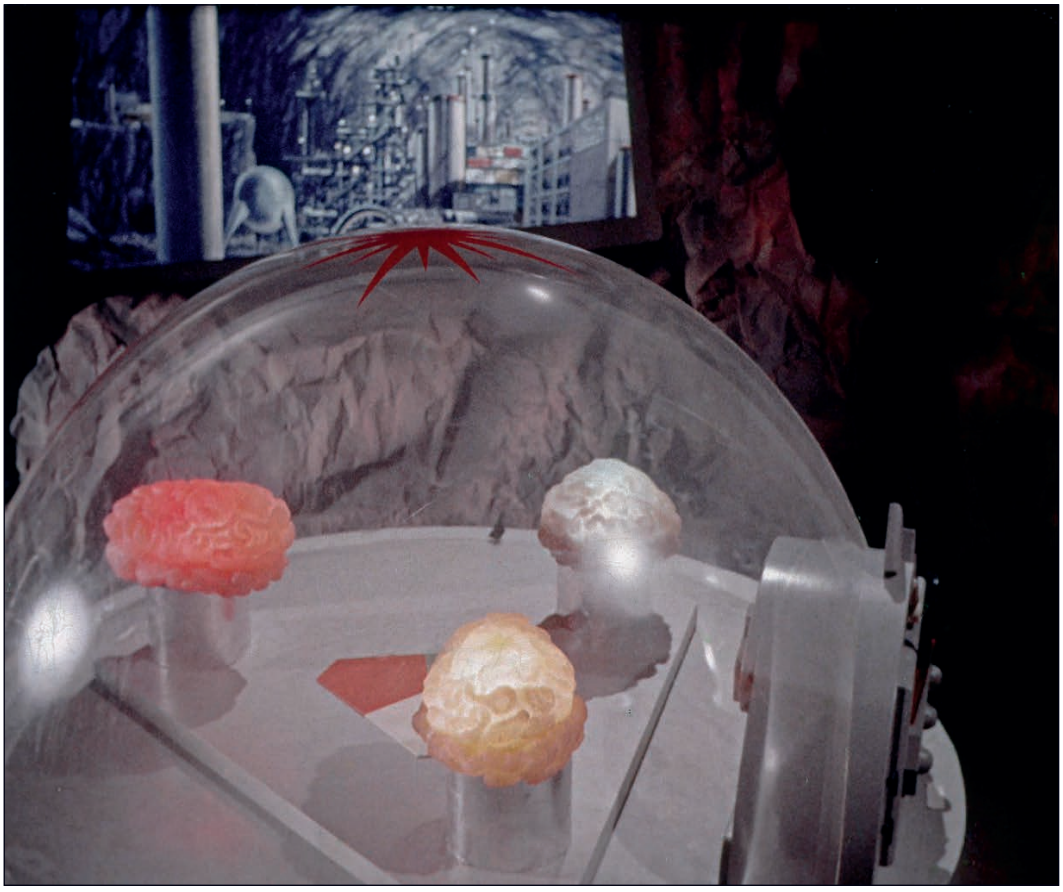

Fig. 5: Brain-based computer in science fiction
Photo taken from Star Trek "*Gamesters of Triskelion*" (Season 2, Episode 16, 1966)[19]. Thanks to Dr Matthew Clark, Charles River Laboratories, for providing this link.

- *Material science and fabrication:* Neural interface breakthroughs will be needed to allow delivery of input signals to organoids, measuring of output signals, and delivery of feedback to the organoid.
- *Computer science, AI, and informatics:* Neural analysis, machine learning, big data, and AI technologies can allow us to understand brain organoids.
- *Electrical and systems engineering:* Systems integration can channel the research into compelling prototypes.
- *Bioethics:* Ethical challenge specification and mitigation for OI is necessary to allow us to identify and address possible ethical challenges facing this emerging field.

It is of utmost importance to embrace the potential of biological computing as a likely future generation of AI. The challenge of interdisciplinary collaboration of brain MPS engineering, electrophysiology and other sensor technologies, big data and machine learning on the one side as enabling technologies, and neuroscience, pharmacology/toxicology, and computer science on the other as the most obvious use areas, requires a bold investment into this emerging field of strategic importance. Often such innovations are enabled by the entrepreneurial state (Mazzucato, 2013), i.e., the investment of public money with a long-term vision as contrasted by the short-term shareholder value-driven perspective of industry.

6 Forming an OI community

Over the last years, a multi-institutional OI community has formed with about 80 researchers from international institutions including universities, start-up companies, major corporations, medical institutions, and large research laboratories. Our emerging OI community combines interests in bioengineered microphysiological organ models and AI. The scientific pillars laid out above will need to be complemented by scientific policy support for policymakers and agencies, public outreach through citizen scientists, massive open online course teaching and curricula development, and engagement toward quality assurance. Our interdisciplinary core team from Johns Hopkins Bloomberg School of Public Health, Whiting School of Engineering, Johns Hopkins Berman Institute of Bioethics and Applied Physics Laboratories brings together a unique combination of biologists,

19 https://www.flickr.com/photos/birdofthegalaxy/3577553434/in/photostream/

electrical engineers, neuroscientists, machine learning experts, and embedded ethicists to tackle this challenge. The community-forming workshop (Morales Pantoja et al., 2023) was to some extent inspired by the Dartmouth summer workshop on AI in 1956, which is widely considered to be the founding event of AI as a field.[20] The creation of a journal, i.e., *Frontiers in OI*, already creates a platform for exchange. The concerted publication of our vision as one of two inaugural articles of Frontiers' new flagship journal *Frontiers in Science* created an article hub[21] and respective visibility. This momentum is being used to form an OI community with a website[22] and newsletter under construction.

It will be critical to find seed funding for this endeavor. The Johns Hopkins Whiting School of Engineering has made OI part of the proposed projects of its philanthropic campaign, and in parallel various grant opportunities with national funding bodies, foundations, and corporate partners are being explored. Discussions with policymakers on both sides of the Atlantic are underway. This unique collaborative community is beyond the scope of traditional government funding models due to the complex multi-institute and multi-disciplinary community. The proposed work is high-risk, high-reward. Such targeted investment will allow development of the key technologies, enable the first wave of neuroscientific discoveries using OI, and cement this emerging community. This represents a scope that can be uniquely supported by large-scale investment.

7 Conclusions

Since the beginning of the computer era, engineering aimed to emulate brain-like functionality, most obviously by striving for AI. Sundar Pichai, CEO of Alphabet, said "*Artificial intelligence could have more profound implications for humanity than electricity or fire*." Still, we are far away from achieving brain functionality *in silico*. At the same time, cell culture aimed to recreate human organ architecture and functionality, for the brain ultimately with cognitive functions. With the advent of human stem cells and the bioengineering of brain MPS, this is now coming into reach with the machinery of learning and memory being realized in organoids and organ-on-chip systems.

OI promises to provide a model of human learning and memory. In an ethical way without animals, we can experimentally study how the brain works with respect to fundamental cellular mechanisms. This allows the study of the many diseases that affect brain function and to exploit this for drug development.

Last, we might be able to improve computer technologies, by either implementing what we learned in our programming and computer architecture or perhaps sometime even by complementing traditional computers with a biological component. This is a bit like living a science fiction movie. To stress this sentiment, Figure 5 shows a scene from Star Trek "*Gamesters of Triskelion*" (Season 2, Episode 16, 1966) with brains at the core of the computer.

Several technologies have recently matured, allowing us to realize OI: the bioengineering of human brain models, sensor technologies to communicate, and AI to analyze the behavior of the organoid. The idea is infectious, and we see more and more neuroscientists, engineers, pharmacologists, and data specialists joining us. It feels like the start of something big. Some are skeptical, and this is understandable. Burt Rutan (1943-), a retired American aerospace engineer and entrepreneur put it best: "*Revolutionary ideas come from nonsense. If an idea is truly a breakthrough, then the day before it was discovered, it must have been considered crazy or nonsense or both—otherwise it wouldn't be a breakthrough*."

References

Alépée, N., Bahinski, A., Daneshian, M. et al. (2014). State-of-the-art of 3D cultures (organs-on-a-chip) in safety testing and pathophysiology. *ALTEX 31*, 441-477. doi:10.14573/altex1406111

Andersen, M. E., Betts, K., Dragan, Y. et al. (2014). Developing microphysiological systems for use as regulatory tools – Challenges and opportunities. *ALTEX 31*, 364-367. doi:10.14573/altex.1405151

Anderson, W. A., Bosak, A., Hogberg, H. T. et al. (2021). Advances in 3D neuronal microphysiological systems: Towards a functional nervous system on a chip. *In Vitro Cell Dev Biol Anim 57*, 191-206. doi:10.1007/s11626-020-00532-8

Bakkum, D. J., Chao, Z. C. and Potter, S. M. (2008). Spatio-temporal electrical stimuli shape behavior of an embodied cortical network in a goal-directed learning task. *J Neural Eng 5*, 310-323. doi:10.1088/17412560/5/3/004

Boers, S. N., van Delden, J. J. M. and Bredenoord, A. L. (2019). Organoids as hybrids: Ethical implications for the exchange of human tissues. *J Med Ethics 45*, 131-139. doi:10.1136/medethics-2018-104846

Buchanan, M. (2018). Organoids of intelligence. *Nat Phys 14*, 634. doi:10.1038/s41567-018-0200-2

Coecke, S., Balls, M., Bowe, G. et al. (2005). Guidance on good cell culture practice – A report of the second ECVAM task force on good cell culture practice. *Altern Lab Anim 33*, 261-287. doi:10.1177/026119290503300313

Demarse, T. B., Wagenaar, D. A., Blau, A. W. et al. (2001). The neurally controlled Animat: Biological brains acting with simulated bodies. *Auton Robots 11*, 305-310. doi:10.1023/a:1012407611130

Efros, A. L., Delehanty, J. B., Huston, A. L. et al. (2018). Evaluating the potential of using quantum dots for monitoring electrical signals in neurons. *Nat Nanotechnol 13*, 278-288. doi:10.1038/s41565-018-0107-1

[20] https://en.wikipedia.org/wiki/Dartmouth_workshop

[21] https://www.frontiersin.org/journals/science/article-hubs/organoid-intelligence-a-new-biocomputing-frontier

[22] https://caat.jhsph.edu/programs/oi/

Friston, K. (2023). The sentient organoid? *Front Sci 1*, 1147911. doi:10.3389/fsci.2023.1147911

Hartung, T. (2007). Food for thought ... on cell culture. *ALTEX 24*, 143-147. doi:10.14573/altex.2007.3.143

Hartung, T. and Zurlo, J. (2012). Alternative approaches for medical countermeasures to biological and chemical terrorism and warfare. *ALTEX 29*, 251-260. doi:10.14573/altex.2012.3.251

Hartung, T. (2016). E-cigarettes and the need and opportunities for alternatives to animal testing. *ALTEX 33*, 211-224. doi:10.14573/altex.1606291

Hartung, T., Smirnova, L., Morales Pantoja, I. E. et al. (2023). The Baltimore declaration toward the exploration of organoid intelligence. *Front Sci 1*, 1017235. doi:10.3389/fsci.2023.1068159

Honegger, P., Lenoir, D. and Favrod, P. (1979). Growth and differentiation of aggregating fetal brain cells in a serum-free defined medium. *Nature 282*, 305-308. doi:10.1038/282305a0

Huang, Q., Tang, B., Romero, J. C. et al. (2022). Shell microelectrode arrays (MEAs) for brain organoids. *Sci Adv 8*, eabq5031. doi:10.1126/sciadv.abq5031

Jun, J. J., Steinmetz, N. A., Siegle, J. H. et al. (2017). Fully integrated silicon probes for high-density recording of neural activity. *Nature 551,* 232-236. doi:10.1038/nature24636

Kagan, B. J., Kitchen, A. C., Tran, N. T. et al. (2022a). In vitro neurons learn and exhibit sentience when embodied in a simulated game-world. *Neuron 110*, 3952-3969.e8. doi:10.1016/j.neuron.2022.09.001

Kagan, B. J., Duc, D., Stevens, I. et al. (2022b). Neurons embodied in a virtual world: Evidence for organoid ethics? *AJOB Neurosci 13*, 114-117. doi:10.1080/21507740.2022.2048731

Koo, B., Choi, B., Park, H. et al. (2019). Past, present, and future of brain organoid technology. *Mol Cells 42*, 617-627. doi:10.14348/molcells.2019.0162

Lancaster, M. A., Renner, M., Martin, C. A. et al. (2013). Cerebral organoids model human brain development and microcephaly. *Nature 501*, 373-379. doi:10.1038/nature12517

Lancaster, M. A. and Knoblich, J. A. (2014). Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc 9*, 2329-2340. doi:10.1038/nprot.2014.158

Lavazza, A. (2019). What (or sometimes who) are organoids? And whose are they? *J Med Ethics 45*, 144-145. doi:10.1136/medethics-2018-105268

Lee, C., Lavoie, A., Liu, J. et al. (2020). Light up the brain: The application of optogenetics in cell-type specific dissection of mouse brain circuits. *Front Neural Circuits 14*, 18. doi:10.3389/fncir.2020.00018

Machairaki, V. (2020). Human pluripotent stem cells as in vitro models of neurodegenerative diseases. *Adv Exp Med Biol 1195*, 93-94. doi:10.1007/978-3-030-32633-3_13

Magliaro, C. and Ahluwalia, A. (2023). To brain or not to brain organoids. *Front Sci 1*, 1148873. doi:10.3389/fsci.2023.1148873

Marom, S. and Shahaf, G. (2002). Development, learning and memory in large random networks of cortical neurons: Lessons beyond anatomy. *Q Rev Biophys 35*, 63-87. doi:10.1017/S0033583501003742

Marx, U., Andersson, T. B., Bahinski, A. et al. (2016). Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. *ALTEX 33*, 272-321. doi:10.14573/altex.1603161

Marx, U., Akabane, T., Andersson, T. et al. (2020). Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development. *ALTEX 37*, 365-394. doi:10.14573/altex.2001241

Mazzucato, M. (2013). *The Entrepreneurial State: Debunking Public vs Private Sector Myths*. 1st edition. Anthem Press.

McDonald, M., Sebinger, D., Brauns, L. et al. (2023). A mesh microelectrode array for non-invasive electrophysiology within neural organoids. *Biosens Bioelectron 228*, 115223. doi:10.1016/j.bios.2023.115223

McLennan, S., Fiske, A., Tigard, D. et al. (2022). Embedded ethics: A proposal for integrating ethics into the development of medical AI. *BMC Med Ethics 23*, 6. doi:10.1186/s12910-022-00746-3

Miller, G. W. (2023). Organoid intelligence: Smarter than the average cell culture. *Front Sci 1*, 1150594. doi:10.3389/fsci.2023.1150594

Modafferi, S., Zhong, X., Kleensang, A. et al. (2021). Gene-environment interactions in developmental neurotoxicity: A case study of synergy between chlorpyrifos and CHD8 knockout in human BrainSpheres. *Environ Health Perspect 129*, 77001. doi:10.1289/EHP8580

Morales Pantoja, I. E., Smirnova, L., Muotri, A. R. et al. (2023). First organoid intelligence (OI) workshop to form an OI community. *Front Artif Intell 6*, 1116870. doi:10.3389/frai.2023.1116870

NASEM – National Academies of Sciences, Engineering, and Medicine (2021). *The Emerging Field of Human Neural Organoids, Transplants, and Chimeras: Science, Ethics, and Governance*. Washington, DC, USA: The National Academies Press. doi:10.17226/26078

Ochalek, A., Mihalik, B., Avci, H. X. et al. (2017). Neurons derived from sporadic Alzheimer's disease iPSCs reveal elevated TAU hyperphosphorylation, increased amyloid levels, and GSK3B activation. *Alzheimers Res Ther 9*, 90. doi:10.1186/s13195-017-0317-z

Pamies, D. and Hartung, T. (2017). 21st century cell culture for 21st century toxicology. *Chem Res Toxicol 30*, 43-52. doi:10.1021/acs.chemrestox.6b00269

Pamies, D., Bal-Price, A., Simeonov, A. et al. (2017). Good cell culture practice for stem cells and stem-cell-derived models. *ALTEX 34*, 95-132. doi:10.14573/altex.1607121

Pamies, D., Bal-Price, A., Chesné, C. et al. (2018). Advanced good cell culture practice for human primary, stem cellderived and organoid models as well as microphysiological systems. *ALTEX 35*, 353-378. doi:10.14573/altex.1710081

Pamies, D., Leist, M., Coecke, S. et al. (2020). Good cell and tissue culture practice 2.0 (GCCP 2.0) – Draft for stakeholder discussion and call for action. *ALTEX 37*, 490-492. doi:10.14573/altex.2007091

Pamies, D., Leist, M., Coecke, S. et al. (2022). Guidance document on good cell and tissue culture practice 2.0 (GCCP 2.0). *ALTEX 39*, 30-70. doi:10.14573/altex.2111011

Qian, X., Song, H. and Ming, G. L. (2019). Brain organoids: Advances, applications and challenges. *Development 146*, dev166074. doi:10.1242/dev.166074

Quirion, R. (2023). Brain organoids: Are they for real? *Front Sci 1*, 1148127. doi:10.3389/fsci.2023.1148127

Reardon, S. (2020). Can lab-grown brains become conscious? *Nature 586*, 658-661. doi:10.1038/d41586-020-02986-y

Roth, A. and MPS-WS Berlin (2021). Human microphysiological systems for drug development. *Science 373*, 1304-1306. doi:10.1126/science.abc3734

Sawai, T., Hayashi, Y., Niikawa, T. et al. (2022). Mapping the ethical issues of brain organoid research and application. *AJOB Neurosci 13*, 81-94. doi:10.1080/21507740.2021.1896603

Schuman, C. D., Kulkarni, S. R., Parsa, M. et al. (2022). Opportunities for neuromorphic computing algorithms and applications. *Nat Comput Sci 2*, 10-19. doi:10.1038/s43588-021-00184-y

Shahaf, G. and Marom, S. (2001). Learning in networks of cortical neurons. *J Neurosci 21*, 8782-8788. doi:10.1523/jneurosci.21-22-08782.2001

Sillé, F. C. M., Karakitsios, S., Kleensang, A. et al. (2020). The exposome – A new approach for risk assessment. *ALTEX 37*, 3-23. doi:10.14573/altex.2001051

Smirnova, L., Kleinstreuer, N., Corvi, R. et al. (2018). 3S – Systematic, systemic, and systems biology and toxicology. *ALTEX 35*, 139-162. doi:10.14573/altex.1804051

Smirnova, L. and Hartung, T. (2022). Neuronal cultures playing Pong – First steps toward advanced screening and biological computing. *Neuron 110*, 3855-3856, doi:10.1016/j.neuron.2022.11.010

Smirnova, L., Caffo, B. S., Gracias, D. H. et al. (2023a). Organoid intelligence (OI): The new frontier in biocomputing and intelligence-in-a-dish. *Front Sci 1*, 1017235. doi:10.3389/fsci.2023.1017235

Smirnova, L., Morales Pantoja, I. and Hartung, T. (2023b). Brain-cell cultures: The future of computers and more? *Front Young Minds 11*, 1049593. doi:10.3389/frym.2023.1049593

Smith, D., Anderson, D., Degryse, A. D. et al. (2018). Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. *Lab Anim 52*, 5-57. doi:10.1177/0023677217744587

Steinmetz, N. A., Aydin, C., Lebedeva, A. et al. (2021). Neuropixels 2.0: A miniaturized high-density probe for stable, long-term brain recordings. *Science 372*, eabf4588. doi:10.1126/science.abf4588

Trujillo, C. A., Gao, R., Negraes, P. D. et al. (2019). Complex oscillatory waves emerging from cortical organoids model early human brain network development. *Cell Stem Cell 25*, 558-569. doi:10.1016/j.stem.2019.08.002

Conflict of interest

T.H. is named inventor on a patent by Johns Hopkins University on the production of mini-brains (also called BrainSpheres), which is licensed to Axo-Sim, New Orleans, LA, USA. T.H. and L.S. are consultants for AxoSim, New Orleans, and T.H. is also a consultant for AstraZeneca and American Type Culture Collection (ATCC) on advanced cell culture methods.

Data availability

No original data was created for this manuscript.

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