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Review

Ethical issues related to brain organoid research

Insoo Hyun[a,*], J.C. Scharf-Deering[a], Jeantine E. Lunshof[b,c,d]

[a] Case Western Reserve University, USA
[b] Wyss Institute for Biologically Inspired Engineering at Harvard, USA
[c] Harvard Center for Bioethics, Harvard Medical School, Boston, MA, USA
[d] University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

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ABSTRACT

This review provides a snapshot of the current ethical issues related to research with human brain organoids. The issues fall into the following main themes: research oversight; human biomaterials procurement and donor consent; translational delivery; animal research; and organoid consciousness and moral status. Each of these areas poses challenges for researchers, bioethicists, regulators, research institutions, and tissue banks. However, progress can be made if these parties build on past experiences with stem cell research, ethics, and policy, but adapted accordingly to new aspects of brain organoid research.

1. Introduction

The purpose of this review is to provide a snapshot of the current ethical issues related to research with human brain organoids. Many of these issues correspond to concerns raised by bioethicists, citizens, and scientists themselves in the field of emerging biomedical technologies, including stem cell research and therapies, human gene transfer research, and organoid research (Hyun, 2013; Hyun, 2011; Hyun, 2007; Cheshire, 2014). While the state of the science is not the focus of this review, we recognize that brain organoid research is moving at a rapid pace, with new approaches continuously evolving (Pașca, 2019; Miura and Pașca, 2019).

The term "brain organoid" is applied to a variety of self-organized cellular structures that evolve to form brain tissue representing aspects of neural development in both typical and diseased states (Bershteyn and Kriegstein, 2012; Lancaster and Renner, 2013; Mansour and Gonçalves, 2018; Editorial, 2018; Kelava and Lancaster, 2016; Buchanan, 2018; Arlotta, 2018; Rossi and Manfrin, 2018; Huch, 2017; Di Lullo and Kriegstein, 2017); and that represent the whole brain or specific regions in 3D (Shuler and Hickman, 2014; Cheah and Mason, 2019). These 3D models can consist of biological material only, biological samples in combination with synthetic biomaterials or bio-printed materials, and brain organoid structures implanted into animal models, regardless of their origin (Karzbrun and Reiner, 2019; Giandomenico et al., 2019; Mahe, 2018; Mariani and Gianfilippo, 2015; Bagley and Reumann, 2017; Bian and Repic, 2018; Hopkins and DeSimone, 2014; Datta and Ayan, 2017; Zhang and Sun, 2018; Lozano and Stevens, 2015; Alessandri and Feyeux, 2016). These types of *in vitro* or *in vivo* brain organoids models make up the vast majority of models from the published scientific literature (Cantley and Chuang, 2018; Amin and Pașca, 2018; Gershlak and Hernandez, 2017; Pajorova and Hluchy, 2018; Farah, 2015).

A review of the ethical literature suggests that there may be novel concerns as brain organoids become more complex (Yeager, 2018). While brain organoid research inches (or leaps) closer to whole brain study and function, there are issues related to consciousness, sentience, and moral status, as we discuss below (Munsie and Hyun, 2017). It is worth noting that there are shared concerns from ethicists working in the field of artificial intelligence (Anderson, 2008; Burton and Goldsmith, 2015; Kaplan, 2004; Giordano, 2014; Greely and Ramos, 2016; Delegates, 2018; Whittlestone and Nyrup, 2019; Yeager, 2018). While we are aware of this intersection, the discussion about brain-computer interface questions is beyond the scope of this review.

2. Ethical themes

We see the various issues related to the ethics of research with brain organoids as falling into the following main themes: research oversight; human biomaterials procurement and donor consent; translational delivery; animal research; and organoid consciousness and moral status. Many of these themes correspond to issues that are pervasive in the ethics literature related to research across the spectrum of biomedical and translational research (Farahany and Greely, 2018; Salles and Bjaalie, 2019). The last area of concern, consciousness and moral status, seems fundamentally more specific to brain organoid research.

* Corresponding author.
E-mail address: insoo.hyun@case.edu (I. Hyun).

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2.1. Theme 1: formal regulatory policy and research guidelines

Public and private institutions in the United States that receive federal funding to conduct research are required to have regulatory review committees in place to assure the safety, ethics, objectivity of the research and stewardship of funds that support research and scholarship (Harris and Gallo, 2017). Many institutions and organizations in the private sector also choose to apply the regulatory framework to all proposed and funded projects, regardless of funding sources. These committees have specific policies or guidelines regarding requirements for committee membership, process for review of research and protocols, and reporting requirements to investigators, the public, and/or funding agencies (Grants, 2020).

The "alphabet soup" of regulatory committees includes institutional or commercial committees for the approval and monitoring of research with animals, human subjects, recombinant or synthetic nucleic acid molecules, human gene therapy, and human/embryonic stem cells. Required committees include the Institutional Animal Care and Research Committee (IACUC), which covers research with vertebrate animal species, the Ethics/Institutional Review Board (E/IRB), for the review of human subjects protocols, and the Institutional Biosafety Committee (IBC), charged with the review of recombinant or synthetic nucleic acid molecules using genetic manipulation and/or viral vectors. IBC review covers the use of recombinant or synthetic nucleic acid molecules used in research with *in vitro* cells and samples, as well as research with *in vivo* models, including human gene therapy (Guidelines, 2020; Updated Guidelines April 2019), vertebrate and invertebrate animal and insect species and plants. For institutions or agencies working with select agents and research materials identified as falling under dual use research of concern, an Institutional Review Entity (IRE) is also required. A myriad of additional required or optional committees may be in place to approve research before it is initiated and monitor research as it progresses.

Investigators conducting brain organoid research may be subject to the review by one or more of these committees, for example IACUC or IRB, as well as review and approval by an Embryonic/Human Stem Cell Research Oversight Committee (E/HSCRO). Currently, E/HSCRO review of brain organoid research is largely confined to the ethical provenance of the human stem cell lines used to generate brain organoids in the lab and studied *in vitro* only (i.e. without subsequent transfer into research animals or human participants). Looking ahead, however, stem cell-specific E/HSCRO review of *in vitro* brain organoid research may expand to other considerations beyond the ethical provenance of cell lines, depending on how complex brain organoid systems can be made to become. For example, according to professional and ethical guidelines issued by the International Society for Stem Cell Research (ISSCR), any research that generates human "embryo-like structures" that "might manifest human organismal potential" must undergo additional review through an embryo research oversight process at an institutional or regional/national level (Guidelines for Stem Cell Science and Clinical Translation, 2016).

Today, no one would claim that brain organoids are biological structures that represent the entire human embryo, much less that they are entities that manifest "human organismal potential," that is, have the biological capacity to generate a fetus in a real or artificial uterine environment. However, there may eventually come a call for a closer review of brain organoid research by embryo research oversight committees, in particular, if mature and complex brain organoids are paired with living or non-living systems *in vitro* that, in their totality, could be thought to generate or to constitute the possibility of nascent human life. While this may now seem to be a very remote possibility, it is our view that, if some researchers proceed in such a heavily bioengineered direction, the regulatory oversight of brain organoid research may expand proportionately in scope as well. Later, we discuss the issue of hypothetical brain organoid consciousness and moral status, that raises regulatory concerns that are somewhat related to the concerns about the human organismal potential of brain organoid-paired systems.

2.2. Theme 2: procurement of human biological materials

Human brain organoid research depends on the procurement of various biomaterials (i.e. pre- implantation embryos, gametes, and somatic cells) necessary for the derivation of stem cell lines used to generate organoids (Boers and Bredenoord, 2018; Boers and van Delden, 2016). The ethical and responsible prospective collection of patient or human samples relies on clearly stating those elements that allow for engagement in informed consent.

Participants should know that they are participating in a research study and what the risks and benefits are to them.

To ensure that procurement of biomaterials for stem cell line derivation is conducted in a manner consistent with current ethical standards for informed consent, and to encourage the implementation of additional stem cell-specific considerations during the consent process, updated professional and ethical guidelines issued by the International Society for Stem Cell Research (ISSCR) recommend the following (Guidelines, 2020).

First, informed consent for the procurement of human biological materials for stem cell derivation should be obtained close to the proposed time in which the materials are to be transferred to the research team. This call for "explicit and contemporaneous consent" requires donors' permission to use their biomaterials to derive immortal stem cell lines. This includes the need to obtain consent from any third-party gamete donors involved in the creation of fertility clinic embryos that may later be used for research. Third-party gamete donors who have provided sperm or eggs for assisted reproductive purposes may object to their inadvertent participation in supporting human embryonic stem cell research, and for this reason they need to be re-contacted and consented specifically for their possible complicit involvement in stem cell research. In practice, however, this call for retroactive third party gamete donors' consent may prove to be too burdensome for research teams and donors, and going forward, the possibility of this type of research should be included in the original consent, maybe with an opt-out for this specific purpose. Also, teams may be more inclined to utilize either human embryonic stem cell lines for which no third party gamete donors were involved, or to utilize induced pluripotent stem (iPS) cell lines to generate brain organoids.

On the other hand, the use of iPS cell lines for brain organoid research comes with its own ethical challenges. Obtaining informed consent from decisionally-competent adult somatic cell donors for brain organoid research is a fairly straightforward process, since donors in these cases would be informed of why they are being asked to contribute their somatic cells – first to generate iPS cells, and then to use the resulting iPS cells to generate brain organoids for the primary research team's defined scientific goals. In cases where the somatic cell donors are either young children or adults with brain disorders (especially those affecting cognition and the capacity to give proper informed consent), research teams must take special care that the appropriate legal guardians are fully engaged in the consent process. All parties must understand that the donors' biomaterials will be used to create genetically-matched brain organoids that might reveal important health information about the donors. The ISSCR recommends having a well-resourced action plan in place just in case clinically relevant incidental findings are discovered during the course of research. If teams have no action plan in place to return incidental findings to somatic cell donors, then this lack of a plan must also be disclosed during the consent process (Guidelines for Stem Cell Science and Clinical Translation, 2016).

While these guidelines set the standards for the professions in stem cell research, their formal jurisdiction is limited, as guidelines do not supersede local laws and regulations. However, they can inform legislation and policy making and provide guidance for good research practice if no regulation exists.

Ethical concerns also arise when research teams generate brain organoids using iPS cell lines derived from anonymized or de-identified tissues samples procured from tissue banks. At this time, it is not a standard practice that the informed consent for tissue collection used by most tissue banks actually discloses to tissue donors the possibility that their biological specimens could be used for iPS cell derivation and use in general, and much less to generate brain organoids. It is currently unknown whether tissue bank donors approve of the use of their biospecimens for brain organoid creation and their subsequent use for nearly limitless future applications, as this is a very recent application and data on donor preferences and objections are lacking. The main ethical concern here is that, while donors' tissue samples can be anonymized or de-identified by a tissue storage facility, it cannot be assumed that tissue donors have given their consent for their participation specifically in brain organoid research. Unless and until tissue banks inform their donors of this potential specific use of their biospecimens, no conclusions can be drawn as to the consent of the people who donated samples for research.

Finally, it remains an ethical challenge across all forms of biomedical research to avoid giving the impression to cell and tissue donors that they or anyone they know could benefit directly from donors' research participation. Researchers should avoid hyping the potential benefits of brain organoid research, and biomaterials donors' understanding should be assessed frequently during the consent process. (Guidelines for Stem Cell Science and Clinical Translation, 2016).

2.3. Theme 3: translational delivery

Brain organoids hold the promise of accelerating discoveries that could lead to novel clinical therapies and diagnostics in the future, but translation is still at the early stage of delivery. The availability as such of new models for brain research can be seen as the first instance of translational delivery, as brain organoids are enabling research that, due to the inaccessibility of the living human brain, has not been possible before (Farahany and Greely, 2018).

There are several ways to model the brain and a recent article by DeGrazia (2009) on the application of brain organoids to clinical problems, provides a comparison of various model systems that are being used to study brain disorders. Brain organoids have clear advantages over the traditional 2-D planar culture systems, over conventional animal models (not including chimera models), and over isolated non-living human brain tissue. While rapid progress is being made, brain organoid models currently still have major limitations because of their developmental immaturity, small size, and the lack of certain cell types. However, approaches for vascularization are being developed that will allow for more mature and larger brain organoids, and missing cell types are increasingly being added through improved methods of stem cell differentiation. Also, neural activity has been demonstrated by Giandomenico and Lancaster (Giandomenico et al., 2019) in a novel brain organoid model where an *in vitro* mouse spinal cord was innervated through the brain organoid, resulting in contraction of connected muscle cells.

These developments are highly relevant for ethics. With increased size, extended viability, and evidence of certain neural activity, these model systems are becoming more realistic, that is, they are increasingly perceived as an instance of the human brain – the brain being the organ most often regarded as key for moral status. This means that with ongoing advances in brain organoid development, a threshold may be reached where questions regarding how to handle these models emerge: what are criteria for continuing or stopping studies, what detection of neural activity could be regarded as morally relevant, and how should brain organoids be disposed of at the conclusion of a research project (Boers and van Delden, 2016)? We will return to some of these issues below when we address consciousness and moral status.

A broad range of neurological disorders has meanwhile been modeled in brain organoids. Chen and Song (2019) discuss Zika virus-associated microcephaly, autism spectrum disorders, and also the possibilities for modeling brain tumors, e.g. glioblastoma multiforme that could be studied in specific glioma organoids representing the tumor heterogeneity that is typical for gliomas (currently a big challenge for therapies). Patient-derived glioma organoids could thus allow for personalized therapy approaches. An even more extensive overview of the translational potential of brain organoids is found in Wang (2018) on the modeling of neurological diseases through brain organoids.

It is important to note that many of the use cases in the reviews by both Chen and Song (2019) and Wang (2018) are described in terms of "would," "could," "might," "in the future" and the like. The translational opportunities that are outlined are clearly significant, but many applications are not realized yet. Currently available applications include disease modeling in microcephaly, in Zika virus-associated microcephaly as well as in three other forms of microcephaly that are not caused by viruses but by genetic mutations.

Disease modeling has also been successful in macrocephaly. And the mechanism of a particular genetic defect (DISC1/Ndel1) was demonstrated in a brain organoid derived from a patient with schizophrenia. In several rare disorders, brain organoid models have led to the understanding of causative mechanisms. Wang (2018) mentions Rett Syndrome, Miller-Dieker Syndrome, and Sandhoff disease. In other more common disorders, the potential of brain organoids is clear, but there have not been concrete models yet. This is the case for a number of common neurodegenerative diseases, and so far there has been no faithful organoid model established for Alzheimer disease, Parkinson disease, and Huntington disease – but, based on the literature available in 2018, Wang (2018) notes that expectations are high for these diseases. While progress in the understanding of rare disorders is recognized - and celebrated - by the relatively few patients and families affected by those diseases, reports of progress, whether true or not, in devastating neurodegenerative disorders that affect millions of people have far-reaching effects on persons at risk for such disorders. For example, understanding the mechanisms of Alzheimer disease and developing a cure or prevention would not only have major impact for individuals but also for society and the health care system. Therefore, as brain organoid research holds the potential to contribute to such impactful developments, the stakes are high.

In some other areas of application, advances in organoid research have already shown the potential of these model systems to contribute to personalized medicine. A concrete example exists in the treatment of cystic fibrosis (CF) where individual response to the novel and very expensive drug ivacaftor (marketed as Kalydeco) has been successfully tested in gut organoids derived from a particular patient, with positive drug response in the organoid being accepted as an indication for therapy with this drug and justification for insurance coverage (Dekkers et al., 2013). This proof of the utility of organoids as personal pharmacogenomic testbeds in the case of CF gives rise to hopes that patient-derived brain organoids might enable lower-risk testing and eventually lead to effective personalized drug treatments for neurodegenerative and neuropsychiatric disorders.

Personalized therapies, however, come with an inherent ethical problem, as such therapies by definition cannot be tested in large cohorts following the standard pathway of a multi-phase clinical trials process. How can one know whether the drugs that have been tested in patient-derived brain organoids will be safe to use in that individual, where it will affect the whole person? This is even more risky when organoids consisting of brain components might be used for implantation into the patient's brain. Initially, these first-in-human uses are only ethically justifiable as interventions of last resort, where any benefit that can be obtained outweighs the potential risks.

In the case of organoids, a substantial part of their application is *in vitro* research, e.g. organoids as a pharmacogenomic model or as a model to understand disease mechanisms. There is no risk of physical harm for anyone at that point, but risk may arise when the *in vitro* findings are applied to the patient. This holds true for findings from

individual-derived organoids and the application to that individual, as well as for generic findings from organoid experiments more broadly used for therapies.

Current ethical criteria for the evaluation of study protocols may not sufficiently apply to organoid research or novel engineered cell therapies in general. Not only new study protocols, but also new approaches for risk–benefit assessment may be needed. Generic use of organoids as models – or for *in vivo* therapies – raises issues related to biobanking, valid donor consent, privacy protection (with the use of biological materials absolute anonymity and privacy cannot be guaranteed), ownership and intellectual property, patenting, and commercialization (Boers and van Delden, 2016; Wang,). These ethical issues are broadly applicable to many areas of bioscience, and it remains to be seen if organoid-specific criteria need to be developed. A key question is whether criteria for brain organoids should differ from those for organoids representing other human tissues.

2.4. Theme 4: animal research

Before brain organoids or brain organoid-derived cells and tissues can be used for therapies in humans, their implantation and integration will be tested in animals. There is a long history of using of animal models in both basic and translational research. As organoid and brain organoid research matures, the capacity for using exclusively human organoid models in lieu of animal testing holds the promise to further reduce, though not fully replace, animal research (Bredenoord and Clevers, 2017). This has potential ethical implications for both organoid and animal research. Despite how far organoid models advance, it is unlikely that the need for testing in a living system will ever become fully obsolete.

Currently, testing of new pharmaceutical agents or compounds remains a required step in translational and clinical research is regulated by the United States Food and Drug Administration (FDA). Prior to first-in-human testing, new agents must be tested in animal models for toxicity and efficacy (Bredenoord and Clevers, 2017). There are limitations on testing in animals. Animal models can fail to fully mimic the agent's effects in humans, due to variability between humans and rodents in terms of metabolism, physiology, and lifespan (Bredenoord and Clevers, 2017).

Responsible research with animals is highly regulated in the United States; there are more regulations regarding the care, treatment, and housing of animals than there are regulations regarding research with human subjects. The regulatory philosophy behind ethical research with animals focuses on designing meaningful research with animals using replacement, refinement, and reduction.

Replacement challenges investigators to consider if alternative experimental designs, such as computer modeling, could be used in lieu of animals. Refinement and reduction focus on ways to limit pain or discomfort to the fewest number of animals.

Despite the regulations, together with the replacement, refinement, and reduction principles, there remain lingering questions about whether the ethical use of animals in research is ever justified (Knuttson and Munthe, 2017; Bonnet and Shine, 2002; DeGrazia, 1991; DeGrazia, 2009; Bradshaw, 2010; DeGrazia, 1999). Animals are sentient beings with the capacity to experience pain and discomfort. During the last few decades, there have been increased calls for movement away from or increased prohibitions on certain types non-human primate research (Bradshaw, 2010). There has also been more concern from the public and animal rights groups related to the use of species of companion animals, focused on dogs and cats, in research.

Most research with animal models focuses on rodents, typically mice or rats. The rodent models provide a sufficient and efficient way to both observe typical development and physiological function, as well as create modified rodent models to approximate diseased states (Bredenoord and Clevers, 2017).

The first transfer of human brain organoids into the brains of adult mice was reported by Fred Gage's team in 2018 (Mansour and Gonçalves, 2018). Since brain organoids lack the vasculature, microenvironment, and neuronal circuits that exist *in vivo*, researchers engrafted 40–50 day-old human brain organoids into immunodeficient mice and observed them for 0.5–8 months to see if any of these missing aspects could be established. The organoid grafts showed good integration, vascularization, and survival in their *in vivo* environment. Gage and colleagues further demonstrated that human brain organoids could integrate and form progressive neuronal differentiation, maturation, gliogenesis, integration of microglia, and axon growth into multiple regions of the mouse host brain. Optogenetic control of the grafts suggested that synaptic connectivity was established between the organoids and their host brains. Finally, the team assessed the spatial learning abilities of the grafted mice in comparison to ungrafted mice using the Barnes maze. There were no observed differences between the two groups, although the grafted mice did not perform as well as their controls when tested for spatial memory. There seemed to be no other observed ill effects (or any benefits) conferred to experimental mice by human brain organoid engraftment.

The overarching scientific rationale for this brain organoid engraftment study was to enable the eventual study of the pathogenesis of neurodevelopmental, neuropsychiatric, and neurodegenerative disorders (and perhaps preclinical drug testing) under physiological conditions of the host animal using human brain organoids derived from patient-specific iPS cells. An important counterweight to the hoped-for scientific benefits of brain organoid engraftment research are ethical concerns about animal welfare and the unknown effects that acute neurological chimerism may have on animal models, especially those that are larger and more complex than rodents.

In 2007 the ISSCR Ethics and Public Policy Committee issued ethical standards for stem cell- based human-to-animal chimera research (Hyun and Taylor, 2007); and these recommendations remain relevant for human brain organoid engraftment studies like the one performed by the Gage team. Any time human stem cells or their direct derivatives are integrated into the central nervous systems of laboratory animals, stem cell oversight review must take place – building on and remaining consistent with animal welfare principles, but with added stem cell specific expertise to consider the further developmental effects on animal welfare of human-to-animal chimerism. Past experience with genetically-altered laboratory animals has shown that reasonable caution might be warranted if genetic changes carry the potential to produce new behaviors and especially new defects and deficits. Best practices dictate that research involving genetically-modified animals must involve the following: (1) the establishment of baseline animal data; (2) ongoing data collection during research concerning any deviation from the norms of species-typical animals; (3) the use of small pilot studies to ascertain any welfare changes in modified animals; and (4) ongoing monitoring and reporting to oversight committees authorized to decide the need for protocol changes and the withdrawal of animal subjects.

Aside from animal welfare concerns and the unpredictable effects that the chimeric grafting of human brain organoids may have on the neurological functioning of laboratory animals, there may be other concerns related to the possibility that a uniquely "human-like consciousness" may emerge in neurological chimeras and that this could raise worries about the enhanced moral status of these chimeric animals (Streiffer, 2005). We address concerns about consciousness and moral status in the following section.

2.5. Theme 5: consciousness and moral status

As brain organoid research advances toward more complex models of mature human cortical regions and their natural *in vivo*-like interconnectedness, some individuals may worry that brain organoids – or more fittingly, complex brain assembloids that combine organoids of multiple cell lineages – may become capable of supporting consciousness (Lavazz and Massimini, 2018). While we believe this consequence

is extremely remote at best, supporters of this concern may attempt to bolster their worries by pointing to a report by Alysson Muotri's group which suggests that six month-old human cortical brain organoids display electroencephalogram (EEG) activity patterns that resemble the electrical activity seen in 25–39 week-old premature infants (Trujilllo and Gao, 2019) – humans with normal brains that can become conscious under the right conditions.

This concern over the possible emergence of consciousness should appropriately motivate a cautious approach to advancing brain organoid research. However, we acknowledge that several important considerations provide good reasons to resist overemphasizing this ethical concern at this time.

First, with respect to the aforementioned study, it is not possible to determine whether the Muotri team's organoids' brain waves are doing exactly the same thing as the brain waves found in premature babies. Currently, too little is known about how babies' brains are actually wired to make solid comparisons between organoids and naturally developing human brains *in utero* and neonatally. (Reardon, 2018) Second, the neural correlates of consciousness – at least those that set the minimal neural mechanisms to support specific conscious precepts – are believed to be distributed across large and diverse anatomical regions of the cerebral cortex and involve multiple cell types. A recent neuroscience review of the peer-reviewed literature suggests that the minimal neural correlates of consciousness are primarily relegated to posterior cerebral cortical regions that include the sensory areas (Koch and Massimini, 2016). These findings were derived from studies involving neuro-imaged participants who could speak about the presence and quality of their conscious experiences. Extending these findings to patients with severe brain injuries, fetuses, newborns – and, we would add here, to brain organoids and assembloids – is very challenging and can only be based on quite tentative inferences at this time.

Third, the term "consciousness" is ambiguous across several possible meanings (Dehaene, 2014); and which particular meaning is presupposed in people's concerns about brain organoid consciousness could make a big ethical difference. If by "consciousness" one means the most basic neuronal activity in a cortical region upon stimulation (what one might call pre-conscious sensory stimulation without subsequent subjective awareness of the sensory input), then this would appear to be ethically innocuous. This is basic brain mechanics. Such mechanisms are precisely what some brain organoid researchers are aiming to model, for example, with respect to cerebral organoids representing the visual cortex. But if by "consciousness" one means something much more complex – for example, in ascending order: conscious access to sensory stimulation; wakefulness; vigilance; focal attention; sentience; and lastly, subjective self-awareness – then the ethical stakes might indeed be raised, although, in our opinion, in inverse proportion to the scientific likelihood that these other forms of consciousness could emerge in brain organoids and assembloids. Each of these more complex conscious states requires, at minimum, the global integration and activation of cortical neurons across long distances and involving multiple brain regions simultaneously (Dehaene, 2014). Brain organoids and assembloids lack this complex network structure, the full complement of cell types, and the sensory inputs necessary to give rise to any discernable subjective experiences.

Lastly, the fact that human brain organoids are derived from human-sourced cell lines may be unduly prejudicing peoples' concerns about the moral status of these *in vitro* models. If, for the sake of argument, complex mouse brain organoids were somehow made to exhibit conscious access to sensory stimulation, or wakefulness, vigilance, focal attention, or sentience (with the appropriate artificial inputs), it is unlikely that people would ethically object to the use of mouse organoids – at least to an extent that would exceed what they typically tolerate for the use of live mice in biomedical research.

Why would human brain organoids displaying comparable levels of "consciousness" be more ethically problematic than neuronally-equivalent mouse organoids with respect to their moral status as research tools? Perhaps the difference maker is that, in the public's imagination, it might be supposed that human brain organoids somehow exhibiting these "lower" forms of consciousness could, under the right circumstances, instantiate the (much more) morally-significant property of conscious self-awareness.

It is worth noting that a similar concern seems to underlie worries about acute neurological chimerism mentioned in the previous section. There the chief worry appears *not* to be that human-to-animal neurological chimeras could gain conscious access to sensory stimulation, or wakefulness, vigilance, focal attention, or sentience through their chimerism – host animals already possess all of these mental capacities. Rather, the lingering ethical concern with chimeras, and here with brain organiods maintained *in vitro*, is that these chimeras and organoids could somehow gain the additional and morally significant characteristic of subjective self-awareness: i.e. a conscious awareness of oneself as a temporally-extended being with experiences, beliefs, and interests, all of which can be mentally reflected upon by oneself. However, as one of us (I.H.) has argued elsewhere, this most complex form of consciousness – that which forms the very basis of the moral life of humans – can only be realized within nurturing social environments and through the acquisition of language that would enable one to have propositional belief systems and reflective beliefs about one's own beliefs (Hyun, 2013). Not even 100% natural human brains found in neonates can develop into recognizably human minds unless they are given the right interactions and social development necessary for their full realization over the span of several years. Since the social support and language-use conditions necessary to support human consciousness in this most robust sense are absent from the laboratory conditions within which neurological chimeras and brain organoids are created and maintained, the threat of conscious self-awareness does not appear to be a serious ethical challenge for biomedical research using either of these types of experimental tools.

3. Concluding thoughts

In this review of the ethical issues related to human brain organoid research, we explored concerns surrounding research oversight, human biomaterials procurement and donor consent, translational delivery, animal research, and organoid consciousness and moral status. Each of these areas poses challenges for researchers, bioethicists, regulators, research institutions, and tissue banks to work through together, building on their past experiences with stem cell research, ethics, and policy, but adapted accordingly to new aspects of brain organoid research. The most practical and productive way forward, we believe, is for these various constituents to work together as the research progresses and takes on new directions. One example of such an integrated approach is to encourage brain organoid researchers and bioethicists to collaborate at the benchside to identify in a bidirectional manner emerging ethical issues in real time during the lifecycle of new protocols. Bioethicists who collaborate in this manner with brain organoid scientists can also act as valuable conduits for facilitating productive dialogue with research review boards and institutional entities, such as the tissue banks that provide the biomaterials to generate brain organoids and the biobanks that may become involved in their future storage and dissemination to other research teams. The ethical issues surrounding brain organoid research today are many, but we think are quite manageable, especially if the various parties work together early and often.

Looking ahead, we encourage leaders in this field to think proactively about issues of social justice and the fair distribution of the downstream benefits of brain organoid research. This is a promising new field of stem cell science, built on the shoulders of human biomaterials donors (most of them patients) and the research teams that had to struggle through the politics of human embryonic stem cell research using non-Federal funding mechanisms. What justified all these past efforts was the future promise of broad social benefit. Brain

organoid researchers and other thought leaders in this field thus have a moral obligation to see to it that the fruits of everyone's labor become reasonably accessible to patients in need.

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