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Publication Date

2023-07-01

DOI

10.1016/j.semcdb.2022.03.020

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Seminars in Cell and Developmental Biology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Seminars in Cell and Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Brain organoids, consciousness, ethics and moral status

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ARTICLE INFO

Keywords:
Brain organoids
Consciousness
Ethics
Stem cells
Brain oscillations
Moral status

ABSTRACT

Advances in the field of human stem cells are often a source of public and ethical controversy. Researchers must frequently balance diverse societal perspectives on questions of morality with the pursuit of medical therapeutics and innovation. Recent developments in brain organoids make this challenge even more acute. Brain organoids are a new class of brain surrogate generated from human pluripotent stem cells (hPSCs). They have gained traction as a model for studying the intricacies of the human brain by using advancements in stem cell biology to recapitulate aspects of the developing human brain *in vitro*. However, recent observation of neural oscillations spontaneously emerging from these organoids raises the question of whether brain organoids are or could become conscious. At the same time, brain organoids offer a potentially unique opportunity to scientifically understand consciousness. To address these issues, experimental biologists, philosophers, and ethicists united to discuss the possibility of consciousness in human brain organoids and the consequent ethical and moral implications.

1. Is this brain organoid conscious?

Brain organoids, a new class of brain surrogates, have gained traction as a model for studying disease of the human brain by using advancements in stem cell biology to recapitulate aspects of the developing human brain *in vitro*. Brain organoids generated from human pluripotent stem cells (hPSCs) offer a means to study human disease. Recent observation of nested, non-random, electroencephalogram (EEG)-like signals from these organoids raises the question of whether further research developments could produce brain organoids that are conscious [1]. These EEG oscillatory waves are ubiquitous in all human living brains but were never before recorded from any human-made *in vitro* system. We discuss the associated ethical and moral concerns.

Consciousness is defined here as any subjective phenomenal experience. Examples of phenomenal experience include the experience of external-triggered sensory percepts, internally-generated body-centered percepts, emotional awareness, self and thought, memories, future planning and dreams. Not all forms of consciousness are of equal normative importance. For instance, experience of sensory input alone may be of less moral significance than the experience of pain and self-awareness. Furthermore, defining consciousness and developing reliable means for detecting it remain a challenge for philosophers and neuroscientists alike. The possibility that brain organoids may develop forms of consciousness that render them entities of moral concern, coupled with the present difficulty of detecting such forms of consciousness, raises questions about how to continue this research in an ethically responsible fashion. While theories of consciousness are debated, scientists are improving experimental models to study human brain development, evolution, and disease pathogenesis. As promising as these experiments are, studying the living brain - without invasive and potentially unethical procedures performed on humans - has generally either been limited to the use of *ex vivo* brain tissue or *in vivo*

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https://doi.org/10.1016/j.semcdb.2022.03.020
Received 4 January 2022; Received in revised form 14 March 2022; Accepted 14 March 2022
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experiments in closely related animal species.

To address rising concerns about the potential for consciousness in brain organoids, a conference was organized at the Sanford Consortium for Regenerative Medicine in La Jolla, California, in October of 2019 to bring philosophers, neuroscientists, ethicists, and the general public together to discuss the implications our current understanding of consciousness might have for the development of functional brain organoid models (**link 1**: https://www.uctv.tv/stem-cell/stem-cell-ethics/). While there are several published discussions of consciousness in brain organoids, and an even larger literature on the ethics of brain organoids, our goal is not to review this literature. Instead, our aim here is to synthesize the discussion and incorporate the results of our meeting.

2. Human brain model systems

Human brain organoids generated from induced hPSCs are emerging as a scaled-down, three-dimensional model of the human brain, mimicking various developmental features at the cellular and molecular levels [2–14]. Human brain organoids have become an essential tool in research, advancing discoveries regarding the development, diseases, and evolution of the human brain[15–17].

Brain organoids can be generated by non-patterned or patterned protocols [17]. Non-patterned differentiation methods rely on the spontaneous morphogenesis and intrinsic differentiation of hPSCs to generate cerebral structures with the most freedom for self-organization. Non-patterned organoids result in a heterogeneity of discrete, though interdependent, brain regions that arise from the stochastic nature of the hPSC differentiation [3]. By contrast, patterned differentiation methods, first pioneered by Sasai's group [18], use external factors and small molecules throughout the differentiation process to guide cell fates more representative of specific and localized brain regions [19–27]. Both protocols can generate laminar structures that mimic the developing neocortex, a region strongly linked to consciousness in humans. Both protocols have limitations intrinsic to the *in vitro* model, including the reduced number of cell types, and lack of non-neural tissues, such as the vascular system [17,28].

Until recently, there was no evidence that these brain organoids could develop sophisticated functional neural network activity resembling the early stages of human brain formation *in vivo* [11,26,29]. Moreover, researchers could not determine whether the bioelectrical activity in brain organoids is a suitable model for developmental normal neural network dynamics [30,31]. However, recent improvements to generate patterned "cortical" organoids exhibit consistent increases in the local field potential (LFP), measured by external electrodes, over several months [1]. Importantly, nested neural oscillatory waves were detected, indicating that some network features observed in premature-newborn brains can be recapitulated in such an *in vitro* system. Although the authors use different acquisition systems, they compared general features of the same electrophysiological entity (LFP). The comparison of different properties of the LFP events is feasible and reasonable, even when acquired using different systems. For example, it was already reported that simultaneous MEA and EEG seizure recordings share common features in the EEG frequency range [32]. Moreover, when comparing the MEA oscillatory and EEG features, the authors cautiously removed any comparison of features that would be explicitly affected by the spatial filtering properties of the skull, and focused exclusively on temporal features, such as event frequency. Thus, they made an objective assessment by considering multiple features, in addition to the fact that oscillations in the LFP are regularly compared to EEG features as in other *in vivo* studies [33–35]. This is not a demonstration of functional equivalence between the organoids and the developing fetal cortex but does represent an *in vitro* model that captures some of the complex, oscillatory spatiotemporal dynamics of the human brain. Thus, ongoing evaluation of cortical organoid activity is expanding our understanding of the emergence of network-level neurodynamics.

Increasingly complex organoids as a model for the human brain raise ethical concerns about their capacity for consciousness, and what ethical lines researchers might unwittingly approach or cross in the future. Can the complexity of these artificially-created, self-organizing cortical networks generate the capacity for some modicum of conscious experience? Could a cortical organoid experience suffering, boredom, pleasure, or pain, or be cognizant of its potential for death? What moral obligations do scientists and society have if minimal consciousness were generated in a cortical organoid?

3. The moral status of the human brain organoids

The ethics of organoids are attracting increased attention [36]. For example, a group of scientists and ethicists recently called for a committee dedicated to overseeing the use of human-brain surrogates [37], and a National Academies of Science and Medicine *ad hoc* committee produced a report on the ethical issues in organoid research (**link 2**: https://www.nationalacademies.org/our-work/ethical-legal-and-regulatory-issues-associated-with-neural-chimeras-and-organoids). The public has competing conceptions of what it means to be human, including theological definitions, folk biological definitions and theories based on capacities. The theory of "What it means to be human" directly impacts belief about moral status. Scientists, bioethicists and American law largely use a definition of a human based on capacities, and thus attributes "personhood" (and thus moral status) to any entity that has enough valued capacities or traits [38]. Thus, humans can lose moral status if they lose enough capacities (such as people with brain death). Living wills typically have a list of capacities for which, if lacking, a person does not want to be kept alive. There is an extensive debate in philosophy about the numerous capacities required for personhood, but all scholars' lists include "consciousness" as a baseline requirement where we can begin to consider personhood as a possibility.

We should not only be concerned with an organoid achieving personhood-level moral status. As others have pointed out, a more immediate scenario is that an organoid could reach the moral status we attribute to various laboratory animals (*e.g.* a mouse), and thus require us to treat them like mice and not like an inanimate object [39]. We would not keep a mouse in a jar the size of its body. While the goal of purposely creating consciousness using organoids is not the focus of most investigators, consciousness might unintentionally emerge as protocols and techniques for disease modeling improve. Depending on underlying assumptions, ethical concerns may arise long before human-level consciousness is achieved. The extent to which organoids are conscious and possess other cognitive capacities will be vital in determining their moral standing.

For example, in the future, brain organoids may be employed in the development and testing of analgesics targeting types of pain that currently evade effective treatment. The more analogous the brain organoid is to the relevant human brain structures, the more useful they are likely to be. Researchers may in fact attempt to create neural states in brain organoids that are similar to those in human brains experiencing pain in order to test the effectiveness of new analgesic treatments. Whether or not the brain organoids experience pain as opposed to merely display neurological features similar to a human brain experiencing pain will be important for determining how to conduct such experiments in an ethical fashion. The possibility that a brain organoid might experience pain will be significant, even if we do not afford the brain organoid moral status equivalent to that of a person.

Similar concerns may arise if organoids are used to study more cognitively complex capacities, like personality, memory, and reasoning ability. Given that brain organoids have been touted as valuable tool for studying neurodegeneration, research of this kind is certain to be on the agenda. It is thus possible that brain organoids will be developed to model varieties of consciousness that we take to be more person-like, such as self-awareness. While organoids do not yet exhibit anything like human levels of consciousness, we cannot rule out that brain

organoids will at some point exhibit features that would be considered evidence of human consciousness if observed in the brain of a human.

In addition, while bioethicists and scientists consider disembodied human tissue to lack independent moral status, at least a sub-set of the public considers human tissue to retain a conceptual if not moral link to the actual human from which it originally came [40–42]. This is undoubtedly part of the public concern about organoids. For these reasons and others, consciousness can be considered a threshold condition for moral status.

4. The neural correlates of consciousness (NCC)

In the last quarter-century, scientists moved from data-poor thought-experiments to actively searching for the physical footprint of consciousness in the brain. Specifically, scientists have pursued the minimal biophysical or neural mechanisms sufficient to produce conscious experience, also known as the neural correlates of consciousness (NCC). At the same time, theories of consciousness have been put forth which make testable predictions about such NCC.

Advances in brain imaging and neuroanatomical understanding have given rise to a plethora of proposed NCC, such as synchronized cortical spiking activity with a broad peak around 40 Hz, corresponding to oscillations in the EEG in the gamma band [43]. Neuroanatomical and brain imaging techniques, using clinical data from patients with disturbances and loss-of-consciousness, imply a dominant role for the neocortex, the intralaminar nuclei thalamus, and re-entrant loops in the thalamocortical systems. Lesion studies in human patients have clinically shown robust and causal evidence that cortical tissue is necessary for the emergence of our thoughts, experiences, and memories. Cortical tissue has also been predicted to be sufficient to elicit the intrinsic causal powers, or subjective experiences, necessary to enable human consciousness. Nevertheless, the preponderance of clinical causal evidence, supported by observational neuroimaging and EEG experiments in neurotypical volunteers, supports the hypothesis that these experiences may be dependent on specific networks within the neocortex.

The Global Neuronal Workspace (GNW) theory and the Integrated Information Theory (IIT) are two theories that have gained attention in building theoretical frameworks to explain consciousness in terms of specific cortical regions. Accordingly, if there are specific cortical regions that are more closely associated with consciousness than other regions, there may similarly be brain organoid systems embodying the relevant cellular dynamics and enabling structures necessary to generate a subjective experience.

GNW theory posits that what we subjectively experience as a conscious state, at any given moment, is the global broadcasting and amplification of information across an interconnected network of prefrontal-parietal areas and many distant high-level sensory cortical areas [44,45]. Unconscious processing occurs in parallel in many localized, modular circuits (*e.g.*, in the ventral visual stream). If this processing ignites the global neuronal workspace (at about 250 ms post-stimulus presentation), some subset of information becomes conscious, being broadcasted and sustained by the workspace. Anatomically, the workspace is believed to be comprised of a network of long-range cortical neurons with reciprocal projections to homologous neurons in other cortical areas, distributed over prefrontal (PFC), parietal-temporal and cingulate associative cortices. These neurons, which are thought to be primarily pyramidal cells of cortical layers 2 and 3, are connected through long-range excitatory axons to high-level sensory processing areas, allowing for flexible, domain-general amplification and distribution exchange of information to various cognitive systems.

Integrated information theory (IIT) is based on a phenomenological axiomatic approach. IIT starts from five essential properties that characterize any phenomenal experience to derive five requirements for a conscious system. The physical substrate of consciousness is thought to be the physical substrate that supports the maximally irreducible intrinsic cause-effect structure. IIT introduces a scalar measure for integrated information (ϕ or phi), defined as the maximum of intrinsic, integrated cause-effect power over this substrate. The NCC are the neuronal mechanisms at the appropriate spatial-temporal level of granularity that maximize ϕ across the brain. Based on theoretical and neuroanatomical considerations, a substrate of maximum ϕ is hypothesized to reside primarily, although possibly not exclusively, in the posterior cerebral cortex, characterized by 'pyramid-of-grids'-like connectivity. These regions, including the parietal, occipital and lateral temporal lobes, are referred to as the *posterior hot zone* [46]. One key difference between IIT and the GNW theory is the predicted location of the NCC – in posterior cortical regions for IIT or in the prefrontal cortex for GNW [47,48]. For reviews and criticisms of the IIT, please refer to the following references [49–51].

It is challenging to distinguish the physical constituents of consciousness from the background or enabling factors that must be present for a system to be conscious (such as a beating heart). Although the cerebral cortex seems to be the favorite core structure for consciousness, many physiological processes are necessary to be in place to give rise to consciousness. While enabling structures are necessary, alone, they do not share the unique cortical structural and network behavior to generate consciousness. The thalamus and its specific sub-regions—the reticular and intralaminar nuclei—are additionally thought to be important contributors to the enabling of consciousness, being the gatekeepers of motor and sensory signals to the cerebral cortex. Another enabling factor is the ascending reticular activating system, a collection of more than forty discrete cellular assemblies [52]. Collectively, they regulate sleep and wakefulness, arousal, breathing and heart rate, temperature, eye movements, and other critical functions. Brainstem neurons enable experience but do not provide the content for anyone's experience. Though cortical neurons are the most prominent players, brainstem neurons also contribute by providing the cortex with neuromodulatory substances, such as dopamine, which can affect attentional or reward mechanisms. Thus, assuming the necessary background conditions are satisfied, specific kinds of cortical activity appear to be sufficient to produce consciousness.

5. Enabling consciousness within a brain organoid

Brain organoids can be generated by non-guided or guided protocols [17]. Non-guided differentiation methods rely on the spontaneous morphogenesis and intrinsic differentiation of hPSCs to generate cerebral structures with the most freedom for self-organization. Non-guided organoids result in a heterogeneity of discrete, though interdependent, brain regions that arise from the stochastic nature of the hPSC differentiation.

While better organoid protocols are evolving, optimized for the different brain regions necessary for consciousness, fused region-specific organoids with distinct domain-specific identities are already a reality. For example, scientists have developed a 3D organoid system that fuses two distinct region-specific organoids representing both the developing thalamus and cortical tissue. The resultant fused organoid also includes reciprocal corticothalamic projections [53]. While we are far from reproducing *in vivo* projections, this "assembloid" approach of fusing region-specific organoids can be used to better model circuit properties present in several neurological disorders. This strategy may be improved to incorporate the different brain region-specific organoids, such as a functional cortex [1], a thalamus [53], and the brainstem [54], enabling anatomical and physiological substrates of consciousness, whether intentionally or not. Thus, the scientific pursuit of a better model for disease modeling and research will eventually lead to organoids more likely to be considered conscious (Fig. 1).

However, it is not known, for example, if the potential for NCC is compromised because brain organoids lack supporting structures such as vascularization, meninges, and other cell types of the central nervous system. These internal differences and intrinsic limitations of the *in vitro*

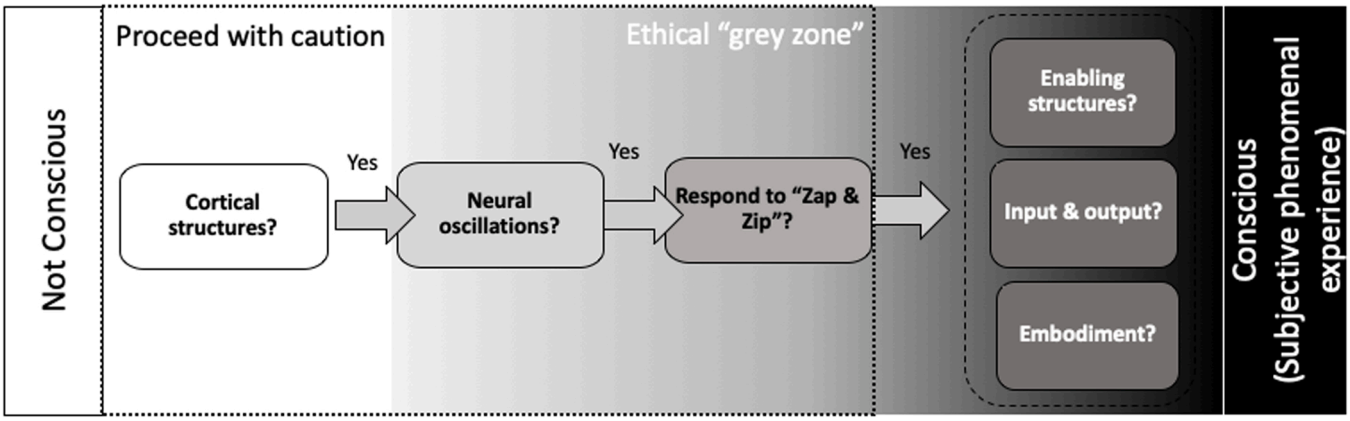

Fig. 1. Decision tree for evidence of consciousness in human brain organoids. The flow chart illustrates major milestones (squares) that are likely necessary to result in a conscious entity. The generation of cortical neural oscillations and the response to "Zap & Zip" warrant caution due to the possibility of dealing with potentially conscious human brain organoids. The more milestones incorporated in the brain organoids, the higher the chances to achieve consciousness. In such a scenario, it would be desirable to have further discussions about the types of consciousness that these brain organoids might be experiencing. In case an investigator reaches any of these milestones, they should at a minimum alert, as appropriate, the Institutional Animal Care and use committee (IACUC) and/or Institutional Review Board (IRB) about the results, and anesthetize the organoids before discarding.

system do affect organoid maturity, size and development, which may affect functionality, such as the capacity to generate consciousness. Is the existing similarity between the primate cortex and organoid cell organization adequate to justify conclusions about organoid consciousness? There is no current basis for a definitive answer. Continued refinement of procedures for growing organoids may well strengthen the case for similarity adequate to justify at least a weak probability claim. It is simply unknown if a perfect cortical, thalamic, and brainstem cellular organization is necessary to generate consciousness, assuming appropriate background conditions. It is plausible that considerable imprecision could still generate a system that feels like something. For example, clearly people can be conscious despite having disorganized or structurally challenged brains (*e.g.*, hydrocephaly, anencephaly, microencephaly). Thus, a human brain organoid does not need to perfectly mimic the neurotypical human brain to achieve consciousness.

Without certainty about what features of a brain organoid would predict minimal NCC, it is worth considering the possibility that brain organoid consciousness could be assessed experimentally, for example, by the Perturbational Complexity Index (PCI). The use of PCI has been previously proposed for human brain organoids [55], although some experimental adjustment might be necessary due to their size. PCI grew from IIT and has been used to distinguish different levels of consciousness in human patients. Briefly, PCI is calculated by repeatedly stimulating or "zapping" the brain with a single magnetic pulse using a transcranial magnetic stimulation (TMS) device placed on the scalp. The effects of the pulse can then be detected by EEG and analyzed or "zipped" with a data-compression algorithm. In fully conscious humans and animals, EEG after "zap-and-zip" reveals prolonged, spreading activity (complexity index), suggesting a reverberating chiming of neuronal activity. In subjects with brain damage, or under anesthesia, the spreading complexity index is limited, or perturbed, resulting in a significantly decreased PCI. This "zap-and-zip test" has been demonstrated as a useful consciousness-meter in multiple clinical studies to measure the spatiotemporal complexity of cortical activity and distinguish between conscious and unconscious states [56]. If brain organoids showed a significant PCI, researcher might be crossing an ethically challenged territory and should proceed with caution towards future experiments. Perhaps that should be a moment of pause for scientists and ethicists to reflect on the future of brain organoid research (Fig. 1).

6. Brain organoid embodiment and experience

A causal or constitutive relationship between the neuronal circuitry and the components of consciousness are two common viewpoints concerning the NCC. However, some scientists argue that consciousness cannot exist divorced of a body, and must be embedded in organismic self-regulation and sensory-motor loops in a complex environment [57]. Even in REM-sleep dreaming, consciousness does not occur entirely independent of sensory stimuli and motor activity; rather, dream imagery often incorporates external sensory stimuli and is linked to bodily self-sampling through muscular activity (*e.g.*, twitching) and vestibular orienting [58]. Nevertheless, whether there can be a minimal form of conscious awareness in the complete absence of sensory input or motor output is unknown. Brain organoid systems have not yet successfully incorporated sensorimotor integration and coupling with the sensorium. Ongoing efforts seek to establish physiological sensory stimuli and motor output for brain organoids. These are ongoing projects that could, ideally, increase brain organoids' maturity [59] and enable the integration of sensorimotor stimuli [60]. However, it is also unknown whether sensory input or motor output, during development if not in adulthood, is required for the human brain to be conscious [51]. Additional ethical challenges will arise once scientists can more effectively integrate brain organoids into live animals [61] or create synthetic autonomous interfaces.

7. Future directions

The possibility that organoids might develop some kind of consciousness is of high ethical and moral importance and certainly merits attention. However, questions about the moral status of brain organoids and the ethical standards governing research are unlikely to have all or none answers. Moreover, research institutions do not normally have a mechanism for addressing questions about human brain organoids. Therefore, it is never too soon for thoughtful dialogue among scientists, philosophers, and the public about the ethical responsibilities of researchers regarding human brain organoids' potential for consciousness. Little can yet be stated with certainty, but we offer two observations that may serve as a useful next step:

1) Based on all we know about the brain basis of consciousness, there is no reason to believe that brain organoids, as of 2022, are conscious in any meaningful sense.
2) It is possible that the rapidly advancing field of stem cell biology could produce brain organoids capable of exhibiting features that, in a human brain, would be considered hallmarks of consciousness.

(Although again, this level of consciousness does not immediately equate to higher moral status.)

To make meaningful progress in developing appropriately nuanced guidelines and regulations, four questions need particular attention:

1) To what extent, if at all, can we find consensus regarding how the presence or absence of characteristics of consciousness with the potential to produce suffering can best be measured?
2) What ethical significance should be given to human brain organoids on the basis that they are human in origin? The answer to this question will have implications for the permissibility of experiments involving chimeras, how tissue ought to be disposed of after experimentation (if at all), and what informed consent procedures need to be followed when acquiring tissues to be used for creating organoids;
3) How should we proceed when there is uncertainty about the ethical standing of a particular organoid? Our ability to reliably and uncontroversially detect cognitive and experiential capacities of organoids is likely to lag behind our ability to construct ever more complex neural organoids. Thus, we propose a series of operational checkpoints to score the potential for a certain organoid to become conscious, in order to assess which milestones should not be crossed in protocols that could unintentionally raise the possibility of consciousness (Fig. 1); and finally;
4) What research guidelines can be adopted that are sufficiently attentive to gradual changes in cognitive and experiential capacities? We propose a "proceed with caution" zone based on incremental advances that might place organoids in an ethical gray zone.

These considerations are essential for moving this exciting scientific field forward in an ethical manner.

Acknowledgements

We would like to thank Drs. Patricia Churchland, Pinar Mesci and Gabriel Silva for their inputs on the manuscript. Dr. Muotri is supported by grants from the California Institute for Regenerative Medicine (CIRM) DISC2-09649, the National Institutes of Health through the R01MH108528, R01MH094753, R01MH109885, R01MH100175, R56MH109587, an SFARI (Simons Foundation Autism Research Initiative) grant #345469, and a NARSAD (National Alliance for Research on Schizophrenia and Depression) Independent Investigator award.

Conflict of Interests

Dr. Muotri is a co-founder and has an equity interest in TISMOO, a company dedicated to genetic analysis and brain organoid modeling focusing on therapeutic applications customized for autism spectrum disorder and other neurological disorders with genetic origins. The terms of this arrangement have been reviewed and approved by the University of California San Diego per its conflict of interest policies.

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Web Resources

link 1: https://www.uctv.tv/stem-cell/stem-cell-ethics/. The UCSD Stem Cell Program and the Institute for Practical Ethics invite you to explore the ethical questions of growing stem-cell-derived human brain organoids and how we as humans define conscious. As the field advances, questions about what these tissues are capable of as they become more sophisticated are now being examined. Join in the stimulating discussion about this technology. Presenting are some of the leading voices in philosophy and neuroscience who will cover topics concerning the different types of consciousness, what it means to be consciousness and when a brain organoid might be considered a sentient entity.

link 2: https://www.nationalacademies.org/our-work/ethical-legal-and-regulatory-issues-associated-with-neural-chimeras-and-organoids. An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine examined the scientific, ethical and regulatory issues associated with neural chimeras and neural organoid research. The committee reviewed the current status of research, considered the benefits and risks of such research, discuss associated ethical issues, and considered what oversight mechanisms might be appropriate in this area. A final report providing the committee's findings was issued at the conclusion of the project and published by the National Academies Press.