RESEARCH ARTICLE

An assessment of the moral value of neuronal cell models and brain organoids [version 1; peer review: 2 approved, 1 approved with reservations]

Sietske A.L. van Till[1], Mariia V. Maksimova[2], Ghislaine J.M.W. van Thiel[2], Eline M. Bunnik[1]

[1]Dept. of Medical Ethics, Philosophy and History of Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, 3015 CN, The Netherlands
[2]Julius Center department of Bioethics and Health Humanities, University Medical Center Utrecht, Utrecht, 3508 GA, The Netherlands

v1 **First published:** 10 Jul 2023, **2**:15
https://doi.org/10.12688/molpsychol.17557.1
Latest published: 10 Jul 2023, **2**:15
https://doi.org/10.12688/molpsychol.17557.1

Abstract

Advances in stem cell technology enable neuroscientists to develop induced pluripotent stem cell (iPSC)-based neuronal models of varying complexity, ranging from single human brain cells to two-dimensional neuronal cell models and three-dimensional brain organoids. While the discussion on the moral status of brain organoids is taking center stage in the bioethical literature and is invariably linked to the presumed capacity of future brain organoids to develop some form of consciousness, analyses of the moral status of other – less complex – iPSC-based neuronal models are lacking. In this paper we aim to clarify the moral value of various types of existing neuronal models, including brain organoids. We show how it is made up of several layers that may encompass various sorts of considerations, including moral values, the results of empirical research, and biological characteristics. We identify four such layers – instrumental, intrinsic, symbolic, and relational – that are relevant for the assessment of the moral value of neuronal models. We demonstrate that it lies not in a capacity to develop some form of consciousness (which is absent in current iPSC-based neuronal models, including brain organoids), but in other considerations, including the genetic links between models and donors, the ability of models to mimic brain (dys)function, and their symbolic value, all of which are often overlooked in the bioethical literature. Also, we demonstrate that the 'thickness' of the layers (i.e., their moral weight) increases when the neuronal model is more complex. Finally, we discuss the practical-ethical implications of our analysis for the use of neuronal models in research settings, for instance in relation to informed consent and biobank governance. Our four-layer framework can be applied also in moral assessments of other iPSC-based models, including emerging and future cell models.

Open Peer Review

Approval Status ✓ ✓ ?

	1	2	3
version 1 10 Jul 2023	✓ view	✓ view	? view

1. **Leonora Buzanska**, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
2. **Robin Ketteler**, Medical School Berlin, Berlin, Germany
3. **J. Lomax Boyd**, Johns Hopkins University, Baltimore, USA
Abigail Cloud, Johns Hopkins University, Baltimore, USA

Any reports and responses or comments on the article can be found at the end of the article.

Keywords
Neuroscience, Organoids, Stem Cell Research, ethics, Bioethics, Brain, Therapeutic development

This article is included in the The Ethics of Brain Organoids collection.

Corresponding author: Sietske A.L. van Till (s.vantill@erasmusmc.nl)

Author roles: van Till SAL: Conceptualization, Investigation, Methodology, Project Administration, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Maksimova MV**: Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **van Thiel GJMW**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing; **Bunnik EM**: Conceptualization, Funding Acquisition, Methodology, Project Administration, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This article is a result of a collaboration of two research projects which are both funded by ZonMw - The Netherlands Organisation for Health Research and Development: 1) 'BRAINMODEL: standardized, IPSC-based medicine for immediate application in monogenic neurodevelopmental disorders', project number 10250022110003 (2021-2027), and 2) 'TAILORED: Towards a human iPSC neuronal platform for neurodevelopmental disorder therapeutic discovery', project number 10250022110002 (2021-2026).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: van Till SAL, Maksimova MV, van Thiel GJMW and Bunnik EM. **An assessment of the moral value of neuronal cell models and brain organoids [version 1; peer review: 2 approved, 1 approved with reservations]** Molecular Psychology: Brain, Behavior, and Society 2023, **2**:15 https://doi.org/10.12688/molpsychol.17557.1

First published: 10 Jul 2023, **2**:15 https://doi.org/10.12688/molpsychol.17557.1

Introduction

Stem cell technology has made significant progress in the past two decades and is now increasingly used in personalized and reproductive medicine research. Scientists can reprogram cells taken from human donors' biological samples to derive induced Pluripotent Stem Cells (iPSCs), and generate specialized human cells, tissues, and organoids with the same genetic profile as their donors. The resulting iPSC-based products can be used for disease modeling and the discovery of innovative (personalized) treatments. In neuroscience, iPSCs enable researchers to model the human brain and investigate the pathophysiological mechanisms underlying neurodegenerative, neurodevelopmental, and psychiatric disorders. In this article we will refer to these stem cell-based entities as neuronal models. IPSC-based neuronal models vary in complexity, ranging from single human brain cells to stem cell-based two-dimensional (2D) neuronal cell models, to complex three-dimensional (3D) self-organizing brain organoids. The developments around neuronal models provoke ethical questions about how they can be used in responsible ways.

In the Netherlands, the so-called Pluripotent Stem cells for Inherited Diseases and Embryonic Research (PSIDER) program has been set up to facilitate research into iPSC technology in the Netherlands. In two PSIDER-funded research projects, iPSC-based neuronal models are used to investigate neurodevelopmental disorders. First, the BRAINmodel project develops 2D neuronal cell models to study the underlying disease mechanisms of rare genetic neurodevelopmental disorders, including Kleefstra syndrome and STXBP1 encephalopathy (Geertjens *et al.*, 2022; https://projecten.zonmw.nl/nl/project/brainmodel-standardized-ipsc-based-medicine-immediate-application-monogenic). More complex neuronal models – 3D brain organoids – are used in the TAILORED project, which focuses on the development of customized antisense oligonucleotide therapy for children with neurodevelopmental disorders, including KCNQ2 encephalopathy and Tuberous sclerosis complex (https://projecten.zonmw.nl/nl/project/towards-human-ipsc-neuronal-platform-neurodevelopmental-disorder-therapeutic-discovery).

Brain organoids, a subgroup of neuronal stem cell entities, have been the subject of special attention in bioethical literature. In the past two decades, brain organoids have come to be seen as morally distinct from other technologies, as having special moral 'status', and as demanding of special conditions for their (responsible) use in research settings (e.g. the setting of limits on chimera research, the development of 'mature' brain organoids, and commercialization of brain organoids) (De Jongh *et al.,* 2022; Greely, 2021; Haselager *et al.*, 2020; Koplin & Savulescu, 2019; Koplin & Massie, 2021; Lavazza & Massimini, 2018). The normative ground for the apparently distinctive position of brain organoids in the bioethical literature, however, has not always been made explicit and is certainly not self-evident. In addition, there is a strong focus on grounding the moral status of brain organoids in their potential (future) ability to develop consciousness (Greely, 2021; Jeziorski *et al.*, 2023; Koplin & Savulescu, 2019; Lavazza, 2019; Lavazza & Massimini, 2018; Zilio & Lavazza, 2023). In contrast, research on the moral status of other – less complex – iPSC-based neuronal models is scarce. Still, in order to develop ethically sound policy for research using iPSC-based neuronal models, it should be clarified to what extent these cell models, too, have 'moral status'.

The term moral status is often reserved for entities that are considered valuable 'for their own sake'. To brain organoids, moral status is attributed mainly because of their presumed potential for developing 'consciousness' or 'sentience'. If brain organoids were to develop consciousness or sentience, they would begin to have interests, for example, an interest not to suffer. Entities that have moral status are taken to engender moral obligations in others, for example, to protect the interests of these entities, or to refrain from unnecessarily harming them. A recent paper argues that consciousness is a sufficient but not a necessary condition for ascribing moral status to brain organoids, as other morally relevant considerations could also provide a basis for the attribution of moral status to brain organoids, such as their human origin and their functional and structural resemblance to the human brain (Zilio & Lavazza, 2023). These other considerations, however, are generally overlooked in the bioethical literature, which is characterized by a binary concept of moral status, in which the presence of (or potential for) consciousness or sentience is seen as the decisive criterium for ascribing moral status to cell-based entities. In the following analysis of the moral status of neuronal models, we aim to clarify the 'moral value' of neuronal models. We prefer the term moral value over moral status to express the view that moral value is a complex and multilayered concept. In our analysis, we use and build on the metaphor of layers introduced by the Argentinian bioethicist Florencia Luna (Luna, 2009).

To bring further the discussion on the moral value of neuronal models, we will focus our analysis on neuronal models that are currently being used in research settings, not on potential future applications of cell technologies that are ultra-complex. We dissect the multiple layers of moral value of various sorts of iPSC-based neuronal models of increasing complexity: from the single cell to 2D and 3D neuronal cell models, including brain organoids. We demonstrate how and to what extent each layer contributes to the moral value of the current range of neuronal models. First, we provide brief descriptions of the biological characteristics of the neuronal models of interest. After that, we will identify the multiple layers of moral considerations that are applicable to this range of neuronal models. Finally, we will discuss the ethical and practical implications of our analysis for neuronal model research.

Current range of neuronal models

To situate our normative analysis in the current state of the art in neuronal model research, we first provide brief descriptions of the biological characteristics of the neuronal models of interest. In our analysis, we distinguish 1D, 2D and 3D neuronal models. It should be noted that this distinction may be

considered a simplified (and possibly even artificial) classification of neuronal models currently in use in biomedical research settings. Yet it serves as a starting point for discussions on the moral value of a broad range of neuronal models.

We will focus on neuronal cell cultures that are derived from iPSCs. As said, iPSCs are generated by reprogramming of somatic cells (e.g., monocytes or fibroblasts) that are taken from blood draws or skin biopsies of human donors. The iPSCs are stimulated by growth factors that lead genes to come to expression that prompt iPSCs to differentiate into mature neurons (Frega *et al.*, 2017). These neurons can be cultured in various ways, resulting in 1D, 2D or 3D neuronal models.

1D neuronal models

In neuroscience research, single neurons can be used to investigate the functional and morphological characteristics of the neuron on a microlevel, for example the membrane potential, ion channels, and spiking activity of the neuron (Vardi *et al.*, 2016). Both physiological (i.e. normal) and pathological neurons can be used for brain modeling to provide information about the neurons' cellular behavior, to test the effects of external stimuli on cellular behavior, and to correlate the activity of the isolated neuron to more comprehensive neuronal networks (Vardi *et al.*, 2016). 1D models can therefore be useful tools in studying disease mechanisms of disorders that find their basis on the cellular level, such as pathologies of the ion channels and other synaptic dysfunctions, which could be related, for example, to epilepsy, autism spectrum disorder, neurodegenerative disorders, and intellectual disability (Linda *et al.,* 2018; Mandolesi *et al.*, 2015; Zoghbi & Bear, 2012).

One approach for the generation of 1D models makes use of so-called astrocyte islands or glia microdot arrays (Meijer *et al.*, 2019). The astrocytes (i.e. glial cells that modulate the communication of neurons) can be derived either from human iPSCs or from animal (usually rat) iPSCs (Frega *et al.*, 2019). On an astrocyte island (i.e. collection of astrocytes), a human iPSC-based single neuron can be placed to help sustain it and allow for its study on an single-cellular level. Once deposited on an astrocyte island, the human neurons are able to grow and live, but unable to multiply. These isolated neurons have the ability to develop ample synapses on themselves to stimulate themselves, and therefore to enable the modeling of synaptic transmission (Bekkers & Stevens, 1991; Meijer *et al.*, 2019). Using patch-clamp electrodes, many synaptic parameters can be measured to characterize the cellular behavior of the neuron, which can ultimately contribute to the generation of hypotheses on disease mechanisms on a network-level and to the development of innovative treatment approaches (Meijer *et al.*, 2019).

2D neuronal model

Neurons can be cultured in a dish, by likewise placing them on an astrocyte-based medium. After a few days, the iPSC-based neurons will start to connect and to develop a neuronal network. These 2D models are self-limiting in size as mature neurons will not, in contrast to their iPSC-precursors, multiply. Depending on the approach that is used, 2D models can be developed in approximately 21 days, and those that are currently used in research laboratories commonly consist of approximately 25.000 to 50.000 neuronal cells (Frega *et al.*, 2017). To fully develop and be kept alive, the 2D model is dependent on frequent renewal of the culture medium for nutrients and growth factors (Frega *et al.*, 2017; Zhang *et al.*, 2013). The culture conditions of the models (e.g. temperature, pH and oxygen-level) should remain stable to allow for reliable measurements (Frega *et al.*, 2017). For practical reasons (i.e. the associated costs and labor-intensity of their maintenance), 2D models are generally only preserved for the time that is necessary to conduct the research experiments of interest. However, it is known that under the right conditions, 2D models can be kept alive for at least 120 days, and the maximum time of survival may be even longer (Habibey *et al.,* 2022).

2D neuronal cell models can show both spontaneous and evoked synaptic activity, i.e., based on external stimuli such as optogenetic simulation (with light) or electrical stimulation with microelectrode arrays, a grid of tens to thousands of microelectrodes that can gather or deliver neural signals. The synaptic phenotypes of neuronal networks can also be displayed, using microelectrode arrays (Pelkonen *et al.*, 2021). Parameters such as the frequency and duration of (network) bursts and the excitation/inhibition balance can be measured in 2D neuronal models to investigate various phenotypes which are associated with, for instance, autism spectrum disorder, schizophrenia, epilepsy, and (rare) developmental disorders (Dolmetsch & Geschwind, 2011; Klein Gunnewiek *et al.*, 2020; Marchetto *et al.*, 2017; Mossink *et al.*, 2021). 2D neuronal models are increasingly used for disease modeling to unravel underlying disease mechanisms and test the efficacy of potential therapeutic compounds (Frega *et al.*, 2019; Klein Gunnewiek *et al.*, 2020; Marchetto *et al.*, 2017). For example, in the BRAINmodel project, 2D neuronal cell models are used to connect network phenotypes with clinical information such as patients' clinical symptoms and EEGs (Geertjens *et al.*, 2022). Thus, 2D neuronal models can assist in overcoming the translational gap between genetic mutations and the heterogeneous clinical manifestations of neurodevelopmental disorders in patients (Dolmetsch & Geschwind, 2011). Furthermore, 2D neuronal models are easily scalable and therefore, they enable researchers to perform drugs screening, on a large scale. This approach can be used to discover personalized treatments that are specifically developed and tested on patient-derived iPSC-based 2D neuronal models. For instance, In the TAILORED project, researchers use neuronal models derived from iPSCs of patients (children with severe neurodevelopmental disorders) to develop and test customized antisense oligonucleotide therapies (https://projecten.zonmw.nl/nl/project/towards-human-ipsc-neuronal-platform-neurodevelopmental-disorder-therapeutic-discovery).

3D neuronal model

Brain organoids, too, are often (but not necessarily) generated out of human iPSCs. The development of 3D models

closely mimics the natural development of the human brain. The development of a brain organoid starts by stimulating iPSCs to form and expand of the three-dimensional aggregates, which usually serve as a common platform to generate specific cell lineages from PSCs (Lin & Chen, 2014). Those aggregates reach the size of 400-600 μm over the course of a week of intensive maintenance with specific cultural media. By day 75 to 90, organoids should have an abundance of mature neurons and grow to 3-5 mm in size (Yakoub & Sadek, 2018). During the process of maturation, the brain organoid forms cortical layering and differentiates into a variety of specialized human brain cell types. This process is often referred to as the self-organizing capacity of the brain organoid. (https://www.stemcell.com/neural-organoid-culture.html#more; Jgamadze *et al.*, 2023). Whereas 2D models can be fully developed within 3 weeks, some 3D model-protocols take up to even 210 days to complete (Mayhew & Singhania, 2022). Brain organoids can be maintained for at least a year (Lancaster & Knoblich, 2014; Sloan *et al.*, 2017).

Researchers may use guided or unguided protocols to develop 3D neuronal models. Organoids developed under unguided protocols contain a variety of cell types related to the forebrain, midbrain and hindbrain, retina, choroid plexus, and mesoderm, which are the same as those observed in human embryos (Camp *et al.*, 2015; Lancaster *et al.*, 2013). The resulting brain organoids are often used to study the natural development of the human brain or to gain a better understanding of how certain disorders develop in individual patients (Lancaster & Knoblich, 2014). However, because the development and structural orientation of the model is minimally steered, these models are, at the same time, heterogenous and less applicable for reliable large-scale drug screening. Scientists can also use guided protocols to develop more predictable region-specific brain organoids that are used to model specific disorders that manifest in specific cell types, such as Zika virus exposure on the forebrain (Zhang *et al.*, 2023; Qian *et al.*, 2016; Qian *et al.*, 2019; Sloan *et al.*, 2017). While such selective 'guidance' results in the right spatial positions of tissue types, it does lack the cell diversity that is necessary to model physiological brain functioning.

Neuronal models can contribute to fundamental research of brain formation by allowing us to observe in vitro the processes that usually eventuate in utero (Arlotta, 2018). Therefore, 3D models are highly applicable in research into disorders that (are considered to be) rooted in the (early) development of the human brain. For instance, iPSC-based 3D models have brought key insights into disease mechanisms of neurodevelopmental disorders, such as microcephaly, autism, and focal epilepsy (Wang *et al.*, 2023).

There have been concerns about the extent to which complex neuronal models could develop forms of consciousness or sentience, following a widely publicized study suggesting that spontaneous electroactivity can be observed in brain organoids that are similar to that observed in preterm babies born between 24 and 37 weeks of gestation (Trujillo *et al.*, 2019). There are different perspectives on what consciousness or sentience means and how it might be measured (Zilio & Lavazza, 2023). However, there is consensus within the scientific community that at current stage of brain organoid research, the higher cognitive functions that would (at minimum) be necessary for an entity to experience any form of consciousness, cannot be developed in current 3D neuronal cell models (Hyun *et al.*, 2022). This consensus is based on several limitations of current brain organoids, including absence of functionally important types of cells that are normally present in the human brain.

Other limitations to the use of 3D models in research settings include non-reproducible heterogeneity within the same batch of organoids (labeled as the 'batch-syndrome') (Lancaster & Knoblich, 2014; Kelava & Lancaster, 2016). Furthermore, the lack of vasculature within organoids severely limits the size of viable organoids, because of insufficient nutrient and oxygen delivery to enable further growth (Kelava & Lancaster, 2016; Sun *et al.*, 2018). As a result, even well-characterized cortical organoids that are region-specific for the neocortex cannot nearly represent its complexity. While cortical organoids can expand at most to about 4mm in diameter, the human neocortex is approximately 15cm in diameter (Qian *et al.*, 2016; Qian *et al.*, 2019). Even though these 3D organoid models are more complex than 1D and 2D models, they are still far from resembling the mature human brain and its functions. Researchers in the field of organoid technology are still looking for solutions to overcome these limitations.

Finally, we have described relevant biological characteristics of neuronal models of varying complexity and shown how these models can contribute to neuroscience research. Still, it should be noted that this whole range of models are highly simplified models of the original human brain, and that research results should always be interpreted with caution. In this stage, research results cannot directly be extrapolated to the clinical context of the donor.

Four layers of moral value applicable to neuronal models

To clarify, and ultimately assess, the moral value of the range of neuronal models used in research settings, we identify and analyze the various considerations that constitute this moral value using the metaphor of layers. The metaphor of layers is used by Luna to explain the complex nature of the concept of vulnerability (Luna, 2009). Luna proposed to consider the concept of vulnerability not as being solid and unique (a fixed label), but rather as dynamic and relational:

> *"The metaphor of layers refers to the functioning of the concept. It suggests that there may be multiple and different strata and that they may be acquired, as well as removed, one by one."* (Luna, 2019)

We believe that this metaphor can assist to clarify the moral value of neuronal models. We do not aim to argue whether or not neuronal models should be granted 'moral status', but instead, we will unravel the layers of considerations that give

rise to the *moral value* of neuronal models. The layer metaphor contributes from both a conceptual and a practical perspective, as, first, it serves in defining how a term or a concept, in this case the moral value of neuronal models, can be understood, and second, it shows how it can be used properly and acted upon (Luna, 2019).

In our analysis we will show how the value of neuronal models is built up by several layers that may encompass various sorts of considerations, including moral values, results of empirical research, and biological characteristics. These considerations can add to and interact with one another within layers, and may lead them to become thicker, and thus to add value to the entity. We identified four layers that should be considered in assessing the moral value of neuronal models; 1) Instrumental layer; 2) Intrinsic layer, 3) Symbolic layer, and 4) Relational layer. Table 1 shows the considerations that constitute these layers. We will now, first of all, discuss these various layers one by one, including their corresponding considerations. Thereafter we will show how these layers can be applied to and be weighted for the different types of models. Lastly, we will reflect on the practical-ethical implications resulting from our assessment.

1. Instrumental layer

Neuronal models have biological characteristics which make them valuable tools in research settings. The instrumentalization of neuronal models may result in scientific progress, patient health benefits, and general healthcare improvements. Neuronal models may therefore assist in achieving important scientific and social goals.

The scientific value of neuronal models on this instrumental layer is rooted in their relation to generating new knowledge that could not be obtained otherwise. It refers to the ability of a study to produce reliable, valid information capable of reaching the stated objectives of the research (Emanuel *et al.*, 2000). First of all, neuronal models are used in fundamental research that aims to better understand the physiology of 'typical' neuronal processes, such as the maturation and self-organizing capacities of neurons. Secondly, the value of neuronal models is mainly emphasized in the context of clinical research: neuronal models can be used to study the origin and disease mechanism of various psychiatric and developmental disorders. Before neuronal cell models became available to researchers, there had been very little opportunity to study brain tissue of individual patients in vitro, as brain cells or brain tissue cannot (easily) be collected from living individuals. This has always limited researchers' opportunities for studying the cellular behavior and network activities of neuronal networks in detail (Kelava & Lancaster, 2016; Quadrato *et al.*, 2016). Through the development of stem cell technology, neuroscientists can now access neuronal cells using merely minimally invasive blood draws from the patient.

The scientific value of neuronal model research does not necessarily increase with the complexity of the models that are used. Instead, it depends on the context in which they are used. Our scientific description of the neuronal models shows that different models can be chosen depending on the research question at hand (Pelkonen *et al.*, 2021). Researchers should choose a model that is most appropriate, and also most efficient and practical for achieving their research aims. Even the most complex 3D models are worthless when they are used in a research project in which the morphological and functional characteristics of the model are not in accordance with those necessary to answer the project's research questions.

The social value of neuronal models refers to the significance of the research results for the well-being of individual patients or health benefits on a societal level. The two ongoing PSIDER-projects in the Netherlands, the BRAINmodel and TAILORED project, aim to contribute to a better understanding of brain disorders and the development of novel treatments using 1D, 2D and 3D models. The targeted patient groups are patients with unmet needs – children with rare (and often severe) neurodevelopmental disorders. Children with developmental disorders can, among all, be affected with serious behavior problems, seizures, speech problems and children's disorder and corresponding care can, as reported by their parents, highly impact various domains of life such as financially, socially, and emotionally (Spindler *et al.*, 2017; Verhage & Sørensen, 2020). Still, for many of these patients, there are no curative or disease-modifying therapies available. In addition, psychiatric disorders, for which neuronal models are often used, are associated with a high disease burden for

Table 1. Four layers giving rise to the moral value of neuronal models.

Instrumental layer	- Social value - Scientific value
Intrinsic layer	- Potential to develop consciousness - Human origin
Symbolic layer	- Representation of the brain as the source of human personhood and the 'self'
Relational layer	- Genetic link with donor - Resemblance with donors' brain functions - Alignment of research aims and personal values

individuals, but also with severe impacts from a global health, economic, and human rights perspective (Collins *et al.*, 2011; Dakić, 2020). If neuronal models can assist in developing more effective treatment options for these patient groups, this could help relieve individual and societal burdens of psychiatric disorders (Zhang *et al.*, 2023). Thus, brain models are instruments that can propagate beneficence and justice (e.g., by increasing equal access to treatment options) for seriously affected patient groups.

The social value of neuronal models depends to a certain extent on their complexity. While 1D models can be used to study the cellular behavior of single neurons, they can only poorly represent brain disorders' complex nature. 2D neural cultures and 3D organoids are often considered preferable for disease modeling and drug screening, because they would better resemble the brain functionally (Pickl & Ries, 2009; Seo *et al.*, 2021). Still, we want to point out that while research using neuronal models has already resulted in potential socially valuable outcomes (Frega *et al.*, 2019; Marchetto *et al.*, 2010; Marchetto *et al.*, 2017; Qian *et al.*, 2016; Samarasinghe *et al.*, 2021), at this point in time, neuronal cell models do not yet have a place in clinical care, and the advances of neuronal cell model research will probably not become widely available to patient groups in the near future. The expected social value brought on by the use of neuronal cell models, even of the most complex models, should therefore be slightly nuanced.

Second, scientific value may not translate into social value per se. To let neuronal cell model research result in social value, it should be ensured that research outcomes contribute to a significant relief of the burdens on patients and their families, and that the aims of research align sufficiently with the perspectives of patient groups. For the past two decades there has been increasing attention to the ethical, legal, and social issues (ELSI) of and stakeholder engagement in research projects, also in research projects using stem cell technologies (Aartsma-Rus *et al.*, 2022). Stakeholder engagement can help in identifying unmet needs and subsequent treatment-prioritizations, and in formulating aims, conditions, and limitations for research projects. For example, in research projects focused on autism, it should be taken into consideration that according to some stakeholder communities, autism is seen as a form of neurodiversity, which should be valued and respected. From this perspective, treatments to 'cure' autism would not result in social value (Barnhart & Dierikx, 2023; Liu, 2017, pp. 394–411). The social value, a significant consideration in the instrumental layer, is thus highly dependent on the context in which neuronal models are used, and do not rely solely on the complexity of the model.

2. Intrinsic layer

In the literature on brain organoids, the moral 'status' of brain organoids is usually equated with their intrinsic value and assumed to result from their potential ability to develop forms of consciousness. However, as we outlined in the introduction, there are other relevant characteristics that can be at play within the intrinsic layer.

The literature on brain organoids highlights sentience or phenomenological experiences as an essential aspect for setting a "*clear* moral threshold" for brain organoids (Goddard *et al.*, 2023). For many scholars, a human brain organoid should be afforded a degree of moral status when it develops some form of sentience or consciousness: if organoids were to experience pain and pleasure, they would have legitimate interests not to be harmed, and should be considered valuable for their own sake (Greely, 2021; Hostiuc *et al.*, 2019; Koplin & Savulescu, 2019; Lavazza & Massimini, 2018; Munsie *et al.,* 2017; Sawai *et al.,* 2019).

As said, neuroscientists agree that currently available brain organoids do not have sentience or consciousness that might lead them to having interests (Hyun *et al.*, 2022). Yet some authors point out that even though current brain organoids may be far from having the potential to develop any form of consciousness, in the future, more complex organoids may arise that actually do have such potential, which can indeed affect their moral value and call for additional ethical requirements for the use of such models in research practice (Greely, 2021; Koplin & Savulescu, 2019; Zilio & Lavazza, 2023). However, in current and emerging neuronal models, consciousness, or even the potential to develop consciousness, does not occur. Furthermore, it is found that the fact that an entity has the potential to develop a morally relevant characteristic (in this case consciousness) is not sufficient to ascribe a layer of value equal to entities that have realized that potential (Koplin & Massie, 2021). Hence, it is not on the basis of such potential that intrinsic value can be ascribed to today's neuronal models.

However, still there might be other considerations that can contribute to the intrinsic layer of the moral value of neuronal models. Some authors consider the 'human origin' of organoids as an important consideration in ascribing intrinsic value to brain organoids (Hostiuc *et al.*, 2019; Zilio & Lavazza, 2023). Brain organoids grown in the laboratory belong to the human species according to genetic and biological criteria. Hostiuc *et al.* consider the human origin of human brain organoids as a non-disputable characteristic as these models consist of human-derived cells (2019, p 120). According to Zilio and Lavazza, human origin is a non-changeable ontological feature of organoids as "*they are grown in the lab from induced pluripotent stem cells taken from an adult human being*" (2023, p 9). Given their human origin, it is argued, brain organoids should at least be treated with the same level of respect as other human or human-derived tissues. This consideration of 'human origin' is applicable to any type of neuronal models derived from human iPSCs.

While, clearly, human tissue does not have the moral value of a person or a subject (Kirchhoffer & Dierickx, 2011) by some authors, it has been placed somewhere on a continuum between human subjects and material objects. For example, Svenaeus refers to human tissue as a "*sobject*", and Boers and colleagues showed how organoids can be attributed both subject-like and object-like values (Boers *et al.*, 2019; Svenaeus, 2016).

However, the current debate on how the intrinsic value of neuronal models should be understood is underdeveloped: can neuronal models really be said to be 'subjects' in any meaningful way, and does 'human origin' really give rise to intrinsic value, or should it rather be seen as a consideration that may contribute to symbolic value or relational value (see below). Also, for instance, it remains unclear whether the intrinsic value of neuronal models should be considered equal to that of other human-derived tissues (e.g., blood samples, skin biopsies, surgical waste), and whether there are reasons – other than their presumed potential ability to develop any form of consciousness – for considering the intrinsic value of brain organoids to be different from that of other organoids, such as gut or pancreatic organoids. In sum, we can conclude that it might be possible to attribute some intrinsic value to neuronal models, based on the human origin of these models, but the extent to which it might, and the arguments that can ground it, should be further scrutinized.

3. Symbolic layer

The third layer of moral value of neuronal models we have identified, is the symbolic layer, which covers sociocultural perspectives on the symbolic value of the human brain and, consequently, of neuronal models.

Symbolic value is often understood as an extrinsic value, not an intrinsic value. People do not necessarily value the entity itself, but rather that which it represents. Symbolic value can be ascribed to signs, language, objects, places, and monuments, and depends on the meanings given to these things by people: it is determined by the context and could thus vary in place and time. Symbolic value could also be at play in the context of neuronal models, and could, for example, be explained as that brain organoids are valuable because they resemble the human brain as an organ that hosts human personhood and the 'self' (Van Till & Bunnik, 2023). As stated, the scientific community agrees that the current range of neuronal models themselves are not capable of developing any form of consciousness, let alone personhood. Still, even if neuronal models do not contain or even model parts of donors' psychological identities or personhood in any reasonable way, they do *represent* the human brain in a way that is deemed valuable by the public.

Until now, only limited empirical research has been conducted on the perspectives of the public and patients on neuronal models. The available empirical studies suggest that patients and general publics might have ethical concerns about the use of brain organoids in research, not only in relation to misuse of organoids (for example when brain organoids are commercialized) or their potential ability to develop forms of consciousness, but also that brain organoids can express aspects of human identity or personality. In an interview study conducted by Bollinger and colleagues in the US, it is reported that patients or parents of pediatric patients find brain organoids *inherently different* from other types of organoids (such as heart or lung organoids), because the brain is characterized, by some respondents, as *"the locus of personhood"* (Bollinger *et al.*, 2021). Also a Dutch interview study of Haselager and colleagues showed that some respondents, including both laymen and patients with neurological or psychiatric disorders, thought that the brain, and so possibly also brain organoids, contains parts of human personality, which makes brain tissue "*more sensitive compared with the scientific use of other types of tissue*" (Haselager *et al.*, 2020). These studies suggest that there may be deeply rooted sociocultural perspectives on the human brain as the source or seat of (moral) reasoning, human behavior, and cognitive functioning, and the 'self'. The perspective from which the brains are considered as the essential source of our 'self' is sometimes referred to as neuroessentialistic thinking:

> "It is not so much that we are not also our genes, our bodies, members of social groups, and so on, but rather that when we conceive of ourselves, when we think of who we are as beings interacting in the world, the *we* that we think of primarily resides in our brains." (Reiner, 2012)

Although different accounts of neuroessentialism exist, its main messages, which is that *we* as human beings, are highly determined by our brains, seems increasingly endorsed by the general public. Human behavior, moral and rational reasoning, and individual identity are often explained by biological processes in our brains. In a context in which the brain is a subject of public attention and deeply valued as the locus our 'selves', it may not be surprising that brain organoids, too, are considered valuable, as they not only model the brain anatomically and functionally, but could also *represent* or *symbolize* the human brain and that what makes us human.

When objects or entities are attributed symbolic value, they deserve to be treated with some level of respect (Steinbock, 2009). The symbolic value of an entity or object can enforce implicit or explicit norms, and even moral obligations that describe how a 'symbol' should or should not be treated (Koplin & Massie, 2021). For example, to some readers, the term 'symbolic value' may be familiar from discussions on the moral status of human embryos. In these discussions, symbolic value refers to the respect that is owed to embryos as they are a symbol of human existence, which entails that they may not be used in 'frivolous' ways (Steinbock, 2009). A similar line of argumentation can be applied to neuronal models, too.

The symbolic value of neuronal models may differ with the extent to which they resemble the human brain. We expect that more symbolic value is attributed to complex 3D neuronal models than to isolated single neurons, as 3D models will more closely resemble the human brain. Also, it is possible that the symbolic value attributed to neuronal models will be affected by the ways in which they are presented to the public: if they are presented as similar, anatomically, and functionally, to the human brain, and, for instance, are called 'mini-brains', this may cause their perceived symbolic value to increase. To assess the symbolic value of neuronal models, we need empirical studies to inform us on the perspectives of relevant

publics on what neuronal models represent for them. Until now, only two empirical studies have been conducted on publics' and patients' perspectives on 3D neuronal models, and none on sociocultural perspectives on 1D or 2D neuronal models (Bollinger *et al.*, 2021; Haselager *et al.*, 2020). Further empirical research is therefore needed to gain a better understanding of the symbolic value of neuronal models of varying complexity.

4. Relational layer

Lastly, some of the moral value of neuronal models lies in their relationships with human donors. The relational layer reflects the personal entanglement of neuronal models with their donors. We distinguish three considerations in the relational layer: 1) neuronal models consist of biological material (i.e., cells) containing the genetic profiles of donors, 2) neuronal models display functions that resemble those of the brain of the donor, and 3) neuronal models consist of biological material (i.e., cells) that reflect donors' personal values.

First of all, neuronal models that originate from human stem cell lines are largely genetically identical to the genetic profile of their donor. Genetic data is a sensitive type of data as it can be related to peoples' personal characteristics, such as their family origin and predispositions to numerous diseases. As every individual has their own unique DNA profile, genetic information derived from biological material can be traced to its donor, even when the sample itself is anonymized. It is often argued that genetic data therefor deserves special protection to protect the privacy of the donor – a view that is sometimes referred to as 'genetic exceptionalism' (Ruiz-Canela *et al.,* 2011). As we noted earlier, the empirical fact that the model consists of biological material that contains the donor's DNA could lead to the attribution of some intrinsic value to the model based on its human origin, but the moral significance of this biological or genetic link could also be understood in relation to the individual donor him- or herself. Neuronal models could be found valuable by their donors as the genetic data they incorporate can reveal *their* personal characteristics. Thus, in the relational layer, based on the genetic link between model and donor, value is contributed to the sum value of neuronal models. This consideration applies even more strongly in the context of research into rare genetic neurodevelopmental disorders, which may afflict or have been diagnosed in only a few patients or families worldwide (Mezinska *et al.,* 2021). Even if information about ultra-rare genetic abnormalities were presented in an anonymized manner, specific individuals or families might be exposed. Furthermore, genetic information relates not only to the donors themselves, but also to their parents, siblings, and children. The protection of genetic data residing in neuronal models therefore serves not only to protect the privacy of the donors, but also that of their family members (Biesecker & Peay, 2003).

Second, neuronal models are often used to simulate neuropsychiatric disorders and/or abnormal developmental physiology in the brain. Several network parameters which can be displayed in 2D and 3D neuronal models, are associated with clinical disorders, including autism, schizophrenia, and bipolar disorder (Quadrato *et al.*, 2016). They may provide additional information about the (dys)functioning of the brain, over and above a genetic diagnosis. In the case of some monogenic neurodevelopmental disorders, for instance, the same genetic mutation can manifest itself in different synaptic and clinical phenotypes (Verhage & Sørensen, 2020). This information can have clinical significance and consequences for the donor. Brain data (i.e. data concerning the neurophysiology and neuropathophysiology of individuals) is often considered one of the most intimate and sensitive categories of personal data, because it can reveal (predictive) information about the mental status, including the cognitive, affective and emotional status, of the individual donor (Ienca & Malgieri, 2022; Minielly *et al.,* 2020; Palermos, 2023). Disclosure of information about (risk of) brain dysfunctioning may have psychological impact on and lead to stigmatization of the donor, more so than disclosure of somatic disease (risks) (Westbrook *et al.*, 1993). Participants in genetic psychiatry research in particular may be concerned about data security and privacy (Lawrence *et al.,* 2016; Rostami *et al.*, 2019). For the donor, neuronal models, and their corresponding brain data, can thus be considered as valuable and worthy of (special) protection.

A third way in which neuronal models relate to people is through their connection to the personal values of donors. Human tissues are described by Svenaeus as *strong-identity-bearing sobjects* (2016). Donors can contribute indirectly to research projects when they donate their biological material, and in that case, the biological material can be considered as a so-called "*stand-in for the values and beliefs of individuals*" (Boers *et al.*, 2019, p 133). By choosing to participate in certain research projects and to withhold from participating in other research projects, participants can support research in a way that they consider meaningful. By donating their tissues to create neuronal models, donors have the opportunity to contribute to scientific and clinical goals that hold personal meaning and align with their personal values. This relational consideration is especially of importance for the informed consent process of research projects. The (potential) donor is informed about the research purposes and provides consent for the use of their biological material for this research project. To respect donors and their personal values, the biological material should only be used for research purposes they explicitly supported.

We have shown that neuronal models may be associated with relational values as they are entangled with donors' genetic profiles, psychiatric and neurodevelopmental (risk) status, and personal values in relation to research. Privacy, non-discrimination, and respect for persons are key ethical considerations within the relational layer. Donors' genetic profiles can theoretically be derived from complex brain organoids or 3D neuronal models just as well as from a single neuron or 1D neuronal model. Consequently, there is equal moral value based on genetic identity in all three neuronal model types. However, information on network phenotypes and the morphologic characteristics of neurons and organoids, which can be associated with clinical psychiatric or neurodevelopmental phenotypes, can be

displayed better in 2D or 3D cell models. This layer of relational value, associated with brain (dys)function, will therefore increase with the increasing complexity of the neuronal model. Last of all, neuronal models derived from donated stem cells can serve as a vehicle of donors' personal values, as through donation, donors can make or withhold contributions to specific research projects in alignment with their personal values. The relational value based on this final consideration does not necessarily increase with the complexity of the neuronal model.

The moral assessment of neuronal models

Now that we have identified these four layers, they can help us to clarify the discussion on the moral value of neuronal models. In Table 2 we provide an overview of the layers, the considerations that apply within these layers, and the extent to which these layers are applicable to the various neuronal models. The table visualizes the relevance of various considerations and the resulting 'thickness' (reflected in the darkness of colors), i.e., moral weight, of the layers. From this table we can deduce that the layers generally become thicker when the neuronal model is more complex. In some respects, 3D neuronal models thus have more moral value than do 1D or 2D models. From our analysis, it is clear that although the majority of the ethical literature on brain organoids is focused on the potential ability to develop a form of consciousness in future brain organoids (labeled as 4D models), the moral value of current neuronal models is made up not of an ability to develop a form of consciousness, for they do not have such ability, but of other considerations, including their genetic links to donors (and their families), their ability to model brain (dys)function, their symbolic value, and the expected benefits of neuronal model research which are often overseen in the literature and stand in need of careful scrutiny.

Now that we have determined the moral value of various types of neuronal models, we will draw out some practical-ethical implications for using neuronal models in research settings. What moral obligations do we owe neuronal models when we use them in research settings? We would like to address five points:

First, researchers may need to understand and be responsive towards sociocultural beliefs about neuronal models. Table 2 visualizes how the symbolic layer of neuronal models is constituted by sociocultural beliefs about the human brain, and how the relational layer, among other considerations, depends on donors' expectations about potential research outcomes and the extent to which these outcomes align with donors' personal values. Given that peoples' perceptions of neuronal models shape their moral considerations, and thereby contribute to the attribution of value to those models, adequate information, and the development of a neutral communicative framework on neuronal model research should be prioritized. For instance, researchers have emphasized the importance of avoiding the label of 'mini-brains' when referring to neuronal models, because it incorrectly suggests that neuronal models contain the same biological and anatomical characteristics as 'real' human brains (Hyun *et al.*, 2022). Presley *et al.* have reported on the risk of dystopian and utopian distortions and misinformation in communication on brain organoid research (Presley *et al.*, 2022). To ensure public support for neuronal model research and sufficient alignment of public (moral) perspectives with the aims and limitations of neuronal

Table 2. Assessment of the layers of moral value of current neuronal models.

	Instrumental layer	Intrinsic layer	Symbolic layer	Relational layer
1D neuronal model	• Social value • Scientific value	• Potential to develop consciousness • Human origin	Representation of the brain as the source of human personhood and the 'self'	• Genetic link with donor • Resemblance with donors' brain functions • Alignment of research aims and personal values
2D neuronal model	• Social value • Scientific value	• Potential to develop consciousness • Human origin	Representation of the brain as the source of human personhood and the 'self'	• Genetic link with donor • Resemblance with donors' brain functions • Alignment of research aims and personal values
3D neuronal model	• Social value • Scientific value	• Potential to develop consciousness • Human origin	Representation of the brain as the source of human personhood and the 'self'	• Genetic link with donor • Resemblance with donors' brain functions • Alignment of research aims and personal values
4D neuronal model	• Social value • Scientific value	• Potential to develop consciousness • Human origin	Representation of the brain as the source of human personhood and the 'self'	• Genetic link with donor • Resemblance with donors' brain functions • Alignment of research aims and personal values

model research, researchers have an important responsibility to be transparent and precise in research communication, and to adequately engage the public in research projects.

Second, from neuronal models, additional genetic or other medical information may be derived on the (future) mental status of the donors. To protect the (sensitive) data inherent in neuronal models, additional measures may be required in responsible biobanking, such as the regulation of the using and sharing of neuronal models between various institutions. Both genetic data and health-related data, including (risk) information pertaining to the (dys)functioning of the brain, are considered 'sensitive' personal data and are protected within Europe under the General Data Protection Regulation (https://www.gdpreu.org/the-regulation/key-concepts/special-categories-personal-data/). It is a matter of debate whether additional sensitive phenotypical information regarding the psychiatric or neurodevelopmental health or risk status of the donor revealed by the neuronal models should be granted additional data protection (Ienca & Malgieri, 2022; Minielly *et al.*, 2020; Palermos, 2023). In any case, careful data and sample governance by research groups and individual researchers is required to prevent (unintentional) disclosure of genetic or psychiatric or other potentially sensitive research results and protect the privacy of donors.

Third, our analysis can be used to help clarify the value of neuronal models in specific cases. An exemplary case is the question whether it is ethically acceptable to commercialize brain organoid biobanks (Boers *et al.*, 2019; Haselager *et al.*, 2020). Commercialization refers to both selling or sharing of biological samples and obtained data with commercial parties, and financial or practical collaboration of scientific institutions with commercial parties (Caulfield *et al.*, 2014). One of the main motivations for donation of biological material to biobanks of academic hospitals and other publicly funded scientific institutions, is altruism or the wish to contribute to scientific improvement (Richter *et al.*, 2021). When these samples are commercialized (e.g. sold to or used by commercial parties), their instrumental value may increase, for instance, when commercial collaborators facilitate the translation of research results into applications for users, such as the development of pharmacological treatments. Yet at the same time, commercialization may take away any control by donors over the neuronal models derived from their donated samples, and it may not be guaranteed that usage of the models will align with the personal values of the donor. A Dutch survey on donors' perspectives (n=12,300) on consent for the secondary use of clinical data and samples for the purposes of research, suggested that the majority of respondents does not support sharing of their donated biological material with parties other than publicly funded scientifical institutions (Patientenfederatie Nederland, 2021). Commercialization of neuronal models could therefor run counter to donors' preferences, and thus conflict with the relational layer of value of the models. Also, the buying and selling of neuronal models might in some ways conflict with their symbolic value and/or intrinsic value (i.e., human origin) (see below).

Fourth, our analysis may serve as a basis for developing recommendations regarding the aims for which neuronal models can or cannot be used. Research projects should ensure that the expected benefits of their research (i.e., the social and scientific value) outweigh the possible risks of research participation for the donor, such as medical risks associated with stem cell collection and privacy risks, and that neuronal models are used to attain social and scientific goals. But also, from the symbolic value of neuronal models, it follows that researchers should ensure that neuronal models are treated with respect and are not used in "frivolous" ways (Steinbock, 2009). Entities that have symbolic value are not precluded from being used, but *if* they are used, this should be done properly:

> "To respect the old-growth forest does not mean that no tree may ever be felled or harvested for human purposes. Respecting the forest may be consistent with using it. But the purposes should be weighty and appropriate to the wondrous nature of the thing." (Sandel, 2004)

As different perspectives exist on the moral duties that we hold towards objects that are attributed symbolic value, we wish to encourage discussion on the implications of this argument (Bortolotti & Harris, 2006; Davis, 2019; Koplin *et al.*, 2022; Steinbock, 2009). For example, the attribution of moral value to neuronal models could require that researchers can only use such models for research purposes that have the potential to yield scientific and social value, such as disease modeling and the development of new treatments. Examples of frivolous usage of neuronal models might include the creation of excess neuronal models that will directly be destroyed, the use of neuronal models for cosmetic purposes, and cowboy science or so-called 'quick and dirty' science. Based on the symbolic value of neuronal models, researchers should refrain from such uses of neuronal models, and should treat the neuronal models with respect.

Fifth and finally, the rapid development of increasingly complex neuronal models can raise ethical questions, for example on the informed consent process (Lavazza & Massimini, 2018). As said, neuronal models can serve as vehicles for donors' identities and personal values. The limited empirical research we mentioned earlier, suggests that patients and laymen may have a wide range of perspectives on brain organoids and the acceptability of certain types of usage of brain organoids (Bollinger *et al.*, 2021; Haselager *et al.*, 2020). For instance, generally respondents support using brain organoids to study disease mechanisms and treatment development but, at the same time, ambivalent perspectives exist on commercialization of organoids and the development of 'mature' brain organoids and connectoids (in which multiple organoids are connected). This is an especially important consideration in developing the informed consent process (Boers *et al.*, 2019; MacDuffie, 2022). Currently most research groups use a broad consent process, in which the donor provides consent for the use of donated material for unspecified current and future research in a specific field (Maloy & Bass, 2020). However, to

respect the donor and their personal perspectives on neuronal model research, more precise informed consent processes may be more appropriate and are sometimes requested by potential donors themselves, in particularly, an informed consent process that ensures that donors are informed about the research projects in which their generated neuronal models will be used, and that donors got the opportunity to withdraw their consent for specific research projects (Haselager *et al.*, 2020).

Conclusions

We have identified four layers of considerations that constitute the moral value of neuronal models: instrumental, intrinsic, symbolic, and relational. Our analysis shows that different considerations apply to different types of neuronal models. Some of these layers are already at stake in 1D models: 1D models contain medical information, both genetic and psychiatric or neurodevelopmental, and should therefore be used in ways that maintain and protect the privacy of the donor. Also, based on their human origin, 1D models should be treated with some level of respect. However, the sum moral value of 1D models is relatively limited as compared to that of 2D and 3D models. Also, the considerations that make up the moral value of 1D neuronal models are not exclusively applicable to neuronal models, as, for instance, other types of human cells, such as gut or heart cells, also contain the donor's genetic information and are of human origin. Thus, 1D neuronal models may need to be treated – at minimum – just like any other human biospecimen.

Furthermore, our analysis suggests that moral value increases with the complexity of the neuronal model, and that more complex neuronal models may be more worthy of ethical consideration than less complex neuronal models. The use of neuronal models yields significant scientific and social benefits, and neuronal models may therefore be considered instrumentally valuable. The more closely a neuronal model mimics (some aspect of) the human brain, the better it can assist, for instance, in understanding the pathophysiology of brain diseases, or the relationships between genetic diagnosis and clinical phenotypes. At the same time, growing complexity of a neuronal model can add to its relational value, as it may reveal more (sensitive) clinical information about donors. Also, more complex neuronal models could provoke more symbolic value as they more closely resemble, and therefore better represent, the human brain that is often considered the source of personhood. However, there is still (very) little intrinsic value in currently available neuronal models.

The field of stem cell technology is rapidly developing, and it can reasonably be expected that in the future, even more complex neuronal models will be available, such as chimeras (organisms composed of cells from two or more species, for example mouses with humanized brains) and assembloids (defined as '*self-organizing 3D cellular systems that result from the integration of multiple organoids or the combination of organoids with missing cell types or primary tissue explants*' (Kanton & Pasca, 2022)); some research groups are developing brain organoids that are connected with neuroprothetics to provide sensory inputs, which raises anew the question whether a potential for sentience or consciousness may emerge and the intrinsic value of such models may increase (Koplin & Savulescu, 2019). Our four-layer framework can be used in the assessment of the moral value of emerging or future neuronal model entities. In these cases, some layers could be thicker/more significant than in the case of current neuronal models, and perhaps even more layers could be identified in relation to these future entities.

Data availability

No data are associated with this article

Acknowledgements

The authors would like to thank Nina Doorn and Anna Bleeck for their input to the scientific description of the neuronal models.

References

Aartsma-Rus A, Vroom E, O'Reilly D: **The Role of Patient Involvement When Developing Therapies.** *Nucleic Acid Ther.* 2022; **32**(2): 118–122.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Arlotta P: **Organoids required! A new path to understanding human brain development and disease.** *Nat Methods.* 2018; **15**(1): 27–29.
PubMed Abstract | **Publisher Full Text**

Barnhart AJ, Dierickx K: **Moving beyond the moral status of organoid-entities.** *Bioethics.* 2023; **37**(2): 103–110.
PubMed Abstract | **Publisher Full Text**

Bekkers JM, Stevens CF: **Excitatory and inhibitory autaptic currents in isolated hippocampal neurons maintained in cell culture.** *Proc Natl Acad Sci U S A.* 1991; **88**(17): 7834–7838.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Biesecker BB, Peay HL: **Ethical issues in psychiatric genetics research: points to consider.** *Psychopharmacology (Berl).* 2003; **171**(1): 27–35.
PubMed Abstract | **Publisher Full Text**

Boers SN, van Delden JJM, Bredenoord AL: **Organoids as hybrids: ethical implications for the exchange of human tissues.** *J Med Ethics.* 2019; **45**(2): 131–139.
PubMed Abstract | **Publisher Full Text**

Bollinger J, May E, Mathews D, *et al.*: **Patients' perspectives on the derivation and use of organoids.** *Stem Cell Reports.* 2021; **16**(8): 1874–1883.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Bortolotti L, Harris J: **Embryos and eagles: symbolic value in research and reproduction.** *Camb Q Healthc Ethics.* 2006; **15**(1): 22–34.
PubMed Abstract | **Publisher Full Text**

Camp JG, Badsha F, Florio M, *et al.*: **Human cerebral organoids recapitulate gene expression programs of fetal neocortex development.** *Proc Natl Acad Sci U S A.* 2015; **112**(51): 15672–15677.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Caulfield T, Burningham S, Joly Y, *et al.*: **A review of the key issues associated with the commercialization of biobanks.** *J Law Biosci.* 2014; **1**(1): 94–110.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Collins PY, Patel V, Joestl SS, *et al.*: **Grand challenges in global mental health.**

Nature. 2011; **475**(7354): 27–30.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Dakić T: **Mental health burden and unmet needs for treatment: a call for justice.** *Br J Psychiatry.* 2020; **216**(5): 241–242.
PubMed Abstract | **Publisher Full Text**

Davis RW: **Symbolic Values.** *J Am Philos Assoc.* 2019; **5**(4): 449–467.
Publisher Full Text

De Jongh D, Massey EK, VANGUARD consortium, *et al.*: **Organoids: a systematic review of ethical issues.** *Stem Cell Res Ther.* 2022; **13**(1): 337.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Dolmetsch R, Geschwind DH: **The human brain in a dish: the promise of iPSC-derived neurons.** *Cell.* 2011; **145**(6): 831–834.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Emanuel EJ, Wendler D, Grady C: **What makes clinical research ethical?** *JAMA.* 2000; **283**(20): 2701–2711.
PubMed Abstract | **Publisher Full Text**

Frega M, Linda K, Keller JM, *et al.*: **Neuronal network dysfunction in a model for Kleefstra syndrome mediated by enhanced NMDAR signaling.** *Nat Commun.* 2019; **10**(1): 4928.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Frega M, van Gestel SHC, Linda K, *et al.*: **Rapid Neuronal Differentiation of Induced Pluripotent Stem Cells for Measuring Network Activity on Micro-electrode Arrays.** *J Vis Exp.* 2017; **1**(119): 54900.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Geertjens L, van Voorst TW, Bouman A, *et al.*: **Following Excitation/Inhibition Ratio Homeostasis from Synapse to EEG in Monogenetic Neurodevelopmental Disorders.** *Genes (Basel).* 2022; **13**(2): 390.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Goddard E, Tomaskovic-Crook E, Crook JM, *et al.*: **Human Brain Organoids and Consciousness: Moral Claims and Epistemic Uncertainty.** *Organoids.* 2023; **2**(1): 50–65.
Publisher Full Text

Greely HT: **Human Brain Surrogates Research: The Onrushing Ethical Dilemma.** *Am J Bioeth.* 2021; **21**(1): 34–45.
PubMed Abstract | **Publisher Full Text**

Habibey R, Striebel J, Schmieder F, *et al.*: **Long-term morphological and functional dynamics of human stem cell-derived neuronal networks on high-density micro-electrode arrays.** *Front Neurosci.* 2022; **16**: 951964.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Haselager DR, Boers SN, Jongsma KR, *et al.*: **Breeding brains? Patients' and laymen's perspectives on cerebral organoids.** *Regen Med.* 2020; **15**(12): 2351–2360.
PubMed Abstract | **Publisher Full Text**

Hostiuc S, Rusu MC, Negoi I, *et al.*: **The moral status of cerebral organoids.** *Regen Ther.* 2019; **10**: 118–122.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Hyun I, Scharf-Deering JC, Sullivan S, *et al.*: **How collaboration between bioethicists and neuroscientists can advance research.** *Nat Neurosci.* 2022; **25**(11): 1399–1401.
PubMed Abstract | **Publisher Full Text**

Ienca M, Malgieri G: **Mental data protection and the GDPR.** *J Law Biosci.* 2022; **9**(1): lsac006.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Jeziorski J, Brandt R, Evans JH, *et al.*: **Brain organoids, consciousness, ethics and moral status.** *Semin Cell Dev Biol.* 2023; **144**: 97–102.
PubMed Abstract | **Publisher Full Text**

Jgamadze D, Lim JT, Zhang Z, *et al.*: **Structural and functional integration of human forebrain organoids with the injured adult rat visual system.** *Cell stem cell.* 2023; **30**(2): 137–152.e7.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Kanton S, Pașca SP: **Human assembloids.** *Development.* 2022; **149**(20): dev201120.
PubMed Abstract | **Publisher Full Text**

Kelava I, Lancaster MA: **Dishing out mini-brains: Current progress and future prospects in brain organoid research.** *Dev Biol.* 2016; **420**(2): 199–209.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Kirchhoffer DG, Dierickx K: **Human dignity and human tissue: a meaningful ethical relationship?** *J Med Ethics.* 2011; **37**(9): 552–556.
PubMed Abstract | **Publisher Full Text**

Klein Gunnewiek TM, Van Hugte EJH, Frega M, *et al.*: **G-Induced Mitochondrial Dysfunction Impairs Human Neuronal Development and Reduces Neuronal Network Activity and Synchronicity.** *Cell Rep.* 2020; **31**(3): 107538.
PubMed Abstract | **Publisher Full Text**

Koplin JJ, Savulescu J: **Moral Limits of Brain Organoid Research.** *J Law Med Ethics.* 2019; **47**(4): 760–767.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Koplin J, Massie J: **Lessons from Frankenstein 200 years on: brain organoids, chimaeras and other 'monsters'.** *J Med Ethics.* 2021; **47**(8): 567–571.
PubMed Abstract | **Publisher Full Text**

Koplin JJ, Skeggs J, Gyngell C: **Ethics of Buying DNA.** *J Bioeth Inq.* 2022; **19**(3): 395–406.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Lancaster MA, Knoblich JA: **Organogenesis in a dish: modeling development and disease using organoid technologies.** *Science.* 2014; **345**(6194): 1247125.
PubMed Abstract | **Publisher Full Text**

Lancaster MA, Renner M, Martin CA, *et al.*: **Cerebral organoids model human brain development and microcephaly.** *Nature.* 2013; **501**(7467): 373–379.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Lavazza A: **What (or sometimes who) are organoids? And whose are they?** *J Med Ethics.* 2019; **45**(2): 144–145.
PubMed Abstract | **Publisher Full Text**

Lavazza A, Massimini M: **Cerebral organoids: ethical issues and consciousness assessment.** *J Med Ethics.* 2018; **44**(9): 606–610.
PubMed Abstract | **Publisher Full Text**

Lawrence RE, Friesen P, Brucato G, *et al.*: **Concerns about Genetic Testing for Schizophrenia among Young Adults at Clinical High Risk for Psychosis.** *AJOB Empir Bioeth.* 2016; **7**(3): 193–198.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Lin Y, Chen G: **Embryoid body formation from human pluripotent stem cells in chemically defined E8 media.** In: StemBook. Harvard Stem Cell Institute, 2014.
PubMed Abstract | **Publisher Full Text**

Linda K, Fiuza C, Nadif Kasri N: **The promise of induced pluripotent stem cells for neurodevelopmental disorders.** *Prog Neuropsychopharmacol Biol Psychiatry.* 2018; **84**(Pt B): 382–391.
PubMed Abstract | **Publisher Full Text**

Liu EY: **Neurodiversity, neuroethics, and the autism spectrum.** In: The Routledge Handbook of Neuroethics. Routledge, 2017; 394–411.
Reference Source

Luna F: **Elucidating the concept of vulnerability: Layers not labels.** *Int J Fem Approaches Bioeth.* 2009; **2**(1): 121–139.
Reference Source

Luna F: **Identifying and evaluating layers of vulnerability - a way forward.** *Dev World Bioeth.* 2019; **19**(2): 86–95.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

MacDuffie KE: **What Brain Organoid Research Can Gain From Engaging Biospecimen Donors.** *AJOB Neurosci.* 2022; **13**(2): 95–97.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Maloy JW, Bass PF: **Understanding Broad Consent.** *Ochsner J.* 2020; **20**(1): 81–86.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Mandolesi G, Gentile A, Musella A, *et al.*: **Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis.** *Nat Rev Neurol.* 2015; **11**(12): 711–724.
PubMed Abstract | **Publisher Full Text**

Marchetto MC, Carromeu C, Acab A, *et al.*: **A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells.** *Cell.* 2010; **143**(4): 527–539.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Marchetto MC, Belinson H, Tian Y, *et al.*: **Altered proliferation and networks in neural cells derived from idiopathic autistic individuals.** *Mol Psychiatry.* 2017; **22**(6): 820–835.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Mayhew CN, Singhania R: **A review of protocols for brain organoids and applications for disease modeling.** *STAR Protoc.* Advance online publication, 2022; **4**(1): 101860.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Meijer M, Rehbach K, Brunner JW, *et al.*: **A Single-Cell Model for Synaptic Transmission and Plasticity in Human iPSC-Derived Neurons.** *Cell Rep.* 2019; **27**(7): 2199–2211.e6.
PubMed Abstract | **Publisher Full Text**

Mezinska S, Gallagher L, Verbrugge M, *et al.*: **Ethical issues in genomics research on neurodevelopmental disorders: a critical interpretive review.** *Hum Genomics.* 2021; **15**(1): 16.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Minielly N, Hrincu V, Illes J: **Privacy Challenges to the Democratization of Brain Data.** *iScience.* 2020; **23**(6): 101134.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Mossink B, Verboven AHA, van Hugte EJH, *et al.*: **Human neuronal networks on micro-electrode arrays are a highly robust tool to study disease-specific genotype-phenotype correlations *in vitro*.** *Stem Cell Reports.* 2021; **16**(9): 2182–2196.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Munsie M, Hyun I, Sugarman J: **Ethical issues in human organoid and gastruloid research.** *Development.* 2017; **144**(6): 942–945.
PubMed Abstract | **Publisher Full Text**

Palermos SO: **Data, Metadata, Mental Data? Privacy and the Extended Mind.** *AJOB Neurosci.* 2023; **14**(2): 84–96.
PubMed Abstract | **Publisher Full Text**

Patientenfederatie Nederland: **Delen van data in de gezondheidszorg.**2021.
Reference Source

Pelkonen A, Pistono C, Klecki P, *et al.*: **Functional Characterization of Human Pluripotent Stem Cell-Derived Models of the Brain with Microelectrode Arrays.** *Cells.* 2021; **11**(1): 106.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Pickl M, Ries CH: **Comparison of 3D and 2D tumor models reveals**

enhanced HER2 activation in 3D associated with an increased response to trastuzumab. *Oncogene.* 2009; **28**(3): 461–468.
PubMed Abstract | **Publisher Full Text**

Presley A, Samsa LA, Dubljević V: **Media portrayal of ethical and social issues in brain organoid research.** *Philos Ethics Humanit Med.* 2022; **17**(1): 8.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Qian X, Nguyen HN, Song MM, *et al.*: **Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure.** *Cell.* 2016; **165**(5): 1238–1254.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Qian X, Song H, Ming GL: **Brain organoids: advances, applications and challenges.** *Development.* 2019; **146**(8): dev166074.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Quadrato G, Brown J, Arlotta P: **The promises and challenges of human brain organoids as models of neuropsychiatric disease.** *Nat Med.* 2016; **22**(11): 1220–1228.
PubMed Abstract | **Publisher Full Text**

Reiner PB: **The Rise of Neuroessentialism.** Oxford Handbook of Neuroethics, 2012.
Reference Source

Rostami M, Dunn LB, Kim JP, *et al.*: **Safeguarding Participants in Psychiatric Genetic Research: Perspectives of Key Stakeholder Groups.** *Ethics Hum Res.* 2019; **41**(6): 12–22.
PubMed Abstract | **Publisher Full Text**

Richter G, Borzikowsky C, Lesch W, *et al.*: **Secondary research use of personal medical data: attitudes from patient and population surveys in The Netherlands and Germany.** *Eur J Hum Genet.* 2021; **29**(3): 495–502.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Ruiz-Canela, M, Valle-Mansilla JI, Sulmasy DP: **What research participants want to know about genetic research results: the impact of "genetic exceptionalism".** *J Empir Res Hum Res Ethics.* 2011; **6**(3): 39–46.
PubMed Abstract | **Publisher Full Text**

Samarasinghe RA, Miranda OA, Buth JE, *et al.*: **Identification of neural oscillations and epileptiform changes in human brain organoids.** *Nat Neurosci.* 2021; **24**(10): 1488–1500.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Sandel MJ: **Embryo ethics--the moral logic of stem-cell research.** *N Engl J Med.* 2004; **351**(3): 207–209.
PubMed Abstract | **Publisher Full Text**

Sawai T, Sakaguchi H, Thomas E, *et al.*: **The Ethics of Cerebral Organoid Research: Being Conscious of Consciousness.** *Stem Cell Reports.* 2019; **13**(3): 440–447.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Seo Y, Bang S, Son J, *et al.*: **Brain physiome: A concept bridging *in vitro* 3D brain models and *in silico* models for predicting drug toxicity in the brain.** *Bioact Mater.* 2021; **13**: 135–148.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Sloan SA, Darmanis S, Huber N, *et al.*: **Human Astrocyte Maturation Captured in 3D Cerebral Cortical Spheroids Derived from Pluripotent Stem Cells.** *Neuron.* 2017; **95**(4): 779–790.e6.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Spindler UP, Hotopp LC, Bach VA, *et al.*: **Seizure disorders and developmental disorders: impact on life of affected families-a structured interview.** *Eur J Pediatr.* 2017; **176**(8): 1121–1129.
PubMed Abstract | **Publisher Full Text**

Steinbock B: **Moral Status, Moral Value, and Human Embryos: Implications for Stem Cell Research.** In: Steinbock B, editor. *The Oxford Handbook of Bioethics*. Oxford: Oxford University Press, 2009.
Publisher Full Text

Sun AX, Ng HH, Tan EK: **Translational potential of human brain organoids.** *Ann Clin Transl Neurol.* 2018; **5**(2): 226–235.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Svenaeus F: **The lived body and personal identity: the ontology of exiled body parts.** In: Malmqvist E, Zeiler K, eds. Bodily exchange, bioethics and border crossing. London and New York: Routledge, 2016; 19–35.
Reference Source

Trujillo CA, Gao R, Negraes PD, *et al.*: **Complex Oscillatory Waves Emerging from Cortical Organoids Model Early Human Brain Network Development.** *Cell Stem Cell.* 2019; **25**(4): 558–569.e7.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Vardi R, Goldental A, Sardi S, *et al.*: **Simultaneous multi-patch-clamp and extracellular-array recordings: Single neuron reflects network activity.** *Sci Rep.* 2016; **6**: 36228.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Van Till SAL, Bunnik EM: **Symbolic Value of Brain Organoids: Shifting the Focus from Consciousness to Sociocultural Perspectives on Resemblance.** *AJOB Neurosci.* 2023; **14**(2): 210–212.
PubMed Abstract | **Publisher Full Text**

Verhage M, Sørensen JB: **SNAREopathies: Diversity in Mechanisms and Symptoms.** *Neuron.* 2020; **107**(1): 22–37.
PubMed Abstract | **Publisher Full Text**

Wang L, Owusu-Hammond C, Sievert D, *et al.*: **Stem Cell-Based Organoid Models of Neurodevelopmental Disorders.** *Biol Psychiatry.* 2023; **93**(7): 622–631.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Westbrook MT, Legge V, Pennay M: **Attitudes towards disabilities in a multicultural society.** *Soc Sci Med.* 1993; **36**(5): 615–623.
PubMed Abstract | **Publisher Full Text**

Yakoub AM, Sadek M: **Development and Characterization of Human Cerebral Organoids: An Optimized Protocol.** *Cell Transplant.* 2018; **27**(3): 393–406.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Zhang Y, Pak C, Han Y, *et al.*: **Rapid single-step induction of functional neurons from human pluripotent stem cells.** *Neuron.* 2013; **78**(5): 785–798.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Zhang Z, Wang X, Park S, *et al.*: **Development and Application of Brain Region-Specific Organoids for Investigating Psychiatric Disorders.** *Biol Psychiatry.* 2023; **93**(7): 594–605.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Zilio F, Lavazza A: **Consciousness in a Rotor? Science and Ethics of Potentially Conscious Human Cerebral Organoids.** *AJOB Neurosci.* 2023; **14**(2): 178–196.
PubMed Abstract | **Publisher Full Text**

Zoghbi HY, Bear MF: **Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities.** *Cold Spring Harb Perspect Biol.* 2012; **4**(3): a009886.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

Current Peer Review Status: ✔ ✔ ?

Version 1

Reviewer Report 13 February 2024

https://doi.org/10.21956/molpsychol.18832.r27313

?

J. Lomax Boyd
Johns Hopkins University, Baltimore, Maryland, USA
Abigail Cloud
Johns Hopkins University, Baltimore, Maryland, USA

The research article from Till et. al. (2023) proposes a novel, and potentially very useful, framework for assessing the moral value of neuronal models. They aim to demonstrate how different kinds of moral consideration can be used to determine the overall moral value of diverse neuronal models, which the authors claim, justifiably, have received significantly less attention than potentially conscious brain organoids of the future. They propose that the moral value of neuronal models, which are identified as 1D, 2D, 3D, or 4D, should be determined from the thickness of four moral layers (instrumental, intrinsic, symbolic, and relational) that represent different sources of moral consideration. The framework provides a scaffold for identifying these factors that reflect the views of donors, researchers, publics, and neuronal models as entities themselves. According to the authors, these considerations are in addition to notions of moral status (founded on consciousness or sentience) that could be raised by 'ultra complex' neuronal models. Although not explicitly mentioned in the article, the 'layer concept' could also provide a helpful conceptual framework for researchers, policymakers, regulators, and ethics committees to capture, and deliberate on, a diversity of views in an anticipatory governance approach toward regulation of emerging neuronal models (10.1080/15265161.2021.2001111[1] and 10.1016/j.biotechadv.2023.108233[2]).

While the layer concept proposed by the authors provides a useful, high-level conceptual framework for identifying factors that contribute to moral value, there are several aspects of the approach that call for clearer argumentation or justification. Our questions primarily focus on principles that group neuronal models, how layer thickness is determined, and the normative implications for decision-making.

First, the authors claim that more complex neuronal models (3D vs 2D, for instance) have greater moral value (p.10) than simpler ones (1D), but do so without clear rationale for how they arrived at these determinations across various the factors that contribute to each layer. Moreover, it remains

unclear what schema is used by the authors to group neuronal models in the first place. What generalized criteria do the authors have in mind for organizing neuronal models into 1D, 2D, 3D, and 4D classifications: morphology or spatial arrangement of cells, complexity of neural network activities, quantity of neurons, presence or absence of external feedback, or other criteria? Neuronal models vary along many dimensions beyond spatial with implications for whether they are considered more or less complex. The authors do provide examples for each group, yet the conceptual/philosophical/empirical framework that led to the proposed classifications could be made more clear. This would be especially helpful when applying the layer concept. For example, should neuronal cell cultures with real-time closed-loop electrical feedback from virtual environments that have been shown to rise to goal-directed learning or criticality (10.1016/j.neuron.2022.09.001[3] and 10.1038/s41467-023-41020-3[4]) be classified as 2D, 3D, or perhaps even, 4D? What about neuronal cultures that exhibit non-negligible PCI scores, which has been proposed as an indicator of consciousness (10.3390/brainsci11111453[5])? Should spheroids and cortical organoids both be considered 3D models despite their internal organization having demonstrated effects on the ability to generate spontaneous nested neural network activities (10.1016/j.stem.2019.08.002[6])? The 4D classification is especially vague. Would Brain-o-ware be classified as 3D or 4D, according to the framework outlined by the authors (10.1038/s41928-023-01069-w[7])?

Second, what method should be used to determine the amount of moral weight to assign a given factor within a layer, or across layers? Should social and scientific value have equal contribution toward overall instrumental layer thickness? How should we compare the degree of thickness across layers that are conceptually very different? Moreover, in pluralistic societies, the assessment of moral weight is likely to vary (significantly), thus, how should a consensus be reached on the appropriate thickness of each layer? In Table 2, the moral weight of the 'human origin' factor would be more significant among communities with certain ontological beliefs. Weight assignment seems very contextual, which may, in fact, be the intention of the authors, but they also presents a challenge when attempting to make generalized claims about the moral thickness of various neuronal models. It is also unclear how the authors arrived at the proposed layer thicknesses illustrated in Table 2. Are these the views of the authors? If so, what was the rationale?

Third, more clarification is requested to delineate the symbolic layer as distinct from others. For instance, we are uncertain whether an individual's ephemeral connection with donated tissue (10.1080/21507740.2022.2048727[8]) as an extension of 'self' would fall under the symbolic or relational layer based on the considerations provided (p.9).

Fourth, the authors claim that layer thickness adds moral value to an entity, yet the implications of thickness across the different layers can lead in different normative directions, as recognized by the authors when making their third point on p.11. A neuronal model with high social and scientific value would produce a very thick instrumental layer in support of exploiting the neuronal model. Yet a very thick relational layer, due to high resemblance with donor brain function or strong mis-alignment with personal values, for example, would suggest more restrictions on using the neuronal model. Given that typical discussions of moral status associate more restrictions or protections for entities with higher values of moral status, the implications of layer thickness on normative decision making is somewhat clouded. Clarification here would strengthen the authors' aim to show how the layer metaphor can be understood acted upon (p.6).

A final a minor point to consider. There are several instances in the article when the authors make broad claims about the views of the scientific community. For example, the authors state, “there is consensus within the scientific community that at the current stage of brain organoid research, the higher cognitive functions that would (at minimum) be necessary for an entity to experience any form of consciousness, cannot be developed in current 3D neuronal cell models”. While there is a lack of empirical evidence that organoids have consciousness, the experiments that support or reject this claim have not been performed but are feasible (10.1007/s12152-023-09538-x[9]). The citation provided by the authors is founded on the views of some (prominent) neuroscientists/neuroethicists, but not a representative sociological sample of researcher beliefs about organoids, which in our view is needed in order to make such a claim. If survey data–qualitative or quantitative–is available then such evidence would support the broad claim being made, otherwise a more qualified statement seems warranted.

References

1. Lysaght T: Anticipatory Governance and Foresight in Regulating for Uncertainty.*Am J Bioeth*. 2022; **22** (1): 51-53 PubMed Abstract | Publisher Full Text
2. Kagan BJ, Gyngell C, Lysaght T, Cole VM, et al.: The technology, opportunities, and challenges of Synthetic Biological Intelligence.*Biotechnol Adv*. 2023; **68**: 108233 PubMed Abstract | Publisher Full Text
3. Kagan BJ, Kitchen AC, Tran NT, Habibollahi F, et al.: In vitro neurons learn and exhibit sentience when embodied in a simulated game-world.*Neuron*. 2022; **110** (23): 3952-3969.e8 PubMed Abstract | Publisher Full Text
4. Habibollahi F, Kagan BJ, Burkitt AN, French C: Critical dynamics arise during structured information presentation within embodied in vitro neuronal networks.*Nat Commun*. 2023; **14** (1): 5287 PubMed Abstract | Publisher Full Text
5. Colombi I, Nieus T, Massimini M, Chiappalone M: Spontaneous and Perturbational Complexity in Cortical Cultures.*Brain Sci*. 2021; **11** (11). PubMed Abstract | Publisher Full Text
6. Trujillo CA, Gao R, Negraes PD, Gu J, et al.: Complex Oscillatory Waves Emerging from Cortical Organoids Model Early Human Brain Network Development.*Cell Stem Cell*. 2019; **25** (4): 558-569.e7 PubMed Abstract | Publisher Full Text
7. Cai H, Ao Z, Tian C, Wu Z, et al.: Brain organoid reservoir computing for artificial intelligence. *Nature Electronics*. 2023; **6** (12): 1032-1039 Publisher Full Text
8. Evans JH: The Public's Ethical Issues with Brain Organoid Research and Application.*AJOB Neurosci*. 2022; **13** (2): 101-103 PubMed Abstract | Publisher Full Text
9. Boyd J, Lipshitz N: Dimensions of Consciousness and the Moral Status of Brain Organoids. *Neuroethics*. 2024; **17** (1). Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and does the work have academic merit?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neurobiology, brain organoids, neuroethics, moral status

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 24 October 2023

https://doi.org/10.21956/molpsychol.18832.r27311

Robin Ketteler
Department of Human Medicine, Medical School Berlin, Berlin, Germany

The manuscript "An assessment of the moral value of neuronal cell models and brain organoids" gives a comprehensive overview of moral aspects relating to the development and use of donor-derived neuronal cell models. The manuscript focuses not so much on the moral aspect of consciousness that such models may or may not obtain at some point, but rather on ethical issues and moral aspects surrounding their existence in itself, their proxy to a human individual, their symbolic nature and other issues related to the use of these models. The article is very well written and it has been very enjoyable to read. For me, as a scientist working with patient-derived iPSC cells and neuronal cells, this has been very insightful and stimulating.

My first comment is that the biology aspects and the description of the neuronal models is very well done. The scientific state-of-the-art is well described.

I have only very minor comments and some thoughts in general about the issue.

1. Table 2: I would recommend not naming the last row "4D". 4D typically refers to time, but here a "future" 3D model is meant. I would recommend "3D+", for instance to make that clear. There is no additional dimension in the future 3D model.

2. Table 2 shading: I would argue that the "potential to develop consciousness" increases from 1D to 3D, so maybe an increased grey scale could reflect that. In the relational layer column, it seems that "Alignment of research aims and personal values" have different shades in 1D

compared to 2D and 3D, but should be the same? Maybe this is just my printout, though.

3. One more aspect that may warrant mentioning is cell therapy. I am certain cell therapy will be done at some point, using either 2D or even 3D models to transplant directly into the brain of patients. These may be derived from the individual itself, but could potentially also be from another individual. If such a transplanted organoid integrates into the brain and takes over new functions, this could have great implications for the definition of self, memory, plasticity, etc.

4. Also, what if a certain brain model results in the development of a new drug for neuronal disease, however later it turns out that this drug has limited or adverse effects in the large majority of patients and is only useful in a very small number? In that case, the donor or model may be taunted and there could be anger and even threats associated with the individual that donated the cells. This is hinted at under the respect and research use aspects, but I just wanted to point this example out. This is somewhat similar to the HeLa cell line, where for a long time it was hailed as a great tool for cancer research, yet, when you're in the lab nowadays there is often a throwaway comment "But your findings are only valid in HeLa cells and have no physiological relevance".

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Stem cell biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 23 August 2023

https://doi.org/10.21956/molpsychol.18832.r27091

Leonora Buzanska
Department of Stem Cell Bioengineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

The research article of van Till *et al.*, "An assessment of the moral value of neuronal cell models and brain organoids" is an interesting conceptual study showing analyses of the moral status of various neuronal models starting from the simplest one cell (one neuron) model, through two dimensional neuronal networks up to more complicated three-dimensional models including brain organoids, resembling some features of early brain development. While the ethical considerations regarded mostly presumed capacity of brain organoids to develop some form of consciousness (which, is not confirmed case), there are many under considered and under investigated moral issues like, e.g. whether the neuronal models are used in responsible ways.

The authors presented the new concept of four layers of moral value applicable to neuronal models. These four-layers, which are identified as: instrumental, intrinsic, symbolic, and relational, allow to encompass other sorts of considerations than only moral values, including the results of empirical research and biological characteristics. They demonstrated how and to what extent each layer contributes to the moral value of the current range of neuronal models and they indicated the possibility to overlap such values.

While this is a research article, but based on conceptual work, not empirical data gathered in the designed experiments, my answer to the questions: "Are sufficient details of methods and analysis provided to allow replication by others?" and "Are all the source data underlying the results available to ensure full reproducibility?" is "Partly", since the section "Materials and Methods" in this article does not exist.
The conclusions are drawn clearly and the paper is scientifically sound in its present way. In my opinion, this article can be indexed in its present form.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neurobiologist and molecular biologist, expert in stem cell research, especially human neural stem cells and brain organoids.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.