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ARTICLE

Ethical Implications in Making Use of Human Cerebral Organoids for Investigating Stress—Related Mechanisms and Disorders

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Abstract

The generation of three-dimensional cerebral organoids from human-induced pluripotent stem cells (hPSC) has facilitated the investigation of mechanisms underlying several neuropsychiatric disorders, including stress-related disorders, namely major depressive disorder and post-traumatic stress disorder. Generating hPSC-derived neurons, cerebral organoids, and even assembloids (or multi-organoid complexes) can facilitate research into biomarkers for stress susceptibility or resilience and may even bring about advances in personalized medicine and biomarker research for stress-related psychiatric disorders. Nevertheless, cerebral organoid research does not come without its own set of ethical considerations. With increased complexity and resemblance to in vivo conditions, discussions of increased moral status for these models are ongoing, including questions about sentience, consciousness, moral status, donor protection, and chimeras. There are, however, unique ethical considerations that arise and are worth looking into in the context of research into stress and stress-related disorders using cerebral organoids. This paper provides stress research-specific ethical considerations in the context of cerebral organoid generation and use for research purposes. The use of stress research as a case study here can help inform other practices of in vitro studies using brain models with high ethical considerations.

Keywords: stress; chimera; animal research; cerebral organoid; research ethics; informed consent; psychiatric disorders

Introduction

Research into stress and stress-related disorders has long been a focus in ethical discussions, on the one hand due to the harm-prone nature of stress, and on the other hand due to several famous, but today considered unethical, cases of stress research covering, for example, Philip Zimbardo's Stanford Prison Study and Stanley Milgram's obedience experiments.[1] The ethics of stress research involves several considerations, including the welfare of research participants,[2] the use of animals in research, and the potential risks and benefits of the research in question.[3] Inflicting stress in animal and human research is a well-known problem in research ethics and is typically considered a harm or burden that needs careful justification and monitoring from a research ethics perspective.[4] One ethical concern in stress research is the welfare of human research participants. Researchers have a responsibility to ensure that participants are informed about the nature of the research and any potential risks, and that they are treated with dignity and respect.[5] This includes obtaining informed consent from participants, ensuring that they are not subjected to any unnecessary harm or discomfort, and protecting their privacy and confidentiality.[6] Another ethical issue, in stress research, is the use of animals. Many stress studies involve the use of animal models, such as rodents or nonhuman primates, to study the effects of stress on the brain and

behavior.[7] Researchers have a responsibility to ensure that animals used in research are treated humanely and with respect and to minimize any suffering or harm, hence the existence of ethics committees.[8] This includes providing appropriate housing, food, and care and using the minimum number of animal necessary to achieve scientific objectives,[9] in addition to justifying the worth of the potential scientific goals themselves. Finally, researchers must consider the potential risks and benefits of stress research. While stress research has the potential to lead to new treatments and therapies for stress-related disorders, it is important to carefully weigh the potential risks and benefits of any research study and to ensure that the research is conducted in an ethical and responsible manner.

Nowadays, investigating aspects of stress and stress mechanisms is possible in human-derived neuronal tissue—without the harm of the donor themselves. Developments in stem cell technology have allowed the differentiation of patient-derived stem cells into both two-dimensional (2D) neuronal cultures and three-dimensional (3D) cerebral organoid cultures in vitro for the study of underlying mechanisms driving brain development in health and disease.[10] This has the potential to provide an improved understanding of molecular mechanisms involved in neurological and psychiatric disorders[11] such as major depressive disorder[12] and post-traumatic stress disorder (PTSD).[13,14,15] For instance, 3D cerebral organoids can be used to investigate the respective impact of key stress-related molecules and stress hormones on processes involved in brain development that are relevant to stress-related disorders, in tissue harvested from different individuals.[16] Thereby, these models can facilitate research into biomarkers for stress susceptibility or resilience and ideally also for stress-related psychiatric disorders[17] and aim at bringing about advances in personalized medicine, because it makes use of human and ideally patient-specific bodily materials. In addition to these scientific and potentially clinical advantages, research on stress biomarkers in laboratory stem cell models may prove to be also ethically advantageous and provide more suitable models for human physiological stress reaction and in some instances serve as a replacement for current rodent experiments.[18]

Nevertheless, this research into molecular stress processing does not come without its own set of ethical considerations.[19] With increased complexity and resemblance to in vivo conditions, increased moral considerations for these human in vitro-based brain models might be warranted. So far, the consensus has been that no specific ethical oversight, by an Institutional Review Board for instance, or protection of such models is required. However, this might change in case these models develop even more complexity, the first steps of which can already be seen in 3D cerebral organoids as compared to 2D neuronal cultures. This might raise new research ethical questions in studies that if conducted in humans would be considered particularly sensitive, such as studies that intentionally inflict stress and hence harm on participants. Here, the question rises on how best to avoid a situation in which the stress induction will require yet again research ethical attention, because these models are becoming "too good" and might themselves be harmed in the process of stress research. In addition, new questions might arise for tissue donors, including the potential need for more vigilance during informed consent procedures, but also issues of reporting research findings back to individual donors might need a place on the ethical agenda of stress research with cerebral organoids. Finally, as research progresses the creation of chimeric cerebral organoid animals might need special attention if stress-induced research is conducted with them, potentially implying that they might not only have to face the harm of stress-induced research as such, but also if somehow "brain enhanced" they might experience the stress exposure even more seriously than typical experimental animals.

This paper will provide an overview of some of the previously discussed themes, including research ethics, donors and biobanks, and animal chimeras in relation to the ethics of stress research with cerebral organoids. Within each theme, novel ethical considerations that arise in relation to research into stress and stress-related disorders will be identified and discussed. Given the harm-prone nature of inducing stress onto an organism, unique ethical issues, not raised in other organoid research, may arise and hence require special attention. The more complex human cell-derived brain models such as cerebral organoids become, and the more they start resembling human- or animal-like in vivo brains, the more the paradoxical situation might emerge that a new kind of sensitive being is brought into existence, putting us in front of the same challenges that in vitro models promised to evade.

Now is a good moment to raise these issues and use the momentum of the ongoing ethical discussions surrounding research ethics of cerebral organoids. Highly complex brain models have been developed already; hence, we are not focusing on a science-fiction field, but to date, they have not achieved a level of complexity that could warrant full sensitivity, a kind of consciousness, or any capacities of suffering. Hence, formulating an ethics agenda on the issues to be considered and formulating initial guidance on what it might mean and what is required to proceed ethically in this area of research are timely endeavors. Unlike currently existing research ethical, frameworks for doing science with human and animal participants have developed only in the aftermath of serious atrocities in medical experimentation[20,21]; proactive thinking about whether, and if so how, we need research ethics for complex brain models and their biobank infrastructures, might prevent avoidable and unnecessary harm from the outset. The aim of this paper is to sketch the current scene and identify conditions where cerebral organoid research into stress and stress-related disorders does or does not raise specific ethical questions.

Cerebral Organoids: Generation and Uses

To understand whether a research ethics framework needs to be set for in vitro brain models among which cerebral organoids, and in particular for their research uses into stress mechanisms, we must first understand the nature of what (or whom) we seek to protect. We will describe cerebral organoids, their origin, how they are generated and developed in vitro, and the myriad of ways they are currently used and hoped to be used in the future. The current state of the art should help inform us on how best to deal with stress research with in vitro brain models, most notably 2D neuronal stem cells and 3D cerebral organoids.

Cerebral organoids are lab-grown 3D structures that mimic the development of the human brain, with great similarities to the cytoarchitecture and cellular and physiological characteristics of the human brain.[22] Despite their complexity as an in vitro model, compared with a real human brain or nervous system, organoids are still rather primitive. However, given their direct linkages to specific individuals, whose somatic cells were used to develop these models, they have increased research potential with, in the not-too-distant future, also clinical and personalized applications. Cerebral organoids are defined as "self-organizing 3D tissue" and are generated through a process known as reprogramming, which involves taking cells from a human donor and inducing them to become pluripotent stem cells (PSCs).[23] Cerebral organoids can originate from a variety of adult somatic cell sources such as connective tissue,[24] blood cells,[25] or even urine[26] taken from donors. These stem cells are then allowed to differentiate into different types of cells found in the human brain, including neurons and glia. The differentiation of cerebral organoids can either be guided, hence leading to specific brain regions (e.g., forebrain, midbrain, or cerebellum), or unguided, hence leading to a self-patterned whole-cerebral organoid with a heterogeneous cell population.[27] Guided region-specific cerebral organoids can be further fused to one another, forming assembloids which can further model interaction between different brain regions and to investigate particular research questions looking into communication between different brain regions.[28]

Organoid models have been developed to better study developmental processes in various organs and to allow the testing of several drugs and compounds onto human-derived tissues and reduce premature testing in humans. Research conducted with neuronal stem cells and cerebral organoids is not new. By now, they have already been used for different purposes across various research fields and for a variety of medical applications. For instance, in the investigation of several human brain developmental functions,[29,30] in health and disease states, to study disease-specific phenotypes of neurodevelopmental disorders,[31,32] or to test drugs[33] and different chemical compounds.[34] Cerebral organoids are also gaining increasing attention in the modeling of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases.[35] Moreover, cerebral organoids have already been implanted into rodents in order to investigate their potential in a more complex and vascularized environment, leading to the creation of chimeras.[36] Eventually and in the long term, researchers hope to transplant cerebral organoids into the brain of stroke and epilepsy patients, as a potential treatment strategy to restore brain function.[37]

Cerebral organoids, however, have been considered special or significantly different from other organoids since their arrival. The reasons, therefore, seem mainly to lie in the special status that the brain is given as an organ, as declared by the Nuffield Council[38] and the close association of the brain with who we are as a person, our self, and personhood. If similar cognitive functions and capacities could be traced back in the dish, doing research with human-derived cerebral organoids might be troublesome and more so than in research with other types of organoids. In so far as this holds for research in general, it is even more applicable in case of potentially harmful research such as research into stress and stress disorders that makes use of the artificial infliction of stress and hence harm. In research with human participants, this would be bound to clear limits and even then requires specific justification as to the potential benefit, in terms of knowledge gain, that might result from this research.[39] This raises the question, whether similar questions for stress research should be posed in the context of brain models as well, and if so whether any differences could and should be made between different levels of complexity in these models.

Ethical Issues in Stress-Related Research

Diving into the research ethics of making use of cerebral organoids, the following three areas are to be investigated: research ethics frameworks for in vitro uses of 3D cerebral organoids, refined and revised biobank research ethics frameworks for the protection of donors, and finally, increased protection for cerebral organoid chimeras.

Research Ethics for Cerebral Organoids

When discussing the ethics of organoids and organoid research, cerebral organoids steal the spotlight. Despite organoid research also carrying more general ethical considerations, there is an inherited belief that cerebral organoids, in particular, deserve increased moral considerations (that other organoids do not possess) given the nature of the organ (and species) they are modeling: the human brain.[40] That of course is due to the fact that the brain is characterized by unique faculties such as consciousness, sentience, experiencing of suffering and pain, decision-making, and other important higher cognitive functions that contribute to make human beings who they are.[41] Many ethicists believe that if cerebral organoids begin to show increased complexity similar to human brains, then research making use of them must undergo a very similar in-depth ethics review similar to animal or human embryo research ethics reviews in order to ensure that the level of pain or discomfort is minimized, and that methods of experimentation and destruction are refined and appropriate,[42,43] especially when paired with other living systems (i.e., chimeras). To date, there are no research ethics guidelines for in vitro research models (except guidelines for research into embryo usage); however, the generation of cerebral organoids might challenge this situation, calling for a further investigation of the situation and an investigation of whether, why, and how specific ethical considerations and regulations for the use of human-derived cerebral organoids and assembloids for research purposes might be required, especially in cases were harm is exercised, such as for research into stress-related mechanisms and disorders.

There is no doubt that cerebral organoids are characterized by increased complexity when compared to their 2D counterparts (human-induced PSC-derived neuronal cultures). From an increased heterogeneous cell population to an improved cytoarchitecture and functional properties, cerebral organoids are, to date, an improved model of other in vitro brain models out there.[44] Increasing literature on cerebral organoids illustrates their increased ability to respond to different stimuli, including the ability to stimulate a skeletal muscle,[45] the ability to respond to a stress hormone (e.g., dexamethasone),[46] among others. Additionally, some scientists claim that cerebral organoids exhibit neuronal activity that resembles human fetuses in the first trimester.[47] However, many question these claims due to our little knowledge of brain activity and functioning in human fetuses at this stage of development.[48] One might assume that increased complexity as manifested with an increase in the number of neurons, number of connections, and number of cell types might lead to increased cognitive complexity. However, we know

from nature that bigger brains do not necessarily translate to increased intelligence or improved cognitive functions.[49] It is inaccurate to immediately assume that increased complexity in cytoarchitecture will lead to or improve the likelihood of conscious-like signatures in cerebral organoid. There are, and will remain, fundamental differences between human cerebral organoids and human adult brains. These differences constitute fundamental building points for the ability to achieve sentience or consciousness.[50] Moreover, many believe that inducing consciousness requires a highly complex network, including a variety of cell types and sensory inputs that lead to subjective experiences, such as pain and discomfort (which current cerebral organoids do not possess). Importantly, scientists have suggested that the consciousness that contributes to the moral life of human beings can only be manifested with the exposure to social nurturing environments and the development of language abilities, something that cerebral organoids will never come to develop or even experience[51] (unless depicted in a science-fiction movie). Moreover, cerebral organoids might or might not need to be regulated depending on what regions of the brain they are modeling (in some cases of guided differentiation). It could be that certain assembloids might not be morally problematic if the collective assembly of certain organoids (representing certain brain regions) does not lead to the creation of sentience. That being said, should we even be discussing moral justifications for the use of cerebral organoids for research purposes?

Conscious awareness of painful sensations and discomfort is another aspect of conscious experience that is argued when discussing cerebral organoids. To date, that cannot be achieved with cerebral organoids are developed in vitro, and more research is needed to better answer this ethical query.[52] For instance, the brain does not have nociceptors (sensory receptors of painful stimuli) and as such a brain alone will not be able to sense painful stimuli, let alone in vitro cerebral organoids. However, some have argued that experiences of stress, sensory deprivation, and conscious discomfort might be possible with cerebral organoids.[53] To date, we do not have the technology capable of assessing these psychological experiences in vitro, and we need to be aware that these experiences do not just "emerge" as the organoids grow larger and more complex. Sentience requires a variety of sensory stimuli and the activation of several processes for it to develop, hence being more complex than what is usually portrayed. What we can currently investigate in cerebral organoids are molecular, cellular, and electrophysiological processes underlying particular genetic variants, and/or in response to drugs and hormones for instance. Many philosophers argue that if there is an uncertainty about whether a particular being is sentient, one should not treat it as lacking moral consideration but instead treat them respectfully and as if they have some moral status.[54] However, given the information we currently have on the nature of cerebral organoids, their capabilities and limitations, and in accordance with the consensus, it is safe to say that cerebral organoids are not sentient and hence do not deserve any moral protection.

It is clear what current established research ethics frameworks seek to protect: Human research ethics aims to protect humans, and animal research ethics aims to protect animals. The first question that a potential research ethics framework (if required in the future) in the context of in vitro brain models will have to answer, however, is what to protect. Should it have to protect: (1) the most complex brain models available, such as current assembloids (consisting of several 3D structures), (2) unguided cerebral organoids with the ability to self-organize with a composition that mostly resembles the developing brain, and/or (3) guided brain organoids that are differentiated into specific regions within the brain. Would oversight and protection be equally needed for different types of cerebral organoid and assembloids? Or would a gradation in the level of protection be more adequate, depending on the levels of complexity and maturation level of these 3D models? The term "cerebral organoids" has been used interchangeably in several ethical discussions and analyses, however, a striking difference exists in these different aforementioned 3D models which warrant separate ethical analysis. For instance, an assembloid composed of different brain regions (e.g., hippocampus and hypothalamus) whose combination cannot form sentience is not deemed ethically problematic, and hence would not require any ethical oversight. The same applies t a guided cerebral organoid differentiated into a hippocampal-like structure. Ethical discussions should clearly state the differences between different cerebral organoids[55] and assembloids and point out those that carry ethical implications and those that do not, which would not only improve our understanding of fundamental differences between different cerebral organoids but will also avoid any unnecessary overgeneralization of all research with cerebral organoids. These

considerations would also apply for research into stress and stress mechanisms, where making use of less complex 2D or 3D structures might be more justifiable over more complex organoids and assembloids.

Even as we talk about investigating stress mechanisms using cerebral organoids, including mechanisms involved in stress susceptibility, with stress requiring increased consideration in animal and human research ethics frameworks, we do not believe that new research ethical questions arise concerning the need to protect cerebral organoids (or research subjects). In vitro, stress itself is not being investigated, however molecular mechanisms involved in stress are. Stress mechanisms have long been investigated in vitro using a variety of neuronal cell lines and by exposing the latter to (synthetic) glucocorticoids or other key stress hormones such as noradrenaline. Cerebral organoids have also been used to investigate the effects of a synthetic glucocorticoid called dexamethasone.[56] For example, a better understanding of the mechanisms that drive susceptibility to stress-related disorders could be facilitated through the generation of cerebral organoids from patient biomaterials. In the context of cerebral organoids or in vitro research in general, the nature of the stressor being used to induce stress-related response is fundamentally different when compared to in vivo research studies on stress where essentially personal experiences of stress and their detrimental effects are playing a role. In vivo, stressors are also of a different type and nature, and they are not only neurochemical but in addition are physical, psychological, or social. Current animal stress models make use of these more complex types of stressors. For example, stress in rodent models can be induced by the administration of the stress hormone glucocorticoids (neurochemical), and it can be induced using (physical) electric shocks or even exposed to social stress among other rodents.[57] In the case of cerebral organoids, the stressors in question can only be neurochemical. This situation might change, however, once cerebral organoids are transplanted into rodents, creating chimeras with humanly adapted rodent brains, especially in cases where the donor is known to be susceptible to stress-related disorders (we will revert to this case later). Given the lack of the capacity to experience stress and be sentient about it in cerebral organoids and given also that stressors in vitro are purely neurochemical, in vitro stress-related research into cerebral organoids currently need not be considered as harmful for cerebral organoids. Therefore, it does not require any specific risk–benefit balance, nor does it require a neat justification of any harm or burden inflicted on structures in-the-dish for the sake of research.

Cerebral organoids have reshaped the way we perform neuroscience research, especially when investigating brain development and diseases implicated in brain development. However, these increasingly sophisticated models do not come without their own ethical considerations. Despite no current evidence pushing for an ethical oversight when conducting research with cerebral organoids, we want to reiterate the continuous justification of making use of cerebral organoids, given that they are not here to replace all models (including 2D neuronal cultures or animal models) but are here as an improved model to answer certain research questions that would otherwise not be easily understood using other less complex models. Reducing the use of cerebral organoids in research, in general, that does not strictly require their use (but particularly in stress-related research) may avoid getting ever entangled in potential ethical considerations that accompany the use of cerebral organoid in research settings.

Donor-Related Ethical Issues

The current state of cerebral organoid research in fact prioritizes ensuring that adult somatic-cell donors for the generation of cerebral organoids are appropriately protected. For instance, donors might have a legitimate interest in not having their materials used in ways they would potentially dismiss (e.g., dual-use purposes),[58] or they might have a preference to receive knowledge and be informed about any research findings, particularly in case these findings can be linked back to themselves and are potentially meaningful.[59] This raises the questions of whether current legislations on consent for biobanks do still fit with the potential that tissue donated might develop into self-organizing cortical structures.[60] Ethical and responsible practices for the collection of patient or human biomaterial (including somatic cells) include transparent disclosure of the benefits versus risk of participation in the research study, in addition to short- or long-term goals of the study in question, as advised by the International Society for

Stem Cell Research.[61] In the case of cerebral organoids, this might include actively engaging the potential participants in the informed consent process (and participant-appropriate alternatives in case of vulnerable groups including children or individuals with cognitive disabilities), clearly informing potential donors that genetically matched cerebral organoids will be generated possibly unraveling peculiar medical-related information about the donor in question, and finally, ensuring that no false hope is transmitted to the donor about directly benefiting from donating their biomaterials for research.[62] Additionally, many limitations remain in that first, these guidelines are not law-abiding and as such do not strictly prevent malpractices; second, the generation of cerebral organoids (or other iPS-derived cells and organoids) is rather challenging when it concerns the use of samples from biobanks and whether tissue bank donors are aware, comfortable with participating, or whether they may even opt out from (future) cerebral organoid-related research.[63]

We do see a peculiar ethical challenge here, in the sense that mainstream media might also influence the informed consent procedure, especially in situations where the research in question relates to investigating stress mechanisms in vitro which involves exposing cerebral organoids to a chemical stressor.[64] With the COVID-19 pandemic, it became clear how fast medical misinformation can spread, and how severe the consequences can be.[65] The overturning of Roe v Wade has also challenged the stance of the scientific community in informing policy and law.[66] Stress research, and ethics of stress research, has always been a sensitive topic, particularly in the context of both human and animal research as we will discuss in the coming section. With a growing number of non-scientific publications reporting research into cerebral organoids,[67] we believe caution should be exercised in the framing and communication of research into cerebral organoids to the public and potential donor participants. The public communication should actively countervail the impression that generating and making use of cerebral organoids to investigate stress-related mechanisms would translate into somehow stressing and hence harming cerebral organoids that are exhibiting signs of sentience, which of course raises considerations as to how research into cerebral organoid must be communicated, including questions on which information should be provided on currently intended research, on research not drafted yet but potentially planned and conducted in the future, but also how detailed should the information about potential sentience or other ethical considerations raised by cerebral organoid generation be. How should and could these issues be discussed with potential donors, in a way that provides them with relevant information to make up their minds on donation in a reasonable way but does not encourage or discourage them to donate their bodily materials for the wrong reasons.

Additionally, in the case of stress-related research, researchers could identify an increased vulnerability to stress-related disorders through screening the cerebral organoids for (epi)genetic variances that have been shown to be associated with increased susceptibility (or even resilience) to stress-related disorders. Identifying susceptibility or resilience to stress-related disorders can also be accompanied with its own set of ethical implications, as it has been previously described.[68,69] Therefore, reporting back to donors about predicted susceptibility or resilience and communicating with them the meaning of such findings are questionable and require careful thought.

This, in turn, invites us to draft guidelines for the ethical communication of cerebral organoid findings for scientists, journalists, science communicators, and other professionals who are involved in the dissemination of findings related to cerebral organoid research in general. This could improve the public understanding of cerebral organoid-related findings without communicating false hope or hype to the general public.

Beyond Organoids In-A-Dish

While cerebral organoids in the dish have raised many ethical questions, further and potentially new and more serious questions might arise if these organoids were no longer kept in an artificial environment but transplanted into a more natural environment. Cerebral organoids are cultured in a dish where they essentially form an island detached rather than connected to a body, but they can also be transplanted into actual living beings. Currently, this has been performed by researchers who have transferred human

cerebral organoids into the brain of rodents and thereby have created humanly adapted chimeras.[70] Performing research, particularly in the context of stress-related research with such chimeras, raises further ethical questions that did not arise in the same way when cerebral organoids were developed and cultured in the dish. For example, the mere transplantation of human cerebral organoids into rodent brains is an invasive procedure and often leads to the rejection of the transplanted organoid, the formation of tumors, faulty integration into the host, and other possible complications that directly harm the receiving animal. While this concern holds in general for the creation of all kinds of chimeric animals, in the current context the additional question arises on how research into stress and stress-related disorders using cerebral organoid chimeras would impact the welfare of hosting animals as compared to non-chimeric animals? While research on cerebral organoid chimeras raises several ethical questions as such, we believe that stress research with cerebral organoid chimeras raises additional and more specific ethical questions.

In order to tackle these questions and in particular questions on the justifiability of stress research in chimeric animals, we should revert to debates in research animal ethics. Since the beginning of the 1980s, the use of animals for research purposes saw a decline with increased public advocacy, awareness among scientists, and the introduction of regulations on animal use. This was accompanied by the implementation of animal ethics committees and a relative improvement in the quality and use of research animals.[71,72] In Europe for instance, according to the directive 2010/63/EU,[73] the performance of animal research must be preceded by an ethical approval by a competent authority. The movement toward the ethical use of animals in research was also inspired by William Russell and Rex Butch and their 3R framework—replace, reduce, and refine. This framework urged scientists to replace animals with alternative models or at least with "lower" species; reduce the sample size of animals by including not more than the minimum number needed for statistical significance; and finally, refine the experimental conditions by minimizing experiences of pain and suffering, in addition to improving quality of care such as housing facilities and welfare.[74] These developments have improved the use of animals in research in a way that ethical justifications of the use of animals for research purposes became a requirement.[75] However, with issues concerning reproducibility and their translational ability to the bedside, the validity of animal models for answering some research questions into human health and disease is becoming increasingly questionable,[76] particularly in the context of psychiatric or mental health issues that relate to human behavior and experiences. Ethical doubts about the justifiability of animal research have further intensified since the advent of organoid research and its great promises in several avenues including personalized medicine, toxicology, drug testing, and improved modeling of human disorders, making them particularly attractive as a suitable alternative to animal research.[77]

Research animals have long been used for the investigation of a number of disorders, even specific disease models have been developed to better represent the underlying pathology. When it comes to stress disorders, by today a number of different stress animal models for major depression, anxiety, and PTSD have been developed. These models cover neurochemical models that induce stress by corticosterone treatment or neuroinflammation. Given the fact that animals unlike organoids are also social beings, stress models in animals do also cover social forms of stress such as early maternal separation, social defeat, social isolation, chronic unpredictable stress, or forms of learned helplessness.[78] The nature of the stressor is also of interest here, while in vitro stressors relevant to stress-related disorders are mainly of a chemical nature, in chimeric animals of cerebral organoids, this limit is no longer needed and stressors theoretically could include chemical, physical, and psychosocial features. The use of animals for stress experiments is itself an ethical concern due to the harm and discomfort to which these animals are subjected. To put it more clearly, the conscious experience and conscious suffering caused by the stressor are of particular interest to researchers because that ensures the validity of their model. And without this conscious suffering, animal stress models are of no relevance for researchers investigating stress in the lab. This raises questions on the harm-benefit balance and the requirements one may put on the relevance of the knowledge gain that might be achieved with such experiments such that it can be proportionate with the harm inflicted. This conscious suffering is also measurable in the form of behavioral tests (including the sucrose preference test, the open-field test, among others). For example, an animal that shows stress symptoms would score low on the sucrose preference test, as compared to a

non-stressed animal. Scientists measure stress effects on animals also by using behavioral output measures such as anhedonia, assuming that animals that exhibit more anhedonia are more stressed than those that show relatively less signs of anhedonia. The use of cerebral organoid chimeras may raise ethical questions as to the meaning and hence justifiability of the suffering inflicted by stress models in both the animals in question and the implanted cerebral organoids.

Today, it has been shown that implanted cerebral organoids integrate with the vascular and nervous system of the host animal and thereby increase complexity of the organoid in several ways.[79] This raises the question of whether and how cerebral organoids transplanted into an animal initiate a humanization of the animal, making it more human and therewith potentially also more protection worthy compared to non-chimeric animals. Related to this, there are growing concerns that introducing human neuronal tissue into animal brains might lead to the development of human-like characteristics, such as self-consciousness, and improved cognitive abilities.[80,81] In so far as these concerns prove reasonable, they would imply that stress research with such chimeric animals would be even harder, or maybe impossible, to justify than similar research with "typical" experimental animals. However, the concept of humanization of cerebral organoid chimeras has been argued against,[82,83] even considered less constructive, first because this has not been demonstrated through behavioral tests in chimera, and second due to other more eminent problems being put forth as more urgent in relation to cerebral organoid research and transplantation, including the welfare of chimeric animals.[84]

Nevertheless, instead of claiming that cerebral organoid chimeras are becoming more human-like in general, another perspective has been put forth concerning chimeric animals transplanted with cerebral organoids, that is, brain enhancement of cerebral organoid chimeras.[85] Brain enhancement in this context ranges, for example, from chimeric cerebral organoid animals exhibiting increased reaction times, improved visual functions, ameliorated learning and memory functions to self-awareness and meta-cognition capabilities.

With the possibility of brain enhancement, a decrease in the welfare of the animals might be at stake beyond the negative effects currently being reported of the transplantation itself. While in certain contexts, cerebral organoid chimeras may lead to brain enhancement, in other cases, such as stress-related research, cerebral organoid chimeras may lead to brain-induced vulnerabilities and susceptibilities to stress-related pathologies, as suggested by Chen et al.[86] For example, humans and nonhuman primates are known to be increasingly susceptible to the negative effects of stress and exposure to stress stimuli, when compared to rodents and other vertebrates.[87] Therefore, stress research with brain organoid chimeric rodents can be considered a double-edged sword. On the one hand, a model that more likely resembles vulnerabilities seen in humans might increase the validity of the animal model and overcome, or at least reduce, current criticisms that argue that animal models for investigating stress-related disorders lack validity and have only poor, if any, reproducibility in humans. On the other hand, however, this very potential advantage also carries the chance of increased negative consequences for the welfare of the cerebral organoid chimeric rodent and leads to increased depressive-like symptoms that they otherwise are unable to experience. This raises the question on whether new research ethical guidelines on cerebral organoid chimera should be developed and whether these should pay particular attention to, or maybe even ban certain kinds of, stress research that might inflict particular suffering on chimeric animals, even though it remains unclear which precise knowledge gain might result from this intensified suffering. However, before such guidelines could ever be developed, first, research is needed that identifies what could count as ethical handling of cerebral organoid chimeras and what it would require. If an improved understanding of the identity and status of such chimeras was available, including ways to attain to their welfare and the limits to their handling should be respected, then guidelines might be developed on how to realize the requested level of protection potentially considering the kind of host animal and the kind of stressor (neurochemical, physical, psychological, or social) intended to be used in any study.

Conclusion

Undeniably, cerebral organoids offer novel and exciting opportunities for the understanding of brain development in a healthy state and in the context of neurological and psychiatric disorders. However, the capacities and promises of cerebral organoids are not limitless (both in case they exhibit or do not exhibit sentient-like features). And it is clear that the use of cerebral organoids does not come without moral considerations; in fact, they raise several unique ethical questions, especially in the context of stress-related disorders. In their current form today, the use of cerebral organoids for research purposes is not ethically problematic from a research ethics perspective, meaning that the potential benefits and knowledge gain resulting outweigh the risks or harm for the organoid itself. Donor-related ethical issues include communication of the promises, limitations, and questions on reporting back research findings on stress susceptibility and resilience, next to issues about the proper protection and information of donors. Ethical debates on stress research in brain models should also pay particular attention to the welfare of chimeric animals with cerebral organoids, because they might experience increased harm and suffering. These ethical considerations of cerebral organoids are more pressing on the ethics agenda than the potential protection worthiness of cerebral organoids.

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