Stem Cell Reports
Perspective
ISSCR
OPEN ACCESS

The Ethics of Cerebral Organoid Research: Being Conscious of Consciousness

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https://doi.org/10.1016/j.stemcr.2019.08.003

Recently, the use of three-dimensional neural tissues cultured *in vitro* and called "cerebral organoids" has advanced recapitulation of neural development and disease modeling studies. Along with such advances, cerebral organoid research, and associated concerns call for the elucidation of two points: (1) how cerebral organoid research is currently progressing and the future directions it is likely to take, especially in functional assessment of organoids, and (2) how we should solve ethical issues of possible consciousness in cerebral organoid research. This paper aims first to explore these two issues, and then to present implications and prospects for future cerebral organoid research.

Introduction

Recently, notable scientific achievements have come from organoid research involving three-dimensional organ-like tissues derived from tissue stem cells or pluripotent stem cells by mimicking the formation process of normal tissues and organs. For example, the use of three-dimensional neural tissues cultured *in vitro* and called "cerebral organoids" has advanced recapitulation of neural development and disease modeling studies of Alzheimer disease, brain tumors, autism, and microencephaly, as well as Zika virus infection-related microencephaly (Dang et al., 2016; Garcez et al., 2016; Kadoshima et al., 2013; Lancaster and Knoblich, 2014; Ogawa et al., 2018; Qian et al., 2016; Quadrato et al., 2017; Watanabe et al., 2017). Along with such advances, some have argued that cerebral organoids themselves may have kinds of mental activity such as consciousness, cognition, and thinking. Concerns have been raised related to the famous "brains in a vat" thought experiment formulated by philosopher Hilary Putnam in 1981—human brains placed in a particular environment (such as connected to a sophisticated computer program) may have the same consciousness as average adult human beings have (Lavazza and Massimini, 2018a; Putnam, 1981).

In contrast to such concerns, despite remarkable progress in their *in vitro* culture methods, cerebral organoids have so far reproduced only local nervous system structures and do not closely reproduce the human neural developmental process over time. It seems highly unlikely then that cerebral organoids do or will soon have consciousness. In the future, however, researchers may be able to culture cerebral organoids with more complicated structures over longer periods of time. We cannot confidently conclude that cerebral organoids will forever continue to have no consciousness. Moreover, in conventional research, the methods for functional assessment of cerebral organoids have been insufficient to detect possible consciousness. In recent years, however, functional assessment methods in cerebral organoid research have progressed, so a sophisticated method may soon be able to show that cerebral organoids have particular types of mental activity.

Issues concerning possible consciousness have thus far been debated philosophically, especially at the intersection of philosophy of mind and medicine/neuroscience. Many agree that it is required to consider what kind or kinds of consciousness warrant moral consideration, rather than that conscious beings should be granted moral consideration because of the possession of *any* kind of consciousness. Moreover, some support a view that consciousness itself is not intrinsically valuable. Thus, on the specific issue of consciousness in cerebral organoid research, it is crucial first to identify which type or types of consciousness are morally significant—that is, what sorts of consciousness would give a being which possesses them moral status, and which would not. From there, we must answer the empirical question of whether cerebral organoids already have or are likely to have such consciousness. Because moral consideration of the produced cerebral organoids depends on the presence or absence of morally significant consciousness, the presence of consciousness in cerebral organoids may become a critical issue in cerebral organoid research.

With this line of thinking in mind, cerebral organoid research and associated concerns call for the elucidation of the following two points: (1) how cerebral organoid research is currently progressing and the directions it is likely to take, especially in functional assessment, and (2) how we should solve ethical issues of consciousness in cerebral organoid research. The present paper aims first to

conduct a concise review of these two issues, and then to present implications and prospects for future cerebral organoid research.

The Current Status of Cerebral Organoid Research and Future Research in Functional Assessment

The pioneering achievement of cerebral organoid generation has its origin in 2005 findings from a group led by Yoshiki Sasai (Watanabe et al., 2005). The group was the first to succeed in creating three-dimensional neural tissues *in vitro* that mimic the mouse cerebrum, by a cell aggregation suspension culture method using mouse embryonic stem cells (ESCs). Three-dimensional differentiation induction of cerebral tissues using human ESCs became possible by an improved differentiation method of cerebral nerve tissue performed by the Sasai group in 2008 (Eiraku et al., 2008). In this study, the group used findings from embryology to guide establishment of a suspension culture method that attempts to reproduce the neural developmental process called the serum-free floating culture of body-like aggregates with quick reaggregation (SFEBq) method.

The group has since used mouse and human ESCs to induce three-dimensional differentiation of many neural areas including the cerebral cortex, hypothalamus, ventral telencephalon, optic cup, anterior pituitary, cerebellum, hippocampus, and thalamus (Danjo et al., 2011; Eiraku et al., 2011; Hasegawa et al., 2016; Ishida et al., 2016; Kadoshima et al., 2013; Kuwahara et al., 2015; Muguruma et al., 2015; Nakano et al., 2012; Nasu et al., 2012; Ozone et al., 2016; Sakaguchi et al., 2015; Shiraishi et al., 2017; Suga et al., 2011; Takata et al., 2017; Wataya et al., 2008). This culture method enables the long-term three-dimensional culture of complex tissues; and, in a 2013 report on the induction of cerebral cortex differentiation, Taisuke Kadoshima and colleagues kept cerebrum tissues induced from human ESCs in three-dimensional culture for up to 90 days (Kadoshima et al., 2013).

Several months before the report by Kadoshima et al., the Jürgen Knoblich's research group reported inducing both human ESCs and human induced pluripotent stem cells (iPSCs) to generate cell aggregates that included tissues from several neural areas, such as the cerebrum, neural retina, and choroid plexus (Lancaster et al., 2013). Knoblich and colleagues called their cerebral tissues cerebral organoids, and proposed employing them as a disease model of microencephaly using iPSCs derived from patients. Since then, cerebral organoid research has focused both on recapitulation of the developmental process and gene expression patterns of the cerebrum, as well as on modeling cerebrum-related disorders.

By elucidating the mechanisms of various cerebrum-related disorders and adopting them into drug discovery screening, it may be possible to develop new therapeutic methods for treating cerebrum-related disorders such as Alzheimer disease, amyotrophic lateral sclerosis, and psychiatric disorders, which have historically been difficult to treat. Indeed, drug candidates for Zika virus-related microcephaly have been identified as a result of cerebral organoid research on the effects of Zika virus on human cerebral tissues (Cugola et al., 2016; Dang et al., 2016; Garcez et al., 2016; Qian et al., 2016; Watanabe et al., 2017).

Despite such progress and advantages, there remain some problems with conventional differentiation induction methods. For example, current cerebral organoids lack desirable supporting structures, such as blood vessels and meninges and other surrounding tissues. Still more challenges exist in the attempt to keep organoids in stable culture for more than one year and in standardizing protocols that can easily induce reproducible differentiation in various laboratories. Various groups have developed on the differentiation induction methods of cerebral organoids from the Sasai and Knoblich groups, and additional advanced research that overcomes aforementioned challenges is on the way.

In addition to disease modeling, the functional assessment of the neural activity of cerebral organoids has also received attention in recent cerebral organoid research. Evaluation of nervous or neuronal activities within cerebral organoids has been conducted using the patch-clamp method, calcium imaging, and recording of extracellular electric potential (multielectrode array [MEA] and silicon multielectrode). Thus far it has remained a challenge to evaluate the comprehensive function of neuronal networks in cerebral organoids because of a limited ability to get into and assess their complexed neural activity.

MEA and calcium imaging may be particularly useful tools toward overcoming this limitation. In MEA, the extracellular potential of neurons that attach to each electrode is measured, and assessment of electrophysiology from multiple recording sites enables the assessment of the function of neuronal networks at high temporal resolution. A weak point of this method is low spatial resolution stemming from the number of electrodes used. To overcome this problem, researchers have recently developed measurement methods that use more electrodes. Additional weak points for MEA are that it can detect only the potential of the part of the organoid in contact with the electrodes, and that the analysis of network activity including recording sites where several neurons contact requires specialized knowledge of computer science. Nevertheless, MEA remains a powerful tool to evaluate functional dynamics in neuronal network activity. Modeling cortical network activity with human late preterm neonatal electroencephalogram features was recently reported using cerebral organoids and MEA measurements (Trujillo et al., 2018), although there has been ongoing discussion concerning its interpretation (Reardon, 2018).

Calcium imaging evaluates time series fluorescence changes induced by the binding of Ca^{2+} ions with calcium indicators using confocal or multi-photon microscopy. Imaging of organoids at a position deep from the surface becomes possible using multi-photon microscopy. Calcium imaging is superior to MEA in this respect, as it does not require direct contact to electrodes for imaging of a region. At the same time, however, calcium imaging has the disadvantage that its time resolution is significantly worse than that of MEA. Comparatively poor time resolution results from its principles as resolution depends on microscopy. Besides, in conventional analytic methods, calcium imaging requires the manual selection of cells for evaluation, and is thought to be suitable primarily for analysis of single neurons or small cell clusters rather than neural networks. Nevertheless, because imaging data include several types of information on activity in a measured field, it has the potential to access activity patterns and dynamics among neural networks. Accordingly, development of comprehensive analytic methods of calcium dynamics in neural networks that do not require manual selection of cells for evaluation is expected to overcome that current weak point.

Toward this end, Hideya Sakaguchi and colleagues (the first author is an alumnus of Sasai's group) conducted a comprehensive functional assessment of networks in cerebral neural circuits through a combination of calcium imaging of neural networks derived from cerebral organoids and a high content analytic method (Sakaguchi et al., 2019). They detected dynamic changes in calcium ion activity of all cells in a single field, and made visualization of cell activity patterns using raster plots with clustering of the activities. These analyses successfully visualized synchronized and non-synchronized clusters and could divide the synchronized clusters into several small clusters with information on the cell distribution. Their work introduced the possibility of a broad assessment of human cell-derived neural activity. By using this method, it may be possible to understand processes by which information is encoded in the brain through the activity of specific cell populations, and the fundamental mechanisms underlying psychiatric diseases arising from abnormalities in these activities.

The findings of Sakaguchi et al. are broadly applicable in three main areas of research. The first is drug discovery research. In this area, it is anticipated that assessment of drug efficacy on abnormal human neural network activities may facilitate the development of medications such as psychotropics and antipsychotics. The evaluation of side effects and optimal concentrations through toxicity evaluation of candidate drugs will be another application in this area. For example, drug-induced convulsion is a frequent side effect in neuropharmacology (Odawara et al., 2018). By adopting the analysis of Sakaguchi et al., it may be possible to detect the minimum dose of a drug that causes abnormal neural network activity. This could bring great advances to pharmacological companies toward replacement of conventional evaluation using animal models. Indeed, drug-neurotoxicity evaluation based on the analysis of Sakaguchi et al. is already being applied by the Consortium for Safety Assessment using Human iPS Cells as a future screening method in an ongoing project.

The second area of research relates to the modeling of neuropsychiatric disorders. In this area, we can expect the generation of pathological models of psychiatric diseases which cause dysregulation of neuronal activity. For example, three monoamines including dopamine, serotonin, and norepinephrine are responsible for controlling neuronal function, and they play critical roles in some psychiatric disorders (Aggarwal and Mortensen, 2017). It has been difficult to model such psychiatric disorders because of the difficulty in detection and evaluation of their atypical neuronal function, but the analysis by Sakaguchi et al. may enable detection of abnormal neuronal activity.

The final research area involves *in vivo* studies of human cerebral organoids using non-human animals. Although this area is preliminary as of now, some studies have suggested that transplantation of cells from mouse cerebral cortex or of mouse ESC- or human iPSC-derived cortical neurons could improve motor dysfunction caused by brain damage (Shinoyama et al., 2013; Péron et al., 2017; Tornero et al., 2013). The precise mechanism of such improvement remains elusive. At present, human cerebral organoids have been successfully transplanted into rodent brains in efforts to further mature *in-vitro*-derived organoids *in vivo* (Daviaud et al., 2018; Mansour et al., 2018). Beyond this basic work, there may someday be translational transplantation of brain organoids, intended to rescue brain dysfunction. The analysis of Sakaguchi et al. could be useful for detecting function in such transplanted grafts.

Ethical Issues Concerning Cerebral Organoid Research

Two ethical concerns have been raised regarding the recent progress of cerebral organoid research. The first issue has to do with whether cerebral organoids created *in vitro* have any kinds of consciousness which should be morally considered, while the second ethical issue relates to how non-human animals with human-like brain functions resulting from the transplantation of human brain organoids should be treated.

Regarding the first ethical issue, Nita Farahany and colleagues suggest that, if cerebral organoids produced *in vitro* become more extensive and sophisticated, they may develop capabilities similar to human sentience (the ability to feel things, such as pleasure, pain, or distress, to store and retrieve memories, and to have a perception of

agency or an awareness of self) (Farahany et al., 2018; see also Lavazza and Massimini, 2018a, 2018b). Current cerebral organoids do not have mature neural networks or "proper" sensory input and motor output necessary for environmental interaction and reaction. Because it is very plausible that these networks and proper inputs and outputs are necessary for cognition, the probability that existing cerebral organoids have thoughts is extremely low (Farahany et al., 2018; Munsie et al., 2017). Nevertheless, Sorin Hostiuc and colleagues suggest that there may be other determinants of whether cerebral organoids have moral status. They attempt to evaluate the moral status of existing cerebral organoids along three dimensions—human origin, sentience, and the potential to generate individual human beings (Hostiuc et al., 2019).

Although skepticism about the possibility of cerebral organoids possessing traits such as consciousness, thoughts, and cognition, is common, the research findings of Sakaguchi et al. demand that these issues be taken seriously. The cerebral neural circuit created in their research was capable of inducing synchronous activity among specific cell populations. According to the Cell Assembly Hypothesis, this kind of neural activity can be the basis for various brain functions including perception and memory, and, from there on, higher brain functions, such as cognition and consciousness (Spatz, 1996). Thus, cerebral organoids produced by more sophisticated culture methods may require moral consideration.

Indeed, even if a cerebral organoid possesses consciousness, thinking, and cognition, it may not necessarily demand moral consideration. The philosophy of mind field classifies and defines different types of consciousness. Although there are others, this paper will examine only self-consciousness, phenomenal consciousness, and access consciousness, as the three forms of consciousness most likely to carry moral status. Self-consciousness entails having a concept of the self and being able to use this concept when thinking about the self, so many people would agree that self-consciousness is morally relevant (Singer, 2011; Tooley, 1972). Beings with self-consciousness have sophisticated cognitive capacity, and are currently thought to include only adult human beings and non-human primates with higher brain functions (Kahane and Savulescu, 2009; Shepherd, 2018a).

Phenomenal consciousness is regarded as the core concept of consciousness in academic literature, and access consciousness is often paired with phenomenal consciousness. Ned Block explored the relationship between phenomenal consciousness and access consciousness, and differentiated between them (Block, 1995). Notoriously, it is thought to be impossible to define phenomenal consciousness (Levy, 2014; Nagel, 1974), but it is characterized as a subjective experience with qualitative content such as pain, pleasure, and perception, and as "the kind of conscious such that there is something it is like to be phenomenally conscious" (Levy, 2014). Phenomenal consciousness is experienced by humans and non-human animals alike. Conversely, access consciousness is described as having "global access to information" (Kahane and Savulescu, 2009) and "a kind of availability of information contents" (Levy, 2014). A zombie, as presented in a well-known thought experiment, is a being which completely lacks phenomenal consciousness but has access consciousness (and self-consciousness) (Siewert, 1998).

We generally use which consciousness(es) a being possesses to decide how the being should be considered morally. The logic there is that a being's interests may differ depending on the being's consciousness. Differences in consciousness between beings reflect differences in interests the beings have. For example, a self-conscious being can have interests to pursue pleasure and avoid pain, fulfill desires, and seek life's objective goods (Kahane and Savulescu, 2009). Many agree as a result that self-conscious beings should be morally considered. Moreover, lower animals do not have self-consciousness but do have phenomenal consciousness. In other words, lower animals have only interests related to experiencing pleasure and avoiding pain. Recently, many people have ascribed moral significance to phenomenal consciousness and assigned moral status as a result of this consciousness. Even so, there is no consensus about the clinical significance of interests tied to a particular kind of consciousness. Some consider the interests of lower animals to warrant fullest moral consideration, while others do not. The picture is complicated by examples such as the hypothetical zombie with no phenomenal consciousness but self-consciousness and access consciousness. It remains controversial as to whether even phenomenal consciousness is morally significant (Kahane and Savulescu, 2009; Levy, 2014). Some have argued that phenomenal consciousness has no intrinsic value (Lee, 2018; Levy, 2014).

In light of the above, how should we consider the problem of consciousness in cerebral organoid research? Cerebral organoids without sensory tissues will not have self-consciousness, phenomenal consciousness, or access consciousness because such forms of consciousness require subjective experience. Without sensory input and motor output, even phenomenal consciousness will not appear, much less access consciousness or self-consciousness. Hence, current cerebral organoids are not be likely to have any kind of interests resulting from consciousness(es). However, if future improvement of culture conditions allows cerebral organoids to acquire mature human brain-like neural function and *in vitro* cerebral organoids develop consciousness requiring moral consideration, the research/clinical use of these cerebral organoids will become a

greater ethical challenge. As just one example, it will likely become necessary to consider how a cerebral organoid with morally relevant consciousness should be treated in comparison with animals who possess those same properties or to humans in persistent vegetative or minimally conscious states (Glannon, 2016; Lavazza and Massimini, 2018b; Levy and Savulescu, 2009; Shepherd, 2018b).

What of the second ethical issue relating to recent cerebral organoid research, that of the moral humanization of transplanted animals? Most non-human animals experience phenomenal consciousness, without human cerebral organoid transplantation. Many view the use of phenomenally conscious animals in scientific and medical research as justified. However, two complicating cases regarding cerebral organoids should be considered: (1) cerebral organoids have consciousness in some sense *in vitro*, and we transplant them into animals, and (2) cerebral organoids do not have consciousness *in vitro*, but they acquire it upon transplantation into animals. In both cases, transplanted animals acquire human-like consciousness. A problem looms as to whether or not to permit transplantation of any type of human cerebral organoids that may experience human-like consciousness before and/or after transplantation, and how to proceed with such studies. It may be morally permissible to *create* self-conscious animals by engrafting human cerebral organoids, but in the case the moral status of such animals should be carefully considered.

Implications for Future *In Vitro* and *In Vivo* Cerebral Organoid Research

We do not believe that all current cerebral organoid research—including basic research, experiments involving transplantation of organoids into non-human animals, and patient-specific modeling research—should be prohibited. However, as the field progresses additional ethical questions may arise. For example, research on fused nerve organoids that would attempt to connect cerebral organoids with other nerve tissues may become technically possible in the near future, and bring forth new ethical challenges (Birey et al., 2017; Xiang et al., 2017). The concept of fusing cerebral and thalamic organoids was recently published by Yangfei Xiang and colleagues (Xiang et al., 2019). They reported establishment of reciprocal thalamocortical projections. As the thalamus is the gate of all sensory input to the cerebrum, such a finding might have the potential to transmit sensory information to the neuronal tissue of the organoid. If the fused organoids are further fused with dorsal spinal cord and peripheral nerves, the organoid might have somatic sensory experience. If such fused organoids are further fused with neural retina and optic nerve tissues, they might recognize light. In that case, such fusion becomes technically achievable, and a problem may arise regarding whether to allow the creation and use of cerebral organoids that possess properties that significantly surpass the conventional framework. Should we permit the generation of cerebral organoids that possess somatosensory or vision capacity? As more sophisticated cerebral organoids are developed, we may also wonder whether it is acceptable to allow the use of cerebral organoids which feel pain for the development of drugs meant to manage or mitigate pain, and, if so, how to handle such organoids.

Besides these fused organoid experiments, ethical issues surrounding the moral status of even conventional *in vitro* cerebral organoids themselves may arise. As *in vitro* culture conditions continue to develop, the neural activity pattern of cerebral organoids may approach that of human brains *in vivo*. In such cases, it will be important to assess whether the *in vitro* organoids themselves have phenomenal or access consciousness. There may be "subpersonal correlates of pain and pleasure" that are responsive to stimulation in brain organoids—an organism may have neural activity corresponding to pain, without having any experience of it feeling like anything (Kahane and Savulescu, 2009). If it becomes possible to assess for these correlates in cerebral organoids, some may argue that such pains and pleasures also demand moral consideration. It may be necessary to consider how much such potential pleasures of an organoid should be taken into account, compared with those of a fetus, for example. Nonetheless, further neuroscientific findings are required to determine whether cell activity patterns are encoded into meaningful information, although there are also strong moral reasons to further develop differentiation induction and functional assessment techniques in brain organoid research.

Finally, *in vivo* experiments need to be conducted in accordance with existing ethical standards and oversight mechanisms. It has been suggested that such transplantation research should be conducted gradually and that any changes in the body and behavior of the host animal should be closely monitored (Farahany et al., 2018; Munsie et al., 2017). At present, all research engrafting any type of human cells into live animals is conducted following scientific and ethical standards for animal research. Indeed, the International Society for Stem Cell Research (ISSCR) has already recommended how to proceed with such studies. Specifically, to minimize unexpected distress and suffering in animal hosts, the ISSCR Ethics and Public Policy Committee recommends four steps: (1) the establishment of baseline animal data; (2) ongoing data collection during research concerning any deviation from the norms of species-typical animals; (3) the use of small pilot studies to ascertain any welfare changes in modified animals; and (4) ongoing monitoring and reporting to oversight committees authorized to decide the need for protocol changes and the withdrawal of animal subjects. These

four steps should also be considered when engrafting human cerebral organoids into animal hosts to proceed with such research in a careful and stepwise manner (Hyun, 2018; Hyun et al., 2007; ISSCR, 2016). Still, however, some might require the research field to more fully discuss why the moral humanization of animals matters morally before proceeding with any research that might result in it. Such conversation may yield additional oversight suggestions and requirements for research that may morally humanize animals.

Concluding Remarks

Similar to the impasse in the debate on the moral status of human embryos and the appropriate scope of their use in research (e.g., the 14-day rule), reaching agreement on the moral status of cerebral organoids and the implications for their use in research will likely be difficult. Difficult though it may be, the recent remarkable advances in organoid studies demand that we should put forth an opinion on the delineation of research, including whether the creation and use of cerebral organoids which possess specific functions is permitted, and which if any organoid functions should warrant discontinuation of a study. In the future, keeping in mind the perspective of the moral significance of consciousness, the moral status of cerebral organoids and the implications for their use *in vitro* must be considered not only by philosophers and bioethicists but by scientists as well.

AUTHOR CONTRIBUTIONS

T.S. and H.S. conducted the initial research. T.S. drafted the initial manuscript and made revisions. H.S., M.F., J.T., and E.T. provided feedback on drafts. T.S. and H.S. prepared the manuscript for submission. All authors approved the final manuscript as submitted.

ACKNOWLEDGMENTS

We are grateful to Neil Levy and César Palacios-González for their comments on earlier drafts of this paper. We would also like to thank a reviewer who gave us very insightful and constructive feedback on the paper. T.S. was funded by the JSPS KAKENHI Grant-in-Aid for Scientific Research (grant number 17K13843) and the Kyoto University Foundation. T.S. and M.F. were funded by the Uehiro Foundation on Ethics and Education. H.S. was funded by the JSPS KAKENHI Grant-in-Aid for JSPS Fellows (JSPS PD research fellowship, grant number 17J10294) and the JSPS KAKENHI Grant-in-Aid for Scientific Research (grant number 18K15046).

REFERENCES

Aggarwal, S., and Mortensen, O.V. (2017). Overview of monoamine transporters. Curr. Protoc. Pharmacol. *79*. 12.16.1–12.16.17. https://doi.org/10.1002/cpph.32.

Birey, F., Andersen, J., Makinson, C.D., Islam, S., Wei, W., Huber, N., Fan, H.C., Metzler, K.R.C., Panagiotakos, G., Thom, N., et al. (2017). Assembly of functionally integrated human forebrain spheroids. Nature *545*, 54–59.

Block, N. (1995). On a confusion about a function of consciousness. Behav. Brain Sci. *18*, 227–247.

Cugola, F.R., Fernandes, I.R., Russo, F.B., Freitas, B.C., Dias, J.L., Guimarães, K.P., Benazzato, C., Almeida, N., Pignatari, G.C., Romero, S., et al. (2016). The Brazilian Zika virus strain causes birth defects in experimental models. Nature *534*, 267–271.

Dang, J., Tiwari, S.K., Lichinchi, G., Qin, Y., Patil, V.S., Eroshkin, A.M., and Rana, T.M. (2016). Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. Cell Stem Cell *19*, 258–265.

Danjo, T., Eiraku, M., Muguruma, K., Watanabe, K., Kawada, M., Yanagawa, Y., Rubenstein, J.L., and Sasai, Y. (2011). Subregional specification of embryonic stem cell-derived ventral telencephalic tissues by timed and combinatory treatment with extrinsic signals. J. Neurosci. *31*, 1919–1933.

Daviaud, N., Friedel, R.H., and Zou, H. (2018). Vascularization and engraftment of transplanted human cerebral organoids in mouse cortex. eNeuro *5*. 0219–18.2018. https://doi.org/10.1523/ENEURO.0219-18.2018.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature *472*, 51–56.

Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K., and Sasai, Y. (2008). Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell *3*, 519–532.

Farahany, N., Greely, H., Hyman, S., Koch, C., Grady, C., Pașca, S.P., Sestan, N., Arlotta, P., Bernat, J.L., Ting, J., et al. (2018). The ethics of experimenting with human brain tissue. Nature *556*, 429–432.

Garcez, P.P., Loiola, E.C., Madeiro da Costa, R., Higa, L.M., Trindade, P., Delvecchio, R., Nascimento, J.M., Brindeiro, R., Tanuri, A., and Rehen, S.K. (2016). Zika virus impairs growth in human neurospheres and brain organoids. Science *352*, 816–818.

Glannon, W. (2016). The value and disvalue of consciousness. Camb. Q. Healthc. Ethics *25*, 600–612.

Hasegawa, Y., Takata, N., Okuda, S., Kawada, M., Eiraku, M., and Sasai, Y. (2016). Emergence of dorsal-ventral polarity in ESC-derived retinal tissue. Development *143*, 3895–3906.

Hostiuc, S., Rusu, M.C., Negoi, I., Perlea, P., Dorobanțu, B., and Drima, E. (2019). The moral status of cerebral organoids. Regen. Ther. *10*, 118–122.

Hyun, I., Taylor, P., Testa, G., Dickens, P., Jung, K.W., McNab, A., Robertson, J., Skene, L., and Zoloth, L. (2007). Ethical standards for human-to-animal chimera experiments in stem cell research. Cell Stem Cell *1*, 159–163.

Hyun, I. (2018). The ethics of chimera creation in stem cell research. Curr. Stem Cell Reports *4*, 235–239.

Ishida, Y., Kawakami, H., Kitajima, H., Nishiyama, A., Sasai, Y., Inoue, H., and Muguruma, K. (2016). Vulnerability of purkinje

cells generated from spinocerebellar ataxia type 6 patient-derived iPSCs. Cell Rep. *17*, 1482–1490.

International Society for Stem Cell Research (ISSCR). (2016). Guidelines for stem cell research and clinical translation. http://www.isscr.org/docs/default-source/all-isscr-guidelines/guidelines-2016/isscr-guidelines-for-stem-cell-research-and-clinical-translation d67119731dff6ddbb37cff0000940c19.pdf?sfvrsn=4.

Kadoshima, T., Sakaguchi, H., Nakano, T., Soen, M., Ando, S., Eiraku, M., and Sasai, Y. (2013). Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. Proc. Natl. Acad. Sci. USA *111*, 20284–20289.

Kahane, G., and Savulescu, J. (2009). Brain damage and the moral significance of consciousness. J. Med. Philos. *34*, 6–26.

Kuwahara, A., Ozone, C., Nakano, T., Saito, K., Eiraku, M., and Sasai, Y. (2015). Generation of a ciliary margin-like stem cell niche from self-organizing human retinal tissue. Nat. Commun. *6*, 6286.

Lancaster, M.A., and Knoblich, J.A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. Science *345*, 1247125.

Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., and Knoblich, J.A. (2013). Cerebral organoids model human brain development and microcephaly. Nature *501*, 373–379.

Lavazza, A., and Massimini, M. (2018a). Cerebral organoids: ethical issues and consciousness assessment. J. Med. Ethics *44*, 606–610.

Lavazza, A., and Massimini, M. (2018b). Cerebral organoids and consciousness: how far are we willing to go? J. Med. Ethics *44*, 613–614.

Lee, A.Y. (2018). Is consciousness intrinsically valuable? Philosophical Stud. *175*, 1–17.

Levy, N. (2014). The value of consciousness. J. Conscious Stud. *21*, 127–138.

Levy, N., and Savulescu, J. (2009). Moral significane of phenomenal consciousness. In Progress in Brain Research, Vol. 177, S. Laureys, N.D. Schiff, and A.M. Owen, eds. (Elsevier), pp. 361–370.

Mansour, A.A., Gonçalves, J.T., Bloyd, C.W., Li, H., Fernandes, S., Quang, D., Johnston, S., Parylak, S.L., Jin, X., and Gage, F.H. (2018). An in vivo model of functional and vascularized human brain organoids. Nat. Biotechnol. *36*, 432–441.

Muguruma, K., Nishiyama, A., Kawakami, H., Hashimoto, K., and Sasai, Y. (2015). Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. Cell Rep. *10*, 537–550.

Munsie, M., Hyun, I., and Sugarman, J. (2017). Ethical issues in human organoid and gastruloid research. Development *144*, 942–945.

Nagel, T. (1974). What is it like to be a bat? Philosophical Rev. *83*, 435–450.

Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M., and Sasai, Y. (2012). Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell *10*, 771–785.

Nasu, M., Takata, N., Danjo, T., Sakaguchi, H., Kadoshima, T., Futaki, S., Sekiguchi, K., Eiraku, M., and Sasai, Y. (2012). Robust formation and maintenance of continuous stratified cortical neuroepithelium by laminin-containing matrix in mouse ES cell culture. PLoS One *7*, e53024.

Odawara, A., Matsuda, N., Ishibashi, Y., Yokoi, R., and Suzuki, I. (2018). Toxicological evaluation of convulsant and anticonvulsant drugs in human induced pluripotent stem cell-derived cortical neuronal networks using an MEA system. Sci. Rep. *8*, 10416.

Ogawa, J., Pao, G.M., Shokhirev, M.N., and Verma, I.M. (2018). Glioblastoma model using human cerebral organoids. Cell Rep. *23*, 1220–1229.

Ozone, C., Suga, H., Eiraku, M., Kadoshima, T., Yonemura, S., Takata, N., Oiso, Y., Tsuji, T., and Sasai, Y. (2016). Functional anterior pituitary generated in self-organizing culture of human embryonic stem cells. Nat. Commun. *7*, 10351.

Péron, S., Droguerre, M., Debarbieux, F., Ballout, N., Benoit-Marand, M., Francheteau, M., Brot, S., Rougon, G., Jaber, M., and Gaillard, A. (2017). A delay between motor cortex lesions and neuronal transplantation enhances graft integration and improves repair and recovery. J. Neurosci. *37*, 1820–1834.

Putnam, H. (1981). Reason, Truth and History (Cambridge University Press).

Qian, X., Nguyen, H.N., Song, M.M., Hadiono, C., Ogden, S.C., Hammack, C., Yao, B., Hamersky, G.R., Jacob, F., Zhong, C., et al. (2016). Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell *165*, 1238–1254.

Quadrato, G., Nguyen, T., Macosko, E.Z., Sherwood, J.L., Min Yang, S., Berger, D.R., Maria, N., Scholvin, J., Goldman, M., Kinney, J.P., et al. (2017). Cell diversity and network dynamics in photosensitive human brain organoids. Nature *545*, 48–53.

Reardon, S. (2018). 'Mini brains' show humanlike activity. Nature *563*, 453.

Sakaguchi, H., Ozaki, Y., Ashida, T., Matsubara, T., Oishi, N., Kihara, S., and Takahashi, J. (2019). Self-organized synchronous calcium transients in a cultured human neural network derived from cerebral organoids. Stem Cell Reports https://doi.org/10.1016/j.stemcr.2019.05.029.

Sakaguchi, H., Kadoshima, T., Soen, M., Narii, N., Ishida, Y., Ohgushi, M., Takahashi, J., Eiraku, M., and Sasai, Y. (2015). Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. Nat. Commun. *6*, 8896.

Shepherd, J. (2018a). Consciousness and Moral Status (Routledge).

Shepherd, J. (2018b). Ethical (and epistemological) issues regarding consciousness in cerebral organoids. J. Med. Ethics *44*, 611–612.

Shinoyama, M., Ideguchi, M., Kida, H., Kajiwara, K., Kagawa, Y., Maeda, Y., Nomura, S., and Suzuki, M. (2013). Cortical region-specific engraftment of embryonic stem cell-derived neural progenitor cells restores axonal sprouting to a subcortical target and achieves motor functional recovery in a mouse model of neonatal hypoxic-ischemic brain injury. Front. Cell Neurosci. *7*, 128.

Shiraishi, A., Muguruma, K., and Sasai, Y. (2017). Generation of thalamic neurons from mouse embryonic stem cells. Development *144*, 1211–1220.

Siewert, C. (1998). The Significance of Consciousness (Princeton University Press).

Singer, P. (2011). Practical Ethics, 3rd Edition (Cambridge University Press).

Spatz, H. (1996). Hebb's concept of synaptic plasticity and neuronal cell assemblies. Behav. Brain Res. *78*, 3–7.

Suga, H., Kadoshima, T., Minaguchi, M., Ohgushi, M., Soen, M., Nakano, T., Takata, N., Wataya, T., Muguruma, K., Miyoshi, H., et al. (2011). Self-formation of functional adenohypophysis in three-dimensional culture. Nature *480*, 57–62.

Takata, N., Sakakura, E., Eiraku, M., Kasukawa, T., and Sasai, Y. (2017). Self-patterning of rostral-caudal neuroectoderm requires dual role of Fgf signaling for localized Wnt antagonism. Nat. Commun. *8*, 1339.

Tooley, M. (1972). Abortion and infanticide. Philos. Public Aff. *2*, 37–65.

Tornero, D., Wattananit, S., Grønning Madsen, M., Koch, P., Wood, J., Tatarishvili, J., Mine, Y., Ge, R., Monni, E., Devaraju, K., et al. (2013). Human induced pluripotent stem cell-derived cortical neurons integrate in stroke-injured cortex and improve functional recovery. Brain *136*, 3561–3577.

Trujillo, C.A., Gao, R., Negraes, P.D., Chaim, I.A., Domissy, A., Vandenberghe, M., Devor, A., Yeo, G.W., Voytek, B., and Muotri, A.R. (2018). Nested oscillatory dynamics in cortical organoids model early human brain network development. bioRxiv https://doi.org/10.1101/358622.

Watanabe, K., Kamiya, D., Nishiyama, A., Katayama, T., Nozaki, S., Kawasaki, H., Watanabe, Y., Mizuseki, K., and Sasai, Y. (2005). Directed differentiation of telencephalic precursors from embryonic stem cells. Nat. Neurosci. *8*, 288–296.

Watanabe, M., Buth, J.E., Vishlaghi, N., de la Torre-Ubieta, L., Taxidis, J., Khakh, B.S., Coppola, G., Pearson, C.A., Yamauchi, K., Gong, D., et al. (2017). Self-organized cerebral organoids with human-specific features predict effective drugs to combat zika virus infection. Cell Rep. *21*, 517–532.

Wataya, T., Ando, S., Muguruma, K., Ikeda, H., Watanabe, K., Eiraku, M., Kawada, M., Takahashi, J., Hashimoto, N., and Sasai, Y. (2008). Minimization of exogenous signals in ES cell culture induces rostral hypothalamic differentiation. Proc. Natl. Acad. Sci. U S A *105*, 11796–11801.

Xiang, Y., Tanaka, Y., Patterson, B., Kang, Y.J., Govindaiah, G., Roselaar, N., Cakir, B., Kim, K.Y., Lombroso, A.P., Hwang, S.M., et al. (2017). Fusion of regionally specified hPSC-derived organoids models human brain development and interneuron migration. Cell Stem Cell *21*, 383–398.e7.

Xiang, Y., Tanaka, Y., Cakir, B., Patterson, B., Kim, K.Y., Sun, P., Kang, Y.J., Zhong, M., Liu, X., Patra, P., et al. (2019). HESC-derived thalamic organoids form reciprocal projections when fused with cortical organoids. Cell Stem Cell *24*, 487–489.e7.