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REVIEW

Human brain organoid transplantation: ethical implications of enhancing specific cerebral functions in small-animal models [version 1; peer review: 1 approved]

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Abstract

Brain organoids are self-organizing, three-dimensional tissues derived from pluripotent stem cells that recapitulate many aspects of the cellular diversity and architectural features of the developing brain. Recently, there has been growing interest in using human brain organoid transplantation in animal models as a means of addressing the limitations of *in vitro* culture, such as the lack of vascularization, and to explore the potential of organoids for neural repair. While there has been substantial debate on the ethical implications of brain organoid research, particularly the potential for organoids to exhibit higher-order brain functions such as consciousness, the impact of human organoid grafts on animal hosts has been less extensively discussed. Enhancement of host animal brain function may not be technically feasible at this time, but it is imperative to carefully consider the moral significance of these potential outcomes. Here, we discuss the ethical implications of enhancing somatosensation, motor processes, memory, and basic socialization in small-animal models.

Open Peer Review

Approval Status ✓

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version 1 06 Jun 2023	✓ view

1. **Bernard Baertschi**, University of Geneva, Geneva, Switzerland

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We consider the moral implications of such outcomes and if safeguards are needed to accommodate any increased moral status of animals transplanted with human brain organoids.

Keywords

Brain organoids, transplantation, enhancement, ethics
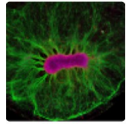
 This article is included in the The Ethics of Brain Organoids collection.

Introduction

Human brain organoids are stem cell-derived three-dimensional tissues that self-organize in a manner that recapitulates the architecture and cell type diversity of the developing human brain[1]. These lab-grown tissues can overcome constraints that have previously limited the study of human neurodevelopment, including the limited availability of human brain tissue and differences in neurogenesis across species that limit generalization from animal models to humans. In addition, the use of patient-derived induced pluripotent stem cells (iPSC) has enabled the modeling of specific diseases, allowing for greater insight into underlying mechanisms and opening the door to the discovery of novel therapeutics[2]. Various directed differentiation protocols have generated a host of brain region-specific organoids, including hippocampal[3], dorsal forebrain[4,5], thalamic[6], hypothalamic[5], cerebellar[7], and midbrain organoids[5,8]. More recently, subregion-specific organoids have been developed such as hypothalamus arcuate nucleus[9], prefrontal[10] and thalamic reticular nucleus organoids[11]. These systems also have enabled studies of comparative biology, revealing functional and developmental differences across species[10]. Furthermore, organoids represent an attractive alternative to scaffold-based grafts as a substrate for transplantation-based neural repair.

Recent studies have advanced from studying brain organoids in a dish to grafting them in small-animal models to observe their maturation and development *in vivo*[12–15]. Transplantation of human brain organoids has become a subject of increased research attention in two broad categories. The first category aims to enhance the study of neurodevelopment and disease modeling, including neuropsychiatric conditions, by facilitating the long-term growth of organoids and overcoming the technical challenges inherent in *in vitro* experiments, such as reduced nutrient supply to the interior of the organoid and limitations in oxygen diffusion[12–14]. The second category focuses on developing organoid-based neural repair strategies with the ultimate goal of translating such therapies for the treatment of conditions such as traumatic brain injury, stroke, and neurodegenerative conditions[15,16] (Figure 1).

Several studies have demonstrated that human brain organoids can structurally and functionally integrate with the native circuitry of the host brain[12–16]. Some studies also suggest that organoid transplantation improves specific brain functions following injury, although the mechanism underlying these changes are unclear[17]. Such advances have made organoids more useful as a platform for studying disease and brain repair. However, these same developments highlight the need for further ethical consideration of the possibility of meaningful gain-of-function in animal hosts related to the introduction of human organoid grafts. In particular, the ability of organoids to mimic later stages of development[18] and replace host tissue with human neural tissue in the case of injury repair[15] raises the potential for some degree of "humanization" of the host animal brain.

In a previous article, we discussed the possible meanings of the term "humanization" in transplantation of human organoids into small animals as well as the physical constraints that make it highly improbable that an organoid graft could instantiate higher-order cerebral function within rodent hosts approaching consciousness[19]. In addition, we described a hierarchy of host function that could potentially be enhanced with basic neurological functions at its base, cognitive functions within the middle tier, and higher-level capacities such as self-awareness at the apex[19]. However, we did not delve into the specific ethical considerations of individual functional enhancements should they become a reality. As the field of organoid transplantation is advancing at a rapid pace, it is prudent to discuss the import of changes in host animal brain function in the context of ethical principles and animal welfare.

In this paper, we examine the ethical implications of the potential "humanization" of specific rodent brain functions resulting from human organoid transplantation. We consider what may be regarded as meaningful host enhancement and discuss metrics by which these changes can be measured. Our focus is primarily on the possibility of affecting functions associated with the bottom and middle tier of the previously described hierarchy, given the much less likely prospect of significantly influencing self-awareness through organoid transplantation. We discuss the consequences of enhancing the somatosensation, motor system, episodic memory, and social behavior of a host animal resulting from integration with a human brain organoid (Figure 2).

Somatosensation

Somatosensation comprises a variety of sensory modalities, including proprioception and the perception of temperature and touch[20]. Studies have shown that these modalities are distributed across multiple regions of the brain[21]. Both humans and mice have primary and secondary somatosensory areas, although the number of subdivisions and complexity of interconnections in the somatosensory cortex differ between species[22]. For example, humans have additional parietal fields for processing sensory input, and more cortico-cortical connections within the somatosensory cortex[23]. Given the distributed nature of sensory processing, particularly in humans, it may be difficult for organoid transplantation alone to meaningfully alter high-level somatosensory processing. Nevertheless, it is instructive to consider the functional and ethical implications of the hypothetical enhancement of somatosensation in rodents because organoid transplantation in rodent somatosensory cortex is already being performed[13] and it may be easier to affect this basic neurological function than other more complex functions.

The human brain can acquire and mediate sensory abilities that exceed those of any other species[24]. Both a larger brain, particularly an increase in the size of the neocortex, and a more complex functional organization of the human somatosensory cortex contribute to the uniquely sophisticated sensory skills of humans relative to other animals[25]. While the somatosensory cortex in humans and small animal models both contain six cortical layers, human supragranular pyramidal neurons feature twice the number of synapses compared to those in rodents[26]. This ability to handle a greater number
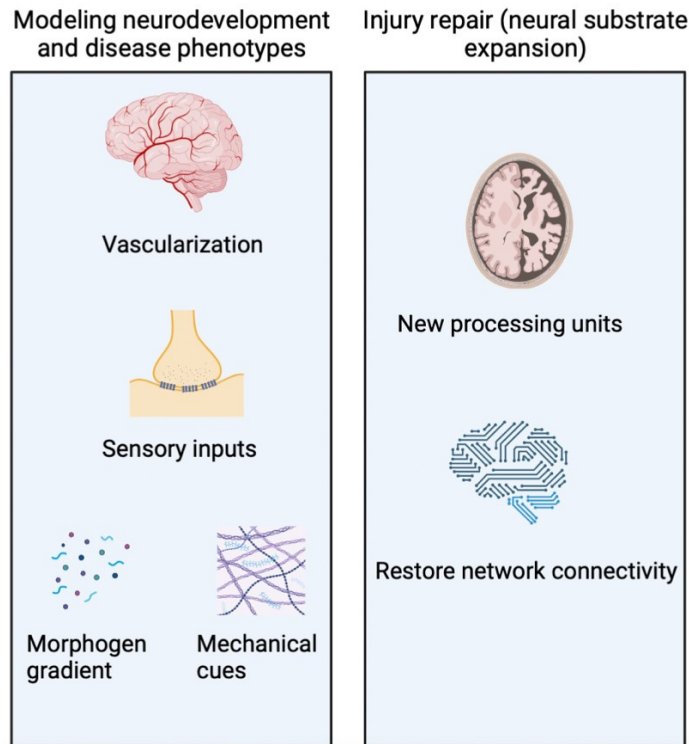
Figure 1. Rationale for human brain organoid transplantation into small-animal models. *Left:* Modeling neurodevelopment and disease phenotypes for therapeutic development and elucidation of disease mechanism. Transplantation of organoids allows for vascularization, provides sensory inputs, and exposes organoids to chemical and mechanical cues from the host. *Right:* Injury repair through neural substrate expansion. Organoids could facilitate neural repair through the addition of new processing units or restoration of network connectivity.
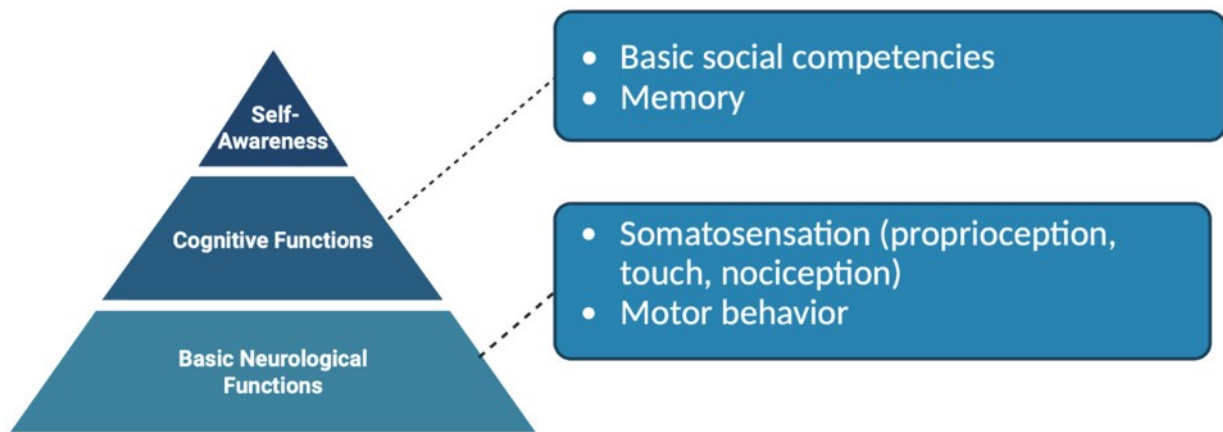
Figure 2. Schematic illustrating the tiers of brain functions that could undergo enhancement by neural transplantation. The ethical implications are enhancing brain functions in the lower- and middle-tiers are discussed in this paper. It is highly improbable that an organoid would contribute to the development of self-awareness and/or consciousness.

of inputs on dendrites offers increased computational capacity in humans compared to rodents within the same volume of brain tissue[27]. Therefore, it could be the case that rodents

with an organoid graft in somatosensory cortex have enhanced information processing capacity of functional inputs. Additionally, the effects of such a transplantation paradigm depend

on the pattern of integration exhibited by the organoid graft. If the organoid graft were to also recapitulate patterns of human somatosensory neuroanatomy, it is likely that it would feature increased organizational subdivisions and communicate with other cortical regions. This combination of increased capacity to integrate multiple inputs per neuron, greater functional delegation, and higher organizational complexity could increase the chances of neurological and behavioral changes based on finer perception of sensory stimuli.

We contend that enhanced processing of sensory stimuli in and of itself would not necessarily be ethically problematic. Sensory sensitivity has not traditionally been used as a criterion for determining moral status. For example, while bats have highly-developed sensorimotor control relative to other animals, they are not given correspondingly greater ethical consideration for that reason. Furthermore, sensory systems are known to change over an organism's life as a result of normal development and aging. Moral status is not adjusted based on such variation. However, one scenario in which enhanced sensory perception in transplanted animals may warrant greater ethical consideration is if the organoid grafts contribute to heightened perception of pain or to the enhancement of other higher-order functions (see below). Increasing a rodent's capacity to perceive pain would almost certainly be maladaptive. Nociceptive pain is thought to have evolved as an "early-warning system" against damaging stimuli and normally plays a protective role[28]. Pain hypersensitivity, however, is generally considered pathological in that it leads to an excessive stress response to typical stimuli[29]. A heightened sensation of pain could discourage normal degrees of physical contact and movement, therefore increasing the likelihood of animal suffering.

An even more severely maladaptive outcome of altering the somatosensory system would be the induction of a pain syndrome. These syndromes encompass a broad category of severely disabling conditions in which pain is felt to a disproportionate degree and duration, often in the absence of stimuli. Prior animal models of pain syndromes such as phantom limb pain are ethically complex because they model pain that occurs in the absence of input from the peripheral nervous system and consistently produce self-mutilation of the affected limb or region in rodents[30]. If somatosensory enhancement with human organoid grafts were to produce this type of severe gain of function in pain sensation, similar objections could be raised.

The ethical principles relevant to heightened pain responses in research animals are well described with Russel and Burch's "3 R's Principle" (i.e., reduction, replacement, and refinement) being among the most well-known[31]. "Refinement," describing the ethical obligation to minimize suffering, may be most applicable to this discussion. If the modification of somatosensory function associated with neural transplantation resulted in extraordinary pain, then the importance of "refinement of scientific procedures and husbandry to minimize suffering" would be elevated. More recently, the "Minimization Principle" has been described, which dictates that researchers not only strive to minimize suffering but also consider the value of the research being conducted relative to the magnitude of pain caused to animals in the research process[32]. In other words, even if animal suffering is reduced to the greatest extent possible in the context of a given experiment, it remains unethical to proceed with the experiment if the significance of the work is questionable. In the case of somatosensory enhancement, proper consideration of these ethical principles would require assessment of the pain experienced by transplanted animals as well as the expected scientific value of the study being performed.

How might pain responses be monitored following neural transplantation? A common challenge in monitoring pain in research animals is the inability to use subjective reports of pain intensity, such as affective and emotional assessments. Furthermore, it has been shown that behavioral expressions of chronic pain may be species-specific; human reactions to pain syndromes may not be generalizable to rodents[33]. Nonetheless, established tools for identifying acute pain in rodents, such as the Rat and Mouse Grimace Scales[34], may be used to quantify acute pain hypersensitivity. In addition, established signs of poor function in rodents remain valuable, including weight loss, hunched appearance, poor grooming, lethargy, and lack of appetite. While less tangible outcomes such as depressive symptoms and anxiety may prove difficult to detect, animal behavior should be carefully monitored and benchmarked against experimental controls.

Motor function

The motor cortex is instrumental in both the execution of movements as well as the learning of motor behaviors. For example, it plays a central role in the well-developed innate manual dexterity of primates by coordinating independent joint and digit movements. Learned skills require repeated rehearsal to organize an individual's existing movement repertoire into novel sequences that can be reproducibly executed to perform a given task[35]. In this context, the motor cortex is often considered to be a "controller" with motor cortical synapses being strengthened to encode skilled movements[36].

Before considering how organoid transplantation could potentially enhance motor function and the ethical import of such changes, it is worthwhile to first review species differences in this domain. Primates in general are capable of an exceptionally wide range of skilled movements compared to rodents. By decomposing a complex motor behavior into a sequence of simple actions, primates are able to learn and refine the execution of elaborate motor actions[35]. For example, athletes and musicians practice long sequences to enable seamless, stereotyped execution. The primary motor cortex (M1) plays a major role in such motor learning with cellular responses changing throughout a learning process[37]. In addition, the medial motor areas in primates, including the supplementary motor area (SMA) and the pre-supplementary motor area (pre-SMA), have been shown to work in concert with M1 to facilitate such movement sequences[38].

Primates also demonstrate a high degree of manual dexterity. The primate M1 has been shown to contain rostral and caudal subdivisions, with neurons in the caudal region making monosynaptic connections with motor neurons in the ventral horn of the spinal cord. In contrast, rodents, cats, and some monkeys lack these subdivisions as well as direct input from M1 to spinal motor neurons. Instead, the descending pathways from the cortex primarily terminate on interneurons[39]. Rodents also lack many of the primate cortical specializations that underpin visuomotor skills and advanced dexterity. In contrast, the rat motor cortex is disproportionally large and comparatively resilient; large lesions are often needed to produce functional motor deficits[40].

If an organoid graft into rodent M1 had direct access to spinal motor neurons, bypassing the interneurons in the spinal cord, the host animal could potentially achieve increased dexterity, enabling the execution of more highly skilled movements. These direct, monosynaptic corticospinal connections are believed to be instrumental in hand dexterity control in higher primates[41] and may therefore contribute significantly to enhancement of fine motor skills in a chimeric animal. For example, studies have shown that rodents have low levels of success on precision grip tasks, which involve retrieval of small pellets using the forepaw[42]. By comparison, macaques have success rates approaching 100% on the same task[43]. In addition, the incorporation of primate-specific motor cortical structures such as the SMA and pre-SMA may increase the capacity for motor learning, particularly conditional behavioral learning[44].

Overall, these considerations describe the conditions necessary to endow rodents with more human-like motor function, such as enhanced motor dexterity and an increased capacity for motor learning. These enhancements could better equip transplanted animals to navigate their environment, an outcome that is not necessarily problematic from an ethical perspective. Enhancement strictly limited to basic motor functions, without effects on other domains, would be remarkable but would not necessarily demand greater moral consideration. We contend that simple dislike of the "unnatural" is not a reasonable ethical objection since it is inherently normative. Furthermore, increases in manual dexterity or motor learning are unlikely to directly contribute to characteristics that are traditionally considered to be most relevant in determining moral status, such as sentience, agency, and self-awareness. In addition, these transplanted animals may *prima facie* have a better life than if they had not had their motor skills enhanced.

Basic motor function should be assessed to ensure that that no undue harm is being done to transplanted animals such as unanticipated loss of function. On the other hand, monitoring motor function is also necessary because changes in basic motor function may be an early indication that other domains such as empathic responses related to mirror neurons[45] (see below) need closer examination. The rotarod, horizontal bar, static rods and parallel bars tests are applicable tasks for assessing motor behaviors in rodents with organoid grafts[46].

Memory

The hippocampus is one of the most extensively studied brain regions and has been the site of many landmark discoveries in neuroscience, including adult neurogenesis[47,48]. In addition, hippocampal dysfunction represents a relatively common sequeula of traumatic brain injury which often has profound functional implications[49]. Hippocampal organoids recently have been created to facilitate disease phenotyping and drug screening[50] as well as cell-based hippocampal repair studies[51]. For example, organoids have been used to identify common pathological phenotypes in neuropsychiatric conditions such as Autism Spectrum Disorder (ASD)[52] and bipolar disorder[53]. Given the possibility of hippocampal transplantation in small animal models, as well as continued progress in the methods for patterning hippocampal organoids[54], it is prudent to consider the implications of enhancing rodent memory with human neurons.

This discussion will focus on episodic memory, as it is a critical component of the human experience[55]. While rodents can encode episodic memory, including "what," "when," and "where" information[56], it is generally accepted that autonoetic awareness is a uniquely human capacity. Autonoetic awareness is the ability to "mentally time travel," contextualizing one's own past and future experiences as well as considering hypothetical alternatives[57]. Although experiments have demonstrated that rodents can engage in retrospective and prospective thinking to make decisions[58], this capacity has only been observed in the context of food reward. The human capacity for autonoetic awareness is much more expansive. However, the subjective nature of episodic memory makes it challenging to study in animal models. The mechanisms underlying mental time travel are therefore not fully understood.

Given the complex and highly distributed nature of memory processes[59], it is improbable that hippocampal organoid transplantation could cause the emergence of autonoetic awareness in rodents. For example, there is evidence that the parietal cortex may be essential to autonoetic memory[60]. It is more likely that a transplanted organoid would participate in circuitry associated with pre-existing memory processes rather than create *de novo* functions. For instance, place memory is considered to be mostly hippocampal-dependent[61] and could be affected in some way by human hippocampal organoid transplantation.

Regardless, let us consider the moral implications of endowing a rodent with autonoetic awareness. The capacity to consciously relive past events and anticipate future ones may open unique avenues for benefit or harm. Enhancement of an animal's ability to remember and process distant experiences may exacerbate trauma associated with repeated experimentation, prompting further consideration of the ethical implications of conducting such research. For example, consider a behavioral task involving a rewarding or aversive stimulus. Instead of a simple implicit expectation, the animal may now experience much more elaborate anticipatory pain or pleasure, essentially re-living sensory aspects of prior exposures.

By providing a rodent with the ability to remember the past and anticipate the future, autonoetic awareness likely would increase an animal's moral significance. The intuitive-level rules of utilitarianism indicate that an increased sense of one's own past and future would increase the moral consideration given to an entity[62]. For example, the ability to consciously remember the past and anticipate the future may contribute an added capacity for happiness or pain in comparison to beings that live entirely in the present. From a utilitarian perspective, the increased complexity of desires and plans for the future may require that the lives of individuals with autonoetic awareness have greater moral significance.

Another possible consequence of organoid transplantation is the enhancement of fear learning and memory. A recent study demonstrated that mice transplanted with organoids demonstrated increased fear conditioning, as reflected in an increased freeze time in fear conditioning tests[14]. The potentiation of fear memory acquisition represents a possibly harmful outcome for transplanted animals, particularly in a laboratory setting. Similar to the case of enhanced autonoetic memory, these animals may suffer more greatly as a result of behavioral experiments and functional testing. Notably, this study observed long subcortical glutamatergic projections from relatively small organoid grafts, indicating that even neural grafts of a limited size are capable of eliciting a degree of behavioral changes in transplanted animals.

It is not possible to directly test the capacity for autonoetic consciousness in non-verbal animals since we are unable to obtain self-reports of their experiences. However, some aspects of episodic memory are certainly testable. Notably, experiments have examined food caching behaviors in scrub jays, indicating that these birds satisfy three of the primary criteria of episodic memory: they are able to recall the "what", "where", and "when" of specific episodes[63]. A more recent study applied this experimental paradigm to investigate episodic-like memory in rats, demonstrating that they are similarly able to discriminate what, where, and when[64]. More difficult versions of such recall tasks may be used to probe for enhancement of episodic memory in rats, although it may remain impossible to directly assess autonoetic awareness. By comparison, the enhancement of fear memory acquisition is more easily measured using conventional fear conditioning tasks[14].

Social behavior

The capacity for complex socialization is a defining characteristic of human nature. Humans engage in uniquely broad and sophisticated forms of social cognition including differentiating between others' motivations and their actions (belief-desire psychology), and learning and using linguistic symbols[65]. Difficulties in social functioning is considered to be a key criterion in many neuropsychiatric disorders such as ASD and schizophrenia, particularly impairment in social cognition[66]. Other species exhibit some of the basic aspects of social cognition, such as recognition of social cues from peers and behavior modification in response to these signals[67]. For example,

a feature that rodents share with humans is a tendency towards prosociality, defined as the preference for outcomes that benefit others[41].

Social cognition is a highly-distributed function involving many brain regions, including the medial prefrontal cortex, superior temporal gyrus, fusiform gyrus, posterior cingulate cortex, and amygdala, among others[68]. Augmentation of existing prosocial behavior following insertion of an organoid graft is therefore more probable than the instantiation of *de novo* social functions. Two brain regions in which engraftment of an organoid could potentially alter social behavior in transplanted animals are the somatosensory and primary motor cortices. The somatosensory system acts as a conduit for processing of sensory information[69] and is shown to play a causal role in prosociality[70]. The wide-ranging cortico-cortical connections that it forms enable the complex integration of somatosensation with higher-order behavioral and cognitive processes[69]. Consequently, transplanted human cortical organoids could elevate the role of somatosensation in similar higher-order cortical processing in a host animal, assuming that appropriate cortico-cortical connections are formed. For example, the human primary somatosensory cortex and its cortico-cortical connections are involved in empathetic processing when witnessing others experiencing emotion[71]. Small-animal models with human organoid grafts in the somatosensory areas could exhibit a pattern of activation that contributes to empathetic behavioral responses.

In the case of motor cortex transplantation, the incorporation of human neurons into rodent motor cortex may result in the enrichment of mirror neurons, assuming that similar connectivity forms between the graft and rodent hosts as in the human brain. This cell type, present in the SMA as well as the dorsal premotor cortex, is believed to map observed movements onto a motor program, thereby contributing to humans' ability to imitate[72]. It is thought that this mirroring mechanism is instrumental in basic forms of empathy, particularly in human infants and macaques[73]. Furthermore, mammalian mirror neurons have been implicated in "neuronal resonance", a phenomenon used to explain why one may cringe when witnessing someone else in pain[74].

Although the prospects of organoid transplantation leading to prosocial and/or more empathetic chimeric animals remain speculative, it is interesting to consider what our moral obligations would be towards these creatures. Promoting prosocial behaviors could be a great benefit to rodents, with individual animals now acting more frequently to benefit the welfare of their cagemates. However, a significant enhancement of empathic capacity may elevate the moral status of transplanted rodents such that they warrant ethical consideration more similar to that currently given to nonhuman primates (NHPs). While many animals possess rudimentary forms of empathy, the capacity for sophisticated empathic behavior has been described as a cornerstone of NHP moral status by some philosophers[75]. More specifically, mice demonstrate limited

forms of empathy while only NHPs and humans have cognitive empathy, which is defined as the capacity to recognize others' mental states[75]. An increased moral standing may necessarily restrict the experiments in which these animals are included. For example, it may be ethically impermissible to perform certain behavioral experiments with empathically enhanced animals. These animals may also feel greater distress by harm to cagemates and experience increased suffering after procedures involving prolonged social separation. Furthermore, compassion and sympathy have often been cited as fundamental moral motivations when differentiating between humans, NHPs, and other animals[76]. As early as 1871, Darwin reported on the remarkable similarities in human and monkey demonstrations of grief[77].

Increases in empathy in rodents can be monitored through several existing behavioral assays. Affective empathy can be observed through pain sensitivity tasks, in which pain response in rodents is measured after witnessing a cagemate displaying pain behavior[78]. Helping behavior has also been studied using tasks that involve a trapped rodent and assess the motivation of a cagemate to open the restrainer door and free the other rodent from the trap[79]. While these tests were originally designed to capture basic forms of empathy that have been observed in rodents, they may also be well-suited to detect increases in baseline empathic behavior following neural transplantation. Transplanted animals should similarly be monitored for changes in prosocial behavior and compassion following neural transplantation. Conventional assessment instruments for compassion rely upon questionnaires[80] posing obvious problems for use with any experimental animal model. However, several recent studies have used measures of neural activity in the medial orbitofrontal cortex, prelimbic cortex, lateral septum, anterior cingulate cortex, insular cortex, and nucleus accumbens as a read-out for prosociality, including electroencephalography[81] and *in vivo* calcium imaging[82]. Selective activation of the anterior cingulate cortex, insular cortex, and nucleus accumbens was highly correlated with helping behavior in rats to measure motivation and social reward[81]. These methods may be applied to brain chimeras to evaluate any changes in prosociality and compassion.

Conclusion

Neural transplantation holds great promise for neurological disease research and therapeutic development with organoids representing a particularly promising substrate. The neurological humanization of animal models has been extensively debated since as early as 2000, when Irving Weissman solicited input about performing experiments to create a "human neuron mouse"[83]. Organoid transplantation represents the next step in the evolution of neural transplantation. Pre-formed neural tissues are more physiologically relevant than dissociated cells, and *in vivo* organoid studies allow for enhanced maturation and the study of therapeutic applications. However, as organoids become more complex, it is essential to consider their potential impact on host brain functions after transplantation. In this article, we explored the ethical implications of changes in the bottom and middle tiers of brain

functions, since the impacts on higher-order function are less likely to result from organoid transplantation in small-animal models (Figure 2).

Given the highly-distributed nature of brain functions and large neuroanatomical differences between humans and rodents, there are inherent limits to how a focal graft such as an organoid could affect host rodent brain function. This consideration is especially relevant for more sophisticated functions, such as memory and social behavior, which involve a wide range of neuroanatomical structures and cell types. Organoid transplantation studies thus far have not demonstrated large-scale changes in host animal behavior, although these behavioral effects are understudied. Transplantation of cortical organoids into the retrosplenial cortex of adult mice resulted in no meaningful changes in spatial learning[12]. More recently, cortical organoids inserted into the somatosensory cortex of early-postnatal rats did not alter in a meaningful manner the memory capacity or fear responses of chimeric rats[13]. However, mice with organoids inserted into their medial prefrontal cortex did exhibit potentiation of fear conditioning responses[14].

Nonetheless, it is important for host animal to be monitored not only for enhancement of basic skills but also possible modification of more sophisticated behaviors. Altering one function, even considered to be basic, may affect another function given the complexity and interconnectedness of the brain. The ethical permissibility of working with these host animals should be predicated not only on the possibility of elevating moral status but also the likelihood of an intrinsically bad outcome such as causing undue pain or trauma. In all experiments that involve transplantation of human brain organoids into small-animal models, animal welfare should be carefully monitored and Institutional Animal Care and Use Committee best practices must be followed to prevent undue suffering.

Other topics will deserve greater attention as brain organoid technology advances and the ethical discussion on organoid transplantation evolves. One area of ethical inquiry concerning brain organoid transplantation is the use of large-animal models as hosts[84]. In larger animals, rather than setting absolute thresholds for "humanization," it may be important to consider changes relative to the baseline ability of the animal model. A second topic for consideration is the potential use of patient-derived stem cell lines to generate disease model organoids for transplantation studies. In such cases, the introduction of organoids with known severe defects in function may adversely affect animal hosts. Collectively, brain organoid transplantation offers meaningful advantages in the study of neurodevelopment and neural repair, but must be pursued with proper consideration for the animal hosts involved.

Data availability

No data are associated with this article.

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Open Peer Review

Current Peer Review Status: ✔

Version 1

Reviewer Report 18 October 2023

✔ **Bernard Baertschi**

French National Institute for Health and Medical Research (INSERM) Ethics Committee, University of Geneva, Geneva, Switzerland

This article examines the ethical implications of transplanting cerebral organoids into animals, asking in particular whether such grafts might constitute enhancements or humanizations. Such transplantation is necessary to vascularize and innervate these organoids, which have only limited survival in vitro.

The idea of improving the animal or making it semi-human easily stimulates the imagination of ethicists and has sometimes contributed to making them lose touch with what is actually feasible or plausible. The authors have avoided this pitfall by examining precisely four areas that are already or will soon be concerned: enhancing the somatosensation, motor system, episodic memory, and social behavior. The criteria for assessing whether or not such enhancements are acceptable are those used in the ethics of animal experimentation: are such enhancements likely to cause the animal suffering, or to alter its moral status? For example, enhancing the processing of sensory stimuli poses no problem for the animal's moral status, but could increase its perception of pain; on the other hand, improving the animal's memory by grafting human neurons could have an impact on its status and therefore on the moral issue of using animals in experimentation.

In the light of these classic ethical criteria (the 3Rs are also mentioned), the authors show that the transplantation of cerebral organoids into animals raises ethical questions, which are also classic, but to which it is necessary to be attentive. The degree of precision with which they are highlighted and argued is welcome and very useful for researchers. However, other issues are glossed over or excluded without much discussion.

In particular, it states: "We contend that simple dislike of the "unnatural" is not a reasonable ethical objection since it is inherently normative". But this is an objection often voiced against biotechnology, and it cannot be reduced to a simple "dislike". For some authors, there is a natural order that we do not respect when we, for example, create chimeras: "The idea of chimeras arouses serious feelings of rejection, suggesting that they would establish something utterly

wrong in the natural order: they should not exist [...]. It disturbs some essential order" (Hübner *et al.*, 2018[1]). That's what it's all about, even if the authors only use the term "chimera" once and seem to want to avoid it. I also don't understand the reason given here: "since it is inherently normative". The fact that an objection is normative in character does not deprive it of being an objection, quite the contrary: many debates are precisely normative in character, even if there are also some that are descriptive or conceptual. In reality, the debate on the natural seems to me to be more of an anthropological or metaphysical character, for what is at stake is the place and role of the human being in the world, and such a debate necessarily has normative consequences in dictating which actions are right and which are not.

Two final remarks:
1. On the subject of the 3Rs, it is stated that: "If the modification of somatosensory function associated with neural transplantation resulted in extraordinary pain, then the importance of "refinement of scientific procedures and husbandry to minimize suffering" would be elevated". This is not disputable, but there is a paradox here: the 3Rs are invoked to suppress or mitigate an effect of animal enhancement, but applying these principles would rather require not enhancing the animal beforehand.

2. It is asserted that: "The ability to consciously remember the past and anticipate the future may contribute an added capacity for happiness or pain in comparison to beings that live entirely in the present", a passage followed by the statement of consequences that a utilitarian might draw from this. But one possible consequence that is not mentioned is that if the felicific calculus were to say that there is more happiness than pain to be expected, then it would be morally required or obligatory to modify the animal to enhance it.

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Is the topic of the review discussed comprehensively in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Moral philosophy and bioethics

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.