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ARTICLE

# A Tale of Two Chimeras: Applying the Six Principles to Human Brain Organoid Xenotransplantation

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**Abstract**

Cerebral organoid models in-of-themselves are considered as an alternative to research animal models. But their developmental and biological limitations currently inhibit the probability that organoids can fully replace animal models. Furthermore, these organoid limitations have, somewhat ironically, brought researchers back to the animal model via xenotransplantation, thus creating hybrids and chimeras. In addition to attempting to study and overcome cerebral organoid limitations, transplanting cerebral organoids into animal models brings an opportunity to observe behavioral changes in the animal itself. Traditional animal ethics frameworks, such as the well-known three Rs (reduce, refine, and replace), have previously addressed chimeras and xenotransplantation of tissue. But these frameworks have yet to completely assess the neural-chimeric possibilities. And while the three Rs framework was a historical landmark in animal ethics, there are identifiable gaps in the framework that require attention. The authors propose to utilize an expanded three Rs framework initially developed by David DeGrazia and Tom L. Beauchamp, known as the Six Principles (6Ps). This framework aims to expand upon the three Rs, fill in the gaps, and be a practical means for assessing animal ethical issues like that of neural-chimeras and cerebral organoid xenotransplantation. The scope of this 6Ps application will focus on two separate but recent studies, which were published in 2019 and 2020. First, they consider a study wherein cerebral organoids were grown from donors with Down syndrome and from neurotypical donors. After these organoids were grown and studied, they were then surgically implanted into mouse models to observe the physiological effects and any behavioral change in the chimera. Second, they consider a separate study wherein neurotypical human embryonic stem cell-derived cerebral organoids were grown and transplanted into mouse and macaque models. The aim was to observe if such a transplantation method would contribute to therapies for brain injury or stroke. The authors place both studies under the lens of the 6Ps framework, assess the relevant contexts of each case, and provide relevant normative conclusions. In this way, they demonstrate how the 6Ps could be applied in future cases of neural-chimeras and cerebral organoid xenotransplantation.

**Keywords:** bioethics; brain organoids; chimeras; Six Principles; xenotransplantation

## Introduction

Calls are growing louder to address concerns over transplanting human cerebral organoids into animal models.[1,2,3] While cerebral organoid models in-of-themselves are considered as an alternative to research animal models, their developmental and biological limitations currently inhibit the probability that organoids can fully replace animal models.[4,5] Furthermore, these organoid limitations have, somewhat ironically, brought researchers back to the animal model via xenotransplantation, thus creating hybrids and chimeras. In addition to attempting to study and overcome cerebral organoid limitations, transplanting cerebral organoids into animal models brings an opportunity to observe behavioral changes in the animal itself.

Traditional animal ethics frameworks, such as the well-known three Rs (3Rs) (reduce, refine, and replace), have previously addressed chimeras and xenotransplantation of tissue.[6] But these frameworks have yet to completely assess, according to Sebastian Porsdam Mann, Rosa Sun, and Göran Hermerén,[7] the neural-chimeric possibilities and the "challenges that have not yet been fully analyzed." And while the 3Rs framework was a historical landmark in animal ethics, there are (as David DeGrazia and Tom L. Beauchamp show) identifiable gaps in the framework that require attention.[8,9]

Here, we propose to utilize an expanded 3Rs framework initially developed by DeGrazia and Beauchamp,[10,11] known as the Six Principles (6Ps). This framework aims to expand upon the 3Rs, fill in the gaps, and be a practical means for assessing animal ethical issues like neural-chimeras and cerebral organoid xenotransplantation. We will first present two studies that involve the use of chimeric models published in 2019 and 2020. We will consider a Rutgers University study wherein cerebral organoids were grown from donors with Down syndrome (DS) and from neurotypical donors. After these organoids were grown and studied, they were surgically engrafted into mouse models to observe the physiological effects and any behavioral change in the chimera. The other study we consider is from Kyoto University wherein neurotypical human embryonic stem cell (hESC)-derived cerebral organoids were grown and transplanted into mouse and macaque models. The aim was to observe if such a transplantation method would contribute to therapies for brain injury or stroke. After presenting these two studies, we will next present an overview of the 6Ps framework. Finally, using the 6Ps framework, we will assess the relevant contexts of each case, provide normative conclusions, and identify any similarities and inconsistencies across the two studies. In this way, we will address the following question: Based on a case comparative analysis with the 6Ps framework, is it morally permissible to transplant human cerebral brain organoids into animal models?

## The Two Chimera Cases

The two studies described here were initially chosen based on certain face value reasons. First, the studies were published recently and in high-impact peer-reviewed scientific journals. Second, one study was conducted in Japan while the other was conducted in the United States. As such, there may be important differences in both scientific governance and culture that ought to be considered. Third, the research animals used in the experiments differed. The study based in Rutgers University used only chimeric mice while the Kyoto University-based study used both mice and monkey chimeras. There may be an important distinction over the moral permissibility of using mouse chimeras versus monkey chimeras due to a difference in moral status. Fourth, the studies focus on different kinds of disability (developmental, in the case of DS, versus acquired, in the case of brain injury). Fifth, related to the previous consideration, the studies focused on different forms of medical solutions (preventative, in the case of DS, versus regenerative, in the case of brain injury). Before assessing these studies under the 6Ps framework, it is unclear whether these face value distinctions will show some form of meaningful impact.

### *The Rutgers Study*

For people with DS, an imbalance of excitatory and inhibitory neurotransmission is often regarded as one of the underlying causes for intellectual disability.[12] Special attention has been given to gamma-aminobutyric acid (GABA)ergic neurons, which produce an amino acid neurotransmitter called GABA.[13,14,15] This GABA neurotransmitter primarily acts as an inhibitory neurotransmitter in a typically developed brain. However, researchers are still debating how GABAergic neuron production is altered in people with DS.[16,17] Some studies, including one which we examine here, suggest OLIG2 gene expression could play a role "as regulators of GABAergic neuron production under normal and DS disease conditions."[18]

**Case 1. The Rutgers Study (Xu et al., 2019).** Ranjie Xu et al.[19] at Rutgers University (along with researchers from the University of Nebraska, University of Texas, Health Science Center at Houston, and Kent State University) made various DS and neurotypical (control) cerebral organoids from human induced pluripotent stem cells (hiPSCs). Furthermore, they transplanted these brain organoids into mice and performed behavioral studies. In particular, the study aimed to uncover "the role of human OLIG genes in regulating interneuron production" with a hope that novel therapies could follow. The findings of the 2019 study "suggest OLIG2 as an excellent potential target for developing personalized prenatal therapy for DS."[20]

After generating, genetically altering, and analyzing the cerebral organoids, they were transplanted into mice. Three behavioral tests were performed in random order: an open field test to measure basal global activity, elevated plus mase test to measure anxiety, and a novel object recognition test to measure learning and memory. The results of these behavioral tests only show a measurable reduction in novel object recognition in the DS chimeric mice, "suggesting that DS chimeric mice had impaired recognition memory."[21] All mice were euthanized after the behavioral tests.

### *The Kyoto Study*

According to Kitahara et al.[22] from Kyoto University, treatments for conditions resulting from brain injury or stroke are limited to medicine and rehabilitation, but neither of these solutions offer full "fundamental cures" to the condition. They do suggest that cell-replacement strategies could offer a "curative treatment for neurological deficits after cerebral cortex injury."[23] More specifically, human cerebral organoid transplantation may be an option for repairing cerebral cortex circuits given that cerebral organoids have a similar developmental process as an embryonic cerebral cortex. In order to know which developmental stage is suitable for transplantation, Kitahara et al.[24] transplanted hESC-derived cerebral organoids into both mice and macaque models and "compared the graft survival and axonal extension to the host."

**Case 2. The Kyoto Study (Kitahara et al., 2020).** The Kyoto study established two cerebral organoid groups based on the stages of callosal projection neurons (CPNs) and how well they represented the cerebral cortical development stages. Organoids 6-weeks after initial differentiation (6w-organoids) represented the developmental stage before CPN production, while organoids 10-weeks after initial differentiation (10w-organoids) represented the late developmental stage with a production of CPNs. Mouse pup populations were then split into two groups, half received 6w-organoids and the other half received 10w-organoids. Two craniotomy windows were opened, a small cavity of 1 mm was made into both sections of the cortex, and the cerebral organoids were placed into the cavities after being cut to dimension. 6-week-old mice underwent two surgeries. The first surgery opened the craniotomy windows, performed the lesioning, and closed the craniotomy window. After one week, the mice then received the 10w-organoids. The other six 6-week-old mice received the 10w-organoids immediately after lesioning.[25]

Researchers also transplanted the hESC-derived brain organoids (10w-organoids) into 3-year-old "purpose-bred male cynomolgus monkeys (*Macca fasclcularis*)" (n = 4). Citing ethical concerns, the Kyoto researchers only transplanted the 10-week organoids into the primary motor cortex ("bilateral precentral gyrus") of macaque brains. According to the researchers, transplanting the 6-week organoids increases the risk of cellular overgrowth, which would result in cranial compression. Furthermore, researchers tried to avoid affecting the higher brain functions by restricting the transplantation sites to the primary motor cortex. Researchers also noted that advancing this form of research would require addressing ethical questions, specifically whether animals can be humanized and if cerebral organoids *in vitro* can have consciousness.[26] All four macaques were euthanized at 12-weeks post-transplantation and their brains were further examined. Euthanasia was performed via transcardial perfusion while under deep anesthesia with pentobarbital. No behavioral studies were performed with either the mice or macaques.

## The 6Ps

### *What are the 6Ps?*

DeGrazia and Beauchamp propose a novel animal ethics framework called the 6Ps. These 6Ps are evenly divided into two core values: social benefits and animal welfare. The social benefit principles focus on the relationship between the possible human benefits an animal experiment may bring and the possible costs associated with the experiment. In all the 6Ps, "costs" should be taken to mean negative projected financial or opportunity impacts and "risks" refer to possible harms to humans. The animal welfare values refer to principles which primarily focus on well-being and possible harms to the animal in question. Each principle within these values is necessary for animal research to be justified. If one of the principles go

unfulfilled, the research may not be considered morally permissible.[27] In the following sections, we will further present each of the 6Ps in more detail. All definitions are provided in the Supplementary Material.

### *Why the 6Ps?*

#### *Moving beyond the 3Rs*

According to DeGrazia and Beauchamp,[28] while the traditional 3Rs themselves are a good first step toward ethical animal research, the framework itself has gaps and limitations. For instance, while the 3Rs certainly, and admirably, heavily consider animal welfare, the context of this focus is limited only to humane experimental technique. But this, according to DeGrazia and Beauchamp, limits the welfare consideration only to the experimental protocols and does not extend to other important elements of animal welfare such as humane transport, housing, feeding, and companionship.[29] Furthermore, DeGrazia and Beauchamp contend that there are omissions of ethical considerations pertaining to human social benefits, such as the genuine possibility of achieving a proposed benefit via animal research or if such a benefit outweighs the anticipated costs and risks associated with the research.[30] It is important to note that while DeGrazia and Beauchamp do criticize the 3Rs, the 6Ps are not meant to be a rebuttal of the framework. Rather, they are meant to incorporate the 3Rs, fill in the gaps, and provide a next step in animal ethics.

#### *The 6Ps in organoid ethics literature*

The 6Ps framework is already being incorporated in organoid ethics literature. Julian Koplin and Julian Savulescu integrate the 6Ps as part of their brain organoid ethical framework. More specifically, they suggest applying a modified version of the 6Ps at a stage when brain organoids (potentially) develop consciousness "(e.g., equivalent to 20 weeks' *in vivo* brain development or more)."[31] This is in addition to regulations and ethical frameworks for stem cells which are applied at earlier stages of brain organoid development. Although the 6Ps framework has been applied to brain organoids, it has yet to be applied to human brain organoid chimeras until now.

## Looking Through the Lens

### *The Principle of No Alternative Method (1st Principle)*

Consistent with the Principle of No Alternative Method, the use of animal models is only acceptable if the knowledge or social benefits derived from the experiment cannot be obtained from another more ethically acceptable alternative method. According to Beauchamp and DeGrazia, sentient animals (and possibly other entities) have a moral status, and because they have a moral status such animals should not needlessly be the subject of experiments that may harm them. This principle is similar to the concept and practice of replacement in the 3Rs. However, researchers have a stronger obligation to not only merely consider possible replacements to animal models but to actively seek out and implement viable alternatives. In this way, the Principle of No Alternative Method goes further and demands more than what replacement in the 3Rs requires.[32] It should be noted that the European Union Directive 2010/63/EU, United States federal legislation such as the Animal Welfare Act, and the Japanese Ministry of Education, Culture, Sports, Science and Technology all afford moral status to animals to some degree.[33,34,35] These degrees may differ for certain animals such as nonhuman primates.

#### *The Rutgers study (1st Principle)*

In terms of no alternative method, the Rutgers study could have curtailed the experiment before xenotransplantation to achieve certain aims of their research, but not all. More specifically, if the researchers' particular focus was at the embryonic stage, there exist embryoid models that could be used in some capacity. Granted, embryoid models are not without ethical controversy. But ethicists have already begun to turn their attention toward these models and provide some guidance.[36,37,38,39,40]

However, certain necessary and valuable aims of the study could not be achieved in any other morally acceptable way. Certainly, studying the behavioral outcomes of a post-brain organoid transplantation

could not be studied otherwise. Furthermore, it would not necessarily be possible to identify if any alterations in OLIG2 gene expression, and the subsequent GABAergic neuron production and neurotransmission, would affect cognitive capabilities associated with intellectual disability in DS.

### *The Kyoto study (1st Principle)*

Based on the stated aims of the Kyoto study, there appears to be no morally acceptable alternative method to assess the viability of a brain organoid transplantation as a technique which could be used for regenerative medicine purposes in the clinic. Indeed, the use of mouse models seemed to be necessary to minimize the risks posed to the monkeys used later in the experiment. Testing the neurophysiological differences between the 6-week organoids and the 10-week organoids after transplantation in mouse models exemplified the risks to cellular overgrowth and cranial compression.

As for the macaque models, the Kyoto researchers do state that, "when considering clinical application, primate transplantation models are necessary."[41] There is some general ethical debate over first-in-human organoid transplantation trials.[42,43,44,45] It may be argued that human brain organoid transplantation for human beings poses more significant risks to persons than other kinds of organoid transplantation due to the possible impacts on behavior, identity, and cognitive functionings. If we want to mitigate the significant risks inherent to brain organoid transplantation for first-in-human trails, then it may well be necessary to utilize chimeric models to an extent to refine the technique.

## *The Principle of Expected Net Benefit (2nd Principle)*

There are several fundamental questions which need to be addressed under the Principle of Expected Net Benefit. First, how significant is the expected benefit to society? Second, how likely is this benefit to be realized and achieved? Estimations of this likelihood might be based on the frequency with which comparable research has previously yielded or failed to yield benefits. An important note mentioned by DeGrazia and Beauchamp is that the cost–benefit analysis that comprises this principle does not fully consider (if at all) the weight of the moral status of animals, even if they are assumed to have a moral standing.[46] This is done out of a matter of simplicity for this principle but is addressed in the next principle. The cost–benefit analysis in question only focuses on costs to human beings while comparing them to the prospective benefits to society. Furthermore, as we assess the studies on their prospective net benefits, we will not take into consideration the reported results of the studies as this would cloud the assessment via *ad hoc* reasoning.

### *The Rutgers study (2nd Principle)*

First, the Rutgers experiment scientifically aims to understand "the mechanisms underlying cognitive deficit in DS" in particular by examining "the role of OLIG genes in regulating interneuron production."[47] What makes this aim significant is the additional stated hope that understanding this OLIG genetic mechanism "may be helpful in devising novel therapeutic strategies."[48] More specifically, these prospective novel therapeutic strategies would be for DS embryos. Broadly speaking, the prospective benefits of the study are two-fold: knowledge and innovative therapy. Specific knowledge from the experiment would add to the greater body of knowledge on DS human brain development and would eventually lead to innovative embryonic therapies for DS. The researchers do not state outright the possible costs or risks to human persons associated with either foregoing the experiment or failing to advance an innovative embryonic therapy for DS, although they do mention cognitive deficits associated with DS.

How significant are the prospective benefits gained from the Rutgers experiment? At face value, it could be argued that if the results of the Rutgers experiment were positive, it could be deeply impactful for prospective parents of infants with DS. But on further examination, even if innovative gene therapies for DS were to eventually result from this work, it is unclear if the whole DS community would view these therapies as significant, meaningful, or warranted. One survey of parents of children with DS indicates that 50.9% of parents hold a positive view on interventions that would "silence" the extra chromosome causing DS.[49] This split-view on silencing the extra chromosome may also apply to

the stated aims of the Rutgers experiment, which further suggests that dialogue between researchers and the DS community is necessary to elucidate the real benefits of gene therapy research for those with DS.

How likely are the knowledge and therapeutic benefits to occur from this experiment? Given that just one experiment, as cited by Xu et al., previously attempted similar aims, it is difficult to fully ascertain the likelihood that these benefits will occur.[50] Any genetic therapy benefit that may arise from the study will need to undergo significant testing and regulatory analysis before introduction into the clinic, which could take many additional years. This research is not aiming to save lives, nor is it an attempt to cure DS. It is aimed at attempting to improve cognitive capacities, and thus improving the quality of life to some degree, of individuals who would be born with DS. But the extent the improvements in both cognitive capacities and quality of life that could occur are unknown, and possibly speculative. In a way, the experiment is assessing the extent to which these possible improvements could be realized—the very knowledge needed for ethical assessment under this principle.

### *The Kyoto study (2nd Principle)*

In the Kyoto study, the stated scientific aim is "to clarify which developmental stage of cerebral organoids is suitable for transplantation" as well as evaluating "the effects of the transplantation site and host brain environment."[51] The researchers hope the study could have an impact on treatment for certain brain injuries or strokes by further developing a method of transplantation. There is no mention of potential costs or risks associated with forgoing these chimeric experiments either.

How significant are the prospective benefits from the Kyoto study? If the Kyoto study is successful in its stated aims, the results would be a meaningful step toward using patient-derived brain organoids as treatments for brain injury or stroke. It would be proof of concept that such a regenerative medicine technique is possible but would still require more experiments (chimeric or otherwise) to move beyond such a stage. The experiment itself would not result in the immediate introduction of the technique into the clinic.

How likely are the prospective benefits to occur from the Kyoto study? While it may be expected that reconstructing neural circuits via cell-replacement or transplantation therapy is on the horizon, it is unlikely that the Kyoto experiment itself will realize this vision.[52] However, given that previous studies reported successful transplantation of mouse embryonic brain tissue into other rodent models, as cited by Kitahara et al., it seems plausible that the Kyoto study would see success,[53,54] thus ensuring the benefit of a step toward the goal of utilizing cell-replacement therapy for brain injury and stroke.

## *The Principle of Sufficient Value to Justify Harm (3rd Principle)*

A challenging definition in this principle is "sufficient value." DeGrazia and Beauchamp defer the tasks of defining the term and assessing what satisfies its conditions to ethics review committees.[55] Indeed, DeGrazia and Beauchamp note that there will be disagreements and difficulties over what constitutes a sufficient value and whether or not this value justifies the expected harms to research animals.[56] In their justification of this principle, Beauchamp and DeGrazia invoke the moral status of animal subjects.[57,58] It is here, in the question of animal moral status, where they foresee most of the discussions about the definition of sufficient value taking place. While the 6Ps framework is built upon the basis that animals have a moral status, it does not give any prescribed weight to the moral status of an animal (sentient or otherwise). By invoking the moral status of animals, DeGrazia and Beauchamp are attempting to fill the "gap in reasoning" between the first and second principles, namely whether the expected net benefits of an otherwise unattainable experimental result can override the interests and moral status of the research animal.

### *The Rutgers study (3rd Principle)*

The rationale behind this principle is based on an animal's moral status and whether the expected net benefits of an otherwise unattainable experimental result can override the harms of the animal in

question. So then, what is the moral status of mice? And are the possible net benefits to the Rutgers chimeric experiment sufficiently justifiable to override their moral standing? The moral status of mice (as well as the concept of moral status itself) is still debated,[59,60] although it is generally accepted that the moral standing of mice is less than human persons.[61]

Even when considering only mice themselves, their moral status can differ. For instance, good mice (i.e., mice used for research purposes) may have a higher moral standing than pest mice (i.e., wild mice that may spread disease).[62] Good mice possess rights and protections of various governing bodies. These rights and protections afforded to good mice are not afforded to pest mice, which are subject to methods of entrapment and extermination that research review committees would find unacceptable in experimental settings.[63] It is best, then, not to consider the moral status of mice in a vacuum. Rather, the moral standing of the mice should be considered as dependent on the relevant context and setting. Given that the mice in the Rutgers study are good mice, they do have a heightened moral status than wild or pest mice alone. Their moral standing is enough such as to afford them the values and principles of animal welfare.

But do the prospects of net benefits from the Rutgers study meet a standard of sufficient value to justify harm to the mice involved? The answer to this question is unclear, in part because it is unclear whether the experiment brings a net benefit that is sufficiently valuable or acceptable to the DS community (as discussed in the Principle of Expected Net Benefit). Furthermore, the harms that were done to the mice were significant as the experiment both altered the mental capacities of the mice and required physical alterations to the mouse itself via surgery (albeit the means of the surgery were humane). An unclear net-benefit value proposition for the DS community is weighed against the moral status of good (chimeric) mice. Arguably, it might be better to err on the side of caution until the net benefits that may come from Rutgers experiment are made more clearly.

### *The Kyoto study (3rd Principle)*

Do the prospective net benefits of the Kyoto study justify the expected harms and override the moral status of the mice and macaques? Again, moral status assumptions are necessary. Here, too, the good mice are afforded certain protections unafforded to pest mice. Given that the net benefits from the Kyoto study are not clearly immense or immediate, it may not be sufficient to justify the harms to the mice. But here too, the issue of an unclear net-benefit value proposition makes it difficult to assess whether that net benefit is of a sufficient value proposition such as to override the moral status of good (chimeric) mice. This becomes even more apparent when considering these supposed net benefits against the moral status of macaques. Most would give macaques a higher moral standing than good mice. Macaques seemingly possess elements of moral agency that may be on par with some human beings.[64] While it is possible to imagine that the results of the chimeric macaque experiment could have a significant scientific impact and inspire further calls to translate the research into clinical applications, it seems unlikely at this stage. In addition, there are still more ethical and safety concerns regarding first-in-human organoid transplant trials that need to be addressed even afterwards.[65,66]

### *The Principle of No Unnecessary Harm (4th Principle)*

Beauchamp and DeGrazia ground this principle in the commonsense notion that doing harm tends to be morally wrong.[67] Instances of necessary harm therefore require moral justification. In many valuable studies, it is often impossible to conduct the experiment without causing some harm. But this Principle of No Unnecessary Harm requires minimizing the harms done while still meeting the scientific objectives. According to DeGrazia and Beauchamp, this principle overlaps with the refinement principle from the 3Rs.[68]

In addition to preventing unnecessary harm directly, it is also necessary "to prevent occurrences of unnecessary harmful conditions,"[69] that is, conditions or environments that may cause the animal to suffer. If unexpected conditions occur that could cause the animal harm, the principle requires that researchers intervene to reduce or eliminate the condition and rectify the harm. For example, if an

unexpected disease spreads through the study population, researchers should intervene, reduce the spread of the disease, and provide reasonable medical care to those animals affected where possible.

### *The Rutgers study (4th Principle)*

The Rutgers study harmed the mouse population in at least two ways. First, it harmed the mouse population physically via surgical transplantation of human cerebral organoids. Second, it harmed at least some mice cognitively by altering and diminishing cognitive capacities. Euthanasia was also performed in the study. However, there are still conflicting philosophical and ethical viewpoints on the meaning of death for nonhuman animals and whether euthanasia (or death itself) should constitute a harm for nonhuman animals.[70] All procedures of euthanasia mentioned in the study are approved in guidance from the American Veterinary Medical Association for humane animal euthanasia.[71] We point out that David DeGrazia and Franklin G. Miller note that the 6Ps framework "remains agnostic on the question of whether or not a premature death counts as a harm—or, equivalently, whether avoidance of premature death is a basic need."[72]

Were the harms done to the mice necessary in order achieve the *scientific* aims of the study? Arguably so. There is no other realistic and morally justifiable way to assess if altering specific genes will bring changes into the cognitive abilities associated with DS. However, an element of the study does raise questions about how well the results are translatable to human beings. The mice themselves did not have DS at the beginning of the experiment, only possessing DS in part of their brain after transplantation. This may reduce the translatability into the clinic as the gene editing technique the researchers are developing is meant for fetuses with DS.

### *The Kyoto study (4th Principle)*

From what can be inferred from the Kyoto study, the mice were harmed in two possible ways. The first is physical harm from the transplantation surgery itself. The second is psychological harm, which is inferred solely on the basis that the cerebral cortex of the mice was significantly altered and most likely affected their mental states. Since the Kyoto study did not include behavioral assessments of the animals, our inference about their psychological suffering is limited.

The monkeys were harmed in a similar fashion as the mice: physiological harm from the transplantation surgeries and possible psychological harm from an altered brain. Again, the psychological harms can only be inferred in limited way. Furthermore, the researchers tried to limit the impairment of higher order brain functioning by transplanting the human brain organoids into the motor cortex of the monkeys, thus showing that the researchers had no intention of doing unnecessary harm to the macaques—although this was not necessarily afforded to the mice. Again, however, the agnosticism of euthanasia as a harm in this principle cannot necessarily lead us to conclude that the macaques were harmed by it.

### *The Principle of Basic Needs (5th Principle)*

It is necessary to satisfy the basic needs of an animal for it to have a minimally acceptable quality of life. Basic needs can include the physiological, psychological, and social needs in certain sentient species, such as those in many mammalian species or nonhuman primates. Some needs include humane transport, shelter, and food. Other needs include certain freedoms such as freedom from disease, injury, or disability, freedom of movement, and freedom from significant harms. While these freedoms and needs are fundamental to a good quality of life for animals, it may sometimes be necessary to deprive the animal of these needs if and only if it is morally justified by scientific purposes. Otherwise, it is necessary to ensure that these minimum standards of needs are met. Although it should be noted that Beauchamp and DeGrazia state the controversial nature of "Freedom from premature death" as constituting a basic need. But no attempt is made within the 6Ps to answer the question of whether premature death constitutes a deprivation of a basic need and is left to ethics committees for debate.[73]

### The Rutgers study (5th Principle)

Aside from the necessary harms of surgical transplantation and its resulting psychological harms, no other basic needs seemed to have been deprived. Although it could be argued that the premature deaths of the mice also constituted a deprivation of a basic need (we will not further mention this here for the sake of repetition). We can assume that given the mice underwent stress examination, and stress factors may unduly influence behavioral results, it can be inferred that the researchers intended the mice to experience as little stress as possible and would therefore not intentionally deprive them of basic needs that would cause stress. Furthermore, while the study does not mention anything about how the basic needs of the mice were met, we can grant the benefit of the doubt that the animal caregivers at Rutgers provided the mice their basic needs in accordance with their published policies.

### The Kyoto study (5th Principle)

According to the reports in the Kyoto study, all animals were subject to a 12 hour light/dark cycle with an *ad libitum* access to food and water. DeGrazia and Beauchamp also state that the Principle of Basic Needs should include fulfilling needs beyond appropriate circadian rhythms, food, and water. It should include needs such as humane transport and companionship if the animal is social in nature. Concerning companionship, certainly both mice and (perhaps more so) macaques are social animals. It is unclear whether this need for companionship was sufficiently met based on what was reported in the Kyoto study. Approval by the Institutional Animal Care and Use Committee does not guarantee that the need of companionship was taken into consideration for the approval. No behavioral studies were performed, and thus no inferences of stress can be concluded. However, Kyoto University's animal research policy guidelines include most of the basic needs that DeGrazia and Beauchamp consider.[74] And given the robust nature of the guidelines, we can reasonably infer that companionship is a consideration for the research animals.

## The Principle of Upper Limits to Harm (6th Principle)

The role of the final principle of the 6Ps is to establish a ceiling of morally acceptable harm. It is not enough to solely meet the basic needs of animals and cause them no unnecessary harm. An animal may be subjected to an unacceptable limit to harm if the severity and intensity of the harm cannot be ameliorated by "appropriate anesthesia, analgesia, sedatives, changes in living conditions, or the like."[75] Beauchamp and DeGrazia suggest that researchers and animal caregivers first establish operational criteria for what may qualify an upper limit to harm.[76]

### The Rutgers study (6th Principle)

Seemingly, the mice in the Rutgers study did not undergo what may constitute severe suffering for an extended period without relief. The study does not note any standards by which researchers or ethics committees would constitute severe, intense harm and what would constitute an "extended period of time." But here again, we point to the behavioral tests as evidence that the chimeric mice were not in significant and intense pain for any prolonged period. Examinations for stress as a factor in behavior were built into the experimental procedures. The only observed change in behavioral outcomes came from a reduction of novel object recognition in DS chimeric mice. This reduction of cognitive functioning of memory would not fit into a category of severe and intense harm on its own.

### The Kyoto study (6th Principle)

The Kyoto researchers did stress that they took precautions to avoid cranial compression and to avoid undue influence on the macaques' higher cognitive functioning. Furthermore, if the animals were suffering, they would have suffered for a period of at least 12 weeks' post-transplantation, as opposed to a longer period. Seemingly, the researchers acted on what they perceived to be a limit to harm that was permissible to the monkeys. However, the researchers do mention that based on their findings, an observation period of 12 weeks is too short for a time frame given that axonal projections extended only

into the subcortical area and the size of the macaque brain itself. They therefore call for a longer observation period in future studies. But a longer observation period opens the risk of further harm to the chimera. The period for observation cannot, as the principle states, be too lengthy to cause undo suffering to the animal.

## Discussion

It is difficult to conclude that the Rutgers and Kyoto studies sufficiently fulfill all the 6Ps, and thus would be morally acceptable under this framework. Certain areas of the analysis require assumptions on prior ethical issues, such as euthanasia, concepts of sufficient value, and moral status. Otherwise, the framework cannot be used to its fullest extent. Here, we further elaborate on some of these reflections, areas that need further clarity in the 6Ps, and how the ethics of both the Rutgers and Kyoto studies are impacted.

### *Ethical Highlights Beyond the Scope*

There are notable elements that are worth highlighting for moral consideration but cannot be examined under the 6Ps framework. For example, Kitahara et al. mention an ethical consideration that ought to be addressed: the question of whether or not cerebral organoids themselves could develop consciousness.[77] Much of the organoid ethics literature centers on this very question.[78,79,80,81] Other important elements include the donor tissue consent process, concerns on biobanking, and the moral distinction between using hiPSCs versus hESCs.[82,83,84,85,86,87] However, even if these initial highlights fall outside of this examining scope, they may be morally relevant in other contexts, frameworks, and examinations.

### *Objections to the 6Ps*

Few objections have thus far been raised over the 6Ps framework. However, Matthias Eggel and Hanno Würbel argue that DeGrazia and Beauchamp mistake the 3Rs as a framework used for ethical evaluation when it really is not. Instead, animal ethics evaluations are based on a full and robust harm-benefits analysis.[88] Moreover, Eggel and Würbel further argue that every principle mentioned by DeGrazia and Beauchamp is already covered in some form in existing ethical and legal frameworks such as legal minimal standards, the 3Rs, and harm-benefits analyses.[89] So rather than providing a new framework or principles, DeGrazia and Beauchamp are actually arguing "for more rigorous implementation of the current principles."[90]

### *Disability and Net Benefit*

The general aims of these studies (knowledge that may lead to future innovative therapies) may not sit well with disability activists—who would rather the priority be on strengthening rights and changing social attitudes, especially attitudes within medicine.[91] While DS individuals were not the direct subjects of the Rutgers study, the results of the study may one day affect them directly. The costs and risks researchers associate with cognitive deficits of DS may be over-estimated, and thus the net benefit from the research is debatable.

Furthermore, the research from both the Rutgers and Kyoto studies are not attempts at significant leaps in developing life-saving drugs or treatments. Rather, they are another basic research step in the gradual perfection of invasive techniques which may lead to a perceived improvement in the quality of life for present or future persons.[92] The researchers in both studies call for more similar chimeric experiments to continue this stepwise progression until a leap is finally made to human experiments (which will require a different ethical framework than the 6Ps).[93,94] This slow and gradual perfection of these invasive techniques, coupled with the social and civil rights concerns of disability advocates, really questions the necessity of such studies.

The debates about these areas of disability bioethics and disability studies are ongoing, and the 6Ps does not need to incorporate a view about disability to provide a normative conclusion on the sufficient value of an animal experiment. However, depending on who is evaluating the sufficient value of chimeric studies, it is certainly possible to argue that the value presented is overstated. Thus a further, disability inclusive, debate would be necessary.

### Of Mice and Monkey Moral Status

The 6Ps does not give particular weight to moral status of animals, even though it does acknowledge, and begins with, the assumption that all animals do have some moral standing and by this standing ought to be afforded certain minimal protections.[95,96] But the weight and significance of the moral status each species possesses are left to committees or other ethicists to consider. In some ways, this lack of a firm stance on animal moral status left a hole in the analysis of the Rutgers and Kyoto studies and possibly opens the framework to criticisms of being too subjective or relative.

This oversight played a role in the analysis of the studies with the Principle of Sufficient Value to Justify Harm. This principle requires that value of the net benefits of a research study should outweigh the moral status of the animals in question. As shown, the moral status of mice and monkeys is not necessarily straightforward, nor is it inherently clear how best to weigh the interests of these animals against the prospective net benefits to society. Here again, there are trade-offs to the flexibility of the framework. The benefit of leaving the moral status question of animals (or chimeras) to ethics committees is that it does allow for deliberation on an unsettled issue. In addition, there is space among both the ethics committees and in the framework for relevant stakeholder input and control from society. But the drawback of this flexibility is that researchers may be less certain if the prospective net benefits of their research do outweigh the interests of the animals in question, nor is there a straightforward way to arrive at an answer. We do not disagree with the initial assumptions of Beauchamp and DeGrazia that animals have a moral status and that their interests ought to be weighed against the prospective net benefits of an experiment. But the 6Ps framework may benefit researchers and committees by providing some guidance on how to weigh these two sides.

Chimeric studies also present the question of humanization. Researchers in the Kyoto study mention possible ethical concerns over humanization, and that this ought to be explored further.[97] This question of humanization may be more pertinent to experiments like the Kyoto study which involve nonhuman primates, as opposed to chimeric human-mouse experiments as in the Rutgers study. Some, such as certain members of the International Society for Stem Cell Research (ISSCR) Task Force subcommittee for stem cell research involving the use of nonhuman embryos and animals, suggest that "these concerns run too far ahead of the actual science, and erroneously conflate higher degrees of *biological* structural humanization with greater *moral* humanization, the latter comprising unique human-like cognitive capacities, such as the emergence of higher-order intellectual processing capabilities and thought, and self-consciousness."[98] Nevertheless, this ISSCR subcommittee provides guidelines for research institutions on chimeric research oversight.[99] Humanization (moral or biological) is not addressed in the 6Ps. Furthermore, it is unclear how humanization would fit into the principles themselves. Likewise with humanization, dehumanization presents a similar conundrum. The change in moral status of "good mice" to "pest mice," if they escape for example, could certainly change how, or even if, the 6Ps is applied. While the 6Ps framework does not give any particular weight or significance to moral status at the onset, it should provide some indication on how to proceed should the moral status of an animal changes during an experiment.

### Animal Euthanasia Agnosticism

Animal euthanasia was another area where the 6Ps remained "agnostic."[100] But despite this supposed agnosticism, moral examiners still need to take a stance on whether euthanasia is an unnecessary harm or even constitutes a violation of the freedom from premature death as a basic need. Beauchamp and

DeGrazia's agnosticism comes with a specific definition of euthanasia, "the rapid and (ideally) painless killing of animals *for their sake* and not merely for the purpose of avoiding costs or inconvenience associated with keeping them alive."[101] Euthanasia can be seen as a harm to the animal itself or it can be seen as a means for preventing further unnecessary harm for their own sake. Here again, we see the double-edged sword of framework flexibility. The agnostic position on animal euthanasia allows ethics committees and researchers room for exploring the relationship between euthanasia and harm. But this agnostic position does not inherently incentivize committees or researchers to examine this euthanasia–harm relationship. Some animal ethics committee members we have spoken to mention that the euthanasia–harm relationship is not heavily examined in comparison to examining the possible social benefits or upper limits to harm. It can even be understood that euthanasia is a necessary means to achieve a scientific aim.

### Ecology Questions for the 6Ps

The use of *Macaca fascicularis*, common long-tailed macaques, in experiments like the Kyoto study may become more controversial over time for ecological and environmental reasons. *M. fascicularis* is listed as a vulnerable species by the International Union for Conservation of Nature and Natural Resources (IUCN) since 2015.[102] The IUCN's assessment found a 30 percent reduction in the species population in the last 36–39 years, with an expected 30 percent further reduction in the following 36–39 years should conservation efforts either fail or are not raised at all.[103] Given their status as a threatened species and forecasts of their continued population decline, it may neither be sustainable nor morally permissible to continue to use long-tailed macaques in experiments. Furthermore, the IUCN specifically identifies that "females are taken into breeding facilities and males are exported internationally for use in laboratory research" as a threat to their population.[104] Demand for common long-tailed macaques to be used in research is contributing toward putting an undue strain on the species' population.

This does further raise a moral question that the 6Ps framework may need to address. Does the scope of the principles extend beyond the individual animal or research animal populations in a study? Certainly, an intended focus of the framework is on the use of laboratory animals. But should the animal welfare considerations extend beyond the laboratory and into nature? Would the framework consider the use of a vulnerable or endangered species morally impermissible, for example, on the grounds of causing unnecessary harm to the greater species? If an experimental protocol were to contribute to the species overall decline, would it be morally impermissible on grounds of an upper limit to harm? We imagine it is possible to answer these questions in the affirmative, since the 6Ps already does take human beings into account regarding their social benefits and not necessarily specific human individuals. While we cannot fully address this question here, it is a worthy point for future discussions as the 6Ps is further applied.

### Ethical Implications for Organoids

What does this cross-comparative case analysis of human brain organoid xenotransplantation imply for the ethics of organoids? It may seem contradictory to use organoids within animal models, given that organoids were created, in part, as a viable alternative and as a way which ought to reduce animal models altogether.[105] However, through this analysis, transplanting human brain organoids into animal models does not necessarily raise new ethical considerations than those of prior experiments, which transplant human stem cells. Furthermore, nothing inherent to brain organoids as alternative models demands that we treat research animals with higher standards of animal welfare.

One interesting, if not speculative, consideration is if brain organoids were to develop to a point of consciousness, it would raise further ethical questions over the permissibility of transplanting one conscious entity into another. This includes questions about the survival of the brain organoid consciousness. But given that neuroscientists are not necessarily concerned about brain organoids developing consciousness themselves, these specific ethical questions are not necessary to answer for the moment.

### Similarity to Other Studies

The Rutgers study is not the first instance of transplanting human DS neuronal cells into mice. A similar Chinese and American collaboration study was published the previous year wherein human DS iPSCs further generated GABAergic progenitor neurons, which were then transplanted into mouse brains.[106] Beyond studying DS, at least two other reports were cited by Kitahara et al. focusing on transplanting human cerebral organoids into animal brains for the purpose of studying brain injury or axonal growth.[107,108,109] And the first instance of transplanting human brain organoids into animal models is reported from the laboratory of Fred Gage in 2018.[110] Certainly then, some of the conclusions of the ethical analysis performed here could be applied to other similar studies.

But the translation of the ethical conclusions of this analysis to similar studies should bear in mind the unique moral contexts and factors of relevant cases. This cross-comparative case analysis also has a limitation in that it is a snapshot of a scientific research continuum. While the first human brain organoids xenotransplantation studies can be traced back to at least 2018, integrating human neuronal cell tissue into animal models has occurred well beforehand. Integrating the whole scientific backdrop and total context into this analysis is not possible or at least reasonably feasible. However, should future cases be viewed under the lens of the 6Ps framework, the cases cannot be thought of in a vacuum, and sufficient relevant contexts and scientific background must be taken into consideration.

## Conclusions

Based on this case comparative analysis with the 6Ps framework, is it morally permissible to transplant human cerebral brain organoids into animal models? Under the scope of 6Ps, both studies reveal multiple, complex, and interrelated moral components that require further in-depth exploration, analysis, and debate. There are more areas of needed discussion that could not be discussed in this paper due to lack of space (e.g., the use of vulnerable species in research and the possible harms of chimeric cognitive enhancement). Furthermore, there are still important ethical issues that need to be addressed in these studies that fall outside the scope of the 6Ps framework such as donor consent, the use of hESCs, and the moral status of cerebral organoids themselves. This comes at a price of increased complexity, interrelations, and nuance. Upon applying the 6Ps to these cases, various shortcomings of the framework appeared, making the analysis challenging to give a definitive conclusion. It is likely that given the higher standards of the 6Ps, experiments such as the Rutgers and Kyoto studies are more likely to be considered morally impermissible—but on which grounds of the 6Ps remain debatable.

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