Commentary

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Ethical considerations for human–animal neurological chimera research: mouse models and beyond

Insoo Hyun

Research that uses stem cell-based chimeras promises to advance our understanding of human developmental biology, as well as new medical interventions, such as generating transplantable human organs in livestock. However, along with these exciting research possibilities come moral concerns about the moral humanization of animals, especially when it comes to the potential effects of human cells in the brains of experimental animals. Recent work involving neurologically chimeric mice may suggest that such worries are reasonable. However, this overlooks the crucial social and neurological conditions for enabling the development of conscious self-awareness, the absence of which leaves us only with animal welfare to monitor and consider.

Chimeras are biological entities with cell populations originating from two or more zygotes of the same or different species. Both in basic research to study human developmental biology, and during translational biomedical research, scientists regularly create what are known as interspecies human–animal chimeras by transferring various types of human cells into an animal. One of the main goals of this research was to biologically humanize animals to study natural human processes or localized human tissues in the animal host.

Despite its significant scientific utility, the aim of partially humanizing research animals carries the unintended consequence of stirring up ethical concerns about the moral limits of this endeavor, especially when it comes to humanizing animals' brains—what I shall hereafter refer to as neurological chimerism. In this commentary, I examine the main ethical concerns surrounding neurological chimerism and the key assumptions underlying these concerns, flagging where these assumptions can get far ahead of the science. But first, I offer some context to position these ethical considerations in a clearer light.

For decades, human–animal chimeras have been used across a broad range of biomedical research to study diseases such as cancer and human immune disorders. For instance, immune-deficient mice enable scientists to engraft pieces of human tumors, turning these chimeric animals into mini cancer patients whose human tumors can be studied and screened with drugs. Mouse models of the human immune system (SCID-hu mice)—created by transplanting human fetal lymphoid tissues into immune-deficient mice—are used to reconstitute and study distinct elements of the human immune system. However, widely used chimeras such as these are created by transplanting human somatic cells that have restricted biological potential; thus, the degree of human–animal chimerism has remained limited. Stem cell-based chimera research aims to overcome this limitation.

In theory, stem cell-based human–animal chimeras can vary widely in degree and kind, depending on the number and type of human stem cells transplanted, the species and developmental stage of the host animal, and the anatomical location where the stem cells are transferred. Owing to the less restricted biological potential of pluripotent and multipotent ("adult") stem cells, the transfer of undifferentiated human stem cells into early animal embryos or fetuses might greatly increase human tissue formation, distribution, and duration compared to transferring more mature, differentiated cells into post-natal animals. For these reasons, stem cell-based human–animal chimeras may provide powerful research tools for developmental biology, preclinical studies of cell-based therapies, and *in vivo* disease modeling and drug screening.

To date, however, researchers' efforts to generate human–animal chimerism with high levels of human cells have been met with a few buoyant successes amidst a sea of frustrations. I begin with the frustrations.

For years, researchers have attempted to generate human–animal chimeras by introducing human pluripotent stem cells into animal embryos and other early stages of development to selectively humanize specific tissues. This approach could, 1 day, be used to create complex disease models and even transplantable human organs in common livestock (Wu *et al*, 2017). Previous studies reported the ability of human pluripotent stem cells to chimerize cultured post-implantation mouse embryos. However, little is known about whether human pluripotent stem cells can engraft in animal embryos and seamlessly undergo further development—prerequisites for organ generation and much disease modeling. To date, various groups have attempted

Department of Bioethics, School of Medicine, Case Western Reserve University, Cleveland, OH, USA. E-mail: insoo.hyun@case.edu
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to produce human pluripotent stem cell-derived chimeras using rodent and pig embryos as the host species (Wu *et al*, 2017). Unfortunately, these studies have suggested either little or nonexistent levels of donor cell contributions, even when means are taken to improve the survival of the transferred human cells.

It is not well understood yet why human pluripotent stem cells do not efficiently integrate into animal embryos, but it is hypothesized that cell death and failure to differentiate post-transfer may be tied to the considerable evolutionary differences between humans and mice and pigs, whose lineages diverged more than 90 million years ago. These include divergence in ligand and receptor amino acid sequences, early post-implantation development, cell adhesion, cell cycles, gestational length, and developmental speed, all of which may reduce not only the efficiency but also the utility of human–mouse or human–pig chimeric studies. While findings from most human–animal chimera research to date are disparate, on balance, they suggest that functional integration of human donor cells occur at either very low efficiency or not at all—with one key exception, as discussed below.

In order to move stem cell chimera research past this biological impasse, some scientists have become interested in exploring the generation of human–monkey embryonic chimeras *in vitro*. Earlier this year, scientists from Stanford University successfully integrated chimpanzee's induced pluripotent stem cells into early monkey embryos in a dish (preprint: Roodgar *et al*, 2019). Prior to this remarkable study, the generation of interspecies chimeras between goat and sheep, wood mice and mice, and rats and mice suggested that the use of hosts and cells that are evolutionarily closer might offer a more successful strategy (Xiang *et al*, 2008).

To follow up on the Stanford study, some believe that the transfer of human pluripotent stem cells into monkey embryos in a dish—*in vitro* human–monkey embryonic chimera experiments—could reveal ways to overcome the xenobarrier that has thwarted previous attempts at stem cell-based human–animal chimerism. Efforts to study human–monkey embryonic chimeras *in vitro* may uncover the best developmental stage of host embryos at which to transplant human pluripotent stem cells including the donor cells' optimum level of pluripotency, critical cell adhesion and growth factors, and the mechanisms through which donor cells are outcompeted in non-human embryos.

Ultimately, *in vitro* research with human–monkey embryonic chimeras might empower strategic approaches to enhance levels of human donor cell contributions within non-primate embryos, such as mice, pigs, and sheep. Since hormonally induced egg procurement is an invasive procedure not allowed in apes for research purposes, scientists would have to use pre-implantation embryos humanely derived from old-world (macaque) and new-world (marmoset) monkeys frequently used for assisted reproductive technologies research, whose last common ancestors with humans lived approximately 29 and 43 million years ago, respectively. From there, researchers can move on to using host embryos of other non-primate species, eventually working their way to pig or sheep embryos for organ-generation studies.

However, if researchers decide to pursue these lines of research in earnest, they will soon have to wrestle with concerns that the resulting chimeras could develop humanized brains. Although the *in vitro* studies proposed above do not entail gestation—which would be needed for embryonic chimeras to get to a stage where brain formation begins—researchers may need to employ strategies that would limit human stem cells' ability to contribute to the animal brain if human–pig or human–sheep chimeras were to be gestated for organ-transplant research. For example, although Japan now permits the creation of human–animal chimeric embryos and their possible transfer into surrogates, new Japanese regulations emphasize a cautious approach in which "research ethics committees and the MEXT (Ministry of Education, Culture, Sport, Science and Technology) should confirm that there is sufficient scientific rationale for the research as well as precautionary protocol measurements, such as differentiation control and step-by-step observation of the developmental process in the womb" (Sawai *et al*, 2019). Such a controlled approach would likely involve the transfer of pluripotent human stem cells that have been genetically modified so as not to contribute to host brain development. Similarly, in order to add further biological insurance against the remote possibility of neurological chimerism, some regulators may request that only lineage-restricted human pluripotent stem cells should be used for *in vitro* studies, even if this extra-cautious approach would make the resulting studies less informative about human stem cell potential.

What is it, one may ask, that is so troubling about neurological chimerism? Human–non-human neurological chimerism could be problematic for people who believe that the human brain is the locus of our unique moral characteristics. For those who maintain this view, the development of large amounts of human neurological matter in non-human animal brains may warrant concerns about the emergence of morally relevant mental properties in chimeric animals. Are there any reasons to believe these concerns have a basis in scientific reality? Perhaps there are, especially in light of recent successes in neurological chimera research.

To date, the most fruitful results of human–animal neurological chimera work have come from the Goldman lab at Rochester Medical Center in New York. In a widely publicized study, Goldman and colleagues reported that human glial progenitor cells (GPCs) successfully integrate into the brains of neonatal immunodeficient mice, where they can generate high levels of human glial progenitors and astrocytes (Han *et al*, 2013). Not only do the transplanted human cells mature *in vivo* to adulthood, but these cells also retain the size and unique structural complexity of human astrocytes and even appear to serve their normal functions of regulating synaptic transmission, plasticity, and learning. Indeed, the experimental outcome that drew the most public attention was the team's claim that their human glial-chimeric mice dramatically outperformed control mice in four different learning tasks: auditory and contextual fear conditioning; Barnes maze; and novel object location. This led Andy Coghlan of *The Washington Post* to declare in a headline, "Mice Injected with Human Brain Cells Get Smarter, Scientists Say." On the other hand, there was no evidence that neurological chimerization had any effect on how these chimeric mice interacted with control mice and littermates: their "sociability" was not affected in any discernable way. This is an important point we shall return to shortly.

Goldman's human glial-chimeras provoke intriguing questions about the role

of human GPCs in cognition. Do human glia influence neural network function in a species-specific manner? Since human astrocytes possess greater fiber complexity than those of non-primate mammals, can human glial-chimera models inform questions about the role of human-specific GPC in human cognitive evolution? As Goldman and colleagues write, the ability to generate high degrees of human glial chimerization in mice—and possibly beyond the mouse model—"should permit us to address these questions, by rigorously evaluating the *in vivo* contributions of both human astrocytes and their progenitor cells to neural network activity, and hence their respective roles in human cognition" (Goldman *et al*, 2015).

These long-term research ambitions may not be so far-fetched, for another major finding of the Goldman lab was that transferred human GPCs tend to thrive in their murine neural environments—so much so that they can developmentally outcompete their hosts' resident GPCs. By the time the chimeric mice reached adulthood, very large proportions of their forebrain glia were comprised of human cells. The remarkable competitive advantage of human GPCs was also shown in some of the Goldman lab's earlier work: 9 months after transplantation, nearly all of the mouse glial progenitors were replaced by human GPCs (Windrem *et al*, 2014).

This ability to generate neurologically chimeric mice containing large populations of aggressively expanding human glial cells opens up exciting new scientific possibilities. For one, this advance makes it feasible to explore the role glial cells might play in hereditary human neurological disorders, as the contribution of these cells to neuropsychiatric pathologies was very challenging to define.

Using human GPCs derived from disease-specific pluripotent stem cell lines, the Goldman team found that intrinsic glial dysfunction was responsible for some of the pathologies observed in Huntington disease and childhood-onset schizophrenia. For instance, mice engrafted with huntingtin-expressing human GPCs showed poor motor performance and suppressed myelination due to reduced expression of the SOX10-regulated myelin regulatory factor MYRF (Benraiss *et al*, 2016; Osipovitch *et al*, 2019). Interestingly, the transplantation of normal glia slowed disease progression and significantly increased survival. And the forced expression of SOX10 and MYRF in hypomyelinated neonatal *shiverer* mice restored myelination in HD-derived glia *in vivo*. Together, these findings suggest that impaired human glial cells seem to play an intrinsic role in the development of Huntington disease, which could lead to better drug and cell-based interventions to help ameliorate symptoms.

In the case of childhood-onset schizophrenia, human glial-chimeric mice engrafted with GPCs from patient-derived induced pluripotent stem cells were found to develop abnormal astrocytic morphology, hypomyelination, and behavioral and sleep abnormalities (Windrem *et al*, 2017). These results suggest again a strong causal contribution of cell-autonomous glial pathology to the development of neurological disease. The chimeric mice's behavioral abnormalities—increased anxiety, antisocial traits, and disturbed sleep—suggest it is impaired glial function itself that may be causing these aberrant patterns. Recall that in Goldman's earlier work, the chimeric mice produced from healthy human GPCs exhibited none of these unusual behaviors. Goldman's healthy glial-chimeric mice could learn faster, but they were not "antisocial."

It would be scientifically valuable to further probe this initial discovery that human glial cells seem to improve learning. What if Goldman's team or others investigate more deeply the impact of human glial cells on cognition and behavior? This could be done either directly using larger animal hosts, such as neonatal pigs chimerized with normal human GPCs, or indirectly during the course of disease modeling in large animals vis-à-vis a control group engrafted with healthy human GPCs. Although human GPCs tend to spread and dominate widely in the mouse forebrain, the total amount of human-derived neural matter that can grow in this particular animal model is naturally limited by the very small size of the mouse brain and skull. What might be the extent of human chimerism in a larger animal's brain? How would it affect the animal, and would we find in its chimeric brain every possible cell type derived from human GPCs? Although it is a matter of debate whether human GPCs are technically stem cells, they do give rise to two main cell types—astrocytes and oligodendrocytes—and may under the right conditions form neurons. What might human GPCs do for animal cognition that non-human GPCs cannot? One does not know until one does the experiments.

This line of inquiry may be deeply disturbing to some people. For some, it could stimulate a deep-seated worry that both the possibility of human–monkey embryonic chimera research, even if directed at the noble goal of organ generation in livestock, and the future research possibilities enabled by Goldman's seminal work may be going "too far." But in order for this concern to carry much substantive normative weight, it must amount to more than an emotive response that this research would be distasteful. There must be some rational basis for limiting how far chimera researchers ought to go: a basis that should be intellectually accessible to people whose personal tastes or sensibilities may vary widely. Thus, the key ethical question appears to be: Could the biological humanization of animal models imbue human–animal chimeras with morally important cognitive attributes? If so, then the moral limits of human–animal chimera research would be demarcated by the possibility of these, yet-to-be-defined, new cognitive attributes arising during the course of, or as the result of, human–animal chimera research.

Obviously, this key ethical question is quite loaded. To address it properly, we must first unpack and disentangle the nuances and assumptions that this question glosses over. The first nuance is what kind of cognitive attributes would be morally important for us to consider? Animals already have many complex cognitive traits. In order for a particular cognitive trait to have any added ethical purchase in a chimeric animal, it would have to be a trait that unchimerized host animals lack but that neurologically chimeric animals would gain through their biological humanization. Most people would probably identify "human-like consciousness" as the most likely contender.

This point brings us to additional important nuances. The term "consciousness" is ambiguous across many possible meanings. Which particular meaning is assumed in claims about chimeras' "human-like consciousness" will matter for ethical judgments. If by "consciousness" one simply means neuronal activation in a cortical region upon stimulation—that is, preconscious sensory stimulation without the subject's awareness of said stimulation—then this would be ethically unproblematic. This is just normal brain mechanics. If by

"consciousness" one means something more complex, such as conscious access to sensory stimulation, or wakefulness, vigilance, focal attention, or sentience and the like, then again we should acknowledge that animal species already have these cognitive features. There is nothing uniquely human about these traits. Chimerism would not give these abilities to host animals anew although it might modulate the degree to which any one of these might be exemplified.

At this point in the discussion, it appears that the chief worry is not that human–animal neurological chimeras could gain conscious access to sensory stimulation, or wakefulness, vigilance, focal attention, or sentience through their chimerism. Rather, the key ethical concern seems to boil down to whether these laboratory animals could somehow gain the additional and morally significant characteristic of human-like subjective self-awareness—that is, the awareness of oneself as a temporally extended being with experiences, beliefs, and interests, all of which can be mentally reflected upon by oneself. At this point, however, we are starting to get far past the science.

Reflective self-awareness is perhaps the most complex version of the many possible meanings of "consciousness." This special cognitive trait forms the very basis of our moral lives as humans. However, we should pause here to acknowledge that this trait is not biologically guaranteed to arise merely by the presence of human brain cells or structures. As developmental psychologists and many philosophers of mind would point out, conscious self-awareness can only be realized within nurturing social environments and through the acquisition of syntactic language that enables humans to have propositional belief systems and reflective beliefs about their own beliefs. Not even the 100% natural brains of human neonates can develop normally to the point of self-awareness unless they are given the right social interactions over the span of several years. These constant interactions and language use conditions necessary to support human-like self-awareness are notably absent from the laboratory conditions within which neurological chimeras would be created and maintained.

Critics of course may still object that neurological chimeras could be imbued at least with the biological *potential* for conscious self-awareness and that this potential would be sufficient to limit how far chimera researchers ought to go. On this view, a chimera's capacity for conscious self-awareness may lie dormant in the brain, even if it is refused the socialization and other external enabling conditions necessary for actualizing it.

It is challenging to know exactly how to reply to this concern, since it would be difficult to know either way whether a chimeric brain has the latent potential for conscious self-awareness just waiting to spring into existence under ideal conditions. A display of faster learning is not enough. Faster learning is not a uniquely human cognitive trait, nor is it necessarily a sign of conscious self-awareness.

Perhaps one response might be to argue that neurological chimerism would have to be widespread throughout the brain for this concern to be taken seriously. The neural correlates of consciousness—in all its forms—are believed to be distributed across large and diverse anatomical regions of the cerebral cortex and involve multiple cell types. A recent review suggests that the minimal neural correlates of consciousness are primarily relegated to posterior cerebral cortical regions that include the sensory areas (Koch *et al*, 2016). These findings were derived from studies involving neuroimaged patients and volunteers who could speak about the presence and quality of their conscious experiences. However, extending these findings directly to neurological chimeras that cannot communicate with humans would be very challenging.

But what if high degrees of human–animal neurological chimerism were found across multiple brain regions, and what if the human cells were appropriately diverse, integrated, and communicating across large distances? Would this be sufficient for making the case that a latent biological potential for conscious self-awareness was present?

Unfortunately, we do not know enough about the neural correlates of consciousness to know what exactly might be going on in the mind of a speechless chimeric animal with the aforementioned brain properties. There does not even appear to be a way to properly image the brains of large animals to discern all the human brain connections therein and the neural work they are actually doing. Until our tools and our knowledge of the brain catch up, now may be good time to pose this frank question: Should the suspected potential for self-awareness alone without a means of verification justify banning certain forms of meritorious neurological chimera research?

I am wary of saying yes, simply because this approach would have to rely solely on inferential reasoning about the inner workings of an imperfectly understood and observed living brain, and inferential reasoning is notoriously open to skeptical attack. We need a more practicable set of considerations to guide chimera research. What would a more tractable approach look like? I believe we have a good precedent.

In 2007, the Ethics and Public Policy Committee for the International Society for Stem Cell Research (ISSCR) issued ethical standards for stem cell-based human-to-animal chimera research, and today, these standards comprise the current ISSCR guidelines for this research (Hyun *et al*, 2007). According to the ISSCR guidelines, any time human stem cells or their direct derivatives are integrated into the central nervous systems of laboratory animals, animal research oversight must take place building on and remaining consistent with animal welfare principles, but with added stem cell-specific expertise to consider the animal welfare of human–animal chimerism. Past experience with genetically altered laboratory animals has shown that reasonable caution is warranted if genetic changes carry the potential to produce new behaviors along with new defects and deficits. Using this past experience as a guide for the future, the ISSCR guidelines recommend that chimera research should involve the following: (i) the establishment of baseline animal data; (ii) ongoing data collection during research concerning any deviation from the norms of species-typical animals; (iii) the use of small pilot studies to ascertain any welfare or behavior changes in modified animals; and (iv) ongoing monitoring and reporting to oversight committees authorized to decide the need for protocol changes and the withdrawal of animal subjects.

These ISSCR recommendations have been echoed in chimera research standards proposed by the US National Institutes of Health (NIH), which has a funding moratorium on certain forms of human stem cell-based chimera research since 2015. The two types of chimera research currently ineligible for Federal funding are (i) the transfer of human pluripotent stem cells into

non-human primate blastocysts and (ii) the breeding of animals where human pluripotent stem cells may contribute to the germ line. While this funding moratorium has remained in place, the NIH also proposes to take a closer look at other forms of chimera research: namely, research that would introduce human pluripotent stem cells into non-human vertebrate embryos up through the end of gastrulation and introduce human cells into post-gastrulation non-human animals (excluding rodents) where there may be substantial contribution or functional modification to the animal brain. In the latter case of neurological chimerism, the NIH would consider (i) the characteristics of the human cells; (ii) the characteristics of the host species; (iii) relevant data on the likely effects on animal cognition, behavior or physical appearance; (iv) planned monitoring and animal welfare assessments; and (v) progressive staging of the research. Like the ISSCR guidelines, these proposed NIH standards emphasize the need for step-by-step monitoring of the research effects on host animals.

It should be pointed out that there are no other comprehensive guidelines at scientific organizations, institutes, or local institutions that are specifically aimed at stem cell-based neurological chimera research. Thus, until any further ethical and professional standards arise, researchers should continue to follow the guidelines proposed by the ISSCR and the NIH for chimera research, since they focus investigators and regulators on issues that are more immediate and tractable, rather than vague concerns about generating moral humanness in animals—namely, animal welfare.

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Conflict of interest

The author declares that he has no conflict of interest.

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