Published in final edited form as:
Am J Bioeth. 2007 May ; 7(5): 27–40.

Thinking About the Human Neuron Mouse

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> Tonight I ask you to pass legislation to prohibit the most egregious abuses of medical research . . . [which include] creating human-animal hybrids.
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> — George W. Bush, 2006 State of the Union Address (2006)

Dr. Irving Weissman, a professor in the departments of pathology and developmental biology at Stanford University (Stanford, CA), approached one of the authors of this article in February 2000 with ethical questions about interesting experiments he was considering. The most interesting experiment would begin with an inbred strain of mouse that begins to form brains during very early fetal development, but, several days before birth, died as a result of the death of most or all of the developing neurons in their brains (the glial cells that make up approximately 90% of the brain are unharmed). Weissman proposed to transplant human brain stem cells into the fetal mice, just before their own neurons died. His hope was to produce a living mouse with a functioning brain made up of mouse glial cells and human-derived neurons. This mouse could then be used to study human neurons *in vivo* in a laboratory animal, similar to the way the severe combined immunodeficiency (SCID)-hu mouse, which Weissman had helped developed in the late 1980s, allowed the study of the human immune system inside laboratory mice.

That conversation led to the creation of a five-person working group that, after meeting for more than one year, in early 2002 reported to Dr. Weissman that it believed his proposed experiments could be performed ethically, subject to some guidelines. The report has never been published and the experiments, for reasons not associated with the report, were never performed. Yet both the experiments and, to a lesser extent, the report have been subjects of discussion and debate (DeWitt 2002; Krieger 2005; Wade 2005; Weiss 2004), and the issue of human/non-human chimeras has only grown more controversial, leading even to proposed criminal legislation that has the “unambiguous” support of the President of the United States (S. 1373; Brownback 2005).

This article is a revised version of our report, updated to reflect nearly five years of debate about the ethical issues surrounding the creation and use of human/non-human chimeras. That debate has taken place in scholarly journals, important policy reports, and the halls of Congress. We believe our analysis has interest as one of the earliest efforts to come to grips with the implications of this scientific research and as an example of a “benchside consult,” an effort

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to provide ethics-based advice on research in progress. More importantly, we also believe that it remains, with slight modifications, a useful approach to such experiments. Our report focuses on transplanting human neural progenitor cells into non-human brains and so falls well within whatever boundaries define "neuroethics," but it also has broader implications for the creation of other kinds of human/non-human chimeras, including some with non-biological components.

This article begins by describing the debate over human/non-human chimeras. It then focuses on our case study, Weissman's proposed experiments aimed at creating what we have called the "human neuron mouse." It provides some background on the experiments and discusses their potential benefits and their risks and costs before providing our recommendations to Dr. Weissman (and, now, others contemplating similar experiments). The article ends with some broader conclusions about the ethics of research with human/non-human chimeras.

Some readers will, no doubt, be disappointed that neither this article, nor the original report, attempts to answer the question whether conferring human-like mental characteristics on non-human animals is, or is not, ethically appropriate. We concluded that this fascinating question just was not plausibly raised by Weissman's proposed experiments. To emphasize that question in the context of these, or similar, experiments would give too much credence to a sensational misreading of this research; as we note in our last section, the question does need further work.

THE DEBATE OVER HUMAN/NON-HUMAN CHIMERAS

Although the definitions and meanings of chimeras are numerous and complex (Greely 2003), for the purposes of this article chimeras are creatures with cells, tissues or organs from individuals of two different species (interspecific chimeras). In spite of President Bush's language, hybrids are not chimeras but are, instead, the result of sexual reproduction involving individuals of different species, as a mule is a hybrid resulting from the mating of a male donkey with a female horse. Human/non-human chimeras can be created in two different directions, by putting human cells or tissues into non-human animals or by putting non-human cells or tissues into humans. This article discusses only the first; the second topic is more often referred to as *xenotransplantation* and is the subject of wide-ranging debates, mainly about its safety. (Interestingly, at least some experiments have transplanted non-human neural cells into human brains with long-term survival [Deacon 1997].) This section of the article reviews the scientific, ethical, and policy discussions that have taken place concerning the first method.

The Continuing Creation of Human/ Non-Human Chimeras

The science and politics of human stem cells have combined to keep human/non-human chimeras a scientifically relevant issue. Weissman hoped to make human neuron mice largely so the mice could serve as model organisms for studying human cells. But as interest, scientific and popular, grows in human stem cell research, human/non-human chimeras are likely to take on broader uses. Before anyone makes new clinical use of human stem cells—or any clinical use of human embryonic stem cells—prudence (and the United States Food and Drug Administration [FDA]) are likely to require preclinical trials with the human cells in non-human animals. The result is likely to be a large number of human/non-human chimeras. When pluripotent embryonic stem cells are used instead of more differentiated stem cells, the concerns potentially become greater; a human embryonic stem cell, even if placed in the liver, might be able to become a neuron, a skin cell, or, ultimately, an egg or sperm.

Although Weissman has not performed the two experiments discussed in the report (he has continued some human/non-human chimera experiments), other researchers have continued to make human/non-human hybrids, in a wide variety of contexts, such as studying human tumor cells by transplanting them into mice. These chimeras receive little or no attention, but two

researchers have received some publicity for work with chimeras, one involving neural cells, one with liver cells.

A group of Yale University (New Haven, CT) researchers led by Dr. Eugene Redmond have been experimenting with transplanting immature human neural cells into the dopamine-producing regions of the brains of green vervet monkeys. Those regions are associated with Parkinson's disease, and Edmond and his group hope that their research may ultimately be useful in understanding and treating humans with Parkinson's disease (Redmond 2002; Shreeve 2005).

Meanwhile, at the University of Nevada (Reno, NV), Dr. Esmail Zanjani has produced chimeras by transplanting human stem cells, mainly human blood-forming stem cells, into sheep. Zanjani has claimed that these human cells have been transformed into a variety of cell types in the sheep, in at least one case producing a sheep with a liver with 40% of the liver cells derived from human cells. According to Zanjani, these livers contained characteristically human structures and produced fully human proteins. Zanjani's work stirred up controversy with reports that the chimeric sheep had been given to a University-owned ranch that let the naïve research sheep out to graze as if they had been raised on the ranch, resulting in dead sheep and happy coyotes (Mullen 2005).

Bioethics

At the time of our report to Weissman, there was effectively no bioethics literature on human/non-human chimeras. That began to change in 2003 with the publication in the *American Journal of Bioethics* of a target article by Jason Scott Robert and Françoise Baylis (Robert and Baylis 2003). Robert and Baylis argued for caution in the creation of human/non-human chimeras, based on the possibility of creating confusion about the moral status of the resulting organism. Their article attracted many comments, of which those by Greely, Streiffer, Cohen, and Karpowicz were particularly interesting (Cohen 2003; Greely 2003; Karpowicz 2003; Streiffer 2003).

Phillip Karpowicz, Cynthia Cohen, and Derek van der Kooy published a useful article in 2005, following up in more detail on a 2004 article (Karpowicz et al. 2004; Karpowicz et al. 2005). They analyze four arguments against human/non-human chimeras: moral taboo, species integrity, unnaturalness, and human dignity. They find only the last argument convincing, but only if the human cells,

> when transplanted into the prenatal mouse or monkey, were to proliferate and develop into a whole human-like brain and if human-like capacities associated with human dignity were to emerge in such animals to some degree . . . (Karpowicz 2005, 123–124).

The article continues to make specific recommendations for limiting chimera research to avoid the risks of "developing whole human-like brains" or "human-like capacities."

Also in 2005, a working group organized by Ruth Faden and Guy McKhann at Johns Hopkins (Baltimore, MD) reported its conclusions concerning transplanting human neural stem cells into the brains of non-human primates (Greene et al. 2005). This working group concluded that such research should try to minimize the risk that the resulting animal would have more human-like cognitive capacities. It argued that such experiments be subjected to special review and that the reviewing bodies should consider six factors:

> (i) the proportion of engrafted human cells, (ii) neural development, (iii) NHP [non-human primate] species, (iv) brain size, (v) site of integration, and (vi) brain pathology (Greene 2005, 386).

The Johns Hopkins working group recognized that there was "no simple relationship between these factors and, thus, no formula for making evaluative judgments." It did use the six factors to argue that grafting a large number of human cells into developing great apes would be more troubling than transplanting small numbers of human cells into the brains of healthy, adult monkeys most distant from humans.

Still later in 2005 Robert Streiffer took the position that the most important ethical considerations in such research should arise out of concern for the chimeras created by such research. He argued that such research *might* significantly enhance the moral status of those chimeras, which he sees as, on its face, good, but that the chimera will not be treated as its higher status demands. Recognizing the very substantial uncertainties about what qualities such research would create—and what ethical significance those qualities would have—he argues that, at this time, policies that require the early termination of such chimeras or that forbid the introduction of pluripotent stem cells into non-human primate blastocysts (the position taken by the National Academy of Sciences [NAS] Guidelines) are appropriate (Streiffer 2005).

Finally, in August 2006 a private organization called the Scottish Council for Human Bioethics published a report on "animal-human mixtures" (Scottish Council 2006). The report covered a wide range of ways in which human genes, cells, or reproductive processes might be mixed with those of non-humans. Two of this group's recommendations are particularly relevant:

> 11. The incorporation of human stem cells into post-natal animals should proceed with extreme caution. Moreover, such a procedure should only take place if it can be demonstrated that the cells cannot contribute to the germline or give rise to specifically human brain functions in the animals.
>
> 12. The incorporation of human stem cells into post-blastocyst stages of non-human embryos should only take place if it can be demonstrated that they cannot contribute to the germline or brain cells of the animal (Scottish Council 2006, 1).

The recommendation concerning embryos is more restrictive (cannot contribute to "brain cells" versus cannot contribute to "specifically human brain functions") because of greater concern about introducing cells at an earlier stage of development. The report makes these recommendations after reviewing many of the discussions of chimeras, which it says demonstrate the special sensitivity of possibly affecting a non-human's brain (or germ) cells.

Policy

The issue of human/non-human chimeras has led to at least two efforts to create guidelines or rules for this activity. The first comes from the National Research Council (NRC) and Institute of Medicine (IOM) in the United States. The second is legislation proposed in the United States. There may appear to be at least one more effort, as Canada prohibited the creation of "chimeras" in 2004 when it adopted its Assisted Human Reproduction Act. That act, however, defines chimeras only as *human* embryos into which cells from other human or non-human entities have been placed, and thus would not cover mice with human cells. (Assisted Human Reproduction Act 2004). (We have not tried to survey all legislation around the world in search of bans on chimeras; other such bans may exist.)

In April 2005 a committee created by the NRC and the IOM produced a report with guidelines for how to conduct human embryonic stem cell research (NRC 2005). The October 2004 meeting of this committee had included testimony from several scholars about the creation of chimeras, including Irving Weissman, David Garbers and Bridgid Hogan on the scientific issues and Henry Greely, Cynthia Cohen and William Hurlbut on the ethical issues (NRC 2005, Appendix C). These guidelines included the recommendation that an embryonic stem cell research oversight committee review and approve all research "involving the introduction of

hES [human embryonic stem] cells into nonhuman animals at any stage of embryonic, fetal, or post-natal development." The guidelines further urged that "particular attention should be paid to the probable pattern and effects of differentiation and integration of the human cells into the nonhuman animal tissues." The guidelines also stated that no animals in which human embryonic stem cells had been introduced should be allowed to breed and no such cells be introduced into the blastocysts of non-human primates (NRC 2005). The text of the report addressed specifically the issue of putting human embryonic stem cells into the brains of non-human animals:

> Perhaps no organ that could be exposed to hES cells raises more sensitive questions than the animal brain, whose biochemistry or architecture might be affected by the presence of human cells. Human diseases, such as Parkinson's disease, might be amenable to stem cell therapy and it is conceivable, although unlikely, that an animal's cognitive abilities could also be affected by such therapy . . . . Protocols should be reviewed to ensure that they take into account those sorts of possibilities and that they include ethically sensitive plans to manage them if they arise (NRC 2005, 50).

The NAS Guidelines and discussion dealt only with human *embryonic* stem cells, but the issues they raise apply to all human stem cells that can give rise to central nervous system cells.

It is not clear how widely the NAS Guidelines are being followed by United States institutions performing human embryonic stem cell research. Those following stem cell research generally believe that the NAS Guidelines are widely used, although firm evidence is lacking. In California, the NAS recommendations on embryonic stem cell research oversight committee review of chimeras have been largely adopted both as regulations by the California Institute for Regenerative Medicine, which manages the stem cell research funding provided by California's Proposition 71, and by the California Department of Health Services Advisory Committee on Human Stem Cell Research, which is charged with recommending guidelines for human embryonic stem cell research in California not funded by California Institute for Regenerative Medicine.

In early 2005, *The New Atlantis*, a conservative journal that calls itself "an effort to clarify the nation's moral and political understanding of all areas of technology," published an editorial entitled "The Bioethics Agenda and the Bush Second Term," in which it called for an aggressive legislative campaign both to ban the creation and destruction of human embryos for research purposes and to "defend and advance the dignity of human procreation and the human family" (Cohen 2005). In March 2005 Senator Sam Brownback (R-KS) proposed legislation that would largely implement the recommendations made by the editorial in pursuant of its second goal—and that would ban the creation of some forms of human/non-human chimeras.

The Human Chimera Prohibition Act of 2005, originally introduced in March 2005 and reintroduced that July as S. 1373, would make it a felony, punishable by 10 years in prison and a civil fine of at least $1 million, to create, attempt to create, transport, or receive for any purpose a "human chimera" (S. 1373, §302(2)). The Act defines "human chimera" in eight different ways, most of which appear to deal with hybrids of various sorts rather than chimeras (S. 1373, §301(1)). The eighth subsection appears to have been aimed directly at Dr. Weissman's proposed experiment:

> (H) a non-human life form engineered such that it contains a human brain or a brain derived wholly or predominantly from human neural tissues (S. 1373, §301(1)(H)).

(The Senator may have missed his target, as Weissman's most ambitious experiment, even if successful, would not affect the 90% of the mouse brain that was made up of glial cells instead of neurons.) It is this legislation that President Bush was endorsing in his 2006 State of the

Union address, legislation that has not been voted on in the Senate or the House of Representatives, or received committee hearings.

BACKGROUND TO THE WORKING GROUP REPORT

When the original report was written for Dr. Weissman, researchers had recently reported finding human brain stem cells—cells that can become all or most of the cell types found in the human brain, including neurons and glial cells. These cells, thought to be very infrequent in adults, were isolated from the brains of human fetuses. This discovery opened the possibility of studying human neurons *in vivo* in laboratory animals by creating mice whose brains were made up, in part or in whole, of human neurons.

Although such a "human neuron mouse" would not stand and talk like a cartoon character, its possible creation raises important and interesting ethical questions about research in human neuroscience. The next section lays the groundwork for evaluating these issues by discussing human brain stem cells, examining completed and planned experiments involving transplantation of these cells into mice, and finally by describing our working group and its general approach to the questions before it.

Human Brain Stem Cells

In 2000, researchers claimed to have isolated human brain stem cells from the brains of fetuses aborted after 12 weeks of development (Uchida 2000). Research with these cells *in vitro* showed that they could form many different kinds of human brain cells—not just neurons in their various types but also other cells that play essential roles in the brain, such as glial cells. They seem, therefore, to be multi-potent cells. The isolation of these cells opened the possibility of growing and transplanting mature brain cells, particularly neurons, into patients with such debilitating neural degenerative disorders as Parkinson's disease. In 2006, the FDA granted an investigative new drug exemption to one firm to perform such transplants for a rare childhood disease (Batten's disease); an institutional research board at the Oregon State Health University (Portland, OR) recently approved a trial of this approach (StemCells, Inc., Palo Alto, CA). Whether this kind of neural regenerative medicine will prove safe or effective remains, of course, unknown. Stem cell therapy with hematopoietic stem cells is regularly used, with frequent success, to build or rebuild a patient's blood and immune systems; it remains of speculative value in other contexts.

Dr. Irving Weissman at Stanford Medical School (Stanford, CA) was one of the researchers who helped isolate these brain stem cells. Weissman had long worked with stem cells and had been instrumental in the isolation of human hematopoietic stem cells. Working with those cells and other human tissues, in 1988 he and Dr. Joseph M. McCune created the so-called "SCID-hu" mouse. This work started with an inbred strain of mouse born with severe combined immune deficiency (SCID). These mice, as a result, had severely impaired immune systems. Weissman and McCune transplanted human hematopoietic cells (in later experiments, human hematopoietic *stem* cells) as well as the tissues that support for the formation of blood and cells of the immune system (human fetal bone, thymus and liver) into these SCID mice. The weak immune systems of the mice did not attack the human cells as alien and those cells were able to colonize the human fetal bone and liver, and later thymus, to create in them a human blood-forming and immune system. The result was a laboratory animal model of the human blood-forming and immune systems, on which experiments could be done that could not ethically be done with the only other creatures with an *in vivo* human immune system, living humans.

Using these mice the human hematopoietic stem cell was first isolated and gained FDA approval for trials that showed these cancer-free stem cells could regenerate the blood-forming and immune systems that had been depleted by cancer therapies. These animals were also used

to infect a human immune system with patient isolates of HIV, the first time one could show definitively that HIV caused the changes that characterize AIDS in humans.

The Mouse Transplant Experiments

As part of the research leading to the isolation of human brain stem cells, Weissman, Uchida and other colleagues at the firm StemCells Inc. began transplanting human brain stem cells into the brains of SCID mice with normal murine brains. (SCID mice were again used to avoid an immune system attack on the human cells.) The human brain stem cells were placed in a brain structure called the *lateral ventricle*, which, in mice, connects to their brains' quite large olfactory bulbs. Weissman's group was able to show that the human neuronal stem cells engrafted in a brain stem cell niche called the *subventricular zone*, near the injections. Those cells also migrated to a second niche, the dentate gyrus of the hippocampus. In these niches the human cells divided and many of them migrated toward the olfactory bulb (Tamaki et al. 2002; Uchida et al. 2000). Samples of the brains of these mice showed that the human neurons had survived and had connected to the mouse brain. Mouse brains have a (relatively) much larger olfactory bulb than human brains and new mouse neurons are regularly migrating to their olfactory bulbs; the human-derived cells did the same. Examination also showed that the human neurons had moved into other areas of the murine brains and made up more than 1% of the neurons in some regions. This research could not, however, determine whether the human neurons were actually functioning as part of the mouse brain, let alone whether they were functioning normally.

Weissman then wanted to do two other experiments transplanting human brain stem cells into mice. These proposed experiments were the subjects of our report.

The first proposed experiment would work with an inbred strain of mouse that lost all the neurons in its cerebellum several weeks after birth. In both mice and men, the cerebellum is involved in fine motor skills, coordination and balance. Other roles of the cerebellum, including any role in consciousness, are unknown. A mouse, or a person, without a functioning cerebellum can survive but with substantial motor deficits. Friedrich's ataxia is a human disease caused by the death of cerebellar cells; this strain of mice displays symptoms similar to those of humans with Friedrich's ataxia. Weissman wanted to transplant human brain stem cells into such a mouse just before its cerebellar neurons began to die. He hoped that the human cells would differentiate into neurons, would migrate into the mouse's cerebellum, and would begin to function. Unlike his earlier experiment with putting brain stem cells into the lateral ventricles of mice, this experiment would be able to determine whether the human neurons were not only surviving but were functioning, in part through seeing whether and to what extent the treated mouse showed signs of normal cerebellum activity. Based on Weissman's previous experiment, he also expected that the human cells would appear at low concentrations in other parts of the mouse's brain.

The second proposed experiment would use a different inbred strain of mice, developed by Fred Alt's laboratory. These mice form the beginnings of the nervous system, creating the structures that support the movement of early stage neural progenitors, but all these developing neurons die, leading to the death of the early stage fetuses. This mouse strain also has a mutation that makes them SCID mice, so that the resulting mice would not reject human cells. For this experiment Weissman planned to transplant the human brain stem cells *in utero* shortly before the murine neurons were expected to begin dying. He hoped that the human cells would differentiate, migrate to the places where the murine neurons are dying, and take their places. The result would be a mouse brain, the neurons of which were mainly human in origin. This experiment could have at least two different end points. In one version, the mice could be aborted as fetuses shortly before birth and have their brains examined on autopsy to see whether the human neurons had populated their brains and, if so, what kinds of brain structures—mouse,

human or mixed—they formed. Alternatively, the mice could be allowed to go to term and, in addition to examination of their brains, by neuroimaging while alive or by autopsy, their functioning and behavior could be observed for variations from the mouse norm. If the mice were viable, they might be the neuronal equivalent to the SCID-hu mouse in terms of being a laboratory animal that could be used for experiments on living, *in vivo*, human neurons.

Although, as subsequently described, the working group's report concluded that these experiments could be undertaken ethically, at this writing neither experiment has been performed. Weissman discovered that the Friedrich's ataxia model mouse did not, in fact, have the characteristics he needed for his experiment. One study, however, did follow up some of the questions involved in the cerebellar experiment. Dr. Fred Gage's laboratory reported in late 2005 that they had transplanted stem cells into the brains of rats and had been able, using patch clamping, to determine that the cells derived from the transplanted human cells were actually firing. The fact that these human-derived neurons were firing does not necessarily mean that those cells were functioning properly, either individually or as part of a larger unit, in the rat. But if it had been the case that the human-derived neurons were *never* firing, they clearly could not be functioning normally (Muotri et al. 2005).

As to the second experiment, there were problems with breeding the mouse strain with complete neuronal death. Weissman has also been occupied with other work, not only with other aspects of his own research but with administrative and advocacy work around human stem cell research. He also needed to find a graduate student or postdoctoral fellow interested in doing the work; the fellow who was interested at the time had gone on to other work. Weiss-man continues to say that he might try the second experiment, but he also from time to time refers to it as a "thought experiment." It is not clear to us, and perhaps not to him, whether or not he will return to this experiment.

The Working Group and Its Approach

Weissman was aware of the sensitivity of these planned experiments, both ethically and in terms of public reaction. He may well have had visions of a headline reading "Stanford Scientist Creates Mouse with Human Brain." As a result he asked one of the authors of this article (Greely) to consider putting together a group to examine the ethical issues in these proposed experiments. Greely pulled together this ad hoc group, with representation from several disciplines. We met several times during 2000 and 2001, interviewed Weiss-man, studied the scientific literature, and discussed the questions—and how we could approach those questions—at length. We concluded that the experiments did raise interesting and important, but manageable, ethical issues

In general, we approached the questions by asking about the potential benefits and the potential costs or risks of the proposed experiments. We first examined the costs to see if any of them might categorically rule out the experiments. We next considered ways in which the experiments might be undertaken to limit the costs of risks involved. We weighed the potential benefits of the research, with or without modifying conditions, against the potential costs or risks. We concluded that the experiments could proceed ethically, subject to careful staging and monitoring.

POTENTIAL BENEFITS

United States government regulations and international agreements on ethical research agree that research on human beings is only permissible if there are potential benefits, to applied or to basic science, from the research that outweigh the potential harms and risks. A similar, though weaker, standard applies in federal law to the use of many laboratory animals, including mice. Researchers obviously can do things to laboratory mice that they may not do to humans,

including routinely maiming or killing them. They may not, however, do such things without a good reason. Both because living animals were to be used and because of the nature of the human cells being used, Weissman's proposed experiments could be justified only if the experiments were likely to offer some benefits.

The most clear potential benefit is the creation of a non-human animal in which human neurons can be studied in a living brain. Many experiments on human neurons, and on the diseases of those neurons, cannot ethically be performed in humans. These experiments involve risks too high to be permissible for a human subject to bear or, in many cases, the killing of the human subject and the subsequent examination of his or her brain. Such research with human subjects is, of course, not morally acceptable.

This benefit, in effect, would come from the creation of a brain equivalent to the SCID-hu mouse. Thousands of SCID-hu mice have been used in research on the human immune system, particularly but not solely with respect to HIV infection. More than 100 grants from the National Institutes of Health (NIH) have involved the use of SCID-hu mice and, over the years, the NIH has contracted for the production of more than 1,200 SCID-hu mice.

Having a laboratory animal for studying human neurons might have substantial benefits, both for basic science and for clinical applications. For example, the methods by which various pathogens or exposures damage human neurons could be directly studied in a living brain without risking harm to a human subject. New drugs or other treatments could be first tested for their effects on human neurons in mice rather than in human subjects. Steps in the *in vivo* functioning of human neurons could be analyzed without risking harm to living people.

None of these benefits is assured. These experiments may fail, or, whether they fail or succeed, a human neuron mouse may prove impossible to create. Given the vast and thus far poorly understood number and type of interactions between cells that take place in the brain, we would be surprised if human neurons could function properly in all the roles necessary to create a properly working mouse brain. Even if a human neuron mouse proved possible, research with it might not be substantially better than existing alternatives. Studies of human neurons outside the brain through *in vitro* research or *in vivo* studies of mouse neurons in mouse brains might prove just as illuminating of human brain function as the study of human neurons in a mouse brain. Nonetheless, the potential for substantial scientific and even medical benefits seemed significant to us. Because of these anticipated benefits, the experiments seemed reasonable and, in the case of the experiment that could create a murine brain composed entirely of human cells, necessary steps to assess that potential.

RISKS AND COSTS

We identified five areas of concern that need to be examined and, if found significant, weighed against the potential benefits. These concerns include: 1) the sources of the human brain stem cells; 2) the potential for pain and suffering to the mice; 3) the propriety of this use of human tissues (particularly brain tissues); 4) the risks of possibly conferring some degree of humanity on another species; and 5) the risks to public support of science.

It is interesting, in retrospect, to compare those concerns with those subsequently expressed in the literature on human/non-human chimeras. Most of the issues that concerned us have been largely or entirely ignored in subsequent discussions. In one form or another, the question of "conferring humanity" has been the focus of the discussion, although generally expressed in terms of either human dignity (Karpowicz 2003) or avoiding moral confusion (Robert 2003). Streiffer's position is more complicated; he argues that the successful conferring of a higher moral status on a human-mouse chimera would not be wrong in itself, but would likely be wrong because we would not treat the chimera in a way consistent with that higher status

(Streiffer 2005). A little has been said on the other issues. The Johns Hopkins group on transplanting human neural tissue to non-human primates did discuss briefly the issue of harm to the subject animals (Greene 2005); Karpowicz did discuss and reject at least one form of the public relations argument (Karpowicz 2005).

We did not discuss in our report some of the moral taboo arguments rejected by Karpowicz, or integrity of species borders and unnaturalness arguments, rejected by both Karpowicz and the Johns Hopkins group. Our internal discussions had already considered and rejected all of those arguments and our report described only arguments we found potentially plausible.

Aborted Fetuses as the Source of the Human Brain Stem Cells

The human brain stem cells that Weissman uses were derived from the brains of human fetuses that had been intentionally aborted. Use of such tissue has been controversial in the United States because of its link to voluntary abortion. The issue of using human fetal tissue in research and medicine was discussed widely in the late 1980s, spurred in part by evidence that transplants of fetal brain tissue into the brains of people with Parkinson's disease could lead to improvement in their condition. (As it happens, this therapeutic application of human fetal tissue has since been shown, at least so far, to be neither safe nor effective.) For research and medical purposes, tissues from intentionally aborted fetuses were greatly preferred to tissues from spontaneous abortions or stillbirths because of the much greater risk that the cells and tissues from the latter had suffered from fatal genetic conditions, had been contaminated by pathogens, or had died in the long period between the *in utero* death of the fetus and the collection of the tissues.

In 1988 the Secretary of Health and Human Services imposed a moratorium on federal funding for research using human fetal tissue pending further consideration. Both government commissions and private commentators debated the morality of such use with an NIH advisory panel recommending in late 1989 that the moratorium be lifted subject to certain restrictions (Greely 1989). The first Bush Administration nonetheless extended the moratorium indefinitely. The Clinton Administration lifted the moratorium in January 1993. On February 1, 1993, the NIH adopted "interim policy guidance" that allowed the use of human fetal tissue in federally funded research under certain conditions (NIH 1993). This guidance was then superseded by very similar provisions in the NIH Revitalization Act of 1993 (NIH Revitalization Act 1993). The NIH conditions sought to ensure that the potential use of the tissue would not induce a woman to have an abortion that she otherwise would not have chosen. Note that at no time has there been a federal ban on the use of human fetal tissue in research *not* funded by the federal government. On the contrary, such research is not even limited by the conditions imposed first by the NIH and then by Congress.

Controversy over research and medical use of human fetal tissue from intentionally aborted fetuses continues in spite of the 1993 legislation. President Bush's August 9, 2001, decision concerning federal funding for human embryonic stem cells does not apply to the human brain stem cells, which are isolated from much older tissues, but it does reflect the continuing debate over the research use of fetal tissue (NIH 2001). The SCID-hu mouse itself has been the subject of a negative article in the conservative publication *Human Events*, focusing on the fact that its creation requires using live tissue from "a human child—and every child who donates tissue to create such mice is first killed by a medical doctor" (Jeffrey 2001, 1). In light of the continuing and high-profile controversy over human embryonic stem cells, it is perhaps surprising that the use of tissue from aborted fetuses has not reappeared as an issue in Congress or the Administration. Certainly, no one can guarantee that it will not return.

The derivation of human brain stem cells from intentional abortions did not raise substantial concerns for this working group, particularly if the tissue is donated in accordance with the

federal funding requirements. Abortion currently is a protected right in the United States and, even if some find that regrettable, the use of fetal tissue is unlikely to affect the number of abortions performed. Our group thus put to one side the issue of the morality of abortion. Nonetheless, we recognize that for some people this issue will be important. Others may and will take a different view. Should human brain stem cells of equal power become available from other sources about which less ethical controversy exists, such as adult humans or spontaneous abortions, researchers might prefer to use them.

Inhumane Treatment of the Mice

Both law and ethics require that laboratory animals not be used wantonly. They should only be used in risky or harmful experiments when the potential benefits outweigh the costs and with due regard to the pain they might experience. Laboratory animals may be killed painlessly if the experiment requires that result and its potential benefits justify the deaths. These animals should not normally be treated in a way that is painful unless both the need for the experiment and the justification for the pain are very strong. (Of course, some have stronger objections to the use of animals in experiments in ways that harm or disable them.)

That mice will be killed in this research, even if the deaths are painless, requires that the experiments have countervailing potential benefits. The effects of these experiments upon the mice, while alive, are, at this point, unknown. Mice killed *in utero* in the second experiment presumably would not experience significant pain. Otherwise, we have no way of knowing whether the mice in the first experiment, which might have cerebellums made of human neurons, or mice brought to term in the second experiment, which might have brains made entirely of human neurons, would feel pain as a result without actually doing the experiments. If the experiments resulted in, for example, constant painful seizures or apparently painful self-destructive behavior, then the continuation of the experiments would have to be reconsidered in light of that finding. (Of course, human consciousness trapped in a mouse's body would truly be cruel treatment, but, as discussed later in text, this possibility seems extremely unlikely.)

Respect for Human Tissue—Particularly Brain Tissue

A third concern arises from the fact that these experiments place living human cells inside a non-human animal. By so doing, some may argue that the researchers show insufficient respect for the human origin of the cells.

Both ethically and legally, we limit the potential uses of human tissues. Human remains are not normally displayed except as part of funeral services; most human organs cannot be sold; corpses and body parts must, by law, be disposed of in a respectful manner; and cannibalism is forbidden. It is not clear whether these prohibitions stem from respect for the individual whose body parts or tissues are involved or from a fear that such uses hold humanity itself in disrespect—and may, in time, lead to even more noxious disrespect for living human persons. Whatever its sources, the demand for respect for the bodies of the dead has deep roots in western culture—consider as one example Sophocles's play *Antigone*—and presumably in many other cultures as well.

In one respect, we do use human tissues for many purposes, at least with the relevant person's permission. Organs are used for transplantation and some human tissues, notably blood, semen and eggs, are bought and sold for medical purposes. Placentas have long been sold for various uses; more than one million each year are sold in the United States to firms that use them to make medications. Extracts from human placenta have even been used in cosmetics, notably skin creams. Corpses, skeletons and organs are used in medical training and in research. These uses are made not only for medical purposes but also for anthropological or historical ones,

including the display of human remains in museums. The line between the educational value of human tissues, for example, mummies displayed in museums, and their function as entertainment is, admittedly, a narrow one. In several places in Europe, that line seems to be fairly clearly crossed as old human skeletal remains are displayed as tourist attractions in catacombs. A newer version of this mixture of education and entertainment includes the touring "plastination" exhibits, which show, in dramatic poses, human corpses preserved through the infusion of plastics into their tissues (Bohannon 2003).

Our working group found little ethical discussion or scholarly literature on the appropriate treatment of human tissues. (Interestingly, there has been some public discussion about the proper uses of brains or brain tissue in two cases; the whole brain of Ishi, a much-studied California Native American who died in 1916 [Starn 2004] and sliced tissue samples from Albert Einstein's brain [Burrell 2004]). The type of tissue involved in these proposed experiments undoubtedly increases the stakes. Whereas both the heart and the liver have been viewed in different cultures as the location of a human's essence, there seems little doubt that in at least Western culture the brain holds a very special place as the seat of consciousness and, for many people, what they view as their souls. Transplanting human thymus or liver tissue into mice does not have the same overtones as transplanting neurons. Part of that heightened concern stems from the possibility of transmitting some human qualities to the mice, which is discussed later in text. But another part of this concern may stem from a sense of "sacredness" about the brain as the site of consciousness.

Our view was that several different considerations are important in analyzing the appropriate use of human tissues, including brain tissue. These considerations include whether the tissues are used with free consent of the proper person, the purposes for which they are used, whether they are treated in a respectful manner, and the tissue's "degree of humanness." After much discussion, we concluded that these considerations appear to support the use of human brain tissue in Weissman's proposed experiments. Human brain stem cells derived from aborted fetuses are not, obviously, used with the consent of the fetus but are used only with the consent of the woman who carried the fetus. No more appropriate source for consent seems plausible. In addition, existing federal law, although it only applies to tissue used in federally funded research, contains provisions to help ensure that the consent is freely given and that no one was coerced into or even influenced toward getting an abortion to acquire fetal tissue; in fact, the woman involved may not even know research use is a possibility before she has committed herself to the abortion (Department of Health and Human Services 2001). The purpose of this use of human tissue is research into the fundamental characteristics of human neurons and the prevention and treatment of neuronal diseases, which seems a worthy use. There seems no reason to believe that the small amounts of human tissue would be treated lightly or without respect.

As to the last point, the human tissues involved here, although they come from arguably the organ most tied to human identity, are small masses of disaggregated cells, suspended in fluid contained in vials. An outsider looking at them would have no idea what they were. They do not have the more obvious humanity of a severed head, a skull or a full skeleton. Human neurons are not human brains, but merely one of their constituent parts. It is not clear—indeed, one of the goals of the proposed experiments was to explore—whether the human neurons in a mouse brain would function in any meaningful way like human neurons rather than mouse neurons. Moreover, it is not obvious to us how "humanity" can be located in a cell or a body part; when we lose skin cells or even limbs, we do not use some of our humanity. If one somehow transplanted—or grew—a full and fully human brain into another animal, the objection about moving special human "tissue" would seem much stronger.

We recognize that the ethical discussion concerning the appropriate treatment of human tissues is not very fully developed and that reasonable people may well disagree with our report on this point. It is certainly clear that different cultures may have different views; respect for human body parts has increased markedly in European and European-derived cultures in the past few centuries as traitors' heads are no longer posted at city gates. At this point, however, our consideration of the apparently relevant factors leads us to conclude that proper respect for human brain tissues does not prohibit the appropriate use of these human brain stem cells in mouse transplantation experiments. (We have not considered how these concerns might ultimately affect successful therapies based on human brain stem cells.) Additional discussion of this issue seems appropriate.

One further issue concerns the appropriate disposition of the brains or bodies of any of the experimental mice after they have died or been killed. Zanjani was criticized for the disposition of his experimental sheep with partially human tissues, particularly heavily humanized livers. The sheep were given to a ranch, which treated them just like its regular sheep and put them out to graze. The research sheep, however, had no experience in the wild and quickly succumbed to its rigors, including coyotes. The press coverage centered on concern that putting these sheep in this situation was inhumane, which to us seems convincing (Mullen 2005). No one would plan to release human neuron mice into the wild, to be slaughtered by local cats. But there is another possible concern raised by the sheep example: it might be inappropriate for partly or fully humanized tissue to be eaten by other animals. For coyotes to eat partially ovine, partially human livers is certainly not cannibalism, but some might argue that, as most cultures strive to avoid letting human corpses be scavenged, we should do the same for human cells or tissues incorporated in non-human animals.

Of course, cultures that practice burial recognize that corpses are consumed, both by generically referenced "worms" as well as by bacteria and other microbes. The Parsi religion continues, at least in some locations, to dispose of their dead on "towers of silence," where the corpses are consumed by vultures (Dugger 2001). And, until now, no culture has had to determine what is the appropriate disposition for the bodies of animals that contain some human cells. In the context of the human neuron mouse, we believe this does not require any more than the treatment of the bodies and tissues of human neuron mice as medical waste, but this issue may need further discussion.

Conferring Humanity on Mice

In Kafka's *Metamorphosis*, Gregor Samsa was transformed into a cockroach; would these experiments, in any relevant way, transform a mouse into a man? Or, to be more precise, into a creature with some aspects of human consciousness or some distinctively human cognitive abilities? This result seems implausible, but we cannot rule it out on a priori grounds.

The mouse brain is significantly smaller than the human brain. In volume it is less than one-thousandth the size of the human brain. Even apart from their smaller size, mouse brains are organized differently from human brains. The proportion of a brain composed of the neocortex, the region most associated in human brains with consciousness, is hugely greater in humans than in mice. The brain is an incredibly complex network of connections. Neuroscientists believe that it is the architecture of the brain that produces consciousness, not the precise nature of the neurons that make it up. As an analogy, architecture determines whether a building is a cathedral or a garage, not whether the bricks used are red or gray. A mouse brain made up entirely of human neurons would still be a mouse brain, in size and architecture, and thus could not have human attributes, including consciousness.

This argument is extremely plausible but, to date, it has not been tested. At least one set of experiments has been done indicating that some behaviors might be transmitted between

species along with brain tissue. As early as 1988, scientists showed that by transplanting embryonic quail tissues, primarily tissues that give rise to the central nervous system, into embryonic chickens, they could produce chickens that exhibited quail-like behavior (Balaban et al. 1988). The resulting chicks "crowed" like baby quail rather than like baby chickens. These experiments involved the transplantation of large quantities of intact tissue, not disaggregated neurons, and much of that tissue remained homogeneously quail-derived in the chick's brains. Thus, the chicks' brains could be viewed as being one building with two different kinds of architecture as well as two different kinds of breaks. In this respect that experiment differs substantially from the experiments in question here. Given how little is still understood about consciousness and its sources, it is not clear whether it differs enough.

The quail-chicken experiments suggest that a crucial question for the human neuron mouse experiments is whether the human neurons become organized in the mouse brain in murine patterns or in human ones. The fact that the mice will have already constructed their own brains with murine neurons before the human cells are transplanted argues that the human cells would follow the existing murine structure, but without doing the experiment, that cannot be assumed. The cerebellar experiment will offer little information on this point. All mammalian cerebella are organized in generally identical, and relatively simple, structures. And, in any event, the cerebellum does not, at this time, appear to be significantly involved in human consciousness.

The whole-brain experiment, however, should offer many opportunities to see whether the brain organization is murine or human. Human and mouse brains are organized differently in many ways, at both large and small scales. The relative sizes of the various parts of the brain are one set of differences. The existence and nature of particular brain structures are others. For example, mouse brains have easily visible structures called "whisker barrels" in their cortexes that appear to receive and manipulate information from their whiskers. Each whisker reports to one and only one whisker barrel. Humans (even mustachioed men) do not have whisker barrels. In contrast, human brains (and other primate brains) have especially complicated visual centers with multiple layers of neurons involved in the processing of visual information. Mouse brains have few layers and less complexity in their primary visual area.

What we called "conferring humanity on mice" seems to be the main concern in the literature on chimeras and, presumably, is the main concern in the "brain clause" of the Brownback chimera bill. The authors have not used our language of "conferring humanity" on the transplanted animal, but the concerns each expresses seem equivalent to the concerns we encompassed in our term.

The Johns Hopkins group took the express position that human/non-human chimera experiments should try to minimize the risk that the resulting animal would have more human-like cognitive capacities. Robert and Baylis (2003) wrote of how chimeras might induce confusion about the moral status of the resulting creature, primarily as a result of the possibility that they would have some human cognitive capabilities.

Karpowicz et al. (2005) find that the only plausible argument against the creation of human/non-human chimeras is based on human dignity:

> By giving nonhumans some of the physical components necessary for development of the capacities associated with human dignity, and encasing these components in a nonhuman body where they would either not be able to function at all or function to a highly diminished degree, those who would create human-nonhuman chimeras would denigrate human dignity (Karpowicz et al. 2005, 121).

It is not clear to us that this is the case, but, in any event, the argument assumes, as the Karpowicz article later makes clear, that the recipient animal would, in fact, develop a "physical

component"—in this case, a brain—"necessary for the development of the capacities associated with human dignity." That would be an extremely unlikely result of these experiments.

And the NAS Guidelines noted the sensitive questions raised by the effects of stem cell transplants on an animal's brain, including the unlikely but conceivable possibility that the human cells might affect its cognitive abilities. The NAS urged embryonic stem cell research oversight committees to pay special attention to the possibilities that embryonic stem cell transplants might give the animal "characteristics that are valued as distinctly human" or "human characteristics that would be ethically unacceptable to find in an animal" (NRC 2005, 50).

We must mention one other way in which these experiments might confer some attributes of humanity on mice. Like hematopoietic stem cells, human brain stem cells are multi-potent. They make many different kinds of brain cells, including both neurons and non-neurons. Some much-contested recent research asserts that, in some circumstances, human hematopoietic stem cells can make cells from other tissues, such as the liver and the brain. If this turns out to be true, which many other researchers strongly dispute, it is conceivable that human brain stem cells could do the same. The possibility that the human neuron mouse would also have a liver or a kidney that was partially made up of human cells seems to add little, if anything, to concerns about its brain. It might be more troubling, though, if the human brain stem cells could become, in the mouse, germ cells—egg and sperm cells that contained wholly human genes.

This result seems almost impossible. Even if human brain stem cells can become germ cells, those cells (or, in the case of sperm, their progenitors) are formed early in mammalian development and would be created and in place long before the human cells arrived. Even if a mouse did produce human sperm or eggs, they could not fertilize or be fertilized by a mouse germ cell in a way that would lead to the production of an embryo. Humans normally have 23 pairs of chromosomes containing their DNA; mice have only 20. This difference, among many others, should forbid the production of any even transiently viable offspring. But, once again, it is difficult to make guarantees before the experiments are done.

This issue was discussed by the NAS guidelines on embryonic stem cell research (NRC 2005, 39–40). It concluded that no animals into which human embryonic stem cells had been transplanted should be allowed to breed. Given that the cells in Weissman's experiment would not be embryonic, and hence pluripotent, but only brain stem cells and thus presumably only able to make cells in the brain lineage, the guidelines would not apply to this research. In another respect, there seems no particular reason to breed human neuron mice; their progeny would not have human-derived brain neurons but regular mouse neurons.

One final issue about humanness is worth noting, even though it is not raised by these proposed experiments. Distinctive humanness does not just reside in the brain and the gonads. Although a chimpanzee with a human gall bladder or a human appendix would not be likely to raise grave concerns, a chimpanzee with a human face, a human skull or human hands and feet might. In addition to concerns about human brain functions and human gametes, giving non-human animals, in whole or in the part, the outward physical appearance of humans, could be deeply unsettling. Whether that is a moral argument or prudential one, such experiments should be undertaken, if at all, only for the most powerful reasons.

Public Reactions

Public reaction to unsettling scientific research has been called everything from "the yuck factor" to Leon Kass's "wisdom of repugnance." Based on our own reactions when we first heard of these experiments and from those of friends and colleagues with whom we have

discussed the experiments, we were confident that some people will have a strong initial reaction against this research. That reaction might be only a passing problem of public relations for the institutions where the research is performed. But it could also be a political problem if it undermined support for this and other useful biomedical research. And if one concluded that such research, aimed ultimately at the relief of human suffering, is not only ethically permissible but ethically compelled, doing experiments with a strong "yuck" factor may itself be unethical.

We could not, in 2002, confidently predict the public reaction to these experiments. Weissman had talked publicly about these experiments, including the completed experiment in mice with normal brains, and they had been discussed, to a very limited and brief extent, in the United States press (Krieger 2002). The news stories did not generate any substantial public reaction. In the United States arguments based on improving health have had great political power; to the extent the human neuron mouse is seen as likely to lead to improved treatments for human disease, we suspected it will not be enormously controversial here.

The British Isles, however, presented a different picture. Weissman's proposed experiments were covered by several prominent newspapers in the United Kingdom and Ireland, including the *Financial Times*, as well as the more populist *Daily Mail* and the *Mirror* (Beattie 2001; Financial Times 2001; Kendall 2001). The experiments were also featured in a small section of a British television documentary on mice in research (Colville 2004). Greater concern about this research in the United Kingdom and Ireland may have been the result of greater cultural concerns about various forms of genetic engineering, as seems to be the case with respect to genetically modified food. It could also stem, in part, from a stronger animal rights movement, particularly with respect to laboratory animals. Or it might just be the result of a more alert press.

In fact, human/non-human chimeras have generated more continued discussion in the United States than we would have expected, particularly in light of the relatively few dramatic cases of such chimeras. News stories have appeared regularly. The NAS guidelines' limited discussion of such chimeras seemed to get more attention than its much broader and more significant recommendations for controlling human embryonic stem cell research. And, in 2005 human/non-human chimeras were both singled out by "bioconservatives" as key part of a "bioethics agenda for the second Bush Administration" (Cohen 2004) and were the subject of Senator Brownback's anti-chimera bill, including a clause (S. 1373 §301(1)(H)), which seems aimed directly at the human neuron mouse. In spite of its endorsement by President Bush in his 2006 State of the Union address, no hearings have been held to date on the Brownback bill. Its chances for passage are uncertain at best. In addition, there seems to have been little attention in the United Kingdom or Ireland to such chimeras since 2003.

RECOMMENDATIONS

In 2002, we told Dr. Weissman that we believed that his two outlined experiments may ethically proceed, but we suggested certain safeguards to minimize any risks.

First, we argued that human brain stem cells only be used if they were obtained pursuant to the procedures required for fetal tissue that may be used with federal research funding. Those procedures help ensure that the donor's consent was freely given.

Second, we urged the experiments should be performed in stages and should be carefully monitored. Disquieting or disturbing results at one stage should lead to discontinuance of the experiments pending further review of the ethical implications of those results. Such results could include the infliction of pain on the mice receiving the transplants, the formation of human-like structures in the mouse brains, or odd and possibly human-like behaviors by the

mice. We believed the cerebellar experiment should be performed first as it seems to have the fewest implications for consciousness. If it proceeded without disturbing surprises, the next stage should be the whole-brain experiment in which the mice are aborted. The mouse brains could then be examined pathologically to determine both whether the experiment worked at all and whether the resulting brain structures were wholly murine, wholly human, or something in between. If the brain appeared functional and its structures appear clearly murine, the experiment could proceed to its next phase and the mice could be born, then observed for unusual behavior.

We recognized that, at each stage, distinguishing between normal and abnormal structures or behaviors might prove difficult. And, in ambiguous cases—for example, a mouse brain with distorted whisker barrels—the decision whether to proceed may prove quite difficult. If the results indicate human brain structures or human behaviors, or even significant ambiguity, the experiments should be stopped and reconsidered in light of the new information. We did not have recommendations about what any such reconsideration should conclude; we did urge that it proceed with great care.

Our third recommendation concerned the possible public reaction to these experiments. We recognized that our belief, based on our study, that these experiments are ethically appropriate did not mean that the public would take the same view. We recommended that these experiments be done in an open manner with information conveyed, when normally appropriate, to the press. The researchers should strive to provide background information about the experiments and the reasons for doing them so that the public's reaction to this work, positive or negative, can be better informed.

In retrospect, we would make two more recommendations for Dr. Weissman. First, the bodies or brains of the dead mice should be disposed of appropriately, such as through incineration as medical waste. Second, unless there is a clear and powerful scientific reason for it, these mice should not be allowed to breed. Although the risk that they would form human gametes seems extremely small, we can see no good reason to take that risk.

Our recommendations were different from those of Karpowicz et al. (2004), the Johns Hopkins working group, or the NAS, but they are consistent with each. Those groups' recommendations sought to avoid the same primary end—the creation of animals with some possibility of human-like cognitive abilities—but focused largely on what cells would be inserted into what creatures, when and how. The Johns Hopkins working group provided six factors to consider in minimizing those risks in experiments with non-human primates. Karpowicz et al. (2005) recommended that as few cells as possible be used in transplants into early non-human embryos, that animals closely related to humans should be avoided, and that dissociated cells be transplanted instead of chunks of human brain. The NAS pointed generally to avoiding the risk of developing human characteristics in the recipient animal and expressly proposed banning the transfer of human embryonic stem cells (the most potent) into blastocysts (the earliest stage) of non-human primates (our closest relatives).

The context for our report made it unnecessary for us to reach those conclusions. We had been asked to give an opinion on transplanting dissociated human brain stem cells into very young mice (the first experiment) or mice in their fetal stages (the second experiment). The Johns Hopkins non-human primate factors were not relevant to these mouse experiments. The proposed experiments met the only relevant Karpowicz guidelines (the second and third guidelines) (Karpowicz et al. 2005). And we believe our analysis was exactly the kind of analysis the NAS guidelines seek from the reviewing embryonic stem cell research oversight committees, an assessment that the experiments are unlikely to result in an animal with human characteristics.

CONCLUSION

This article, and the report it was based on, tried to describe and discuss the ethical issues raised by one narrow set of proposed experiments, but the analysis may have broader implications. Three points deserve special mention.

First, the discussion of the ethical significance of transferring some aspects of human consciousness or some human cognitive abilities clearly needs to be taken further. Our report, and this article, do not conclude that it would be a clearly bad act to confer such capabilities on non-humans. We conclude only that it needs further discussion. We can note that, as far as we can see, the concern must be about specific kinds of human characteristics. A mouse with the human brain's sense of vision does not seem particularly troubling. Even a mouse with a memory of human quality might not be a concern. But a mouse with human language capabilities or that seemed to have a human level of self-consciousness would be, at the least, troubling. The thought experiment of considering mice (or other animals) with specific kinds of human cognitive or emotional capacities may prove one useful way to explore these problems.

And we further note that this issue is not limited to the human neuron mouse or even to biology. Some of the same issues would be raised by the creation of machines, as computers or as androids, with something approaching human consciousness. The creation of non-biological human/non-human chimeras with human-like intelligence may well be much more realistic than biological chimeras; after all, computers already have some human cognitive abilities, including some abilities that exceed ours, such as chess playing. Work looking at both the biological and the non-biological contexts seems likely to be important.

Second, our discussion of the appropriate uses of human tissue noted that human brain tissues, and perhaps particularly neurons, raise special issues. Many believe the field of neuroscience is entering a golden age of increased understanding of brain function. The extent to which the brain or tissues from the brain are given special, quasi-sacred status may have major effects on brain research and treatment. This is a particularly ripe issue for consideration in neuroethics.

Finally, and most important, all the specific issues noted in this article need to be watched. We tried our best in our initial report to predict what would seem ethically important about the human neuron mouse experiments and, almost 5 years later, we think we were largely, but not entirely, right. In the coming years, we are confident that our predictions will, in still other ways, small or perhaps large, prove to be wrong. The results of the experiments, the ongoing ethical discussion, and the interactions of the two need to be monitored to make sure that what now appears to be ethically permissible remains so. For, as noted by Robert Burns in his poem, *To a Mouse on Turning Up Her Nest with the Plough*,

> But, Mousie, thou art no thy lane In proving foresight may be in vain: The best laid schemes o' mice an' men Gang aft agley, An' lea'e us naught but grief an' pain For promis'd joy.
>
> —Robert Burns, *To a Mouse on Turning Up Her Nest with the Plough* (1795)

Acknowledgements

The authors wish to thank Professor Bill Newsome, our colleague on the working group and an extremely valuable contributor to the initial report; Dr. Irving Weissman, for giving us this opportunity and for his subsequent comments; an anonymous (and kind) referee; and Professor Greely's research assistant, Sean Rodriguez.

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