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The Other Stem-Cell Debate

By JAMIE SHREEVE

Except for the three million human brain cells injected into his cranium, XO47 is just an average green vervet monkey. He weighs about 12 pounds and measures 34 inches from the tip of his tail to the sutured incision on the top of his head. His fur is a melange of black, yellow and olive, with white underparts and a coal-black face. Until his operation, two days before I met him, he was skittering about an open-air enclosure on the grounds of a biomedical facility on the Caribbean island of St. Kitts. Afterward, he was caged in a hut shared with half a dozen other experimental monkeys, all of whom bore identical incisions in their scalps. Judging from the results of previous experiments, the human neural stem cells inserted into their brains would soon take hold and begin to grow, their fibers reaching out to shake hands with their monkey counterparts. The green vervets' behavior was, and will remain, all monkey. To a vervet, eye contact signals aggression, and when I peered into XO47's cage, he took umbrage, vigorously bobbing his head in a stereotypical threat display. Still, it was hard not to stare.

By virtue of the human material added to his brain, XO47 is a chimera -- that is, an organism assembled out of living parts taken from more than one biological species. The word comes from the monstrous creature of Greek mythology -- part lion, part serpent and part goat -- that is slain by the hero Bellerophon. Less fearsome chimeras occur naturally -- lichen, for instance, is a mix of fungus and algae. Most, however, are created in the laboratory by scientists like Dr. Eugene Redmond of Yale University, the soft-spoken, 65-year-old psychiatrist and neurosurgeon who operated on XO47. He set up the St. Kitts Biomedical Foundation on this island because that is where the monkeys are -- an overabundant feral population of them, ideally suited for research. Redmond has transplanted immature human brain cells into a region of XO47's brain that produces dopamine, a neurochemical that is depleted in the brains of people with Parkinson's disease. If the human cells can take hold and differentiate and bolster the monkey's own dopamine-producing machinery, a similar operation on a Parkinson's patient, the reasoning goes, should have an even greater chance of success.

Redmond is of the opinion that the insertion of a few human cells into a monkey brain is no big deal, and most biologists would agree. But many bioethicists and policy makers are alarmed by recent research developments that have made chimeric experiments more common and increasingly capable of producing human-animal amalgamations that are more ambitious, more "unnatural" -- and thus more troubling -- than Redmond's vervets.

Driving the surge in chimeric experimentation is the enormous but still untested promise of human stem cells. In theory, stem cells isolated from an early human embryo can transform themselves into virtually any kind of cell in the body, kindling hope that one day they may be transplanted into human patients to provide new tissue wherever it is needed -- heart muscle for cardiac patients, insulin-producing cells for diabetics, nerve cells to repair crushed spinal cords and so on. But there are serious hurdles to overcome before this dream can be realized, including figuring out what controls the differentiation of stem cells and combating their tendency to form tumors. Clearly it is unethical to study the unknown actions of stem cells in human subjects. One obvious solution is to insert the cells into animals and watch how they develop. Depending on what kind of stem cells are used and where they are put in the animal, it may also be possible to pluck some particular human biological feature or disease trait out of its natural context and recreate it in an animal model, where it can be examined and manipulated at will.

While the objections to stem-cell research have largely revolved around the ethics of using human embryos, there is another debate bubbling to the surface: how "human" are chimeric creatures made from human stem cells? Fueling the anxiety has been the lack of coherent regulations in the United States governing the creation of chimeras. The President's Council on Bioethics has twice taken up the issue in recent weeks, and Senator Sam Brownback, the Kansas Republican and outspoken social conservative, has introduced legislation to restrict chimeric experiments. Meanwhile, the National Academy of Sciences is expected to issue guidelines later this month as part of a widely anticipated report on the proper use of human stem cells. While the academy's recommendations will carry considerable clout, compliance will be voluntary.

Few people argue that all experiments mixing human and animal material should be banned outright. But where should the lines be drawn? "Some scientists are completely upset with even a single human cell in a monkey brain," says Evan Snyder, a neurobiologist who has conducted chimeric experiments with Redmond. "I don't have problems with putting in a large percentage of cells -10 or 20 percent -- if I felt it could help a patient. It comes down to what percentage of human cells starts making you squirm."

Francoise Baylis, a bioethicist at Dalhousie University in Halifax, Nova Scotia, and a co-author of Canada's stem-cell guidelines, squirms not at a percentage of human cells but at the place where awareness begins. "We have to be sure we are not creating beings with consciousness," she says. The very existence of biologically ambiguous creatures could lead to "inexorable moral confusion" in a society with two ancient and irreconcilable codes of conduct governing the treatment of humans and animals. That said, all modern genetic research, including the sequencing of the human genome itself, underscores how trivial the biological difference really is between a human being and the rest of life. Ninety-nine percent of our genome is shared with chimpanzees. Thirty-one percent of our genes are interchangeable with those of yeast. Does the nearness of our kinship with the rest of nature make the prospect of a quasi-human chimera among us less of a threat to our collective psyche or more of one?

Chimeras have been with us for some time. In 1988, Dr. Irving Weissman and his colleagues at Stanford University created a lab model for AIDS by endowing a mouse with an entirely human immune system. Since then, scientists have tailored mice and other animals with human kidneys, blood, skin, muscles and various other components. Baboon and chimp hearts have been transplanted into human chest cavities, pig cells into the brains of Parkinson's disease patients and, more routinely, pig heart valves into people with heart disease, including Jesse Helms, the former U.S. senator.

For most of us, a senator with a partly porcine heart or a mouse with a human immune system is not sufficient to provoke the kind of instinctive queasiness known among ethicists as "the yuck factor." The man most identified with that term, Dr. Leon Kass, the bioethicist and current chairman of the President's Council on Bioethics, is of the opinion that widespread feelings of repugnance may be an alarm that something is morally wrong, even if you are not able to articulate precisely why. The mouse and the senator may not trigger a yuck because they look just like a rodent and a person. But what about a normal-looking mouse with a headful of human brain cells or a human-animal embryo that is only briefly alive and never seen?

If you want to get a peek at a real live chimera, drive about five miles east from downtown Reno, Nev., until you come to a farm that looks pretty much like any other farm. The gate will be locked, but from the road you can see some pens holding sheep that look pretty much like any other sheep. Pound for pound, however, these may be the most thoroughly humanized animals on the planet. They are the work of Esmail Zanjani, a hematologist in the College of Agriculture, Biotechnology and Natural Resources at the University of Nevada at Reno. Several years ago, Zanjani and his colleagues began injecting fetal lambs with human stem cells, mostly ones derived from human bone marrow. He said he hoped that the cells would transform into blood cells so that he could use the sheep to study the human blood system. According to Zanjani, when he examined the sheep he discovered that the human cells had traveled with their lymphatic system throughout the sheep's body, developing into blood, bone, liver, heart and assorted other cells, including some in the brain. While some scientists are skeptical of his findings, Zanjani told me that some have livers that are as much as 40 percent humanized, with distinct human structural units pumping out uniquely human proteins.

While the idea of partly humanized sheep might make some people a little uncomfortable, it isn't easy to see where they trespass across some unambiguous ethical line. But according to Dr. William Hurlbut, a physician and consulting professor in human biology at Stanford, who serves with Kass on the President's Council for Bioethics, the seeing is exactly the point. What if, instead of internal human organs, Zanjani's sheep sported recognizably human parts on the outside -- human limbs or genitals, for instance, ready for transplant should the need arise? Hurlbut maintains that this is scientifically plausible. But it would be wrong. Every living thing has a natural trajectory through its life beginning at conception, and in Hurlbut's view, a visible chimera would veer dangerously off course.

"It has to do with the relationship between signs and their meaning," he told me. "Human

appearance is something we should reserve for humans. Anything else that looks human debases the coinage of truth."

Understanding the world as divided into distinct categories is a fundamental organizing principle of civilization. We conceive of the living aspect of that world as separated into species, with boundaries around them that should not be purposively muddled. The underlying validity of our categorical constructs is not as important as how we use them to make sense of the world. Our minds have evolved to be hypersensitive to the borders between species, just as we see a rainbow as composed of six or seven distinct colors when it is really a continuum of wavelengths of light. "When we start to blend the edges of things, we're uneasy," Hurlbut says. "That's why chimeric creatures are monsters in mythology in the first place."

It is easy to marshal rational arguments to counter this thinking. The limitations of a typological concept of species, which goes back to Aristotle, are well known. Some species interbreed with closely related ones on the borders of their habitats. Evolutionary biologists cannot agree on how to define what a species really is in the first place, so it is hard to see how the boundaries between them can be absolute. Even if species boundaries do have a natural integrity, how alarming is it to find that those walls can be perforated by artificial means? We have been engaging in unnatural acts upon nature for centuries, grafting plants onto one another or breeding dogs in visible shapes and sizes that diverge wildly from their natural state -- let alone performing heart transplants and in vitro fertilizations. I'm not sure I would undergo a crisis of truth at the sight of a sheep with a human arm, especially if it were the best means available for replacing a lost one. But everyone has a squirm threshold. What would you make of a sheep with a human face?

The reason Zanjani's chimeras look like perfectly ordinary sheep is that he injected them with stem cells in a late stage of their fetal development, when their body plans were already laid down. The reason he was allowed to conduct the experiment at all is that he works in the United States, as opposed to Canada or Great Britain where such chimeric research is restricted. Older fetuses are not as impressionable as younger ones, and embryos are the most vulnerable of all. And the younger the human stem cell you insert, the more powerful an influence it can have on the body and brain of the host animal. The way to produce the most homogenous blend of human and animal would thus be to inject fully potent human embryonic stem cells into the very early embryo of, say, a mouse. This is the experiment that policies in those countries are most keen to prevent.

It is also the one that Ali Brivanlou is poised to begin. For several years, Brivanlou, a 45-year-old developmental biologist at Rockefeller University in New York, has been arguing that one of the best ways to understand the usefulness of stem cells for regenerative medicine is to first insert them in an animal embryo and see how they divide and differentiate in a living system. The experiment is explicitly prohibited by the institutions that supply the stem-cell lines approved by the Bush administration, so he is using private funds to develop his own lines. He plans to insert them into 3-to-5-day-old

mouse embryos, which he will then implant in the wombs of female mice. Brivanlou is anxiously awaiting the publication of the National Academy of Sciences guidelines before proceeding, but he says he doubts that they will prove an impediment. In his view, showing the potency of stem cells only in a petri dish is like testing the power of a new car by revving its engine in the garage. He wants to take the car out on the track and see how it might perform some day on the open road.

"This experiment must be done," he says. "We can't go directly from culture to a patient. That would be extremely dangerous."

But his experiment is one that most are very reluctant to undertake, even in the private sector. When I inquired at Geron Corporation, a biotechnology company in California, whether scientists there were considering such work, I received a terse e-mail reply that "the company is not, has not and will not pursue inter-species stem-cell chimeras."

Robert Lanza, vice president for medical and scientific development at Advanced Cell Technology in Worcester, Mass., says much the same thing. "I personally don't want to engage in those kinds of experiments, and I won't have any of my scientists do that work," he says. "Sure, we could reach our endpoints quicker that way. But it takes you into very murky water."

Why all the shuddering? For starters, there is the gonad quandary. If the experiment really works, the human cells should differentiate into all of the embryo's cell lineages, including the one that eventually forms the animal's reproductive cells. If the mouse were male, some of its sperm might thus be human, and if it were female, some of its eggs might be human eggs. If two such creatures were to mate, there would be a chance that a human embryo could be conceived and begin to grow in a mouse uterus -- a sort of Stuart Little scenario, but in reverse and not so cute.

"Literally nobody wants to see an experiment where two mice that have eggs and sperm of human origin have the opportunity to mate and produce human offspring," says Dr. Norman Fost, professor of pediatrics and director of the bioethics program at the University of Wisconsin and a member of the National Academy of Sciences committee reviewing stem-cell research policies. "That's beyond anybody's wildest nightmare."

Is the concern over the reproductive issue overblown? It is, of course, biologically impossible for a human fetus to be delivered from a rodent uterus. Moreover, for a human embryo to be conceived, the chimeras would have to be born first in order to mate, and Brivanlou says he has no intention of allowing them to come to term. He plans to terminate them and examine the fate of the human cells after a week. Still, there remains the question of what kind of being would be present during those seven days. Nobody knows. Does even the fleeting, prenatal existence of a chimera of unknown aspect cross a moral line -- not because of what it might look like or become but simply for what it is?

Brivanlou is not troubled by that question. He sees the other methods of testing the stem cells' power -- in vitro or in the body of an older fetus or of a fully developed animal -- as

inadequate, and he says he wants the science to be allowed to follow its natural course. "One thing that is important to remember -- we've been here before," he says. "In the 70's, there was a huge debate around whether recombinant DNA should be allowed. Now they do it in high-school labs. For any new technology that emerges, the first reaction is fear. Time will take care of that. When people take the time to think, it becomes routine."

During my visit to St. Kitts, I watched as Gene Redmond, dressed in blue surgical scrubs in the operating room, drilled into the skull of a vervet monkey. Once he penetrated the skull, Redmond positioned a four-inch hypodermic needle on a mount over the hole and ever so slowly lowered it into the monkey's cerebral cortex, down through structures associated with emotion and on until it reached its target in the basal ganglia at the base of the brain. He let the brain settle around the needle for a while and then injected a solution of donor cells into the target.

If he were performing this operation on a human patient, the procedure would be more or less the same. But he would need a much longer needle. If it is not some categorical essentialism that draws a bright line between us and the rest of the animals, surely it is the size and power of our brains. They are the physical address of everything we think of as uniquely human -- our rational thinking, intelligence, language, complex emotions and unparalleled ability to imagine a future and remember the past. Not surprisingly, chimeric experiments that seed the brain of an animal with a little neural matter of our own are uniquely suspect, especially those that meddle with the sites of higher function in the cortex.

"If you create stem-cell lines that might produce dopamine and want to put them in an animal first to see if they retained their stability, that's not problematic," Norman Fost maintains. "But what if you want to study brain cortex? You'd want to create a stem-cell line that looks and acts like cortex and put this in an animal. In the toughest case, you'd want to put it in a very early stage of development. This is extremely hypothetical, but suppose these cells completely took over the brain of the animal? A goat or a pig with a purely human brain. Unlikely, but imaginable. That would certainly raise questions about what experiences that animal was having. Is it a very smart pig? Or something having human experiences? These are interesting questions that no one has thought about before because they haven't had to."

The scientist most responsible for making people think about those questions -- and squirm and fume -- is Irving Weissman. Several years ago, Weissman and his colleagues at Stanford and at StemCells Inc., a private company he helped to found, transplanted human neural stem cells into the brains of newborn mice. The human cells spread throughout the mouse brain, piggybacking on the host's developmental pathways to eventually make up as much as 1 percent of some parts of the host's neural tissue. Once again, the ultimate purpose of the chimera was to create a research model for human brain function and disease. While somewhat successful in this regard, Weissman said he felt his model was hampered by the 99 percent of it that was still mouse. So he came up with an ingenious idea: why not make a mouse with a brain composed entirely of human neurons? In theory, at least, this could be achieved by transplanting human neural stem

cells into the fetal brain of a strain of mouse whose own neurons happen to die off just before birth. If the human stem cells took up the slack and differentiated along the same lines as in the earlier experiment, you might just end up with a living newborn mouse controlled by a functioning brain that just happened to be composed of human cells.

Before proceeding with this experiment, Weissman said he thought it might be a good idea to solicit some ethical input. He contacted Hank Greely, a bioethicist at Stanford's law school, who put together a committee to review the benefits and risks involved. The members agreed that the human neuronal mouse could be an extremely beneficial tool to study the effects of pathogens and disease in the human brain and the action of new drugs. They identified several areas of risk. The most difficult one to articulate, as Greely told the National Academy of Sciences panel reviewing the use of human stem cells, was the "nontrivial chance of conferring significant aspects of humanness on the nonhuman organism."

"Though exceedingly remote, we thought this possibility was reason for caution and concern," Greely told me recently. His committee, which has yet to publish its report, did not find that risk alone was sufficient grounds for canceling the experiment. Instead, the members suggested that Weissman incorporate into the experimental protocol a series of "stopping points." Some of the fetal mice should be terminated and examined before birth, and if there should appear any "disquieting or disturbing results," the experiment should be suspended pending further ethical review. Results deemed troubling would include any evidence that the transplant was shaping the architecture of the mouse's neural edifice, as opposed to just contributing the bricks. Mice have sensory structures in their brains called "whisker barrels," for instance, which we lack, while we have a far more complicated visual cortex. Shrunken whisker barrels or swollen visual cortex in the fetal mice brains would be a red flag. If everything appeared normal, the remaining animals could be brought to term and monitored for the appearance of any odd, and especially humanlike, behavior, which would again warrant stopping the experiment and seeking additional input from the ethical community.

Weissman is still months or even years away from actually trying his human neuron mouse experiment, and it has already drawn "This shall not stand" rhetoric from Jeremy Rifkin, the anti-biotech activist, Bill O'Reilly and numerous religious commentators and bloggers.

The real problem with Weissman's proposed mouse, however, may turn out to be not that it is too human but that it is not human enough. The basic structure of our nerve cells is not all that different from those of any other mammal, including a mouse's. But because our brains are so much bigger, the cells that compose them reach across greater distances, and the timing of their development is much longer. How likely is it that human nerve cells will develop into a whole functioning brain in the tiny arena of a fetal mouse's skull? Weissman concedes that his proposed chimeric experiment may not succeed. But, hypothetically speaking, what if you could conduct the analogous experiment in an animal with a brain more like our own, like a monkey or a chimpanzee? Strictly from a biomedical perspective, a human-ape chimera could be the ultimate research model for

human biology and disease -- one that is completely human in everything but its humanity.

"If someone were to try Irv's mouse experiment with a great ape or even a monkey, I'd get real worried," Greely says. "I'd want to make sure people thought long and hard about that."

The danger, of course, is in how difficult it would be to know when you've slipped over the edge. While Greely's committee has been brooding over Weissman's mouse and the National Academy has been pondering its recommendations for the use of embryonic stem cells, another ethics group has been meeting at the Phoebe R. Berman Bioethics Institute at Johns Hopkins University to grapple with the especially dicey issue of human/primate chimeras. Could the introduction of human cells into nonhuman primate brains cause changes that would make them more humanlike? How would one tell? Would it be morally problematic to create a chimera with a significant degree of humanlike consciousness, cognition or emotion? Should such experimentation be banned? If such chimeras were to be created, what legal rights and protections should they have, distinct from other animals?

The report of the Working Group on Interspecific Chimeric Brains is expected to be published later this spring in a scientific journal. While the group's recommendations remain confidential until then, a rough idea of the boundary they might draw between allowable and prohibited research is suggested by two experiments that have already been conducted. One was carried out in 2001 by Evan Snyder, then at Harvard University and now director of the stem-cell program at the Burnham Institute in La Jolla, Calif. Snyder and his colleagues implanted human neural stem cells into the brains of 12-week-old fetal bonnet monkeys, aborted them four weeks later and found that the human cells had migrated and differentiated into both cerebral hemispheres, including into regions of the developing monkey cortex. Like Redmond, Snyder discounts any possibility that had the monkeys been brought to term the relatively small number of human cells in their brain would have had any effect on their normal cognition and behavior.

"Even if I were to make a monkey with a hippocampus composed entirely of human cells, it's not going to stand up and quote Shakespeare," Snyder says. "Those sophisticated in human functioning know that it's more than the cellular components that make a human brain. It's the connections, the blood vessels that feed them; it's the various surfaces on which they migrate, the timing by which various synaptic molecules are released and impact other things, like molecules from the bloodstream and from the bone."

It's quite likely that the members of the Johns Hopkins committee (it includes distinguished philosophers, bioethicists, neuroscientists, primatologists and stem-cell researchers) will conclude that an experiment like Snyder's is ethically safe. A relatively small scattering of human cells could be introduced into a primate brain, late in its development when there would be no chance the human cells could influence its fundamental architecture. But a result of another experiment, performed in the late 1980's by Evan Balaban, who is now at McGill University in Montreal, might give the group

pause about mixing human and primate tissue in a very early fetus. Balaban removed a section from the midbrain of a chick embryo, grafting in its place the corresponding piece of proto-brain from an embryonic quail. While many of the embryos failed to develop, a few matured and eventually hatched. The newborn chicks were normal in most respects -- except they crowed like quails.

"One could imagine that if you took a human embryonic midbrain and spliced it into a developing chimpanzee, you could get a chimp with many of our automatic vocalizations," says Terrence Deacon, a biological anthropologist at the University of California at Berkeley and a member of the Johns Hopkins committee. "It wouldn't be able to talk. But it might laugh or sob, instead of pant-hoot."

Of course, Deacon adds quickly, such an experiment would be highly unethical. The notion of a chimpanzee normal except for its human sobbing would probably exceed the squirm threshold of the other members of the Johns Hopkins group. Perhaps it is not what a human-animal chimera would be that violates some fundamental categorical construct in our minds, or what it would look like, as William Hurlbut maintains, as much as what it could do -- whether it would have a brain that makes it act in a way that is uncomfortably familiar. "Humanness" surely resides in the emergent layers building the vastly complex architecture of the human brain.

But is there a clear biological distinction between us and the rest of creation, one that should never be confounded by the scuffling of strange new feet in laboratory basements? Deacon has devoted a great deal of thought and research to such questions. While his is hardly the only view, after a career spent comparing the brains of living primates and the skulls of fossilized hominids, he says that there is little evidence for the sudden appearance of some new thing -- a uniquely human gene, a completely novel brain structure in the hominid lineage -- that sets us distinctly apart. Obviously, there has been an overall increase in brain size. But the telling difference is in more subtle shifts in proportion and connections between regions of the brain, "a gerrymandering of the system" that corresponds to a growing reliance on the use of language and other symbolic behavior as a means of survival. This shift, which Deacon believes began as long as two and a half million years ago, is reflected most prominently in the swollen human prefrontal cortex.

"We humans have been shaped by the use of symbols," he says. "We are embedded in a world of human creation, where demands for success and reproduction are all powerfully dependent on how well we swim through our symbolic niche."

This raises some fascinating questions, not just about the chimeras we might create with our scalpels and stem cells but also about the ones we may already have fashioned by coaxing humanlike behaviors from animals who have the latent capacity to express them. In the wild, chimpanzees and other apes do not engage in any symbolic behavior remotely comparable to what humans have evolved. But in the laboratory they can learn to communicate with sign language and other means on a par with the skills of a toddler. The difference is that the toddler's symbolic behavior becomes increasingly enriched,

while the chimpanzee hits a wall. How much further could a bioengineered chimera go? Could it swim in our symbolic niche well enough to communicate what is going on inside its hybrid mind? What could it teach us about animals? What could it teach us about us? And what is the price of the knowing?

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