Human Brain Disease Recreated In Mice

Transferring a mutant prion gene into mice mimics a human brain degeneration in the animals, thus supporting the idea that the prion protein can cause disease.

IN THE EARLY 1980S, NEUROLOGIST Stanley Prusiner made one of the more provocative proposals of the decade. He suggested that scrapie, an apparently infectious degenerative brain disease of sheep, could be transmitted by something he called "prions," infectious particles made just of protein—and containing no nucleic acids. Although researchers already knew that the scrapie agent had to be unconventional—it simply didn't behave like a typical virus—few people were prepared to accept anything as unconventional as prions.

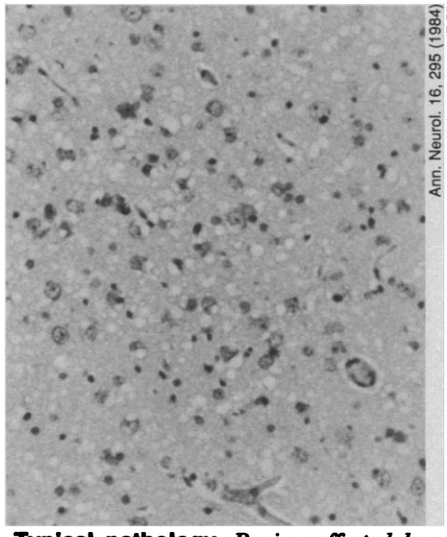
But prion research has come a long way since then. In 1985, the cloning of the gene encoding the prion protein proved that it does in fact exist. And somewhat surprisingly, the gene turned out to be widely expressed in the brains of higher organisms, a result suggesting that the prion protein has a normal brain function that can somehow be subverted, leading to brain degeneration. Then studies done during the past 2 years suggested that specific mutations in the prion gene might cause two similar human brain diseases, Gerstmann-Sträussler-Scheinker syndrome (GSS) and Creutzfelt-Jakob disease.

Now, Prusiner's group at the University of California, San Francisco, has used genetic engineering techniques to recreate GSS by transplanting the mutated prion gene into mice (also see report on p. 1587). "It's a fantastic piece of work," says Dmitry Goldgaber, an expert on neurodegenerative diseases at the State University of New York in Stony Brook. "It shows very clearly the major role of the prion protein in causing pathological changes in the brain."

Not only will the animal model help neurobiologists answer the many remaining questions about prions and how they work, but it may also shed some light on other neurodegenerative diseases as well. Although GSS is extremely rare—it strikes only one in about 10 to 100 million people—some of its pathological features resemble those of Alzheimer's disease, for which there is no good animal model. Still lacking, however, is formal proof that the prion protein can by itself form an infectious agent, capable of transmitting disease to other animals.

Prusiner and his colleagues originally implicated the prion protein in the etiology of GSS about 2 years ago. The finding that GSS, although it is a genetic disease in humans, can be transmitted to nonhuman primates and rodents by injecting their brains with material extracted from the brains of human patients, had already raised suspicions that prions might be involved. In that regard GSS behaves very much like scrapie and Creutzfelt-Jakob disease. Moreover, the brain changes in all three conditions are similar, another indication that they might have a common cause.

In any event, once the prion gene had been cloned, which was achieved by Prusiner's group in collaboration with those of Charles Weissmann at the University of Zurich and Lee Hood at the California Institute of Technology, it became possible to ask whether the gene could be defective in GSS. And in 1989, Prusiner and Karen Hsiao, also of UCSF, together with Tim Crow of the Clinical Research Center in Harrow, England, and Jurg Ott of Columbia University, showed that GSS patients indeed have a specific mutation in the prion gene that does not occur in family members who do not have the disease.



Typical pathology. *Brains affected by scrapie, GSS, and Creutzfelt-Jakob disease develop a spongy appearance.*

But showing a genetic linkage between a mutation and a disease does not necessarily prove that the mutation causes the pathology. That's where the current experiment comes in. In it, Hsiao and Prusiner, with UCSF colleagues Michael Scott and Stephen DeArmond, transferred a prion gene with the GSS mutation into mice. The result: The animals that carry the mutant "transgene" develop neurological symptoms and brain changes very much like those seen in GSS patients. "I think it's really extraordinary," says neurobiologist Donald Price of Johns Hopkins University School of Medicine. "A single mutation in a transgene, when put in a mouse, can cause clinical disease and brain pathology."

Moreover, the same thing may be true for Creutzfelt-Jakob disease. This past summer, Lev Goldfarb, Paul Brown, D. Carleton Gajdusek, and their colleagues at the National Institute of Neurological Disease and Stroke identified another mutation in the prion protein gene in families with hereditary Creutzfelt-Jakob disease from two very different ethnic backgrounds, Slavs and Sephardic Jews.

Nevertheless, all this work still leaves a perplexing question: Do the mutated prion proteins work alone in causing the diseases? That question was the source of much of the skepticism about Prusiner's original suggestion that a protein particle, with no nucleic acid, could transmit disease. The scrapie, GSS, and Creutzfelt-Jakob agents clearly replicate, as the diseases can be passed from one animal to another. But replicate without genetic material?

Both Brown and the Prusiner group note that the current experiments have not completely ruled out the possibility that the mutant prion gene does not actually cause disease by itself but instead renders those who carry it susceptible to the real culprit, perhaps an as yet unidentified virus. But they consider that prospect unlikely, for despite years of searching, no one has ever detected evidence of nucleic acid as an active ingredient, either in purified prions or in brain extracts that transmit the diseases in question.

Brown also points out that the disparate backgrounds of the Slavs and Sephardic Jews make it unlikely that both groups were exposed to some common virus or other environmental influence that causes Creutzfelt-

Jakob disease. He observes, however, that some family members in the NIH group's study have the mutation but don't appear to be getting sick, including one woman who is 75 years old, well beyond the age when Creutzfelt-Jakob disease usually develops. "She's a crucial lady, but we don't know what to think about her," Brown says. She might be an indication that something besides the mutated gene is required. Or she might just be an example of a well-known phenomenon that geneticists call "incomplete penetrance," meaning that a disease gene just doesn't cause its ill effects in everybody who inherits it.

Prusiner's group is already working on an experiment that may clarify the issue of whether the prion protein works alone. The researchers are attempting to show whether brain extracts from the sick transgenic mice can transmit the disease to other mice. That would indicate that the presence of the mutant transgene is all that it takes to get infectious prions.

It's been 220 days since the animals were injected with the brain extracts, however, and so far they are doing just fine. The transgenic mice generally develop their symptoms before they are 200 days old, but, says Price, it may still be too early to conclude that the transmission experiment is negative. Depending on conditions, he notes, it can take more than a year for neurological symptoms to develop in animals injected with scrapie brain extracts.

A good many other questions remain as well. Researchers want to know what the prion protein does normally, as well as what might cause it to go awry and produce disease. And then, of course, there is the question of how prions can reproduce without nucleic acid.

Prusiner and his colleagues addressed this issue in the 16 November *Cell,* in which they show that an interaction between the scrapie prion protein and the normal cell protein is involved. Proteins can induce changes in each other's shapes when they bind to one another, and the Prusiner group suggests that the scrapie prion protein has an abnormal three-dimensional configuration that can be transmitted to any normal prion protein with which it interacts, and so on in a sort of chain reaction ultimately leading to disruption of brain cell function and nerve degeneration.

Nevertheless, neither the replication issue nor the other remaining questions about prions are likely to be resolved soon. But the identification of the mutations in GSS and Creutzfelt-Jakob disease and, just as important, the ability to create transgenic mice in which to study the effects of the prion protein genes at least open the door to addressing those questions. ■ JEAN MARX

Viewing the Universe as a Coat of Chain Mail

New calculations have pointed the way to quantum gravity and suggested a novel structure for the sub-sub-microscopic world

IT MIGHT SEEM LIKE QUITE A philosophical journey to get from a medieval coat of chain mail to the fundamental nature of reality. But according to theoretical physicists Abhay Ashtekar and Lee Smolin of Syracuse University, and Carlo Rovelli of the University of Pittsburgh, it's not very far at all.

If their latest calculations are correct—and the three physicists put a heavy emphasis on the "if"—then the sub-sub-microscopic fabric of space and time is best understood as a densely woven skein of loops and coils and braids, all described by a branch of mathematics known as knot theory—precisely the same mathematics that describes the intricate linkages of chain mail or the tangled snarls in a garden hose.

These intertwining loops are *not* superstrings, they are quick to point out. Those hypothetical strands of energy got a lot of press a few years back as a promising Theory of Everything, but since then they have bogged down in mathematical intractability. The loops that Ashtekar, Smolin, and Rovelli are talking about are much simpler entities, roughly analogous to the "lines of force" that surround a bar magnet, and that cause compass needles to align with the magnetic field of the earth.

Ashtekar and his colleagues say their model of loopy space-time suggests a whole new way of searching for a truly unified theory of all the fundamental forces, including gravity. And it goes a long way toward answering such previously unanswerable questions as "What happens at the center of a black hole?" and "Where did the Big Bang come from?"

All of which makes it rather startling to realize that Ashtekar, Smolin, and Rovelli have actually been quite conservative in their theorizing. "If there is strength in what we're doing," explains Rovelli, "it's that we're not just pushing some idea that we like. We're sticking on the solid ground of general relativity [Einstein's theory of gravity] and quantum mechanics. All the results are forced on us from those two." Indeed, everything they've done so far comes from looking at exactly the same equations that physicists have been staring at for generations—but looking at them in a totally new way.

Like many other physicists before him, Ashtekar had been struggling with the equations of general relativity, which express Einstein's insight that gravity is actually the result of subtle distortions in the shape of space. General relativity is widely considered to be one of the most conceptually elegant theories in physics. It successfully describes phenomena ranging from Big Bang cosmology to tiny variations in the ticking of atomic clocks. But it is also one of the most mathematically difficult theories to work with. When the equations are expressed in their conventional form—the way Einstein first wrote them in 1915—they are a tangled, nonlinear mess.

This mathematical muddiness is one reason that it's been almost impossible to reconcile Einsteinian gravity with the quantum theory of atoms and elementary particles, says Ashtekar. Physicists have been trying to achieve that feat for nearly 70 years now, ever since they first started talking about quantum jumps and probability waves back in the 1920s. It just didn't seem reasonable that quantum principles should govern everything in nature except gravity. And yet the mathematics of the two theories went together about as well as oil and water.

Until 1986, that is. What Ashtekar discovered in that year was that Einstein's equations could be simplified enormously, just by rewriting them in terms of a certain set of mathematical variables related to the intensity of the gravitational field. The effect was magical: In their new, streamlined form, Einstein's equations looked almost exactly like the equations that govern electric and magnetic fields. And if there is one thing that physicists know how to quantize, it is electricity and magnetism. "Quantum elec-