The Prion Heretic

For 30 years, Laura Manuelidis has rejected the dominant theory that misfolded proteins cause infection. Sticking to a minority view has become a career in itself

NEW HAVEN, CONNECTICUT—Laura Manuelidis has an acrobatic mind. Her train of thought evokes a circus performance. She soars up and drops down, she twirls, she swoops in one direction and swerves back to where she started. She titled her volume of poetry, published in 2007, *Out of Order*. "Isn't that fitting?" she says with a laugh.

Manuelidis has spent her whole adult life here at the Yale School of Medicine, first as one of a half-dozen women in her 1967 graduating class and then as a parser of brain tissue. At 24, she ignored taboo and wed her professor, 48-year-old Elias Manuelidis. Despite naysayers who declared the marriage doomed, it turned out to be a lively and passionate one, lasting until her husband's death from a stroke in 1992. Since then Manuelidis has pursued the work they began together: challenging the now-entrenched view that prions, which are misshapen proteins, transmit disease. Skeptics linger but rarely speak out. Now 68 years old, Manuelidis has become the de facto representative for doubters worldwide.

She knows that the history of science is littered with heretics who reject conventional wisdom, insisting that their experiments reveal the truth while others' do not. Often they turn out to be wrong and either abandon their view when the evidence against it grows overwhelming or go to their grave still believing. Sometimes they're right. Manuelidis, comfortable in the role of dissenter, likes to quote 20th century mathematician and philosopher Bertrand Russell: "Doubt is the essence of science," she says.

Her seeds of doubt—or rather, tall, flourishing plants—germinated years ago. In 1982, Stanley Prusiner, a neurologist at the University of California, San Francisco, gave prions their name. He described them as infectious particles made up mainly of a protein, PrP, that misfolds and goes awry in the brain, causing a cluster of rare, transmissible, and fatal brain diseases. Mad cow disease (officially known as bovine spongiform encephalopathy) is arguably the most famous.

When first proposed, Prusiner's theory was widely dismissed as bizarre. Biology then held that infectious disease was caused by organisms built from DNA, RNA, or both, like viruses and bacteria—something containing a nucleic acid sequence that can replicate and spread through a cell. Proteins lack these sequences. But Prusiner promoted his thesis, snagging millions of dollars in grants and publishing his experiments widely. In 1997, he won the Nobel Prize. Today, Prusiner's view dominates. "It's the dogma," says Adriano Aguzzi, a neuropathologist at the University Hospital of Zurich in Switzerland, who counts himself a believer.

Manuelidis takes an opposing stance that we don't need to rewrite the book on infectious disease to accommodate prions. She regards the protein not as the cause of infection but as a pathological reaction to it and believes mad cow disease and others like it are triggered by viruses. What—and where—those viruses are, Manuelidis isn't sure. No one has found them. Whether that means they don't exist depends on whom you ask.

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She's not the only prion doubter, but her voice is by far the loudest. In part that's because she's safe. Long a tenured professor at Yale, Manuelidis still commands vast lab space she no longer uses. Her unpopularity among top prion scientists leaves her unfazed. "What can they do to me?" she says. "If I don't say it, nobody's going to say it."

Beginnings

In her youth, Manuelidis aspired to be a poet. She enrolled at Sarah Lawrence College, a small liberal arts school outside New York City known for its strong focus on the arts and literature. Her older brother, with whom she'd always been close, was attending Harvard Medical School at the time. "He said, 'I don't see any want ads in *The New York Times* for poets. … How long are you going to be a parasite on Mom and Dad?' "

Manuelidis considered this problem and settled on medical school as an alternative. "My brother said, 'Why don't you become a nurse?' and I said, 'Why don't *you* become a nurse?' " she remembers. "He wanted a normal sister."

In medical school she knew from the start that she would focus on the brain, hoping to cure schizophrenia. Manuelidis had been deeply affected by college summers spent volunteering at Waltham State Hospital outside Boston, where she says patients were rarely seen by physicians. ("I looked in the charts" to find out.) They were so heavily drugged that they reeked of Thorazine, an antipsychotic drug. "I saw people with frontal lobotomies," she says. "I felt there was a certain arrogance in medicine. It was very good to see before I went to medical school."

Manuelidis embraced pathology, encouraged by a supportive department chair. Another draw was her neuropathologist husband-to-be, a confirmed bachelor when she met him. Their relationship was scandalous, she says. They lived together openly and threw large parties at his house attended by many of her friends.

The two began exploring a class of deadly diseases called transmissible spongiform encephalopathies (TSEs), so named because once symptoms surface, the brain turns into spongy tissue with alarming speed. TSEs in people are rare; the most prevalent, sporadic Creutzfeldt-Jakob disease, strikes about one person in 1 million each year. A curious feature is that TSEs, like viral diseases, can be passed from one animal to another by injecting affected brain tissue. TSEs also have a long latency period in animals. The first guinea pigs Elias Manuelidis exposed to TSE thrived for 500 days before getting sick.

But the virus, if there was one, was elusive. Some experiments indicated that there couldn't be a virus at all. A radiobiologist named Tikvah Alper showed in the 1960s that blasting infected tissue with radiation didn't destroy its ability to infect. And exposing tissue to high heat failed to prevent transmission of a TSE called scrapie, which kills sheep and goats. Strategies that annihilate the usual viruses didn't do much to halt disease.

In the early 1980s, Prusiner began to advocate a different theory to explain where the "transmissible" in TSEs came from. The answer, he argued, was a particle he called a prion, in effect the first infectious protein. The evidence, "one has to admit, was very shaky" to start, Aguzzi says. But the work slowly advanced. Prusiner reported that purifying bits of scrapie-laden brain down to their infectious components always left behind a protein that resisted chemical breakdown, suggesting it might be misfolded. In 1985, the gene that generates the prion protein was cloned, and researchers were shocked to discover that the healthy prion protein, PrP, was naturally abundant in the brain tissue of normal people.

Aguzzi became convinced of the prion hypothesis in the early 1990s, when he was working with prion biologist Charles Weissmann, then at the Institute of Molecular Biology in Zurich, and saw that mice genetically engineered to lack PrP didn't get sick when injected with infected material. This suggested to Aguzzi that the disease is transmitted by a misfolded PrP protein and that it targets healthy PrP in the brain and turns it toxic, killing neurons. "That was really the tipping point" for me, he says.

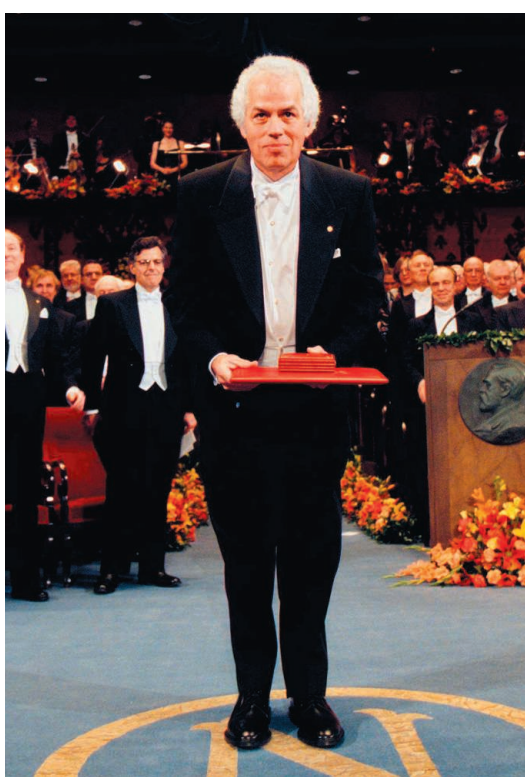
Another tipping point came when Prusiner won the Nobel Prize in physiology or medicine in 1997 for "his discovery of prions—a new biological principle of infection," the Nobel Committee announced. "Numerous attempts to disprove the prion hypothesis over the past 15 years have failed," Prusiner declared in his Nobel lecture on 8 December of that year, shortly before accepting the prize in Stockholm, Sweden. For him, the case was settled.

Days after the award was announced, Manuelidis shared her blunt assessment with *The New York Times*. "My fear is that debate is going to be stifled," she told a reporter. "That's the problem with Nobel prizes. If people feel everything is decided, you can't possibly risk going against the grain." Manuelidis keeps a yellowed clipping of that article pinned up outside her office door.

Prusiner's Nobel came as fear of TSEs was running high. In 1995, the first person died of what would later be called variant Creutzfeldt-Jakob disease (vCJD): a version of the neurological ailment, transmitted by eating beef from affected cows. Panic ensued, especially in the United Kingdom, where 175 people were eventually diagnosed as having the lethal disease. But uncertainty about its cause hasn't been "an impediment to making sensible public health decisions," says David Asher, chief of the laboratory of Bacterial and TSE Agents at the U.S. Food and Drug Administration (FDA). Governments simply took steps to keep meat from sick cows out of the food supply. Recently, cases of vCJD have dropped, and fear has subsided.

Battle lines

In the years since Prusiner won his Nobel, even some who have wavered on the prion hypothesis say that evidence has mounted steadily in its favor. About 10 years ago, neuroscientist Claudio Soto, who trained in Chile and is now at the University of



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Nobelist. The views of neurologist Stanley Prusiner, at the Nobel Prize ceremony in 1997, are now dogma in the field.

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Texas Medical School at Houston, developed a new technology called protein misfolding cyclic amplification (PMCA). As its name suggests, PMCA is designed to boost the concentration of prion protein in a sample and eliminate living cells (along with viruses they may contain). To test for infectivity, researchers run samples of brain homogenate through PMCA and inject the concentrated product into mice. Because the mice get sick, many believe this points to prions as the infectious culprit.

The results have been difficult to argue with. Bruce Chesebro, who has long been on the fence about the prion hypothesis, is leaning in its favor. Chief of the Laboratory of Persistent Viral Diseases at the U.S. National Institute of Allergy and Infectious Diseases, based in Montana, Chesebro now says, "PMCA suggests it may not be a virus" that triggers these maladies.

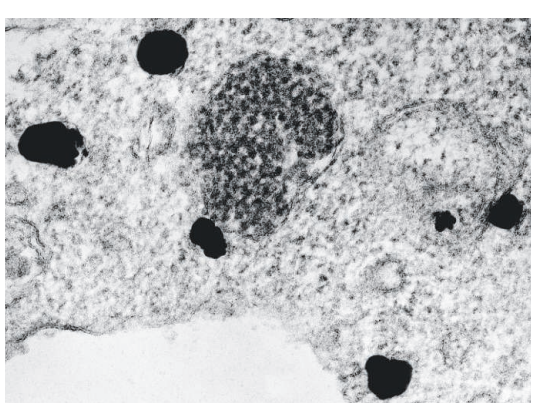
Biochemist Jiyan Ma of Ohio State University in Columbus and his colleagues reported online in *Science* on 28 January 2010 (http://scim.ag/prion_ma) that mixing PrP with various lipid molecules, which force it to misfold, and then injecting the mixture into the brains of healthy mice gave them prion disease. "The data show that prions exist," says Surachai Supattapone of Dartmouth College, who published one of the landmark experiments in a 2007 issue of the *Proceedings of the National Academy of Sciences* (*PNAS*). "That's I think now clear." And, he adds, prions are "not viruses."

This all sounds unambiguous, but even backers of the prion hypothesis admit to some gaps in the evidence. Misfolded PrP is sometimes found in noninfectious tissue, and sometimes it is not found in tissue that can infect other animals. Brain homogenates from people and animals afflicted by prion disease—even prion diseases from the same species—can have wildly different effects when injected into animals, including genetically identical ones. Some develop symptoms after a few months, others not until years later. Prion supporters attribute the variation to different "strains" of PrP, suggesting that the protein can misfold into different chemical conformations that have different levels of toxicity. Not everyone buys it.

Another festering worry, Manuelidis and some others say, is that prion experiments are easily contaminated. Anyone examining the brains of animals with TSEs risks winding up with the infectious agent on their equipment, Chesebro says—on their test-tube racks, their benchtops, the hoods under which they work for protection. Although great care is taken to keep stray bits of brain tissue at bay, there isn't an airtight solution short of switching to a new lab each time. What does this mean, practically speaking? If an unidentified infectious agent exists, it could be a stealthy actor even in the best-controlled experiments.

Family ties. Laura and Elias Manuelidis built their careers together, and their family—here in 1967 with their infant son Manoli.

The Ma study, many argue, has come the closest to definitively proving the prion hypothesis. Researchers synthesized misfolded PrP in the lab, injected it into normal animals, and watched them develop symptoms of TSEs. But no one has replicated this result. Instead, researchers often use genetically engineered mice that overproduce healthy PrP because they develop symptoms faster once infected. This makes for cheaper and easier experiments. Normal mice can take an eternity to show symptoms. It "might take longer than the life span of the mouse," Aguzzi says. "So it helps to have an overexpresser."



Minority view. Viruslike particles cluster as small circles inside a large bundle, while PrP protein (labeled by large black dots) scatter nearby. Manuelidis discovered the particles and says they're likely causing prion diseases.

Casting a long shadow over the field is Prusiner, who is as tight-lipped in public as Manuelidis is loquacious. He almost never talks to the press and declined, through his correspondence manager, to be interviewed for this article.

Minority views

Asking Manuelidis to elaborate on her prion skepticism can be an exercise in frustration. Encouraging her to talk is not the problem—but grasping her case against prions isn't easy. Manuelidis is aware of this. "I can't think in a straight sentence," she says, soon after parking her low-slung Mazda convertible and sitting down for dinner at a Portuguese restaurant. "My brain goes into literature."

Many prion biologists—even some with questions about the current dogma—are disappointed by the evidence she's turned up. In 2007, Manuelidis published a paper in *PNAS*, describing small viruslike particles in TSE-infected tissue but not in uninfected samples. That's a correlation, however, not proof that the particles are causing disease, Chesebro says. "I'm sympathetic of her battle," he notes. "I wish she were more convincing."

Weissmann, Aguzzi's colleague, who's now at the Scripps Research Institute in Palm Beach, Florida, has been both friend and foil of Manuelidis over the years and was the only one to mention her unprompted in conversation. (More often there is a long pause after her name is brought up.) "The work itself is sound; she's done some interesting work with cell cultures," Weissmann says. "But then she tries to force it into the viral hypothesis," going through "contortions" to interpret the data. His language is nearly identical to Manuelidis's descriptions of the work of the "prion cabal."

Manuelidis and others say she has paid a price for holding so tightly and so publicly to her virus theory. She describes a prominent prion scientist walking out when she took the lectern at a meeting, and another screaming at her in a room full of people. Anonymous reviews of her papers have sometimes been caustic and personal, she says. "She's had a very tough time scientifically, but she also has many friends and allies," says Robert

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Somerville, who studies TSEs at the Roslin Institute at the University of Edinburgh in the United Kingdom. He's known Manuelidis since 1980 and considers himself a good friend. "The divisions run perhaps deeper in our field than others and are longer lasting. Which is sad, really—it can be difficult to have a useful, constructive discussion."

Like Somerville, Asher of FDA worries about the path prion biology has taken. "I'm still left with this nagging concern that the abnormal protein, important though it may be, has not been demonstrated to be the infectious agent," he says. "The field has been very forgiving of failures of the prion hypothesis to predict things that are found in the laboratory." Asher also complains that prion studies are almost never repeated—scientists just move on to a new one. And, he says, papers that fit the dogma are more readily published than those that do not.

Maurizio Pocchiari, a neurologist at the Istituto Superiore di Sanità in Rome, agrees that siding with the vocal majority can certainly help one's career. "If you are aligned with the prion hypothesis, it is very easy to publish, … [and] it's easier to get a good result" experimentally, he thinks, thanks partly to the genetically modified mice churning out PrP that are so popular.

Pocchiari is another member of the Manuelidis fan club. He disagrees that a conventional virus is lurking behind TSEs, believing it would have been found by now—but he isn't satisfied with the protein-only dogma, either. "We now are pretty aware that when we try to purify infectivity, we purify the pathological prion protein, but we also purify something else," he says. "There is a something else, … [but] we have no idea what we are looking for." Some, like Somerville, wonder about a "virino," a small viral particle that doesn't code for proteins on its own and acts in conjunction with PrP. Virinos fit the bill in part because of their size, which is useful because of long-ago studies suggesting that nothing as large as a virus was hiding in TSE-infected tissue. But virinos are a concept invented to fit the experimental data that haven't been found anywhere else. Then again, many argued before the prion theory took hold that prions shared this feature, too.

Tenacity

If there is a virus behind TSEs, how could it have stayed hidden for so long? Isolating these tiny snippets, Manuelidis argues, is just plain difficult because copies are so scarce, even in brain tissue where disease concentrates. Most people disagree with her, but not everyone. "The fact that we can't detect it is, I would argue, not a statement of what's present or not, but maybe more a statement about our abilities as scientists to discover it," Somerville says. Asher hopes that Manuelidis will continue her work with the viruslike particles she identified in the 2007 *PNAS* paper. "Far too little has been done with it," he says.

Manuelidis perseveres, working near the morgue in the basement of the surgery building, where she's been since the mid-1970s. The labs are old; her husband's office remains largely untouched. Like everywhere else, precautions against infection were an afterthought when they first began this work. "My husband was determined you had to feel the stuff; nobody put on gloves and then [we] ate lunch," Manuelidis says. A guinea pig injected with CJD that mysteriously didn't get sick was christened Harold and lived for 12 years in the lab's biohazard facility. Manuelidis accidentally squirted herself once in the eye with an infectious brain sample and took out a $1 million life insurance policy for 10 years for her two sons. She never developed the disease, reinforcing her view that TSEs are not particularly virulent and may need to be injected or ingested in large quantities for someone to get sick.

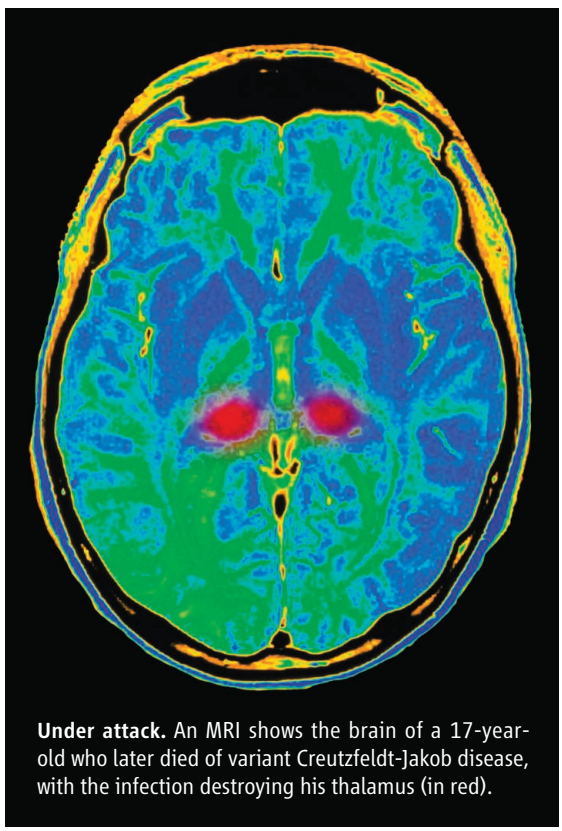
In the late 1990s, Manuelidis began downsizing her lab, frustrated by how grueling her struggle for money had become—a shift that began after Prusiner's Nobel, she says. Now she has three recent Yale undergraduates working for her, as they bridge time between college and medical or graduate school. Her students learned the prion gospel in biology class and are fascinated by what, to them, is a new controversy. They're not sure where they stand. As they tell it, Manuelidis doesn't push them. "The data doesn't seem to contradict" the virus theory, says Terry Kipkorir, a thoughtful 2010 graduate who grew up in Kenya and slouches at a desk, a blue stocking cap on his head. Kipkorir and the others are looking for viral particles in infected tissue and trying to determine which components are infectious. But "I don't want to dismiss the prion theory" either, he says. If there's one thing he's realized as he ends an academic year in Manuelidis's lab, it is to prize autonomy. "I don't think I'm going to work under a lot of direction" going forward, he says.

Manuelidis attributes the slow pace of her never-ending virus hunt to technical challenges and a shortage of money. She has the same CJD grant from the National Institutes of Health that she's had for more than 30 years; it now brings in $539,000 a year, nearly $200,000 of which goes to Yale for overhead costs. Multimillion-dollar awards—the kind that allow you to work with hundreds of animals—are not in her future, she thinks.

Should they be? The same researchers who lament how incomplete her work is say no. "To be quite frank, I don't think it's worth funding," Weissmann says. "In the 19th century, there were still people who thought that life could originate from boiled hay. These people just die out—there's always fewer and fewer of them." He believes that Manuelidis will never relinquish a theory to which she's held tight for decades, no matter what story the data tell.

Asked if letting go would be hard for her, Manuelidis is unambiguous. "No, no," she says. "All I know is that my experiments don't show" that prion protein is causing disease. She's hopeful now that she's on the cusp of something new. "I think I can crack this stuff," she says.

–JENNIFER COUZIN-FRANKEL



Under attack. An MRI shows the brain of a 17-year-old who later died of variant Creutzfeldt-Jakob disease, with the infection destroying his thalamus (in red).

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