NEUROPATHOLOGY

Protein Studies Try to Puzzle Out Alzheimer's Tangles

Amidst the knot of unanswered questions surrounding the causes of Alzheimer's disease is the puzzle of the Alzheimer's tangles. These twisted filaments, which scar the brains of Alzheimer's victims, are one of the two major pathological features of the disease. But their origin and role in the cascade of events that causes neuronal death in Alzheimer's has been unclear. Indeed, many researchers have concentrated their efforts on the other major Alzheimer's feature, the plaques, which contain a potentially neurotoxic protein called β-amyloid.

Now, a series of recent studies is helping explain the creation of these neurofibrillary tangles and how they might contribute to neuronal death. The explanations focus on the major component of the snarls: an abnormal form of a protein called tau. In the January *Annals of the New York Academy of Sciences* neuropathologist John Trojanowski, neuroscientist Virginia Lee, and their colleagues at the University of Pennsylvania School of Medicine summarize experiments indicating that phosphatases, enzymes that remove phosphate groups from tau, are suppressed in the neurons of Alzheimer's victims.

Carrying more phosphates than it should, the researchers suggest, keeps the protein from performing its normal role of securing vital parts of the neuronal cytoskeleton and thus harms the cell. "In time," says Trojanowski, "hyperphosphorylated tau would precipitate like crud in the plumbing." Other work by the Penn researchers, as well as by Brandeis University biochemist Gerald Fasman, suggests that the aggregation of this crud into scarring tangles may be aided and abetted by an old Alzheimer's suspect: aluminum.

Dennis Selkoe, professor of neurology and neuroscience at Harvard Medical School in Boston, says these studies are "the first to show that decreased dephosphorylation ... has to be carefully considered as a mechanism" in the alteration of tau during Alzheimer's. Nevertheless, Selkoe and other scientists caution that the work doesn't prove decreased phosphatase activity is the primary cause of Alzheimer's, as other factors may trigger the phosphatase regulation problem in the first place. The remainder of the Penn researchers' scenario, particularly the role of aluminum, also remains to be tested.

Tau's ties to Alzheimer's were highlighted in 1991, when Trojanowski and Lee showed it made up the paired helical filaments (PHFs) that comprise the neurofibrillary tangles. They also found this "PHF-tau" carries many more phosphate groups than cover the normal protein, including many at sites not ordinarily phosphorylated (*Science*, 8 February 1991, p. 675).

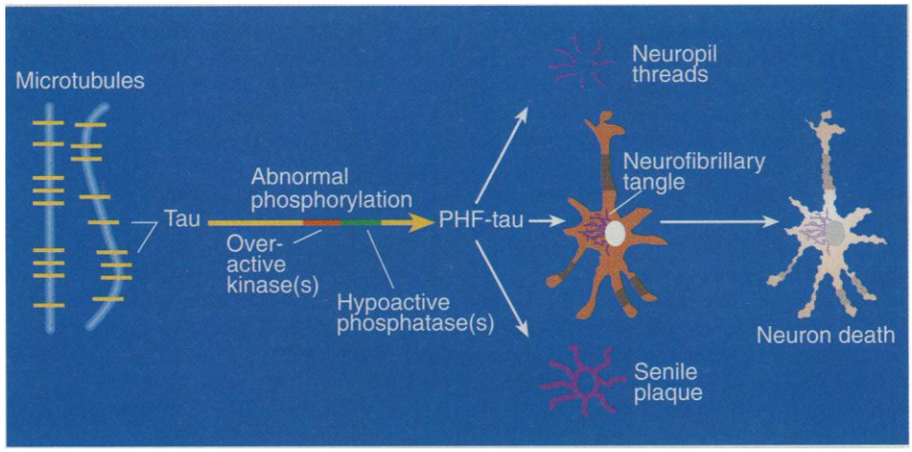
Animal studies indicated that extra phosphates might lead to neuronal damage, even before tangles form, by interfering with a crucial function of tau. The protein is supposed to assemble and stabilize the microtubules, filaments that convey cell organelles, glycoproteins, and other vital materials through the neuron. Tau's ability to bind to microtubule segments is partly determined by the number of phosphate groups attached to it. Extra phosphates might derail this process.

These ideas spurred a search for the mechanisms that change normal tau into PHF-tau. Researchers turned first to kinases, enzymes that add phosphate groups to proteins. But no one could definitively catch a particular overactive kinase in the act.

Yet the studies that had mapped the locations of phosphate groups on both normal human tau and PHF-tau had been done on proteins taken from post-mortem brains. Perhaps normal brains had enzyme activity not found in Alzheimer's brains that was stripping off phosphates after death.

That's exactly what the researchers found in experiments described in the October issue of the journal *Neuron*. Along with Eriko Matsuo of Penn and four other researchers, Lee and Trojanowski mapped the phosphorylation state of tau taken from the brains of epileptic but otherwise normal surgical patients. The analyses showed that tau in living neurons possesses phosphate groups at many of the same sites as PHF-tau. But after progressively longer postsurgical intervals, tau lost more and more phosphates. "Within an hour or two normal tau is almost completely dephosphorylated," says Trojanowski.

The phosphatases, in other words, were still doing their jobs in cells from normal brains. PHF-tau in Alzheimer's brains, in contrast, remains just as phosphorylated many hours after death. This suggests that phosphatases are underproduced in the diseased neurons or that their action is somehow being inhibited. And that spells trouble for PHF-tau's ability to fasten microtubules. These structures serve as the neuron's railway track for material transport, and normally tau functions as the ties on this track. Says Trojanowski, "Flip a couple of ties off and the trains will still go around, but knock a lot of them off and the train will crash."



SOURCE: J. TROJANOWSKI AND V. LEE

Troubled tau. This simplified model shows how normal tau (rectangles on microtubules) might be changed to PHF-tau through abnormal accumulation of phosphate groups. PHF-tau then cannot bind microtubules and instead infests the neuron as tangles, harming and killing the cell.

According to Selkoe, that's what makes this study important. "There is great interest in how this protein that normally promotes integration of ... microtubules becomes diverted, and how these neurons ultimately die and leave behind 'ghost' tangles," he says.

That latter stage is addressed by other Penn studies. Once formed into tangles, researchers believe, the masses of PHF-tau further obstruct cellular transport and damage the neuron. But what prevents protein-digesting enzymes, whose job is to get rid of such useless protein tangles, from eliminating them? In a study reported in November's *Journal of Neuroscience*, Lee, Trojanowski, and Penn colleague Ryong-Woon Shin pointed to an environmental factor—aluminum—as a possible culprit.

The researchers injected the brains of a group of rats with a combination of human PHF-tau and aluminum salts. They compared the brains of these animals with those from rodents injected with PHF-tau and one of several other proteins associated with Alzheimer's disease, including ApoE4 and β-

amyloid. (Duke University's Allen Roses has argued that ApoE4 contributes to the hyperphosphorylation of tau.) The scientists found that PHF-tau injected along with aluminum resisted breakdown for the longest period. The researchers suggest that aluminum, which binds avidly to phosphate groups, may change PHF-tau's molecular conformation so that it is less accessible to the protein-digesting enzymes.

Trojanowski and Lee's suggestions are "very reasonable, very significant," says Fasman of Brandeis, whose own test-tube experiments have shown that the more phosphate groups that are attached to synthetic neurofilament fragments, the more aluminum ions are able to bind and cross-link neurofilaments, rendering them less soluble. The activity of aluminum may thus be "the crucial step" opening the route to tangle formation, Fasman says.

Other scientists, however, say the "crucial step" may occur even earlier, before phosphatases or aluminum come into play. In the first place, no one knows whether the modest buildups of aluminum found in the brains of Alzheimer's patients contribute to, cause, or result from tangle formation, says neuroscientist Zaven Khachaturian, director of the Office of Alzheimer's Disease Research at the National Institute on Aging. As for the post-mortem stability of PHF-tau, Michel Goedert, a molecular neurobiologist at the Medical Research Council's Laboratory of Molecular Biology in Cambridge, U.K., says it could simply be caused by some other "upstream" events that give the filaments their particularly insoluble structure.

Harvard's Selkoe thinks these upstream events may be genetic and may involve β-amyloid, the main component of the other characteristic Alzheimer's lesion, senile plaques. Increased neuronal secretion of β-amyloid, possibly as a result of a genetic defect, may eventually produce neurotoxic effects that alter the phosphorylation state of tau protein, Selkoe says. (New studies of mice that express the human gene for β-amyloid precursor protein, reported this week in *Nature*, may allow tests of this possibility.) "Many people in the field now believe that tangles are a step in the degeneration of neurons, not the cause," Selkoe says.

Khachaturian points out that Alzheimer's research has long been polarized between labs focusing on β-amyloid and those interested in tau—or the "BAPtists" and the "Tauists." But he notes that both tangles and plaques apparently result from breakdowns in the balance between protein synthesis and degradation in the neuron, and that the new findings may point to a common path during at least part of this process. And perhaps along that common path will lie a way to untangle the puzzle of the disease.

–Wade Roush

At Math Meetings, Enormous Theorem Eclipses Fermat

Hardly a word was said about Fermat's Last Theorem at the joint meetings of the American Mathematical Society and the Mathematical Association of America, held this year from 4 to 7 January in San Francisco. For Andrew Wiles's proof, no news is good news: There are no reports of mistakes. But mathematicians found plenty of other topics to discuss. Among them: a computational breakthrough in the study of turbulent diffusion and progress in slimming down the proof of an important result in group theory, whose original size makes checking the proof of Fermat's Last Theorem look like an afternoon's pastime.

Slimming an Outsized Theorem

What use is a proof so long that no one mathematician can plow through the whole thing? That's been a problem facing group theorists for the last decade. The reason is that one of their most important theorems, describing the taxonomy of the mathematical objects known as simple groups, has a proof that runs an estimated 15,000 pages, spread over upwards of a thousand separate papers written in widely varying styles by hundreds of researchers. But the Enormous Theorem, as it's affectionately called, is in for some downsizing. Two mathematicians—Richard Lyons at Rutgers University and Ron Solomon at Ohio State University—are leading an effort to tame it.

As befits this mathematical monster, the job isn't going to be finished in a day. After a dozen years, Lyons and Solomon have completed only a fraction of the job, they told their colleagues at the San Francisco meetings, and they expect the task to stretch well into the next century. It's not just the length of the original proof that's so time-consuming, they say, but the need to rework its logic to simplify and shorten it. The wait should be worth it, however. "We've proved some old theorems in considerably greater generality than they were proved the first time," Lyons notes. When they are finished, the result should be a proof that a single individual can comprehend—which should give comfort to some mathematicians who are now hesitant to base their own work on the Enormous Theorem because they can't read it all.

Many researchers in group theory, as well as "customers" who come across groups in other areas of mathematics, rely heavily on the Enormous Theorem, known more prosaically as the classification theorem for finite simple groups. Groups are fundamental algebraic objects that describe various kinds of symmetry. (The rotations of a pentagon by multiples of 72 degrees make up one example of a finite group.) Simple groups are the building blocks from which other groups are assembled, much as atoms are the building blocks of molecules. And just as chemists organize the elements into eight columns, the classification theorem says each finite simple group belongs to one of four categories: cyclic, alternating, Lie-type, or sporadic. The four categories are as different as Heraclitus's earth, air, water, and fire, but knowing that every finite group represents some combination of just four types of simple groups is itself an enormous simplification.

Part of the reason the original proof turned out to be so long is that the four categories have widely varying properties, so that unifying concepts are hard to come by. Group theorists chipped away at the classification problem for nearly 30 years, from 1950 to 1980, slowly building up an arsenal of techniques and proving results for specific cases. In 1972, Daniel Gorenstein of Rutgers spelled out a 16-point program for attacking the problem, but there were few who thought the effort would be successful until Michael Aschbacher at the California Institute of Technology made a series of breakthroughs in the early 1970s. By 1980, it was clear to the experts that their collective effort had solved all the problems of the classification; Gorenstein declared victory in what he called the Thirty Years' War.

The proof, though, was unlike anything mathematicians had ever called a proof before. A traditional mathematical proof is one that an individual can sit down, read, and check for him- or herself. But the proof of the Enormous Theorem has so many pieces that even the experts who produced it rely on one another for assurance that the pieces—some still unpublished—fit together. As Solomon puts it, "If the generation of people who worked on the proof were to vanish, it would be very hard for future generations to reconstruct the proof out of the literature. It wouldn't be impossible, but it would be quite a scramble."

To some mathematicians, it's worrisome that they can't check the theorem on their own. Shreeram Abhyankar of Purdue University, for example, tries to avoid citing the Enormous Theorem in his work on algebraic