Alzheimer's Debate Boils Over

The role of β-amyloid in Alzheimer's disease has triggered plenty of controversy—but never before has it required an entire issue of a major journal to clear the air in the community

Last summer, the world of Alzheimer's research was stirred when Harvard researchers Bruce Yankner and Neil Kowall and their colleagues showed that the protein β-amyloid, injected into the brains of rats, could cause nerve cell degeneration very much like that seen in the brains of Alzheimer's patients. There was good reason for excitement: Not only did the work apparently help clarify β-amyloid's role in the disease's pathology, an issue that has long bedeviled Alzheimer's researchers, it also appeared to provide a much-needed animal model for screening new Alzheimer's drugs. The Yankner group found, for example, that another brain peptide, called substance P, could protect against β-amyloid's neurotoxic effects in the brain, suggesting that substance P, or related compounds, might be useful as Alzheimer's therapies.

But when it comes to Alzheimer's research, nothing is as simple, or as encouraging, as it first appears. Even before the year was out, Yankner's hopeful results had triggered a raging controversy—because some researchers were finding that they couldn't reproduce the work in their own labs. But the story goes beyond the scientific question of β-amyloid's neurotoxicity to the sociology of researcher interactions in a field where the stakes are high both intellectually and commercially. Indeed, Yankner himself seems to have attracted an unusual amount of hostility. "The degree of rancor around him is really extraordinary," says one Alzheimer's researcher familiar with the controversy.

The reasons for the hostility aren't completely clear, although some observers suggest Yankner's personal "style" raised hackles, especially among researchers who thought he wasn't being cooperative in sharing all the experimental details they needed to reproduce his work. Yankner denies that charge and notes that much of the criticism comes from researchers who are allied with companies trying to develop Alzheimer's drugs. Although Yankner himself doesn't have commercial ties, the implication is that those researchers aren't happy with the idea of a competitor moving ahead in producing an animal model, which would give a big edge to anyone trying to get an Alzheimer's drug to market.

Most controversies like this one—involving a volatile mix of issues concerning science, collegiality, and personality—get played out over time in many different journals and in hallway conversations at meetings. But this one became so intense that it's about to boil over in an unusual way: A prominent journal, *Neurobiology of Aging*, decided to devote an entire issue to papers on the question of β-amyloid's neurotoxicity. As journal editor Paul Coleman of the University of Rochester told *Science*: "Clearly this is a topic of consuming interest to the community, and that was the major consideration [behind the decision to produce the special issue]."

Evidence for (and Against) β-Amyloid's Neurotoxicity

The controversy now raging among Alzheimer's researchers centers on a seemingly straightforward question: Is the protein β-amyloid toxic to nerve cells and thus a prime suspect in the degeneration seen in the brains of Alzheimer's patients? If the answer is yes, as work by Harvard researcher Bruce Yankner suggests, scientists will have understood a fundamental feature of Alzheimer's pathology—and also, perhaps, have come up with an animal model for the disease. But as an entire issue of the journal *Neurobiology of Aging* and extensive interviews by *Science* show, experiments to date have yielded equivocal results concerning this key question.

In experiments done on neurons growing in lab culture, "it's quite clear that the peptide [β-amyloid] does cause toxicity. That is reproducible," says Ivan Lieberburg of Athena Neurosciences in South San Francisco. But the animal experiments offer far less consistency. While some groups have seen neuronal degeneration following β-amyloid injections into animal brains, others haven't. And even when the injections do cause degeneration, the lesions display a great deal of variability, depending on such factors as the source of the β-amyloid and the solvent used to dissolve the protein.

Why the variability in the animal results? For one thing, says Mark Mattson of the University of Kentucky Medical Center in Lexington, such *in vivo* studies are "very hard to interpret, because you get a lot of damage from the injection itself." That difficulty touches directly on a key piece of data that the Yankner group interpreted as supporting their conclusion that β-amyloid induces Alzheimer's-like pathology in animal brains.

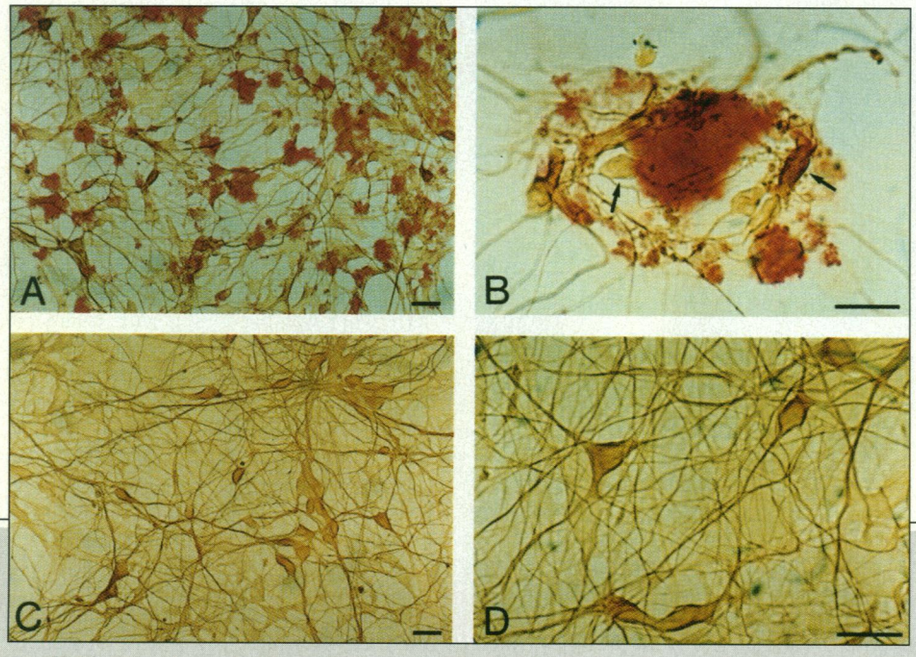
Yankner's group found that the animal lesions stain with an antibody called Alz-50, which recognizes an abnormal form of the tau protein found in the neurofibrillary tangles that are characteristic of Alzheimer's brains. But Dennis Selkoe's group at Harvard detected the same staining wherever a blood vessel was accidentally damaged during injection—regardless of whether the solution being administered contained β-amyloid or an innocuous control. "We're concerned," Selkoe explains, "that Alz-50-positive neurons aren't just in response to [β-amyloid] peptide, but to other injuries."

Even Yankner concedes Selkoe has a point. But he argues that more recent work on monkeys, being done with Neil Kowall and Ann McKee of Harvard's Massachusetts General Hospital, is yielding electron micrographs showing structures that look very much like neurofibrillary tangles at β-amyloid injection sites. The researchers haven't yet analyzed the structures to show biochemically that they are in fact typical Alzheimer's tangles, however.

And that's not the only uncertainty. Gregory Cole of the University of California, San Diego, notes that the type of solvent used to dissolve β-amyloid and the control peptides can influence the results. In experiments on rats, Leon Thal, also of San Diego, Cole, and their colleagues found that they could see increased toxicity with β-amyloid injections when the peptide was dissolved in acetonitrile, which was used by several researchers, including Yankner. But they saw no effect when it was dissolved in another common solvent, a simple salt solution. Since the salt solution dissolves less β-amyloid than acetonitrile does, Cole says he suspects that the difference may in fact reflect the higher doses delivered by the acetonitrile. However, Selkoe, Marcia Podlisny, also of Harvard, and their colleagues didn't see any

On the central scientific issue at stake—the idea that β-amyloid's neurotoxicity can be demonstrated simply by injecting the peptide into brains, as Yankner's results suggest—the special issue, which will appear on 28 September, has produced an evenly split decision. Four groups report evidence for neurotoxicity, at least under some conditions of β-amyloid injection, and four others report that they couldn't detect the neurotoxicity (see box on page 1336).

And that leaves the hope for an Alzheimer's model pretty much in tatters, at least for now. As Sam Sisodia and Donald Price of Johns Hopkins University School of Medicine and their colleagues write in their summary paper for the *Neurobiology of Aging* special issue: "If neurotoxicity cannot be produced reliably and unequivocally, then it seems unlikely that this model will be useful for analyzing pathogenetic mechanisms of [Alzheimer's disease] or for testing therapeutic strategies." Nor do most researchers see any protective effects of substance P in their test systems, although Yankner continues to stand firmly behind that result, as well as the others. Also in the special issue, however, are results obtained with nerve cells growing in culture that may eventually provide a way out of the current morass—as well as some comfort for Yankner.



YANKNER, BUSCIGLIO, AND LORENZO

Making a difference. Bruce Yankner's β-amyloid peptide formed aggregates over the cultured nerve cells *(upper micrographs)*, and they degenerated. But Dennis Selkoe's peptide didn't aggregate and the neurons remained healthy *(below)*.

The β-amyloid roller coaster

Scientifically, the current controversy throws into high relief just how frustratingly difficult it's been for researchers to get a handle on the role β-amyloid plays in Alzheimer's pathology. And, in the absence of a stable consensus, the community's view of the peptide's significance has gone up and down like a roller coaster, a ride that has left researchers divided into amyloid partisans and amyloid skeptics. In fact, the question of whether β-amyloid is directly neurotoxic is, in the words of Alzheimer's expert Dennis Selkoe of Harvard, one of the "thorniest issues in Alzheimer's neuropathology."

The roller coaster ride began in 1984, when George Glenner and C. W. Wong of the University of California, San Diego, discovered that the protein cores of the "senile plaques" found in the brains of Alzheimer's patients are made mainly of β-amyloid. Since then, the roller coaster has gone swooping from sky to ground as investigators argue about whether β-amyloid deposition is the cause—or just the effect—of the neuronal degeneration in Alzheimer's.

neurotoxicity with β-amyloid, no matter what solvent they used.

But there may a good explanation for that, Yankner says, if the differing abilities of the two peptides to aggregate are taken into account. In work beginning 3 years ago, Carl Cotman's group at the University of California, Irvine, found that β-amyloid could be either toxic or stimulating for nerve cells, depending on whether or not it is aggregated. In more recent work, Yankner and his colleagues Jorge Busciglio and Alfredo Lorenzo compared the effects on cultured nerve cells of the β-amyloid preparation his group used for their positive animal studies with the β-amyloid preparation the Selkoe group used for their negative studies. The result: The Yankner peptide formed insoluble aggregates over the nerve cells—which subsequently degenerated; the Selkoe peptide didn't form aggregates, and "these neurons are perfectly happy," Yankner says. "Dennis' peptide doesn't have activity in the cell cultures, so I wouldn't expect it to have any in the animals."

Still unclear is what makes Yankner's peptide more prone to aggregate than Selkoe's since a variety of tests have verified that the peptides are indeed chemically identical, Yankner says. But the idea that aggregation is important fits with the fact that Alzheimer's neuropathology takes a long time to develop. Researchers see diffuse β-amyloid deposits, for example, years before the dense insoluble deposits in the plaques form. In the intervening time, one thing that may be occurring is β-amyloid aggregation. The long time course may also be influenced by another factor, however. Cotman and Mattson have evidence that β-amyloid isn't necessarily neurotoxic on its own, but may act by making neurons more susceptible to the effects of other toxins, such as the excitatory amino acids, or to damage caused by oxygen or glucose deprivation—just the kinds of insults that would accumulate with age.

Finally, the need for β-amyloid to aggregate to cause toxicity may help explain another discrepancy between the Yankner group's results and those of other researchers. Practically everyone *Science* interviewed pointed out that protective effects of substance P haven't been replicated, even by researchers who duplicated other aspects of the Yankner group's work. "We can see the toxicity that the Yankner group sees [on cultured neurons]," says Cole, "but we couldn't see the protective effects of substance P." Yankner suggests that substance P works by preventing β-amyloid aggregation, and the failure to take that into account may explain why many researchers fail to see the substance P effect.

In spite of these—and other—uncertainties, nobody is ready to give up on the idea that β-amyloid is important for Alzheimer's pathology. And everyone agrees that better animal models are needed for studying the peptide's role, as well as potential Alzheimer's therapies. But many in the field remain skeptical that Bruce Yankner's direct injection method is the best way of modeling a disease that develops over many years, as Alzheimer's does.

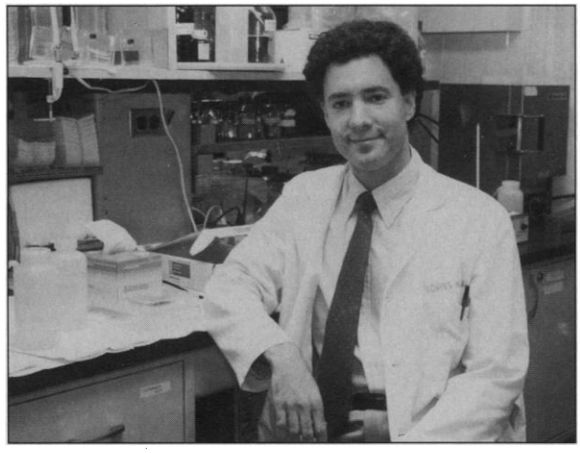
–J.M.

By last year, the roller coaster was definitely ascending, propelled by the discovery that some cases of hereditary Alzheimer's can be linked to specific mutations in the gene encoded β-amyloid, as well as by Yankner's work. "Ultimately, we have to find out what the consequences of having these [β-amyloid] deposits are clinically," says Zaven Khachaturian, who heads up the Alzheimer's program at the National Institute on Aging. "And when Yankner came along with this finding that β-amyloid might have neurotoxic effects, I thought we might be addressing that question."

But the researchers who were struggling to duplicate Yankner's results weren't at all sure that they agreed with that assessment. The results of the Yankner group's original experiments, in which the researchers found that β-amyloid is toxic to cultured neurons, were published in the 12 October 1990 issue of *Science*. The subsequent work with β-amyloid injections into rat brains appeared in the 15 August 1991 issue of the *Proceedings of the National Academy of Sciences*. Throughout the period between those publications, Yankner had been discussing the results at meetings, and several groups, among them Selkoe's, Robert Sapolsky's at Stanford University, and Ivan Lieberburg's at Athena Neuroscience in South San Francisco, California, were trying to replicate the results at their own lab benches—without success. "The basic story," says Sisodia, "is that the effects originally seen by Yankner, Kowall, and coworkers have been far from reproducible."

It's not all that surprising that the inability to reproduce the results raised frustration levels and tempers. "A lot of people spent a lot of time and money and effort in going after this thing. You'd be mad, too," Sisodia says. Sapolsky, for example, says that it took "two highly trained people in my lab 9 months and a whole lot of unnecessary dead animals" to get his group's negative results.

Yet frustration at the lab bench is a common feature of science—and rarely does it rise to the level of the anger directed at Yankner. "Some of the rancor that comes through is not really warranted by the science," notes Price. So why should Bruce Yankner's peers be so angry at him? One "stylistic" reason is what some of his peers contend is a tendency to overstate the significance of his results before they've been absolutely nailed down. Two researchers who requested anonymity, for instance, mentioned a talk Yankner gave at the 1990 Neuroscience Society annual meeting, which he ended by paraphrasing the famous concluding line of the paper in which James Watson and Francis Crick reported the double helical structure of DNA: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Yankner responds that if he did seem to "overstate" his results, it was because he was very enthusiastic and optimistic—especially when he started seeing neurotoxicity in animals.



Eye of the storm. Reports by Harvard's Bruce Yankner and his colleagues that β-amyloid is neurotoxic have stirred an unusual degree of controversy.

Some researchers in the field also complain that Yankner wasn't giving them enough help in replicating his results. Specifically, they told *Science*, they found it difficult to get out of him the precise experimental details they needed to reproduce the work. Yankner thinks those critics are wrong. "I've sent our peptides out to over 30 investigators with detailed protocols," he says. And, indeed, during preparation of the *Neurobiology of Aging* special issue Yankner won high marks for his cooperation. "Bruce really bent over backwards trying to be open and fair. You couldn't have wanted a more collegial response," says Price. Yankner does concede, however, that he was more protective—and less forthcoming—before his results were published.

But whatever Yankner's—real or imagined—offenses against collegiality, even his critics concede the emotional response directed at him seems excessive. One reason for that could be that the controversy goes beyond discussion of the value of specific Alzheimer's models and is complicated by what Price refers to as "issues related to commercial commitments and priorities for discoveries in the amyloid field." He was referring to the fact that Yankner has been particularly challenged by investigators allied with Athena Neuroscience, who have a stake in developing their own Alzheimer's models. Among the four groups reporting negative animal studies in the *Neurobiology of Aging* special issue are Selkoe's (a major Athena stockholder), Lieberburg's (an Athena employee), and that of James Clemens at Eli Lilly and Co. (a financial underwriter for Athena). And those investments could raise the emotional stakes in the affair as well—although everyone agrees that there's absolutely no suggestion that anyone on either side of the controversy has found anything other than the results actually reported. Yankner himself has tested Selkoe's peptide, for example, and says it doesn't show neurotoxicity.

Stepping in to mediate

Whatever the "currents" underneath the controversy's surface, by the time of last year's neuroscience meeting in New Orleans in October, tempers were running so high that some of those responsible for the field's welfare began to be concerned enough to step in and mediate. Khachaturian, for example, says, "When I learned there was that kind of skepticism, rather than have [Yankner] ostracized, I thought I'd try to get senior people, like Price, Selkoe, [Kenneth] Kosik, and [John] Trojanowski, together" to try to sort out exactly what was happening with β-amyloid neurotoxicity. (Kosik is another Alzheimer's researcher whose lab is at Harvard, and Trojanowski is at the University of Pennsylvania in Philadelphia.)

But it turned out that Khachaturian's plan didn't need to be put into action, because Coleman was already considering a special issue on Alzheimer's research, and, at Kosik's instigation, Coleman decided to have the issue focus on β-amyloid neurotoxicity. "This area has just generated a lot of controversy and there were so many negative reports that are hard to publish," Kosik explains. The idea was to air the negative data, as well as the positive, with a heavy emphasis on the experimental details that could help the researchers figure out why β-amyloid sometimes seemed neurotoxic and other times not in their experiments.

That special issue will be out shortly, putting the matter squarely before the community, and every Alzheimer's investigator will have a chance to read and evaluate the studies. And that's all to the good, because the β-amyloid story is hardly at an end. As Selkoe puts it: "Very few of us doubt that in the human brain β-amyloid is responsible for toxicity to nerve cells. The question is whether any of the experiments so far have replicated the condition in the human brain." But even before that question is resolved, it's clear that this flap has offered an intriguing way to begin resolving a dispute in a hot scientific field. While the *Neurobiology of Aging* special issue leaves the main scientific question up in the air, it has already helped clear the air in the community and put the emphasis back onto the nitty-gritty details of research—at least for the time being.

–Jean Marx