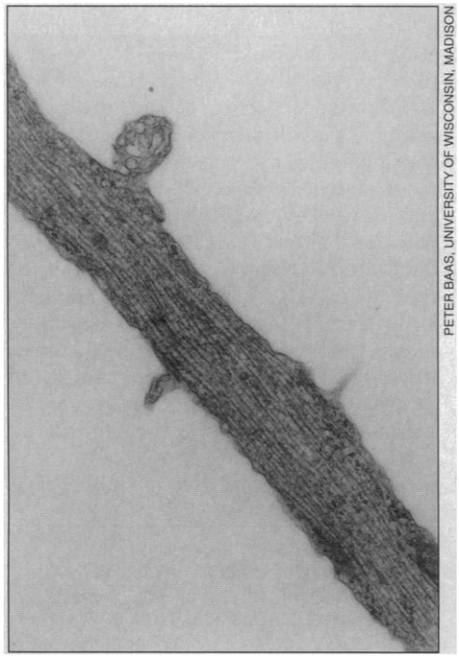
Neuroscientists Reach a Critical Mass in Washington

Last week, more than 22,000 neuroscientists converged on Washington, D.C. for the annual meeting of the Society for Neuroscience. The record number of participants—up from 14,000 at last year's meeting—meant standing-room only at most sessions and led to speculation that the society may be forced by its sheer size to split up. But there were unifying forces at the meeting as well, including presentations exciting enough to draw attendees from all across neuroscience.

New Alzheimer's Theory Stirs Controversy

If the neuroscience society gave an award for sparking controversy, Duke University researchers Allen Roses and Warren Strittmatter would surely win hands down this year. The reason: their radical new hypothesis about the cause of late-onset Alzheimer's—by far the most common form, accounting for about 80% of the estimated 4 million cases in the United States. If Roses and Strittmatter are right, their work could show how to prevent the devastating neurodegenerative disease.



PETER BAAS, UNIVERSITY OF WISCONSIN, MADISON

At risk? The fine lines are microtubules running through the axon of a cultured neuron.

But that's only if they're right in holding that Alzheimer's is fostered by the absence of a protein that helps protect the structure of the microtubules, protein filaments needed for normal neuronal functioning. And although the idea has received considerable attention from the press and the neuroscience community—when Roses and Strittmatter presented their idea at a seminar sponsored by the National Institute on Aging (NIA), the room was packed—skepticism in the Alzheimer's community runs high. "It's not a meaningful result," said neurogeneticist Rudolph Tanzi of Harvard's Massachusetts General Hospital. In his view, the work the Duke group has done so far to provide evidence for their hypothesis amounts to little more than a "pilot study for a pilot study."

The starting point for the Roses team's hypothesis is not in dispute: their recent identification of a previously unsuspected risk factor for late-onset Alzheimer's (*Science*, 13 August, p. 828). In genetic linkage studies, the researchers found that people who inherit the gene for a particular variant of the cholesterol-carrying protein apolipoprotein E are much more likely to develop the disease than people who don't inherit that variant: ApoE4. That association has been confirmed by several other researchers.

What has Alzheimer's researchers buzzing is the model that Roses and Strittmatter have put forward to explain how inheritance of ApoE4 might predispose to Alzheimer's disease. Their mechanism isn't direct. Rather than assuming that ApoE4 causes Alzheimer's, they propose that the other two ApoE variants—ApoE2 and ApoE3—help to *protect* against the disease and that people who inherit the ApoE4 gene lose all or part of their protection.

Roses and Strittmatter suggest that ApoE2 and E3 protect against Alzheimer's by shielding a protein called tau from changes that could be dangerous to nerve cells. Tau binds to microtubules, helping to form and stabilize them. Evidence suggests that if tau undergoes abnormal addition of phosphate groups, it binds less well to the microtubules, presumably allowing them to degenerate—a process that could ultimately lead to the death of the neurons. Indeed, some researchers think such tau phosphorylation may contribute to Alzheimer's development.

Roses and Strittmatter propose that ApoE3—but not ApoE4—can prevent the abnormal phosphorylation by binding to tau. They based this conclusion partly on test-tube experiments in which Strittmatter compared the binding of the two ApoEs to tau. The result: "ApoE3 avidly binds to tau in vitro and forms a complex that resists dissociation by boiling. But ApoE4 doesn't form such a complex," Strittmatter said.

The idea that people might get Alzheimer's because their brain neurons lack a protective factor suggests an encouraging scenario for therapy, Roses said: "You don't have to correct something that is wrong, but you have to supply something that isn't there." He hastens to add, however, that the new model for Alzheimer's development "is just a hypothesis; it isn't proven."

Not only is it not proven, many in the field don't think it's even plausible, partly because it leaves no room in Alzheimer's for the protein called β-amyloid, which many researchers think causes the neurodegeneration of the disease (*Science*, 4 September, p. 1336). Beyond that, both tau and β-amyloid experts were critical of the binding experiments on which the hypothesis is based. "[The hypothesis] is potentially very provocative, but we require evidence that ApoE-tau binding is happening in living cells," said Ken Kosik of Harvard's Brigham and Women's Hospital, who has been studying tau's role in Alzheimer's for more than a decade.

Kosik, like Tanzi and several other researchers contacted by *Science*, was particularly concerned because Strittmatter used protein concentrations for the binding studies much higher than those likely to be found in the cell. What's more, these critics assert, he didn't measure a dissociation constant, which would have given an indication of whether the binding might occur at cellular concentrations. "That's not a fair criticism," responds Strittmatter. He points out that ApoE3 binding to tau is irreversible, making measurement of a dissociation constant impossible. He used the high concentrations, he said, to get measurable binding in a short, experimentally feasible time.

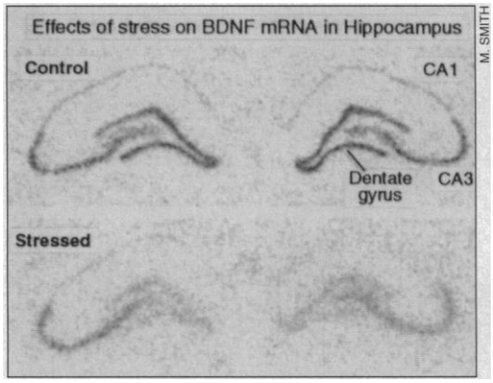
Given all the doubts, some Alzheimer's researchers faulted NIA for holding a press conference after the seminar, netting what many considered to be premature stories on television and in the popular press. But Zaven Khachaturian, associate director of the Neuroscience and Neuropsychology of Aging Program at NIA and the seminar organizer, thinks the publicity may be salutary. He notes that he is already recruiting several of the skeptics to join with Roses in testing his hypothesis. Khachaturian also points out that research on β-amyloid has been dominating the field for the past several years, to the point where other theories of what causes Alzheimer's may not be getting the attention they deserve. "Such scientific orthodoxy is pretty dangerous,"

Khachaturian says. "By putting forth such a provocative idea, Allen shook the field, and I think that's healthy."

–Jean Marx

This Is Your Brain on Stress

It's well known that difficult experiences reshape people's lives. Some of that reshaping no doubt goes on in the brain itself. Bruce McEwen of Rockefeller University and other neuroscientists have shown, for instance, that prolonged exposure to stress hormones causes dendrites—the spines that receive signals from adjacent neurons—to atrophy in the hippocampus, a brain region involved in learning and memory. But stress hormones may not be the only agents of change. At the



A message of stress. In the rat hippocampus, less messenger RNA for brain-derived neurotrophic factor *(dark specks)* is expressed when the animal is stressed.

meeting, psychiatrist Mark Smith from the National Institute of Mental Health (NIMH) and his co-workers presented evidence that neurons themselves can drive change: Stress alters the production of the "trophic factors" that neurons synthesize to protect themselves from damage.

Smith and his colleagues found that, in adult rats, long-term stress seems to lower production of the protein called brain-derived neurotrophic factor (BDNF). The scientists stressed out their rats by immobilizing them for 2 hours a day over 7 consecutive days. To determine whether this regimen affected the expression of neurotrophic factors, the researchers looked for the messenger RNAs (mRNAs) corresponding to the factors, which would indicate that the genes were being expressed. They found that the expression of BDNF was decreased throughout the brain, but particularly in a region of the hippocampus called the dentate gyrus, where there was a 50% to 60% drop compared to a group of nonstressed rats. The drop, Smith says, could explain how stress is able to prune away the dendrites and eventually kill off the neurons themselves.

Paradoxically, the researchers also found that stress increases the expression of another trophic factor, neurotrophin-3 (NT-3). In the stressed rats, NT-3 mRNA jumped by about 50%, again in the dentate gyrus. That, says Smith, is harder to understand, but it could be that the neurons are compensating for the stress hormones by producing more of a protective factor. Indeed, it's possible that stress, by increasing NT-3 levels, might at times actually strengthen neurons. Smith says, "It gets back to the old idea that there's good stress and bad stress."

For the moment, that's little more than a suggestion, since this research hasn't shown that the changes in gene expression actually produce functional changes in the neurons, points out Ned Kalin, a psychiatrist at the University of Wisconsin, Madison. The changes in trophic factors do, however, add another dimension to the process by which the brain copes with, and responds to, stress. "This ties stress into brain plasticity in a way we haven't thought of before," he says.

–Joshua Fischman

The Mice That Roared

Searches for the biochemical roots of aggression have turned up many candidate substances whose presence or absence might make human beings abnormally violent. In that fearsome group is serotonin: Some pathologically aggressive people have been found to have reduced levels of this neurotransmitter in their brains. Researchers have been at a loss, however, about where to start trying to understand serotonin's actual role in blunting aggression. Now the focus of the search has been sharpened by a most unmeek mouse that lacks not serotonin itself, but a certain serotonin receptor.

Part of the difficulty in sorting out the effects of serotonin stems from the fact that it is active in many parts of the brain, interacting with 14 different receptors and affecting a variety of mental states. Different receptors are probably involved in the various effects, but few tools were available to tease out those relationships; drugs weren't much help, for example, since few drugs selectively activate or inactivate just one type of receptor. Faced with this problem, Rene Hen of INSERM in Strasbourg, France, and his colleagues decided to take a genetic approach, creating strains of knockout mice, each missing the gene for one serotonin receptor, then testing the mice for behavioral abnormalities. They hit pay dirt on the first try.

Their target was the serotonin 1B receptor, found in several parts of the mouse brain, including the limbic system, which governs emotion. Mice lacking the receptor have no obvious defects, says Hen: "They develop normally, breed normally, and eat normally." They also seem normal in tests for anxiety and activity, two states that can be affected by serotonin levels. But when male mice missing the gene were subjected to a test in which the mouse is isolated for 4 weeks, and then faced with an intruder to its cage (a situation that makes even normal mice aggressive), they attacked the hapless visitor with twice the vigor of a normal mouse.

Whether the nasty behavior really reflects an increase in "pure aggression" is not yet clear, says Hen. Muddying the picture are results of a second behavioral test in which the mice were first stressed with a loud noise, then placed in the middle of a large open enclosure. Normal mice, fearful of open spaces, spend most of their time near the walls. But the serotonin-receptor knockouts spent much more time in the open area, suggesting they are less fearful under stress than normal mice. That finding, says Hen, made him wonder whether the intruder-attacks in the first test were motivated not by an increase in aggression but by a lack of fear of the intruding mouse, a question he plans to address with further behavioral tests.

Regardless of its cause, the abnormal aggression finding is "certainly very interesting," says John Mann of the University of Pittsburgh, who studies serotonin and aggression. "We now have a gene deletion that correlates with some of the behaviors in patients we are seeing." The finding will spur a more intensive study of the corresponding receptor in humans and could lead to a strategy for treatment, if any aggressive humans turn out to have defects in this receptor. "I think there is a good chance that some human pathologies will be associated with this gene," Hen says. But the problem of aggression is far from solved, he adds, because "this isn't the only gene that will be associated with aggressive behavior."

–Marcia Barinaga

New Accomplices in Cell Death

As far as neurons are concerned, glutamate is a Jekyll and Hyde molecule. When it shows its benign face, it is among the brain's most important neurotransmitters, carrying excitatory messages from neuron to neuron across synapses. On the darker side, glutamate can be deadly: In strokes, head trauma, and some neurodegenerative diseases, glutamate becomes an "excitotoxin," flooding synapses in amounts that excite neurons to death. And like most villains, glutamate in its Hyde role has accomplices, as several reports at last week's meeting made clear.

For years researchers have known that excitotoxicity must involve more than a mere increase in glutamate release, because under normal conditions, cell-surface proteins known as glutamate transporters can mop up a flood of glutamate and save neurons from damage. During excitotoxicity, they

reasoned, the transporter must be somehow crippled. But how? The new findings may hold an answer: They show that glutamate-stimulated cells produce substances that sabotage the transporters, leaving the destructive flood of glutamate to linger in the synapse.

Sakire Pogun and Michael Kuhar of the National Institute of Drug Abuse reported that one accomplice is nitric oxide (NO), a volatile and reactive gas that got lots of attention several years ago as the newest (and weirdest) neural signaling molecule. NO is made when glutamate activates a receptor called the NMDA receptor, something that happens during both learning and excitotoxicity. Researchers had already found one way in which NO release might abet excitotoxicity: As a glutamate-stimulated cell makes more of the gas, the NO feeds back on the glutamate-producing cell, boosting glutamate release.

But Pogun thought that NO might inhibit the glutamate transporter as well. She decided to check, by treating a preparation of neuron membranes with nitric oxide, and found that NO reduced glutamate uptake by half. Pogun has not yet discovered how the nitric oxide exerts its effects. It could be acting directly on the transporter-containing cells, but some researchers suggest that NO may be converted into an even more reactive free-radical molecule called peroxynitrate, which actually does the damage. In support of that possibility, Andrea Volterra, of the University of Milan, reported that highly reactive oxygen radicals like peroxynitrate have an inhibiting effect on the transporter.

Whether NO acts directly or not, it doesn't act alone. Volterra also reported a second, apparently independent, avenue of self-destructive behavior in glutamate overstimulated cells. Along with NO, they produce arachidonic acid, a fatty acid that, like NO, diffuses through membranes and can affect nearby cells. Pak Chan, of the University of California, San Francisco, and David Attwell, of University College, London, had already found that arachidonic acid inhibits the glutamate transporter. But Volterra reported that it does so by a mechanism separate from that used by free radicals.

These reports fill in a missing piece in the picture of excitotoxicity, says Johns Hopkins University neurologist Jeff Rothstein, who studies glutamate transporters: "Now we find you have direct transporter toxins that are made as part of the excitotoxic process." That knowledge, he adds, coupled with Volterra's finding of separate mechanisms of action, offers not one but two potential new targets for drugs for stroke and head-trauma patients. By unshackling the glutamate transporter, such drugs might limit excitotoxicity's toll.

–M.B.

IMMUNOLOGY

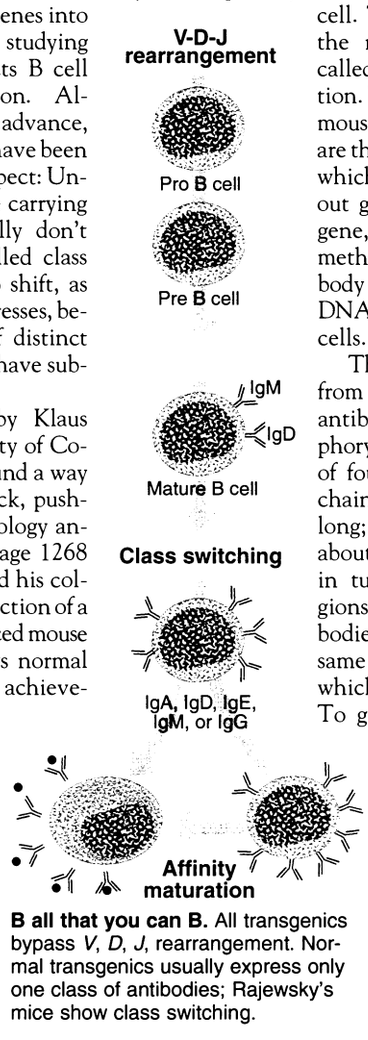
Transgenic Mice Display a Class (Switching) Act

Over the past decade, genetically engineered mice have transformed immunology. Using transgenic animals, researchers have, for example, helped unravel many mysteries of the development of B cells—the immune system cells that produce antibodies—by inserting specific antibody genes into fertilized mouse eggs and studying how their presence affects B cell and antibody production. Although that was a great advance, these transgenic animals have been deficient in one major respect: Unlike normal B cells, those carrying the inserted genes usually don't show a phenomenon called class switching—the ability to shift, as an immune response progresses, between the production of distinct classes of antibodies that have subtly different functions.

Now, a group led by Klaus Rajewsky of the University of Cologne in Germany has found a way to overcome this drawback, pushing the study of B cell biology another step forward. On page 1268 of this issue, Rajewsky and his colleagues describe the production of a mouse carrying an introduced mouse antibody gene that shows normal class switching. Their achievement opens the way to investigate how class switching is controlled. "It allows you to study, in vivo, the biology of the B cell response," says immunologist Dennis Loh of Washington University in St. Louis.

And the ramifications of this work aren't limited to fundamental science, since the technique could have clinical applications. In a second paper on page 1271, Rajewsky's team reports the creation of a mouse in which an antibody gene is replaced by the corresponding gene from human cells. This animal can produce part-mouse, part-human antibodies against any antigen—and may provide a short cut in the production of "humanized" monoclonal antibodies for clinical use.

The key to these achievements is the technique that is usually employed to make knockout mice: gene targeting. Conventional transgenic techniques, which rely on microinjecting foreign DNA into a fertilized egg, introduce genes randomly into the genome. Knockout mice, on the other hand, are made by cloning a particular gene, disrupting it by introducing a short DNA sequence, and inserting it into a mouse stem cell. The disrupted gene replaces the normal gene by a process called homologous recombination. These cells are injected into mouse embryos, yielding mice that are then bred to produce a strain in which all cells carry the knocked-out gene. Instead of disrupting a gene, Rajewsky's group used this method to directly replace an antibody gene with the corresponding DNA from a particular clone of B cells.



B all that you can B. All transgenics bypass *V, D, J,* rearrangement. Normal transgenics usually express only one class of antibodies; Rajewsky's mice show class switching.

ILLUSTRATION: K. SUTLIFF

The introduced gene comes from a B cell line that produces antibodies to the chemical phosphorylcholine. Antibodies consist of four polypeptides: Two heavy chains, each some 440 amino acids long; and two light chains, of about half that length. Both types, in turn, consist of constant regions, which are identical for antibodies of the same class from the same animal; and variable regions, which differ from B cell to B cell. To generate a huge diversity of antibodies, the immune system takes the gene fragments that code for these variable regions—called *V*, *J*, and *D* segments—and rearranges them in different combinations. The Cologne researchers isolated a particular rearrangement of these fragments—coding for the heavy chain variable region of an antibody to phosphorylcholine—and used gene targeting to make a mouse in which all cells carry this sequence in place of the DNA containing the unrearranged gene segments.

In homozygous animals, which carry two copies of the inserted gene, the result is a mouse all of whose B cells make antibodies with antiphosphorylcholine heavy chains. During the course of an immune response, these cells in the transgenic mouse switch class—just as in a normal mouse—to produce antibodies that recognize the same antigen but which carry different heavy chain