Neurosciences Advance in Basic and Clinical Realms

More than 10,500 participants gathered last month in Washington, D.C., at the 16th Annual Society for Neuroscience meeting to present new results over a wide range of subjects in both basic and clinical research, a sampling of which is reported below.

A Possible Diagnostic Test for Alzheimer's?

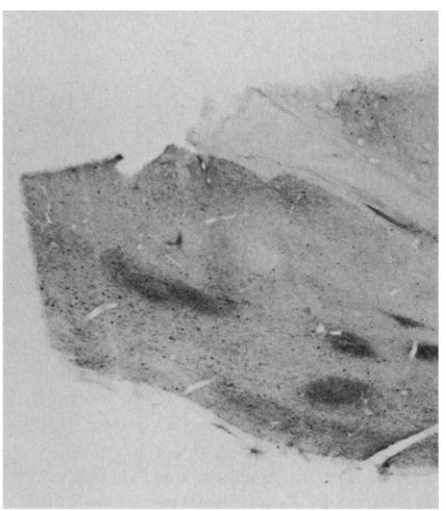
New information from neuroscientists at the Albert Einstein College of Medicine in New York indicates that A68, an abnormal brain protein, may be a diagnostic marker for Alzheimer's disease. Peter Davies and Benjamin Wolozin first described A68 last year, and they now report that the protein is an enzyme present in the cerebrospinal fluid (CSF) of presumptive Alzheimer's dementia patients.

Davies and Wolozin, in collaboration with Robert Harbaugh of the Dartmouth-Hitchcock Medical Center in Hanover, New Hampshire, find that A68 accumulates in neurites in the brains of living Alzheimer's patients early in the course of their disease. In addition, the Einstein researchers detect the abnormal protein in postmortem brain tissue of aged patients with Down's syndrome. Although high levels of A68 in brain tissue seem to be specific to Alzheimer's disease, very low levels of the protein may be present in other neurodegenerative disorders. And some researchers question whether low levels of A68 also exist in normal brain tissue.

Davies and Wolozin detect A68 in brain and in CSF with an antibody called Alz 50 that binds to the abnormal protein. In Alzheimer's brains, but not in normal brains, Alz 50 labels those regions in which plaques and tangles frequently occur, including the nucleus basalis, hippocampus, and parts of the neocortex.

Davies and Wolozin tested nine living patients whose physicians diagnosed them as having Alzheimer's and found the protein in the cerebrospinal fluid of eight of them, which gives the system good diagnostic potential. The researchers are now working to make the assay for A68 more sensitive and efficient. If their present results are confirmed in a large number of subjects, and if they modify their techniques, then the spinal fluid assay for A68 may be the first real diagnostic test for Alzheimer's disease in living patients. To date, diagnosis of Alzheimer's, which affects about 7% of the population over 65 years of age, is certain only after the patient dies, at which time a microscopic examination of brain tissue reveals the neuritic plaques and tangles characteristic of the disease.

Wolozin speculates that A68, in addition to being an enzyme that phosphorylates itself, may also turn out to be a kinase for *tau* proteins, which are associated with the paired helical filaments of neurofibrillary tangles. At least four other research groups have reported that *tau* proteins are abnormally phosphorylated in Alzheimer's. If A68 is the kinase that phosphorylates *tau*, then it might explain why Davies and Wolozin see A68 staining associated with the paired helical filaments of Alzheimer's tangles.



The nucleus basalis *from the brain of an Alzheimer's patient shows antibody staining (gray masses of fibers and small darkly stained neurons) for the abnormal protein, A68. [Photo courtesy of Peter Davies]*

Dennis Selkoe, of Harvard's Brigham and Women's Hospital, takes issue with Davies' notion that there is a specific antigen, such as A68, for Alzheimer's. "What we're talking about is higher levels of an antigen in Alzheimer's brain than is present in normal brain," he says. He thinks that A68 may be a kinase, possibly for microtubule-associated proteins including *tau*, but that it is also likely to be present in normal brain and CSF.

Another important new result from the Einstein group is that postmortem tissue from the brains of aged Down's syndrome patients shows about the same level of staining for Alz 50 as Alzheimer's brains do. "This is consistent with the hypothesis that all elderly individuals with Down's syndrome develop Alzheimer's disease," says Wolozin. This finding, that an abnormal protein may be common to both conditions, strengthens the hypothesis that the brain degeneration in Alzheimer's disease and in late Down's syndrome may be due to the same genetic defects.

Davies and Wolozin are also collaborating with Tsunao Saitoh, of the University of California School of Medicine at San Diego, to clone the A68 gene and ultimately to identify the amino acid sequence of the protein. "We want to know why this protein is produced in Alzheimer's brains and not in normal brains," says Davies. "Is it the product of a gene that is not turned on in normal brain or is it an abnormal product of a gene that is normally turned on?"

Davies thinks that it should take about 3 to 4 months to simplify the procedure for testing A68 in the cerebrospinal fluid and make it more efficient. He indicates that there is "an enormous amount of commercial interest" in the technique and has filed for a patent. "Given the fact that A68 is present in the CSF of most Alzheimer's patients," Davies says, the potential for a diagnostic test based on A68 "looks very promising."

Ion Channels That May Underlie Learning

Neuroscientists who study learning and memory constantly search for changes in individual nerve cells—even in single molecules—that underlie behavioral changes in the entire organism. Charles Stevens and Craig Jahr of Yale University School of Medicine have just reported that an ion channel complex in brain neurons is regulated directly by some of the same signals that are thought to be important in learning and memory. They find that excitatory neurotransmitter compounds and electrical potential work together to control both the activity of ion channels and the species of ions that flow through them in membrane patches from hippocampal neurons. The molecular changes that result may contribute to the increased communication among brain neurons that is essential to the learning process.

By binding to specific receptor molecules in nerve cell membranes, neurotransmitters

cause ion channels to open and thus change the electrical potential across the membrane, either increasing or decreasing the activity of the cell. Many neuroscientists think that the amino acid glutamate is the major excitatory neurotransmitter in the mammalian brain and that it is an important signal molecule for learning. The ion channel–receptor complex studied by Stevens and Jahr probably binds glutamate in vivo, but the researchers tease out different properties of the complex by using compounds that activate it in specific ways.

For instance two compounds, quisqualate and kainate, open the ion channel to a low level of activity, a low conductance state of about 10 picosiemens. A different compound, *N*-methyl-D-aspartate (NMDA), activates the channel much more, inducing a large (50-picosiemens) conductance state. In addition, NMDA allows calcium ions, as well as sodium and potassium ions, to flow through the channel. Although it is possible that separate channels account for the small conductance, Stevens and Jahr think that the same ion channel complex may be responsible for all the currents they measure. In addition to these two conductance states, the researchers also have evidence that the ion channels in their membrane patches have at least three other major states and even more substates.

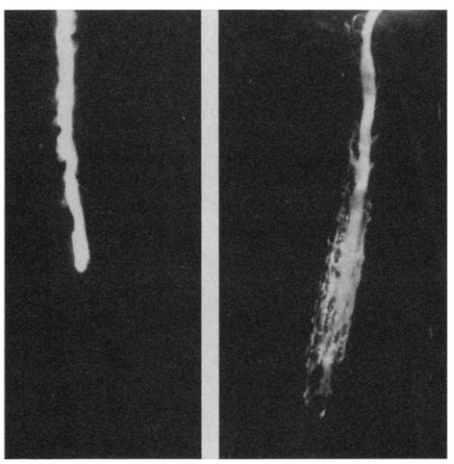
"We find that these channels are very complex, much more complex than we expected them to be," says Stevens. In the intact animal, an excitatory neurotransmitter (probably glutamate) normally activates these ion channel complexes. But, as other research groups have shown, they are also regulated by magnesium ions, which are present in the space surrounding neurons and which normally block the channel from going to its large conductance state.

"What we think is that the small conductance levels are used for synaptic transmission," says Stevens. "Each time one is open, you get a small depolarization. If you activate enough of them, it produces a threshold depolarization that activates the large conductance. This depolarization releases the magnesium block, causes larger postsynaptic potentials, and allows calcium influx."

Thus, if two things happen at once in an animal's brain—if glutamate binds to its receptor sites at the same time as the postsynaptic membrane is sufficiently depolarized—then the magnesium block is removed, the NMDA ion channels in the postsynaptic membrane go to a large conductance state, and calcium ions flow through them into the cell. These specifically timed, spatially coordinated events have been proposed to occur during learning.

Neurotransmitters Regulate Growth Cones

The first processes a developing or regenerating nerve cell extends toward its ultimate target are headed by specialized structures called growth cones. The idea that neurotransmitters somehow regulate this important growth phenomenon is a recent one, and only within the past 3 years have neuroscientists been able to show that specific neurotransmitters influence growth cone elongation in invertebrate neurons in tissue culture. New evidence indicates that neurotransmitters may regulate growth cone extension in vivo; that neurotransmitters modulate the growth of some vertebrate, as well as invertebrate, neurons; and that second messengers and calcium may determine growth cone activity versus stability.



Control Glutamate

Glutamate *induces neurite outgrowth from a severed* Heliosoma *neuron in culture.* [*Photo courtesy of Andrew Bulloch*]

Andrew Bulloch and Peter Jones of the University of Calgary in Alberta have just shown that the amino acid glutamate enhances neurite outgrowth in an identified snail neuron. By making a series of observations, the Canadian group discovered that physiological stress for *Heliosoma* induces neurons to send out new processes and that the active agent for neuron 5 appears to be the neurotransmitter glutamate.

The researchers stress *Heliosoma* by putting them into 20% seawater, which induces neuron 5 of the buccal (feeding) ganglia to sprout. "We had a clue that a blood-borne factor activated sprouting and we started looking for it," says Bulloch. He and his colleagues found that a transient four- to fivefold increase in blood glutamate occurred 1 to 2 days after the stress, at the same time the neurons began to sprout.

The Calgary group finds that when they apply glutamate to isolated ganglia in vitro from which the axons have been severed, the amino acid produces two kinds of responses in neuron 5—it hyperpolarizes the cell bodies dramatically and it induces sprouting in 80% of the neurons tested. Interestingly, a phorbol ester known to activate the enzyme protein kinase C also induces sprouting in these cells. Bulloch sees this as evidence that glutamate may activate the phosphatidyl inositol–mediated second messenger system that stimulates protein kinase C.

William Klein, Karen Lankford, and Fernando De Mello of Northwestern University in Evanston, Illinois, find that a different neurotransmitter, dopamine, inhibits rather than stimulates the outgrowth of certain vertebrate neurons. They previously reported that dopamine transiently stimulates adenylate cyclase to make a second messenger compound, cyclic adenosine monophosphate (cAMP), in cultured embryonic chick retinal neurons several days before synapses form. Now, they show that dopamine inhibits growth cone elongation in some retinal neurons and that the effect is reversible.

The Northwestern group also finds that forskolin, which stimulates adenylate cyclase directly, induces neurite retraction in the cells that are sensitive to dopamine, but that its effects are less pronounced than those of dopamine. The researchers propose that a transient population of D1 dopamine receptors appears on these retinal neurons during development and that it mediates the growth-inhibiting effects of dopamine.

Stuart Lipton, Matthew Frosch, Micheal Phillips, Elias Aizenman, and their colleagues of Harvard's Children's Hospital have evidence that still another neurotransmitter, acetylcholine, may inhibit neurite extension in cultured rat retinal ganglion cells. "This is a regenerating system rather than a developmental one," says Lipton, who established the cultures from 1- to 2-week-old rats.

Lipton and his co-workers find that drugs that block the so-called nicotinic responses of the acetylcholine receptor, including *d*-tubocurarine and mecamylamine, enhance neurite outgrowth from about 70% of the ganglion cells. They hypothesize that a "spontaneous leak" of acetylcholine from cells in the culture is responsible for inhibiting outgrowth, and that, by blocking nicotinic receptors, they can remove the inhibition to allow growth.

The researchers also find that acetylcholine induces electrophysiological responses in retinal neurons that resemble those of sympathetic ganglion neurons. But just because a neuron has receptors for a neurotransmitter and responds to it electrophysiologically does not mean that the transmitter

also affects its growth. For instance, γ-aminobutyric acid (GABA) inhibits the retinal neurons electrophysiologically, but Lipton has no evidence that GABA affects neurite outgrowth. Nevertheless, the Harvard researchers find that acetylcholine acts at nicotinic receptors as a tonic growth inhibitor for most retinal ganglion neurons.

Stanley Kater and Christopher Cohan, formerly of the University of Iowa in Iowa City, and John Connor of Bell Laboratories in Murray Hill, New Jersey, have just demonstrated that the level of intracellular calcium may be the determining factor in whether growth cones will elongate or stabilize. Last year Kater and his co-workers showed that two kinds of signals—action potentials and serotonin—inhibit the outgrowth of invertebrate neurons cultured from the buccal ganglion of *Heliosoma*. Using a calcium-sensitive dye to measure changes in the concentration of the ion in the growth cones of neuron 19, they now find that serotonin or action potentials raise calcium levels from 100 to 130 nanomolar to several hundred nanomolar, causing growth to cease.

It seems that even within the same animal, different neurotransmitters regulate the growth of different neurons. Bulloch and his colleagues find that glutamate regulates growth cone elongation in neuron 5 from *Heliosoma*, and Kater and his co-workers show that neuron 19 responds to serotonin. According to Cohan, "signals that inhibit growth cone motility also increase the calcium concentration inside the growth cones."

But Cohan also notes that, "after some time in culture, growth cones spontaneously stop elongating." To their surprise, the researchers find that calcium levels in spontaneously stable growth cones are low, at about 50 nanomolar, not high.

What does it all mean? "A neuron has a calcium set point," Kater proposes, which in *Heliosoma* seems to be about 100 nanomolar. "On either side of that set point is the realm of no growth. Other biochemical processes required for growth, such as the rearrangement of cytoskeletal proteins, also require strict calcium concentrations." So it is not surprising to Kater and his colleagues that a narrow range of calcium concentrations promotes growth.

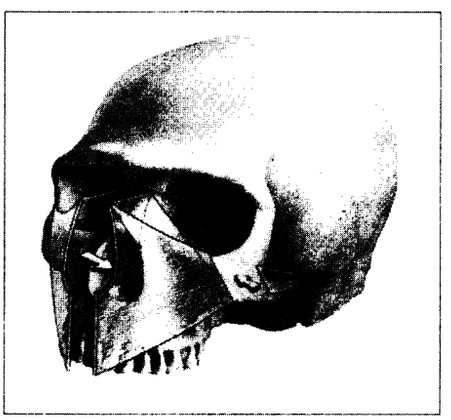
Kater also raises the question of whether neurons that are regarded as stable, such as those in the mature brain of a mammal, may be subject to some of the same tropic effects of neurotransmitters that developing or regenerating neurons are. Bulloch speculates along the same lines. "One of the reasons we are so excited about growth cones is that they may play a role in the synaptic changes that occur during learning." ■

DEBORAH M. BARNES

A New Look at an Old Fossil Face

Someone once said that if you were to take a Neanderthal individual, shave, wash and clothe him, he would be virtually indistinguishable from many of the denizens of the New York subway. That may be true, but Neanderthal anatomy is not as close to that of modern humans as this whimsy might imply. One major difference was the face: it was extraordinarily big. Why the face was so big has long been a matter of speculation among anthropologists, and in a recent publication Yoel Rak, of Tel Aviv University, adds his interpretation to the debate. The reason, he suggests, is mechanical: the face was built to counteract the considerable forces that Neanderthals developed between their upper and lower front teeth.

If you were to take hold of the nose of a plastic, western European face and tug mightily, you would finish up with a very Neanderthal-like face. Specifically, the mid-



Making a Neanderthal face. *By swinging forward—like opening double doors—the sheets of bone beneath the eye regions of a modern human skull one forms the mid-facial projection and large nasal aperture of the Neanderthal (shown by the "transparent" overlay).*

dle of the face would protrude dramatically; and the nose would be very big. Viewed from the top, the head forms quite a steep triangle, with a wide base lining up from ear to ear, the two long sides running along the cheeks, and the apex being formed by the nose. By contrast, the modern human head would look like a truncated triangle: the face is relatively flat from top to bottom.

This very peculiar facial architecture has therefore become something of a hallmark of the classic Neanderthals, who lived in western Europe between 100,000 and 35,000 years ago. One explanation for the anatomy, which was first developed during the 1950's, was that the enlarged nasal chamber formed what Rak describes as "an immense radiator that would warm and humidify dry cold air." Neanderthals, remember, lived through much of the last major glaciation in Europe, although some populations were in relatively temperate regions. In essence, this hypothesis argues that the nose led the way and the rest of the facial structure followed.

A second proposal, which was first put forward in the 1960's and is the one that Rak's latest contribution extends, invokes dental biomechanics as the selective agent of the protruding face. Neanderthals have very large front teeth (incisors and canines) relative to the back teeth (the premolars and molars). Moreover, Neanderthal individuals typically show very heavy wear on the front teeth, sometimes going down to the roots. Whether these people were processing tough food between their front teeth or manipulating hide or some other material, the forces developed there were clearly great.

Rak's contribution is to look in detail at the functional aspects of the facial architecture in a way that has not been done before. He shows how the sheets of bone beneath the eye region in modern humans are swung forward "as in the opening of double doors." The effect is to thrust the face forward and create a very large nasal opening. Mechanically, however, now that these sheets of bone are in much more of a forward plane, they can resist the forces created by heavy biting on the front teeth.

Specifically, biting on these teeth will tend to cause rotation of the front of the upper jaw. Sheets of bone that are deep vertically at the point of bite will be an effective counter to the rotation, bending, and torsion that is generated there. Other factors contribute to the shape of the face, of course, not least of which is the space required in the front of the face and beneath the nose for the roots of the unusually large front teeth. Nevertheless, in this hypothesis it is the face that led the way, and the nose was carried on before it.

The Neanderthal face, according to Rak, is quite distinct from that of modern humans. "The facial morphology of *Homo* specimens preceding the classic Neanderthal is more similar . . . to the morphology of those following it than either is to the Neanderthal," he notes. The clear implication is that the Neanderthals were not directly ancestral to modern European populations, the debate over which is becoming one of the hottest topics in human origins research. ■ ROGER LEWIN

ADDITIONAL READING

Y. Rak, "The Neanderthal: A new look at an old face," *J. Hum. Evol.* 15, 151 (1986).