NMDA Receptors Trigger Excitement

NMDA receptors seem to regulate many normal changes in the brain of an adult or developing animal, but their overstimulation may cause nerve cell death in heart attack or stroke patients

As recently as 5 or 6 years ago, neuroscientists had little understanding of the mechanisms that regulate many important changes at brain synapses. They did not know, for example, what cellular and molecular processes might underlie learning and memory in an adult animal or what events regulate the formation of nerve cell contacts in a developing animal. And until 2 or 3 years ago, researchers had little knowledge of what actually causes brain damage in a heart attack or stroke victim. Today, neuroscientists are beginning to view NMDA receptors in the brain as regulators of all of these processes—good and bad.

"Each one of the processes in itself is an area of great interest, so when you have a nice unitary mechanism that seems to underlie all of these events, it is very exciting," says Charles Stevens of Yale University School of Medicine. In his presentation at the annual meeting of the Society for Neuroscience,* Stevens highlighted new data on mechanisms that regulate NMDA receptor activity. He stressed the emerging concept that appropriate stimulation of NMDA receptors may promote beneficial changes in the brain, whereas overstimulation of the receptors can cause nerve cell damage or death.

The NMDA receptors take their name from the fact that *N*-methyl-D-aspartate (NMDA) excites them under experimental conditions. NMDA itself does not exist in the brain, however. It is simply a chemical tool used to probe the activity of this class of receptors. In an intact animal, amino acid neurotransmitters such as glutamate or aspartate probably stimulate NMDA receptors.

During the past several years, neuroscientists have pursued the roles of NMDA receptors from at least three major perspectives. First, some study the mechanisms that regulate the activity of NMDA receptors and their ion channels. Second, some focus on the role of NMDA receptors in normal brain processes that depend on long-lasting increases in nerve cell communication at synapses. These include changes in brain development as well as changes that underlie learning and memory. And third, neuroscientists monitor the role of NMDA in brain disease or trauma. For example, NMDA receptors may participate in the hyperactivation of certain brain circuits in epilepsy or Huntington's disease. And increasing evidence indicates that overstimulation of NMDA receptors may cause damage to brain neurons in an ischemic attack when the blood supply to the brain is blocked during a heart attack or stroke.

"Excitotoxicity comes into play if there is too much glutamate and too much calcium."

Stevens summarized recent findings in the first research area, namely, the structure and activity of the NMDA receptor and the membrane pore (ion channel) that it regulates. During the past several years, researchers have learned that the receptor is part of a large molecular aggregate that includes binding sites for certain chemical substances. When glutamate or NMDA binds, the ion channels open and allow positively charged ions to flow into the nerve cell. This excites the neuron.

Stevens and others now postulate that the NMDA receptor complex is a member of a superfamily of proteins—a group that includes the receptors and ion channels for the neurotransmitters acetylcholine, glycine, and γ-aminobutyric acid. Several researchers are now trying to isolate and clone the receptor complex for NMDA and glutamate, which should yield specific information about its organization.

Although the NMDA receptor complex seems to be physically similar to other receptor complexes, Stevens notes that regulation of the NMDA ion channel is unique. Most ion channels are governed by one major kind of signal—either a chemical transmitter or the electrical potential across the nerve cell membrane. But the NMDA channel is unusual because both chemical and electrical signals affect its activity.

Even the chemical regulation of the NMDA receptor is complex. Within the past 2 years, researchers have discovered that at least two different categories of chemical signals regulate NMDA receptor activity. One kind is an excitatory amino acid transmitter, probably glutamate or aspartate in vivo, or NMDA under experimental conditions. Now, according to Stevens, recent data indicate that glutamate binds to at least two kinds of receptors in nerve cell membranes, and it activates at least three kinds of ion channels—small, medium, and large. Whether each receptor type is coupled to a different sort of channel has yet to be resolved, however.

Last year, Jon Johnson and Philippe Ascher of the Ecole Normale Supérieure in Paris reported that the amino acid glycine serves as a second category of chemical signal that increases NMDA receptor activity. This surprised many neuroscientists because glycine is a well-known inhibitory transmitter, particularly in the spinal cord. The idea that glycine somehow enhances excitatory transmission in the brain was completely novel.

"If glycine is not present, glutamate will not open NMDA-gated channels unless the concentration of glutamate is raised," says Stevens. Glycine apparently binds to a site on the NMDA receptor complex that is different from the NMDA binding site, but its precise mechanism of action is not known. And because the normal level of glycine in brain tissue may be sufficient to increase NMDA receptor activity, it is also not clear if the glycine effect is an ever-present one or whether it occurs only under specific conditions.

In addition to chemical signals, the electrical potential associated with the nerve cell membrane also regulates NMDA receptors and ion channel activity. For instance, depolarization (making the normal negative electrical charge across the nerve cell membrane more positive) allows a bigger response to glutamate or NMDA. This is so because depolarization somehow dislodges magnesium ions that block NMDA channel activity when the cell is at rest.

Ascher and Linda Nowak of the New

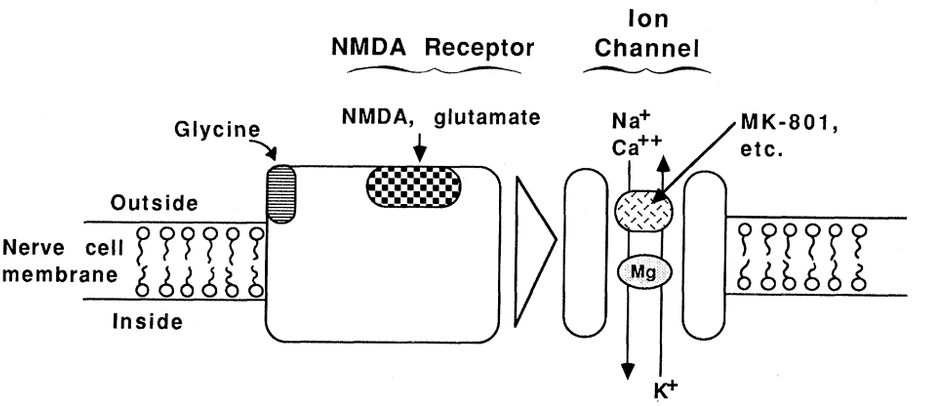
*The annual meeting of the Society for Neuroscience was held from 16 to 21 November 1987 in New Orleans, Louisiana.

York State College of Veterinary Medicine at Cornell University at Ithaca, are continuing to explore the means by which magnesium ions block the NMDA channel. "It appears that magnesium ions sense the electrical field of the membrane and enter the NMDA ion channel," says Nowak. "Magnesium is very hygroscopic; it attracts water strongly." Other ions, including sodium and calcium, also attract water but can go through the ion channel easily because they lose their water readily. Nowak theorizes that because it takes much more energy to strip the water from magnesium than from calcium, magnesium ions tend to retain water. And, in this bulky state, they clog the NMDA ion channel.

Thus, it requires a combination of chemical stimuli and the right electrical environment to activate NMDA receptors fully. With maximum stimulation, NMDA ion channels open and allow sodium, potassium, and calcium ions to flow through the nerve cell membrane. Of these, calcium seems to be the most critical. Apparently, the right amount of calcium influx leads to beneficial changes in brain synapses but too much intracellular calcium causes nerve cell damage. A growing number of neuroscientists are actively pursuing these second and third areas of research on NMDA receptors.

For example, within the past year, several groups of researchers have shown that activation of NMDA receptors may regulate synaptic changes that are crucial to normal brain development—particularly of neural pathways that carry sensory information. Wolf Singer of the Max Planck Institute for Brain Research in Frankfurt, West Germany, and his colleagues find that stimulation of NMDA receptors is important in the development of brain pathways that carry visual information in the kitten. Martha Constantine-Paton of Yale University in New Haven, Connecticut, and her co-workers describe a role for NMDA receptors in visual system development in the experimental three-eyed tadpole. And Carl Cotman of the University of California at Irvine and his collaborators report that NMDA receptors are important for the development of the olfactory system in the rat.

Perhaps the most exciting aspect of NMDA receptor function in the adult brain is its potential role in learning and memory. Several research teams propose that two molecular events must occur simultaneously in the brain of an intact animal in order for it to learn. The first is that NMDA receptors must be excited chemically, and the second is that the nerve cells receiving the excitatory NMDA stimulus must be depolarized sufficiently to trigger calcium influx. This combination of events, the theory goes, will lead to long-lasting increases in nerve cell communication at synapses.



How the NMDA receptor complex might work. *A combination of factors are thought to regulate the activity of the NMDA receptor and its ion channel—an agonist, such as NMDA or glutamate, membrane potential, magnesium (Mg) ions, and glycine. When the NMDA ion channel is activated, as shown here, sodium (Na^+) and calcium (Ca^{++}) ions flow into the nerve cell and potassium (K^+) ions flow out.* [*Adapted from J. A. Kemp* et al., Trends Neurosci. ***10****, 297 (1987)*]

Tim Bliss of the National Institute for Medical Research in London, for example, sees a persistent increase in synaptic strength as one of the mechanisms that underlie learning. "There is no doubt that the NMDA receptor is involved in long-term potentiation in the hippocampus," he says. "But we are not certain if it is exclusively an effect on postsynaptic neurons or whether NMDA receptors on the terminals of presynaptic neurons are involved as well." Postsynaptic cells receive signals from other neurons. Presynaptic neurons send the signals in the form of chemical transmitters such as glutamate. The hippocampus is a primitive part of the cerebral cortex that plays an important role in certain kinds of learning and memory.

Within the past few years, other investigators showed that long-term potentiation can induce changes in the physical structure of a synapse. Some of these changes, which probably involve NMDA receptor activation, are on the spiny protrusions of dendrites, the fine neuronal processes that typically receive chemical signals.

Now, Bliss and his colleagues have new data indicating that NMDA may also stimulate another kind of synaptic change. Their results indicate that NMDA induces postsynaptic neurons to release derivatives of arachidonic acid, a kind of fatty acid. Bliss proposes that these metabolites might travel backwards across synapses to presynaptic neurons and increase their output of neurotransmitter. Such a change, he says, would cause a larger response from the postsynaptic neuron and be interpreted as an increase in synaptic strength. Whether these events occur in an animal when it learns, as the London researchers have shown it does under experimental conditions, is not yet known.

Additional recent data indicate that activation of NMDA receptors may stimulate protein kinase C, an intracellular enzyme that adds phosphate groups to certain proteins. With stimulation, the enzyme apparently moves from the cytoplasm of the nerve cell to its membrane where it may phosphorylate proteins thought to be inportant in long-term potentiation and certain growth processes. Like other events regulated by NMDA receptors, this response also seems to be linked to the influx of calcium.

Thus, accumulating evidence points to the fact that NMDA receptors play an important role in long-lasting changes at brain synapses. But Stevens, Nowak, and Bliss all question whether NMDA receptors play a role in ordinary communication among nerve cells in the brain. They postulate that normal synaptic transmission may be controlled by non-NMDA receptors, which trigger small currents carried by sodium ions flowing into a neuron. In contrast, NMDA receptors are more likely to be important for inducing larger ionic signals that depend on calcium influx, thereby inducing increases in nerve cell communication that underlie long-lasting changes at synapses.

NMDA receptors not only regulate beneficial synaptic changes in the brain, their overstimulation can be toxic to nerve cells and kill them. This is the subject of a third major area of research. "Excitotoxicity comes into play if there is too much glutamate and too much calcium," says Stevens. Under these conditions cells can no longer regulate their internal concentration of calcium, leading to very high levels of calcium that are toxic. "It is not known what the actual mechanism of cell death is, but a current guess is that calcium stimulates pro-

tein- and fat-digesting enzymes within the cell that destroy it," says Stevens.

The idea that overstimulation of NMDA receptors might cause brain damage has prompted researchers to develop compounds that might block the activity of the receptors and prevent nerve cell death. For instance, a class of compounds, including MK-801 and the hallucinogen phencyclidine ("angel dust"), appears to block the NMDA ion channel. Last year, Leslie Iverson of Merck, Sharpe & Dohme Research Laboratories in Harlow, England, and his colleagues proposed that MK-801 may be a treatment for preventing brain damage during a heart attack or stroke.

Several other research groups are trying to unravel the precise mechanisms by which MK-801 works. Among them are Stuart Lipton and Elias Aizenman of Harvard Medical School in Boston and their collaborator Andreas Karschin of the Max Planck Institute for Brain Research in Frankfurt. They are pursuing an earlier observation that MK-801 works best when the NMDA ion channel is already open. "We find that the action of MK-801 is dependent on the concentration of agonist," says Lipton. "The more NMDA you put on a cell, the better MK-801 blocks its effects. And, if you think about it, this is exactly the way you would want the drug to work."

Like others, Lipton reasons that in the brain of a stroke patient, damage to nerve cells increases with the release of more and more excitatory neurotransmitter. If MK-801 works in vivo as it does in the Harvard group's in vitro system, then the drug should have its maximal blocking effect in a patient with escalating levels of excitatory neurotransmitter in the brain.

The past 5 years have produced an explosion of information about NMDA receptors. Complex chemical and electrical mechanisms regulate their activity. NMDA receptors, in turn, regulate synaptic changes that may underlie learning and memory. They also appear to participate in normal synapse structuring in the brains of developing animals. But the same receptors probably cause some of the brain damage that occurs during ischemia. Despite the progress in understanding NMDA receptors and ion channels, key questions remain about their precise structure, regulation, location, and function. These issues will most certainly sustain this research field as one of the most exciting in neuroscience today. ■

DEBORAH M. BARNES

ADDITIONAL READING

(Special issue) "Excitatory amino acids in the brain—focus on NMDA receptors," *Trends Neurosci.* **10**, 263 (1987).

Corals Remain Baffling

Marine scientists are still baffled by the mysterious coral "bleaching" that swept through the Caribbean last summer and early fall (*Science*, 27 November, page 1228). The reef-building corals have not died in large numbers, as feared, and many are recovering, but the episode appears to be more widespread and more complicated than previously believed.

Bleaching occurs when corals expel the algae, known as zooxanthallae, that normally reside within their tissues, providing energy and oxygen in exchange for nutrients. It is called bleaching because when the brown algae are expelled, the corals turn a startling white. If bleaching is severe enough, corals may die, and the reef ecosystem itself becomes vulnerable to erosion and physical devastation.

Isolated bleaching events are fairly common in response to environmental stress, such as extremes in water temperature or pollutants. But starting last June, corals throughout the Caribbean basin became bleached at almost the same time, in an area stretching from the Florida Keys to Jamaica and as far east as the Virgin Islands. Bleaching on this scale had never been observed in the Caribbean before. However, an even more massive bleaching event devastated coral reefs in the eastern Pacific in 1983, leading to mass mortality.

Alarmed that the Caribbean bleaching, too, might portend mass mortality, some 50 researchers met at a hastily convened workshop in St. Croix in December. The picture that emerged is somewhat mixed, though prospects for recovery seem brighter than they did a month or two ago. "There are definite signs that it is getting better," says John Ogden, director of the West Indies Laboratory of Fairleigh Dickinson University in the Virgin Islands and one of the organizers of the meeting. Most of the corals in St. Croix seem to be recovering, Ogden says, but some colonies, especially brain coral, have died. In Jamaica and Looe Key, there is evidence of recovery as well, he says.

But the picture is not universally rosy. In all, some 60 species of coral and coral-like animals have been affected, although not all species at all locations, says Ernest Williams of the University of Puerto Rico, who is compiling data on the event. "Although most of the corals are recovering, some are getting worse. It has become more complicated than it originally appeared. And I'm not sure the episode is over yet," says Williams, who has new reports of a second bout of bleaching in previously unaffected areas in the southern Caribbean, off the coasts of Venezuela, Panama, and Colombia. The Caribbean episode looks "more and more similar to what happened in the Pacific," he says. "There were a number of bouts. Some species were bleached at one time, others at another."

Efforts are now under way to collect data in a more standardized way on exactly what species are affected, and to what degree. By comparing those data with information on water temperature, salinity, solar irradiation, and wind speed, researchers hope to get a firmer fix on the cause of the bleaching.

The evidence still points to the slightly elevated water temperatures of last summer, researchers generally agree, but not in isolation. Only temperature explains the near simultaneous bleaching over such a wide area, but "temperature alone does not explain all the bleaching in all the locations," says Ogden. For example, the apparent second bout of bleaching, which began in the fall when waters were cooler, is hard to explain by temperature alone. "There is a problem with every simple cause that has been suggested," says Williams. The favored hypothesis is now temperature in combination with changes in ultraviolet light, either decreased light penetration due to sedimentation, or increased light from unusually calm waters.

What is particularly troubling about the bleaching episode is that it is the latest in a series of unexplained mass ecological disturbances in the Caribbean that include the massive fish kill of 1980, the 1983–84 sea urchin (Diadema) die off, in which 95 to 99% of these sea urchins were killed, and a slowly spreading disease, white band disease, that is now devastating elkhorn coral, the principal reef-building coral in the Caribbean.

"Are all these events warning signs of something bigger that we are not aware of?" asks Ogden. "We are treating them as isolated events. But when you put them all together, are we looking at a bellwether for something we should be getting hot about?" ■ **LESLIE ROBERTS**