From Spreading Depression to the Memory Trace

BY ANTHONIE VAN HARREVELD

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One characteristic of animals in general, and of man in particular, is the ability to learn from changes in the environment and to react with changes in behavior. This "memory," which is so important for the survival of the individual, is believed by many biologists to be due to more or less permanent changes in the brain itself.

There are several possible mechanisms that could explain such changes. They could be alterations of the chemistry of the brain, with the formation of a specific compound in the brain for each individual memory. Or perhaps the amount of an existing compound involved in the transmission of an impulse from one nerve cell to the next (the synaptic activity) is increased. Another possibility is that brain structures become more sensitive when they are activated by such transmitter compounds. One theory is that structural changes occur in the brain when a memory is established: for instance, new synapses may be formed.

During the last decade we have been making investigations in this laboratory into the unstable nature of the water and electrolyte (salts that can split in charged particles) distribution in the central nervous system. These studies have suggested a possible mechanism underlying the phenomenon of memory. Our research group (consisting Dr. Eva Fifkova, myself, and several research fellows) has been particularly interested in the effect of a specific amino acid—glutamate—on the water and electrolyte distribution, and how it is involved in the phenomenon of "spreading depression," a condition in which electrical and other activities are temporarily turned off and which spreads at a rate of two to four millimeters a minute in the cerebral cortex. This has recently led us to do research into the role of glutamate in the formation of memory.

In the lower mammals, particularly rabbits and rats, spreading depression especially affects the cerebral cortex, the most complex and highly developed part of the nervous system. When it occurs, all the cortical functions —except metabolic processes and oxygen uptake—are temporarily suspended.

Although glutamate is a natural component of the brain and is present in all the proteins of the body, it can become highly toxic when present in the nervous system in an abnormal distribution. What appears to happen during spreading depression is that glutamate is released from cells and fibers in the cortex into the material between the cells, where it increases the permeability of the membrane surrounding the nerve cells. The increased permeability allows electrolytes and water from the extracellular space to diffuse into the cells and fibers. The electrical potential of the cells is decreased by this effect of glutamate, making it impossible for them to receive or transmit electrical impulses. This explains the loss of function of the cerebral cortex in spreading depression.

Furthermore, this released glutamate causes the nerve cells to release more glutamate, which can then spread and stimulate other nerve cells to release their glutamate. This may be the mechanism by which spreading depression is transmitted through the cortex.

After some minutes the process is reversed, electrolytes and water leave the cells, the membrane permeability returns to normal, the membrane's electrical potential is reestablished, and function is restored. The total blackout persists for five to ten minutes in rabbits.

Wondering what the biological significance of the release of glutamate during spreading depression could be, we

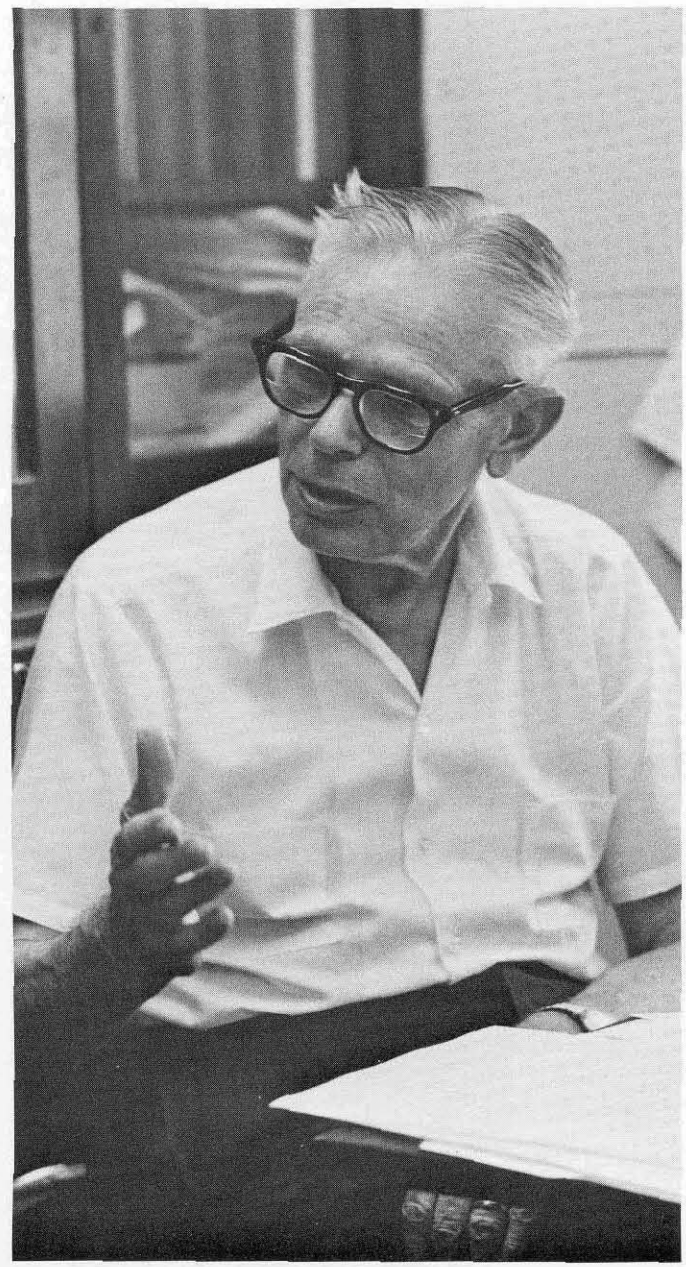
proposed many years ago that spreading depression could be a mechanism to protect the tissue against overactivity and overstimulation. We based this idea on the observation in our laboratories that, in the rabbit, convulsive activity triggers spreading depression, which then abolishes all electrical activity for some time. However, since this reaction was not observed in higher mammals (cats and monkeys), we eventually had to assume that protection against overactivity does not seem to be the true function of spreading depression.

These observations, however, led to the conclusion that neural activity causes a release of glutamate, which in the rabbit may then result in spreading depression. This suggested to Dr. Fifkova and me that spreading depression itself may indeed have no real function in the normal operation of the brain and nervous system, but that what we observed as spreading depression may be an abnormal large-scale activity of a mechanism that has its real functional meaning on a microscale.

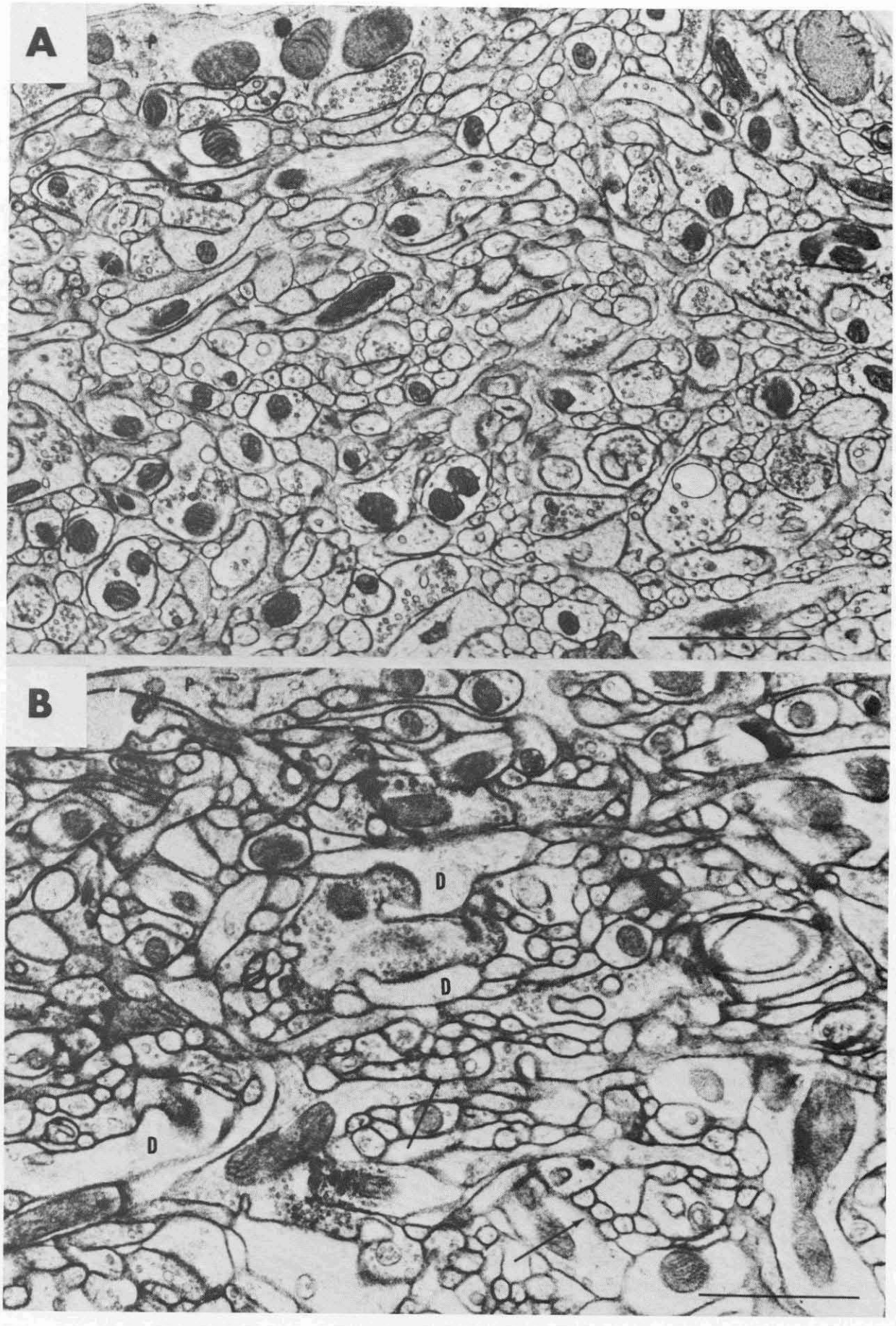
We therefore suggest that glutamate's true role is at the neuronal level, where it may be involved in the mechanisms by which the brain processes information. The activity of the brain could cause a release of glutamate in a pattern determined and shaped by incoming electrical impulses from the sense organs. This would cause localized changes in the configuration of the nerve-cell elements and of the extracellular space, which could change the flow of information in the cortical tissue.

If such a function exists, detecting it would be greatly facilitated by the availability of an antagonist of the glutamate action, because deficiencies in the behavior of an animal caused by administration of such a compound would be a clue to the role played by glutamate in the activities of the brain.

With this in mind, we looked for a compound that could counteract the effect of glutamate. We found that isolated chicken retina (which is really a part of the chicken's brain and can exhibit spreading depression) was a preparation singularly suited to such a search. Using it as a test object,



Anthonie Van Harreveld, professor of physiology, is interested in mammalian physiology, particularly the structural and physiological interactions of neurons in the central nervous system.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we investigated the effects of a large number of amino acids and drugs on the retina's response to stimulation with glutamate. None of the drugs suppressed the glutamate action on the retina, and most of the amino acids did not have any effect. (Some amino acids had an injurious effect, or were undesirable in other ways that prevented their use as glutamate antagonists.)

Only the amino acid, proline, seemed to have features that would make it acceptable as an antagonist to the uptake of extracellular material caused by glutamate. Because it appeared to prevent the structural changes postulated to be involved in the mechanism associated with spreading depression, we decided to examine its effect on animal behavior.

The study of the effects of compounds like proline on the brain function is hampered by the blood-brain barrier which prevents many amino acids and other compounds from passing from the blood into the brain. However, in embryos and in very young animals this barrier is not yet fully developed. From previous experience we knew that in chickens the blood-brain barrier remains permeable for amino acids during the first day after hatching. In such chicks the injection of proline caused no changes in behavior or in brain waves—the most easily noticeable effect of neuronal activity. We therefore looked for less obvious behavioral changes, which led us to investigation of the initial phase in the formation of memory, a function that could well be based on a mechanism involving glutamate. In this early phase, transient neuronal patterns must be transcribed into a more enduring change in the tissue. A release of glutamate patterned by the neuronal activity could well cause such a structural change.

The newly hatched chicken is a favorable subject for such an investigation. These animals tend to peck at small shiny objects, but after they had been offered such a lure moistened with a bad-tasting substance, only a small percentage peck again in later tests. In an experiment in which chickens were avoidance-trained during proline administration, about 58 percent pecked at the lure when tested 45 minutes later, as compared with 24 percent in control groups that had not been injected with proline. This shows that proline somehow inhibits the formation of the memory trace, perhaps by its effect on the action of glutamate on the neurons.

The path that leads from an initial impulse pattern to a more or less permanent memory is a complicated one, in which several phases have been distinguished that can be affected by different procedures. Such experiments have indicated that memory formation is a multistage affair, spread over a considerable range of time, and probably involving several mechanisms. The extensive literature on this subject suggests that the impulse pattern evoked by the experience to be remembered is first transcribed into a "short-term memory trace" that fades in a few hours, and in turn is replaced by a "long-term memory trace" that may remain for the duration of the animal's life.

The formation of the memory trace can be prevented by an electrically induced convulsion given within 30 seconds after the presentation of the bad-tasting lure. The convulsion may interfere with the electrical activity of the brain which mediates the memory. The long-term memory trace is affected by compounds that inhibit protein synthesis in the brain. There is evidence that proline acts neither on the protein synthesis, nor on the electrical activity, but affects the transformation of the impulse pattern into the short-term memory trace.

This concept of the nature of the short-term memory trace opens the interesting possibility that a structural change in the central nervous system tissue is involved in memory and that this change may be detectable by microscopic examination. It should be kept in mind, however, that the effect of proline on the memory—although suggestive—is no proof that glutamate is involved in memory consolidation. Proline may have effects that interfere with the formation of the memory other than the inhibition of the response of nerve-cell elements to glutamate.

Whatever the mechanism of its action, proline is of interest because it seems to affect a different phase of memory consolidation than that acted upon by electroconvulsive shock or by inhibitors of protein synthesis. Therefore, it supplies a new tool in the search for the explanation of the mystery of memory. □

Opposite: Two photographs taken with an electron microscope show the difference in the amount of extracellular space in the cortex of a mouse brain under normal conditions (A) and during spreading depression (B). The electron density of the various tissue elements is rather uniform. In B the spaces between the small profiles have disappeared, and the plasma membranes look thicker and blacker than in A because they are close together. Certain tissue elements that can be recognized as dendrites (D) are electron transparent and seem swollen. The arrows in both A and B indicate axons, which are nerve fibers that carry impulses away from the neurons.