Molecular Theories of Memory

Any theory of memory in the nervous system must consider structure and function in the entire neuron.

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Recently there has been a surge of interest, both theoretical and experimental, in what might be called "the molecular basis of memory." The spectacular success of recent investigations of the molecular basis of transmission of genetic information has suggested that there may be an analogous molecular mechanism for storing and utilizing experiential information during the life of the individual—that is, that the memory of an experiential event is stored in the nervous system by the formation or alteration of a particular molecule or set of molecules, which may be regarded as a molecular engram or memory trace. Various types of molecules, including DNA, RNA, proteins, and lipids, have been suggested as the actual engram. This article is an attempt to provide a critique, rather than a comprehensive review, of certain theoretical and experimental approaches to this general hypothesis (*1*). Since the particular hypothesis that specific changes in neuronal RNA represent the molecular engram of memory has received special attention of late, we consider it in some detail here. Our aim is to use this particular molecular theory to illustrate the problems that are fundamental to all purely molecular theories which fail to consider the cellular environment within which molecules exist.

RNA and Memory

A large number of experiments have now been performed which support the view that RNA metabolism may be intimately connected with memory storage and learning. Although there is still definite controversy about some of the methods and techniques that have been used in these investigations, we limit this discussion to interpretations of experimental data and do not discuss experimental methods. The most direct suggestion that RNA metabolism is involved in memory storage is the report (*2*) that a significant change in the base composition of nuclear RNA of Deiters' nerve cells occurs when a rat learns a balancing task (the adenine-to-uracil ratio of the nuclear RNA of these cells was reported to be increased significantly) and that this change persists for at least 48 hours after the end of the learning experiment. Changes in the base composition of RNA in associated glial cells were also reported in these studies (*3*).

The formation of an epileptogenic mirror focus, a neurophysiological model of memory, has been shown to be correlated with an increase in the total amount of neuronal RNA in the cells involved (*4*). Furthermore, studies on planarians have indicated that ribonuclease blocks the retention of a conditioned response in regenerating planarian tails (*5*), and it has been claimed that learning is transferable from one planarian to another by way of cannibalistic ingestion (*6*). However, the interpretation of the cannibalism data is by no means straightforward, since it appears that in these experiments it was transfer of the general capacity to learn, rather than transfer of the specific learning of a particular task, that was being measured. 8-Azaguanine, a purine analog which can cause formation of nonfunctional RNA (*7*), has been found to depress a rat's ability to learn a new maze without impairing its ability to traverse and recall a previously well-learned maze (*8*). This same antimetabolite was also shown to prolong the interval required for "fixation of experience" in an assay in which the spinal cord of rats was used (*9*); moreover, in the latter report (*9*) it was noted that 1,-1,3-tricyano-2-amino-1-propene, a drug believed to increase the RNA concentration of neurons (*10*), shortens the interval required for "fixation of experience." Finally, long-term administration of yeast RNA has been reported to improve memory function in human subjects with cerebral arteriosclerotic and presenile dementia (*11*), and, in animal experiments, long-term treatment with yeast RNA increased the rate at which the animal acquired a behavioral response motivated by shock (*12*).

Criteria for a Permanent Memory Trace

None of the experiments just described directly test the proposition that an RNA molecule, or set of molecules, represents the molecular engram which is the permanent memory trace; they merely stress the fact that RNA metabolism is an important parameter of neuronal function. In order to prove that a given molecule or set of molecules may be regarded as a permanent memory trace, a more rigorous set of criteria should be met.

We suggest that the following criteria must be satisfied in order to demonstrate that a given molecule, set of molecules, structure, or set of structures is indeed a permanent memory trace: (i) It must undergo a change of state in response to the experience to be remembered. (ii) The altered state must persist as long as the memory can be demonstrated. (iii) Specific destruction of the altered state must result in permanent loss of the memory.

If these criteria are applied to the experimental data relating RNA and memory, it is apparent that the evidence that RNA molecules are specific memory traces is highly circumstantial at present. In particular, a change in the base composition of nuclear RNA in cells involved in a learning task does not necessarily signify that these RNA molecules are permanent memory traces; it might signify that they are transient intermediates in the formation of permanent memory traces, or merely that

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changes in RNA occur concomitantly with learning. The effects of ribonuclease on learning in planarians may also be regarded in this fashion, since this enzyme was applied during the time the "trained" tail was regenerating a head, and presumably, then, during the process of formation of permanent memory traces in the regenerating head. Likewise, in the experiments in which drugs were used to affect RNA metabolism during the fixation of experience, the drugs were active at the time the proper functioning of transient intermediates in the formation of memory traces would be expected to be important.

In summary, not all the experiments cited have yet satisfied criteria ii and iii for establishing a set of molecules as a permanent memory trace. Indeed, any attempt to show that *specific* destruction of a *particular* set of molecules results in the permanent loss of an already established memory trace would appear to be beset with great experimental difficulties. Thus, in experimental work on the RNA hypothesis it has not yet been possible to distinguish between the following alternatives.

1) RNA molecules, like many other types of molecules, are important constituents of the nervous system whose structural and functional state may change dramatically during a learning experience, but they do not function as permanent memory traces; or

2) RNA molecules do have a unique role in the nervous system, that of serving as the final engram of experiential memory, the permanent memory trace.

A Restatement of the Problem

It should be apparent that there is now an abundance of data which suggests some relationship between RNA metabolism in brain and the process of memory storage. What, however, is known about the specificity of this relationship? The major function of all known types of RNA is participation in protein synthesis (*13*); no other function has thus far been demonstrated for RNA in brain. Since protein synthesis is one of the most fundamental of all cellular processes, and since the proteins of a cell are largely responsible for its behavior, one would expect that the process of memory storage in a neuron might well involve some participation of the protein-synthesizing mechanism (*14*). It is not surprising to find that this mechanism may undergo some change of state during cellular activity, or that interference with this mechanism may cause changes in the overall behavior of a cell. Indeed, it would be more surprising if it could be unequivocally demonstrated that RNA function is in no way involved in memory storage. The important point is that proponents of the RNA hypothesis have yet to demonstrate that a unique set of RNA molecules functions as specific permanent memory traces. Criticisms of the RNA hypothesis similar to this one have also been made by Briggs and Kitto (*15*).

At this point one might raise the question: Is there perhaps an inherent difficulty in *any* hypothesis which attempts to explain the encoding of memory solely in terms of *one* set of molecules? Cellular metabolism is not merely a rigid hierarchy whereby DNA controls the synthesis of RNA, RNA controls the synthesis of proteins, and proteins control the synthesis of other metabolites in the cell. Rather, the cell has many regulatory mechanisms, whereby proteins, hormones, and metabolites of low molecular weight may regulate the synthesis of RNA by DNA, as well as many feedback mechanisms for the regulation of functional activity of enzymes (*16*). Thus one cannot logically specify one set of molecules as totally controlling the activities of another set. Furthermore, in the neuron, in which certain functional activities (for example, RNA synthesis) are localized in the cell body and other functional activities (for example, synaptic transmission) are localized in peripheral processes of the cell, the cell body and peripheral synaptic structures exert mutual regulatory effects. Consequently, proper functioning of the nucleic-acid- and protein-synthesizing mechanisms of the cell body is necessary for the proper maintenance of synaptic structure (*17*), and the phenomenon of axoplasmic flow would appear to provide the necessary communication channel whereby centrally synthesized metabolites reach the peripheral synaptic regions (*18*). Moreover, proper synaptic function is necessary for the proper performance of the nucleic-acid- and protein-synthesizing structures of the cell body. A great deal of experimental work indicates that pronounced changes in the state of RNA and proteins occur in the cell body of a neuron that is actively stimulated (*19*), and conversely, that removal of afferent stimulation of a neuron can also cause marked changes in its cell body (*20*). The latter phenomenon is dramatically illustrated by the extreme degeneration of cell bodies of retinal ganglion cells of rabbits that were born and raised in darkness and never received visual stimulation (*21*). Moreover, there is much evidence, from the neuroembryological literature, which indicates that the nature of efferent connections of neurons may influence the structure and function of the cell body (*22*). Whereas we now understand the details of some aspects of the synthetic mechanisms (for example, RNA and protein synthesis) whereby the metabolism of the cell body may control synaptic function, we have almost no understanding of the mechanisms whereby synaptic function may control the metabolism of the cell body.

Thus we may perhaps more adequately investigate the structural basis of the permanent memory trace if we seek to answer the following questions: What permanent changes in neuronal structure and function result from stimulation of the neuron, and what is the mechanism of production of these changes? In such an approach an attempt is made to bridge the gap between current investigations, which emphasize the importance of particular molecules in memory storage, and the more cytologically and physiologically oriented theories of Ramon y Cajal (*23*), Hebb (*24*), and Sholl (*25*), in which emphasis is on the importance of synaptic interrelationships between neurons. These older theories stressed the role of growth of new axonal and dendritic connections as a fundamental process in memory storage and learning. At the time they were formulated little was known of the molecular biochemistry of nucleic acid and protein synthesis, and thus there is an incompleteness in these formulations. It is now apparent that the molecular and the cytological approaches to the problem of memory are by no means mutually exclusive, especially if one postulates that a major function of the synthetic mechanisms of the cell body is to provide molecules necessary for the growth and maintenance of axonal and dendritic connections. Weiss (*26*) has stressed that the adult neuron, as well

as the immature neuron, appears to be in a perpetual state of growth or regeneration, or both, and he has emphasized the importance of axoplasmic flow for this process. The axonal termination of a synapse is essentially devoid of ribosomes (*27*), which are necessary for protein synthesis; hence any new proteins required for new axonal growth would, presumably, have to be synthesized in the cell body and reach new synapses by the process of axoplasmic flow. The major advantage of including synaptic structure and function in *any* hypothesis of memory storage is that one thereby takes into consideration a unique cytological feature of the neuron—namely, the fact that such a vast amount of its surface area (*25*) and functional mass (*28*) is located a great distance from the central cell body. The picture on the cover, taken by Stanley Jacobson (*29*), illustrates these properties of the neuron. As Sholl (*25*) has noted, "The activity of a single cortical neuron may well affect that of 4000 other neurons, [while] a single neuron may have more than 50 dendritic branches." No other type of cell in the body has thus become specialized for direct intercellular communication. Moreover, consideration of possible changes in synaptic structure during memory storage may provide an experimental approach to test for satisfaction of criteria ii and iii; hypotheses which consider memory storage solely at the molecular level have been weakest at this point. Therefore, we may be able to achieve a more comprehensive understanding of the phenomenon of memory if we regard this process as a property of a neuron or set of neurons rather than solely as a property of individual molecules. The molecular approach to the problem has already elucidated certain crucial biochemical processes which might underlie this phenomenon, but the picture is by no means complete at present.

Some Future Problems

In biochemical studies of memory, little attention has been paid, so far, to the lipids of the nervous system, in spite of the fact that lipids are such an important constituent of synaptic membranes. Little is known about the turnover of phospholipids and sphingolipids in such cell membranes. Are such lipids synthesized peripherally, or must they, too, reach the synapse by axoplasmic flow after being synthesized in the cell body? Are new membranes formed as part of the establishment of the memory trace? The recent description of specific inhibitors of fatty acid synthesis (*30*) should make possible an experimental approach to some of these problems.

The kinetics of the behavioral effects of drugs which have been used to produce a specific inhibition or acceleration of synthesis of essential metabolites is another problem which has so far received scant attention. If synthesis of certain necessary metabolites for synaptic growth occurs in the cell body, inhibition or acceleration of such synthetic activities may not be immediately reflected at the synapse. The rate of axoplasmic flow has been estimated to be of the order of 1 to several millimeters per day (*31*); thus, in neurons with long processes there may be a considerable delay between the time a molecule is synthesized and the time it reaches peripheral regions of the neuron. It is thus suggested that, in studies of the kinetics of memory-trace formation, both the initial learning and the later retention trials should be carried out at varying intervals after administration of drugs whose principal mechanism of action is upon synthetic activities in the cell body, since such drugs may fail to produce an immediate behavioral effect but may have a pronounced delayed effect. Some of these problems have been approached in the recent and intriguing investigations of Flexner *et al.* (*32*) on the effects of puromycin (an inhibitor of protein synthesis) on learning and memory in mice. These workers investigated the effect of injecting the drug at various sites and the effect of varying the interval between the initial learning experience and the subsequent administration of puromycin and they found that under certain conditions puromycin caused loss of memory. Further experiments, on the effect of varying the interval between an initial injection of puromycin and a subsequent learning experience, would be of interest in evaluating the hypothesis that axo-plasmic transport of newly synthesized proteins to synaptic terminals is necessary for the fixation of new experiences by means of synaptic growth.

The mechanism of synaptic influence on the metabolism of the cell body is yet another major problem to be solved. It has been suggested (*33*) that the phenomenon of enzyme induction brought about by synaptic stimulation may be important in establishing memory traces, but experimental evidence is scanty. The finding of changes in base ratios of RNA in response to learning situations does not prove that there has been induction of a new type of RNA; since there are many types of RNA in the cell, a change in the relative proportions of the different types being synthesized could produce the same result as induction of a new type. Further studies on the specificity of any such evoked changes in the metabolism of the cell body are critically needed.

Summary

If one establishes a rigorous set of criteria for defining a given type of molecule as a memory trace in the nervous system, then no one type of molecule may at present be regarded as the sole engram of a permanent memory trace. Much evidence already exists that RNA and protein metabolism are intimately involved in the process of memory storage, but the role of other molecules, such as lipids, must also be considered. Sophisticated techniques of molecular biology and enzymology will undoubtedly provide valuable data on biochemical processes involved in memory storage. However, a comprehensive theory of the structural basis of memory must also consider the function of the entire neuron, with consequent emphasis on the reciprocal relationships between the cell body and the synapse, as well as the complex functional interrelationships between neurons.

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SCIENCE AND PUBLIC AFFAIRS

Scientific Advice for Congress

A veteran legislator suggests that current proposals are overlooking some realities of legislative life.

Clinton P. Anderson

One of the results of the growing federal involvement in science and technology has been a growing uneasiness in Congress about its own ability to oversee programs in these areas effectively. The number of inquiries into the general state of science-government relationships undertaken recently is a measure of this unrest, as is the variety of proposals put forth to improve Congress's capacity to judge scientific programs. There is no doubt that Congress does have to make some adjustments to changing patterns of federal expenditure, and all the proposals deserve to be taken seriously. But before a wholly new system for dealing with science is created, it would be well to examine both the source of Congressional interest in science and the kind of advisory structure best suited to its needs.

There are at least three reasons for the interest of Congress in improving its grasp of science and technology. The first is cost consciousness—this year's federal R&D budget is about $15 billion. Congress is concerned, however, not only about the amount of money spent on research and development (which has multiplied 100-fold since 1940) but about the relationship of cost to performance. How can Congress make intelligent decisions when budget costs are based on estimates which fail to hold true? The Air Force, for example, estimated in 1960 that Project Skybolt would cost $893 million; in 1961 the estimated cost had reached $1.9 billion, and by the summer of 1962—when Skybolt was scrapped—not only had the cost estimate climbed to $2.3 billion, but Skybolt was a year and a half behind schedule. Another example is the project for the nuclear-powered airplane (ANP). In November 1951, one contractor estimated that it would take $188 million to deliver the nuclear power plant for mounting in an aircraft by May 1956. By 1961, when the project was cancelled, the costs of that one company had reached over $527 million and the power plant had never been delivered. The total cost of ANP, when it was ended, exceeded $1 billion.

It is true that the money supposedly "wasted" on the nuclear-powered plane may yet pay valuable dividends when some of its positive findings in metallurgy and instrumentation are applied to some future project, such as the supersonic airliner. Knowledge, however useless at the moment of its discovery, will someday find its place in the scheme of things and make its contribution. Nonetheless, a better way must be found to estimate the long-range costs of R&D programs; more accurate target dates for their completion must be determined. And Congress needs to be more accurately informed on both, not only for their implications for the budget and the sensible allocation of funds for R&D, but for their frequent implications for national defense as well.

Legislative Control

A second reason for Congressional attention to what Vannevar Bush has called the "endless frontier" is the belief among some members that Congress has lost the ability to oversee effectively the vast diffusion of R&D activities for

The author, U.S. Senator from New Mexico, is chairman of the Senate Aeronautical and Space Sciences Committee, and is a member of the Joint Committee on Atomic Energy and the Interior and Insular Affairs Committee. This article is based, in part, on an address delivered 20 November to the Atomic Industrial Forum.