Memory in Mice Analyzed with Antibiotics

Antibiotics are useful to study stages of memory and to indicate molecular events which sustain memory.

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Memory is thought to consist of overlapping stages. In the first stage the essential process is believed to be the electrical activity of those nerve cells which participate in a learning procedure. In this stage memory can be destroyed by electroconvulsive shock which disrupts this selective electrical activity. The period when memory is vulnerable to electroconvulsive shock in the mammal varies greatly, with a minimal value of less than 1 minute (*1*).

The learning process also leads to changes of a permanent kind so that in man, for example, memory of an event in childhood may persist for life. Thus long-term memory appears to be a relatively stable condition reached as the outcome of events occurring in a period of consolidation. In this period electrical activity is transformed into a more permanent record. Halstead (*1*) in 1951 suggested that the durability of memory may depend upon changes in neuronal nucleoprotein. The past several years have seen a surge of interest in this area and numerous efforts are being made to evaluate the roles of RNA and protein in the function of the brain.

Further clues to the nature of the learning process and memory can be obtained by considering instinctive or inherited behavior. Such behavior must be attributed to certain stable patterns of gene expression which become established during the development of the individual. These patterns of gene expression are dictated by the sequence of nucleotides in the DNA and are manifested during the complicated and mysterious process known as differentiation.

Behavioral patterns acquired by learning or training are so similar to instinctive ones that they are often difficult to distinguish. Accordingly it is reasonable to assume that well consolidated, long-term memory has the same fundamental basis as instinctive behavior, that is, it is the manifestation of a stable pattern of gene expression. Nature frequently uses the same mechanism for a variety of purposes. According to this view, the difference between the two situations is that the instinctive pattern develops from precursor patterns in response to some of the multitude of interactions which comprise differentiation, but the learned pattern is derived from an earlier quasi-stable pattern in response to the chemical events which are initiated by the learning experience.

Although the detailed mechanisms of differentiation remain obscure, there is little doubt that they involve repression and derepression of genes, as differences in the RNA components have been demonstrated in different organs and in different stages of development. Control of the rate of protein synthesis and of the final behavior of the proteins themselves is also likely to play an important role. Interactions within the cell, between one cell and its neighbors, and with distant organs are all parts of the process. Furthermore, the stability of the patterns which persist in the adult organism depends on the stability of a dynamic state. Individual molecules, cellular substructures, or complete cells can be degraded and replaced if synthesis and degradation remain in balance.

In accord with these principles it seems reasonable that the changes in the patterns of gene expression which result from learning will be accompanied by changes in the kinds and quantities of RNA and proteins (as well as small molecules) which are produced by the brain cells. Furthermore, interference with these synthetic processes by inhibitors might prevent the establishment of new patterns of expression or might upset patterns which were partially (or even completely) established.

Whether or not these broad speculations are valid, it is desirable to identify what, if any, macromolecular events are essential for the maintenance of memory. We hoped to approach this goal by injecting into the brain antibiotics which inhibit the synthesis of a specific macromolecule and then testing the effect of this inhibition on established memory. An antibiotic may also provide a way of differentiating different stages in the formation of memory and of indicating molecular events necessary for learning and for its fixation. This article will be concerned with these several aspects of memory and learning in mice.

Procedure

We use a simple behavioral situation. Mice are trained in a Y-maze with a grid floor through which shock can be applied. The mouse is placed in the stem of the Y. If it fails to move out of the stem within 5 seconds (error of avoidance) it is shocked. If it fails to enter the selected arm of the Y (error of discrimination) it receives shock until it moves to the correct arm. Training is continued in one session (usually lasting 15 to 20 minutes) until the mouse has achieved nine correct responses out of ten attempts (the criterion). The same procedure is used to test for memory of the training experience (retention testing); shock is given for errors of performance. Memory is evaluated in the retention tests in terms of the percentage savings of trials and errors. These percentages are calculated by subtracting the number of trials or errors to criterion in the retention tests from the number to criterion in training, dividing by the number in training, and multiplying by 100. Savings of 100 percent indicate perfect memory; zero savings, complete loss of memory.

In our biochemical studies we have so far been concerned only with changes in the rate of cerebral protein synthe-

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sis after injection of antibiotics. At various times after treatment, a constant amount of radiovaline is injected subcutaneously. The mouse is killed 40 minutes later, since the rate of incorporation of labeled essential amino acids into cerebral proteins is practically constant during this interval. Protein precipitates are prepared from the following parts of the brain which are separated by dissection: the hippocampus, amygdala, thalamus, corpus striatum, temporal cortex (including entorhinal cortex), and the parietal and frontal portions of the neocortex (Fig. 1). The rate of synthesis of protein is calculated from the amount of radiovaline incorporated into protein and from the specific radioactivity of the valine pool.

Intracerebral injections are placed so as to expose the hippocampus, the entorhinal, and the neocortex to relatively high concentrations of the antibiotics. In our early efforts the spread of injected material was estimated from intracerebral injections of fluorescein, which is easily identified with ultraviolet light. These injections, each of 12 microliters, were made through small holes in the skull and at a depth of 2 mm from the surface of the skull. From one to three injections were made in each hemisphere. Bilateral injections, designated frontal injections, were made near the midline in the forward part of the skull. Ventricular injections were made near the midline well behind the frontal injections. Temporal injections were made below and behind the ventricular injections (*2*). Frontal injections of fluorescein heavily stained the forward third of the neocortex; ventricular injections stained all of the hippocampus and the caudal half of most of the neocortex, but importantly, spared the entorhinal cortex; and temporal injections stained all of the hippocampus and the caudal third of the cortex including the entorhinal cortex (Fig. 1). The staining obtained from combinations of these three types of injections was essentially additive.

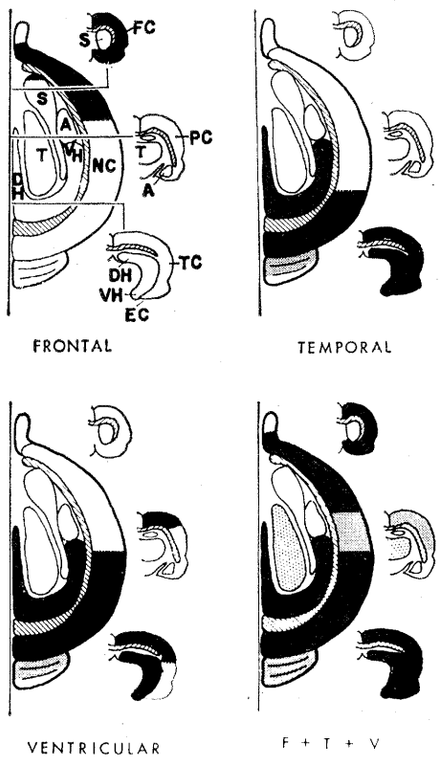

Fig. 1. Spread of fluorescein after it is injected intracerebrally. The diagrams at the left indicate structures viewed from the top after removal of a horizontal section of the hemisphere; at the right, cross (frontal) sections of the hemisphere at the level indicated in the diagram for frontal injections. Relative intensity of staining is indicated by relative density of stippling. *A*, Amygdaloid nucleus; *DH*, dorsal hippocampus; *EC*, entorhinal cortex; *FC*, frontal cortex; *NC*, neocortex; *PC*, parietal cortex; *S*, corpus striatum; *T*, thalamus; *TC*, temporal cortex; *VH*, ventral hippocampus; *F* + *T* + *V*, frontal + temporal + ventricular injections. From Flexner, Flexner, and Stellar (*2*).

Effects of Puromycin

Our initial choice of an antibiotic was determined by the possibility that maintenance of memory might depend upon protein sustained above a critical level by continuing synthesis. We proposed to drastically reduce the rate of synthesis of cerebral protein for several hours and then to test the ability of mice to remember their training in the Y-maze. At that time Yarmolinsky and de la Haba (*3*) had found that puromycin is a powerful inhibitor of protein synthesis. Intracerebral injections of puromycin were made with the same procedure used with fluorescein.

Puromycin is used with caution. Its intracerebral injection in our albino mice causes toxic symptoms. There are often lethargy and loss of alertness followed by hyperexcitability, as well as loss of weight due to failure to eat and drink normally. If sufficient time is not given for recovery, there is the possibility that apparent loss of memory may be due to illness with an attendant impairment of motivation and performance. We delay tests for memory until weight is recovered and behavior is normal, usually 3 to 4 days after treatment. In addition, there is the possibility that an antibiotic may interfere with several cellular functions and so give a misleading answer to the question for which it was chosen. It may consequently be important to use several antibiotics before making firm interpretations of the effects of any one of them. This has proved to be the case with puromycin.

The effects on memory produced by puromycin 1 day or 11 to 60 days after training are given in Table 1. The table shows, after various types of intracerebral injections, the number of mice in which memory was lost, impaired, or retained. The first series of experiments were made with mice trained to criterion and injected 1 day later with puromycin. After six injections (bilateral temporal, ventricular, and frontal), each of 30 to 60 micrograms of puromycin, retention tests showed that memory of the training experience had been lost completely and permanently (memory was absent when tested 3 months after puromycin). An effort was then made to localize this effect. Memory was also consistently lost with bitemporal injections of 90 micrograms of puromycin. By contrast bilateral frontal or ventricular or combined frontal plus ventricular injections were essentially without effect. The next series of experiments was made with mice injected with puromycin 11 to 60 days after training to criterion. In these mice only bilateral temporal plus ventricular plus frontal injections quite consistently destroyed memory. Bitemporal injections, which destroyed 1-day memory, were ineffective.

What do these results indicate about the parts of the brain concerned with recent (1-day-old) and longer-term (11- to 60-day-old) memory? Recent memory was lost when puromycin was given by temporal injections, involving, on the basis of the distribution of fluorescein, the hippocampal area (hippocampus plus entorhinal cortex), while loss of longer-term memory required puromycin additionally in a substantial part of the neocortex. The conclusion from these observations that the hippocampal area is concerned with recent memory and an enlarged area of the neocortex with older memory is supported by the evidence that has come from neurosurgical and autopsy findings on man and from ablation experiments on animals (*4*).

As indicated by our method, how long does it require after learning for

an enlarged area of the neocortex to participate in the effective memory trace? Bitemporal injections consistently destroyed memory 2 days after training (Table 2), but they were consistently without effect 6 days after training. Results were variable at 3, 4, and 5 days. Thus it appears that the enlarged locus of longer-term memory in the type of training experience we have used with mice becomes effective in from 3 to 6 days, depending upon the individual.

We have put these observations on recent and longer-term memory to an additional test by means of reversal training. A mouse was first trained, for example, to move from the stem of the Y into its left arm; then 3 weeks later it was retrained to move into the right arm. Puromycin was injected bitemporally 24 hours later. Would recent memory be destroyed by this treatment and longer-term memory be preserved? Shock was omitted in the retention trials 3 days after injection of puromycin since there was, within the design of the test, no right or wrong response. As shown in Table 3, when they were tested for memory, the first choice of all mice was consistent with the first learning experience, as were the large majority of subsequent choices. Untreated mice, in contrast to the experimental group, made choices consistent with their recent or reversal training. The results fit our evidence for the difference in the parts of the brain concerned with recent and longer-term memory.

We had chosen to use puromycin to test the possibility that continuing protein synthesis is essential for the maintenance of memory. We were encouraged in this view by the destructive effects of puromycin on memory. As has been mentioned, however, our results might have been due to some side effect not related to protein synthesis and it was consequently essential to test our tentative interpretation in other ways. We have done this by correlating the effects on memory and cerebral protein synthesis of consistently destructive and of smaller intracerebral doses of puromycin, of puromycin subcutaneously injected, of several substances related to puromycin, and of other antibiotics which are known to be inhibitors of protein synthesis (*5*).

Figure 2 gives the percentage of inhibition of protein synthesis in six areas of the brain as a function of time after bitemporal injections of 90 micrograms of puromycin, a treatment which uniformly leads to loss of recent memory. The figure shows that puromycin, unlike fluorescein, spreads widely from the site of the injection to other parts of the brain, but inhibition is most drastic in the hippocampus and temporal cortex (including entorhinal cortex). Inhibition in both of these areas with one exception was maintained at a level in excess of about 80 percent from the first to the tenth hour after the injection. On the supposition that destruction of memory by puromycin is related to its effect on protein synthesis, we tentatively concluded that to produce consistent loss of recent memory in our experimental situation, protein synthesis must be inhibited in the hippocampus and temporal cortex for about 9 hours at a level exceeding 80 percent.

Table 1. Effects of different sites of injection of puromycin on short and longer-term memory. L, lost; I, impaired; R, retained; Days, days after learning. T, V, and F refer, respectively, to temporal, ventricular, and frontal injections, all given bilaterally. For the mice with loss of memory, the means and standard deviations for percentages of savings of trials and of errors were respectively 1 ± 3 and 2 ± 6; for those with impaired memory, 26 ± 29 and 39 ± 12; for those with retention of memory, 90 ± 14 and 90 ± 9. From Flexner, Flexner, and Stellar (*2*, *5*).

Puromycin injections			No. of mice in which memory was		
Site	Days	Dose (mg)	L	I	R
Short-term memory					
T+V+F	1	0.03–.06	7	0	0
T	1	.09	10	0	0
V	1	.09	0	0	5
F	1	.09	0	0	5
V+F	1	.09	0	1	2
Longer-term memory					
T+V+F	11–60	0.03	17	2	0
T	11–35	0.06–.09	0	0	7
V	12–38	.06–.09	0	0	3
F	16–27	.06–.09	0	0	3
V+F	28	.06–.09	0	2	2
V+T	28–43	0.09	1	1	2
T+F	28	.09	0	0	3

Table 2. Effect of bilateral temporal injections of puromycin on memory of increasing age. Each injection contained 0.09 milligram of puromycin. For the seven mice with loss of memory, the means and standard deviations for percentages of savings of trials and of errors were respectively 1 ± 4 and 0 ± 0; for the seven mice with retention of memory, 85 ± 19 and 93 ± 7. In one mouse with impaired memory the percentages of savings for trials and errors were respectively 38 and 20; for the other, 39 and 55. From Flexner, Flexner, and Stellar (*2*).

Injections: days after learning	No. of mice in which memory was		
	Lost	Impaired	Retained
2	3	0	0
3	4	0	1
4	0	1	1
5	0	1	2
6	0	0	3

Inhibition of protein synthesis in six areas of the brain was also measured after six injections (bilateral, temporal, ventricular, and frontal) each of 30 micrograms of puromycin. This dose leads to loss of longer-term (greater than 5-day-old) memory. The inhibitory effects of these combined injections on protein synthesis is most pronounced in the hippocampus and temporal cortex. In these two areas inhibition exceeded 80 percent from at the most 1.7 hours to more than 11 hours after the injection. Inhibition in the frontal cortex was somewhat less over this period with a minimum of about 70 percent. The parietal cortex, thalamus, and corpus striatum showed with time a greater decrement, reaching 35 to 50 percent inhibition 11.7 hours after the injection. Again on the supposition that destruction of memory by puromycin is related to its effect on protein synthesis, we tentatively concluded that longer-term memory is destroyed by injections which inhibit protein synthesis in the hippocampus and temporal cortex by at least 80 percent for 10 hours, and in a substantial part of the remaining neocortex to a minimum of 70 percent for the same period of time.

The relationship between puromycin's effect on memory and on protein synthesis was studied further by injecting graded amounts of the antibiotic into the mouse brain. The amounts were smaller than required to consistently destroy memory. As the amount of puromycin was reduced it became progressively less effective in destroying memory; there was a similar trend in its effect on the degree and duration of inhibition of protein synthesis.

In studying the effects of subcutaneous injections of puromycin we used the highest amount of the antibiotic which could be tolerated. We could not detect any interference with memory in these experiments. Again, biochemical measurements showed that protein synthesis was inhibited at a substantially lower level and for a shorter time with the subcutaneous than with the effective intracerebral injections.

A series of substances of interest because of their chemical relationship to puromycin or because they were known inhibitors of protein synthesis were also tested. These substances, in-

Table 3. Differential effect of bilateral temporal injections of puromycin on recent and longer-term memory. Each injection had a volume of 0.012 milliliter and contained 0.06 or 0.09 milligram (mouse 49) of puromycin. Choices of the arm of the Y-maze by an animal after injection were scored as 1 if consistent with initial learning, and as 2 if consistent with reversal learning. Trials were continued irregularly beyond the ten originally planned. From Flexner, Flexner and Stellar (*2*). Learning was always to criterion, and reversal learning occurred 3 weeks after initial learning.

Mouse	Initial learning (No. trials)	Reversal learning (No. trials)	Choice of arm of Y-maze
		Experimental animals	
26A	13	22	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1
24A	7	10	1, 1, 2, 1, 1, 1, 1, 2, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 2, 1, 1, 1
25A	8	10	1, 1, 1, 1, 2, 1, 1, 1, 1, 2, 1, 2, 2, 1, 2
22A	9	8	1, 2, 2, 1, 1, 2, 1, 2, 1, 2, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1
23A	13	4	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1
49	22	9	1, 1, 2, 2, 1, 1, 1, 2, 1, 1, 1, 1, 2, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1
27A	12	5	1, 1, 1, 1, 1, 1, 1, 1, 1, 2, 1
		Control animals	
58A	10	14	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
60A	10	12	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2

jected intracerebrally, were puromycin hydrolyzed at the glycosidic bond, the aminonucleoside of puromycin, the D- and L-isomers of phenylalanyl puromycin, and chloramphenicol. All were without effect on memory. The biochemical studies showed that all failed to produce the severe, sustained inhibition of protein synthesis obtained with puromycin. At this time there was consequently nothing in our experience to contradict the view that memory depends upon protein maintained above a critical level by continuing synthesis.

Before proceeding to experiments with acetoxycycloheximide, designed further to test this oversimplified working hypothesis, several unpublished observations will be briefly mentioned to give a more complete picture of the effects of intracerebral injections of puromycin. (i) To obtain consistent destruction of memory, the volume of puromycin which is injected intracerebrally must be increased with increased skull size. Our routine procedure is designed for mice that weigh 28 to 32 grams. In addition, injections must promptly follow one another. With bitemporal injections, for example, irregularities of response occur if the injections are made more than 5 minutes apart. (ii) In mice trained to criterion, both recent and longer-term memory are maintained for 10 to 20 hours after injection of puromycin, then they disappear permanently (the longest time at which memory has been tested after injection of puromycin is 3 months; at this time, memory was absent). (iii) If mice are run through the maze a sufficient number of times after reaching criterion (that is, over-trained), puromycin, as we inject it, has no effect on memory. About 60 trials beyond criterion on the average are needed to give this protection against puromycin. (iv) Dorsal hippocampal lesions and ventricular dilatation, varying from slight to moderately severe, may be found after injections of puromycin. Damage to other parts of the brain, including the entorhinal cortex, has not been seen except in areas of the neocortex surrounding the needle tracks. Under our conditions the effects of puromycin on memory are unrelated to the degree of severity of the hippocampal lesions. Indeed, ventricular injections cause damage to the hippocampus in the same way as temporal injections, but they have no effect on memory. (v) After treatment with puromycin, all mice are capable of relearning the maze, are capable of reversal learning, and retain memory of their last training indefinitely. Some reach criterion on second learning in practically the same number of trials with the same number of errors as on first learning; in others, second learning is substantially more difficult than first learning. No correlation has been found between this difference on second learning and the degree of hippocampal damage. (vi) Mice which had their memory destroyed by puromycin were retrained. In most instances the standard treatment with puromycin then failed to destroy memory and in addition had relatively little effect on protein synthesis. We have shown with tritiated puromycin that the antibiotic is lost more rapidly from the brain after the second injections, probably because of vascular changes which persist after the first injections. A similar resistance to puromycin often develops after any procedure in which the skull is entered.

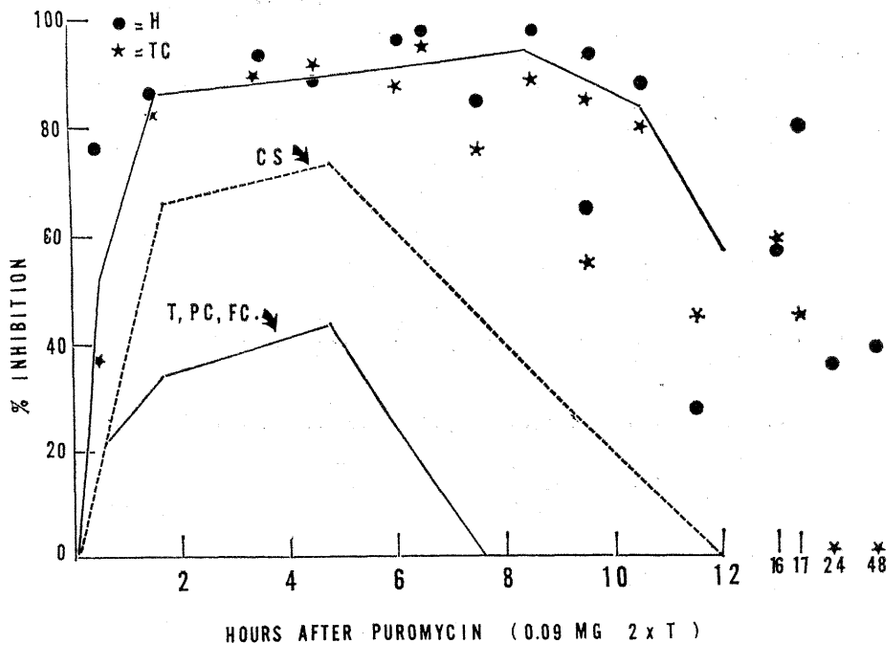

Fig. 2. Changes with time in the inhibition of incorporation of radiovaline into protein of the hippocampus (*H*), temporal cortex (*TC*), corpus striatum (*CS*), thalamus (*T*), parietal cortex (*PC*), and frontal cortex (*FC*) after bitemporal injections each with 90 micrograms of puromycin in 12 microliters. From Flexner, Flexner, Roberts, and de la Haba (*5*).

Effects of Acetoxycycloheximide

The antibiotic acetoxycycloheximide became available to us at about the time we had completed these experiments with puromycin. It is a power-

ful inhibitor of protein synthesis and, importantly for us, suppresses protein synthesis by a mechanism different from that of puromycin. Puromycin produces its effect by being incorporated into the carboxyl ends of growing polypeptide chains and causing their premature release from ribosomes (*6*). Acetoxycycloheximide, by contrast, inhibits the transfer of amino acids from s-RNA to polypeptide (*6*). Thus, unlike puromycin, the heximide suppresses the formation of peptide bonds. Could we destroy memory with the heximide and, as with puromycin, correlate this effect with severe inhibition of protein synthesis? If this proved to be the case, our tentative view that memory depends upon the continuing synthesis of protein would receive strong support.

Figure 3 shows the drastic and sustained effect of bitemporal injections of the heximide, up to 10 hours after treatment, on rate of protein synthesis in the hippocampus, an effect at least equal to that produced by puromycin (Fig. 2). Unlike puromycin, however, the most severe inhibitory effect of bitemporal injections of the heximide is not limited to the hippocampus and temporal cortex. Suppression of protein synthesis in the other six areas of the brain which were studied over the first 10 hours after the injections was as severe as in the hippocampus. Acetoxycycloheximide provided just the agent which we needed to test our working hypothesis.

Table 4 gives the results of the behavioral studies with acetoxycycloheximide. They, like the biochemical results, were unequivocal. Memory was not affected by the heximide in spite of its profound suppression of protein synthesis. Thus it was clear that the simplified version of our working hypothesis was inadequate to explain the destruction of memory by puromycin.

What explanation might be given for the differences between puromycin and acetoxycycloheximide in their effects on memory? One possibility is that the heximide also inhibits the degradation of protein; as a result continued synthesis would not be required to maintain the quantity above a critical level necessary for the expression of memory. That peptide bond formation occurs at a normal rate with puromycin but is suppressed by the heximide suggests two other possibilities. With puromycin it was possible that small, abnormal peptides are synthesized which are toxic and which somehow destroy memory. The second possibility rests upon the assumption, to be stated fully later, that memory depends in part upon the preservation of certain species of messenger RNA (mRNA) which are produced by a learning experience. It is also assumed that puromycin destroys memory because this essential mRNA decays without replacement, while with heximide memory is maintained because essential mRNA is preserved. In support of this possibility it has been found that mRNA is degraded at a normal rate in the presence of puromycin and that puromycin inhibits the synthesis of RNA (*9*). By contrast, the rate of decay of mRNA is decreased with suppression of peptide bond formation as occurs with acetoxycycloheximide (*9*).

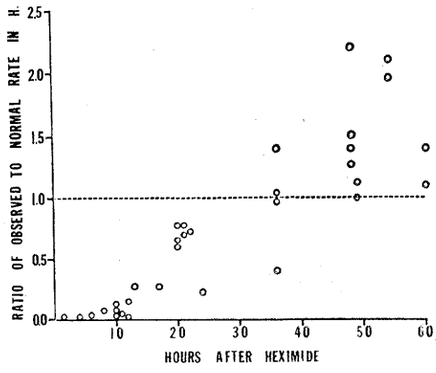

Fig. 3. Rate of protein synthesis in the hippocampus after bitemporal injections of 60 micrograms of acetoxycycloheximide. Values below the dotted line show inhibition; values above, increase of rate over normal level. From Flexner and Flexner (*7*), and Flexner, Flexner, and Roberts (*8*).

If either of these latter explanations were valid, it could be predicted that puromycin would have no effect on memory in the presence of an agent which adequately suppresses the formation of peptide bonds. This prediction was tested (*7*) by using intracerebral injections of puromycin in mixture with acetoxycycloheximide, as well as with cycloheximide or chloramphenicol, which also interfere with transfer of amino acids to protein. All of these antibiotics protected memory against puromycin (Table 4).

Our attempts to demonstrate the presence of small, abnormal polypeptides was based on identification of the puromycin which they would be expected to contain in terminal position. Tritiated puromycin was injected intracerebrally. We were unable to demonstrate radioactivity in protein precipitates prepared from appropriate areas of the brain (*5*). Chromatographs of the supernatant fluid had significant radioactivity only in the spot occupied by free puromycin. However, marked effects on memory have been reported after the injection of small quantities of peptide (*10*). Accordingly, we do not consider that our failure to find an accumulation of abnormal peptides is conclusive evidence that they are not involved in the loss of memory. This possibility remains open.

Self-Inducing System

Understanding of an experimental test which we have made of the alternate possibility involving mRNA depends upon a more complete presentation of our working hypothesis than has thus far been given. We assume that an established memory of long duration depends, not on the continued presence of any protein or nucleic acid molecules, but on the establishment of a self-sustaining system for their synthesis. Such a system can occur whenever some of the products of a gene's expression act as inducers (or derepressors) of that gene. If the gene is repressed, inducers are not synthesized and the gene stays repressed. On the other hand, if the gene is induced for a sufficient time, inducers will ac-

Table 4. Lack of effect of acetoxycycloheximide (A) and of a mixture of it and puromycin (P) on recent (1 day) and longer-term (12 to 35 days) memory. T, V, and F refer, respectively, to temporal, ventricular, and frontal injections, all given bilaterally. For the 30 mice with retention of memory, the means and standard deviations for percentages of savings of trials and errors were, respectively, 90 ± 15 and 92 ± 10; for the three mice with impaired memory, the corresponding means were 45 and 68. From Flexner and Flexner (*7*).

Substance	Injection site	Dose (μg)	Days after learning	No. of mice in which memory was		
				Lost	Impaired	Retained
A	T	60	1	0	1	8
A	T	120	1	0	1	3
A	T+V+F	15–30	1	0	0	2
A	T+V+F	15–30	12–35	0	0	5
A+P	T	120 A + 120 P	1	0	1	6
A+P	T+V+F	8 or 15 A + 30 P	14	0	0	6

cumulate above a critical level and the gene will stay induced. If, however, the synthetic processes are inhibited for a sufficient time, the level of inducers will fall below the critical level and the gene will revert to its repressed state. (A more quantitative description of the self-inducing system is given in *11*.)

The processes involved in the establishment of a long-term memory can be described in terms of the self-inducing system. We assume that the initial learning experience triggers the synthesis of one or more species of mRNA. This mRNA alters the synthetic rate of one or more proteins which are essential for the expression of memory. These proteins are thought to modify the characteristics of synapses concerned in a learning process so that the passage of impulses between nerve cells is facilitated. In turn, the proteins or their products act as inducers of their related mRNA; in this way the concentration of the inducer proteins is maintained. In this view, expression of memory depends upon changes in proteins, changes which are initiated and sustained by qualitative and quantitative changes in mRNA produced by a learning experience. Loss of this mRNA would lead to loss of essential protein with consequent permanent loss of memory. In the presence of an inhibitor of protein synthesis, the concentration of essential protein could fall to levels too low for expression of memory, but loss of memory would be temporary if mRNA were conserved to direct the synthesis of protein when the inhibitor had disappeared (*7*).

Such a loss and recovery of memory has been observed in the behavior of mice at various times after training conducted (i) during or (ii) immediately before the severe suppression of protein synthesis which follows treatment with acetoxycycloheximide. Both sets of experiments showed an initial period in which memory was retained, an intermediate period in which memory was temporarily lost, and a final period during which expression of memory returned (*8*).

The duration of the initial period during which memory is retained in spite of severe inhibition of protein synthesis seems to vary with the conditions of learning and the inhibiting agent. Barondes and Cohen (*12*) observed that when mice are trained to a Y-maze in the presence of puromycin, they retain their memory of the maze at a high level for less than 45 minutes. In our mice trained immediately before treatment with acetoxycycloheximide, the initial period lasted for more than 14 hours. Memory of training in the presence of the heximide appeared to persist for between 3 to 5 hours, though the reliability of the upper limit is questionable because of the relatively poor condition of the mice at this time. In any event, there is a period in which memory is retained in spite of drastic inhibition of protein synthesis throughout the brain. Similar observations have been made on goldfish by Davis and Agranoff (*12*). Memory during the initial period may be based on changes in concentrations of ions or small molecules or in the configuration or location of preexisting macromolecules.

The intermediate period is characterized by failure of the mice to perform the training procedure. Our observations seem to indicate that the temporary loss of memory is not due to a general, nonspecific failure of performance or recall. Memory of training immediately before injection of heximide was expressed during a period when memory of training after injection of heximide could not be demonstrated. Furthermore, relearning occurred in both groups at the time when mice with loss of memory were given retention tests; this relearning indicated again an adequate capacity for performance. During the intermediate period memory appears to reside in a form which cannot be expressed until protein synthesis has been restored.

The final period is characterized by the return of memory to a condition where it can control performance. In essentially all mice in both experimental situations memory returned at a high level 58 to 96 hours after training. This period is at least 20 hours after protein synthesis was found to have returned to normal or higher than normal rates (Fig. 3).

Clearly only a beginning has been made in testing the hypothesis based on a self-sustaining system. The hypothesis is consistent with the results of Hydén and collaborators (*12*) who demonstrated an increase in nuclear RNA following training. It is also consistent with the recent finding by Zemp *et al.* (*12*) that rate of synthesis of nuclear RNA is increased in a learning situation. There is, however, as yet no completely convincing demonstration that changes in RNA and protein are fundamental to memory.

Conclusion

It is apparent that antibiotics are useful in differentiating different stages in the formation of memory. Puromycin gave the first indication that very early memory can be established and survive, for a short period at least, in spite of inhibition of protein synthesis (*12*). Injection of actinomycin D indicates that RNA synthesis is not essential during this early stage (*13*). The duration of this early period seems to vary with the inhibiting agent; with puromycin memory was notably degraded in less than an hour, but with actinomycin D or with acetoxycycloheximide it persisted for several hours or more.

The fixation or consolidation of memory involves whatever processes give permanence to memory. These processes are disrupted when electroconvulsive shock is administered shortly after a learning experience, presumably because of the interference with organized patterns of neuronal electrical activity. Memory acquired in the presence of antibiotics appears to proceed to a stage beyond that based purely on electrical activity because the memory persists beyond the period usually reported as sensitive to electroconvulsive shock. Further work should show whether this stage is truly insensitive to electroconvulsive shock. Memory acquired in the presence of puromycin does not seem to achieve any durable consolidation. In contrast, memory acquired in the presence of or immediately before injection of acetoxycycloheximide does appear to initiate the later stages of consolidation, as permanent memory reappears some days after the initial stages have become ineffective in controlling performance.

Finally, puromycin has provided evidence of the enlarged area of the neocortex which participates as memory matures. Puromycin also indicates the time required for this maturation process.

Since antibiotics have also been useful in studying learning and memory in goldfish (*14*), this approach seems to have general applicability in defining various stages in the process of memory formation.

The initial purpose of these investigations was to determine the molecular basis of the "memory trace." This goal still remains distant, although there are some indications that protein synthesizing systems are involved. This objective, though of enormous interest,

is to be regarded as only a necessary first step. Whether new proteins or some other molecules cause the changes in synapses thought to underlie memory, this knowledge of itself will contribute only a beginning to our understanding of the events which account for the functioning of the brain. A determination of the composition of computer components would provide very little information towards unraveling their function.

As the experiments proceeded, however, information of a more general nature was being obtained. The identification of different stages of consolidation show how injections of antibiotics can supplement electroconvulsive shock as a way of disrupting the establishment of memory and how it can supplement ablation in destroying memory already laid down in a permanent mode. Applied to larger animals the localization of various regions sensitive or insensitive to the action of the drugs should become more definitive. We hope that such experiments will contribute increasingly to the general problem of brain function.

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Basic and Applied Research: A Meaningful Distinction?

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One of the noticeable recent themes in the literature on federal support for science is that the budget for basic research should be separated from budgets for applied research and development. This assumes what is in fact dubious: that operational definitions of these phrases exist. Further, the definitions offered by scientists may afford significant clues to their thinking in a larger context, clues to their assumptions about the nature of the basic-applied-developmental spectrum and about the social meaning of each portion of the spectrum. What does one find by an impressionistic review of recent statements about the basic-applied relationship?

As one reads attempt after attempt to define "basic" and "applied" research, and establish a clear distinction between them, one's sympathy increases for Charles V. Kidd's conclusion (*1*) that "it is not possible to define basic research operationally." Although natural scientists are professionally engaged in exploring empirical phenomena with great precision, and place great emphasis upon defining their concepts so that they can be handled objectively, most of them provide essentially intangible, imprecise, subjective definitions of research itself.

Whether one agrees with the mystical tone adopted by Edward Teller (*2*) (pure research "is a game, is play, led by curiosity, by taste, style, judgment, intangibles") or the more common descriptions used by Leland J. Haworth (*3*) (basic research "seeks an understanding of the laws of nature without regard to the ultimate applicability of the results") or Glenn T. Seaborg (*3*, p. 66) ("intellectual curiosity" is the foundation of basic research; "the motivating force is not utilitarian goals, but a search for a deeper understanding of the universe and of the phenomena within it"), it is apparent that basic research depends on the psychological motivation of the man performing it.

Motivation, however, is not the easiest concept to make operational, to use as a basis for gathering statistics on the amount of federal support going to basic research. Is the National Science Foundation to ask each grantee what inner need of his soul is to be met by the research he proposes? One quickly agrees with Frederick Seitz (*2*, p. 283) that "when one reaches a point where one is dealing with incentives, motives, you need a good psychologist, perhaps even a psychiatrist to decide what the goals are." Furthermore, to define basic research by the emotional state of the researcher logically leads to the conclusion that the exploration of space, including manned flight to the moon, is "basic research" to those who look upon the space program as founded in human curiosity and the "game" of attacking the unknown; yet a good portion of academic scientists who would endorse the motivational definition have also been castigating that program for some time as being unscientific, or at best, marginally scientific. Perhaps, then, a more objective definition would stress the qualities of the thing being done rather than the motives of the doer.

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