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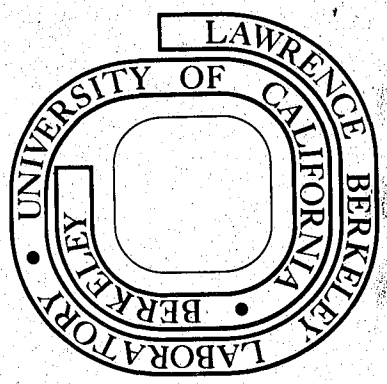

ROLE OF NEUROTRANSMITTERS AND PROTEIN SYNTHESIS IN SHORT- AND LONG-TERM MEMORY

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ABSTRACT

Anisomycin is an effective inhibitor of cerebral protein synthesis in mice and is also an effective amnestic agent for both passive and active behavioral tasks. From use of anisomycin in combination with a variety of stimulant and depressant drugs, we conclude that the level of arousal following acquisition plays an important role in determining the duration and the rate of the biosynthetic phase of memory formation. While we have interpreted the experiments with anisomycin as evidence for an essential role of protein in memory storage, others have suggested that side effects of inhibitors of protein synthesis on catecholamine metabolism are the main cause of amnesia. Several experiments were therefore done to compare the effects of anisomycin and catecholamine inhibitors on memory. We conclude that anisomycin's principal amnestic mechanism does not involve inhibition of the catecholamine system. The results strengthen our conclusion that protein synthesis is an essential component for longterm memory trace formation. Also, it is suggested that proteins synthesized in the neuronal cell body are used, in conjunction with other molecules, to produce permanent and semi-permanent anatomical changes.

The search for the elusive memory trace has long intrigued scientists, but until approximately 1950 research in this field was primarily limited to anatomists such as Raymon y Cajal or psychologists such as D. O. Hebb. In 1950 Katz and Halstead (1) proposed a chemical theory of memory when they suggested that "an essential feature in the genesis of the memory trace is the formation, as a result of individual experience, of geometrically ordered protein molecules in the neurons of the cerebrum. These new ordered molecules then function as template molecules and thus now have the same relationship to the cell as do the original genes." With the discovery in the early 1950's of the double-helical structure of DNA and the evolving theories of RNA in information processing, investigation of the

involvement of macromolecules in memory began in earnest. At that time, a few scientists, notably Professor Holger Hydén, began experiments on possible changes in RNA associated with the training of the laboratory rat. His studies, which have continued to the present, served as the catalyst for numerous attempts to demonstrate the elusive role of nucleic acids in memory. Out of these studies, it is generally accepted that increased RNA synthesis occurs during and shortly after training, but not too much more can be said of the role of this RNA in memory-trace formation except in general terms that it leads to the synthesis of new protein.

The presumed involvement of proteins in the formation of long-term memory naturally led to experiments to test this relationship. In the early 1960's, the Flexners initiated an approach which, with refinements, is still in use. This approach will be the main focus of this paper.

The Flexners began their classic experiments with the inhibitor of protein synthesis, puromycin. They showed that puromycin, when injected intracerebrally into the mouse, led to amnesia for a recently learned task. It was suggested that the maintenance of memory depends upon the continuing synthesis of protein (2). The initial reports of the Flexners encouraged others to investigate the possible relationship of protein synthesis to long-term memory formation, and during the remainder of the 1960's it was shown that other inhibitors of protein synthesis would produce amnesia in a variety of species including goldfish, mouse, and rat if administered shortly before or after training. Nevertheless, problems of interpretation still remained, and many investigators in this field have been or are still unwilling to agree that the experiments with protein synthesis inhibitors have demonstrated a crucial role for protein in memory trace formation.

Each of the inhibitors of protein synthesis being used at that time--puromycin, cycloheximide and acetoxycycloheximide--had certain disadvantages. Puromycin had to be injected intracerebrally, and further research showed that it leads to hippocampal seizures in the mouse. It also produces changes in the concentration and turnover of catecholamines and has been shown to produce a long-lasting peptidyl-puromycin. For all of these and other reasons, the Flexners are still hesitant to conclude that experiments with puromycin have demonstrated a requirement for protein synthesis to establish long-term memory (3). Cycloheximide had to be used at near toxic doses in the mouse in order to obtain 80% or more inhibition of protein synthesis lasting about 2 hours. This limited experimental flexibility and also raised questions of interpretation. Acetoxycycloheximide, which was much more potent and produced a much longer inhibition of protein synthesis, became unavailable.

It was with these concerns in mind that approximately 1970 we began a search for another inhibitor of cerebral protein synthesis--one that would not show many of the disadvantages cited above. Rather fortuitously we found one--anisomycin (ANI) (4). ANI is a relatively simple pyrollidine derivative isolated from a

streptomyces. It is a potent inhibitor of protein synthesis through its interference with transpeptidation (5).

In the mouse, we found that subcutaneous injections of ANI at a dosage of 20 mg/kg inhibited protein synthesis by at least 80% for approximately 2 hours. Also, ANI was relatively non-toxic in the mouse and injections could be repeated many times. Thus, the duration and time course of inhibition of protein synthesis can be controlled (6).

We found that ANI is an effective amnestic agent for a variety of passive and active behavioral tasks. The most used experimental tasks fall into two broad categories--passive avoidance and active avoidance. Typical passive avoidance tasks are the step-down and step-thru tasks. If the animal steps down from the platform or steps thru the hole, it receives a slight electrical shock to its feet until it returns (usually very quickly) to its original position. Typically, only one training trial is required in order that upon a subsequent test some days later, the animal will demonstrate "memory" of the training by not stepping down from the platform or by not going thru the hole into another section of the box. An animal that quickly steps down or thru is said to be "amnesic". Two types of active avoidance training procedures have been used in our studies. In the T-maze, the animal is trained to go to the correct arm (for example, the right arm) to escape or avoid shock. In the pole-jump, which also has been frequently used by Prof. DeWied's group, the animal must cling to the wire mesh on the pole to escape or avoid shock. These active avoidance tasks typically are more difficult for the animal to learn than passive avoidance; frequently, they take 5 or more training trials (7,8).

Using all of these tasks, we showed that ANI was an effective amnestic agent. In addition, in the course of these investigations, we began to appreciate the many factors that can control how well a behavioral task is learned and indirectly the degree of amnesia for that task with a given treatment with ANI. These factors, which now seen rather obvious, include the following: species and strain of animals, shock intensity, duration of shock, latency of animal to enter shock compartment, number of training trials, interval from training to testing, and difficulty of behavioral task.

It was found that for a given training task, the greater the training strength, the less the amnesia. It was also found that for a given training strength, the longer the duration of protein synthesis inhibition, the greater the amnesia (4).

From these and other studies we have derived a rather simplistic model of the formation of a memory trace. The general idea is that memories of different strengths are formed and decay in a manner shown in Fig. 1 which depends upon many factors designated here simply as "training strength."

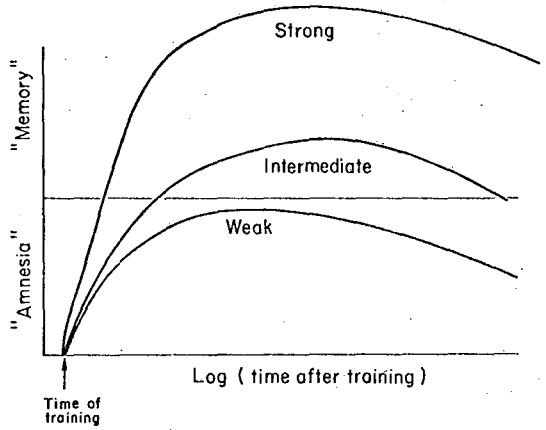

Fig. 1. Schematic representation of memory trace formation as a function of training strength. Animals with a training strength near the dividing line between "memory" and "amnesia" can be used to study the effect of numerous drugs on long-term memory trace formation.

These concepts indicate that the ANI treatment might provide a generally useful and sensitive experimental design to determine the effects of numerous other drugs on long-term memory formation. With a suitable program of training and administration of ANI we can place the subjects near a "balance point" that is, near the behavioral dividing line which classifies an animal as having either "memory" or "amnesia". Here the effects of the other drug can be measured very sensitively. The general procedure is to administer ANI 15 min prior to training, train the mouse, and at a suitable interval after training administer the drug under investigation, and perhaps administer one or more additional doses of ANI in order to place the "memory strength" near the balance point. A week or so later the animals are tested. This procedure permits the administration of the drug under investigation at an extended time--30 min to 1 hr--after training and thus serves to eliminate possible effects of the drug on the training itself.

Results of an experiment using ANI and d-amphetamine are shown in Fig. 2. d-Amphetamine administered either 30 or 90 min after training attenuated the amnesic effect of two successive doses of ANI, but if d-amphatamine administration was delayed until 150 min after training, little attenuating effect was seen.

Using this procedure, we have shown that a variety of drugs generally classed as stimulants--nicotine, picrotoxin, d-amphetamine, strychnine, and caffeine--can overcome the amnesia produced by several injections of ANI (8). It is of interest to note two recent reports of improvement of long-term memory in normal humans by physostigmine, arecholine, and choline (9,10).

On the other hand, depressants had the opposite action; chloral hydrate and phenobarbital administered 30 min after training increased the percentage of amnesic mice (Fig. 3). Similar results were obtained with meprobamate and chlorpromazine.

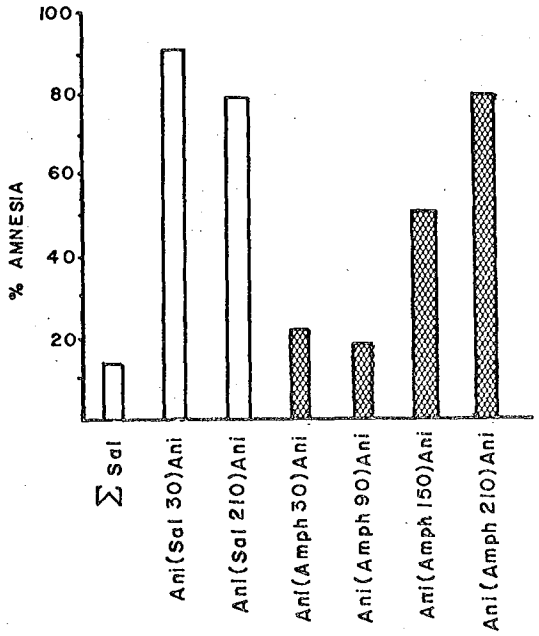

Fig. 2. Time-dependent effect of a stimulant on ANI-induced amnesia. d-Amphetamine blocked amnesia caused by anisomycin when administered 30 or 90 min after training, but was ineffective when administered 210 min after passive avoidance training. (N = 20/group).

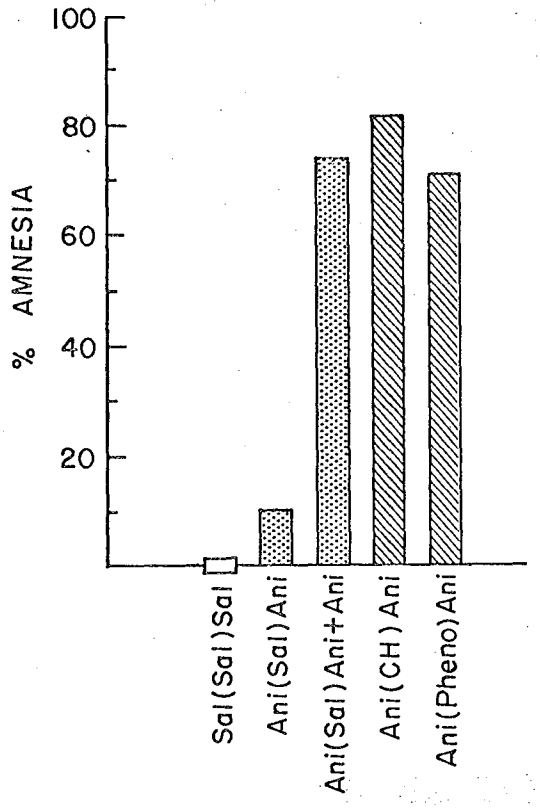

Fig. 3. Chloral hydrate (CH) and phenobarbital (Pheno) increased ANI-induced amnesia for passive avoidance training. The level of amnesia obtained with 2 successive injections of ANI and one of the depressants was comparable to that obtained with 3 injections of ANI.

We believe these data and other data both from our laboratories and those of others are consistent with the hypothesis that the level of arousal following aquisition plays an important role in determining the length of time and the rate of the biosynthetic phase of memory formation.

While we and several other groups of investigators have interpreted experiments with ANI and cycloheximide as evidence for an essential role of protein in memory storage, other investigators, principally Flexner and coworkers and Quartermain and coworkers, have suggested that side effects of inhibitors of protein synthesis on catecholamine metabolism are the main cause of amnesia (3,11,12). They point out that administration of protein synthesis inhibitors appears to modify concentrations of catecholamines and that catecholamine inhibitors can produce amnesia. A further reason for critically determining whether protein synthesis inhibition produces amnesia because of effects on catecholamines comes from observations of Flood.

Flood has recently investigated some 20 drugs that modify neurotransmitter-receptor interaction. These experiments have demonstrated that post-trial administration of drugs that increase catecholamine receptor activity, such as apomorphine,

clonidine, and desmethyl imipramine improve retention of a T-maze avoidance task. Drugs that decrease catecholamine receptor interaction such as pimozide, γ-hydroxybutyric acid, and propranolol impair retention. It is concluded that dopamine and norepinephrine are both involved in memory formation (13,14).

We recently have completed a series of experiments in which the amnesic effects of ANI were compared with those of the catcholamine inhibitors diethyldithiocarbamic acid, tetrabenazine, and α-methyl-p-tyrosine. These agents each have rather different effects on cerebral catecholamines; diethyldithiocarbamate lowers norepinephrine levels in brain by inhibiting dopamine-β-hydroxylase but does not affect serotonin or dopamine; tetrabenazine disrupts storage of dopamine, norepinephrine, norepinephrine, and serotonin, and α-methyl-p-tyrosine decreases norepinephrine and dopamine but does not affect serotonin (Fig. 4).

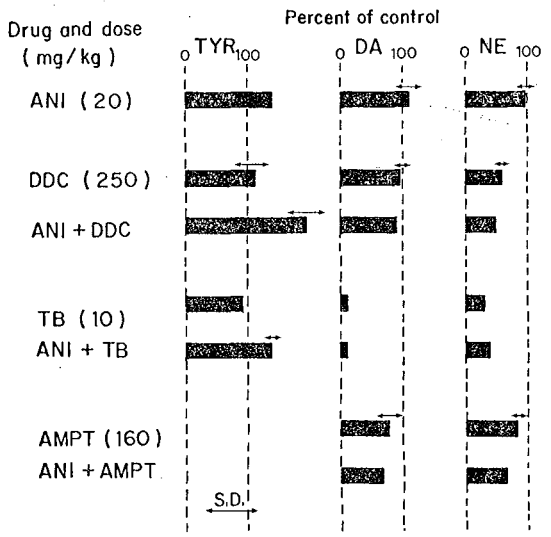

Fig. 4. Effects of the catecholamine inhibitors diethyldithiocarbamate (DDC) tetrabenazine (TB), and -methyl paratyrosine (AMPT) alone and in combination with anisomycin (ANI) on cerebral concentration of tyrosine (TYR) dopamine (DA), and norepinephrine (NE). The CAI's were administered 1 hr after the ANI and the mice were sacrificed 1 hr later. Drugs were administered by subcutaneous injection.

The rationale of our experiments was this: If the major cause of the amnesic action of ANI was due to its effects on catecholamines, rather than its actions as an inhibitor of protein synthesis, then catecholamine synthesis inhibitors such as diethyldithiocarbamic acid, tetrabenazine, and α-methyl-p-yrosine could be substituted for ANI and should be equally or more effective as amnestic agents. If, however, a correspondence of behavioral effects was not obtained over a variety of experimental paradigms, and if ANI was more effective than the catecholamine inhibitors (CAI's), this would provide strong support for our conclusion that protein synthesis is a necessary and distinct component of long-term memory formation.

In one experiment designed to test the "catecholamine hypothesis", we compared the effectiveness of a series of three injections of each of these drugs in producing amnesia in well-trained mice. A moderately high shock intensity and a step-thru passive avoidance task were used. We have noted earlier that longer inhibition of protein synthesis leads to amnesia even in better trained animals. The CAI's were first administered 60 min prior to training to allow sufficient time for them to exert their effects on neurotransmitters. ANI was administered 15 min

prior to training. We have shown that ANI inhibits brain protein synthesis in less than 5 min (15). The second and third injections were 2 and 4 hrs after the first. None of the three CAI's was effective as an amnestic agent, whereas extended inhibition of protein synthesis did produce amnesia (Fig. 5). Thus, this experiment provided clear evidence that inhibition of protein synthesis, achieved by administration of ANI produced amnesia whereas inhibition of catecholamines was ineffective.

A further test of the "catecholamine hypothesis" was made as follows: If the the mode of action of ANI as an amnestic agent is a result of its inhibition of catecholamine synthesis, then one should be able to replace one of the series of three ANI injections with an injection of a CAI and still obtain a high percentage of amnesic subjects. To test this hypothesis, subjects were trained as in the previous experiments on the one-trial passive avoidance task. ANI was administered to all groups (except the saline control) 15 min prior to training. The second injection was either ANI or cycloheximide administered 1 3/4 hr after training, or tetrabenazine, α-methyl-p-tyrosine, diethyldithiocarbamate or saline 3/4 hr after training. All groups except the saline control received ANI at 3 3/4 hr after training. The CAI's could not be substituted for ANI, while cycloheximide, another protein synthesis inhibitor, could (Fig. 6).

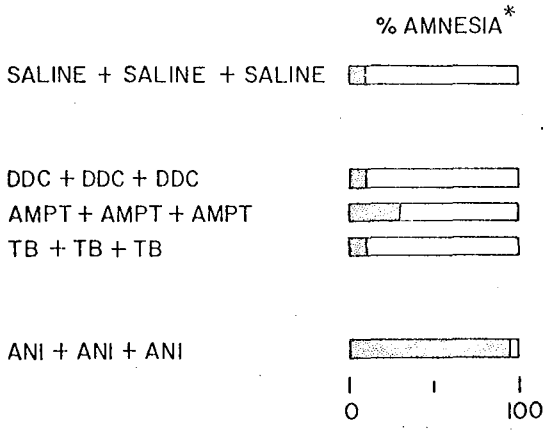

Fig. 5. This experiment compared the effect of three successive injections of ANI or the catecholamine inhibitors on retention of a passive avoidance task. When tested one one week later, ANI-injected mice were amnesic, but the mice administered catecholamine inhibitors were not. (N = 20/group).

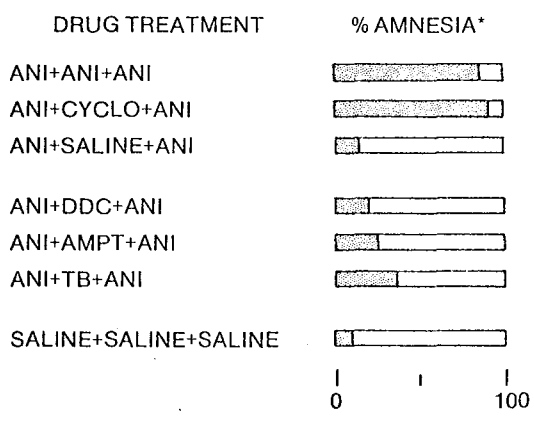

Fig. 6. With the one-trial passive avoidance task, no amnesia was obtained when a catecholamine inhibitor was substituted for the second of a series of ANI injections. However, amnesia was obtained when cycloheximide, another protein synthesis inhibitor, was substituted for anisomycin. (N = 20/group).

The results of these and other experiments indicate that while catecholamine inhibitors can impair long-term memory formation, in a variety of conditions they cannot be substituted for ANI. It is clear that ANI's principal amnestic mechanism does not involve inhibition of the catecholamine system. The results of these

experiments strengthen our conclusion that protein synthesis is an essential component for long-term memory trace formation.

At this point, it perhaps is appropriate to speculate and indicate what we believe to be the role of neurotransmitters and proteins for memory.

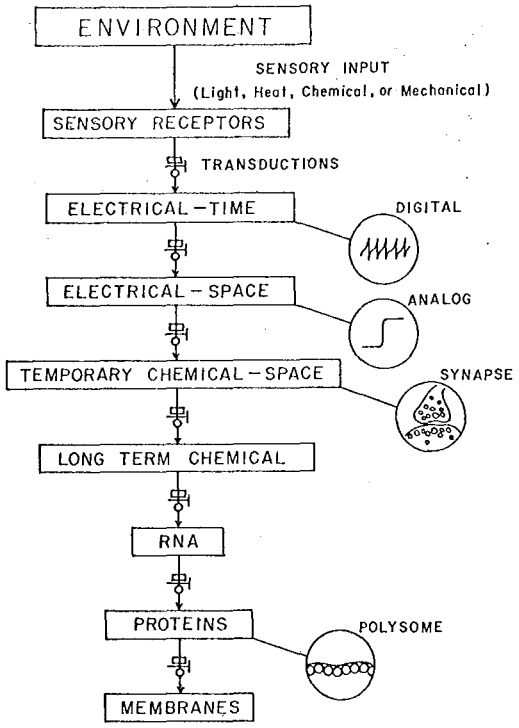

Fig. 7. A theoretical model for memory storage (16). In this paper we are concerned primarily with the biochemical steps occurring after the block labeled "Temporary Chemical-Space". Proteins and other macromolecules transported down the axon are thought to lead to long-lasting changes in membranes at the synapse.

Let us use a schema proposed by Shashoua some years ago (Fig. 7) (16). In the lower portion of this schema, we see the neurotransmitters as important at the stage labeled "Temporary Chemical-Space". In the excited neuronal pathways involved in a specific sensory event, the neurotransmitters can modulate the activity of RNA to produce additional proteins in the cell-body. Under the heading of "neurotransmitters" we can include not only the more common ones we all recognize but also molecules such as ACTH peptides which are usually classed as hormones, and cyclic nucleotides (second messengers). The proteins formed by this activity are then transported down the axons or the dendrites to either synaptic endings or post-synaptic receptors. Ultimately, the transported molecules lead to alteration of synapses and perhaps even formation of new dendritic branching and synaptic endings. In other words, protein synthesized in the neuronal cell body is used, in conjunction with other molecules, to produce permanent and semi-permanent anatomical changes. Evidence is being obtained from many laboratories for the anatomical plasticity of brain in response to environmental stimuli. Thus, the speculations of Cajal and those of Katz and Halstead were both correct--learning does modify the brain in ways that can now be measured, both anatomically and biochemically.

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REFERENCES

1 J.J. Katz and W.G. Halstead, Protein Organization and Mental Function. Comp. Psychol. Monog., 20(1950)1-38.

2 L.B. Flexner, J.B. Flexner, G. de la Haba and R.B. Roberts, Loss of memory as related to inhibition of cerebral protein synthesis. J. Neurochem., 12(1965)535-541.

3 L.B. Flexner and R.H. Goodman, Studies on memory: Inhibitors of protein synthesis also inhibit catecholamine synthesis. Proc. Natn. Acad. Sci. USA, 72(1975)4660-4663.

4 J.F. Flood, E.L. Bennett, A.E. Orme, M.R. Rosenzweig and M.E. Jarvik, The influence of duration of protein synthesis inhibition on memory. Physiol. Behav., 10(1973)555-562.

5 D. Vasquez, Twenty Five Years of Research on Inhibitors of Protein Synthesis: Prospects for Future Developments in the Subject. In A. Kornberg, B.L. Horecker, L. Cornudella and J. Oro (Eds.), Reflections in Biochemistry, Pergamon Press, New York, 1976, pp 347-356.

6 J.F. Flood, E.L. Bennett, A.E. Orme and M.R. Rosenzweig, Relation of Memory formation to controlled amounts of brain protein synthesis., Physiol. Behav., 15(1975) 97-102.

7 J.F. Flood, E.L. Bennett, A.E. Orme and M.R. Rosenzweig, Effects of protein synthesis inhibition on memory for active avoidance training. Physiol. Behav. 14(1975)177-184.

8 J.F. Flood, M.E. Jarvik, E.L. Bennett, A.E. Orme and M.R. Rosenzweig, Protein synthesis and memory for pole jump active avoidance and extinction. Pharmacol. Biochem. Behav., 7(1977)71-77.

9 K.L. Davis, R.C. Mohs, J.R. Tinklenberg, A. Pfefferbaum, L.E. Hollister and B.S. Kopell, Physostigmine: Improvement of long-term memory processes in normal humans. Science, 20(1978)272-274.

10 N. Sitaram, H. Weingartner and J.C. Gillin, Human serial learning: Enhancement with arecholine and choline and impairment with scopolamine. Science, 201(1978)274-276.

11 L.B. Flexner, F.G. Serota and R.H. Goodman, Cycloheximide and acetoxycycloheximide: Inhibition of tyrosine hydroxylase activity and amnestic effects. Proc. Natn. Acad. Sci. USA 70(1973)354-356.

12 D. Quartermain and C.Y. Botwinick, The role of biogenic amines in the reversal of cycloheximide-induced amnesia. J. comp. physiol Psychol. 88(1975)386-401.

13 J.F. Flood, G.E. Smith, E.L. Bennett, A.E. Orme and M.E. Jarvik, Comparison of effects on memory of catecholamine and protein synthesis inhibitors. Pharmac. Biochem. Behav. (submitted).

14 J.F. Flood, G.E. Smith and M.E. Jarvik, Catecholamine neurotransmitter-receptor interaction and its effects on long-term memory processing. (in preparation.

15 E.L. Bennett, J.F. Flood, A. Orme, M.R. Rosenzweig and M. Jarvik, Minimum duration of protein synthesis needed to establish long-term memory. Fifth Intl. meeting of the International Society for Neurochemistry, Barcelona, September 2-6, 1975, Abstract 382.

16 V.E. Shashoua, RNA Metabolism in the Brain. In C.C. Pfeiffer and J.R. Smythies (Eds.), Intl. Review of Neurobiology, Vol. 16, Academic Press, New York, 1974.

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