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The Neurochemistry of Learning and Memory

by Adrian J. Dunn*

The neurochemical basis of memory has been approached experimentally in four different ways: the bioassay; the interventive approach; the interventive-correlative approach; and the correlative approach. These approaches are fundamentally similar to those used for the study of the chemistry of any behavior. Each of these approaches has serious limitations, and significant progress has only been made by combinations of more than one. I shall discuss each of these approaches in turn.

The problem of the neurochemical basis of memory has been reviewed extensively (*1–6*). It can be investigated experimentally by a variety of approaches.

The Bioassay

The bioassay assumes that when an animal is behaving in a particular way or has learned something, there is a chemical in its brain that can reproduce the same behavior or learning if it is transferred to a second animal. This concept does have some experimental support. For example, a peptide is secreted during sleep that has soporific properties (*7*). Thus, a brain extract of a sleeping animal may contain more of the peptide, and this extract may then induce sleep in a second animal. However, despite numerous claims to have "transferred memory" in this manner (*8*), the concept has not received general support. This is because the idea of "memory molecules" does not fit well into our present ideas of how the brain works. Moreover, the concept imposes severe restraints on models of memory and there are no good empirical reasons to do this. Furthermore, the data submitted in support of such a hypothesis are not unequivocal (*9*). There is little question that behavior can be affected by brain extracts, but it is not at all clear that memories or learning are transferred. Currently this approach is not favored by most researchers.

Interventive Approach

Essentially, the interventive approach is to study treatments that either impair or enhance the formation memory. Such treatments may be subdivided into those that have specific and those that have nonspecific chemical or cellular actions (Table 1), but it is not clear that this distinction is meaningful. Nonspecific treatments that disrupt memory include hypothermia, hypoxia, hypercapnia, and almost any agent that induces seizures, including electroconvulsive shock or drugs such as pentylenetetrazol (Metrazol) or fluorothyl (Indoklon). I would also include local electrical stimulation of brain structures as nonspecific. Most of these treatments induce a multiplicity of biochemical responses, generally commencing with alterations of energy metabolism and a depression of ATP levels. Consequently, these nonspecific treatments result in inhibitions of RNA and protein synthesis among other things, but these effects are at least partially

Table 1. Interventive agents in learning.

Inhibitory	Stimulatory
Nonspecific	
Hypothermia	Strychnine
Hypoxia	Pentylenetetrazol
Hypercapnia	Arousal (tailpinch, etc.)
Electroconvulsive shock (ECS)	ACTH and related peptides
Convulsant drugs (e.g., Metrazol)	Vasopressin
Local electrical stimulation	Glucocorticoids
Specific	
Protein synthesis inhibitors (puromycin, cycloheximide, anisomycin)	Amphetamine
Catecholaminergic blockers (diethyldithiocarbamate, reserpine, α-methyl-p-tyrosine)	

* Department of Neuroscience, University of Florida College of Medicine, Gainesville, Florida 32610.

secondary to the effects on energy metabolism (*10*). Thus despite initial hopes, it has proved very difficult to perform serious analyses of what neurochemical consequences of the treatments cause the amnesia.

Potentially, the study of stimulants of learning should be more useful, because they must act on a rate-limiting process. However, problems similar to those discussed above have confounded the analysis of nonspecific stimulants (*11*). Stimulants of learning include low doses of convulsant drugs such as strychnine or pentylenetetrazol, or such stimulants as a prior footshock or tailpinch.

There has been considerably more success with the specific interventive agents. Many biochemical processes such as DNA synthesis have been ruled out in this way (*12*). Others, such as RNA synthesis inhibitors, have produced equivocal results; about as many studies have found inhibitors of RNA synthesis to be amnestic as have not (*3, 4*). One problem is that most known RNA synthesis inhibitors (e.g., actinomycin D, α-amanitin) are extremely toxic and often lethal. The most important studies have concerned inhibitors of protein synthesis (*13*). Somewhat surprisingly, inhibition of protein synthesis does not seem to affect learning (acquisition), and very high inhibitions (90% or more) are needed to affect retention (*14*). Even then, amnesia can often be overcome by training to more stringent criteria, or by administering hormones such as glucorticoids (*15*), ACTH (*16*), vasopressin (*17*), or adrenergic stimulants such as amphetamine (*15*). True, many of these agents will enhance learning in the absence of protein inhibitors (*11, 16, 18*), but the learning is clearly remarkably robust.

Although no protein synthesis inhibitor has been shown not to be amnestic, many have argued that the amnestic effects of protein synthesis inhibitors are not due to the inhibition of protein synthesis. It seems unlikely that the amnesia is due to the production of seizures as observed with puromycin, since acetoxycycloheximide is amnestic but does not cause seizures (*19*). The lack of a glucocorticoid response by the adrenals (*20*) also appears not to be causative, since other adrenocortical blockers are not amnestic (*21, 22*). However, the amnesia might well be due to an elevation of the brain free amino acid concentration (*23*) or to interference with catecholamine metabolism (*24*).

Similar problems have been encountered with the adrenergic drugs. At the simplest level, it has been found that drugs that inhibit noradrenergic activity tend to impair memory and those that stimulate noradrenergic systems can enhance it (*11*), although there is not universal agreement on this. However, the most effective treatments are the least specific; thus, of the inhibitors, diethyldithiocarbamate (DDC) was the only inhibitor effective when administered after training (*25*).

The Interventive-Correlative Approach

To solve these problems, a combined interventive-correlative approach has been used. That is to say that, following intervention biochemical measures are made and correlations with the behavioral performance sought. Thus, a good correlation between the extent of protein synthesis inhibition and the degree of amnesia was observed following cycloheximide treatment in mice (*26*). However, an equally good correlation can be found between the degree of behavioral impairment and the synthesis of catecholamines (*27*). This emphasizes the weakness of the correlative approach; only correlations and not causes can be established. Nevertheless, correlative techniques can be useful. Gold (*28*) has shown an excellent correlation between cerebral norepinephrine content and performance altered by a variety of agents. On the other hand, Iuvone et al. (*29*) were able to rule out seizures as the causative agent in pentylenetetrazol-induced amnesia, although protein synthesis inhibition correlated well. Table 2 summarizes the ex-

Table 2. Interventive-correlative findings.

Agent—correlation	Researcher
ECS—protein synthesis	Cotman et al. (*30*), Dunn (*31*)
Pentylenetetrazol—seizures/protein synthesis	Iuvone et al. (*29*)
Puromycin/cycloheximide/acetoxycycloheximide—seizures/protein synthesis	Cohen and Barondes (*19*)
Puromycin—RNA synthesis	Shashoua (*32*)
Cycloheximide—protein synthesis	Quinton (*26*)
Anisomycin—protein synthesis	Flood et al. (*33*)
Cycloheximide—catecholamine synthesis	Bloom and Quinton (*27*)
Catecholaminergic Drugs—catecholamine metabolism	Haycock et al. (*25*)
ACTH/epinephrine—cerebral norepinephrine	Gold et al. (*28*)

periments that have been performed using this approach.

The Correlative Approach

A wealth of data has been gathered correlating neurochemical changes with learning, and this has been reviewed extensively (*2–5*). The chemicals with which correlations have been found are listed in Table 3 with appropriate references. Contrary to classical wisdom it now appears that cerebral metabolism is remarkably responsive to peripheral events, and it has not proved very difficult to find neurochemical changes consequent on learning. In general, metabolic changes have been found in most cellular structural components except DNA (*3, 4*).

An important problem of biochemical interpretation has arisen in many of these studies, because the synthesis of macromolecules has been estimated from the incorporation of radiolabeled tracers. While this technique is very sensitive and technically easy, it is in practice almost impossible to correct adequately for variation in the cellular uptake of the labeled precursor, and the potential variability in this uptake between cells. The ideal of a uniformly labeled "pool" of precursor in all cells for a reasonable time period is never attained. In one case an increased labeling of RNA, formerly interpreted to indicate an increase in RNA synthesis, has now been reinterpreted in terms of changes in the metabolic pool of precursor nucleotides (*47*).

Another important problem has been to devise adequate behavioral controls to distinguish the changes that are associated specifically with learning itself with those that are associated with other aspects of the learning process. Serious doubt has arisen as to whether it is even possible to do such a behavioral dissection (*4, 6, 51*); the components of a behavior are intimately integrated. It has been shown, for example, that the effects of the reception of a visual stimulus in primary visual system structures may depend on the significance of the stimulus to the animal (*52*). Ultimately, the biggest problem will be to show how the neurochemical changes relate to memory itself.

After a decade and a half of biochemistry of learning what do we know? We know that DNA is not directly involved, that protein synthesis probably is, and that catecholamines have important influences on learning although we do not understand them.

Table 3. Neurochemicals correlated with learning.

Chemical	Experimenters
RNA	Hydén (*34*)
	Glassman and Wilson (*35*)
	Shashoua (*32*)
	Bateson et al. (*36*)
	Matthies (*37*)
	Izquierdo (*38*)
Proteins	Beach et al. (*39*)
	Hydén and Lange (*40*)
	Bateson et al. (*36*)
	Rees et al. (*41*)
	Matthies (*37*)
	Shashoua (*42*)
Glycoproteins	Bogoch (*43*)
	Damstra-Entingh et al.(*44*)
Nuclear phosphoproteins	Machlus et al. (*45*)
Synaptic phosphoproteins	Perumal et al. (*46*)
Nucleotides	Entingh et al. (*47*)
Gangliosides	Dunn and Hogan (*48*)
Acetylcholine	Deusch (*49*)
Catecholamines	Seiden, Brown, and Lewy (*50*)

Hormones and Learning

It is possible that the catecholaminergic influences on learning reflect a physiological role of adrenal medullary epinephrine. It is interesting that other hormones of the pituitary-adrenal axis also influence learning. Surprisingly, not only do ACTH and glucocorticoids have important effects, but vasopressin (antidiuretic hormone) also does. Since all these hormones are normally secreted during stress this may emphasize the physiological role of stress in learning. The important effects of ACTH and ADH are the restoration of learning deficits caused by hypophysectomy (*53*), the enhancement of retention for weakly learned tasks (*16*) and the reversal of amnesia induced by a variety of agents (*16, 54, 55*).

Interestingly, ACTH itself has direct effects on cerebral protein synthesis (*56*) and on catecholamine metabolism (*57–59*). There is even evidence that ACTH mediates some of the neurochemical effects of footshock or other stressors (*60*). Thus, ACTH may directly mediate effects of learning. The complexity is that the hormones are not essential to the learning process, often merely facilitating it. The problem then is to distinguish these peripheral effects from the learning proper.

Neurochemical Thoughts on Environmental Toxicology

A question before us is whether neurochemical parameters should be monitored as indices of toxicity. Except in cases where there are prior reasons for suspecting direct nervous system activity, I would argue against using neurochemical techniques. In general, behavior is a far more sensitive index of nervous system function. Moreover, be-

havioral measures are sensitive to disruption of the complex interactions between the nervous system and the periphery which make up behavior.

If one were to study a neurochemical parameter, I would advise against studying neurotransmitters. Despite a very considerable amount of work, our understanding of the relationships between parameters of neurotransmitter metabolism and behavior is very shallow. A casual survey of the literature suggests that the metabolism of neurotransmitters, particularly the catecholamines and serotonin, is extremely sensitive to environmental chemical influences. We simply would not know how to interpret any changes detected in response to potential toxic agents.

To determine selective effects on specific brain structures, there is now an excellent technique. Glucose consumption is monitored by the uptake of the analog, 2-deoxyglucose (2DG). This compound is taken up into the brain just as glucose and is phosphorylated to 2-deoxyglucose-6-phosphate which is a stable metabolite and accumulates (*61*). The accumulation of 2DG phosphate is then an index of the glucose uptake. Since in the brain glucose uptake is related to functional activity (*61*), using radioactive 2DG one can devise a functional map of the brain by autoradiography. Such maps highlight structures that are hyperactive or hypoactive. Thus lesions of the visual system drastically reduce 2DG uptake in visual system structures, and conversely visual stimulation increases the uptake (*61*). Autoradiographically, this appears as a loss or an increase of silver grains respectively.

If I were to choose a neurochemical parameter for screening purposes, I would seriously consider protein synthesis. The reasons for this are that it is relatively easy to assay by using the incorporation of radioactive precursors, and is also rather sensitive to gross dysfunction of the nervous system. This is because it is a complex process requiring the integrity of a number of components, and is very dependent upon a continuous supply of energy in the form of ATP and GTP. When demand for these substances for other processes, such as ion pumps, is high, protein synthesis and other long-term anabolic reactions suffer first. A few examples of toxins that do effect cerebral protein synthesis are acrylamide (*62*) and methylmercury (*63*). However, I would advocate using this approach only after there was a suspicion of a neural toxicity.

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