A Molecular Basis for Learning and Memory

(transmitter transfer/presynaptic alterations/synaptomeric protein/
short-term memory and disulfide bonds/acetylcholine sensitivity)

EDWARD M. KOSOWER

Institute of Chemistry, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel; and Department of Chemistry,
State University of New York, Stony Brook, N.Y. 11790

Communicated by Andrew Streitwieser, July 13, 1972

ABSTRACT Three stages in memory (electrical, short-term, and long-term) are reviewed. The short computing time of organized neural systems favors synapses as loci for storage of memory. Transfer of neuronal excitation depends upon transfer of transmitter, involving the steps: vesicle attachment to presynaptic vesicle-release sites, contraction at dithiolate structures of these sites, exocytosis of transmitter, movement of transmitter across synaptic cleft, and reception at postsynaptic sites. Disulfide formation from dithiolates (calcium dithiolate salt) occurs during excitation and can represent a short-term alteration in properties of vesicle-release sites and, thus, short-term memory. Repair by one mechanism of the altered vesicle-release sites through reduction of the disulfide bond returns the system to its original state or, by a second mechanism, enlarges the presynaptic area covered by these sites. Such enlargement is a stable, permanent mode: long-term memory. Suitable concentrations of transmitter at postsynaptic receptor sites lead to mobilization of additional receptor sites through polymerization of monomeric receptor units. Postsynaptic expansion constitutes a metastable long-term storage, readily reconstituted under appropriate stimuli. Reverberations at the electrical stage of memory are suggested as a necessary link to the chemical stage of memory. These ideas constitute the elements of a molecular theory of learning and memory.

In spite of considerable effort (1-4), our understanding of learning and memory is still rather limited. Knowledge about neuronal activity (5, 6) and numerous behavioral experiments (1, 2) have provided the base for a scheme involving at least three stages of information storage. The first stage is electrical, with a time-scale between 2 and 500 msec. It is highly likely that the first stage is lengthened by reverberatory action (7), i.e., that repetitive firing of active neural networks occurs. The nature of the second stage (variously called labile, intermediate, or temporary) is unknown; the time-scale extends from perhaps 10 msec to a few hours (8, 9). The third stage, permanent memory, consolidates the information preserved through the first two stages; its activity becomes evident after minutes or even hours.

Synapses have long been thought of as the most suitable location for memory elements, regardless of their nature (10, 11). Now that we have a clear idea of the time required to generate a protein or an RNA molecule, we can be properly skeptical of proposals for DNA or RNA as ultimate, readily readable, storage sites for memory. Furthermore, data on

intraneuronal transport (not very fast to very slow) imply that neuronal modifications, which have to be communicated to a following cell, must be made close to the site at which communication takes place. Thus, the short computing times (tens of milliseconds) of small organized neural systems lead to a natural preference for synapses as the location at which information storage takes place.

The communication between most nerve cells is effected by *transmitter transfer*, an overall description within which we may include the following steps: (1) Approach of and accumulation of vesicles at presynaptic regions; (2) attachment of vesicles to presynaptic vesicle-release sites (VRS); (3) rearrangement of VRS (initiated by local contraction), leading to the possibility of transmitter release from the vesicle; (4) exocytosis, expulsion of vesicle contents into the synaptic cleft; (5) movement of transmitter across synaptic cleft; and (6) transmitter reception at postsynaptic membranes.

High concentrations of vesicles are found near synapses on the presynaptic side. A relationship of some kind between the nature of synapses and the concentration of vesicles would contribute to the efficiency with which information stored at synapses could be expressed.

Vesicles do become attached to the presynaptic membrane, probably at specific sites, which I shall call vesicle-release sites. Electron micrographs of electric cells from the elasmobranch *Torpedo* show vesicles with necks fused to the presynaptic membrane (12). A grid-like structure is seen in electron micrographs of presynaptic membranes (13, 14), and the spacing of the dense projections of that grid would allow vesicles to rest between the projections.

The VRS must be activated in some way to promote release of transmitter. According to Werman *et al.* (15), formation of glutathione disulfide (GSSG) within neurons of a neuromuscular junction promotes release of vesicles, with increases in the rate of appearance of miniature end plate potentials. Kosower and Werman (16) formulated a theory to explain this result, proposing that dithiol sites in presynaptic membranes are converted to disulfides. The local contraction resulting from the chemical change leads to release of transmitter. A parallel process involving calcium ion is written for normal release through neuron depolarization by an action potential except that four (17, 18) or five (19) calcium ions are necessary for normal release. We now believe that VRS activation initiates a sequence of events, which ends in exocytosis. The upper portion of Fig. 1 illus-

Abbreviations: VRS, vesicle-release sites; ACh, acetylcholine; GSH, glutathione; GSSG, glutathione disulfide; AChase, acetylcholinesterase; GABA, γ -aminobutyric acid.

trates these molecular events. The significance of the disulfides in the VRS is discussed below.

Exocytosis (a process by which material can be conveyed from the inside of cells to the outside through an opening) now appears to be the probable mechanism for introducing transmitter into the synaptic cleft. There are small holes, termed "synaptopores," found in presynaptic membranes through electron microscopy on freeze-etched preparation, that may represent the openings through which transmitter is released from vesicles (20). There are also several examples of moderate-sized molecules (e.g., horseradish peroxidase, diameter around 50 Å) being introduced into vesicles in a discharge region. Multiple steps in the appearance of acetylcholine (ACh) vesicles with respect to exhaustion and recovery in different forms containing horseradish peroxidase have been reported by Heuser (21); Holtzman *et al.*, (22) have shown that horseradish peroxidase is incorporated into glutamic acid [or γ -aminobutyric acid] vesicles, and Douglas and coworkers earlier described similar phenomena for the neurosecretory terminals of posterior pituitary glands (23, 24). Release processes for neurohormones and neurotransmitters are thought to be similar (25, 26).

Although electron microscopy has shown thread-like structures in the synaptic cleft between the presynaptic side and the postsynaptic side, there is no evidence of any special process controlling the movement of transmitter across the synaptic cleft.

Transmitter reception is achieved through receptor molecules. For ACh, the receptor molecule has been identified with α -bungarotoxin binding activity. Since the latter is separable from acetylcholinesterase (AChase) activity, the two functions are considered to belong to different molecules (27-29), although some opinion in favor of AChase as the receptor still exists (30a). Receptors that are not accompanied by AChase have been reported (30b).

Whatever the (unknown) mechanism of increased conductance change is at the postsynaptic membrane, a relationship between the degree of conductance change and the number of receptor sites can be expected (31). Thus, the dynamics of the distribution of receptor molecules are important. After denervation, ACh receptors do not remain localized either at muscle-fiber end plates or at nerve-cell synapses, but spread throughout the membrane of the fiber or cell (32-34). Conversely, delocalized ACh sensitivity becomes focused at the synapse upon reinnervation (33, 35).

A simple scheme can account for the gross features of this interesting phenomenon. The ACh receptor is composed of subunits (27), perhaps two to four, judging from the molecular weight and the behavior on treatment with detergent [AChase is a dimer (36)]. Neurophysiological evidence suggests that there are two types of ACh receptors, those at the synapse being fast and those that are delocalized being slow, the kinetic terms referring to the nature of the response to added ACh (37). Let us postulate that the fast receptor is a polymer (dimer, trimer, or tetramer) of the slow receptor, a monomer. ACh (or another as yet unidentified substance

at the place where it was formed. In the absence of sufficient ACh, the polymer dissociates to receptor monomer. The monomer is mobile, either within the membrane or within the cytoplasm, and spreads. Presumably, there is also synthesis of new receptor monomer at a low rate [observed in embryological tissue after inhibition of old receptor with α -bungarotoxin (38)]. It is interesting that ACh stimulates synthesis of a phosphatidyl inositol, which has a high affinity for isolated ACh receptor phospholipid (39), without necessarily being specific for any particular membrane within the cell (40, 41). The whole question of the dynamics of the formation and distribution of receptor molecules is currently the object of intense investigation in the laboratory of Hartzell and Fambrough, Miledi, and others.

The simple hypothesis of receptor polymer slowly interconverting with monomer at a rate influenced by the concentration of ACh (or another substance) accounts for delocalization of sensitivity after denervation and for relocation of sensitivity after reinnervation (33), and is a straightforward way of explaining the formation of synapses. The relationship to memory will be discussed shortly. The findings that three molecules of the transmitter GABA (γ -aminobutyric acid) (43) are required for conductance changes at one receptor site and three molecules of ACh are needed at a molluscan receptor site (44) may be relevant to the concept of a polymeric receptor. The concept of molecular mobility within the membrane is strongly reinforced by immunological experiments and expressed clearly in the fluid mosaic theory of membranes developed by Singer (42).

With the foregoing discussion of normal transmitter transfer as background (including the new theory of receptor behavior), we may now consider the nature of alterations that increase the effectiveness of the transfer of the signal through the synapse. In Fig. 1, formation of a dithiolate salt with calcium ion is shown as concomitant with normal depolarization (16). The neuron contains a limited amount of glutathione disulfide (GSSG), which can react with the dianion to produce a disulfide and GSH. The calcium-independent production of miniature endplate potentials has been explained as the final consequence of the reaction of the undissociated dithiol site with the intracellular GSSG (16). The reaction rate of the dithiolate with GSSG should be considerably faster than that of the dithiol. The rate constants for such interchange reactions have not been studied to the extent their significance in chemistry and biochemistry deserves, but a probable value for the rate constant of the thiol anion-disulfide interchange reaction (45) is about $1 \text{ M}^{-1} \text{ sec}^{-1}$. The interchange reaction must go in two steps (Eq. 1).

where P = protein. The fraction of the dithiolate sites reacting per msec is given by Eq. 2, taking the equilibrium GSSG concentration as $3 \mu\text{M}$ (46).

$$\frac{\text{Fraction}}{\text{msec}} = \frac{\text{Rate constant (M}^{-1} \text{ sec}^{-1}) \times \text{Dithiolate sites (M)} \times \text{GSSG (M)} \times 10^{-3} \text{ (sec/msec)}}{\text{Dithiolate sites (M)}} \quad [2]$$

released from the presynaptic terminal) stimulates conversion of the monomer into polymer by a relatively slow process. The polymer is immobile in the membrane and remains

The fraction of dithiolate sites reacting with GSSG during a single depolarization (time taken as 1 msec) is about 3×10^{-9} . The total number of disulfide bonds formed per initial

FIG. 1. A schematic illustration of some aspects of important processes at a neural synapse, including depolarization, acetylcholine (ACh) release, disulfide-bond formation (miniature endplate potential production, labile memory), and synaptic site expansion (permanent memory). Protein is represented by squares; only two pairs of protein molecules are illustrated, even though the fact that 4-5 Ca^{++} ions are required for each quantum of transmitter released implies that 4-5 pairs of protein thiols would function in each release step.

The upper left portion of the diagram shows the "normal" dithiol state of the vesicle-release site (VRS) of the presynaptic membrane. During depolarization, Ca^{++} interacts with the dithiol to form the calcium dithiolate. The sulfur-sulfur distance in the dithiolate is less than that in the dithiol, and formation of the Ca^{++} salt causes a contraction of the membrane at the dithiol site (upper right). The vesicles interact with the protein at a VRS, and the contraction at the dithiol site should produce, in this model, a strain in the vesicle initiating the sequence leading to exocytosis and release of transmitter. The emptied vesicle is lost from the VRS in some unspecified way.

Glutathione disulfide (GSSG) oxidizes a small part of the dithiolate to a disulfide (upper right, second row). Miniature endplate potentials may be produced in a Ca^{++} -independent GSSG oxidation (ref. 16) to disulfide as shown by the arrow from the original dithiol state to the disulfide at a rate of about 1/sec. The estimate for disulfide bond formation for the labile store corresponds to about 15/sec. The sulfur-sulfur distance in the disulfide would be even shorter than that in the dithiolate, producing greater contraction at the dithiol site. Interaction of vesicles at the VRS of the disulfide form could lead to strained vesicles, and thus eventually to the release of acetylcholine. The disulfide form is a labile store and can, by one VRS "repair" mechanism, be returned by reaction with glutathione (GSH) to the normal, dithiol state. The lifetime of the labile store is affected by the efficiency of the GSH reaction.

These transformations should operate for all transmitters. The upper forms illustrate the theory of normal transmitter release, and an explanation for the dramatic increase in miniature endplate potentials after treatment of a myoneural junction with

input into a given neuron can be estimated as in Eq. 3. Reverberations (7) are cyclic pathways that lead to the recurrence of action potentials in a neuron. The electrical stage of neuron actions would otherwise be too short for conversion of the activity into a more stable (chemical) form.

Disulfide bonds/neurone = $L =$

$$3 \times 10^{-9} \times 5 \times 10^2 \times \frac{2}{\text{msec/reverberation}} \times \frac{10^2}{\text{No. reverberations}} \times \frac{10^4}{\text{No. synapses/neurone}} = 3 \quad [3]$$

Initiation of the input into a neuron results eventually in formation of a small number of disulfide bonds (Eq. 1), and one might expect similar numbers of disulfide bonds to be produced in all of the other neurons involved in a neuronal chain response arising from the initial event.

I believe it reasonable to identify the disulfide bonds as the labile form of information storage, that which is expressed as short-term memory. The disulfide bonds act as information stores by making the VRS more effective as release sites. In the absence of other reactions, interchange with GSH would return the disulfide to the dithiol form, and thus erase the labile store. Reviewing the steps in transmitter transfer, one might well choose the VRS as a logical place for a temporary information store. Our previous finding that disulfide bond formation (in our view, at the VRS) promoted transmitter transfer makes the disulfide bond a prime candidate as the molecular basis of short-term memory. Based on the rate constants for thiol-disulfide interchange reactions and the concentrations of the reactants, the half-life for short-term memory can be between 10 sec and 30 hr.

The disulfide bond represents a distortion in the equilibrium form of the VRS. We may postulate that cells possess two repair mechanisms for the distortion. One, simple reduction, returns the VRS to its original dithiol state. The second depends upon the presence of a cytoplasmic component, which we shall call *synaptomeric protein*. The synaptomeric protein should be a dimer of the thiol-bearing unit component of the VRS. Insertion of the synaptomeric protein into the distorted VRS would lead eventually to an expanded VRS, or to the creation of new VRS, and thus to the expansion of the region of presynaptic transmitter release. An alternative formulation might involve insertion of neurofibrils next to a dithiol-bearing site, which might be a "control protein." The participation of neurofibrils in transmitter release has been adduced through experiments with cytochalasin-B (47). An excellent model for the dithiol carrier is the Ca^{++} -responsive protein investigated by Fuchs (48). Calcium-dependent release of histamine from mast cells is strongly inhibited by cytochalasins A and B (49) (see Fig. 1). An increase in synaptic size would increase synaptic effectiveness, and would represent a stable information store, i.e., long-term memory. Greater synaptic size does correspond to greater release of transmitter as measured by the size of the excitatory postsynaptic potential in endplates of different sizes (50). The same

the thiol-oxidizing agent, diamide (refs. 17 and 18). The disulfide form is recognized by a second VRS "repair" system which incorporates a synaptomeric protein; which, after rearrangement and reduction with GSH (lower center $\rightarrow$ lower left); produces an expanded synapse, containing an additional dithiol site. Expansion of the synapse should lead to more effective release of transmitter, and such expansion represents a permanent store for information. The postsynaptic consequences of an expanded release region are discussed in the text.

mechanisms might well operate for all transmitters, with direct evidence for this from the observation of diamide-induced increases in rates of miniature endplate potentials for preparations of crayfish dactyls in which the transmitter is almost certainly glutamic acid (R. Hoy and E. M. Kosower, unpublished results).

The efficiency with which the labile store is converted into the permanent store may well be less than one and may also depend upon the physiological state of the system, the availability of synaptomeric protein, etc. Assuming that synaptomeric protein has a molecular weight of 10^4 , the total amount of protein incorporated in the permanent memory store of the brain over a lifetime of 10^9 sec would be only a few grams, allowing 0.1 sec for a trace expressed through a chain of 10^9 neurons.

The discussion given previously concerning the two types of ACh receptor allows a simple way of explaining how the postsynaptic receptor region can respond to an increase in the quantity of transmitter released by the presynaptic side of a synapse. An increase in the average quantity of transmitter (or other activating substance released from the VRS) arriving at the postsynaptic side over an extended period of time (minutes to days) should lead to an augmentation in the number of receptor sites and an expansion of the postsynaptic receptor region, through conversion of receptor monomers into receptor polymers and perhaps some increase in the synthesis of monomers. [None of these ideas bears upon the chemical basis for depolarization induced by acquisition of transmitter by receptor. There is evidence that disulfide links are near the receptor sites (51, 52).] The possibility for partial degradation of the postsynaptic receptor region through depolymerization of receptors allows for apparent inactivation of synaptic pathways. Reactivation of previously learned behavior could occur rather easily, since the permanent store is still present in the presynaptic membranes of the pathways involved in the learning. The expansion of synapses in response to learning has been reported (53-56).

The molecular basis for short-term and long-term memories is set forth in this article. Much of our information about transmitter transfer comes from work on ACh synapses, and it is not yet known whether the details are applicable to all other types of synapses. The present theory provides a basis for learning at all synapses (indeed, at all membranes carrying the appropriate molecular apparatus for specifying release of some compound) and a natural way for explaining how the complexity of the neural system reinforces the ability of the system to learn and respond. The use of the stored information (i.e., the computing done on the stored information) is a subject that is clearly beyond the scope of this theory. However, the mechanism of storage would be consistent with holographic operation (57, 58) of the brain, and fits in neatly with diffuse storage of information (59, 60). The fate of transmitter vesicles, which remain distinct from the synaptic membrane in protein composition, is not considered, since there is no reason at present to think that they are directly involved in information storage (61-63). Another hypothesis for short-term memory, basically a mechanism for extending the electrical stage of memory, has been proposed by Bass and Moore (64).

New staining techniques for synapses have revealed an increase in the size and number of presynaptic dense projections (65) parallel to the development of the rat brain. This

change is consistent with the role proposed for these structures in the VRS.

Certain aspects of the presently proposed theory are open to experimental test, most notably the nature of the proposed dithiolate sites. It is hoped that such research can be undertaken in the near future.

I am grateful to my wife, Prof. N. S. Kosower, and my colleague, Prof. R. Werman, of the Institute of Zoology, Hebrew University, Jerusalem, for many useful discussions. The work was supported by the National Institutes of Health and the Army Research Office (Durham).

- Byrne, W. L. (1970) *Molecular Approaches to Learning and Memory* (Academic Press, New York).
- Unger G. (ed.) (1970) *Molecular Mechanisms in Memory and Learning* (Plenum Press, New York).
- Roberts, E. & Matthysse, S. (1970) *Annu. Rev. Biochem.* **39**, 777-820.
- Rose, S. P. R. (1969) *FEBS Lett.* **5**, 305-312.
- Hodgkin, A. L. (1967) *The Conduction of the Nervous Impulse* (Liverpool Univ. Press, Liverpool).
- Eccles, J. C. (1964) *The Physiology of Synapses* (Springer-Verlag, Berlin).
- Eccles, J. C. (1964) in *The Physiology of Synapses* (Springer-Verlag, Berlin), p. 260.
- John, E. R. (1970) in *Molecular Approaches to Learning and Memory* (Academic Press, New York), p. 335.
- Halstead, W. C. & Rucher, W. B. (1970) in *Molecular Approaches to Learning and Memory* (Academic Press, New York), p. 1; Fishbein, W., McGaugh, J. L. & Swarz, J. R. (1971) *Science* **172**, 80-82.
- Deutsch, J. A. (1971) *Science* **174**, 788-794.
- Hebb, D. O. (1949) *The Organization of Behavior* (John Wiley and Sons, New York).
- Nickel, E. & Potter, L. T. (1971) *Phil. Trans. Roy. Soc. London Ser. B* **261**, 383-391.
- Gray, E. G. (1963) *J. Anat.* **97**, 101-114.
- Akert, K. & Pfenninger, K. (1969) in *Cellular Dynamics of the Neuron*, ed. Barondes, S. H. (Academic Press, New York), p. 245.
- Werman, R., Carlen, P. L., Kushnir, M. & Kosower, E. M. (1971) *Nature New Biol.* **233**, 120-121.
- Kosower, E. M. & Werman, R. (1971) *Nature New Biol.* **233**, 121-122.
- Dodge, F. A. & Rahamimoff, R. (1967) *J. Physiol.* **193**, 419-429.
- Rahamimoff, R. (1970) in *Calcium and Cellular Function*, ed. Cuthbert, A. W. (McMillan, London), p. 131.
- Werman, R. (1971) *Comp. Gen. Pharmacol.* **2**, 129-138.
- Pfenninger, K., Akert, K., Moor, H. & Sandri, C. (1971) *Phil. Trans. Roy. Soc. London Ser. B* **261**, 387; *Experientia* **27**, 1117.
- Heuser, J. (1971) *Soc. Neurosci., First Annu. Mtng.*, Washington, D.C., Oct. 27-30, Abstract 13.16, Abstracts, p. 112.
- Holtzman, E., Freeman, A. R. & Kashner, L. A. (1971) *Science* **173**, 733-736.
- Douglas, W. W., Nagasawa, J. & Schulz, R. A. (1971) *Nature* **232**, 340-341.
- Nagasawa, J., Douglas, W. W. & Schulz, R. A. (1971) *Nature* **232**, 341-342.
- Douglas, W. W. (1968) *Brit. J. Pharmacol.* **34**, 451-462.
- Dreifuss, J. J., Grau, J. D. & Nordman, J. J. (1971) *Experientia* **27**, 1110.
- Miledi, R., Molinoff, P. & Potter, L. T. (1971) *Nature* **229**, 554-557.
- de Robertis, E. (1971) *Science* **171**, 963-968.
- Changeux, J. P., Kasai, M. & Lee, C.-Y. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1241-1247.
- (a) Belleau, B. & DiTullio, V. (1971) *Can. J. Biochem.* **49**, 1131-1133; (b) Barnard, E. A., Wieckowski, J. & Chiu, T. H. (1971) *Nature*, **234**, 207-209.
- Werman, R. (1969) *Comp. Biochem. Physiol.* **30**, 997-1017.
- Kuffler, S. (1971) *Lecture at Soc. Neurosci., First Annu. Mtng.*, Washington, D.C., Oct. 27-30.
- Miledi, R. & Potter, L. T. (1971) *Nature* **233**, 599-603.

34. Lømo, T. & Rosenthal, J. (1971) *J. Physiol.* **216**, 52P.
35. Kano, M. & Shimadu, Y. (1971) *J. Cell. Physiol.* **78**, 233-244.
36. Leuzinger, W. (1971) *Biochem. J.* **123**, 139-141.
37. Feltz, A. & Mallart, A. (1971) *J. Physiol.* **218**, 85-100, 101-116.
38. Hartzell, H. C. & Fambrough, D. M. (1971) *Soc. Neurosci., First Annu. Mtng.*, Washington, D.C., Oct. 27-30, Abstract 23.1, Abstracts p. 161.
39. Lunt, G. G., Canessa, O. M. & de Robertis, E. (1971) *Nature New Biol.* **230**, 187-190.
40. Miller, E. K. & Dawson, R. M. C. (1972) *Biochem. J.* **126**, 805-822, 823-834.
41. Lapetina, E. G. & Michell, R. H. (1972) *Biochem. J.* **126**, 1141-1149.
42. Singer, S. J. & Nicolson, G. L. (1972) *Science* **175**, 720-731.
43. Werman, R., Brookes, N. & Blank, M. (1971) *Experientia* **27**, 1120.
44. Ziskind, L. & Werman, R. (1972) *Isr. J. Med. Sci.*, in press.
45. Fava, A., Illiceto, A. & Carnera, E., (1957) *J. Amer. Chem. Soc.* **79**, 833-838.
46. Prins, H. K. & Loos, J. A. (1969) in *Biochemical Methods in Red Cell Genetics*, ed. Yunis, J. J. (Academic Press, New York), pp. 115-142.
47. Thoa, N. G., Worten, G. F., Axelrod, J. & Kopin, I. J. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 520-523; Zigmond, S. H. & Hirsch, J. G. (1972) *Exp. Cell Res.* **73**, 383-393.
48. Fuchs, F. (1971) *Biochim. Biophys. Acta* **226**, 453-458; Greaser, M. L., Gergely, J., Han, M. H. & Benson, E. S. (1972) *Biochem. Biophys. Res. Commun.* **48**, 358-361.
49. Orr, T. S. C., Hall, D. E. & Allison, A. C. (1972) *Nature* **236**, 350-351.
50. Kuno, M. (1971) *Experientia* **27**, 1115.
51. Karlin, A. & Bartels E., (1966) *Biochem. Biophys. Acta* **126**, 525-535.
52. Bartels, E., Deal, W., Karlin, A. & Mautner, H. G. (1970) *Biochem. Biophys. Acta* **203**, 568-571.
53. Greenough, W. T., West, R. & Fleischman, T. B. (1971) *Soc. Neurosci., First Annu. Mtng.*, Washington, D.C., Oct. 27-30, Abstract 13.13, Abstracts p. 111.
54. Cragg, B. G. (1967) *Nature* **215**, 251-253.
55. Illis, L. S. (1969) *Nature* **223**, 76-77.
56. Schapiro, S. & Vukovich, K. R. (1970) *Science* **167**, 292-294.
57. Longuet-Higgins, H. C. (1968) *Nature* **217**, 104-106.
58. Gabor, D. (1969) *IBM J. Res. Develop.* **13**, 156-163.
59. Lashley, K. D. (1929) *Brain Mechanisms and Intelligence* (University of Chicago Press, Chicago).
60. Penfield, W. & Roberts, L. (1966) *Speech and Brain Mechanism*, (Atheneum, New York).
61. Chalazonitis, W. (1969) in *Cellular Dynamics of the Neuron*, ed. Barondes, S. H. (Academic Press, New York), p. 299.
62. Clark, A. W., Mauro, A., Longenecker, H. E. & Hurlbut, W. P. (1970) *Nature* **225**, 703-706.
63. Mahler, H. R. & Cotman, C. W. (1970) in *Protein Metabolism in the Nervous System*, ed. Latjtha, A. (Plenum Press, New York), pp. 356-365.
64. Bass, L. & Moore, W. J. (1971) *Brain Res.* **33**, 451-472.
65. Bloom, F. E. (1972) in *Structure and Function of Synapses*, eds. Pappas, G. D. & Purpura, D. P. (Raven Press, New York), pp. 101-120.