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THEORETICAL NOTE

NEGATIVE AS WELL AS POSITIVE SYNAPTIC CHANGES MAY STORE MEMORY[1]

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Hypotheses concerning synaptic changes that might store memory have been appearing ever since E. Tanzi proposed the first one in 1893. Most of them have been cast in terms of positive changes with learning, that is, either growth of new synaptic junctions or changes in size or effectiveness of existing ones. In the last decade, a few theorists have proposed that negative changes—decreases in the number or effectiveness of contacts—could store memory. Little data have been available to test either hypothesis. We propose that structural synaptic changes underlying long-term memory need not be of a single form but may include increase in number of synapses, increase in size, decrease in number, and decrease in size. Recent electron microscopic data are reviewed to show that each of these four types of change has been observed as a consequence of differential experience.

Shortly after the neuron doctrine was enunciated, the psychiatrist Eugenio Tanzi (1893) surveyed recent advances in neurohistology and hypothesized that learning produces anatomical changes in interneuronal contacts (which were to be named "synapses" by Foster & Sherrington, 1897), and that these changes serve as a mechanism of memory. Tanzi was confident that investigators would soon be able to test by direct inspection the changes that he hypothesized to occur with age and training. Now, almost 80 years later, researchers are just beginning to be able to perform such tests (Bloom, 1970; Kandel & Spencer, 1968). While some have become pessimistic ("No major breakthrough in the synaptic connectivity hypothesis of memory has been achieved, despite extensive work during the past 10 years . . . [Baumgarten, 1970, p. 264]"), others decry that more has not been done to link various cerebral changes in learning to synaptic mechanisms (Anonymous, 1971).

Most theorists during the intervening period, whether or not they knew of Tanzi, have supposed with him that learning involves either the formation of new interneuronal connections or the strengthening of existing ones; few have thought of learning in terms of elimination or weakening of synapses. Foster and Sherrington (1897, Pt. III), also related this structure to learning:

> Shut off from all opportunity of reproducing itself and adding to its number by mitosis or otherwise, the nerve cell directs its pent-up energy towards amplifying its connections with its fellows, in response to the events which stir it up. Hence, it is capable of an education unknown to other tissues [p. 1117].

Such concepts were taken up by a number of theorists during the present century and were brought to prominence in the late 1940s by Konorski (1948) and Hebb (1949). Hebb assumed explicitly that "the changed facilitation that constitutes learning" is based on "the growth of synaptic knobs [p. 65]." Formulations of some authors have not made clear whether growth in the nervous system with learning involves formation of new synapses or changes in size or other dimensions of existing synapses. Other workers have discriminated between these possibilities, as when Eccles (1965) stated his belief that learning and memory storage involve "growth just of bigger and better synapses that are already there, not growth of new connections."

The elimination or weakening of synaptic connections as a mechanism of learning and memory storage has been considered much less frequently than have positive changes. Concerning embryological development of the nerv-

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ous system, Ramón y Cajal (cited by Jacobson, 1970) did propose that there is a selection among somewhat random original connections "due to atrophy of certain collaterals and the progressive disappearance of disconnected or useless neurones [p. 125]." A large decrease in spinal neurons during embryology has been demonstrated in later work (Jacobson, 1970). While it has been suggested that "synaptic atrophy" (Konorski, 1948) or "synaptic decay" (Hebb, 1949) may occur with disuse even in the adult, this was proposed as a correlate of forgetting, the converse of acquisition of memory. Even Harlow (1959; Harlow & Hicks, 1957), who has suggested that learning is accomplished largely through the reduction of errors, conceived of this as occurring through the establishment of inhibitory processes rather than through elimination of excitatory ones (see Harlow & Hicks, 1957, pp. 108–109). Perhaps the prevalence of notions of positive synaptic changes with learning stems from the concept of "consolidation of memory traces" formulated in 1900 by Müller and Pilzecker. The first to hypothesize negative changes to account for learning may have been Thesleff (1962). He suggested that, in analogy to facts established for muscle cells, the chemoreceptor area of a neuron might become restricted to sites activated frequently, and that other synaptic connections would thereby be rendered inoperative. Young (1964, p. 285) suggested that learning occurs through the reduction of unused redundant connections, but he did not elaborate this hypothesis. Recently, Dawkins (1971) proposed that selective death of neurons may represent a mechanism of memory storage. These suggestions have remained speculative because, until very recently, there were no data against which to test them.

We hypothesize that structural synaptic changes underlying learning and memory storage need not be of a single form, but rather may take one or more of these four forms: (*a*) increase in number of synapses, (*b*) increase in size of contact areas, (*c*) decrease in number, and (*d*) decrease in size of contact. It would be expected that with any particular instance of learning, such changes would be limited to particular brain regions and to particular types of contacts; in fact, different changes may occur simultaneously in different sets of synapses in the same brain.

A few examples from recent studies will serve to demonstrate that changes of each of these sorts are beginning to be reported and that more than one type of change may occur within the same region of the brain. Cragg (1967, 1968, 1969) has made several reports on measures of axon terminals in rats kept permanently in the dark or removed for various periods of light exposure. In 1967, he reported findings suggestive of all four types of synaptic changes: When dark-reared rats were exposed to daylight, axon terminals in the upper half of the occipital cortex became larger and less numerous, whereas in the lower half they became smaller and more numerous. His 1969 report states that axon terminals in the lateral geniculate nucleus are 15% greater in diameter in dark-raised rats, but 34% less in density per unit of tissue volume. When he measured length of synaptic contacts rather than axon terminals, the contacts "did not show any regular difference between the light- and dark-exposed rats of successive pairs [p. 61]." Fifková (1970) measured axodendritic synapses in Layers II–IV of occipital cortex of rats after six weeks of unilateral lid suture; synapses in the hemisphere contralateral to the sutured eye were 20% fewer in number but 7.5% larger in cross-section than in the control hemisphere.

We have been using quite a different procedure, including enriched or impoverished experience, which induces a variety of chemical and anatomical changes in the rodent brain (Rosenzweig, Bennett, & Diamond, in press), and we have recently found that this produces clear synaptic changes (Møllgaard, Diamond, Bennett, Rosenzweig, & Lindner, 1971). In our procedure, the main factor inducing cerebral effects is experience with a variety of stimulus objects in the enriched condition; the impoverished-experience animals are isolated but are not deprived of visual stimulation. The experimental conditions present opportunities for differential experience over several weeks. Control experiments have shown that the cerebral effects previously measured are not attributable to stress, locomotion, altered rates of maturation, endocrine involvement, handling, or to any brief irrelevant stimulation. Visual experience is not required to produce these effects, since they occur with groups run completely in the dark and also in blinded rats (Rosenzweig, Bennett, Diamond, Wu, Slagle, & Saffran, 1969). The effects are not closely age dependent, since some at least have been induced in 300-day-old rats (Riege, 1971).

Significant differences in size and number of synaptic junctions were found between littermate rats assigned at weaning (25 days of age) to enriched or impoverished environments and kept there for 30 days. The synapses measured were asymmetrical axodendritic junc-

tions in the neuropil of Layer III of the occipital cortex. Rats from the enriched condition showed, in comparison to littermates from the impoverished condition, synapses that averaged 48% greater in length but that were only 67% as numerous. The enriched rats had more large synapses as well as fewer small synapses than did impoverished rats, so the enriched condition size distribution could not have been derived simply by loss of small synapses from the impoverished condition distribution. The total area of synapses in the enriched group, taking both size and number of contacts into account, was 40% greater than in the impoverished group. It should be noted that the inverse effects between size and number of synapses in this study is similar to the relation between synaptic size and number reported by Cragg and Fifková.

In another study,[3] evidence of a positive enriched-impoverished difference in a number of dendritic spines (and thus presumably of synaptic contacts) on pyramidal cells in the occipital cortex of rats was obtained. Counts of spines were made on enriched-impoverished littermate pairs from four experiments with behavioral conditions similar to those of the preceding paragraph. On basal dendrites there was a 9.7% enriched-impoverished difference ($p < .01$) in the number of spines per unit of length along the dendrite. The counts of spines by light microscopy on cells stained by the Golgi-Cox method cannot be assigned to cortical layers for direct comparison with the preceding electron microscopic results. It is possible that electron microscopic counts in other cortical layers will reveal an increase in the number of junctions, or the relation between electron microscopic synaptic counts and Golgi-Cox spine counts may be more complex; only further research can answer this question. When enriched-experience rats are compared with impoverished-experience littermates, they appear to have a lesser number but greater size in one population of synapses and to have a greater number in another population. Experimental evidence from several laboratories is thus consistent with the old hypotheses that stimulation and learning may induce increases in synaptic size and number, and it also suggests that negative as well as positive changes occur.

Our measures of synapses and of dendritic spines were made in occipital cortex because that is where we have found the largest changes in other anatomical and biochemical measures after several weeks of differential experience (Rosenzweig et al., 1969). Experiments with brief training by other investigators have shown chemical changes located especially in the hypothalamus (Beach, Emmens, Kimble, & Lickey, 1969; Kahan, Kriginan, Wilson, & Glassman, 1970). Some groups (e.g., Halas, Beardsley, & Sandlie, 1970) are recording the activity of single neurons at various levels of the brain during conditioning. Results indicate that the earliest changes during training occur in the reticular formation and in basal reward areas, with changes occurring eventually in the cerebral cortex. Thus, the chemical and electrophysiological observations both suggest a shift of locus from short-term effects involving motivational or reinforcing areas in basal regions of the brain to long-term storage, especially in the cerebral cortex. The chemical and anatomical changes that we find at the cortex may well represent the cumulation of innumerable small effects of learning during the prolonged differential experience.

It should be clear that we are not hypothesizing that the four types of synaptic changes are the only mechanisms for storing memory. In particular, some memories are formed very rapidly, and at least the initial type of storage is unlikely to be a structural change of synapses (although a substantial increase in the proportion of large axon terminals was found after 65 minutes of stimulation in the spinal cord of cats; Illis, 1969). Long-term memory storage, however, could well involve the positive and negative synaptic changes described here.[4]

[3] A. Globus, M. R. Rosenzweig, E. L. Bennett, and M. C. Diamond. Effects of differential environment on dendritic spine counts. In preparation.

[4] After completion of this paper we found somewhat related hypotheses in Albus (1971). Albus holds that in order for the learning process to be stable in cerebellar cells, and possibly elsewhere in the nervous system, "pattern storage must be accomplished principally by weakening synaptic weights rather than by strengthening them [p. 25]." He notes that "The argument synaptic weights are weakened by learning rather than strengthened is counterintuitive and contrary to most, if not all, theories of synaptic learning that have appeared in the literature [p. 50]," and he therefore examines it in detail. Later, he holds that synaptic facilitation is a mechanism of learning in stellate cells and elsewhere (p. 52). It should be noted that the physiological or anatomical mechanisms by which "synaptic weights" are expressed or altered are never defined. Nevertheless, it is interesting that Albus, arguing from quite different material, suggests as we do that both negative nad positive synaptic changes may store memory.

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