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Reactive Synaptogenesis in Aging and Alzheimer's Disease: Lessons Learned in the Cotman Laboratory*

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Early experiments resulting in partial deafferentation of the rodent hippocampus demonstrated a robust reactive plasticity response that includes the replacement of lost synaptic contacts. Similar experiments carried out in the hippocampus of aged animals produced an alteration in the temporal sequence of the reactive plasticity response and a slowing of synaptic replacement. In Alzheimer's disease, one observes a marked reduction in the number of synaptic contacts in important association areas of the cortex and hippocampus. This reduction may be the result of an altered reactive plasticity response.

KEY WORDS: Synaptogenesis; hyppocampus; Alzheimer's Disease.

As a young graduate student who had previously spent several years in the functional recovery laboratory of Stan Finger at Washington University in St. Louis, I remember very well Geoffry Raisman's paper (1) reporting replacement of lost synaptic contacts in the denervated septal region of the rat. What distinguished this study from previous claims of CNS plasticity was the fact that it occurred in the adult CNS in response to a lesion. Restructuring of synaptic connectivity and replacement of lost contacts could help explain, in part, the behavioral recovery we had studied in the Finger laboratory. Raisman followed this initial report with a more complete study (2), and the neuroscience community was buzzing with the idea that such dynamic synaptic changes could occur in the adult nervous system. Shortly after Raisman's report, the research team of Carl Cotman and Gary Lynch went to work in this area and published several very notable papers. In 1972, they published the first of many papers in *Brain Research* (3) demonstrating cholinergic plasticity in the hippocampus. At the time, few would predict the impressive research endeavors this group would undertake to probe the dynamics of this injury-induced response. By 1973, when Cotman published the first ultrastructural study of synaptic rearrangement in the hippocampus (4), his laboratory had already initiated a combined morphological, neurochemical, and neurophysiological assault on the area. When I arrived in Carl's laboratory late in the summer of 1974 as a postdoctoral fellow, his group was already established as one of the pioneers in the young field of lesion-induced plasticity. The atmosphere in the Department of Psychobiology at the University of California at Irvine was exciting and the dream of any young neuroscientist. Numerous new discoveries were being made weekly, and laboratory meetings created a forum for the discussion of many innovative and diverse ideas. Gary Lynch's laboratory was on the same floor as Carls's and only a short walk down the corridor. The two laboratories interacted routinely and on many levels, often sharing authorship

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on important manuscripts. In 1976, Carl and Gary coined the term "reactive synaptogenesis" to describe the injury-induced replacement of synapses (5). The intent was to differentiate the synapse formation that occurs following injury from that occurring during normal development. Both groups recognized that axon sprouting was an extremely important component of lesion-induced hippocampal reorganization and the process they were studying was different from regeneration.

One of the early goals in Carl's laboratory was to try and understand the maximum extent of axon sprouting/reactive synaptogenesis cascade and what conditions possibly control the rate and magnitude of the response. Every student in the Cotman laboratory was encouraged to think on a broad scale and to probe numerous aspects of the plasticity response. Some of the work I was pursuing at the time was morphological in nature and directed at controlling axonal sprouting in the hippocampal dentate gyrus. Victor Nadler, another postdoctoral fellow in Carl's laboratory, demonstrated extremely robust plasticity in the hippocampus if the injury was inflicted at a very early stage of postnatal development. This plasticity was significantly greater than we had observed in the adult nervous system (6). An undergraduate, Larry Benardo, now a professor at SUNY Health Science Center, and I speculated that the nervous system might continue to diminish its plasticity response with advanced aging. To explore this idea, we housed young adult animals in the vivarium for an extended period of time (20 months) and subjected them to a unilateral entorhinal cortex lesion. Fortunately the housing costs were only a fraction of what they are today, but the animals did grow to an enormous size. At one of Carl's laboratory meetings, Larry and I reported a decline in axon sprouting in these aged animals compared to young adult controls. Carl's enthusiasm and excitement about these findings was contagious, and he directed more resources at this project. A significant loss of plasticity in the aged nervous system could have far-reaching implications, especially in regards to functional recovery or the retention of normal function after injury. The diminished plasticity we subsequently observed (7–9) could be attributed to a number of different possibilities such as a reduction in the ability of neurons to synthesize the materials necessary for growth, inability of the target cells to accept new synapses, and the loss of a growth promoting signal or a change in the signal threshold. One of the major concepts in age-dependent plasticity was the finding that the aged nervous system still retains a significant plasticity response.

Many discussions in the laboratory centered around the possibility that reactive synaptogenesis and axon sprouting may be a recapitulation of developmental processes. The synapses that were formed following the injury appeared morphologically identical to those in the neuropil of the naïve animal. Perhaps the developmental machinery necessary for forming synapses was simply dormant and awaiting the right stimulus to engage it once again. Early ultrastructural studies (10,11) showed that although both young and aged rodents are capable of replacing lost synaptic contacts to the same magnitude, the time course of the reactive process is very different. (Fig. 1). Not only was there a delay in the initiation of the synaptic replacement but there was also a delay in the time-dependent loss of synapses, indicative of a fundamental age-dependent change in the neuropil. This idea was supported by the fact that whereas the numerical density of synapses was identical between naïve young and aged animals, the number of reactive astrocytes was significantly elevated in the hippocampus of aged rats (9). In addition, the vascular supply of the hippocampus also showed age-related changes possibly contributing to alterations in essential energy supply and demand.

One possible reason for the age-dependent changes in the plasticity response could be due to age-dependent changes in circulating hormone levels. Several very early studies noted that the hypothalamic neuroendocrine regulatory system was dramatically altered in aging, and that

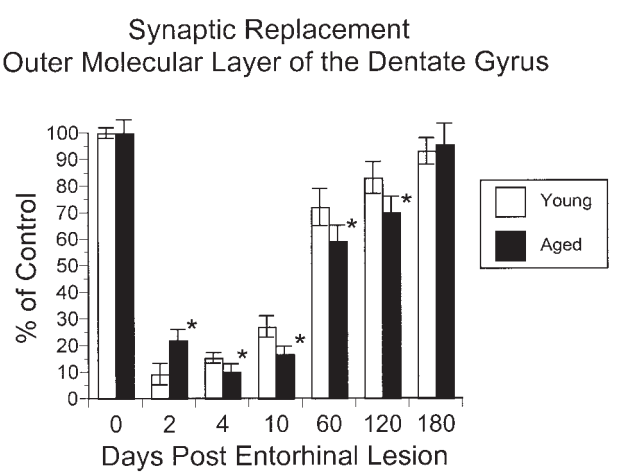

Fig. 1. Groups of young (3 mo) and aged (24–30 mo) Sprague-Dawley rats were subjected to a unilateral removal of the entorhinal cortex. The outer molecular layer of the hippocampal dentate gyrus ispsilateral to the lesion was assessed for changes in synaptic numbers at different days post injury. Data were normalized to nullify any possible age-related differences in preinjury synaptic numbers. Although there are age-related differences at specific survival times, the magnitude of the replacement response is equivalent by 180 days post injury.
Data were adapted from Hof et al. (11). Bars represent group means (±SEM). *$p < 0.01$.

circulating levels of glucocorticoids significantly altered the hippocampal neuropil (12–18). We subsequently determined that the serum corticosterone levels were significantly elevated throughout the diurnal cycle in old animals with initial changes seen even in middle-age animals (19). To explore this possible relationship, we treated young adult rats with different levels of corticosterone and assessed both axon sprouting and reactive synaptogenesis. Animals treated chronically with high levels of steroids demonstrated lesion-induced responses similar to those of aged animals. Both axon sprouting (19–23) and reactive synaptogenesis (24) showed significant diminution. Animals treated with the high corticosterone levels also showed a delay in the clearance of degenerating synapses, suggesting that the failure to replace lost synapses may be linked to clearance of degenerative debris from the neuropil. Nevertheless, animals treated with the high levels of glucocorticoids did attain synaptic densities equivalent to control animals, indicating that although steroids can alter the plasticity response they do not totally inhibit it.

In 1984, Brad Hyman and colleagues published a paper in *Science* demonstrating that a major problem in Alzheimer's disease (AD) was pathology in the entorhinal cortex that effectively isolated a large portion of the hippocampus (25). These findings suggested that in the early stages of the disease the hippocampal dentate gyrus was selectively denervated, paralleling the model system that Carl had used to investigate hipppocampal plasticity. In an interesting paper published in *Science* (26), Carl's group reported a marked increase in acetylcholinesterase (AChE) activity in the outer molecular layer of the dentate gyrus in AD patients compared to age-matched control subjects. These results were interpreted as evidence for a plasticity response in AD that occurred along with the degenerative events normally associated with the disease. A subsequent study reported an indirect measurement of plasticity in the commissural/associational fibers system in AD patients (27). Their working model was that even a slow and fractional loss of a neuronal assembly could trigger reactive axonal growth and synaptic replacement thereby enhancing neuronal stability. Although the true functional significance of these findings had not been determined, they provided initial evidence of synaptic plasticity in human adult aging.

As a faculty member at the University of Kentucky, my laboratory was interested in the question of AD plasticity. We initially followed the idea that the problem in AD might originate in the cholinergic system and the cause of the dementia was related to a loss of cholinergic input to the hippocampus and neocortex, particularly the association cortex. Impaired cholinergic metabolism had been reported in AD (28,29) In a rather labor-intensive study (30), my colleague Steven DeKosky and I monitored changes in cholinergic enzyme activity in the frontal cortex of AD and control subjects. We found the greatest choline acetyltransferase (CAT) losses in the upper laminae, although all lamina showed significant declines in AD. Loss of AChE activity was also significantly lower in all laminae. Based on these results we initiated an ultrastructural study of synaptic density in the frontal cortex (Brodmann area 9).

If Cotman and colleagues were correct in their assumption that a significant amount of plasticity occurs in AD, and the aged nervous system still maintains significant plasticity, then we might not observe any change in synaptic numbers in the cortex. Previously a few studies had investigated possible synapse loss in this brain region, but the results were mixed (31–33). We found that both lamina III and V of the frontal cortex had significantly fewer synapses in AD subjects compared to age- and postmortem-matched controls (34). These results did not support the plasticity hypothesis at least for end-stage AD subjects. On the contrary, it appeared that one problem associated with AD is perhaps a marked reduction in reactive synaptogenesis. This reduction of synapse number in the frontal cortex is closely correlated with the individual's overall cognitive function (35). One of the surprising findings was a change in the synaptic apposition length or active zone of the synaptic contact in AD brains. A regression analysis revealed an important relationship between the synaptic density and the size of the remaining synapses (Fig. 2). As the number of synapses decreased, the apposition length of the residual synaptic contacts increased (34,35). Subsequent studies ruled out the possibility that the change in synaptic size was not simply due to a loss of small synapses in the neuropil (36). We speculated that this enlargement in synaptic size was a general compensatory mechanism that had not been fully explored in regard to maintenance or restoration of neural function. A similar phenomenon had been observed with experimental animals (37). By combining the number of residual synapses and the mean apposition length, we calculated the mean total synaptic contact area per unit volume of cortex and used this as a measure of synaptic plasticity (34,35). We were surprised by the finding that the synaptic enlargement had nullified most of the synaptic loss in terms of total synaptic contact area. This phenomenon was replicated in a subsequent investigation of the superior and middle temporal gyrus (36). More importantly, this unique form of plasticity could be observed not only in AD material but also cognitively normal age-matched controls. Thus our findings

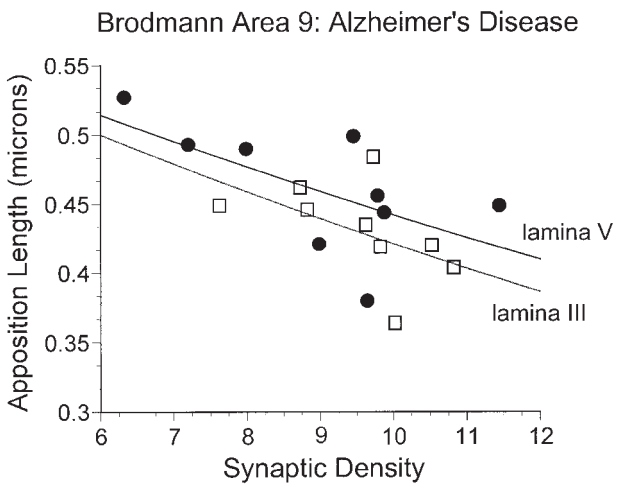

Fig. 2. Relationship between the number of synapses (synaptic density) and the synapse size (apposition length) in lamina III and V of the frontal cortex (Brodmann area 9) in patients with Alzheimer's disease. Regression analysis revealed an interesting relationship between the synaptic density and the apposition length. As the number of synapses declines, the synaptic size of the residual synapses increases.
Data adapted from Scheff et al. (34). Points indicate individual subjects with open boxes representing lamina III and closed circles lamina V.

supported Carl's initial suggestion of plasticity in the adult human CNS.

The AD plasticity work of Carl's concentrated on the hippocampus, and it was possible that this structure responded quite differently than regions of the neocortex. Because of the well-established pathological hallmarks of AD found in the hippocampus and its association with cognitive decline in the disease, we assessed possible changes in synaptic numbers in this brain region. Initial studies were concentrated in the outer molecular layer of the dentate gyrus, the terminal zone of the entorhinal afferents. Several immunohistochemical studies had reported a loss of synaptophysin staining (38–42) in the hippocampus, indicative of a loss of plasticity. Our ultrastructural studies (43) found a highly significant loss of synaptic numbers accompanied by an increase in apposition length, that again appeared to negate any loss in total synaptic contact area. This apparent compensatory response needed to be viewed in a relative manner because the hippocampal formation demonstrates considerable atrophy in the course of the disease. Thus the plasticity response we observed could not totally compensate for the *total loss* of synaptic input. In fact, we found a significant decrease in the size of the dentate gyrus molecular layer in the AD subjects. Restoration of total synaptic contact area was also observed in the inner molecular layer of the dentate gyrus (44), a region that does not directly receive entorhinal afferents but does demonstrate a loss synaptic input in AD.

The inability of hippocampal and neocortical regions to maintain plasticity in terms of normal synaptic numbers may reflect a problem with the primary afferents or the dendritic arbor of the target neuron. A loss of target cells, such as in the hippocampus (45,46), would present fewer opportunities for residual afferents to form appropriate synaptic contacts. Alternatively, the maintenance of total synaptic contact area may reflect a different type of plasticity in AD. There has been much discussion in the plasticity literature regarding the specificity of synaptic reorganization. The target neuron is usually incriminated with dictating the specificity of the reorganization. Proximity of residual afferents may also be important. We propose a model in which a denervated target neuron plays an important role primarily by signaling a need for synaptic input. When maximally denervated, the signal is strongest when attracting synaptic input, and as new synaptic complexes are formed the signal diminishes in intensity until adequate reinnervation occurs or the signal is too weak to attract new input (Fig. 3). In this model, the total area of synaptic contact is one of the significant variables dictating the intensity of the signal. The target neuron may not be extremely particular as to the source of the innervation but more concerned with the net area of the contact. The interaction between the residual afferents and target neurons may be salubrious, because the enlargement of the active zone of the synapse would boost weakened signals of a particular synaptic influence and the target cell would approach signal homeostasis. This process would also maintain functional stability for a particular synaptic input. Eventually one might envision a situation where the pathological cascades overcomes the ability of the target cells to produce the appropriate signal, even the enlargement of residual complexes. Finally, the source of the residual afferents is also severely affected resulting in a significant loss of total plasticity.

The functional significance of these synaptic changes in AD could be quite devastating. If the objective of a therapy is to enhance specific connectivity by providing neurotrophic agents, presumably taken up at synaptic complexes, enlarged synapses would have a distinct advantage. In this situation, it is unclear whether or not the enlarged synapses are the most appropriate. Such a therapy might result in unwanted functional outcome. Alternatively, such agents might further strengthen a specific type of synaptic complex and provide stability of not only the synapse itself but also the neuronal process resulting in stability of neuron numbers. Our ability to even speculate about possible functional significance is a tribute to Carl Cotman and the

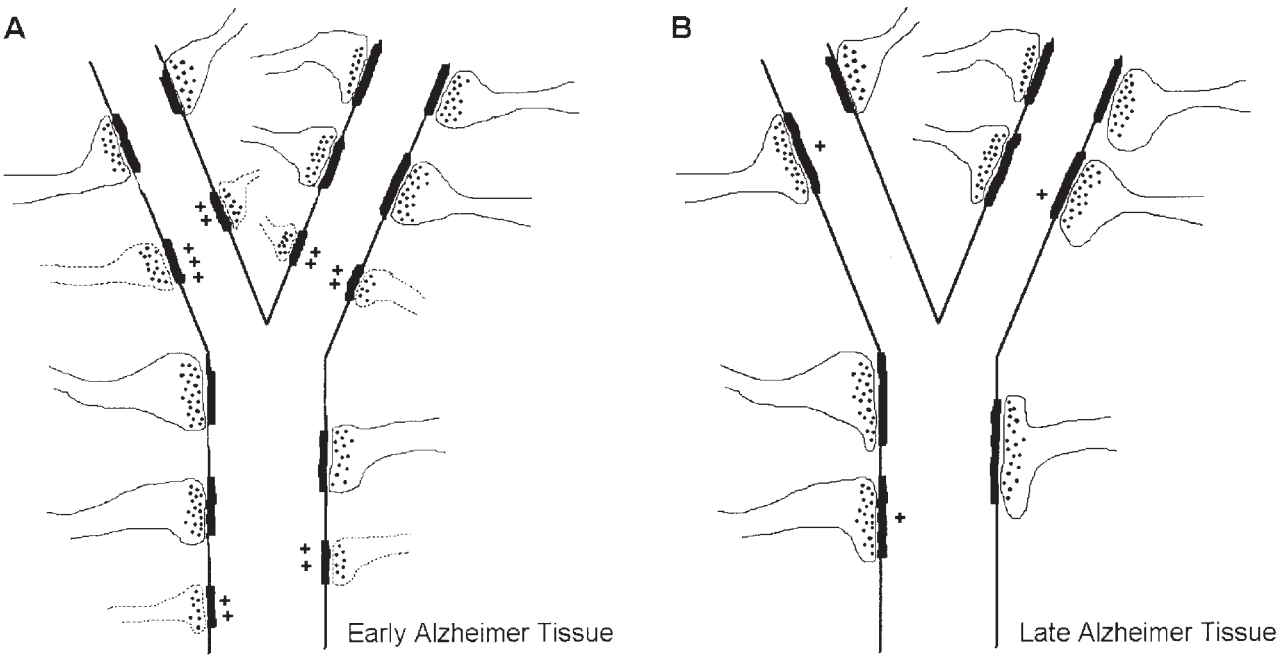

Fig. 3. Schematic diagram of a model of possible dynamic change in synaptic complexes observed in Alzheimer's disease (AD) neuropil. The loss of synaptic complexes (A) (dashed outlines) in early stages of AD initiates a signal (+) by the target neuron for increased contact area. In the later stages of AD (B) the residual afferent inputs to the denervated target neuron increase the size of their complexes in response to this signal resulting in increased synaptic contact area. The increased total contact area of the residual afferents significantly decreases the signal of the target neuron by approximating the total contact area in the earlier stages of AD.

tremendous energy he used to focus on the study of brain plasticity. His enthusiasm for this field and the support and encouragement he provided those that worked with him in this area have provided the entire neuroscience community with a new appreciation of how the nervous system functions.

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