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Structural Plasticity of Synapses in Alzheimer's Disease

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Contents

Abstract

Plasticity of the synaptic contact zone was previously observed following loss of synapses in the cerebral cortex of normal aging humans. The present study was undertaken to determine if there was quantitative evidence of synapse loss and synapse plasticity in the inferior temporal, superior parietal, parieto-occipital, and superior frontal cortical regions in Alzheimer's disease (AD), and how such changes related to the neurofibrillary tangles and amyloid plaques. The results showed that age at autopsy did not correlate with the numbers of synapses, plaques, or tangles. However, the numbers of synapses strongly reflected the pathology of AD; in all four brain regions, there were fewer synapses as the numbers of plaques and tangles increased. In the inferior temporal and superior parietal cortices, the loss of synapses was accompanied by an increase in the synaptic contact length. The results suggest that, in some cerebral cortical brain regions, synapses are capable of plasticity changes, even when the pathology of AD and loss of synapses are severe.

Index Entries: Alzheimer's disease; synapses; plasticity; aging.

Introduction

Neural plasticity has been defined as adaptive changes in structure and function of the nervous system, occurring during all stages of development and adulthood, as a result of experience or following injury (Phelps, 1990). Morphologically, plasticity may involve axonal sprouting, dendritic growth, synaptic remodeling, and glial hypertrophy. Strong support for new synapse formation and reorganization in the mature brain comes from mechanically and chemically induced lesion studies (Bjorklund and Stenevi, 1979; Goldowitz et al., 1979). There is also increasing evidence that the mature mammalian brain possesses considerable capacity for continued growth and reorganization, and that synapses are being lost and replaced throughout life as part of a normal process (Adams and Jones, 1982; Geinisman et al., 1988).

Dendritic and synaptic plasticities have also been observed in the normal aging brain (for reviews, *see* Adams and Jones, 1987; Flood and Coleman, 1990). In the normal aging human brain, plasticity of the synaptic contact zone has been observed following loss of synapses (Adams, 1987a,b). As synapses were lost, there was a significant increase in the length of the postsynaptic contact zone of the remaining synapses. This synaptic plasticity was thought to represent a compensatory response by the aged brain, since it was not apparent in brain regions where there was no age-related loss of synapses.

Currently, the role of plasticity in the pathology of Alzheimer's disease is a topic of considerable interest. Dystrophic neurites and terminals have been implicated in Alzheimer's disease pathology for many years. However, there have been few quantitative ultrastructural studies that have investigated the loss of synaptic contacts and synaptic plasticity in the AD brain.

Of the current literature on Synapses in AD, two studies found no significant decline in the number of synapses of the frontal cortex (Gibson, 1983: Paula-Barbosa et al., 1986), and two studies found a signficant decrease in the number of synapses of the temporal and frontal cortices (Davies et al., 1987; Scheff et al., 1990). The latter study also found that cortical samples with fewer synapses had larger synapses.

The present study was undertaken to assess:

1. The numerical density of synapses in various regions of the cerebral cortex from tissue obtained at autopsy of patients with Alzheimer's disease;
2. Structural evidence of synaptic plasticity by quantitative measurement of the length of the postsynaptic density (referred to as synapse length); and
3. The relationship of these synaptic parameters to the neuritic amyloid plaques and neurofibrillary tangles of Alzheimer's disease.

Materials and Methods

The material used in this study was obtained from brains examined at the Department of Neuropathology, Royal Perth Hospital (by kind courtesy of C. Masters), and subjected for neuropathological evaluation to confirm Alzheimer's disease. The criteria used were based on easily identifiable amyloid plaques and neurofibrillary tangles (Khachturian, 1985). However, the histological data was not correlated to the clinical condition, other than the fact that all patients had been diagnosed as having Alzheimer's disease prior to death.

Neocortical blocks were taken from ten autopsy cases as follows: superior frontal gyrus (SFG, Brodmann area 8), inferior temporal gyrus (ITG, Brodmann area 36,20), superior parietal lobule (SPL, Brodmann area 7), and parieto-occipital lobe (POG, Brodmann area 40).

Immunocytochemical detection of amyloid βA4 protein (kindly donated by C. Masters) utilized formalin-fixed free-floating vibratome tissue sections (50 μm) and a standard peroxidase-antiperoxidase technique. These tissue sections were used to locate the immunoreactive amyloid plaques and cortical layer 1. Subsequently, the identified areas were selectively cut from the thick sections and prepared for electron microscopy using ethanolic phosphotungstic acid

(E-PTA) staining (Adams, 1987b). Electron micrographs (×150 and ×43,000 magnification) of representative areas were systematically photographed and analyzed for each brain region.

The following parameters, expressed as counts per unit tissue, were investigated: number of synapses, and number of amyloid plaques (plaques), and neurofibrillary tangles (tangles). A Kontron MOP semiautomated image analyzer was used to determine the length of the postsynaptic contact density (synapse length). The usual precautions concerning morphometric evaluations and postmortem changes were taken, as recorded in previous studies (Adams, 1987a,b).

Results

Data for Individual Alzheimer's Patients (Table 1), Depicting Linear Regressions in the Age Spectrum of Alzheimer's Disease

For purposes of correlating the measured parameters specifically with age in Alzheimer's disease, all data from the four cortical regions were pooled, and mean ± SEM for each parameter was determined (Table 1). Linear regression analyses were then performed to determined if the morphometric values—number of synapses, synapse length, and plaque and tangle counts—showed significant age-related changes over the combined cerebral cortical regions examined.

From Table 1, it can be seen that a significant negative correlation ($r = -0.66, p < 0.05$) with age was apparent only for synapse length. There were no correlations between age and number of synapses, plaques, or tangles. This suggests that age alone is a poor indicator of AD pathology. However, there was considerable heterogeneity among the individuals. The individual (aged 76 yr) with the least plaques and tangles had the shortest, but highest number of synapses, and the AD individual (aged 68 yr) with the most plaques and tangles had fewer, but longer synapses.

Correlations of the Various Parameters in the Different Cerebro-Cortical Regions

Number of Synapses vs Synapse Length

There was a significant negative correlation ($r = -0.77, p < 0.01$) when the mean values for the combined four brain regions were analyzed. When the data were analyzed separately for the four different brain regions (Fig. 1), significant negative correlations were found in the superior parietal and inferior temporal lobes; i.e., as the number of synapses became fewer, the remaining synapses became longer in synaptic contact length. No significant trends were apparent in the parieto-occipital or superior frontal gyrii.

Number of Synapses vs Number of Plaques

There was a significant negative correlation ($r = -0.89, p < 0.01$) when mean values for the combined four brain regions were analyzed by linear regression. When analyzed by regions, all four brain regions showed significant negative correlations (Fig. 2); i.e., as the number of synapses decreased, the number of plaques increased.

Number of Synapses vs Number of Tangles

There was a significant negative correlation ($r = -0.89, p < 0.01$) when mean values for the combined four brain regions were analyzed by linear regression. This correlation was apparent in all four cortical regions when analyzed by region (Fig. 3); i.e., as the number of synapses decreased, the number of tangles increased.

Length of Synapses vs Tangle and Plaque Counts (Not Graphed)

There were significant positive correlations for the mean values for the combined four brain regions for both the tangle and plaque counts ($r = 0.68, p < 0.05$ and $r = 0.69, p < 0.05$, respectively); i.e., the length of the synaptic contact zone increased as the number of tangles and plaques

Table 1
Data for Individual Alzheimer's Patients

Age, yr	Number syn/100 μm²	Length syn, μm	Plaques/mm²	Tangles/1000μm²
60	10.1 ± 0.9	.307 ± .101	1.3 ± 0.5	18.6 ± 3.8
68	10.0 ± 1.0	.313 ± .010	1.9 ± 0.6	23.4 ± 4.4
73	10.8 ± 0.1	.289 ± 0.11	0.6 ± 0.2	10.8 ± 2.3
74	12.4 ± 0.7	.289 ± 0.005	0.6 ± 0.2	9.5 ± 3.8
76	13.5 ± 0.8	.270 ± .002	0.1 ± 0.03	0.9 ± 0.1
77	12.0 ± 0.4	.273 ± .003	0.5 ± 0.3	10.0 ± 4.0
78	11.0 ± 0.6	.279 ± .005	1.1 ± 0.01	15.5 ± 3.0
82	11.9 ± 0.7	.277 ± .004	1.0 ± 0.3	14.9 ± 5.0
93	11.6 ± 0.4	.277 ± .008	1.3 ± 0.4	14.5 ± 3.7
	$r = 0.47$	$r = 0.66^*$	$r = -0.16$	$r = -0.29$

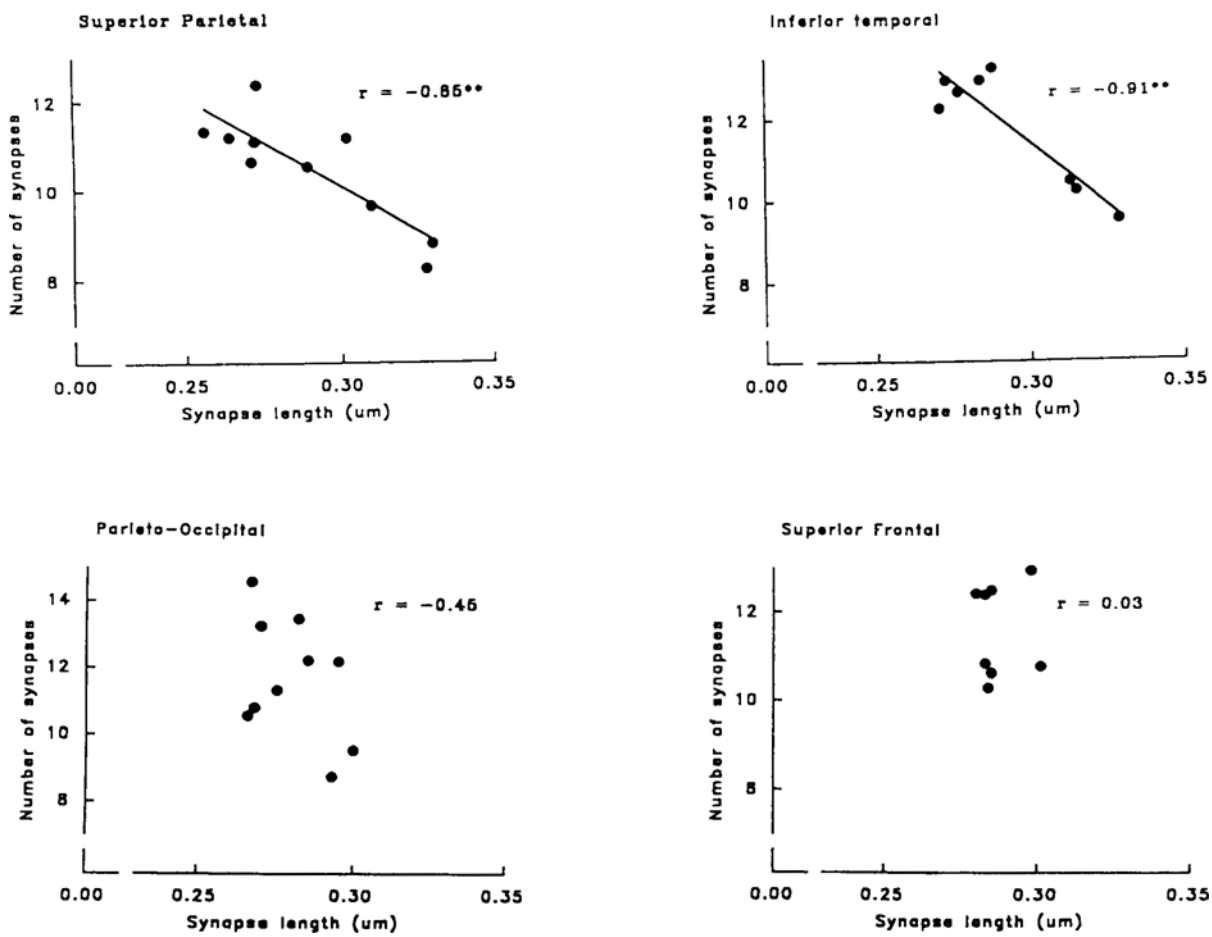

Fig. 1. Relationship between synapse length (μm) and the number of synapses per 100 μm² of tissue in E-PTA-stained tissue; r = correlation coefficient.

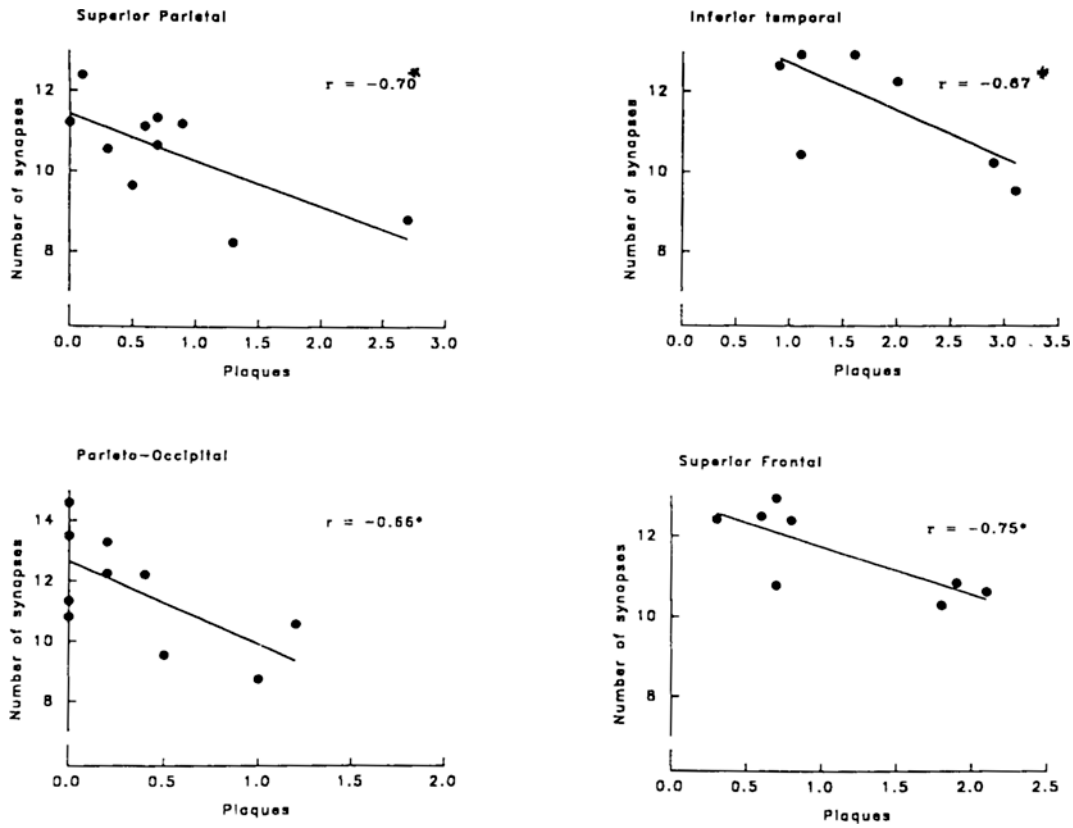

Fig. 2. Relationship between number of synapses per 100 μm^2 of tissue and number of amyloid plaques per mm^2; r = correlation coefficient.

increased. When analyzed according to each brain region, significant correlations were seen in the superior parietal lobule (tangles: $r = 0.71, p < 0.01$ and plaques: $r = 0.84, p < 0.01$) and inferior temporal gyrus (tangles: $r = 0.66$, $p < 0.05$ and plaques; $r = 0.67, p < 0.05$).

Discussion

In the present study, age was found to be a poor indicator of AD pathology, numerical density of synapses, and synaptic plasticity. However, there was a strong correlation between number of synapses and number of tangles and plaques; i.e., the loss of synapses was reflected by a corresponding increase in the number of plaques and tangles. In the superior parietal and inferior temporal cortices, this was accompanied by an increase in the length of the synapse.

The marginally significant negative correlation ($p < 0.05$) between age and synapse length for pooled cortical data (Table 1) was in contrast to the age-related increase in synapse length seen in normal aging (Adams, 1987a,b). This may reflect the different cortical regions examined, or that other factors, such as plaques and tangles, may override age effects in Alzheimer's disease with respect to synapse loss and synaptic plasticity. This is supported by the strong correlation seen between number of synapses and number of tangles and plaques in both the pooled and individual cortical data. Similarly, dendritic sprouting, although present in AD, was found to be reduced or aberrant when compared with normal aging (Flood and Coleman, 1990).

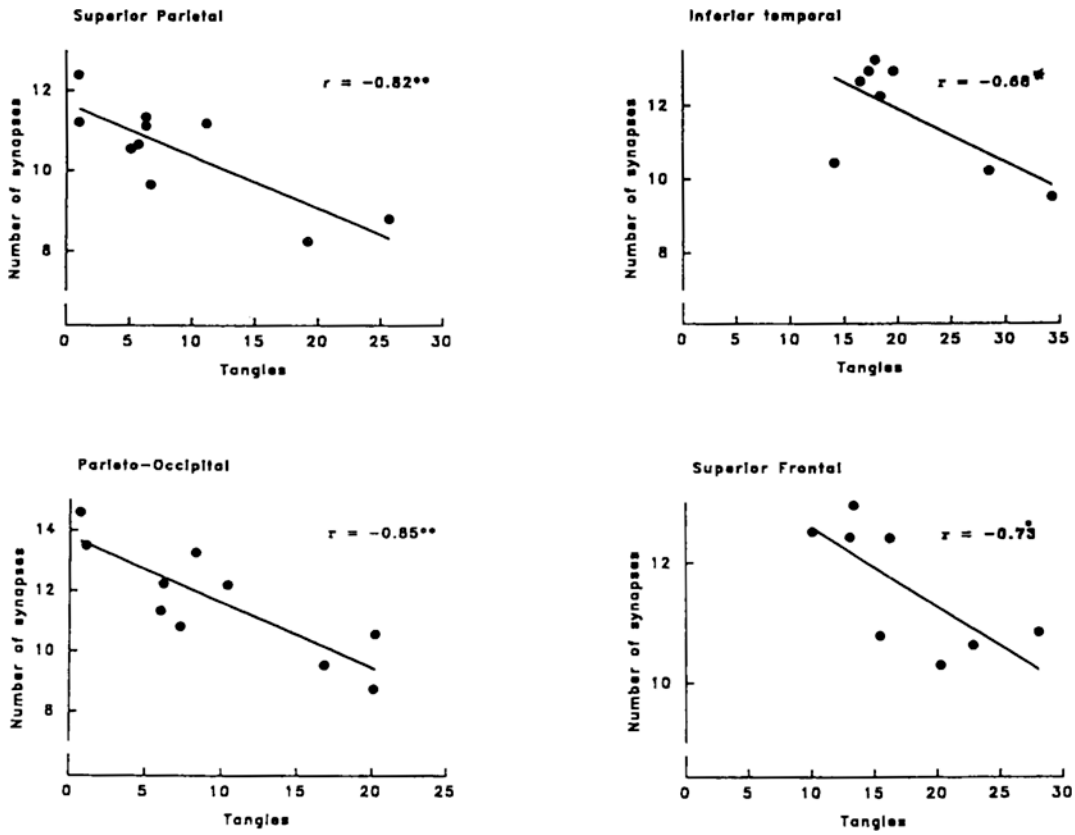

Fig. 3. The relationship between number of synapses per 100 μm^2 of tissue and number of neurofibrillary tangles per 1000 μm^2; r = correlation coefficient.

This study and others (Davies et al., 1988, Jorm, 1985) suggest that it is difficult to differentiate older and younger patients with Alzheimer's disease solely on the criterion of severity of tangle and plaque lesions. Similarly, the results do not support the once-held notion that the occcurrence of neurofibrillary tangles and amyloid plaques rises dramatically with increasing age (for a review, *see* Terry and Hansen, 1988) or the view that the more severe pathology is seen in the younger AD patient (Hansen et al., 1988). However, the present study had only a limited number of cases, especially in the sixth and ninth decades.

There was a strong negative correlation between synapse number and synapse length. The data suggest that, especially in the superior parietal and inferior temporal regions, the increase in synapse length may be a compensatory response to the loss of synapses. Recently, Scheff et al. (1990) also reported a negative correlation between synapse number and synapse size in area 8 of the frontal cortex in AD.

This is the first ultrastructural study to report quantitatively that the number of synapses decreased as the number of plaques and tangles increased in four cerebral cortical brain regions known to be vulnerable in AD. However, another recent

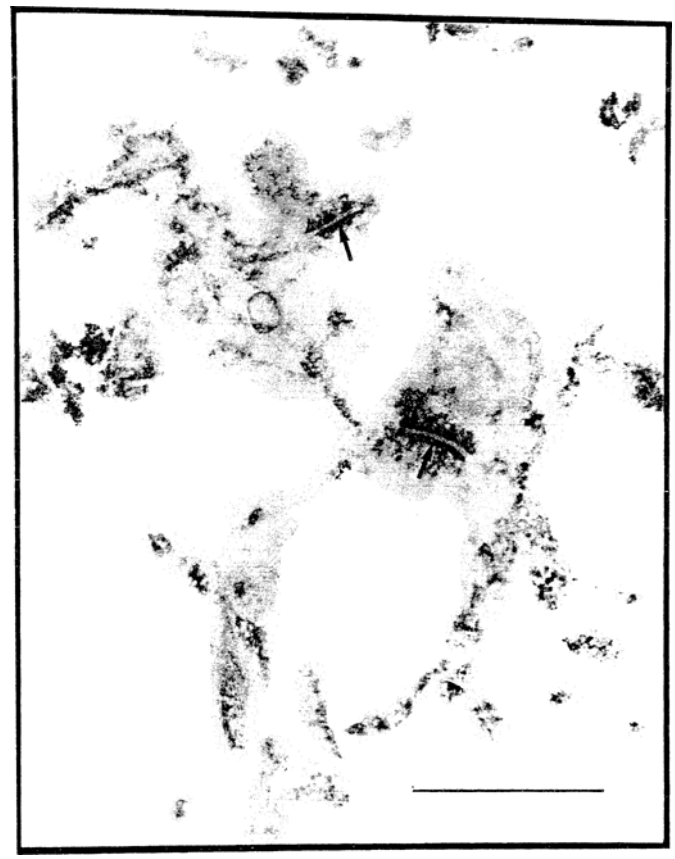

Fig. 4. Electron micrograph of layer 1 of inferior temporal cortex from human subject with Alzheimer's disease, fixed by immersion and stained with E-PTA. Several synapses (arrows) sectioned perpendicular to the cleft are clearly visible. Bar = 1 μm.

study found that an increase in the number of plaques was accompanied by an increase in the numerical synaptic volume density in layer 3 of the frontal cortex (Scheff et al., 1990). However, the latter study did not utilize βA4 immunocytochemistry for the identification of amyloid plaques. In addition, the different results may reflect the different cortical laminae examined. The present study examined lamina 1, and Scheff et al. found the postive correlation in lamina 3, but no such relationship in lamina 5.

The mechanisms underlying synapse loss in AD are unknown, but peptides related to the amyloid precursor protein (APP) may play a substantial role. APP has been found in neocortical neurons from the brains of both control subjects and patients with AD (Lewis et al., 1988), suggesting that the protein precursor of the pathological amyloid material in AD is a natural constituent of some cortical neurons. It has been postulated that damage to the synapse may stimulate abnormal cleavage of the nonamyloidogenic APP at the transmembrane site, causing generation and release of the βA4 amyloid protein seen in AD plaques (Masters and Beyreuther, 1988; Beyreuther and Masters, 1991; Pasternack et al., 1991). What causes the initial damage to the synaptic region remains obscure. Some stud-

ies have shown that βA4 protein and different fragments of the APP may have neurotoxic effects on neurons (Masters and Beyreuther, 1988).

It remains to be determined whether the synapses are responding in a compensatory manner, or whether the plasticity response is retarded by the pathology of AD and is largely aberrant. Compensatory functioning implies that remaining brain structures, in this case the synapses, can be used to carry out the functions previously undertaken by the lost or damaged structures (Hannay and Levin, 1987). This would necessitate some neuronal reorganization and plasticity mechanisms, such as reactive synaptogenesis, rerouting of axons and dendrites, axonal and dendritic retraction, and sprouting and denervation supersensitivity. The latter suggests that, following loss of input from damaged neurons, a synapse may have increased sensitivity to chemical signals produced by the intact neurons, damaged neurons, or glia. This process may involve molecular and structural plasticity changes and some recovery of neuronal functioning. If exploited, it may be a pallitative treatment strategy in AD.

However, in the AD brain, the relationship of synaptic plasticity and synaptic loss to the increase in plaques and tangles is a complex one and largely hypothetical at this stage. AD brain extracts have been isolated that enhance survival of rat cortical neurons in vitro (Uchida et al., 1988; Whitson et al., 1989), suggesting that the AD brain is not devoid of neurotrophic factors. However, neurotrophic agents may in fact exacerbate the pathology of AD (Butcher and Woolf, 1989). It has recently been reported that growth-inhibiting factor (GIF), found in astrocytes, is downregulated in AD brain and that the loss of GIF correlated with the density of plaques (Ihara, 1988, 1991).

The unraveling of AD usually assumes that the protein amyloid depositions and neurofibrillary tangles are of primary significance. However, these lesions of AD may represent the endpoint of damage in AD. Normal synaptic remodeling may be compromised early in the disease by factors of unknown etiology, precipitating a cascade of events that may include plasticity changes, loss of synapses and neurons, and culminating in the readily identifiable features of AD, the amyloid plaques and neurofibrillary tangles.

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