Commentary

Staying Connected: Synapses in Alzheimer Disease

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Careful dissection is continuing to reveal the complexity and importance of synapse alterations in Alzheimer disease (AD). Synaptic alterations are the best biological correlate to the extent of cognitive loss^{1,2} and have become the gold standard of meaningful biomarkers of AD. Not neurofibrillary tangles (NFT), senile plaques, or even neuronal loss show such strong statistical correlation with dementia.²⁻⁴

Connections are the hallmark of neurons and without functional synapses, neurons are in "solitary." Recent findings showing that amyloid- β ($A\beta$) affects and accumulates in synapses⁵ and that amyloid- β protein precursor ($A\beta$ PP) over-expressing mice show synaptic alterations^{6,7} have provided additional evidence that synapses are a critical readout of the biology of AD.

In this issue, Gyls and co-workers⁸ provide an elegant and precise dissection of synaptic alterations in AD using synaptosomal preparations. The results are both consistent with the synaptic alteration hypothesis proposed by Terry and colleagues²⁻⁴ and refine it. In previous studies synapses have been quantitated by synaptophysin immunoreactivity,^{1,9} a protein belonging to a subclass of synaptic vesicles. However, when synapses were quantitated by precise ultrastructural morphometric/stereological approaches, reduction in synapses was much less striking than the reduction in synaptophysin.¹⁰ Furthermore, synapse alterations included major remodeling of morphology¹¹ as noted in some of the earliest studies of AD.¹² These established findings lead one to consider whether the alterations in synapses in AD are limited to global reduction in vesicles rather than synapse changes. Gyls and collaborators⁸ used a broad range of pre- and postsynaptic markers to precisely analyze synaptosomes by flow cytometry. They found that the synaptosomes in AD show no reduction in synaptophysin and only a slight reduction in the overall number of synaptosomes. To account for the undoubted major reduction in synaptophysin while synapse number and synaptic vesicle number are only slightly reduced, brings to mind the seminal findings of Suzuki and Terry¹³ who reported substantial axonal transport abnormalities in

AD. Consistently, one finds vesicle pile-up in cell bodies similar to transport blockage models.¹⁴ Recent findings that microtubules are greatly reduced in AD¹⁵ and that $A\beta$ PP/ $A\beta$ play important roles in vesicular transport¹⁶⁻²⁰ further link Terry's transport and Gyls' synaptic hypotheses. Not answered is where the extrasynaptic synaptophysin is located. One of the most attractive possibilities is that many of the synaptophysin vesicles are located in axons; for that reason, Terry's initial insight to focus on vesicles was particularly fruitful because it marked the reduction most specific to AD. Additional work either by cell fractionation studies, as Gyls and collaborators⁸ have so elegantly performed, or by immunoelectron microscopy is called for to dissect this issue more clearly.

Synaptic Degeneration by $A\beta$

Investigators studying the primary culprit responsible for AD have primarily focused on $A\beta$ as the predominant factor responsible for the disease²¹ and, according to the amyloid cascade hypothesis, the accumulation and toxicity of $A\beta$ leads to neuronal and synaptic loss in AD.²² $A\beta$ is derived by proteolytic cleavage of $A\beta$ PP, a protein of unknown cellular function that has the general properties of a cell surface receptor.²³ The regulatory activity of three different proteolytic enzymes, α , β , and γ -secretases, at their specific cleavage sites yields a number of different products, including $A\beta_{1-40}$ and $A\beta_{1-42}$.²⁴ While $A\beta_{1-40}$ is the predominant product of this proteolytic pathway, $A\beta_{1-42}$ is far more fibrillogenic *in vitro* and is the major $A\beta$ species present in the core of senile plaques (both AD and non-AD related).^{25,26} Although many new questions have arisen regarding which $A\beta$ molecule, oligomer or fibril, is the real culprit in AD pathophysiology, $A\beta$ is inherently toxic in cell culture models.^{27,28} However, *in vivo* studies show contradictory results. $A\beta$ PP transgenic mice, which possess high levels of $A\beta$ and deposition of $A\beta$ plaques, presented marked deficits in spatial learning by the age of 9 months.²⁹ Although no neurotoxicity was observed in these mice, it is thought that their impaired spatial learning, which is correlated with long-term

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potentiation, is related to synaptic loss marked by synaptophysin. Furthermore, human studies show that, while amyloid plaque deposition is increased in brains of AD patients when compared to age-matched controls, the presence and density of amyloid deposition correlate poorly with AD symptoms and severity.^{30,31} In light of these findings, the amyloid cascade hypothesis has been “forced” to update, ie, instead of neuronal degeneration (neurotoxicity), $A\beta$ induces synaptic degeneration (synaptotoxicity).²² In this updated hypothesis, it is suggested that oligomeric $A\beta$ rather than fibrillized $A\beta$, may accumulate in synaptic terminals causing synaptic degeneration.⁵ Although no direct evidence showing whether oligomeric $A\beta$ causes morphological and biochemical changes in synapses exists, correlation and electrophysiological studies support this updated hypothesis.

Studies performed with $A\beta$ PP transgenic mouse lines showed that synaptophysin immunoreactivity is significantly decreased when compared with non-transgenic controls at 2 to 3 months old, an age where $A\beta$ plaque formation is nonexistent in $A\beta$ PP transgenic mouse lines.³² Indeed, decreases in presynaptic terminals are critically dependent on cortical $A\beta$ levels and not on $A\beta$ plaque burden or $A\beta$ PP levels.⁷ In accordance, presynaptic terminals are already significantly depleted in 2- to 4-month-old $A\beta$ PP transgenic mice, which present high levels of soluble $A\beta$ levels, but have no $A\beta$ plaque deposition. Furthermore, electrophysiological studies of young $A\beta$ PP transgenic mice presenting AD-causing mutations have revealed significant deficits in basal synaptic transmission and/or long-term potentiation (LTP) in the hippocampus, well before the development of $A\beta$ deposits. For example, transgenic mice possessing the V717F $A\beta$ PP mutation show relatively smaller excitatory postsynaptic potentials and rapid decay of LTP when compared with age-matched non-transgenic mice.³³ Similarly, intracerebroventricular microinjection of cell medium containing $A\beta$ oligomers and abundant monomers, but without fibrillized $A\beta$, potentially inhibited LTP in adult rats.³⁴ These findings support data obtained from cell culture studies that show that oligomers of synthetic $A\beta$ can cause acute electrophysiological changes in cultured neurons or hippocampal slices.^{35,36}

Furthermore, it is important to emphasize the potential role of oxidative stress in synaptic degeneration, since $A\beta$ is known to either increase or decrease oxidative stress dependent on its presentation. In fact, a recent study demonstrates that while the correlation between oxidative stress and synaptic degeneration does not reach statistical significance, the tendency of the correlation is consistent with the idea that an increase in oxidative stress may adversely affect maintenance of synaptic density.¹¹

Good Face of $A\beta$: $A\beta$ May Not Be the Cause of Synaptic Degeneration but Rather a Repair

It is important to emphasize that different types of cells such as neurons, astrocytes, neuroblastoma cells, hepatoma cells, fibroblasts, and platelets produce $A\beta$.^{37–39}

These data suggest that $A\beta$ should have an important function in normal cell development and maintenance. Recently, it has been shown that nanomolar concentrations of $A\beta$ can block neuronal apoptosis following oxidative damage, which suggests that $A\beta$ is essential for neuronal survival.^{40,41} These findings are consistent with the trophic and neuroprotective actions of $A\beta$ at physiological concentrations in deprived conditions and neonatal cells reported during the last decade.^{27,42–47} Moreover, studies *in vivo*^{48,49} show that $A\beta$ deposition occurs after oxidative stress insults, suggesting that $A\beta$ may have a protective role against oxidative stress.⁵⁰ Indeed, we observed that in brains of AD and Down syndrome patients oxidative stress markers precede $A\beta$ deposition and, oxidative stress seems to be inversely correlated with $A\beta$ deposition.^{48,49} It is unclear whether oxidative stress also precedes synaptic degeneration and further studies are required to clarify this issue.

Together, these data provide a plausible physiological explanation for the increased generation of $A\beta$ in AD, ie, the presence of $A\beta$ in degenerating synapses may represent a compensatory response aimed to fight oxidative stress and promote neurite outgrowth and prevent neuronal degeneration. Therefore, in a chronological study, we can easily imagine a scenario where an initial oxidative stress event causes a neuronal defense response, such as the increase in $A\beta$ monomer and oligomeric species,^{51–53} aimed to neutralize the initial cellular stress.^{48,49} This situation may give the “wrong” idea that $A\beta$ is present before or in the absence of oxidative stress and, consequently, to misinterpretations that $A\beta$ actually precedes oxidative injury and causes synaptic degeneration.

Conclusions

Synaptic degeneration is one of the most prominent events occurring in brains of AD patients. However, its causes and mechanistic basis remains unclear. While $A\beta$ accumulation in synaptic termini may play an important role in synaptic degeneration, as suggested by previous studies, including the elegant one by Gyls et al,⁸ the protective role of $A\beta$ strongly also suggests that accumulation of $A\beta$ may be a compensatory reaction against pathological stress (eg, oxidative stress) and aimed to maintain normal synaptic functions. Furthermore, the importance of NFT in synaptic degeneration should not be ignored. Since a previous study suggested that NFT mediate starvation of synapses⁵⁴ followed by synaptic degeneration, it would be extremely interesting to analyze NFT (phospho-tau) in synaptosome model, like $A\beta$ analysis done by Gyls et al.⁸ Interestingly, synaptic degeneration is not only found in AD but also in other neurodegenerative diseases such as prion diseases and Huntington disease.^{55,56} Therefore, the information obtained from AD research may also help in understanding the mechanisms of synaptic degeneration in other neurodegenerative diseases.

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