## Review

# Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease

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**Abstract**. Alzheimer's disease (AD) is a devastating neurodegenerative disorder without a cure. Most AD cases are sporadic where age represents the greatest risk factor. Lack of understanding of the disease mechanism hinders the development of efficacious therapeutic approaches. The loss of synapses in the affected brain regions correlates best with cognitive impairment in AD patients and has been considered as the early mechanism that precedes neuronal loss. Oxidative stress has been recognized as a contributing factor in aging and in the progression of multiple neurodegenerative diseases including AD. Increased production of reactive oxygen species (ROS) associated with age- and disease-dependent loss of mitochondrial function, altered metal homeostasis, and reduced antioxidant defense directly affect synaptic activity and neurotransmission in neurons leading to cognitive dysfunction. In addition, molecular targets affected by ROS include nuclear and mitochondrial DNA, lipids, proteins, calcium homeostasis, mitochondrial dynamics and function, cellular architecture, receptor trafficking and endocytosis, and energy homeostasis. Abnormal cellular metabolism in turn could affect the production and accumulation of amyloid-β (Aβ) and hyperphosphorylated Tau protein, which independently could exacerbate mitochondrial dysfunction and ROS production, thereby contributing to a vicious cycle. While mounting evidence implicates ROS in the AD etiology, clinical trials with antioxidant therapies have not produced consistent results. In this review, we will discuss the role of oxidative stress in synaptic dysfunction in AD, innovative therapeutic strategies evolved based on a better understanding of the complexity of molecular mechanisms of AD, and the dual role ROS play in health and disease.

Keywords: Alzheimer's disease, amyloid-β, antioxidants, caloric restriction, exercise, mitochondria, mitohormesis, neurotransmission, oxidative stress, synaptic function, tau protein

## MOLECULAR HALLMARKS OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) affects more than 5 million Americans, with numbers expected to grow as the population ages [1, 2]. Most AD cases are sporadic where the origin of the disease is not known but might be influenced by multiple factors including environmental exposure, genetic risk factors, mitochondrial

haplotypes, age, and sex [2–4]. About 1% of cases are associated with familial mutations in the genes that encode either a transmembrane amyloid-β protein precursor (AβPP), or proteins presenilin 1 (PS1) and presenilin 2 (PS2), which are directly involved in the AβPP processing. While cleavage of AβPP at the plasma membrane by the α-secretase occurs without formation of pathologic amyloid-β (Aβ) peptides, cleavage with β- and γ-secretases leads to the release in the extracellular space of Aβ peptides with 40 or 42 residues where $Aβ_{42}$ is more prone to aggregation and is the major component of extracellular amyloid plaques [5, 6]. Along with

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the formation of extracellular aggregates, Aβ peptides are present in neurons [5, 7]. Multiple studies conducted *in vitro* and *in vivo* using human tissue and transgenic mice demonstrated that intracellular Aβ accumulates prior to the development of extracellular plaques where it specifically affects synaptic function leading to a profound memory deficit [8–10]. The existence of intraneuronal Aβ could be explained by multiple mechanisms. Besides the plasma membrane, AβPP is present at several intracellular sites including the trans-Golgi network [11], endoplasmic reticulum (ER), and endosomal, lysosomal [12], and mitochondrial membranes [13] where Aβ could be generated via β- and γ-secretase cleavage. In addition, secreted Aβ peptides could be internalized via receptor-mediated or/and receptor-independent endocytosis [14–16]. Extensive studies also support the notion that soluble Aβ oligomers represent the most toxic species that affect multiple early molecular mechanisms leading to synaptic dysfunction in AD [15].

Intracellular neurofibrillary tangles (NFT) represent another hallmark of AD. Tau is a microtubule stabilizing protein. When it becomes hyperphosphorylated, it dislocates from the microtubules leading to their destabilization and a disruption of neuronal trafficking machinery [17]. Aβ-induced translocation of Tau to neuronal spines is associated with synaptic dysfunction early in AD pathogenesis [18]. The definitive diagnosis of AD can only be done by examining the postmortem brain tissue based on the presence of extracellular plaques formed by Aβ peptides, intracellular NFTs comprised of hyperphosphorylated Tau protein (pTau), Aβ deposits in blood vessels, neuronal and synaptic loss, and significant atrophy in selective brain regions involved in cognitive function (hippocampus, entorhinal and frontal cortices) [19]. The identification of familial AD mutations in *APP*, *PS1*, and *PS2* genes gave rise to the amyloid cascade hypothesis that considered the formation of Aβ a culprit of the disease. While excessive production of Aβ peptides is observed early in patients that develop AD and is essential for AD pathology [20], it is not sufficient. Some aged individuals have significant Aβ load, but do not develop cognitive impairment [21, 22]. Recent studies conducted using positron-emission tomography (PET) and novel tracers that allow imaging of both amyloid and Tau distribution in the brain of living individuals suggest that there is a relationship between Tau protein deposition, Aβ plaques, and neurodegeneration [23]. Based on the pattern distribution and the

manifestation of cognitive symptoms, it appears that the widespread presence of Aβ in the brain does not lead to the development of AD without Tau being present in the affected areas. These observations support the idea that the synergistic interaction between Aβ and Tau is essential to trigger neurodegeneration in AD [24, 25]. While this provides important insights into AD patient's diagnostic and prognostic criteria, early molecular mechanisms leading to the accumulation of Aβ and pTau or driving factors that promote their spreading in the brain remain poorly understood hindering the development of efficacious therapeutic interventions [26–29].

## THE ROLE OF OXIDATIVE STRESS IN ALZHEIMER'S DISEASE

In search for the underlying mechanisms of AD, the amyloid cascade hypothesis that dominated the field of AD research for the past decades has been challenged [30–32]. An alternative explanation of the disease mechanism has emerged from the observations linking mitochondrial dysfunction and increased production of reactive oxygen species (ROS) to the development of AD. The mitochondrial cascade hypothesis states that in sporadic, late-onset AD, loss of mitochondrial function associated with age affects the expression and processing of AβPP initiating Aβ accumulation [33]. Mitochondrial dysfunction has been well documented in AD [34, 35]. Abnormal mitochondrial axonal trafficking is already observed in embryonic neurons from multiple transgenic mouse models of familial AD with additional abnormalities in fission, fusion, and function detected prior to the development of amyloid plaques or memory impairment [36–50]. Brain glucose metabolism measured using fluorodeoxyglucose-positron emission tomography (FDG-PET) is reduced prior to the onset of disease in several groups of at-risk individuals including patients with mild cognitive impairment (MCI), a prodromal stage of AD, and in carriers of the apolipoprotein E epsilon-4 (ApoE4) allele, a strong genetic risk factor for late-onset AD. However, this hypometabolism does not correlate with an increase in brain Aβ deposition [51–53]. Furthermore, disruption in glucose metabolism associated with early mitochondrial dysfunction detected in multiple animal models and AD patients [38, 41, 43, 48, 54–60] may also be a direct determinant of oxidative stress and synaptic dysfunction that contribute to early disease mechanisms before any evidence of Aβ or

Tau pathology [48, 61–63]. In the brain, the free energy necessary to drive most cellular reactions is primarily produced in mitochondria from the oxidation of glucose under aerobic conditions (Fig. 1). Oxidative stress, which is defined as 'an imbalance in pro-oxidants and antioxidants with associated disruption of redox circuitry and macromolecular damage' [64], is associated with increased production of ROS and reactive nitrogen species (RNS) including superoxide radical anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($HO^-$), nitric oxide (NO), and peroxynitrite ($ONOO^-$). While there are multiple sources of ROS production in the cell including ER, peroxisomes, a family of NADH oxidases, and other enzymes such as monoamine oxidases [65, 66], mitochondria are the largest contributor to ROS production (Fig. 1) [67, 68]. During oxidative phosphorylation, $H_2O_2$ and $O_2^-$ are produced as byproducts in mitochondria primarily by complexes I and III [69]. Under normal conditions, the antioxidant enzymes acting as free radical scavengers mediate levels of ROS. These include superoxide dismutases (SOD), glutathione peroxidase (GPX), glutaredoxins, thioredoxins, and catalase (Fig. 1). Additional mechanism of protection against oxidative stress involves the activation of nuclear factor erythroid-2-related factor 2 (Nrf2). Nrf2 is a transcription factor negatively regulated by its binding to the cytoplasmic repressor and stress sensor Kelch-like ECH associated protein 1 (KEAP1), which acts as a substrate adaptor to mediate ubiquitination and degradation of Nrf2 by the E3 ubiquitin ligase Cullin-3 [70]. In the presence of electrophiles and oxidants, KEAP1 releases Nrf2 with its subsequent translocation to the nucleus where it activates transcription of cytoprotective genes via promoter sequences containing conserved antioxidant response elements (AREs) [71, 72]. This increases levels of antioxidant enzymes
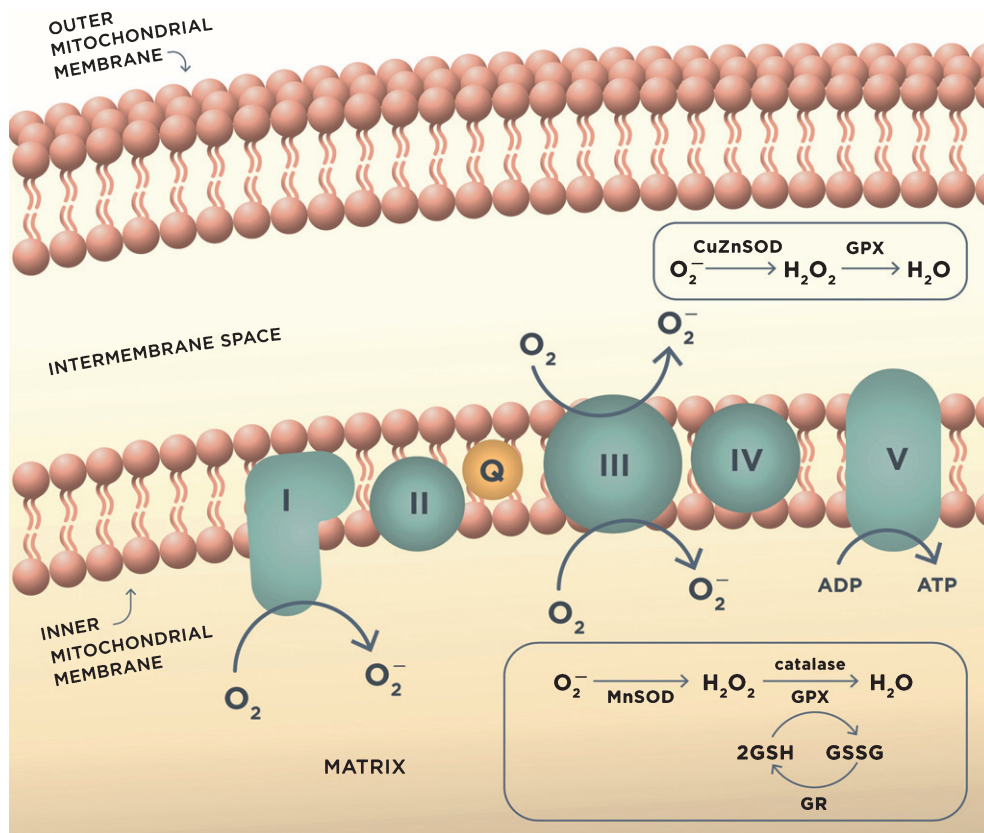


Fig. 1. ROS production in mitochondria during oxidative phosphorylation and antioxidant mechanisms. Complex I and complex III of the mitochondrial electron transport chain are the major sites of superoxide anion ($O_2^-$) production during aerobic respiration. $O_2^-$ is converted to $H_2O_2$ by MnSOD or CuZnSOD in the intermembrane mitochondrial space. $H_2O_2$ is further reduced to water by detoxifying enzymes glutathione peroxidase (GPX) or catalase. GPX uses reduced glutathione (GSH) as the reductant, and the resulting oxidized glutathione reacts with another glutathione molecule to form glutathione disulfide (GSSG), which is restored to GSH by the enzyme glutathione reductase (GR). These reactions occur in mitochondrial matrix.

and proteins such as glutathione-S-transferase, NAD(P)H: quinone oxidoreductase-1, SOD, GPX, heme oxygenase-1 (HO-1), glutamate cysteine ligase, thioredoxin, and catalase, and also promotes mitochondrial biogenesis ensuring a replacement of damaged organelles [73, 74]. However, there is conflicting evidence on whether Nrf2 is activated in AD. In one study, levels of Nrf2 expression were found to be decreased in AD patients despite the presence of oxidative stress [75]. Other studies reported an increase in the expression of the ARE-related genes in patients with MCI and AD [76, 77]. While the exact mechanism is presently unknown, these discrepancies could be associated with the variations in the levels of Nrf2 expression that could be influenced by aging and the disease mechanisms [78].

The balance between ROS production and the antioxidant defense is essential for normal cellular function. However, in AD, the activity of antioxidant enzymes is altered, thereby contributing to the unconstrained accumulation of oxidative damage [79]. When unbalanced, overproduction of ROS combined with the insufficient antioxidant defense leads to oxidative stress [80]. There is evidence that mitochondrial damage resulting in increased production of ROS contributes to the early stages of AD prior to the onset of clinical symptoms and the appearance of the Aβ pathology [80]. In support, markers of oxidative stress including high levels of oxidized proteins, glycosylated products, extensive lipid peroxidation, formation of alcohols, aldehydes, free carbonyls, ketones, cholestenone, and oxidative modifications in RNA and nuclear and mitochondrial DNA were found in postmortem brain tissue and in peripheral systems including cells and isolated mitochondria from people with preclinical or early stages of AD and ApoE4 carriers (Fig. 2) [58, 81–97]. Mitochondrial ROS could collapse mitochondrial membrane potential accelerating ROS production within the same organelle (Fig. 3). As a result, an increase in ROS production in a small subset of organelles could be sufficient to propagate ROS damage to other mitochondria eventually affecting the whole cell [66].

Compelling data demonstrate that in addition to mitochondrial ROS production, abnormal homeostasis of bioactive metals including iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), and aluminum (Al) could be involved in free radical production and oxidative stress influencing Aβ and Tau aggregation [35, 98–100]. Increased levels of Fe, Cu, and Zn were detected using proton
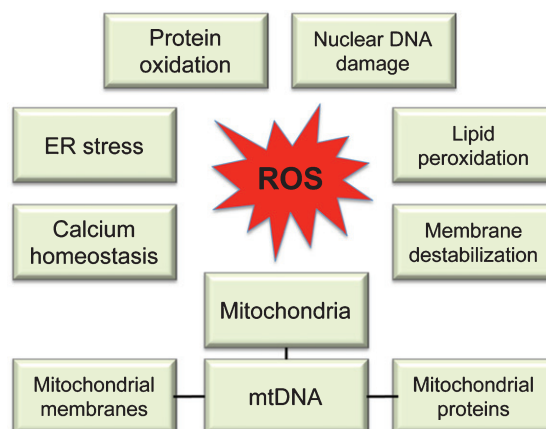


Fig. 2. Molecular targets of ROS. While multiple sites in the cell can contribute to ROS production, uncontrolled ROS generation in mitochondria could impair a major source of energy in the cell resulting in detrimental consequences to the whole cellular environment. Intermediate levels of ROS can gradually affect multiple cellular functions including loss of synaptic activity, while critically damaged mitochondria can trigger a release of cytochrome *c* activating apoptosis.

induced X-ray emission, immunohistochemistry, and synchrotron X-ray fluorescence in close proximity to the amyloid plaques in the brain tissue of AD patients and transgenic mouse models of AD [101–108]. The accumulation of these metals in the first place is thought to originate from the impaired neuronal metal homeostasis affected by aging, and exacerbated by amyloid and Tau pathologies in case of AD [109–111] There is a tight connection between protein misfolding, aggregation, and metal ion homeostasis. In particular, Zn directly affects AβPP processing by binding to the protein [112], and Al, Zn, Fe, and Cu directly bind Aβ promoting its aggregation [113–115]. Similarly, the redox metals could promote Tau phosphorylation, its release from the microtubules, and formation of NFTs [116, 117]. ROS production is facilitated by the redox-active metals including Cu, Fe, and Mn using catalytic reactions similar to the Fenton reaction where metals convert $O_2^-$ and $H_2O_2$ to $HO^-$ species that are involved in lipid peroxidation [118]. Moreover, a direct binding of Aβ peptides to Cu or Fe has been shown to generate $H_2O_2$ [119]. Thus, the transition metals and Aβ could synergistically contribute to an increase in oxidative stress and extra-mitochondrial production of ROS. In agreement with a role of metal ions in pathology, restoration of metal dyshomeostasis after application of metal chelators [120–122] reduced levels of amyloid plaques and Aβ aggregation, and improved
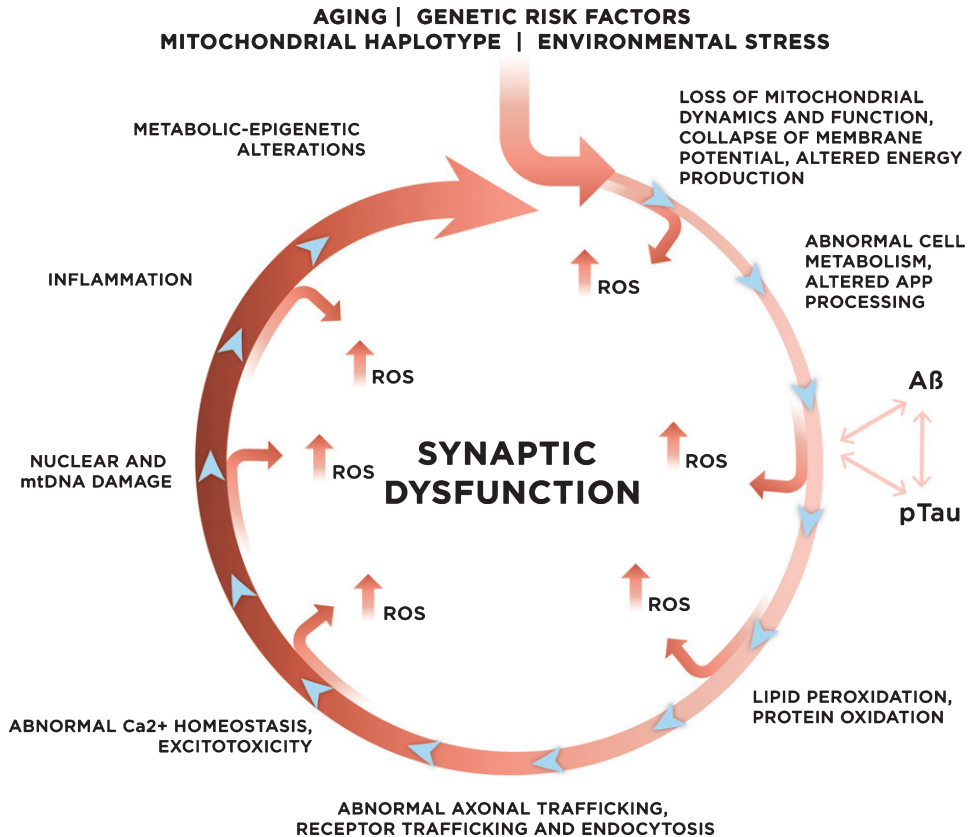
Fig. 3. Genetic and environmental risk factors contribute to the development of late onset sporadic AD. With age, increased mitochondrial dysfunction and ROS production could initiate a vicious cycle where multiple systems and mechanisms affected by ROS exacerbate ROS production, accelerating cellular damage, and leading to synaptic dysfunction.

cognitive performance in humans and mouse models of AD [123].

Another source of ROS production directly mediated by Aβ involves microglia activated in the brain during an inflammatory response to the deposition of extracellular amyloid plaques [124]. Further, increased levels of Aβ could accelerate a production of ROS by directly binding to mitochondrial membranes, altering mitochondrial dynamics and function, ultimately leading to the abnormal energy metabolism and the loss of synaptic function [35, 37, 39, 46, 62, 125, 126]. Membrane-associated oxidative stress induced by Aβ peptides perturbs ceramide and cholesterol metabolism that, in turn, triggers a neurodegenerative cascade leading to additional Aβ accumulation, Tau phosphorylation, and clinical disease (Fig. 3) [127–135]. Furthermore, there is a direct link between altered membrane lipids and mitochondrial function, which is detrimental for brain bioenergetics [136, 137]. Strong data generated in animal models and humans suggest an intimate

relationship between oxidative stress, Aβ accumulation, and abnormal Tau phosphorylation, where pTau specifically affects the activity of complex I synergistically contributing to the Aβ-mediated mitochondrial dysfunction and ROS production [138]. This could explain why accumulation of both Aβ and pTau may be required to initiate neurodegeneration in AD patients [23–25]. Moreover, emerging data suggest that mitochondria-mediated cellular bioenergetics could independently affect AβPP processing and Aβ production (Fig. 2) [139–145]. However, the details of causal relationship between oxidative stress, mitochondrial dysfunction, and Aβ and pTau accumulation in AD remain to be elucidated. Taken together, these data suggest that altered mitochondrial function, increased oxidative stress, exhausted antioxidant defense, production of Aβ and pTau, which furthermore affects mitochondrial function and ROS production, could represent a "vicious cycle" that with time exacerbates the disease process, eventually leading to neuronal death [47] (Fig. 3).

## OXIDATIVE STRESS AND SYNAPTIC DYSFUNCTION IN ALZHEIMER'S DISEASE

Synapses are structurally specialized regions in neurons that propagate an electrical or chemical signal from one cell to another. During neurotransmission, signaling molecules such as glutamate, acetylcholine, dopamine, and others released from the active zones of a presynaptic neuron bind to and activate receptors on a postsynaptic neuron (Fig. 4) [146]. The strength of synaptic transmission depends on changes in neuronal activity where the dynamic nature of synaptic plasticity including long-term potentiation (LTP) and long-term depression (LTD) represents the fundamental mechanism of learning and memory [147, 148]. Neurons have a unique cellular architecture where formation or pruning and maintenance of dendritic spines are essential for neurotransmission and synaptic function

(Fig. 4). Synaptic transmission critically relies on the fidelity of multiple cellular mechanisms including biosynthesis of neurotransmitters from amino acids to ensure their availability; the delivery of neurotransmitters to the sites of synapses requiring intact microtubule tracts and vesicle trafficking machinery; formation of synaptic vesicles that encapsulate neurotransmitters preparing for their release via exocytosis; binding of the neurotransmitter to the receptor on the postsynaptic neuron with subsequent activation of cellular response; and the removal of the neurotransmitter from the synaptic cleft after the release (Fig. 4) [149]. In addition, $Ca^{2+}$ plays an essential role in mediating basal synaptic transmission, where an increase of its conductance through voltage gated $Ca^{2+}$-channels clustered in the presynaptic membrane at the active zone triggers the release of synaptic vesicles [146]. Given the complexity of neurotransmission machinery, factors that affect any step of the process could have a detrimental effect on synaptic
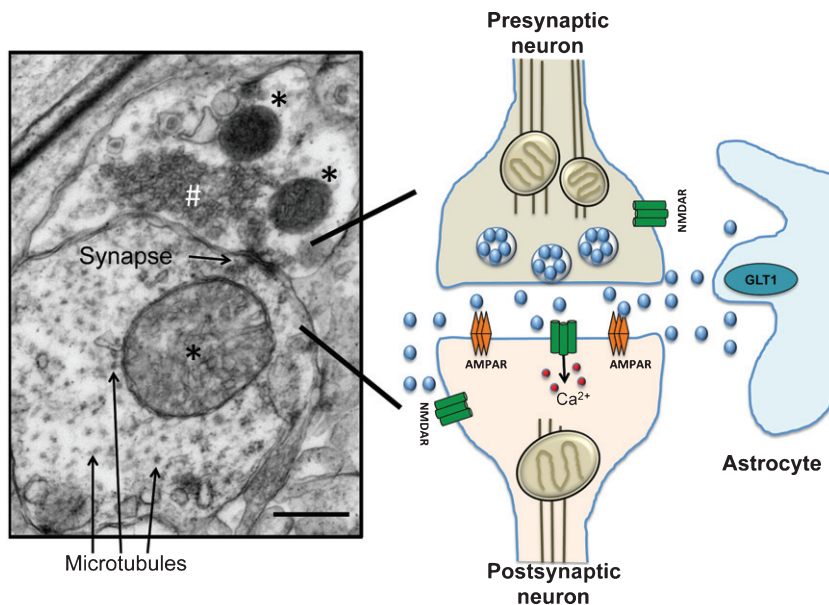


Fig. 4. Structure of a synapse. Left: synapse between two neurons observed in the brain tissue of a wild type C57/Bl6 mouse using transmission electron microscopy (generated in Dr. Trushina laboratory [231]). An arrow indicates electron dense plasma membrane at the synapse. Presynaptic neurons contain a large number of synaptic vesicles (#). Both presynaptic and postsynaptic neurons have mitochondria at the site of synapse (*), which are delivered along the microtubule tracks (indicated with arrows). Scale bar, 0.5 μm. Right: a simplified cartoon of a synapse. Glutamate (blue spheres) released from the presynaptic neuron in a voltage dependent manner, activates the NMDA glutamate receptors present on pre- and postsynaptic neurons. These include AMPA (orange) and NMDA (green) receptors among others. Glutamate is cleared from the synaptic cleft primarily by the glial cells transporters (GLT-1). It is then recycled to neurons, repackaged into synaptic vesicles, and used in another synapse. An inadequate glutamate clearance could lead to the spillover and activation of extrasynaptic NMDA receptors. Memantine is believed to prevent this particular activation. Excessive entry of $Ca^{2+}$ into presynaptic neuron (red spheres) could damage synaptic mitochondria leading to ROS production, altered synaptic transmission and neuronal dysfunction. This phenomenon is called excitotoxicity. Note that mitochondria are delivered to the site of synapse along the Tau-containing microtubule tracks. Destabilization of microtubules could affect mitochondrial localization and energy supply required for proper synaptic function.

function in neurons and, ultimately, on cognitive function.

AD is characterized by progressive memory impairment, which is associated with the inhibition of LTP and enhancement of LTD in the hippocampus [150]. Loss of synapses in the affected brain regions correlates best with cognitive impairment in AD patients and has been considered as the early mechanism that precedes neuronal loss [151–157]. Extensive studies conducted *in vivo* and *in vitro* support a direct relationship between oxidative stress and synaptic dysfunction in AD [39, 126, 158, 159]. In particular, it has been shown that independently and synergistically, ROS, Aβ, and pTau affect the activity of *N*-methyl-D-aspartate (NMDA) receptors. The NMDA receptors belong to the ionotropic family of glutamate receptors, which in coordination with $\alpha$-amino-3-hydroxy-5-methyl-4-isoxa-zolepropionic acid (AMPA) receptors regulate the excitatory synaptic transmission and plasticity in the brain playing an essential role in learning and memory [160, 161]. Activation of NMDA receptors allows $Ca^{2+}$ to enter the postsynaptic cells initiating a cascade of events that is critically involved in establishing LTP. The function of NMDA receptors declines with age, which could explain memory alterations associated with chronological aging. However, in AD, in addition to age-related changes, the expression of neurotoxic Aβ has been shown to reduce the amount of surface NMDA receptors in neurons and in brain tissue of AD mice [162], trigger NMDA-mediated $Ca^{2+}$ influx inducing excitotoxicity and stress-related signaling pathways, exacerbating aging-related increase in oxidative stress, impaired energy metabolism, defective $Ca^{2+}$ homeostasis, and altered regulation of transcription of genes important for neuronal development and plasticity [47, 163]. Memantine, the only FDA-approved drug for AD that is not an acetyl cholinesterase inhibitor, is a noncompetitive, low-affinity antagonist of NMDA receptors. Importantly, memantine has greater affinity to non-synaptic NMDA receptors, which are implicated in excitotoxicity associated with the glutamate spillover and have distinctly different composition of receptor subunits [164, 165]. In addition to the effect on NMDAR, soluble Aβ species have been shown to bind to AMPA receptors promoting their internalization via clathrin-mediated endocytosis after $Ca^{2+}$-induced activation of calcineurin [166]. Altered internalization of AMPA receptors affects synaptic plasticity inducing synaptic dysfunction and loss of dendritic spines (Fig. 4).

Another type of synapses in the central nervous system utilizes $\gamma$-aminobutyric acid (GABA), which is a major neurotransmitter that induces inhibitory effect. In AD, levels of GABA are decreased with disease progression, and reduced levels of expression of GABAergic receptors has also been noted [167]. Degeneration of basal forebrain cholinergic cells that directly project to the cortex and hippocampus is well-documented in AD [168]. The cholinergic system is also implicated in cognitive functioning, especially in attention, memory, and emotion. Extensive data generated in human tissue and multiple animal models of AD demonstrated severe deficit in the activity of multiple acetylcholine synthesizing and degrading enzymes, acetylcholine transporters and receptors involved in synaptic signaling, along with reduction of presynaptic cholinergic markers. These investigations provided compelling evidence for the development of one of the few therapeutic approaches currently FDA-approved for AD, cholinesterase inhibitors. This approach allows increasing levels of acetylcholine at synapses by blocking the activity of acetylcholinesterase and butyrylcholinesterase enzymes, which are involved in acetylcholine hydrolysis [169].

Among numerous mechanisms that connect neurotoxic Aβ, Tau, oxidative stress, and synaptic dysfunction in AD are excitotoxicity, oxidation of proteins, and lipid peroxidation (Figs. 2, 3). Application of Systems Biology approaches including metabolomics and epigenetics to study early changes associated with AD progression in plasma, CSF, and brain tissue from individuals with different severity of AD and multiple animal models of AD confirmed that major alterations in metabolic networks identified early in disease are directly relevant to changes in neurotransmitter, lipid, and energy metabolism [48, 63, 170, 171]. Aβ-induced excitotoxicity associated with an excessive influx of calcium in postsynaptic neurons can lead to a cascade of events that increases ROS production, oxidative stress, Tau phosphorylation, and lipid peroxidation, ultimately leading to synaptic dysfunction (Fig. 3) [172–174]. Alteration of structure and fluidity of plasma membrane associated with lipid peroxidation could affect the organization and function of dendritic spines, signaling pathways, receptor trafficking, and localization [175]. Indeed, alterations in lipid trafficking and metabolism affect membrane fluidity and lipid homeostasis early in ApoE4 carriers [92, 137, 176, 177]. Moreover, lipid peroxidation of mitochondrial membranes could directly affect the dynamic and function

of the organelle leading to reduced energy support at the sites of synapses, which is detrimental for brain bioenergetics [136, 137]. Altered mitochondrial fission, fusion, axonal motility, and function in turn could contribute to ROS production exacerbating synaptic function. As was mentioned earlier, Aβ-induced hyperphosphorylation of Tau destabilizes microtubule tracks, which alters axonal trafficking of mitochondria and their synaptic docking, and translocation of Tau to dendritic spines also may have a synergistic effect contributing to NMDA receptor destabilization, excitotoxicity, and increased oxidative stress with detrimental effect on synaptic function (Fig. 3). The role of protein oxidation in the mechanism of AD has been recently reviewed in [4].

## THERAPEUTIC STRATEGIES FOR ALZHEIMER'S DISEASE

Currently approved treatments for AD are limited to three cholinesterase inhibitors, donepezil, rivastigmine, and galantamine, and a low affinity NMDA receptor antagonist, memantine. None of these approaches are disease modifying; they do not provide a "cure" but rather symptomatic treatment for some individuals [178]. Moreover, failure of the recent clinical trials focused on production or clearance of Aβ peptides emphasizes the urgency to consider alternative molecular mechanisms in order to design interventions that will delay or alleviate the development of AD [179]. While compelling evidence implicates oxidative stress in the early molecular mechanisms of AD [180], there is no FDA-approved antioxidant therapy for AD. Moreover, while antioxidant experimental therapeutics produced promising results in animal models of AD [181–183], clinical trials either failed or delivered inconclusive results [184]. For example, multiple trials assayed the effect of a strong antioxidant vitamin E (alpha tocopherol) on cognitive function in cognitively normal and generally healthy women 65 years or older [185], in cognitively normal women with preexisting cardiovascular disease or cardiovascular disease risk factors 40 years or older [186], in people with MCI [187], in patients with moderate to severe AD [188], and in individuals with mild to moderate AD [189]. Positive results where statistically significant changes in cognitive performance were achieved after vitamin E administration compared to placebo were found only in people with mild to moderate AD [189]. Importantly, there were

no significant differences in the groups receiving memantine alone or memantine plus alpha tocopherol as a combination therapy. Moreover, meta-analysis of 19 randomized trials with vitamin E demonstrated its high toxicity and all-cause mortality at high doses [190]. Inconclusive results were also achieved in an open clinical trial where AD patients stably taking a cholinesterase inhibitor were supplemented with vitamin C and E over 1 year [191]. While oxidation of CSF lipids was significantly reduced after 1 year of the supplementation, the clinical course of AD did not differ between the vitamin-supplemented and the control group. Another failed trial involved the supplementation with vitamin E, C, and α-lipoic acid in patients with mild to moderate AD [192]. Despite a detection of reduced levels of markers of oxidative stress in CSF, a rapid cognitive decline observed in treated group raised significant safety concerns. Similar results were obtained in clinical trials with curcumin, a polyphenolic compound that has been demonstrated to have antioxidant and anti-inflammatory effects in preclinical studies [193]. Comprehensive update on the outcomes of the antioxidant treatments in recent clinical trials was provided in recent reviews [194, 195].

Multiple challenges associated with the design of clinical trials in elderly and the lack of a complete understanding of the molecular mechanism of antioxidant therapy may account for such diverse outcomes. First of all, there is no definitive test to diagnose AD in living individuals. The conclusive diagnosis of AD can only be done after the examination of postmortem brain tissue for the presence of amyloid plaques and NFTs. This introduces some ambiguity in the etiology behind cognitive impairment in the subjects recruited for clinical trials. Next, it is important at what stage of the disease the treatment is administered since some of the interactions may be efficacious only at the early stages. Furthermore, clinical trials in elderly are associated with the relatively small number of participants and short period of treatment, high frequency of death, inconsistent use of medication, and a lack of a follow-up data. However, there are clinical trials in progress that have greater number of participants and extended periods of treatment that may provide better results on the effect of the antioxidant therapy in AD [196, 197]. Along with the trials designed to test efficacy of a single compound found beneficial in preclinical trials, combination therapy for AD may hold a promise [198]. This approach includes treatment with multiple compounds with diverse properties that could improve several mechanisms and functions

altered in AD without adverse side effects. In one of such trials, the administration of a nutraceutical formulation that included folate, alpha-tocopherol, B12, S-adenosyl methioinine, N-acetyl cysteine, and acetyl-L-carnitine to the AD patients over 1 year resulted in stabilization of cognitive function [199]. Similar antioxidant cocktails were shown beneficial in improving memory and cognitive performance in community-dwelling adults without dementia [200, 201].

In recent years, it has become apparent that strategies designed to target total ROS in the organism might not be productive since ROS have dual function. On one hand, increased ROS production contributes to age-related chronic conditions and neurodegeneration [47]. On the other, oxidant species, such as superoxide and hydrogen peroxide, can function as signaling molecules in a broad array of essential redox-dependent signaling pathways that are critical for the organismal survival including epidermal growth factor receptor signaling [202], inactivation of the tumor suppressor PTEN [203], circadian rhythms [204], the inflammatory response [205], and hormetic stress response [206–209]. Redox homeostasis with tight control over levels of ROS production is essential to protect cells from oxidative stress and, at the same time, to ensure presence of the important signaling molecules [210]. Thus, understanding how the dual role of ROS is maintained with age and in the context of different stages of the disease is important for the development of therapeutic approaches that target ROS production and clearance.

Based on the recognized contribution of mitochondria to cellular ROS, the development of novel antioxidants that directly target mitochondria represent a promising approach to mitigate local ROS production compared to the reduction of global levels of ROS. These compounds include coenzyme Q10, idebenone, creatine, MitoQ, MitoVitE, MitoTEMPOL, latrepirdine, methyleneblue, triterpenoids, series of Szeto-Schiller (SS) peptides, curcumin, Ginkgo biloba, and omega-3 polyunsaturated fatty acids. These mitochondria-targeted compounds have been extensively evaluated in multiple laboratories using various *in vivo* and *in vitro* models of AD where some of them including a peptide, 6'-dimethyltyrosine-Lys-Phe-NH$_2$ (SS31), have been shown very efficacious in protecting against Aβ-induced oxidative stress, synaptic loss, mitochondrial dysfunction, and abnormal calcium homeostasis [62]. Some of these compounds demonstrated

promising results in clinical trials [211, 212]. Moreover, emerging data demonstrate that partial inhibition of OXPHOS with pharmacological inhibitors is beneficial in preventing obesity and type II diabetes, another risk factors contributing to AD [213–216], and promoting longevity in model organisms and in humans [217–220]. In particular, modulation of mitochondrial Complex I activity with small molecules was found efficacious in cognitive protection in multiple mouse models of AD [221] and in extending lifespan [222]. However, the details of molecular mechanism remain to be determined.

While supplementation with antioxidants so far appears to produce little modifying effect on AD development, non-pharmacological treatments and lifestyle interventions including exercise and caloric restriction have gained significant attention due to their overall positive effect on health and life span [223]. Specifically, grounded on a population-based perspective, the Alzheimer's Association has identified regular physical exercise as one of the strategies to reduce the risk of cognitive decline and the development of dementia [224]. Indeed, regular physical activity was associated with reduced oxidative stress, increased antioxidant capacity, increased anti-inflammatory effects, reduced levels of ceramides that are elevated in AD, improved Aβ clearance associated with the upregulating Aβ transporters, and induced neurogenesis [223, 225, 226]. The molecular mechanisms implicated in the beneficial effect of exercise are not fully understood. One of the explanations is based on the concept of mitohormesis, which suggests that an exposure to low continuous or higher intermittent sub-lethal doses of exercise-associated stress could lead to a mitochondrial adaptation by inducing changes in gene expression through exercise-sensitive transcription factors such as PGC1α, mtTFA, NF-κB, HIF-1, and p53. Downstream effects result in increase in mitochondrial biogenesis and antioxidant response. Potential signaling factors that mediate this mitochondria-nuclear communication may include ROS, calcium, mitochondrial unfolded protein response, mitochondrial metabolites, and mitokines [66, 227]. In addition to exercise, modulation of diet, especially caloric restriction, has been shown not only to extend lifespan, but also to protect against cognitive decline [228, 229]. However, a recent study demonstrated that meals rich in saturated fat and foods with a high glycemic index have differential effect in adults with and without cognitive impairment [230]. In individuals without cognitive impairment,

a consumption of high caloric food worsened cognitive performance, whereas consumption of high caloric food was beneficial in adults with cognitive impairment or the ApoE4 carriers. The authors also found that levels of Aβ in plasma were affected by meal type, suggesting a relationship between metabolic response and amyloid regulation. Therefore, a better understanding of the effect of diet modifications and exercise on metabolism, mitochondrial function and ROS production during different stages of disease progression is needed to develop safe and efficacious therapeutic strategies for AD.

## CONCLUSIONS

Multiple lines of evidence provide strong support for the involvement of oxidative stress in the development of AD. At the same time, limited success of antioxidant therapies achieved to date emphasizes the need for better understanding of molecular mechanisms associated with different stages of AD development. Moreover, the dual role of ROS in essential neuroprotective cellular mechanisms versus detrimental effects of increased uncontrolled ROS production should be carefully considered while developing strategies to mitigate oxidative stress in neurodegenerative diseases.

## ACKNOWLEDGMENTS

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