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Mitochondrial Dysfunction and Synaptic Transmission Failure in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a chronic neurodegenerative disorder, in which multiple risk factors converge. Despite the complexity of the etiology of the disease, synaptic failure is the pathological basis of cognitive impairment, the cardinal sign of AD. Decreased synaptic density, compromised synaptic transmission, and defected synaptic plasticity are hallmark synaptic pathologies accompanying AD. However, the mechanisms by which synapses are injured in AD-related conditions have not been fully elucidated yet. Mitochondria are a critical organelle in neurons. The pivotal role of mitochondria in supporting synaptic function and the concomitant occurrence of mitochondrial dysfunction with synaptic stress in postmortem AD brains as well as AD animal models seem to lend the credibility to the hypothesis that mitochondrial defects underlie synaptic failure in AD. This concept is further strengthened by the protective effect of mitochondrial medicine on synaptic function against the toxicity of amyloid- β , a key player in the pathogenesis of AD. In this review, we focus on the association between mitochondrial dysfunction and synaptic transmission deficits in AD. Impaired mitochondrial energy production, deregulated mitochondrial calcium handling, excess mitochondrial reactive oxygen species generation, and release play a crucial role in mediating synaptic transmission deregulation in AD. The understanding of the role of mitochondrial dysfunction in synaptic stress may lead to novel therapeutic strategies for the treatment of AD through the protection of synaptic transmission by targeting to mitochondrial deficits.

Keywords

Alzheimer's disease; mitochondrial dysfunction; synaptic injury; synaptic mitochondria; synaptic transmission

Introduction

Characterized by progressive cognitive decline, Alzheimer's disease (AD) is the most common type of dementia attacking the aged population [1]. Among the known risk factors,

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age is the leading one in particular for the sporadic form of AD, with the incidence of this neurological disorder dramatically rising in an exponential pattern after 65 years of age [2–4]. The pathological hallmarks of AD include extracellular and intracellular deposition of amyloid beta (A β) [5–9]. Characterized by progressive cognitive decline, Alzheimer's disease (AD) is the most common type of dementia attacking the aged population [1]. Among the known risk factors, age is the leading one in particular for the sporadic form of AD, with the incidence of this neurological disorder dramatically rising in an exponential pattern after 65 years of age [2–4]. The pathological hallmarks of AD include extracellular and intracellular deposition of amyloid beta (A β) [5–9], neurofibrillary tangles (NFTs) [10–12], synaptic failure [13–16], and neuronal loss [14, 17] in AD sensitive brain regions such as the hippocampus, neocortex, and nucleus basalis of Meynert [10, 18]. Clinical and pathological studies have shown a strong correlation between synaptic deficits and the degree of memory loss in AD [13–16]. Of note, discernible changes of synapses have been detected in the neocortex of patients, even in the very early stage of AD or with mild cognitive impairment (MCI), who have little or mild A β plaques and neurofibrillary tangles as well as neuronal death [19]. Such findings indicate that AD is a disease of synaptic dysfunction [20–25].

In the central nervous system (CNS), synapses are the contact sites of neurons to pass signals via synaptic transmission, also known as neurotransmission. Electric neurotransmission plays a critical role in the development of the nervous system, while the communications between CNS neurons in fully developed adults are predominantly dependent on the function of chemical synapses, at which neurotransmitters are released from the presynapses and target postsynaptic receptors to induce the downstream cascades related to synaptic activity [21, 26]. In recent years, increasing lines of evidence have implicated the essential and unique role of mitochondria in synaptic transmission. It is proposed that mitochondria are involved in each and every stage of neurotransmission including the synthesis and storage of neurotransmitters, the trafficking of synaptic vesicles (SVs), and the release of neurotransmitters from the presynapses, as well as the recycling of SVs [27–30]. It is generally accepted that mitochondria support synaptic transmission primarily via their functions in maintaining calcium homeostasis, providing energy, and regulating the production of reactive oxygen species (ROS), as well as synthesizing essential intermediates or final products of several neurotransmitters [27–30]. Conceivably, mitochondrial deficits impair synaptic activity at pathological states [30–33]. Indeed, the concomitant occurrence of synaptic stress and mitochondrial dysfunction has been repeatedly identified in many neurodegenerative diseases including AD [33, 34]. Furthermore, the protection of mitochondrial function preserves synaptic activity in AD-related pathophysiological settings in both basic and clinical platforms [35, 36], reinforcing the direct link between the two pathological processes accompanying AD. Thus, elucidating the relationship between mitochondrial abnormalities and synaptic injury in AD will foster us a better understanding of the pathogenesis of this neurodegenerative disease and hopefully lead to the development of therapeutic strategies for the protection of synaptic activity and the subsequent cognitive function.

Here, we focus on mitochondrial pathology in AD and discuss the link between mitochondrial dysfunction and chemical synaptic transmission impairment in this chronic neurological disorder.

Synaptic Transmission Changes in AD

It is a well-documented notion that synaptic activity is the basis of cognition and weakened synapses are closely associated with cognitive deficits [37]. In the past decades, histological studies have determined that synaptic alterations including synaptic loss, altered synaptic architecture, and compromised synaptic transmission as well as defected synaptic plasticity are characteristics of AD and the aging brains [21, 38–41]. Importantly, the degree of these synaptic changes correlates with the severity of cognitive decline in AD [21]. Although the molecular mechanisms causing synaptic failure in AD have not been elucidated in detail, A β toxicity, tau hyperphosphorylation, and mitochondrial dysfunction have been implicated as critical involving factors conjointly disrupting synapses [22, 25, 34, 42–44].

Alterations of neurotransmitters in AD

Neurotransmitters are a group of small molecules serve as the chemical messengers to transmit signals from one neuron to another or to other cell types. Ever since the identification of acetylcholine (ACh) and norepinephrine (NE) as neurotransmitters, currently there are more than 100 types of neurotransmitters on the list, which is still expanding [45]. The classic and very simplified definition of a neurotransmitter is a chemical that has been synthesized, then packaged and stored in SVs in presynapses, and ready to be released to synaptic clefts to regulate the excitatory state of other cells by acting on the receptors at postsynapses. By their influence on the excitatory state of the cells, neurotransmitters can be categorized into two clear-cut groups, excitatory and inhibitory [45]. It should be noted that some neurotransmitters such as ACh may have both excitatory and inhibitory effects depending on the types of receptors at the postsynapses [46, 47].

Owing to the progress in neurochemistry, neurotransmitter deregulation in AD brains has been intensively studied since the mid-twentieth century and still remains a target of great interest to date. The study on cholinergic system in AD dates back to the discovery of ACh [48–53]. Pathological studies of AD brains have found that severe lesions in nucleus basalis of Meynert, hippocampus, and neocortex are prominent in postmortem AD brains [54]. The hypothesis of cholinergic system deregulation in AD is built on the abundance and critical function of cholinergic neurons in these brain regions. This concept has been subsequently supported by the findings of severe degeneration of cholinergic neurons, reduced ACh synthesis and levels, decreased choline levels as well as down-regulated activity of choline acetyltransferase (ChAT), and the deregulations of ACh receptors in AD brains [47, 55–61]. Moreover, the concentration of ACh in the cerebrospinal fluid (CSF) extracted from AD patients is significantly reduced, and the levels of CSF ACh are proportionate to the severity of cognitive deficits in AD [62–64]. These observations have confirmed the perturbations of cholinergic system in the development of AD and further implicated the possibility of using CSF ACh as a diagnostic biomarker of AD. Indeed, efforts to rescue ACh deficits in AD by the application of acetylcholinesterase inhibitors such as donepezil, rivastigmine, and galantamine have demonstrated benefits for the treatment of AD [65, 66]. Despite their modest benefits and side-effects, acetylcholinesterase inhibitors are among the very few agents currently used as the first-line treatment for AD.

Another critical neurotransmitter associated with AD is glutamate, which is a member of amino-acid neurotransmitter family. Glutamate is the most plentiful excitatory signal messenger for a majority of synapses in neocortex and hippocampus [67, 68]. The ionotropic glutamate receptors, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and n-methyl-D-aspartate receptor (NMDAR), play a key role in mediating synaptic transmission and synaptic plasticity [69, 70]. Although glutamate carries important physiological function, excess glutamatergic activation causes prolonged calcium overloading at postsynapses that perturbs mitochondrial function and some key intra-cellular signaling transduction pathways, eventually leading to severe synaptic injury. The devastating effects of glutamate excitotoxicity on synaptic activity and neuronal survival have been discerned in a variety of pathological scenarios including AD [71, 72]. A study using magnetic resonance spectroscopy measured glutamate plus glutamine (Gx) levels in bilateral posterior cingulate gyrus of AD and MCI patients as well as the age-matched non-AD controls, and found significant decreased Gx levels in AD brains; while the levels of glutamate in MCI brains were comparable to those in the control subjects [73]. However, another study showed no change in the levels of Gx in the frontal lobes from probable AD [74]. The results seem to suggest that the cerebral Gx levels are significantly changed at the late stage of AD. But it cannot be excluded that the changes of Gx in AD brains is region-specific. This needs further investigation. Interestingly, the studies on glutamate and glutamine in CSF from AD patients have found decreased glutamine [75] while significantly increased glutamate levels [76–79], which does not match the results collected from the AD brains. Although the mechanisms of this disparity are not clear, the aforementioned evidence at least suggests the dysmetabolism of glutamate/glutamine in AD-related pathological settings. Other than the changes of glutamate, alterations of glutamate receptor, NMDAR in particular, have also been observed in AD brains. Evidence has shown the hypersensitivity of NMDAR to glutamate in AD brains and the resultant glutamatergic toxicity leads to synaptic injury. Such NMDAR hypersensitivity might be the result of A β -induced intracellular calcium elevation and magnesium removal from the receptor [71, 80]. Apart from the direct role of A β in enhancing NMDAR sensitivity, A β may also impose indirect disruptive regulation on NMDAR activity, thus reducing the NMDAR-associated long-term potentiation. In addition, mitochondrial dysfunction-associated neuronal oxidative stress, calcium dysmetabolism, and ATP deficiency are as well proposed to contribute to the glutamate excitotoxicity of AD neurons [81–84].

Interestingly, a significant reduction of the density of NMDAR has been detected in AD neocortex and hippocampus, where A β deposition is heavy [85–87], which may underlie NMDAR hyposensitivity in the late stage of AD. In consistent with the influence of glutamatergic toxicity on synaptic function, memantine, a glutamate receptor antagonist has demonstrated some modest protection of memory loss in AD patients [88–90]. In addition to glutamate, gamma-aminobutyric acid (GABA) is another amino-acid neurotransmitter whose deregulation has been linked to synaptic stress in AD brains. Decreased levels of GABA, an inhibitory neurotransmitter, has been reported in patients with severe AD [91]. The deficits of GABAergic system are thought to contribute to synaptic perturbations through the interrupted crosstalk between GABAergic, cholinergic, and glutamatergic synapses due to the loss of inhibitory function of GABAergic neurons [92].

Alterations in monoamine neurotransmitters have also been reported in AD patients. The lesions in locus coeruleus, the major brain area for the synthesis of NE, and reduced brain NE levels have been implicated in memory loss and psychiatric symptoms in AD [93, 94]. The supplementation of NE has exhibited protection against A β -induced mitochondrial dysfunction and neuronal stress in cultured mouse neurons. Furthermore, the administration of adrenergic receptor agonists attenuates A β toxicity-associated cognitive impairment in mice; such protective effects are thought to be the result of enhanced NE release [95, 96]. Another example of deregulated monoamine neurotransmitter in AD is dopamine. Dopaminergic system defects, which are generally implicated in the pathogenesis of Parkinson's disease (PD), have as well been linked to AD [97, 98]. A previous study has compared the difference of dopaminergic system deficits in PD, AD, and AD with parkinsonism and found that although all of them have loss of presynaptic dopamine receptor D2 (DRD2) and decreased levels of tyrosine hydroxylase, these three groups demonstrated distinct patterns of lesions sites, which may explain the difference in extrapyramidal symptoms between each of them [99]. In addition, the dopaminergic changes were almost restricted to the nucleus accumbens in AD patients absent of PD-like symptoms, while the AD with parkinsonism group showed more severe damages in rostral caudate and putamen. In sharp contrast, the PD group had more vulnerability in the substantia nigra and ventral tegmental region [99]. Intriguingly, when they compared the levels of postsynaptic DRD2, DRD2 was preserved in the examined extrapyramidal system in AD groups but not the AD with parkinsonism group [100]. A more recent study has suggested that dopamine may mitigate cognitive impairment in AD at least in part through the interaction of dopaminergic and cholinergic systems [101]. However, although emerging evidence has highlighted the function of the dopaminergic system in the consolidation of memory [102, 103], the correlation between dopaminergic system deficits and AD cognitive impairment has not yet been comprehensively investigated.

Neurotransmitter alterations and their contribution to the development of AD are still a fast growing topic. Importantly, current pharmaceutical therapies for AD treatment are predominantly stemmed from the studies on neurotransmitters. With the identification of new neurotransmitters and the progress in AD research, in-depth studies on neurotransmitters in AD still hold promise for the future development of novel therapeutic strategies to protect synaptic function and memory in AD.

Deficits of synaptic vesicle cycling in AD

Neurotransmitter release and reuse are through SV cycling. Stepwise, SV cycling includes the loading of neurotransmitters into SVs, the SV trafficking to and docking at the active zone of presynapses, the releasing of neurotransmitters, and at last the endocytosis and recycling of SVs [104]. In addition to synaptic loss, dramatically decreased expression levels of SV proteins and their coding mRNAs have been discerned in vast areas of postmortem AD brains including most part of the neocortex, limbic system, basal ganglia, and cerebellum; and the changes have a strong correlation to cognitive impairment [19, 105–111]. It should be mentioned that the severity of these changes varies; and the relatively AD sensitive brain regions such as hippocampus, frontal, temporal, and parietal lobes, where there are more severe A β deposition, tau pathology, and mitochondrial damages, are early

affected areas and demonstrate a greater loss of SV proteins and their coding mRNAs [108–111]. Another prominent change potentially leading to defected SV cycling in AD is the perturbations of signaling transduction pathways including cyclic-AMP-dependent protein kinase A (PKA), Ca²⁺/calmodulin-dependent kinase II (CaMKII), and protein kinase C (PKC). Those signaling transduction pathways play critical roles in modulating the functional status of several SV and synaptic plasma membrane proteins via phosphorylation, thus potentiating presynaptic activity. In fact, deregulated activities of CaMKII [112–115], PKA [116, 117], and PKC [118, 119] have been repeatedly linked to compromised synaptic activity and neuronal death in AD. The dysfunction of these transduction pathways is at least in part associated with calcium dysmetabolism, ATP deficiency, and oxidative stress in AD neurons [112, 120].

Direct evidence of altered SV cycling in AD-relevant conditions was obtained predominantly in cultured neurons given the difficulty in recording SV exocytosis and endocytosis in an *in vivo* setting in AD patients or AD animal models. In a previous study, A β at nanomolar level induces severely impaired synaptic transmission in cultured mouse hippocampal neurons. With A β -induced reduction in the expression levels of SV proteins and postsynaptic receptors, the exposure of A β significantly disrupts SV depletion and recycling [121]. Furthermore, the effects of tau abnormalities on SV exo- and endocytosis through the damages on the integrity and functionality of microtubules have also been proposed to underlie SV mobilization in AD [122].

Therefore, a large number of direct and indirect evidence have pointed to the deregulation of neurotransmitters and SV exo- and endocytosis in AD-relevant pathological settings. To elucidate the mechanisms underlying the synaptic transmission deficits is of paramount importance for our understanding of synaptic injury and cognitive decline in AD.

Mitochondria and Synaptic Transmission

Mitochondria are a key organelle in eukaryotic cells. In addition to their role in energy metabolism, mitochondria have multiple traits of critical functions including maintaining appropriate regulating intracellular calcium homeostasis, intracellular redox balance, and mediating cell apoptosis and necrosis. Thus, mitochondria have a fundamental role in the life and death of cells. Of note, strong evidence has suggested the multiplicity of mitochondrial functions. Some mitochondrial functions are even cell-type specific. For example, mitochondria in steroidogenic cells carry the function of steroid hormone biosynthesis [123]; and mitochondria are essential sites for fatty acid synthesis in adipose and liver tissues [124–126]. Moreover, mitochondria have been found to be involved in RNA metabolism [127]. Chen and colleagues have reported the role of mitochondria in cell innate immune system mediating cellular anti-viral pathways [128]. It is therefore not surprising that more and more investigators have embraced the concept of mitochondrial heterogeneity. It is said that mitochondria are morphologically and functionally heterogeneous in different cell types as well as in different compartments of a single cell. Neurons represent a typical pattern of mitochondrial heterogeneity. Based on their physical position and functionality, neuronal mitochondria are categorized into several different subgroups, among which

mitochondria at synapses or namely synaptic mitochondria are generally accepted to play an important role in supporting synaptic activity.

Synaptic mitochondria are a subpopulation of neuronal mitochondria specifically residing at synapses. Due to their extremely physical proximity to synapses, synaptic mitochondria are critical in assisting synaptic transmission.

Mitochondria and neurotransmitter synthesis and storage

The synthesis and packaging of some key neurotransmitters significantly rely on mitochondria. For example, ACh is synthesized from choline and acetyl co-enzyme A (Acetyl CoA); and the reaction is catalyzed by the enzyme called ChAT. Humans directly obtain choline from the diet; while Acetyl CoA is synthesized at mitochondria by using pyruvate from the glycolysis [129–136]. The synthesized ACh is subsequently transported into SVs through ACh transporter (VACHT) [137] via an ATP consuming process and then stored in SVs [138]. Another example is glutamate, which is critical for glutamatergic synapses. Due to the deficiency of pyruvate carboxylase in neurons [139–141], glutamate is synthesized in astrocytes [142–144] and then delivered into neurons by glutamate transporters [144, 145]. The synthesis of glutamate is at astrocyte mitochondria through multi-steps to convert oxaloacetate to α -ketoglutarate and eventually the final product, glutamate [142–144]. Other than their function in the synthesis of glutamate, the transport of glutamate from astrocytes into neurons and the packaging of glutamate into SVs are energy consuming processes significantly relying on the ATP producing function of mitochondria [144, 145]. In fact, neuronal mitochondria are also known to play a critical role in the *de novo* synthesis of several other key neurotransmitters such as NE [146–148], dopamine [148, 149], GABA [150, 151], and serotonin [151, 152]. Again, mitochondria are indispensable for the packaging of these neurotransmitters into SVs by providing ATP. In addition, mitochondria also have indirect influence on the production of non-classical neurotransmitters including adenosine, ATP, and nitric oxide via the function of mitochondria in the metabolism of these molecules. Therefore, mitochondria are extremely essential for the synthesis, packaging and storage of a number of neurotransmitters, the dysfunction of which are thought to be associated with AD.

Mitochondria and synaptic vesicle cycling

The process of synaptic transmission (that is, SV exocytosis) is initiated by calcium entry into presynapses via the voltage-gated calcium channels, which triggers the depolarization of the plasma membrane [153, 154]. By measuring the large synapses of the Calyx of Held, it is estimated that the depolarization-driven calcium transients last for around 400–500 μ s [155]. The time course of calcium pulse may vary in different types of neurons depending on the size of the synapses. But in general the rise of calcium levels in the cytoplasm is transitory followed by a quick dissipation of the calcium sparks, which brings to an end of the neurotransmitter release [155, 156]. The aforementioned synaptic calcium handling mechanisms require the coordination of calcium influx and fast removal. The involving role of mitochondria in the two processes has received considerable attention. First, the activation of the voltage-gated calcium channel-forming Ca^{2+} -ATPases predominantly relies on mitochondrial ATP provision [157, 158]. On the other hand, mitochondria are the major

calcium sequestering unit at synapses and play a key role in maintaining the intra-synaptic calcium homeostasis [158–160]. Previous studies have shown that mitochondria at synapses buffer calcium following the depolarization-evoked calcium influx and dissipate intra-synaptic Ca^{2+} to resting levels in mouse retinal bipolar cells [158] and at the rat Calyx of Held [161] as well as the mouse motor nerve terminals [162]. Another important piece of evidence showing mitochondrial role in regulating calcium during synaptic transmission was collected from a study on mitochondrial conductance during the activation of the giant synapses in squid. Jonas and the colleagues found that the elevation of mitochondrial conductance is closely associated with the synaptic stimulation-driven calcium transients in the presynapses and lasts throughout the whole process of post-tetanic potentiation [163]. It could be argued that mitochondria are not the only calcium storing organelle at synapses and the calcium-retention capacity of endoplasmic reticulum (ER) should not be overlooked. Indeed, it has been suggested that mitochondria and ER vesicles near the active zone have similar extent of effect in regulating intra-synaptic calcium levels during synaptic activation [164]; while notably other studies indicated that the calcium uptake by mitochondria is more rapid than that by ER [165–167]. More than their role as the calcium bank, mitochondria and ER also release calcium, which has been identified to contribute to the production of miniature excitatory post synaptic currents in mouse hippocampal and neocortical neurons [168, 169] and the sustainment of prolonged SV exocytosis [165].

Of note, synapses have a high demand of ATP to energize the trafficking, exocytosis, and endocytosis of SVs. To meet the synaptic energy demand, mitochondria are delivered to dock at synapses, and these synaptic mitochondria serve as the *in situ* energy warehouse to fuel synapses [27, 170–173].

Furthermore, mitochondria are the major source of ROS in neurons. It has been suggested that superoxide at physiological levels may serve as a key regulator for synaptic transmission- and plasticity-related signaling molecules, receptors and channels, which are critical for long-term potentiation induction and memory formation. For example, scavenging superoxide through the overexpression of extracellular superoxide dismutase (SOD) in mice surprisingly inhibits long-term potentiation and long-term memory [174, 175]. Moreover, genetic mutation on or pharmaceutical inhibition of NADPH oxidase suppresses NMDAR activation-dependent ERK signaling transduction pathway in mouse CA1 region, which further implicates the critical role of ROS in synaptic activity [176]. However, the information of the physiological role of ROS in synaptic transmission and plasticity is still extremely limited, which is in sharp contrast to our knowledge on the disruptive effect of excess ROS on synaptic activity at pathological states.

In addition, it should not be neglected the indirect impacts of mitochondrial dysfunction on the functions of synaptic activity-modulating signaling pathways through ATP deficiency, calcium perturbations, and oxidative stress [112, 120].

Put together, mitochondrial function in ATP production, calcium homeostasis regulation, and redox balance maintenance is pivotal for the potentiation of synaptic transmission. Deficits in mitochondrial particularly synaptic mitochondrial function are detrimental to synaptic activity and strength.

Mitochondrial Dysfunction in AD: The Link To Synaptic Transmission Failure

The role of mitochondrial dysfunction in synaptic injury in AD has received considerable attention [177–180]. Mitochondrial dysfunction has been found in AD patients [181–185] and AD animal models [8, 9, 186–190] as well as in A β -insulted cells [191–193]. Importantly, despite the coincidence of mitochondrial dysfunction and synaptic stress in AD-related conditions, increasing evidence has shown that synaptic mitochondrial dysfunction occurs prior to severe synaptic injury in early stage AD [34, 194, 195] as well as in young AD animal models [187, 188, 196–199]. This further implicates the promoting role of mitochondrial deregulation in potentiating synaptic stress in AD.

Synaptic transmission has a very intensive energy demand and the majority of brain ATP is consumed to support synaptic activity [200]. Neuronal ATP is almost exclusively provided by mitochondria through oxidative phosphorylation (OXPHOS). It is noteworthy that mitochondrial OXPHOS deficits and ATP deficiency are hallmark pathologies in AD brains [201, 202]. Much work has been done on the defects of electron transfer chain (ETC) in AD mitochondria. Decreased activities of mitochondrial complexes I through IV have been detected in the neocortex and hippocampus from postmortem AD brains; while the deactivation of mitochondrial complex IV is more prominent with altered steady-state composition of this enzyme [203–207]. The deregulation of mitochondrial complex IV in neocortex has also been determined in AD animal models overexpressing human form A β [9, 187, 196, 208, 209]. Furthermore, mitochondrial ETC deficits are not just confined in AD sensitive brain regions. Decreased mitochondrial complex IV activity has been detected in platelets from AD patients [210, 211] and MCI subjects [211], suggesting mitochondrial ETC deficits are a systemic change in AD. In addition to the studies on mitochondrial complexes I to IV in AD, the functional status of mitochondrial complex V also known as F1Fo ATP synthase in AD has long been overlooked. This enzyme locates in the inner mitochondrial membrane and constitutes the primary site for OXPHOS [212]. In our recent study, we have found alterations of mitochondrial complex V in postmortem MCI and AD temporal lobes as well as in synaptic mitochondria from an AD mouse model [187]. The deregulation of mitochondrial complex V in AD-related conditions has a strong association with loss of its oligomycin sensitivity conferring protein (OSCP) subunit and the interplay of OSCP with A β . The deregulation of mitochondrial complex V leads to severe mitochondrial dysfunction and compromised synaptic transmission. Noteworthy, the restoration of OSCP rescues synaptic transmission from A β toxicity in cultured mouse neurons [187]. Put together, impaired mitochondrial ETC and defected mitochondrial OXPHOS are a primary pathology in AD, which results in insufficient ATP provision to fuel synaptic transmission.

Calcium influx is the initiative step of SV exocytosis. As discussed above (“Mitochondria and synaptic vesicle cycling”), normal synaptic mitochondrial calcium handling capacity is essential for synaptic transmission. Mitochondrial permeability transition pore (mPTP) is a non-selective trans-inner mitochondrial membrane pore. Over-activation of mPTP has been proposed to be a critical mechanism underlying compromised mitochondrial calcium buffering capacity in AD [208, 209, 213–217]. Excess mPTP leads to decreased

mitochondrial calcium retention, collapsed mitochondrial membrane potential, reduced OXPHOS efficiency, and elevated ROS production and release, as well as ruptured mitochondrial membrane, eventually cell death [218, 219]. Although the exact molecular identity of mPTP still remains to be elucidated, a mitochondrial matrix protein called cyclophilin D(CypD) is a determined key regulator of mPTP formation [218, 219]. In our previous studies, we have found that the blockade of mPTP by genetic depletion of CypD confers protection against A β -mediated mitochondrial dysfunction as well as synaptic loss and synaptic transmission deficits in an AD mouse model [191, 197, 208], suggesting the deleterious impact of mPTP overactivation on synaptic transmission. This concept has further been supported by our recent study. Based on recent studies showing the role of mitochondrial F1Fo ATP synthase in the formation of mPTP [220, 221], we studied F1Fo ATP synthase (mitochondrial complex V) dysfunction in AD. We have determined the correlation of F1Fo ATP synthase deregulation and the activation of mPTP in AD-related conditions [187]. Of note, the restoration of F1Fo ATP synthase mitigates synaptic transmission injury in A β -insulted neurons [187]. Given the dual roles of F1Fo ATP synthase deregulation in ATP production and mPTP formation as well as the function of CypD, these studies suggest that defected synaptic transmission in AD-relevant conditions is associated, at least in part, with mPTP activation-induced mitochondrial calcium perturbations.

Impaired mitochondrial function results in excess ROS production and the resultant oxidative damages to neurons; and oxidative stress is characteristic of AD brains [222]. Several studies have found that lipid oxidation in presynaptic cytoplasm membrane obstructs the fusion pore opening, thus blocking the SV exocytosis leading to the abnormal detention of SVs in the active zone of presynapses [223, 224]. Moreover, oxidative stress induces hyperphosphorylation of tau through the inactivation of protein phosphatase 1 and 2A, causing abnormal tau aggregation [120], which blocks SV trafficking. Since mitochondria are known as the major source of ROS in neurons, previous attempts to scavenge mitochondrial ROS have shown significant protection against A β -induced synaptic injury. Dumont and the colleagues found that the overexpression of mitochondrial SOD significantly ameliorates mouse synaptic dysfunction and cognitive impairment in an AD mouse model expressing human form A β [225]. This is in agreement with Massaad and the colleagues' study [23]. These observations suggest the protective effect of reducing mitochondria-generated ROS on synaptic function in an AD mouse model and also serve as indirect evidence of the devastating role of excess ROS in mediating synaptic stress in AD-related pathological settings. Furthermore, similar protection has been reported by eliminating ROS using pharmaceutical approaches. The application of antioxidants, in particular mitochondria-targeted antioxidants such as mitoQ, SS peptides, mitochondrial SOD mimetic, and many others prevent synaptic degeneration from A β toxicity [226–228], which further confirms the contributing role of mitochondria-mediated oxidative stress in AD synaptic failure. Therefore, considering the physiological function of ROS in synaptic transmission and synaptic plasticity and the disruptive effect of excess ROS in particular mitochondrial ROS, it has been proposed that ROS act as a “double-edged sword” with respect to synaptic plasticity and memory consolidation [229]. To this end, how to eliminate

excess mitochondrial ROS production while maintaining a physiological level of ROS would be an intriguing and critical scientific issue for the treatment of synaptic failure in AD.

To exert mitochondrial function in supporting synaptic transmission, it is a prerequisite that mitochondria accumulate at presynapses. Mitochondrial motility and dynamics are critical aspects of mitochondrial biology in neurons. Neurons are highly polarized cells and soma are the primary sites for mitochondrial generation. Newborn mitochondria are delivered to the terminal ends of neurites and anchor at synapses. During the transportation, mitochondria constantly change their morphology via the fission and fusion processes to accommodate their host cell's requirement. Indeed, deregulated mitochondrial motility and dynamics have deleterious impacts on synaptic transmission in diseases including AD [230] evidenced by the observations that suppressed neuronal mitochondrial movement and imbalanced mitochondrial fusion/fission are prominent in AD-related conditions. Zhu and the colleagues performed pioneer and comprehensive studies on alterations of neuronal mitochondrial dynamics in AD brains and A β -overexpressing cell lines [231]. They have found significantly decreased expression levels of optical atrophy 1, mitofusin 1 and 2, and dynamin-like protein 1 (Dlp1) with the concomitant elevation of mitochondrial fission 1 protein in the postmortem hippocampal tissues from AD patients. These results serve as solid evidence of neuronal mitochondrial fusion/fission imbalance toward increased mitochondrial fragmentation in AD brains. Importantly, they also observed increased mitochondrial accumulation in soma and lessened mitochondrial distribution in the neurites in AD hippocampal neurons, implicating the altered patterns of neuronal mitochondrial motility with increased mitochondrial anterograde transport in AD conditions [231]. These findings were soon verified in AD brains and AD animal models as well as A β -insulted cell lines by many other groups [42, 188, 191, 196, 232]. Notably, in an A β -rich environment, anterograde movement of axonal mitochondria is more vulnerable than retrograde movement [42, 191], which helps to interpret the evacuation of mitochondria from synapses and increased retrieval of axonal mitochondria to soma in AD neurons. In addition to the changes of expression levels of mitochondrial fusion and fission proteins, two other groups have found increased Dlp1 S-nitrosylation in AD [233], and Dlp1 interaction with A β [193], which may also play critical roles in promoting axonal mitochondrial fragmentation in AD-related environment. In our previous study, we have found the inhibition of mPTP by CypD depletion ameliorates intra-axonal calcium perturbations and oxidative stress in A β -treated neurons. The blockade of mPTP protects axonal mitochondrial motility and dynamics, and importantly preserves synaptic transmission from A β toxicity. In view of the critical role of normal axonal mitochondrial distribution and morphology in maintaining synaptic activity, the protection of synaptic transmission by mPTP inhibition has the implication to be associated with the ameliorated axonal mitochondrial motility and dynamics [191].

Conclusions and Perspectives

Synaptic deficits form the pathological basis of cognitive impairment, the cardinal sign of AD. Synaptic loss, synaptic transmission reduction, and synaptic plasticity impairment are early pathology in AD brains and exacerbate with the progress of cognitive decline in AD. Therefore, to understand the molecular basis of synaptic injury is of paramount importance for our understanding of the pathogenesis of AD as well as for the development of

preventive and therapeutic strategies for the treatment. However, our knowledge is still limited for a full description of the mechanisms underlying synaptic failure in AD, given the complicated nature of the potentiation and regulation of synaptic activity and, more importantly, the many unknowns about the pathogenesis of AD.

Indeed, despite the determined genetic risk factors for familial AD, the etiology of sporadic AD still remains enigmatic. Mitochondrial cascade hypothesis as an important supplementation to the prevailing A β cascade and tauopathy hypotheses has provided us an alternative avenue to understand the pathogenesis of AD, in particular its sporadic form [234]. Of note, the role of mitochondrial deficits in synaptic injury in AD has been firmly built on a large amount of, and still emerging, evidence. Mitochondria are actively involved in each and every step of synaptic transmission via their critical functions in providing energy, keeping intracellular redox balance, and maintaining intrasynaptic calcium homeostasis, as well as regulating key signaling transduction pathways, and synthesizing the intermediates and/or final products of several key neurotransmitters. The pivotal role of mitochondrial in synaptic transmission, the early mitochondrial dysfunction, and the concomitant mitochondrial and synaptic injury in postmortem AD brains and the AD animal models, as well as the protective effect of mitochondrial medicine [35, 227], have lent credibility to the hypothesis that mitochondrial deficits confer susceptibility to synaptic impairment in AD (Fig. 1). However, to fully address this concept, there are still many critical questions to be answered, e.g., which one happens first in AD, synaptic injury or mitochondrial dysfunction? What are the detailed mechanisms causing mitochondrial defects in AD-related conditions? Does synaptic injury in turn reinforces mitochondrial dysfunction in AD, thus forming a vicious cycle? The answers to these questions will foster a better understanding of synaptic failure and memory loss in AD. It is true that treatments targeting mitochondria are not likely to be the miraculous cure to stop synaptic injury and restore cognitive function in AD, but protecting mitochondria still holds very high promise as of now, not only because of the critical role mitochondrial dysfunction plays in synaptic injury in AD, but also because of the lack of the definite cause of synaptic failure in this chronic neurodegenerative disorder.

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AD-relevant conditions

Fig. 1.

Working hypothesis. In Alzheimer's disease (AD)-related conditions, the convergent effects of amyloid- β ($A\beta$), tauopathy, and other unknown factors induce mitochondrial dysfunction, thus leading to reduced ATP production, deregulated calcium homeostasis, and excess reactive oxygen species (ROS) generation, decreased production of some key neurotransmitters and their intermediates, as well as perturbed cell signaling cascades. These changes affect synaptic transmission, eventually causing synaptic failure in AD. PKA, protein kinase A; PKC, protein kinase C; CAMKII, Ca^{2+} /calmodulin-dependent kinase II; SV, synaptic vesicle.