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Review

Probable Reasons for Neuron Copper Deficiency in the Brain of Patients with Alzheimer's Disease: The Complex Role of Amyloid

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Abstract: Alzheimer's disease is a progressive neurodegenerative disorder that eventually leads the affected patients to die. The appearance of senile plaques in the brains of Alzheimer's patients is known as a main symptom of this disease. The plaques consist of different components, and according to numerous reports, their main components include beta-amyloid peptide and transition metals such as copper. In this disease, metal dyshomeostasis leads the number of copper ions to simultaneously increase in the plaques and decrease in neurons. Copper ions are essential for proper brain functioning, and one of the possible mechanisms of neuronal death in Alzheimer's disease is the copper depletion of neurons. However, the reason for the copper depletion is as yet unknown. Based on the available evidence, we suggest two possible reasons: the first is copper released from neurons (along with beta-amyloid peptides), which is deposited outside the neurons, and the second is the uptake of copper ions by activated microglia.

Keywords: beta-amyloid peptide; microglia; copper deficiency; inflammatory cytokines; NMDA receptor

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1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder related to aging [1]. The number of Alzheimer's patients in 2019 was estimated to be 50 million, and that figure is expected to exceed 152 million by 2050. It was approximated that about US$1 trillion was spent on AD in 2019 [2]. Despite the deadly nature of this disease and its high prevalence as well as the consequent economic burden, after more than a hundred years have passed since its discovery, no effective treatment has been found up to now. The complexity of the disease means its recognition and treatment development have been similar to the story of the "elephant in the dark", with various researchers attributing various causes to the disease as well as studying wide-ranging and overlapping options for treatment of different aspects of the condition.

There are several hypotheses concerning the cause of AD based on the available experimental data [3]. Yet, the main accredited hypothesis of the 'amyloid cascade' has been challenged in the last decade [4]. Metal dyshomeostasis is among the relatively new proposed mechanisms for AD onset [5–7]. Considering that Aβ is produced in the brain under physiological conditions (as a soluble component), the "metal hypothesis" has also been put forward, in which Aβ binding to metal ions, especially copper and zinc ions, causes pathogenic Aβ deposits to form that eventually lead to AD [8].

In the past 10 years, a body of evidence has been collated suggesting that copper imbalance specifically affects a certain percentage of AD patients [9–11]. Copper imbalance appears to be typified by an increased level of copper in the general circulation, which can be attributed to expansion of the non-ceruloplasmin-bound (non-cp) copper fraction in serum as well as a decreased level of copper in the brain [12–19], though the proportion of labile copper (i.e., exchangeable copper relative to the copper content of the tissue) increases in the brain [20].

Though copper is highly concentrated in amyloid plaques, its concentration reduces in neurons [12,14–19,21]. A body of evidence suggests that intracellular copper deficiency in neurons could represent a possible cause of neuronal death in AD (reviewed in [22]). Beyond describing the processes of copper content dysregulation in the bloodstream (recently reviewed in [5]), it remains unclear why the concentration and distribution of copper in the brains of AD patients change, though a number of attempts have been made to interpret this complex phenomenon (reviewed in [9]). Determining how this happens may significantly contribute toward finding appropriate methods for intervening in the progression of the disease and identifying its starting point [23,24]. In this review, we compile and discuss the latest results available on one aspect of the complex puzzle of copper imbalance in AD—the brain copper deficiency.

2. Copper Physiology Focusing on the Brain

Copper in the gut is mainly absorbed by copper transporter 1 (CTR1) [25], and also by divalent metal transporter 1 (DMT1) to a lesser extent [26,27]. In the blood, approximately 75–95% of the absorbed copper ions bind to ceruloplasmin. Inside the cells, these are transported to different destinations using different chaperones [28]. It was found that essential metals for the body, such as copper and zinc, play major structural and catalytic roles in metalloenzymes; however, their high levels are fatal to cells [29]. Eukaryotes can activate certain mechanisms to store these metals inside intracellular organelles, and use them when needed. For instance, macrophages use Zn^{2+} and Cu^{+1} to attack bacteria, which is referred to as the brass dagger [30–32]. In fact, there is less than one free copper ion per cell under physiological conditions [33].

Copper, as a cofactor or structural component of several important enzymes, is known as an essential metal in brain functioning that plays key roles in various pathways, including neurotransmitter and neuropeptide synthesis, energy metabolism, antioxidative defense, and iron metabolism [34]. The brain barrier system (the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier) plays an essential role in modulating copper homeostasis in the brain. The related evidence suggests that copper is principally transported to the brain through the BBB using both CTR1 and DMT1. Correspondingly, excess copper is transported into the CSF in the ventricles of the brain by the choroidal epithelial microvilli [35,36]. Some major changes to the cerebral vasculature, including increased permeability and weakened detoxification and repair functions, occur in people following aging. Subsequently, these changes can significantly affect the function of the brain barrier system [37]. Moreover, some studies have previously shown that exposure to other metals such as lead [38] or manganese [36,39], by altering the activity of copper transporters in the brain barrier system, can also alter the copper homeostasis in the brain.

The imported copper into the brain cells is likely to be detached by glutathione (GSH), stored as an metallothionein-copper complex, or transported by copper chaperones to some specific target cell locations [34]. Of note, the intracellular copper concentration in the brain is estimated to be 100–1000 times more than that of extracellular copper [40]. The approximate amount of copper in the human brain varies from 3.1 to 5.1 μg/g wet weight. In this regard, numerous studies have shown that copper is distributed unequally in the brain [41,42]. Overall, the copper concentration is higher in grey matter compared to white matter [43]. Notably, two-dimensional copper maps of human brain slices have shown that those areas heavily enriched in copper are found in the hippocampus [41]. Both the amount and distribution of copper in the brain also change with aging and in those affected by neu-

rodegenerative diseases [44–46]. Brain copper levels naturally decrease with aging [47–49]. In addition, brain copper decreases in those with the neurological diseases, including early-onset familial AD [50], Menkes' disease [51], Parkinson's disease [52–54], transmissible spongiform encephalopathies, Lewy body dementia, Creutzfeldt–Jakob disease [53], and Huntington's disease [54].

3. Connections between Copper and Aβ

Copper is an essential trace element in the nervous system's development, and its dyshomeostasis causes neurodegenerative phenotypes of both Menkes' and Wilson's diseases [55]. As multivalent cations are highly concentrated in senile plaques in the brains of patients with AD, many studies have previously reported dyshomeostasis of copper and of zinc as the main causes of AD pathogenesis [22]. Both these metals were observed to affect the oxidative status of the brain. As well as this, the Cu(II)/Cu(I) cycle could primarily generate free radicals in Fenton-like reactions (reviewed in [9]) outside the cells, in the form of labile copper [20]. Furthermore, combined evidence suggested that a reduced copper content in the brain cells results in greater production of Aβ peptide, copper migration to lipid rafts, and copper-amyloid complex formation in lipid rafts [22]. It was demonstrated that binding to copper [56] and proximity to the cell membranes [57] change the folding of Aβ peptides, creating channels in the membrane [58]. It has been postulated that the created channels could lead to apoptosis [59,60]; however, the causative factor behind the copper deficiency of neurons remains unknown.

APP belongs to the mammalian APP gene family. APP family members have various biological functions, including nervous system development, synaptogenesis, axonal growth and guidance, and synaptic functions [61]. APP is a transmembrane protein strongly expressed in the brain and very rapidly metabolized by a series of proteases [62,63]. Several paths have been found for APP proteolysis, some of which end in Aβ production while others do not [62]. APP can be cleaved by α-secretase and then γ-secretase without leading to Aβ production; however, Aβ is produced if APP is cleaved by β-secretase (instead of α-secretase). Aβ is released into the extracellular space or degraded in lysosomes [62]. It has a number of significant functions in both synaptic physiology and regulation. In addition, Aβ accumulates in the brains of patients with AD for an unknown reason [64,65].

Copper transportation to the brain is complex and involves many factors, including APP, Aβ peptide, and cholesterol [66]. APP has two copper binding sites, one of which is included in the Aβ peptide sequence [67,68]. Though APP has no equivalent in yeast, overexpression of APP in yeast consequently causes copper efflux [69]. In transgenic mice, on the other hand, overexpression of APP induces reduced copper levels in the murine brain [70]. Therefore, based on this evidence, it can be proposed that APP may be a specific copper transporter in mammals [66] as it displays a high affinity for copper [71–73]. In addition, a recent study has indicated that Aβ scavenges copper from the synaptic space [74].

4. Copper's Importance in the Hippocampus

Glutamate is the main excitatory neurotransmitter in the brain, acting on ionotropic and metabotropic receptors. The ionotropic glutamate receptors (iGluRs) are known to be responsible for rapid neural communication in excitatory synapses, which include the following three subfamilies: kainate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and ***N***-methyl-D-aspartate (NMDA) receptors [75]. Nevertheless, extreme stimulation of glutamatergic signaling during excitotoxicity, causes neuronal cell death [76–78]. Excitotoxicity through NMDA receptors is primarily mediated by excessive entry of Ca^{2+} [79,80] since NMDA receptors are highly permeable to calcium ions compared to the other iGluRs [81].

The NMDA receptors play important roles in synaptic transmission and plasticity, which are known to form the basis for learning and memory processes [82]. A previous study conducted on older healthy adults showed that higher hippocampal activity is associated with increased Aβ deposition as well as decreased memory function [83]. Another

study indicated that patients with mild cognitive impairment (MCI) and Aβ deposits had smaller hippocampal volume, higher hippocampal activity, and lower Mini-Mental State Examination (MMSE) scores compared to Aβ-negative patients. In addition, they showed higher rates of hippocampal atrophy and disease progression as well as a greater decrease in MMSE score over time, despite high levels of hippocampal activity [84]. Recently, a study showed that abnormal NMDA receptor activation is associated with synaptic dysfunction in AD [85]. Indeed, disruption of Ca^{2+} signaling results in a gradual loss of synaptic function, which eventually leads to neuronal cell death; this is clinically associated with a gradual decrease in cognition or memory [86].

The hippocampus, which is the brain part associated with neurogenesis and long-term memory, is one of the first affected brain areas in AD, and suffers from the most damage [87–89]. It was shown that about 15 μM of copper is released from post-synaptic vesicles into the glutamatergic synaptic cleft following neuronal depolarization through excitation of NMDA receptors in the hippocampal area [90,91]. This copper concentration (15 μM) has been obtained from two previously-performed independent studies, one that measured the emission of fluorescent dye (sensitive to copper binding) from synaptosomes derived from bovine chromaffin cells [91], and another that measured the ^{67}Cu radioactivity of cells taken from a rat's hypothalamus [90]. However, a study conducted on the synaptosomes of a rat's cerebral cortex that used the atomic absorption technique calculated the amount of released copper to be at least 100 μM [92]. Thus, this process appears to be a protective mechanism for neurons against NMDA excitotoxicity.

High ATP7A expression can be observed in hippocampal neurons. Some studies on primary cultures of murine hippocampal neurons showed that after activation of the NMDA receptor, but not AMPA or kainate-type glutamate receptors, ATP7A trafficking occurs in the hippocampal neurons along with the rapid release of copper [93]. Copper chelation exacerbates cell death due to overactivation of the NMDA receptor, while the addition of copper protects the cell and then leads to a significant reduction in cytoplasmic Ca^{2+} levels after the NMDA receptor's activation [94]. In this regard, further evidence showed that PrP interacts with the NMDA receptor, which then decreases its interaction with glycine (as a receptor activator), leading to reduced receptor activation [95]. PrP is involved in S-nitrosylation of the NMDA receptor, and to perform this activity, it is dependent on copper [96,97]. S-nitrosylation is a chemical post-translational modification process through which a nitric oxide group is added to the protein cysteine, playing a role in inhibiting the NMDA receptor [96]. The NMDA receptor is a highly calcium-permeable glutamate receptor and its hyperactivity leads to cell death [95]. It was shown that copper decreases the cytoplasmic Ca^{2+} concentration after the NMDA receptor's activation and consequently attenuates NMDA-induced excitotoxicity [93,95,96,98,99].

5. Roles of Microglia in Both Healthy and AD Brains

Microglia are brain-specific macrophages known as the first line of defense in the central nervous system [100]. Increased microglia activation has been reported in dementia. Related data indicate that this is associated with white matter maintenance and preserved cognition in dementia with Lewy bodies; however, it is not associated with reduced grey matter volume [101]. While a study indicated that microglial activation is negatively correlated with hippocampal volume in patients with Parkinson's disease dementia [102], another study reported that microglial activation is associated with white matter maintenance as well as preserved cognition in Parkinson's disease dementia [103]. In a case with AD, microglia markers were repeatedly reported as being overexpressed in the brains of patients. Among the different regions of the brain, the white matter and cerebellum appear to be more resistant to this increase [104]. New findings suggest that microglial activation in MCI patients is correlated with greater grey matter and hippocampal volumes [105], whereas previously reported data indicated that microglia activation is inversely associated with the hippocampal volume in patients with AD [102]. In fact, microglia play a dual role in AD, either inducing nerve survival (by clearing Aβ deposits) or disturbing the neuronal

function and then leading to cell death (by releasing cytotoxic mediators). The magnitude and context of microglial activation lead different molecules to be secreted with different effects [94,106,107] (Figure 1).

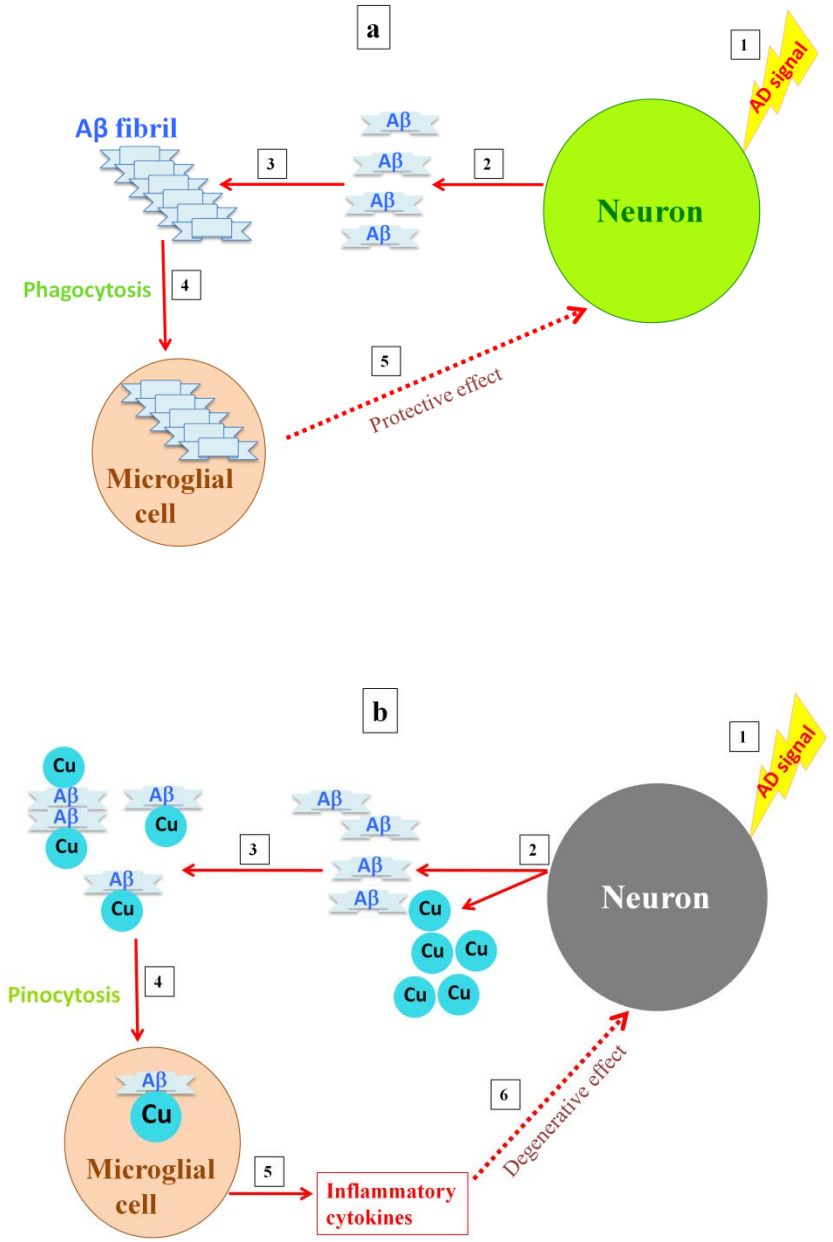

Figure 1. Postulated differences in microglia activity with either the absence or presence of a high copper concentration. When high levels of amyloid are released into the synapse, amyloid fibrils are formed and the microglia are activated, clearing the fibrils by phagocytosis (**a**). Releasing high levels of copper into the synapse, as copper inhibits the NMDA receptor, results in soluble Aβ deposits, which are then taken up by microglia via pinocytosis, causing overactivation of the microglia and inflammation (**b**).

6. Interplay of Copper, Aβ, and Microglia Activation in Mediating NMDA Receptor-Induced Excitotoxicity

The ATP7A protein is a copper pump located in the Golgi network, which can transport cytoplasmic copper into secretory compartments to make it available for copper-dependent enzymes. ATP7A also exports copper from the cytoplasm to the post-Golgi vesicles or the plasma membrane via trafficking [108,109]. Overexpression of ATP7A has been reported in

activated microglial cells observed around amyloid plaques in a TgCRND8 mouse model. In addition, after interferon gamma (IFN-γ) stimulation (but not tumor necrosis factor-α (TNF-α) or interleukin 1 beta), the cultured microglial cells showed a significant increase in both ATP7A and CTR1 expression [110]. It was shown that in IFN-γ-stimulated microglial cells, despite ATP7A trafficking into cytoplasmic vesicles, copper export did not happen. Copper accumulation was instead observed as a result of increased copper uptake due to the expression of copper importer CTR1 [110]. Murine macrophages accumulate copper in intracellular vesicles in response to infection with *S. Typhimurium* or treatment with lipopolysaccharides (LPS) [111]. Previously performed studies showed that both IFN-γ and TNF-α cause copper accumulation in phagosomes of infected macrophages [112]. The innate immune response uses copper to attack infectious microorganisms [113]. In the presence of copper, IFN-γ–activated macrophages have a higher bactericidal activity [114]. Moreover, it has been demonstrated that copper ions in culture medium induce the transformation of the macrophage phenotype to the proinflammatory M1 state by activating copper transport signaling [115]. Furthermore, a recent report shows that copper changes the microglia phenotype to a degenerative phenotype [116].

Findings on the NLRP3 inflammasome (NACHT, LRR, and PYD domains-containing protein 3) can be considered as additional evidence demonstrating the involvement of copper in the activity of microglia. Inflammasomes are multiprotein complexes mediating the proteolytic activation of caspase-1 and cytokines via a NOD-like receptor [117]. It was also indicated that the NLRP3 inflammasome is involved in various human disorders, including metabolic diseases, chronic inflammation, autoinflammatory syndromes, and infections [118]. Additionally, inflammasome activation is important in neuroinflammation induced by microglia [119], and it has been indicated that Aβ oligomers can activate NLRP3 inflammasomes in microglia [120,121]. The activation of NLRP3 inflammasome requires intracellular copper, and removing copper from superoxide dismutase-1 suspends the activity of inflammasomes. Notably, copper regulation of the NLRP3 inflammasome is only specific to macrophages [122]. Given that microglia are a type of macrophage, this mechanism may also be applied to them, as studies have previously confirmed [123].

High densities of the activated microglia are located around the Aβ plaques in the hippocampi of patients with AD [105,124], which contain Aβ deposits [125]. Aβ deposits can activate microglial cells (Figure 1a), which is regarded as an important pathological event in the brains of patients with AD [126–128], via various innate immune receptors expressed on their membranes [106]. Furthermore, it has been reported that Aβ deposits cause rapid ATP release as well as overexpression of the P2Y2 receptor gene, which consequently causes microglial activation to increase Aβ clearance [129]. It has been shown that ATP effectively induces pinocytosis in microglia with the involvement of P2Y4 receptors, which results in Aβ clearance by microglia [130]. Both phagosomes and pinosomes are membrane vesicles formed when phagocytes swallow extracellular material; phagosomes are formed when the solid material swallowing process occurs and pinosomes are formed when liquid is ingested [131]. Small soluble Aβ deposits are taken up nonspecifically by pinocytosis, and directed to late endosomes for degradation [132], while Aβ fibrils' uptake is mediated by scavenger receptors, which results in localization to distinct subcellular compartments called phagosomes [133]. Aβ fibrils can increase the phagocytic capacity of microglia. Aβ oligomers cannot only reduce the phagocytosis of fluorescent microspheres by microglia, but can also significantly reduce Aβ fibril phagocytosis. The treatment of microglia with Aβ oligomers causes a rapid and temporary increase in interleukin-1β as well as production of more inflammatory mediators—including TNF-α, nitric oxide, prostaglandin E2 (PGE2), and intracellular superoxide anion—compared to Aβ fibrils. More data on this subject showed that inflammatory markers could reduce fibril-induced phagocytic potency. Altogether, these results indicated that oligomers disrupt microglia phagocytosis and fibril clearing by inducing a strong inflammatory response [134] (Figure 1b). High copper concentrations can also be found in amyloid plaques in the hippocampus [135]. Of note, copper ions play important roles in Aβ aggregation and neurotoxicity [136–138]. Aβ

has been shown to be less toxic to neurons in the absence of copper ions, but these levels of copper ions (in control studies) in the absence of Aβ have not been found to be cytotoxic. In other words, copper ions can significantly increase Aβ's toxicity to neurons [137]. Moreover, copper ions have been shown to induce toxic non-amyloidogenic aggregates of amyloid peptides [139]. In addition, the toxicity of Aβ is attributed to the production of reactive oxygen species, among which copper is known as one of the metals involved in this process [140]. Copper ions have also been shown to have the ability to increase the effect of Aβ on microglial activation as well as the subsequent neurotoxicity (Figure 2). Indeed, a copper-Aβ complex (but not copper or Aβ alone) at subneurotoxic concentrations can activate microglial cells and then induce both TNF-α and NO production in microglia that ultimately result in neuronal death [141]. It was demonstrated that copper could significantly decrease the phagocytic property of microglial cells activated by Aβ fibrils or LPS as well as reduce the intracellular degradation of Aβ, whereas it increases the secretion of proinflammatory cytokines [142,143].

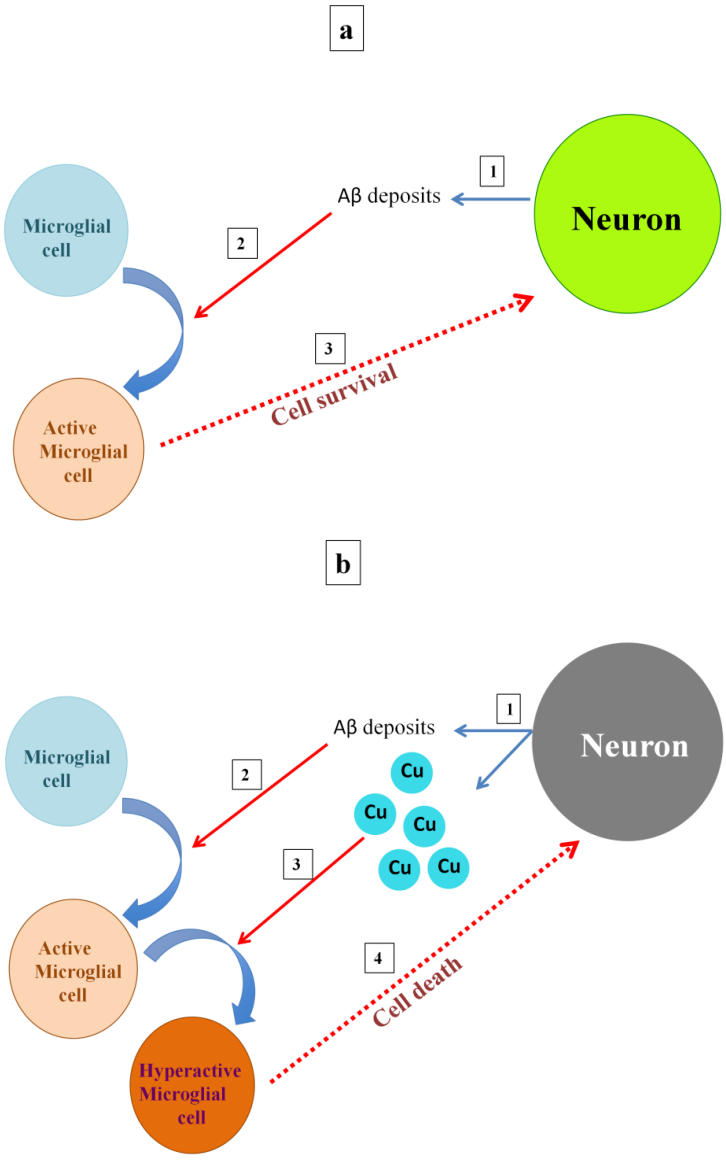

Figure 2. Model of microglia's dual role in AD. The activation of microglia is key to neuronal survival (**a**). The presence of large amounts of copper in the synapse changes the role of microglia from protective to lethal and also induces neurons' copper deficiency (**b**).

Different concentrations of copper have the ability to increase the formation of fibrillar amyloids [137]. At substoichiometric levels of copper, fibril formation increases [137], and in contrast, when the ratio of copper to Aβ increases, fibril formation decreases [144]. Moreover, the addition of metal chelators to amorphous Aβ deposits converted them into fibrillar forms [140]. Furthermore, some previously-performed structural studies showed that at the ratio of 1:1 copper and Aβ, the β-sheet structure reaches a maximum and then decreases with an increased amount of copper [145], which is in line with other studies stating that copper at high concentrations reduces fibril formation [139]. In general, the role of copper in mediating Aβ between oligomeric and fibril forms mostly depends on the ratio of copper to Aβ. In this way, fibril formation up to a ratio of 1:1 of copper/Aβ can be facilitated; with an increasing copper/Aβ ratio, fibril formation decreases, while vice versa, the situation favors the generation of non-fibrillar forms.

7. Insights and Concluding Remarks

The grey matter and certain areas of the brain like the hippocampus that are the most damaged in AD have been reported to contain the highest levels of copper in a healthy brain [41,43]. The brains of patients with AD are copper deficient [12,14–19,146], and a dataset led to the hypothesis that this deficiency can consequently lead to channel creation in the neuron membrane, which results in apoptosis [22,146]; however, the cause of this deficiency is not yet known.

Recently, a critical, location-dependent copper dissociation constant (K_{dc}) was proposed as a new mechanism, featuring a shift from physiological bound metal ion pools to loose toxic pools in copper imbalance (reviewed in [9]). This hypothetical mechanism provided some clues on the key decreased copper enzymes and transporters in the AD brain that can majorly affect copper buffering and functioning in synapses during the glutamatergic transmission process. The concept proposed is applicable to Aβ and APP as well as other copper proteins relevant to the AD cascade, including the prion protein and α-synuclein [9].

Another putative mechanism of copper deficiency in neuronal cells lies in Aβ sorting and segregation within lipid rafts. Of note, Aβ is produced in lipid rafts [147,148] before entering the synaptic space or being digested inside the neuron [62]. In AD, it seems that Aβ production increases or its clearance decreases, and binding of copper ions to this peptide (with a high affinity for copper ions) or deposition outside the cell can lead to copper deficiency in neurons. The findings indicate that the Aβ is greatly deposited in areas of the brain with the most damage. As well as this, greater deposition may cause more intense apoptosis due to greater copper deficiency.

Certain evidence suggested that microglia activation can be considered as another possible cause of copper deficiency in neurons. Copper is unevenly distributed in the brain, and some brain areas contain greater amounts of copper. One such area is the hippocampus, which is related to memory and becomes severely damaged in AD [41,42]. Neurons of this area routinely use copper to prevent excitotoxicity by NMDA receptors, and also release copper at the micromolar level after each depolarization to synapses [90,91]. Aβ deposits have also been shown to activate microglia, and the activated microglia then clear amyloid from the environment [129]. Meanwhile, the activated microglia increase the expression of copper-related proteins, which consequently causes copper uptake into the microglia [110]. Also, a new study confirmed that microglia increase their intracellular copper in response to the inflammatory stimuli [149]. Therefore, it seems that if microglia are activated in the areas using copper to prevent excitotoxicity by NMDA receptors, by microglia absorbing copper from the synaptic space, copper re-uptake by neurons can be disrupted, which consequently causes a serious copper deficiency in neurons. Correspondingly, this phenomenon can cause overactivation of NMDA receptors and subsequently lead to neurodegeneration. Moreover, it was found that copper imbalance in the heart is dangerous. Loss of copper from the heart occurs in myocardial ischemia [150,151]. Recently, in an animal study, it was shown that upregulation of an intracellular copper exporter, such as copper metabolism MURR domain 1 (COMMD1), in the heart is key to exporting copper from the heart to the

blood on ischemic insult [152]. Accordingly, this mechanism can also take place in the brain in AD, especially in the hippocampus. In addition, the upregulation of CTR1 in microglia can function to absorb the copper released from neurons.

NMDA receptors seem to regulate different processes in various brain regions [153,154]. Accordingly, they have different distributions in the central nervous system in terms of their type of subunit [155]. For instance, NR2A and NR2B are overexpressed in the cortex and hippocampal areas, respectively, while high NR2C expression is specific to the cerebellum area [155,156]. On the one hand, the earliest instance and highest level of damage were found to be related to the cortex and hippocampal areas, respectively, while the lowest was related to the cerebellum area [157,158]. On the other hand, the cortex and hippocampus have the highest levels of copper in the brain. Taken together, this evidence suggests that activation of microglia in the presence of copper-regulated NMDA receptors may be a significant factor in copper deficiency in neuronal cells.

In addition, the activation of microglia in areas using copper to prevent excitotoxicity by NMDA receptors can lead to copper deficiency through another mechanism. Copper reduces the phagocytic properties of microglia, which can consequently result in greater Aβ deposition [142,143]. Logically, a greater increase of Aβ deposition in this area would lead greater amounts of copper to be deposited out of reach of neurons. Otherwise, evidence has shown that copper intensifies Aβ-mediated microglia activation, and subsequently, highly activated microglia do not play a protective role, which leads to neuronal death [141] (Figure 2b). However, previous studies have shown that microglia form a barrier around small amyloid plaques, and slowing of the dystrophic neural process can be detected in areas with microglial coverage, suggesting peptide clearance by microglia protects neurons against Aβ toxicity [159].

In general, copper imbalance in AD, similar to the disease itself, is a complex phenomenon. In this review article, we attempted to specifically address the possible causes of copper depletion in neurons. We hypothesized that there may be two possible causes of copper depletion in neurons: first, the release of amyloid (as a copper transfer protein with a high affinity for copper) from neurons and its deposition outside neurons can trap copper outside neurons, which in turn, causes copper deficiency in neurons. Second, the uptake of copper by the activated microglia makes copper inaccessible to neurons.

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