Review

Neuro-regeneration Therapeutic for Alzheimer’s Dementia: Perspectives on Neurotrophic Activity

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Alzheimer’s disease (AD), the leading disorder of memory impairment in our aging population, is increasing at an alarming rate. AD is currently identified by three ‘gold standard criteria’: (i) dementia in life, (ii) amyloid plaques at autopsy, and (iii) neurofibrillary tangles at autopsy. Several autopsy studies have indicated that dementia in life is a consequence of lost synaptic networks in the brain, while many clinical trials targeting neurotoxic amyloid beta (Aβ) have consistently failed to produce therapeutic effects on memory function in AD patients. Restoring cognitive function(s) by activating endogenous repairing/regenerating mechanisms that are synaptogenic and antiapoptotic (preventing neuronal death), however, is emerging as a necessary disease-modifying therapeutic strategy against AD and possibly for other degenerative dementias, such as Parkinson’s disease and multi-infarct dementia.

Alzheimer’s Disease

Alzheimer’s disease (AD) (see Glossary), a chronic disorder of neurodegeneration, is characterized by memory impairment, formation of senile plaques and neurofibrillary tangles (NFTs), and the loss of synaptic networks. The majority (>95%) of AD cases occur sporadically, without a specific family link, but with age as the single greatest risk factor. After decades of extensive research, there is still no cure for AD, as demonstrated by consistently failed efficacy in clinical trials [1,2]. The four FDA-approved AD drugs currently available (donepezil, galantamine, rivastigmine, and memantine) provide symptomatic short-term benefit at best [2]. A recent analysis of data from 2242 individuals, clinically diagnosed with MCI-AD (mild cognitive impairment due to AD) or with mild ADm (mild AD dementia), available from the National Alzheimer’s Coordinating Center’s Uniform Data Set, reveals that in the patients who used cholinesterase inhibitors (e.g., Aricept) to treat their cognitive impairment, their cognitive decline became greater after the treatment initiation in both groups [3].

Several different pathologies have been proposed as the underlying cause(s) in AD, including cholinergic deficits, formation/accumulation of neurotoxic substances, oxidative/vascular stress, neuroinflammation, and mitochondrial dysfunction. Amyloid beta (Aβ) accumulation, especially the soluble neurotoxic oligomers, is commonly viewed as the initiator, inducing tau hyperphosphorylation, neuroinflammation, oxidative stress, and mitochondrial dysfunction through its downstream molecular cascades. Aβ and NFTs can activate microglia and induce oxidants and inflammatory factors, which in turn promote further Aβ and NFT formation. The vicious cascade cycle includes interactions between neurotoxic substances and neuroinflammation: toxic Aβ promoting inflammation, which promotes more toxic Aβ (i.e., inflammasome activation is connected to seeding and spreading of Aβ in AD patients) [4,5]. This popular amyloid hypothesis (Figure 1) has, however, two main problems that are not consistent with its validity. First, the

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Amyloid plaque load does not correlate well with cognitive impairment in AD patients [6], although the plaque load may not accurately reflect the exact level of neurotoxic Aβ in the brain. Second, reducing the neurotoxic amyloid accumulation or its vicious cycle component(s) does not produce the expected therapeutic outcomes in AD patients (see below).

Early amyloid accumulation starts intracellularly and leads to tauopathy, which is more proximal to the disease progression. In a single 3D human neural cell culture system, inhibition of Aβ generation with β- or γ-secretase inhibitors was reported to not only decrease Aβ pathology, but also attenuate tauopathy [7]. Aβ42 induces cultured human stem cells to lose plasticity, a deficit that can be rescued with interleukin (IL)-4 [8]. However, problems with multiple clinical trials of amyloid-targeting therapeutics, including an effective removal of neurotoxic Aβ species, and licensed AD therapeutic drugs are their lack of effectiveness in targeting the pathophysiological core underlying the dementia: functional deficits/loss of synapses and neurons beyond the brain’s ability to repair. Synapses permit a neuron to pass a signal, chemical or electric, to another cell and are adaptive in operation and structure according to functional demands. Both acquisition of memory and its consolidation/reconsolidation involve restructuring of synapses and their operations [9]. For all the unsettled issues in AD, one finding is clear: the synaptic deficit/loss, revealed among many other pathologies, is highly correlated with the levels of dementia in AD patients [10–12] (Box 1). Within 2–4 years of AD onset, brain biopsies are reported to have a decrease in the number of synapses by 25–30% in the frontal and temporal cortices [13], most severe (by 44–55%) in the hippocampus [11,14], both in the degenerating and surviving neurons (about 38% loss [12,13]). These correlations strongly indicate that removal of the uncorrelated/weakly correlated pathological factors for a disorder, such as the Aβ for AD or Lewy bodies for Parkinson’s disease, is unlikely to produce dramatic therapeutic impacts on cognition. Nilvadipine, an antihypertensive drug that has demonstrated antiamyloid, anti-inflammatory, and antiauropathy activity, for example, failed to show therapeutic benefit for mild-to-moderate AD patients in a recent Phase III clinical trial [15]. Verubecestat, an oral β-secretase-1 inhibitor, produced near-maximal reduction of the soluble Aβ in cerebrospinal fluid (by up to 94%) but did not reduce cognitive decline in patients with mild-to-moderate AD [16], and instead worsened cognition in a 2-year, double-blind, placebo-controlled trial of 1454 prodromal AD subjects with a positive amyloid positron emission tomography (PET) scan at baseline [17]. The failed efficacy also includes recent Phase III clinical trials with Aducanumab, a human monoclonal antibody to Aβ oligomers in early AD patients. While tauopathy correlates more closely than amyloid pathology with neuron loss and cognitive decline in AD, obvious tauopathy throughout the

Figure 1. Pathogenesis of Alzheimer’s Disease (AD): Amyloid Hypothesis (A) and Synaptic Deficiency Hypothesis (B). (A) The amyloid cascade hypothesis: the deposition of amyloid beta (Aβ) initiates pathological events in the brain: formation of senile plaques and neurofibrillary tangles, oxidative stress, inflammation, synaptic and neuronal injuries, and loss with functional consequences of cognitive impairment and dementia. Toxic Aβ can directly induce synaptic/neuronal damage and produce damages through other mediators, such as hyperphosphorylated tau, excitotoxicity, oxidative, and inflammation. For instance, Aβ oligomers/soluble Aβ bind to glutamate receptors and other components, resulting in influx of extracellular Ca2+, calcium homeostatic disruption, actin depolymerization, actin dynamic disruption through impairing the PI3K/Akt/mTOR pathway, and oxidative stress with the consequence of synaptic failure. The hypothesis predicts that: (i) the pathological cascade can be blocked or arrested by effective removal of Aβ or blocking its formation; and (ii) such effective therapeutics (drugs, antibodies, etc.) would be disease-modifying. (B) The synaptic deficiency hypothesis is consistent with the evidence that formation of Aβ and tau is mainly reactive (secondary) to cell injuries and roles of neurotrophins in synaptic and neuronal survival. In development, the neurons that fail to receive a sufficient level of retrogradely transported neurotrophins from the targeted cells are eliminated through a rapid degeneration mechanism, while similar mechanisms operate in the adult brains but with a rather chronic and slow process, a degeneration process, especially when widespread (primary). The hypothesis predicts that: (i) antiamyloid treatment(s) can only produce very limited therapeutic effects in AD, and (ii) maintaining synaptic efficacy would be AD disease-modifying. Abbreviations: BDNF, brain-derived neurotrophic factor; PKC, protein kinase C.
Box 1. Synaptic Loss in Alzheimer’s Disease

Synaptic loss and abnormality occur early in AD and are very highly correlated with severity of the cognitive deficits in AD patients [8]. The synapses in both the degenerated and surviving neurons are affected. The correlations between the cognitive functions in AD patients and plaques and tangles are rather weak [8]. Figure I shows correlated alterations in the number of synapses and neuropil volume in the hippocampal CA1 regions of AD patients with the levels of cognitive deficits: decreases in subjects with mild cognitive impairment and further decreases in mild AD subjects (postmortem human brains [11]).

Figure I. The Total Number of Synapses (A) and Neuropil Volume (B) in the Stratum Radiatum Subregion of the Hippocampal CA1 of Three Different Diagnostic Groups of Human Subjects Examined. Figure reproduced and adapted with permission from Wolters Kluwer Health, Inc. [11]. Abbreviations: mAD, Mild Alzheimer’s disease; MCI, mild cognitive impairment; NCI, no cognitive impairment.

brain is present only at later AD stages. Most of the initial antitau therapies have been discontinued because of toxicity and/or lack of efficacy [18], in addition to the observation that the existence of NFTs does not necessarily impair neuronal function [2]. However, the existing endogenous neural regeneration/repair mechanisms in adult mammalian brains (see below), though insufficient by itself, raises hopes that AD may be eventually cured through restoring the brains’ capacity for neural regeneration. In this short review, we shall discuss the roles of neural and synaptic regeneration in cognition, in cognitive impairments of AD, and as an essential AD therapeutic target.

Synaptogenesis and Remodeling

Mammalian brains operate on an efficiency principal of keeping the number of synapses modest for a particular function, since signal transfer through synapses is rather expensive in energy cost
Powered through plasticity, the brain, however, can remodel its structure and operation of synapses/network for new challenges. The downside of this efficiency, the lack of abundance in neural connections, is its vulnerability to injury/damage, especially when this remodeling/regeneration ability is compromised.

Cognitive functions are guarded constantly through active repairing/regeneration against any injury/damage to the synapses/networks. Neural regeneration, or neuro-regeneration, refers to an action or process of regenerating/restoring neuronal structures [19], such as synapses or neurons, from injury/damage, to its preinjury state. A successful neuro-regeneration can be achieved through endogenously or exogenously (neuronal replacement) methods. The endogenous process involves increasing the expression of genes and activity of proteins, such as neurotrophins, growth associated protein 43 (GAP-43), and many other signaling molecules.

Neurotrophins are a family of proteins, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin 3 (NT-3), and neurotrophin 4 (NT-4/5), in mammals. NGF, NT3, and NT4 are secreted mainly through constitutive pathways. BDNF, a synaptogenic neurotrophin, is unique for its activity-dependent activation and high level of expression in the brains. In cultured neurons, transient tropomyosin receptor kinase B (TrkB); a BDNF receptor) activation promotes dendritic growth and spine morphogenesis, while sustained TrkB activation facilitates neuronal dendritic arborization and spinogenesis. BDNF plays essential roles in cell proliferation [20], neurogenesis [19,21,22], cognition, and synaptic integrity, including regulation of synaptic transmission, synaptic plasticity, and synaptic growth in developing and adult brains. Activity-dependent BDNF activation in the brain is required for maintenance of mature spine phenotype [23] and involves activity-driven Ca²⁺ signals [24], as well as demethylation of the BDNF promoter IV in postmitotic neurons. This demethylation is sufficient to activate BDNF expression [25].

The BDNF signaling pathways in the brains are regulated by specific protein kinase C (PKC) isoforms. The PKC-BDNF signaling pathways play essential roles in maintaining synaptic functions and structures and a variety of memory tasks. PKC activation, with bryostatin-1 or other specific agents, promotes BDNF expression and secretion, synaptogenesis, and neurogenesis in the hippocampus and related cortices and provides protective effects against a variety of neurotoxic events/factors, such as amyloidosis, tauopathy, apoptosis, neuroinflammation, and oxidants (Figure 2). Common pathways, for example, the mammalian target of rapamycin (mTOR) signaling pathway, operate during developmental axon regrowth and axonal regeneration [26]. mTOR, a serine/threonine kinase for cell survival and growth, integrates messages to regulate transcription, translation, and other cellular functions [27] and may also serve as one of the therapeutic targets.

Impaired Neuro-regeneration in AD

Cognition depends on integrity and operational efficacy of synapses, both structural and functional, and both are vulnerable to injuries and disorders. Increasing evidence indicates that synaptic deficiency is a key pathophysiological hallmark in AD. Deficits in BDNF mRNA and spine pathology (synaptic degeneration) are the earliest pathologies in AD [28,29], before any neuronal loss and perhaps prior to Aβ deposition [30,31]. Pathological damage and injury to the brain result in often irreversible dysfunction of the brain, mainly due to the insufficient ability of the brain to regenerate or repair [32]. Thus, a key pathogenic mechanism underlying AD progression is long-term deficits in neurotrophic signaling, deficits observed in preclinical AD [33]: reduction in the PKC-BDNF levels, BDNF transport [34],...
Protective functions
- Protects synapses/network from synaptic deficiency against injury and damage
- Protects synapses against neurotoxic Aβ oligomers
- Protects against neuronal death antiapoptosis
- Prevents oxidative stress and mitochondrial dysfunction
- Normalizes GSK3-β and inhibits hyperphosphorylated tau formation and transformation to neurofibrillary tangles
- Blocks APOE4’s reduction of BDNF via HDAC inhibition
- Reduces neuroinflammation and promotes remyelination

Activation functions
- Experience mediated remodeling of synaptic operations and structures in memory acquisition and consolidation
- Synaptic growth factors (BDNF, NGF, IGF, etc.)
- Synaptic regeneration, remodeling/repairing, and maturation
- α-Secretase activity, which reduces neurotoxic Aβ formation through BACE and γ-secretase
- Amyloid degrading enzymes (EC, neprilysin, and IDE)
- Inhibition of GSK3-β hyperphosphorylation of tau

Figure 2. Effects of Protein Kinase Cε (PKCε). PKCε is a key signaling enzyme that correlates synaptic growth and neuronal revitalization and also blocks formation of amyloid beta (Aβ) and abnormal tau. The isoform can be selectively activated by bradykinin-1 or other agents. Through a dynamic regulation of neurotrophin activity and other signaling molecules, such as GAP-43, extracellular-signaling-regulated kinases (ERK), and myristoylated alanine-rich C-kinase substrate (MARCKS), the PKC-BDNF (brain-derived neurotrophic factor) signaling pathways play essential roles in maintaining synaptic functions and structures and a variety of memory tasks, such as Pavlovian conditioning of the subdivisions in the hippocampus, spatial learning and memory in rats, recognition memory, learning, and memory of eyeblink conditioning in rabbits, olfactory discrimination learning, conditioned avoidance, contextual fear memory, and substance-associated reward memory in other mammalian animal models. Appropriate levels of PKCε activity in the brain sustain synaptic integrity and capacity and reduces prevalence and effects of neurotoxic forms of Aβ and tauopathy. Structure of PKCε based on Pymol rendering of Protein Data Bank 1gm1 and adapted from https://commons.wikimedia.org/w/index.php?curid=88211044. Abbreviations: BACE, β-site APP-cleaving enzyme1; BDNF, brain-derived neurotrophic factor; HDAC, Histone deacetylase; NGF, nerve growth factor.

and/or deficits of BDNF downstream receptors and signaling pathways in the hippocampal and related cortices.

Mammalian brains have a certain capacity to regenerate/remodel synapses/neural networks when facing injury, disorders, and cognitive challenges. Cognitive impairment becomes evident only when injury/damage/cognitive demands reach a threshold by which the brain can no longer initiate and sustain the required responses. This threshold is lower with neurotrophic hypoactivity.

The brain’s responses to injury also involve reactions from its immune system, mainly through microglia, the immune cells of the nervous system. Inflammation can be chronic and systemic in the absence of overt infection. Evidence suggests that microglia might be the mediator of synaptic loss [35]. Upon injury, the immune system in the brain responds quickly, resulting in activation and proliferation of microglia [36–38], inducing the release of associated inflammatory factors, as well as attracting additional immune cells from the blood. Microglia release BDNF and have a strong pro-regenerative role in the nervous system, as in other organs/tissues [39], but also promote activity-dependent synaptic pruning and inflammation (see below). In AD, microglia exert a neuroprotective role through phagocytosis of debris and...
toxins, including Aβ, at least initially. However, Aβ affects glial function, leading to neurotoxic consequences, especially when the reaction becomes a chronic and more damaging proinflammatory state [40]. During development, microglia-mediated synapse removal is an important process for proper brain maturation during development. Active microglia promote phagocytosis of neuronal, in particular, synaptic structures [41]. Microglia can also release soluble synaptotoxic factors, such as tumor necrosis factor-α, nitric oxide, and IL-6, promoting synapse loss. Microglia and complement (C1q, the initiating protein of the classical complement cascade, and C3, the microglial complement 3), might be involved in early synaptic loss in AD models [35]. In mammalian brains, microglia are the dominant source of C1q [42]. Diffusible Aβ increases C1q, which promotes activation of C3 [12], which in turn opsonizes subsets of synapses for elimination in developing brains. Activated microglia can also induce astrocytes to become neurotoxic [43]. The neurotoxic astrocytes lose the ability to promote neuronal survival, outgrowth, and synaptogenesis, but rather induce the death of neurons and oligodendrocytes [43]. By using a colony stimulating factor 1 receptor inhibitor PLX56622, which specifically depleted microglia, a recent study indicated that microglia were not essential in retinal ganglion cell degeneration or axonal regeneration after central nervous system (CNS) injury [44], although the removal of death-labeled retinal ganglion cells was impaired after microglia depletion.

Microglia-mediated cellular damage and synaptic loss are mainly secondary, further worsening the sustained low repairing/regeneration ability in AD brains. Associated with the early stage of AD is a reduced signal pathway (the PKC-BDNF-TrkB signalling pathway), PKCε activity, and neurotrophic activity in the brain [34,45], which may account for the alterations in the memory deficits, neuronal cell death, and synaptic deficiency in AD [46].

**Neuro-regeneration Targeting Interventions for Memory Impairment**

Memory impairment and dementia are the consequences of synaptic/neuronal deficits. The intrinsic neuro-regeneration capacity in the mammalian brain has been shown to be very limited, even after eliminating multiple known inhibitory signals [47]. In the adult mouse brain, most axons cannot regenerate sufficiently, even with precise laser-mediated lesions that produce minor scarring [48]. This low endogenous capacity for neuro-regeneration in mammalian brains does not mean, however, that this capacity cannot be enhanced to achieve dramatic outcomes. Spines are highly dynamic and capable of remodeling and restoring their original structure, location, and function [49], when triggered with appropriate therapeutics, such as neurotrophic activators (see below). Therapeutic strategies aimed at the reactivation of these pathways in injured CNS neurons might be successful in enhancing our capacity to revitalize neurons and regenerate synapses.

**Nonpharmacological Intervention**

A wide range of nonpharmacological approaches have been tried to treat AD models and AD patients. Some have been reported to promote neurotrophic activity and neuro-regeneration and exhibit therapeutic value against cognitive impairments, but their clinical utility is still preliminary. Readers are referred to a recent review for their utility and limitations [50].

**Environmental Enrichment and Exercise**

Increasing evidence suggests that manipulating neuronal activity [51] might be an approach for enhancing intrinsic neuronal growth ability. Calcium influx into the axoplasm is one of the first signals caused by injury, a back-propagating signal required to activate endogenous protein synthesis for sealing the membranes, assembly of growth cone, and enzymatic histone acetylation [52]. Environmental enrichment and physical exercise are the least invasive
approaches that enhance endogenous neuro-regeneration, although they are mild and limited [53], they produce impacts better than social enrichment for reducing memory deficits in AD rats [54].

**Brain Stimulation**

Brain stimulation has generated some hope for symptomatic relief in AD. Transcranial current stimulation, a noninvasive technique, induces BDNF expression and secretion and enhances passive avoidance learning, an effect blocked by TrkB inhibitor [55]. Deep brain stimulation, an invasive neurosurgical procedure, involves driving a small burr hole into the skull and inserting thin electrodes deep into specific brain targets to stimulate the tissue electrically. Thus, brain activity accessible under surgery can also be directly measured. In a recent clinical trial with mild AD cases, a fornix deep brain stimulation increased glucose metabolism but produced no differences in cognitive outcomes [56]. A direct electric pulse may cause an acute depolarization and a disruption in memory formation and recall [57]. Brain stimulation thus has risks of interfering with memory functions, resulting in adverse reaction in cognition [58].

**Neural Stem Cells**

Loss of neurons could be significant, especially at the late AD stage. Repopulation of the lost neuronal circuitry with stem cells and regeneration of the lost structures/synapses are rational strategies in therapy for patients with late-stage AD. Several exogenous sources are available [59]. The therapeutic effects of stem cells rely on two developments: an incorporation of the cells into the neural network and an increased secretion of neurotrophins, which promote endogenous neuro-regeneration [60]. Alternatively, the endogenous source can also be activated through neurogenesis and/or through direct reprogramming [61]. In preclinical studies, most hippocampus transplanted neonatal mouse subventricular zone-derived neural stem cells are reported to differentiate into neurons [62], resulting in cognitive improvements in animal models [63], but see [64]. The mesenchymal stem cells (MSCs), for instance, have been reported to produce neurotherapeutic effects in animal models [65,66], but not in AD patients [59] (i.e., they caused no slowing of cognitive decline and AD pathology over the 24 months of follow-up). While still not precisely defined, the therapeutic mechanism(s) may include inducing neurorepairing activity through BDNF in addition to a potential integration into the existing brain network of the host. In a recent study, BDNF was used to modify human umbilical cord MSCs and transplantation of these cells to the hippocampal area of Aβ-impaired rats significantly improved their spatial learning and memory abilities, enhanced the activation of astrocytes and microglia, reduced the expression of Aβ and recombinant human β-site APP-cleaving enzyme1 (BACE1), inhibited neuronal apoptosis, and promoted neurogenesis [60]. An enhanced BDNF activity may underlie the improved outcome [60]. The future of such cell therapy in AD patients, of course, depends on its cognitive efficacy and long-term safety. This is particularly true since there have been few demonstrations that exogenously introduced stem cells form functioning synaptic networks that are capable of meaningful information processing.

**Pharmacological Interventions**

As mentioned above, whilst adult mammalian brains have capacity for synaptic/neuronal repair and remodeling, this regenerative capacity is insufficient for replacing the number of synapses/neurons lost due to injury or neurodegenerative disorders, even after enhancement through physical exercise or environmental enrichment. The question is thus how to address the insufficient neural regeneration through synaptic/neuronal pharmacology. Several compounds, such as bryostatin-1 and DCP-LA (see below and Figure 3), show promising therapeutic potential against AD. These agents, including those promising and less so, tend to possess a similar pattern of
Figure 3. Enhancement of Brain Protein Kinase (PK)Cα-BDNF (Brain-Derived Neurotrophic Factor) Signaling Activity Produces Therapeutic Effects on Learning and Memory in 5xFAD Mice and Advanced Alzheimer’s Disease (AD) Patients. (A) DCP-LA, a selective PKCα activator, rescues, through blind-evaluation of synapses with electron microscopy (A1), the ability to maintain the number of synapses (A2) and the capacity in formation mushroom synapses (A3) in the hippocampus of 5xFAD mice. Yellow highlights mushroom spine synapses, and red, synapses. (B) DCP-LA prevents deficits in water-maze spatial learning (B1, shown as means ± SEM (standard error of the mean), using the daily three trials as a block) and memory performance (B2, target quadrant ratios) in 5xFAD mice. Data are shown as means ± SEM; *, P ≤ 0.05; **, P ≤ 0.01. Figure reproduced, with permission, from [7]. (C) Bryostatin-1 produces a significant improvement in cognition, evaluated with Severe Impairment Battery (SIB) in advanced AD patients without memantine. The traces show that SIB improves throughout the trial in the bryostatin-1 group and that SIB declines in the placebo group. Figure reproduced, with permission, from [105]. Abbreviations: Bry, bryostatin-1; DCP, DCP-LA; Veh, vehicle; 5x, 5xFAD transgenic mice.
multiple pharmacological profiles, such as antioxidant, anti-inflammatory, pro-BDNF, and pro-synaptic remodeling/regeneration.

**Antioxidants**

Oxidants may play an important role in AD pathology, synaptic dysfunction, and cognitive impairment [67]. Resveratrol (3,5,4′-trihydroxy-trans-stilbene), a polyphenolic compound (in grapes, peanuts, berries, and more than 70 other plant species), can activate SirTuin 1 (SIRT1, silent information regulator-1), an NAD+-dependent protein deacetylase, and produce pro-BDNF, antioxidative, anti-inflammatory, and antiapoptotic processes, autophagy regulation, and neuroprotection against cognitive impairment [68,69]. Sirt1 knockout mice exhibited defects in dendritic development and synaptic function [70].

Curcumin, (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a component of Indian spice turmeric, can bind directly to Aβ and prevent Aβ aggregation [71], in addition to its antioxidant and anti-inflammatory action [72,73]. Clinical studies of curcumin in mild AD, however, found no significant differences in cognition between placebo and intervention groups [74]. It is not clear whether the failure is due to its poor absorption [75].

**Anti-inflammatory Agents**

Two types of observations suggest involvement of inflammation in AD. First, early studies reveal the existence of reactive microglia surrounding amyloid plaques in AD brains [76]. Second, the prevalence of AD in patients with rheumatoid arthritis is unexpectedly low [77], probably due to the usage of anti-inflammatory therapy, as in the long-term users of nonsteroidal anti-inflammatory drugs (NSAIDs) [78]. However, it has not been actually well established whether this overt plaque-related inflammatory/immune process is helpful or destructive in the context of AD progression. There is abundant evidence that anti-inflammatory treatments can produce anti-AD impacts in AD models [79]. But, the importance of neuroinflammation in AD pathogenesis and progression has been questioned by an equal number of observations. Donepezil, for instance, can effectively inhibit microglial activation in APP/PS1 mice and produce cognitive improvements [80], but does not appear to modify AD progression. Anti-inflammatory cytokines have also been reported to increase AD plaque burden and worsen cognitive outcomes in AD mice [81,82]. The controversy does urge caution in focusing on the therapeutic strategies through inhibiting microglial activation before we fully understand the role of inflammation in AD. Although there are a large number of failed clinical trials with anti-inflammatory agents in AD patients [43,83,84], including some classical NSAIDs, such as ibuprofen, rofecoxib, celecoxib, and R-flurbiprofen, and other anti-inflammatories, such as pioglitazone (acting on PPAR-γ), steroids, and aspirin, these trials do not necessarily rule out the involvement of neuroinflammation in preclinical AD pathology [85]. The late AD inflammation may just represent a tissue resolution [86], less relevant in AD pathology since it wanes with age [87].

**Histone Deacetylase (HDAC) Inhibitors**

Histone deacetylation has been implicated in contributing to the AD-like phenotype. A high level of HDAC3 expression is seen in the hippocampus and cortical areas. An increased nuclear translocation of HDACs is associated with BDNF downregulation in human neurons, a response that can be evoked by Aβ oligomers or ApoE4 but reversed with PKCζ activator bryostatin-1 [88]. HDAC inhibitors have been tested for their therapeutic effects in AD [89]. RGFP-966, a brain-penetrant and selective HDAC3 inhibitor, has been found to increase histone H3 and H4 acetylation and BDNF expression, decrease tauopathy and Aβ1–42 accumulation, and improve spatial learning and memory in 3xTg-AD mice [90].
Enhancing PKC-BDNF Signaling

Several signaling pathways play critical roles in synaptogenesis, synaptic maturation, and synaptic repair, the endogenous mechanisms to maintain synaptic/neuronal integrity against injury/damage/cognitive challenges. Their actions can be modulated through pharmacological treatment and neurotrophic activation.

Synaptic repair and behavioral normalization can be achieved with an enhanced neurotrophic activity without targeting Aβ and tauopathy [91,92]. In a recent study, a combination of BDNF and induced neuregulation is reported to reduce cognitive impairment in 5xFAD mice [93]. More recently, conditional BDNF delivery from astrocytes through overexpressing BDNF under the GFAP promoter, has been found to rescue memory deficits, spine density, and synaptic properties in 5xFAD mice [94]. The authors concluded that the effects of conditional BDNF did not result from reduction in amyloidosis or neurogenesis improvement, but rather from changes in structural and functional synaptic properties [94]. With a focus on reversing synaptic and neuronal loss in AD, we have developed a therapeutic strategy that has shown a neurorestorative potential (i.e., to restore lost synapses in AD brains in preclinical studies) [9,95], as well as the concomitant potential to prevent apoposis [9,96–98], reduce Aβ oligomers, lower hyperphosphorylated tau [9,97–100], miRNA stabilization of growth factor mRNAs, and reduce oxidative stress [101]. Activators of PKCε (Figure 2) with bryostatin-1, a relatively selective and powerful PKCε activator with clinical safety profile at appropriate doses [102], and DCP-LA (Figure 3) have been shown to increase synaptic numbers via synaptic growth factors [103,104]. Bryostatin-1, a macrocyclic lactone, enhances BDNF expression/secretion and synaptic remodeling/synaptogenesis in the brain and produces several other anti-AD effects, such as antiapoptosis, anti-inflammation, anti-amyloidosis, antitaupathy, and antioxidant, at therapeutic doses [10,105].

Clinical trials using recombinant BDNF are disappointing so far, most likely due to poor delivery and a short half-life of BDNF in vivo. Chronic administration of bryostatin-1, however, has been shown to improve cognitive functions in advanced AD patients in the absence of memantine (Figure 3) [105]. An optimal prodrug of the BDNF mimetic compound 7,8-dihydroxyflavone (7,8-DHF), a potent small molecular TrkB agonist with poor oral bioavailability, has recently been found to prevent Aβ deposition and synaptic loss in the hippocampus in 5xFAD mice [106]. ROCK (Rho kinase) inhibitors, such as FSD-C10 [107], have also been found to promote BDNF and GDNF activity, reduce Aβ and taupathy, and improve cognition in APP/PS1 mice [108].

Concluding Remarks

It has been well established that pathological changes in the AD brain occur early, many years before any clinical symptoms. Aβ could accumulate in the brains for 15–20 years before any AD clinical symptoms [109,110]. Evidence supports the notion that cognitive deficits occur only when synapses/neural network cannot be appropriately maintained through neuronal/synaptic repair and synaptogenesis/neuro-regeneration to meet cognitive demands, indicating that synaptic deficiency should be the focused therapeutic target. The synaptic deficiency hypothesis (Figure 1), consistent with enormous evidence that an early failed maintenance in synaptic integrity triggers neurodegeneration in the brain and cognitive decline, does not rule out the pathological contribution of neurotoxic Aβ and tauopathy to synaptic/cognitive deficits in AD and potential therapeutic benefits of anti-Aβ and antitaupathy. Along this line is also the evidence that oral Porphyromonas gingivalis infection results in accumulation of neurotoxic gingipains in AD brains and amyloidosis [111]. Gingipain inhibitors could be valuable for treating P. gingivalis and stopping neurodegeneration in AD. The possibility also exists that Aβ and tau synergize to impair the functional integrity of neural circuits [112]. However, evidence is abundant that formation of amyloid and...
hyperphosphorylated tau is reactive (secondary; [60,113,114,119]) to brain injury, hypoactive BDNF [102], and deficient BDNF-associated degenerative processes, as are microglia and neurotoxic astrocytes. Amyloid accumulation and tauopathy can reduce BDNF levels and secretion [115], probably contributing to a further (secondary) reduction in neurotrophic activity in the brain (Figure 1B).

Accumulating evidence, to date, suggests that structural and functional deficits of synapses are at the core of the underlying pathophysiology in AD (see Outstanding Questions). In clinical trials, AD therapeutics that target synaptic loss and dysfunction (i.e., to slow, halt, or reverse progression of the disorders at the level of synapses), via synaptogenic molecular cascades such as the PKC-BDNF signaling pathway, show promising results [105]. This differs from the failure of 300–400 AD drug candidates in recent years [116]. The key to effective neurotrophic therapy in AD appears to require a sustained and appropriate PKC-BDNF activity in the brain, guiding against the aging/disease-related neurodegenerative process. A too-high level of PKC-BDNF activity can also be neurotoxic [117,118]. Overcoming the self-repair limitation in the mammalian brain would transform how AD patients, and others with memory disorders, are treated clinically.

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