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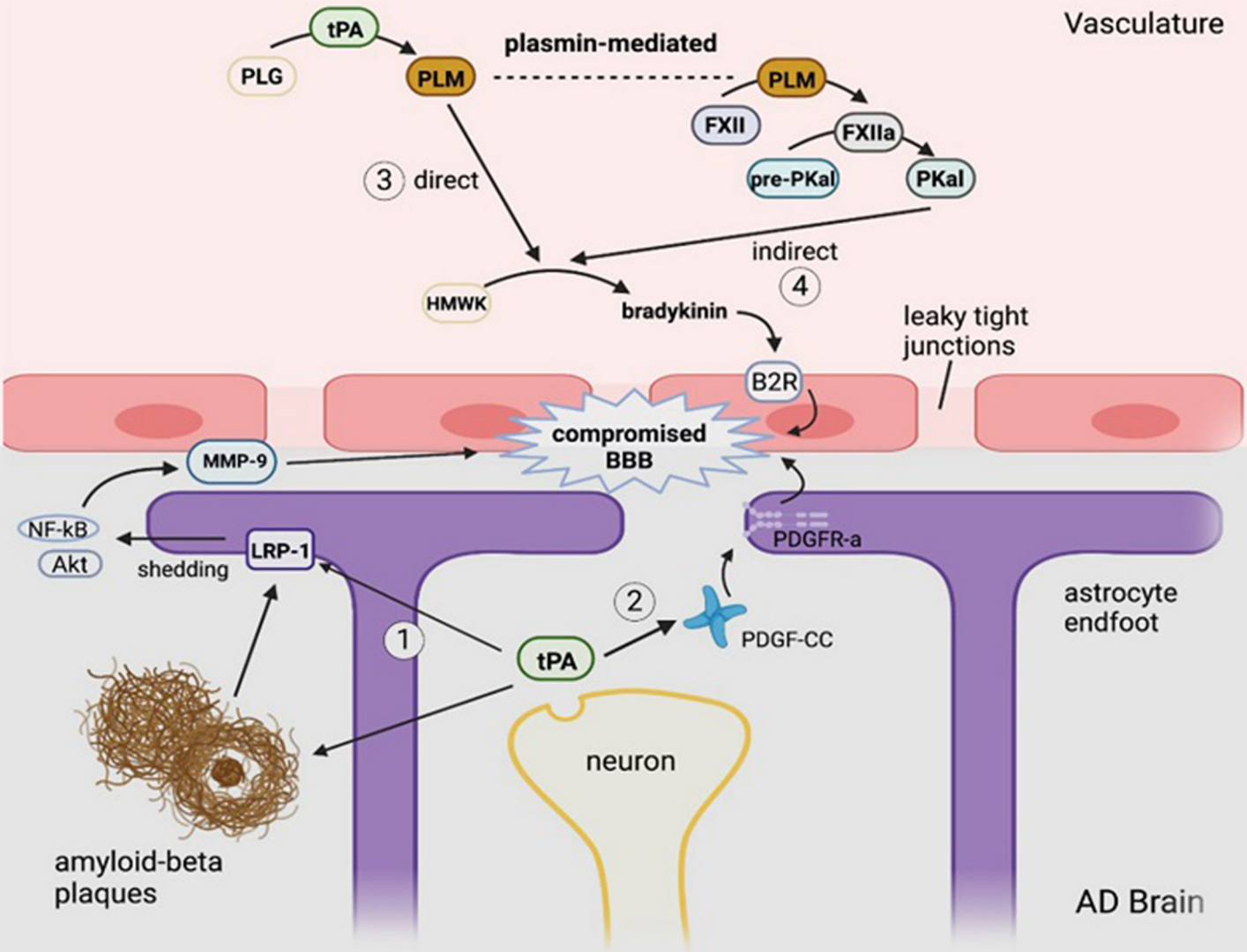
AGEING AND NEURODEGENERATIVE DISEASES



Review of evidence implicating the plasminogen activator system in blood-brain barrier dysfunction associated with Alzheimer's disease

Mei-Yun Tang, Fredric A. Gorin, Pamela J. Lein

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High-quality original articles, reviews, case reports, commentaries are welcomed from ageing and neurodegenerative diseases, particularly on the basic mechanisms of ageing and their roles in the onset and progression of neurodegenerative diseases. The journal aims to report innovative research advances on the following topics:

1. The cellular and molecular mechanisms of ageing and the pathogenesis of neurodegenerative diseases;

2. The associations between neurodegenerative diseases and the biological bases of ageing with a focus on: genomic instability, epigenetic alterations, telomere attrition, protein degradation system failure, mitochondrial dysfunction, cellular senescence, nutrient sensing deregulation, stem cell exhaustion, intercellular communication impairment, etc.;

3. Translational research into prevention and treatment of age-related neurodegenerative diseases;

4. Mechanistic bases for epidemiological observations in aging-related neurodegenerative diseases.

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AND is a peer-reviewed and open access multidisciplinary journal that publishes high-quality original articles, reviews, case reports, commentaries, letters to editor, etc. Ageing is a major risk factor for neurodegeneration, and the prevalence of ageing-related neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, etc. continues to rise with the increased ageing population. Unfortunately, there are no effective treatments available for the age-related neurodegenerative diseases. Thus, to develop successful interventions, it is important to investigate the basic mechanisms of ageing and their roles in the onset and progression of neurodegenerative diseases. Therefore, we plan to launch this new journal, which is aimed to report innovative research advances on the following topics:

1. The cellular and molecular mechanisms of ageing and the pathogenesis of neurodegenerative diseases;
2. The associations between neurodegenerative diseases and the biological bases of ageing with a focus on: genomic instability, epigenetic alterations, telomere attrition, protein degradation system failure, mitochondrial dysfunction, cellular senescence, nutrient sensing deregulation, stem cell exhaustion, intercellular communication impairment, etc.;
3. Translational research into prevention and treatment of age-related neurodegenerative diseases;
4. Mechanistic bases for epidemiological observations in aging-related neurodegenerative diseases;
5. Psychology and Geriatric Mental Illness;
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Ageing and Neurodegenerative Diseases

Review

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A novel neuroprotective cholinesterase-monoamine oxidase inhibitor for treatment of dementia and depression in Parkinson's disease

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Abstract

The current novel therapeutic approach suggests that multi-targeted compounds, with diverse biological activities but a single set of bioavailability and pharmacokinetics, will be significantly more advantageous in the treatment of the complex pathology of Parkinson's diseases (PD) than traditional therapies. This review introduces a novel cholinesterase (ChE)-monoamine oxidase (MAO) inhibitor, namely MT-031, which was designed by amalgamating the propargyl moiety of the irreversible selective MAO-B inhibitor and neuroprotective/neurorestorative anti-Parkinsonian drug, rasagiline, into the methylamino position of the ChE inhibitor anti-AD drug, rivastigmine. MT-031 possesses neuroprotective, cognition enhancing, anti-depressant, and anti-inflammatory properties both *in vitro* and *in vivo*. Altogether, these findings suggest that MT-031 may be a potential treatment for combating PD and associated dementia and depression.

Keywords: Parkinson's disease, dementia, cholinesterase, monoamine oxidase, multi-targeted drugs

INTRODUCTION

With aging and the increasing life span of the population, Parkinson's disease (PD), an age-related neurodegenerative disorder, is receiving increased attention. It is estimated that the number of PD patients will reach more than 12 million by 2040, doubling the cases seen in 2016[1]. The motor deficits of PD are emphasized in both making the initial diagnosis and in tracking the progression of the disease[2]. As understanding of the symptoms and pathogenesis deepens, however, it has been suggested that the non-motor features of PD, including cognitive impairment, i.e., dementia, should be more attended to[3,4]. A previous study indicated that approximately 25.8% of individuals with PD exhibit mild cognitive impairment[5], and longitudinal studies have documented that up to 70% of these patients will progress to dementia after ten years of symptoms[3]. In addition to cognitive impairment, other symptoms, e.g., depression, may emerge regularly throughout the development of PD[6-8], and this symptom may worsen the severity of dementia as the disease progresses. Since dementia in both Alzheimer's disease (AD) and PD patients generally presents with similar features, present treatments for Parkinson's disease dementia (PDD) are mostly derived from drugs utilized in AD, such as cholinesterase inhibitors (ChEIs) and memantine, which was initially developed for the treatment of AD. To date, rivastigmine is the only FDA-approved therapy that is currently licensed for PDD.

It is well known that neurodegenerative diseases, such as AD, PD, amyotrophic lateral sclerosis, and Huntington's disease, are possibly triggered by a group of pathologies, characterized by separate etiologies with distinct morphological and pathophysiological features, including iron accumulation[9-11], generation of reactive oxygen[11] and nitrogen species[12], inflammation[13-15], mitochondrial (complex I) deficiency[16], ubiquitin-proteasome system dysfunction[17], and abnormal protein folding and aggregation[18,19]. This suggests that the "cocktail of drugs" strategy, i.e., mixing different targeted molecules as drug combinations, may offer theoretically feasible treatment for these diseases. Nonetheless, compared to using a single effective compound, the cocktail strategy increases the risk of side effects and ups the difficulty of managing drug-drug interactions, safe dosing, and metabolic shunt effects[20,21]. A single drug with multiple targets - one compound conjugating two or more diverse biological properties - thus has a pronounced advantage over single-target drugs or drug cocktails[22,23]. An attractive example of a multi-targeted drug is ladostigil (TV3326), a cholinesterase (ChE)-monoamine oxidase (MAO) inhibitor, indicated to target various pathogenic mechanisms of neurodegenerative diseases[24-27]. The underlying principle in the design of ladostigil was to join the carbamate ChE inhibitory moiety of the anti-AD drug, rivastigmine, to the irreversible selective MAO-B inhibitor, rasagiline[24]. Ladostigil has shown positive results in a phase II clinical trial evaluating its safety and efficacy in patients diagnosed with MCI[28].

Based on a similar rationale, a novel ChE-MAO inhibitor, namely MT-031 [Figure 1], was designed and synthesized for the treatment of AD. MT-031 amalgamates the propargyl moiety of the irreversible selective MAO-B inhibitor and neuroprotective/neurorestorative drug, rasagiline, into the methylamino position of the ChE inhibitor, rivastigmine[29]. Since AD and PD share similar pharmacological treatment demands, this review discusses the potential use of this novel multi-targeted drug, MT-031, for dementia and depression in PD.

INHIBITORY EFFECT OF MT-031 ON MAO

Rasagiline (Azilect®) is an anti-Parkinsonian MAO-B inhibitor drug, which presented neuroprotective and neurorescue activities in animal models and neuronal cell models of neurodegeneration[30] and exerted

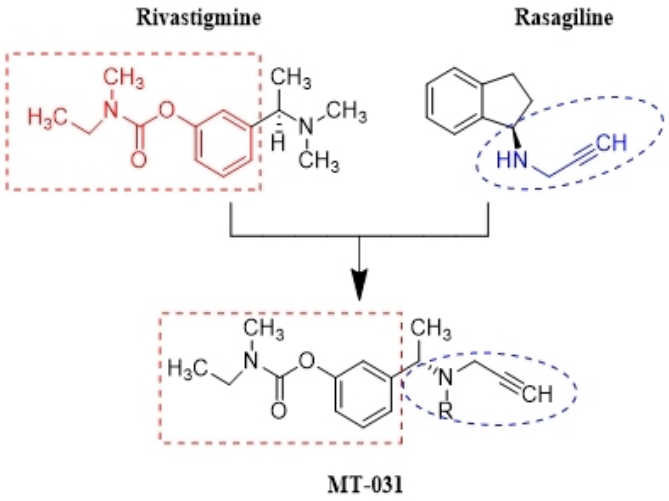

Figure 1. The chemical structure of the novel ChE-MAO inhibitor, MT-031[(*S*)-3-(1-(Methyl(prop-2-yn-1-yl)amino)ethyl)phenyl ethyl(methyl)carbamate], designed by amalgamating the active propargyl moiety of the anti-Parkinsonian drug, rasagiline, a brain selective MAO-B inhibitor, into the "N-methyl" position of the anti-AD drug ChE inhibitor, rivastigmine. AD: Alzheimer's disease; ChE: cholinesterase; MAO: monoamine oxidase; MAO-B: monoamine oxidase-B.

disease-modifying effects in PD patients[30-32]. The propargyl moiety of rasagiline has been proven to be an important active functional group for its MAO inhibitory activity[33,34] and neuroprotective/neurorestorative effects[35,36]. By retaining the active propargyl moiety, the inhibition of MAO in the brain is associated with neuroprotective effects in the neurodegenerative and age-related disturbances of homeostasis, and the products of the MAO-catalyzed reaction (e.g., aldehydes and hydrogen peroxide) are compelling inducers of lipid peroxidation and the generation of free radicals in the involution of the nervous system[37,38]. By retaining the propargyl moiety of rasagiline, MT-031 was found to be a selective MAO-A inhibitor (selectivity of MAO-A/B > 500-fold, Table 1); interestingly, this is different from its parent drug, rasagiline, which is a selective MAO-B inhibitor (selectivity of MAO-B/A = 100-fold, Table 1)[29]. In humans, MAO-A is found within the outer mitochondrial membrane of both neuronal and glial cells, where it participates in the inactivation of dopamine (DA) in the primate and human brain[39]. As dopamine depletion in the striatum causes the core motor manifestations of PD, a selective MAO-A inhibitor might provide an anti-Parkinsonian benefit[40,41].

Additionally, depression has also been reported to be one of the most common symptoms of PD, occuring in around 40% of patients with PD, and it is often persistent[42]. The efficacy of MAO-A inhibitors has been proven effective in the treatment of atypical depression, high levels of anxiety, anergic bipolar depression, and treatment-resistant depression for decades[43-45]. MAO-A mainly metabolizes serotonin (5-HT) and norepinephrine (NE), and a reduction in the 5-HT major metabolite, 5-hydroxyindoleacetic acid, in the cerebrospinal fluid was reported to be associated with violent and impulsive behavior, including violent suicide attempts[46]. The antidepressant effects of MAOIs were hypothesized to be based on a deficiency in catecholamines, specifically NE and DA, as well as possibly the indolamine 5-HT[47]; the mechanisms of action of MAOIs as antidepressants were thus thought to be because they directly resulted in increased levels of neurotransmitter amines at nerve terminals[48,49]. Selective MAO-B inhibitors may not be effective as antidepressants because MAO-B has no direct effect on either 5-HT or NE metabolism. A dual MAO-A/B inhibitor may rapidly increase DA levels to heighten feelings of pleasure, but abnormal surges in DA are linked to serious side effects[50-53]. Therefore, a drug with selective MAO-A inhibition could potentially be a safer and more effective treatment for depression in PD patients.

Table 1. The inhibitory effect (IC_{50}) of MT-031 and its parent drugs, rasagiline and rivastigmine, on MAO and ChE *in vitro*

Compound	Inhibition (IC_{50} μM[a])					
	MAO-A	MAO-B	MAO selectivity (A/B)	AChE	BuChE	ChE selectivity (AChE/BuChE)
MT-031	0.71 ± 0.04	> 1000	> 500	58.3 ± 6.3	34.6 ± 8.3	0.59
Rasagiline	0.41	0.0044	0.01	NA	NA	-
Rivastigmine	NA[b]	NA	-[c]	2.07	0.37	0.18

[a]IC_{50}, micromolar (μM) concentration at which compound inhibits 50% of the enzyme activity; [b]NA, no activity; [c]-, not tested. MAO: Monoamine oxidase; MAO-A: monoamine oxidase-A; MAO-B: monoamine oxidase-B; ChE: cholinesterase; AChE: acetylcholinesterase; BuChE: butyrylcholinesterase.

Moreover, an important finding is that, following administration of MT-031, there is little inhibition of MAO-A in the liver and small intestine[29,54]. Irreversible, high degrees of MAO-A inhibition in peripheral tissues is associated with potentiation of tyramine-induced cardiovascular activity[55], namely the "cheese effect"[56,57]. These data indicate that MT-031 may produce only limited potentiation of blood pressure in response to oral tyramine, as previously described for rasagiline[57,58] and other propargyl containing drugs, such as ladostigil[59], M30[60], and VAR-10303[61].

INHIBITORY EFFECT OF MT-031 ON CHE

To date, acetylcholinesterase inhibitors (AChEIs) have been the mainstay of therapeutic approaches for AD. AChEIs are used to increase synaptic levels of acetylcholine (ACh) and block the breakdown of ACh by inhibiting AChE[62]. Some reports suggest that cortical cholinergic deficits are more pronounced in PDD and that they are strongly correlated with cognitive decline and neuropsychiatric disturbances in PD[63,64]. The efficacy of the only FDA approved dual AChE and butyrylcholinesterase (BuChE) inhibitor, rivastigmine [Figure 1 and Table 1], one of the parent drugs of MT-031, has been proved in various clinical trials in the treatment of PDD[65]. Rivastigmine exerts its therapeutic effects by increasing the levels of acetylcholine in the brain via reversible inhibition of its hydrolysis[66]. It has been proposed that the effects of rivastigmine might reflect an additional property of BuChE inhibition, which is implicated in symptom progression and thus can provide some patients supplementary benefits over AChE selectivity[67]. In humans, AChE predominates (80%) and BuChE is considered to play a minor role in regulating ACh levels in the healthy brain[68]. Especially, BuChE activity rises while AChE activity remains unchanged or declines in the AD brain[68-70], thereby supporting the key role of BuChE in regulating brain acetylcholine levels[71]. Therefore, both enzymes are likely to be involved in regulating ACh levels and represent legitimate therapeutic targets to ameliorate cholinergic deficits[72]. MT-031 was found to significantly inhibit both AChE and BuChE activities *in vitro*, although with a lower IC_{50} than that of its parent drug, rivastigmine [Table 1][29]. Accordingly, our previous study showed that MT-031 treatment prevented cognitive deficits induced by scopolamine and improved spatial learning and memory. These results may be attributed to MT-031 being able attenuate scopolamine-induced ChE disturbance by inhibition of ChE activity. In addition, after acute treatment in rats, MT-031 inhibited cortical and hippocampal AChE/BuChE by 50%-70% at doses ranging from 5 to 10 mg/kg[29]. The high inhibitory effect of ChE activity is very crucial, as the fact that the clinical study of ladostigil (clinicaltrials.gov/ct2/show/NCT01354691) in the treatment of AD did not achieve its primary outcome may be due to its low inhibitory ratio on AChE (ladostigil inhibited an average of 21.3% of AChE)[28,73]. Furthermore, 24 h after the last dose was given to mice in a chronic administration model, MT-031 still caused dose-dependent antagonism of the spatial memory deficits induced by scopolamine in mice[54]. These results may suggest that MT-031 is a reversible but long-term ChE inhibitor, and that it is able to increase brain ACh levels sufficiently to compete with scopolamine for the muscarinic receptors subserving memory[74].

NEUROPROTECTIVE ACTIVITY OF MT-031

One aspect of the neuroprotective activity of MT-031 is that it directly scavenges free radicals over-produced in hydrogen peroxide (H_2O_2)-treated SH-SY5Y cells[29]. H_2O_2 is a major source of free radicals; it is produced during the redox process and considered to be a messenger in intracellular signaling cascades, including cellular metabolism and proliferation[75,76]. The predominant sources of H_2O_2 in the brain are spontaneous superoxide dismutation catalyzed by the enzyme superoxide dismutase[77] and MAO activity[78]. MAO-A and -B, in particular, catalyze the oxidative deamination of DA, 5-HT, and NE[39] and yield metabolic products, aldehydes, and reactive oxygen species (ROS) such as H_2O_2. Therefore, the neuroprotective abilities of MAO inhibitors in the treatment of PD may be through reducing ROS production[39,79,80]. In addition, several lines of evidence suggest that AChE and BuChE activation may be involved in the apoptosis associated with H_2O_2[81,82]. The link between cholinergic signaling and oxidative stress provides an additional therapeutic target for ChEIs in PD. Indeed, the ChEls, tacrine[81], huperzine A[83], and rivastigmine[84] were demonstrated to significantly protect cells against H_2O_2 insult. Moreover, MT-031 was found to enhance the mRNA expression levels of neurotrophins, anti-apoptotic molecules (Bcl-2 like 1 and Bcl-2), and an anti-oxidative enzyme (catalase) in the mouse striatum, further demonstrating the significant neuroprotective and anti-oxidative actions of this drug[54]. Multiple studies with various apoptotic paradigms have shown that Bcl-2 can protect cells against oxidative insults[85-88]. Measurements of ROS levels including H_2O_2 have shown that Bcl-2 expression is correlated with reduced levels of oxidative stress in cells exposed to oxidative damage. Additionally, increased synaptic ACh levels resulting from AChE inhibition may potentiate the effect of neurotrophins, neuronal growth factor and brain-derived neurotrophic factor, which was previously demonstrated to induce neuroprotection against free radical insults[89,90].

Increasing evidence suggests that neuroinflammation contributes to the cascade leading to progressive neuronal damage in PD[15,91]. The major pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-2, IL-6, IL-17, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), lead to increased production of inducible oxidative stress, neuronal stress, and further neuronal dysfunction and death in the AD brain[92-95]. The anti-inflammatory effect of MT-031 was found to be associated with elevation of the levels of one of the major cytokines, IL-10, which limits inflammation by reducing the synthesis of pro-inflammatory cytokines such as IL-1, IL-6, IFN-γ, and TNF-α[54]. The anti-inflammatory effect of MT-031 was also demonstrated in proliferated splenocytes activated by anti-CD3, in which MT-031 did not affect the viability of the unstimulated splenocytes, indicating that the anti-proliferative effect was not associated with a protective effect against cytotoxicity[54]. In addition to proliferation, splenocytes and microglia cells can also be activated to produce cytokines, multi-functional soluble factors with pro- and anti-inflammatory activities[96,97]. MT-031 suppressed the elevation of IL-17 and INF-γ in anti-CD3-activated splenocytes, possibly by increasing the generation of IL-2, although the exact mechanism needs to be addressed by further study. Inconsistent with the anti-inflammatory effects seen in cell cultures, MT-031 upregulated the mRNA expression levels of the anti-inflammatory cytokine neurotrophic tyrosine kinase receptor and reduced levels of the pro-inflammatory cytokine IL-6 in a scopolamine mouse model[54].

EFFECTS OF MT-031 ON SCOPOLAMINE-INDUCED DEMENTIA

It has been shown that scopolamine exerts its effects through antagonizing muscarinic acetylcholine receptors[98,99]. A previous study confirmed that MT-031 treatment prevented cognitive deficits induced by scopolamine and improved spatial learning and memory, as examined in the Y maze task and Morris water maze test[54]. This effect may be attributed to an increase of amine contents, NE, 5-HT, and DA, as well as to the direct effect on scopolamine-induced ChE disturbance through inhibition of ChE activity. MT-031 exerted a significant inhibitory effect on ChE in the hippocampus and frontal cortex of mice[54]. This is an

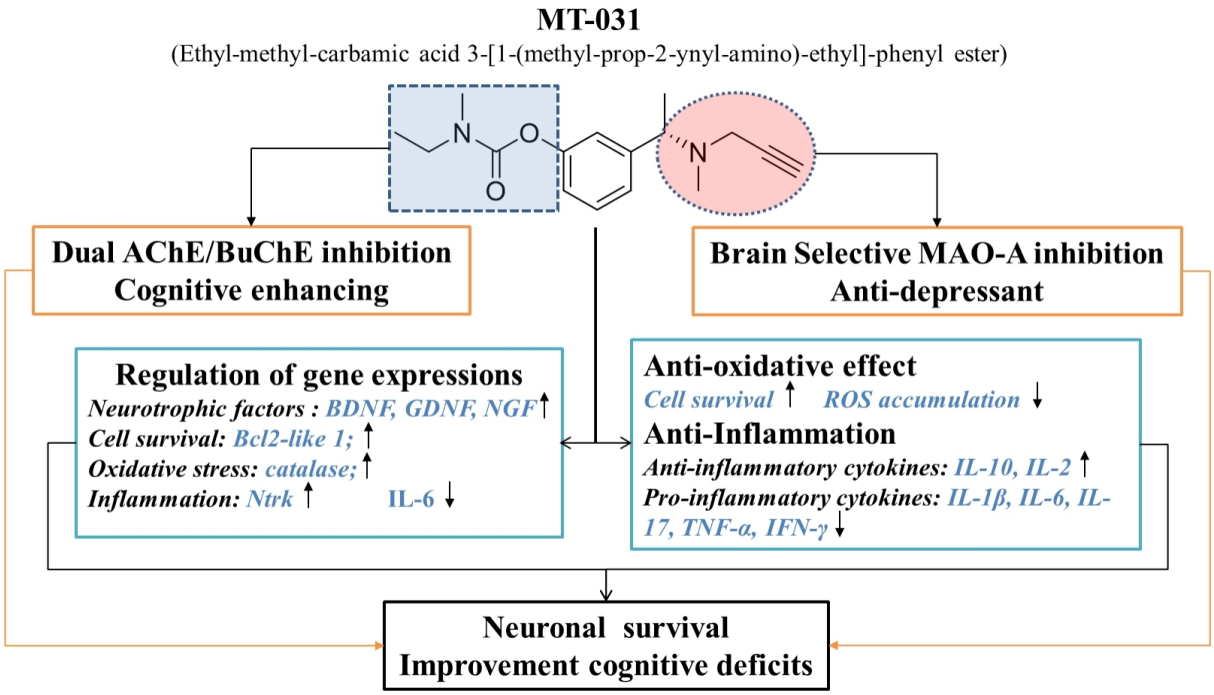

Figure 2. Suggestive schematic illustration for the mechanism of multifunctional brain permeable drug, MT-031, as a potential therapeutic approach of dementia and depression in PD. PD: Parkinson's disease; AChE: acetylcholinesterase; BuChE: butyrylcholinesterase; MAO-A: monoamine oxidase-A; ROS: reactive oxygen species; IL: interleukin; TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma; TNF-α: tumor necrosis factor-alpha; Ntrk: tyrosine kinase receptor; NGF: neuronal growth factor; BDNF: brain-derived neurotrophic factor; GDNF: glial cell-derived neurotrophic factor; Bcl-2 like 1: B-cell lymphoma 2 like 1.

advantageous property of MT-031, as previous data show that, when ChE inhibitors are less effective in the hippocampus, other brain regions may produce insufficient amounts of ACh to displace scopolamine from receptors, which results in dysfunctional mediation of working memory[100]. Our data are in line with the reported protective effects of rivastigmine[101] and ladostigil[102] in a scopolamine mouse model, suggesting the importance of inhibiting both AChE and BuChE activities in ameliorating cognitive impairments[65,101]. There are more and more studies that support the idea that multi-targeted brain selective MAO and ChE inhibitors may exert better treatment effects than single ChE inhibitors in the treatment of dementia in neurodegenerative disorders such as AD and PD[22,26,80].

CONCLUSION AND PERSPECTIVE

Available treatments for PDD are limited in both number and quality, and they only provide symptomatic relief for cognitive impairment. The multi-factorial causes of the disease make the development of new drugs a difficult task. The rational design of incorporating two or more distinct functional pharmacophores into one molecule has been suggested to be feasible[22,103]. A single target molecule may have greater affinity towards a specific target than a molecule with multiple targets; however, a multi-target strategy creates compounds with a balanced affinity for treating the multifactorial causes of multiple neurodegenerative diseases. To date, none of the cholinesterase inhibitors in the clinic has been proved to possess neuroprotective activity or anti-depressant action. The design of the novel drug candidate, MT-031, was aimed at targeting multiple neurodegenerative processes. MT-031 is a brain selective MAO-A and AChE/BuChE inhibitor and has been found to exert a wide range of neuroprotective activities [Figure 2], including anti-oxidative activity, clearance of ROS accumulation, prevention of neuronal death, and increasing levels of neurotrophic factors. MT-031 also possesses anti-inflammatory capabilities including

preventing cellular proliferation, upregulating anti-inflammatory cytokines, and downregulating pro-inflammatory cytokines[29,54]. There is evidence that MT-031 inherited the neuroprotective potency described for propargylamine derivatives in neurodegenerative animal models[29,54,104]. Similar to its other parent compound rivastigmine[101] at a dose that inhibited ChE in the cortex and hippocampus by approximately 70%, MT-031 was effective in antagonizing the working and reference memory deficits induced by scopolamine[54]. These miscellaneous pharmacological properties of MT-031 [Figure 2], accompanied by its ability to improve cognitive deficits, make this compound valuable as a novel drug candidate for the treatment of dementia and depression in PD.

DECLARATIONS

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Authors' contributions

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Checked the review paper: Wang Y, Youdim MBH

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Ageing and Neurodegenerative Diseases

Review

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Review of evidence implicating the plasminogen activator system in blood-brain barrier dysfunction associated with Alzheimer's disease

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Abstract

Elucidating the pathogenic mechanisms of Alzheimer's disease (AD) to identify therapeutic targets has been the focus of many decades of research. While deposition of extracellular amyloid-beta plaques and intraneuronal neurofibrillary tangles of hyperphosphorylated tau have historically been the two characteristic hallmarks of AD pathology, therapeutic strategies targeting these proteinopathies have not been successful in the clinics. Neuroinflammation has been gaining more attention as a therapeutic target because increasing evidence implicates neuroinflammation as a key factor in the early onset of AD disease progression. The peripheral immune response has emerged as an important contributor to the chronic neuroinflammation associated with AD pathophysiology. In this context, the plasminogen activator system (PAS), also referred to as the vasculature's fibrinolytic system, is emerging as a potential factor in AD pathogenesis. Evolving evidence suggests that the PAS plays a role in linking chronic peripheral inflammatory conditions to neuroinflammation in the brain. While the PAS is better known for its peripheral functions, components of the PAS are expressed in the brain and have been demonstrated to alter neuroinflammation and blood-brain barrier (BBB) permeation. Here, we review plasmin-dependent and -independent mechanisms by which the PAS modulates the BBB in AD pathogenesis and discuss therapeutic implications of these observations.

Keywords: Bradykinin, matrix metalloproteinase, neuroinflammation, neurovascular unit, plasmin, tissue-type plasminogen activator

INTRODUCTION

Alzheimer's disease (AD) is recognized as the most common cause of dementia in the elderly, and over 6 million Americans are currently living with this disorder. In the United States, AD is the sixth leading single cause of death and the second most common contributing cause of death. The hallmark neuropathologic characteristic of AD is abnormal extracellular protein accumulation in the brain, notably the extracellular deposition of amyloid-β (Aβ) peptide generated from the improper cleavage of amyloid precursor protein (APP) that gives rise to Aβ monomers that aggregate into oligomeric Aβ fibrils and plaques, and intraneuronal neurofibrillary tangles (NF) comprised largely of hyperphosphorylated tau. These proteinopathies are associated with the loss of synapses and subsequent neuronal cell loss in the entorhinal cortex, hippocampus, and frontal cortex[1-3], and currently, the biomarkers most commonly used in human AD studies are beta-amyloid 42, tau, and phospho-tau proteins in the cerebrospinal fluid. More recently, blood p-tau181 has been reported as being a useful biomarker for distinguishing AD from other dementias[4]. Thus, it has been widely posited that Aβ plaques and/or abnormal hyperphosphorylated tau protein accumulation are causally linked to the behavioral and neurologic symptoms of AD. However, therapeutic strategies for decreasing Aβ plaque load[5,6], reducing Aβ production with BACE-1 inhibitors[7], or inhibiting hyperphosphorylated tau aggregation[8], have been largely unsuccessful in clinical trials over the past several years[3]. These failed clinical trials coupled with observations of age-related increases in Aβ deposition in cognitively intact individuals as well as evidence that Aβ plaque load does not closely correspond with cognitive decline in AD patients[1,9] and neurofibrillary tangles are associated with severe cognitive impairment characteristic of late stages of AD[10,11], have prompted research into alternative pathogenic mechanisms of AD.

It is now recognized that the extracellular deposition of Aβ and hyperphosphorylated tau triggers pro-inflammatory responses in microglia and astrocytes[12-14]. The neuroinflammatory response in AD has been described in detail in several recent reviews[14,15], and it appears that neuroinflammation plays an important role in the early progression of AD[16,17]. Multiple investigators have shown that Aβ monofibrils, oligomers, and plaques activate gene expression of pro-inflammatory mediators in microglia and astrocytes[13,16,18,19]. While microglial phagocytosis of amyloid may be neuroprotective in the early stages of AD by promoting Aβ clearance[20,21], microglial activation in later stages may promote the progression of AD[1,16]. Network-based integrative analysis of whole-genome gene-expression profiling and genotypic data obtained from late-onset AD and non-demented control brains identified the immune/microglia module as the molecular system most strongly associated with the pathophysiology of AD, and in particular, late-onset AD[22]. Microglial activation is thought to promote AD progression by (1) complement-mediated phagocytosis of synaptic structures to promote synapse loss; and/or (2) release of nitric oxide (NO) and proinflammatory cytokines, including TNF-α, IL-6, and IL-1β, that act as soluble synaptotoxic factors and induce "A1" neurotoxic astrocytes[23-26]. In support of these proposed mechanisms, microglial activation has been linked to increased synaptic loss and neurodegeneration in AD[2,24,27], and pharmacologic inhibition of microglial proliferation in the APP/PS1 mouse effectively shifted microglia to an anti-inflammatory phenotype that was associated with decreased synaptic degeneration and improved memory[28]. In Alzheimer mouse models, early synaptic loss is associated with C1q complement tightly bound to AB plaques surrounded by neuronal atrophy from microglial phagocytosis[29]. Mononuclear phagocytes enter the central nervous system (CNS) signaled by chemokines (CXCL1), while the innate immune system also appears to contribute to the neuroinflammatory response to activated microglia in AD models[30].

While the initial focus on the role of the immune response in AD pathogenesis has been on the brain's intrinsic neuroinflammatory response, attention is now being directed to multiple systemic inflammatory disorders that accelerate or in some instances may be the primary trigger for neuroinflammatory responses that initiate and/or promote AD and other dementias[31-34]. Some of the observations that have stimulated this shift in focus include reports that young children chronically exposed to high levels of air pollution were found to have neuropathological hallmarks of AD upon incidental autopsy[35,36], and evidence that type 2 diabetes/ metabolic syndrome and inflammatory bowel disease are associated with increased risk of developing AD[15,37,38]. The causal factors linking peripheral inflammatory conditions to AD are likely multi-factorial and have not yet been clearly delineated; however, several mechanisms are emerging. Peripheral inflammatory conditions have been shown to (1) generate inflammatory cytokines that facilitate access of peripheral inflammatory lymphocytes into the CNS, most notably TNFα, IL-1β, and IL-6; (2) cause dysfunction of the blood-brain barrier (BBB); and (3) activate the plasminogen activator system (PAS), which has direct effects on the CNS and further facilitates BBB dysfunction. The remainder of this review will investigate the role of the PAS in mediating inflammatory crosstalk between the periphery and the brain and its potential role in AD pathogenesis.

PLASMINOGEN ACTIVATOR SYSTEM

The plasminogen activator system (PAS) is comprised of a group of serine proteases, inhibitors, and binding proteins that control the activity of the serine protease plasmin [Figure 1][39]. Plasmin plays a key role in the fibrinolysis cascade, catalyzing the final degradation of fibrin and various extracellular matrix proteins[40,41]. The zymogen plasminogen (PlG) is converted to activated plasmin by plasmin activators, which include tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). tPA is primarily involved in intravascular fibrinolysis, activating plasminogen that is bound to polymerized fibrin. In contrast, uPA is secreted as a pro-enzyme whose active form is primarily localized on cell surfaces where it binds to the uPA receptor (uPAR). Plasminogen conversion by tPA and uPA in both the periphery and the CNS is tightly regulated by serine protease inhibitors (serpins). Serpins represent a superfamily of proteins with similar structures. Most relevant to this discussion are plasminogen activator inhibitor type 1 (PAI-1) and neuroserpin (NSP). PAI-1 irreversibly inhibits uPA or tPA by undergoing a large conformational change upon binding uPA or tPA that disrupts the active site of the plasmin activator and of PAI-1. In contrast, NSP preferentially inhibits tPA by forming an unstable complex that can release active tPA[42]. Reflecting the need for stringent regulation of the plasminogen cascade, free forms of activated plasmin activators, PAI-1, and NSP exist at very low concentrations with half lives in the order of minutes[43,44].

PAS in the periphery

The peripheral PAS plays a central role in mediating fibrinolysis, extracellular migration, cell signaling, cellular migration, and tumor growth, which has been reviewed in detail elsewhere[45,46]. The PAS converts inactive plasminogen to plasmin, a trypsin-like serine protease, via the catalytic activity of PA[41]. Plasminogen is primarily present in platelets in the plasma and liver. However, in mice, plasminogen mRNA has been found in the adrenal, kidney, brain, testis, heart, lung, uterus, spleen, thymus, and gut[40,47]. In the periphery, PAI-1 serves as the main suppressor of plasma fibrinolytic activity[40]. In the bloodstream, PAI-1 exists on its own in an active form, or as part of a complex with tPA or vitronectin, a glycoprotein that can convert PAI-1 into its active form. Elevated levels of PAI-1 are associated with metabolic syndrome and associated with increased risk of atherothrombosis and stroke[48,49].

PAS in the CNS

In the CNS, plasminogen is expressed at low levels by neurons in the hippocampus, cortex, cerebellum, as well as neuroendocrine tissues, but it is primarily transported to the brain via systemic circulation[12,50,51]. Plasminogen has been localized to the extracellular space, while the plasmin activators, tPA and uPA, have

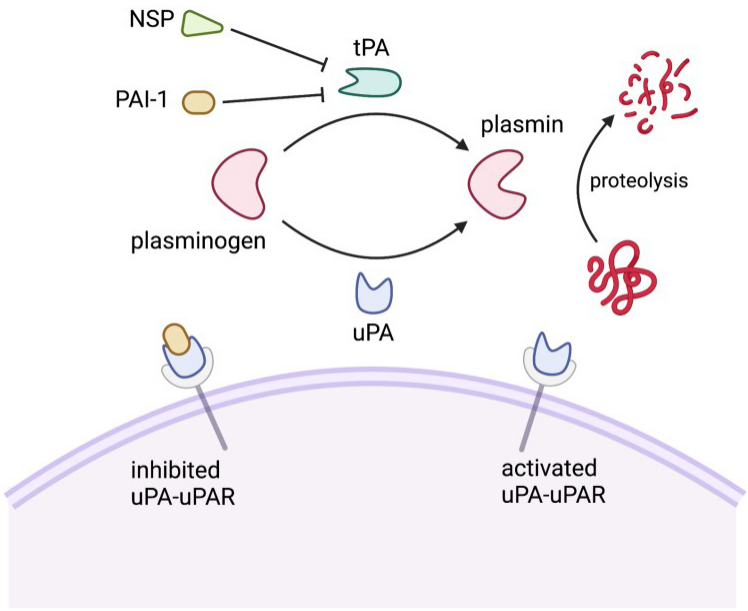

Figure 1. Schematic diagram of the molecular mechanisms of the plasminogen activator system. PAI-1: Plasminogen activator inhibitor-1; NSP: neuroserpin; uPA: urokinase-type plasminogen activator; tPA: tissue-type plasminogen activator; PLG: plasminogen; PLM: plasmin. Created with BioRender.com.

been localized to not only the extracellular space, but also to neurons and astrocytes. Both plasmin activators have been shown to modulate synaptic function when released into the synaptic cleft[52-54]. Membrane depolarization induces the rapid release of tPA from cerebral cortical neurons, which modulates neuronal plasticity, learning, stress-induced anxiety, and visual cortex plasticity[55]. tPA and uPA activities have been localized to well-defined areas of the brain[56-59] and shown to participate in intracellular signaling that is independent of plasminogen activation (see below). tPA is the principal plasmin activator in the CNS with PAI-1 regulating its activity primarily in the extracellular space. NSP is primarily localized in neurons in the developing brain with very low levels detected in the mature CNS[60], where it preferentially binds to and inhibits tPA[61]. Interestingly, mutations of NSP are associated with rare familial dementia characterized by neuronal inclusion bodies that are biochemically comprised of polymers of NSP[62].

Plasmin activity has been shown to be upregulated in axonal growth and synaptic pruning, suggesting a role in brain development and regeneration that is not yet well understood[50]. While both tPA and uPA can mediate plasminogen activation in the CNS, plasminogen activation is primarily controlled by the tight regulation between tPA and PAI-1[51]. uPA has a low baseline expression in specific neurons and astrocytes in the normal brain, but is upregulated in pathologically inflammatory environments, such as multiple sclerosis and epilepsy[50,51]. Endothelial cells of microvessels in the brain contribute to the production of tPA, but tPA can also be expressed by glial cells, neurons, and infiltrating leukocytes, implicating a broad spectrum of tPA involvement in the brain. While tPA in the mature brain is detected primarily in neurons, its enzymatic activity is primarily restricted to the hippocampus, amygdala and hypothalamus[63,64]. The discrepancy between the expression of tPA mRNA and its areas of enzymatic activity is consistent with its trafficking and transport to mossy fiber tracts[63,64].

The plasmin activators, tPA and uPA have been shown to play an important role in CNS function and dysfunction with some of their functions being independent of plasminogen[65,66]. Extracellular tPA participates in cerebellar motor learning[67], remodeling in various nonneural tissues[67], and neuronal

regeneration following ischemic injury[68]. tPA also participates in the regulation of BBB permeability[69,70]. Neuronal uPA is present in lower levels than tPA, participating in neurogenesis in the developing brain[71]. Its release in the mature central nervous system triggers astrocytic activation[53] and, like tPA, uPA promotes axonal and synaptic recovery following different forms of injury[72]. Both tPA and uPA are found in pre-synaptic vesicles that are released by calcium-dependent mechanisms[52,54,55].

The PAS is altered in AD

There has been longstanding interest in the role of the PAS in AD beginning with early reports that active plasmin efficiently digests Aβ peptides[73-77] both *in vitro* and in rodent AD models[19,73,74,76-81]. In the AD brain, tPA is highly expressed in regions of AD plaques, and in AD models where tPA is genetically inactivated, there is an increased accumulation of Aβ, synaptic dysfunction and memory deficits[78]. However, the enzymatic ability of brain tPA and uPA to activate plasmin *in vivo* is thought to be prevented by irreversible binding to high levels of extracellular PAI-1 secreted by immune-activated microglia and astrocytes[18]. PAI-1 is minimally expressed in the normal brain or cerebral vasculature, but does increase with senescence[82-84]. Brain levels of PAI-1 are also markedly increased in APP/PS1 mice[66] and the serum levels of PAI-1 are positively correlated with cognitive impairment in AD patients[85]. Consistent with the hypothesis that PAI-1 promotes AD pathology, genetic knockdown or small molecule inhibitors of PAI-1 reduced plaque formation in AD rodent models, and the small molecule PAI-1 inhibitor, PAZ-417, was shown to significantly improve hippocampal LTP and cognitive function in AD mice[73,74,86,87]. This finding was confirmed recently in an APP/PS1 AD mouse model using another small molecule PAI-1 inhibitor[86].

Whether tPA primarily plays a beneficial or detrimental role in AD progression is debated. Several studies have demonstrated that tPA activation of plasmin enzymatically reduces Aβ accumulation[78]. Conversely, tPA has been shown to mediate excitotoxic neurodegeneration by activating plasmin and causing subsequent laminin degradation[66,78]. Independent of plasmin activation, tPA causes GSK3 activation, tau hyperphosphorylation, microtubule destabilization, and neurotoxicity in rodent hippocampal neurons[88]. It has also been shown to mediate amyloid-induced microglial activation[89]. Based on such observations, it has been proposed that tPA contributes to neurotoxicity, microglial activation, and tau hyperphosphorylation as part of a feed-forward inflammatory pathway[73,88,89].

PAI-1 expression has been reported to be increased in the plasma[85,90,91] and brain tissues of AD patients[76]. PAI-1 expression is not detected in normal healthy human brains but is sporadically present in aged brains[84,92], and possibly linked to cerebrovascular disease. PAI-1 is the primary regulator of tPA in the CNS extracellular space and is a proinflammatory biomarker. Cytokines upregulate PAI-1 expression in microglia and astrocytes in human and animal models of AD[18,93]. The PAI-1 promoter is activated by TNF-α via an NFκB 5' upstream element and directly activated by TGF-β1 via SMAD2/3 promoter binding sites[82,94,95]. When PAI-1 is complexed with low density lipoprotein receptor-related protein-1 (LRP-1), it signals changes in microglial morphology and motility that are consistent with microglial activation[96-98]. In patients with AD, plasminogen activator activity is reduced while PAI-1 and NSP are upregulated[99]. However, there are contradictory findings regarding measurements of PAI-1 and tPA in the CSF and serum of patients with AD[76,92,100].

Congophilic amyloid angiopathy (CAA) is a vascular complication of AD in which Aβ40 plaques accumulate within the brain endothelium of cerebral arteries, arterioles and capillaries[101]. CAA can result in intracranial hemorrhages, cognitive impairment, or subacute inflammatory encephalopathy. tPA activation of endothelial NMDA receptors has been shown to regulate neurovascular coupling via nitric oxide-mediated regulation of cerebral blood flow. Elevated levels of brain PAI-1 impairs this tPA-dependent

neurovascular coupling in Tg2576 AD mice, and pharmacologic inhibition of PAI-1 was shown to improve cognition in this animal model by selectively restoring neurovascular function while not affecting cortical amyloid plaques[102].

PAS modulates BBB integrity in AD

There is increasing evidence identifying BBB leakage as an early sign of cognitive dysfunction, as well as evidence linking BBB dysfunction to AD pathogenesis[103,104] and its neuroinflammatory pathology[33,105]. However, the mechanisms underlying BBB dysfunction in AD are currently not well-elucidated. The BBB is part of the neurovascular unit (NVU) in the brain, which consists of endothelial cells (ECs), mural cells, including vascular smooth muscle cells and pericytes, basement membrane, glia cells including astrocytes and microglia, and neurons [Figure 2]. The ECs of the BBB are a distinct characteristic of the NVU due to their tight junctions and lack of fenestrae. This allows the ECs to regulate the selective transport and metabolism of substances from blood to brain and vice versa, thereby separating the microenvironment of the brain parenchyma from changes in circulating ion and metabolite concentrations in the systemic circulation[105].

In CNS injury, there are several potential mechanisms by which tPA is able to mediate changes in the permeability of the BBB [Figure 3], which in turn further exacerbates CNS injury by promoting neuroinflammation. AD is associated with BBB dysfunction in humans and animal models. Amyloid deposition activates gliosis that can alter the morphology of astrocytic endfeet, which are integral to the integrity of the neurovascular unit. As described previously with CAA, amyloid deposition can also injure the brain endothelium, which can additionally impair BBB integrity[106]. Finally, Aβ oligomers stimulate fibrin production that complexes with amyloid plaques, and fibrin has been shown to be increased in the parenchyma and vasculature of AD brains[107,108]. This fibrin-Aβ complex promotes further neuroinflammation and neurodegeneration. tPA is conformationally activated by fibrin deposition, but its enzymatic activity is inhibited by the elevated levels of PAI-1 found in AD parenchyma. However, as summarized in Figure 3, activated tPA has multiple plasmin-independent mechanisms by which it can compromise BBB integrity.

tPA in the CNS directly alters BBB integrity

tPA has long been known to play a significant role in the NVU, mostly in the context of stroke[109-111]. tPA has been reported to directly alter the BBB integrity by triggering activation of LRP-1 on the surface of astrocytes[12]. LRP-1 is a multifunctional signaling receptor that functions in receptor-mediated endocytosis and cellular signaling. LRP-1 binds many ligands, including tPA and amyloid-beta[112], which thereby facilitates Aβ endocytosis across endothelial cells of the BBB[113]. Aβ oligomers may compromise BBB integrity via activation of matrix metalloproteinases (MMPs)[113]. Alternatively, tPA may cleave LRP-1 at its substrate binding ectodomain, activating NF-κB, which promotes the synthesis of matrix metalloproteinases MMP-3 and MMP-9, leading to matrix protein degradation and BBB leakage[12]. tPA-induced activation of LRP-1 shedding from astrocytic endfeet also promotes detachment of endfeet projections from tight junctions of the endothelial cells of the neurovascular unit, further compromising the BBB[12]. Additionally, tPA can directly alter BBB integrity via platelet-derived growth factor PDGF-CC[114]. Upregulated neuronal expression of tPA expression induced by CNS disease or injury results in the release of tPA into the extracellular matrix of the brain, where it cleaves complement subcomponents C1r/C1s, urchin EGF-like protein, and bone-morphogenic protein-1 (CUB) from PDGF-CC forming an active ligand that binds to PDGF receptor-α (PDGFR-α). PDGFR-α promotes BBB leakage that worsens cerebral edema, neuroinflammation and neuronal death[114]. One study found this tPA-mediated activation of PDGF-CC to be inefficient in an *in vitro* stroke model[115]. However, *in vivo*, the Mac-1 integrin expressed on microglia works cooperatively with the endocytic receptor LRP-1 to promote tPA-mediated activation of PDGF-

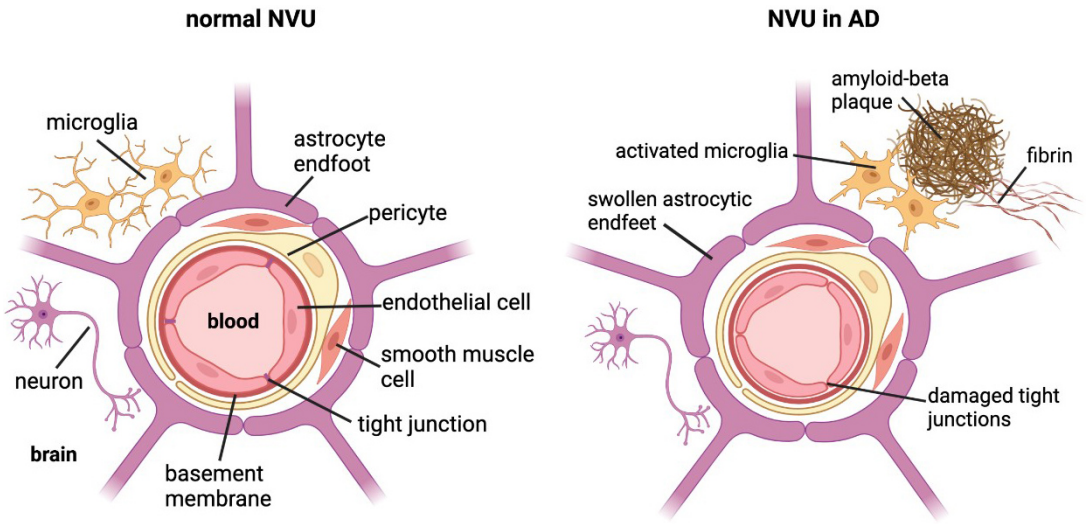

Figure 2. Cross-section of the neurovascular unit (NVU) in a normal brain *vs.* an Alzheimer's disease (AD) brain. The blood-brain barrier (BBB) consists of endothelial cells joined by tight junctions, basement membrane, mural cells (i.e., pericytes and vascular smooth muscle cells), enclosed by astrocytic endfeet. Neurons and microglia closely associate with the BBB. In the AD brain, the NVU undergoes morphological and structural changes due to AD pathology. Amyloid-beta plaques complexed to fibrin result in neuroinflammation and BBB disruption, including activated microglia, swollen astrocytic endfeet, and compromised tight junctions. Created with BioRender.com.

CC[115]. Multiple studies have also implicated tPA in binding amyloid-beta, thereby facilitating Aβ endocytosis across endothelial cells of the BBB[113].

Peripheral tPA alters BBB

In addition to its endogenous effects within the CNS, peripheral tPA can cross the intact BBB[116], phosphorylate claudin-5 and occludin, thereby weakening endothelial tight junctions and increasing BBB permeability by plasmin-independent mechanisms[117,118]. Chronic release of plasma tPA can induce a hyperfibrinolytic state that also directly increases vascular permeability of the BBB. Resultant plasmin activation by tPA also triggers bradykinin (BK) production[119,120]. BK is a peptide mediator generated from its circulating precursor, high molecular weight kininogen (HMWK), and is known for its ability to induce vascular permeability and cause vasodilation of arteries and veins[119]. It is a pro-inflammatory mediator, and its role as a neuromediator was identified in clinical conditions including AD[119]. While it is still debated as to how the PAS triggers BK generation, two primary pathways have been proposed [Figure 3]. A direct mechanism identified using an *in vitro* model involves tPA-mediated conversion of plasminogen to plasmin, which then cleaves HMWK into BK. BK acts through the bradykinin 2 receptor (B2R) on endothelial cells, triggering a signaling cascade that promotes intracellular calcium release and downregulation of claudin-5, a critical protein involved in maintaining EC tight junctions[120]. B2R activation can additionally induce tPA release from endothelial cells, further amplifying additional BK generation[121]. The PAS alternatively can indirectly trigger BK formation through a plasmin-dependent pathway where plasmin activated by tPA then converts Factor XII (FXII) into Factor XIIa (FXIIa), which then converts plasma pre-kallikrein into plasma kallikrein (PKal)[121]. PKal then serves to cleave HMWK, leading to BK formation and B2R signaling activation [Figure 3]. This indirect mechanism was demonstrated *ex vivo* and *in vivo* with the former using human plasma incubated with tPA, which resulted in the formation of active PKal; the latter demonstrating that intravenous injection of tPA in mice increased PKal activity[121,122].

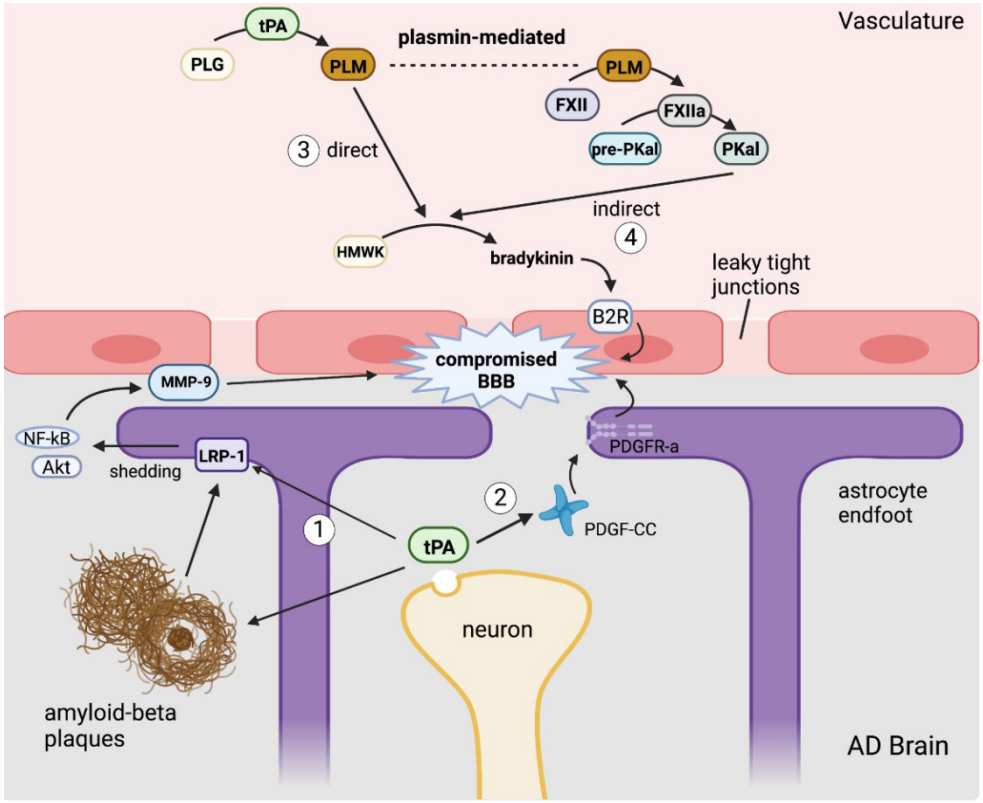

Figure 3. Mechanisms by which tPA may disrupt the blood-brain barrier. (1) tissue-type plasminogen activator(tPA) released from neurons cleaves lipoprotein receptor-related protein-1 (LRP-1) to activate an NF-κB signaling cascade resulting in the production of MMP-9. tPA and LRP-1 can bind amyloid beta, which facilitates Aβ endocytosis across the blood-brain barrier (BBB). (2) Neuronal tPA degrades platelet-derived growth factor-CC (PDGF-CC) to release the active ligand for PDGF receptor-α (PDGFR-α) on astrocytic endfeet, causing them to retract from endothelial cells. (3) Plasma tPA activates plasmin to directly produce bradykinin that activates bradykinin 2 receptor (B2R) receptor on endothelial cells. (4) Plasma tPA cleaves plasminogen to generate plasmin that indirectly upregulates bradykinin expression through plasma kallikrein (PKal). Created with BioRender.com.

AD has been shown to produce BBB dysfunction in humans and animal models. Amyloid deposition activates gliosis that can alter the morphology of astrocytic endfeet, which are integral to the integrity of the neurovascular unit. As described previously with CAA, amyloid deposition can injure the brain endothelium, which can additionally impair BBB integrity[106]. Finally, Aβ oligomers stimulate fibrin production that complexes with amyloid plaques and has been shown to be increased in the parenchyma and vasculature of AD brains[107]. This fibrin-Aβ complex promotes further neuroinflammation and neurodegeneration. tPA is conformationally activated by fibrin deposition, but its enzymatic activity is inhibited by the elevated levels of PAI-1 found in AD parenchyma. However, as summarized in Figure 3, activated tPA has multiple plasmin-independent mechanisms by which it can compromise BBB integrity.

CONCLUSION

Over the past two decades following initial reports of histologic evidence of Aβ deposition in the brains of children chronically exposed to severe air pollution[123], it has become clear that chronic peripheral inflammatory conditions, including those that involve lung, gut, liver, and metabolic syndrome, exacerbate or initiate neuroinflammatory disorders. This has been supported by epidemiologic findings of a positive association between chronic peripheral inflammatory conditions and increased incidence of dementia, including AD. More recently, there has been increased interest in the contribution of the peripheral PAS to the neuroinflammatory component of AD. Recently, it has become recognized that the risk of blood clots,

increased mortality, and persistent neuroinflammatory complications of COVID 19 viral infections are also associated with pre-existing systemic inflammatory disorders shown to chronically activate components of the PAS[124]. With respect to AD, the available evidence suggests that the peripheral PAS may modulate the neuroinflammatory response via multiple mechanisms[12,51]. Besides fostering the transcytosis of inflammatory cells across the BBB, components of the PAS have been shown to decrease BBB integrity and increase BBB permeability, consequences that have been independently linked to early cognitive dysfunction[125] including progressive stages of AD[126] perhaps in association with concomitant vascular disease[127]. Overall, the means by which the PAS modulates BBB integrity by tPA and plasmin-dependent mechanisms is complex and requires further validation and investigation. tPA in the CNS has been shown to alter BBB permeability by LRP-1 and PDGF-CC-dependent mechanisms, while tPA produced from peripheral inflammation can cross the BBB where it may work in tandem with the kinin system to directly generate BK via plasmin, or indirectly by increased PKal. It is likely that tPA works multifactorially and that these mechanisms are not mutually exclusive [Figure 2][118]. Based on what is currently known, further studies investigating the role of the PAS in AD and other dementias are certainly warranted.

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Authors' contributions

Made substantial contributions to conception and outline of the review: Tang MY, Gorin FA, Lein PJ
Performed literature search to identify relevant publications: Tang MY, Gorin FA
Wrote the initial draft of the manuscript and created figures: Tang MY
Significantly edited early versions of the manuscript: Gorin FA
Made final edits to the manuscript: Lein PJ
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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Ageing and Neurodegenerative Diseases

Review

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Recent developments in understanding brain aging: sex differences, mechanisms, and implications in diseases

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Abstract

Exemplified by the disproportionate cases of Alzheimer's disease among women, many diseases show considerable sexual disparity in the aging process. Given that such a sex bias varies significantly in different neurological conditions, considering sex differences is necessary for the diagnosis as well as the treatment of neurological disorders. However, currently, relatively few studies have specifically focused on sex differences in brain aging or the general aging process, which has prevented the development of precision medicine for these sex-different diseases. Here, we summarize age-related disparities relating to cognitive function and dysfunction for males and females from human cross-sectional and longitudinal studies. By discussing potential anatomical and physiological bases underlying such differences, we highlight the importance of sex for aging studies in this review, which may hopefully shed light on understanding the precise causes of different brain diseases.

Keywords: Sex difference, brain aging, dementia, cognitive decline

INTRODUCTION

According to figures from the World Health Organization (WHO), the number and proportion of people aged 60 and older are expanding and estimated to increase to 1.4 billion by 2030 and 2.1 billion by 2050, up from 1 billion in 2020[1]. The process of aging is associated with both normal and pathologic cognitive changes, which significantly affect older adults' daily life and society. The most recent data suggest that the prevalence of dementia will double in Europe and triple worldwide by 2050. Economic costs for dementia reached 957.56 billion dollars and are set to increase to 2.54 trillion dollars worldwide[2]. Alzheimer's disease (AD) is a main cause of dementia, the related total costs of which will reach 507.49 billion dollars by 2030 in China and 1.89 trillion by 2050[2]. Despite this, the clinical diagnosis of AD still faces many problems. There is a clear lack of precise gold standards for both diagnosis and treatment, and scientists have yet to develop multiple effective therapies for AD, especially for patients suffering in the later stages of the disease. Hence, it is clear that, for AD and other broader age-related conditions, research on aging and age-related diseases requires urgent attention.

During the human aging process, females show longer lifespans overall[3,4] but often also display more frailty than males[5]. For example, women aged 45-79 had a higher frailty index based on standards[6] including 28 variables on function, cognition, co-morbidity, health attitudes and practices, and physical performance measures[7]. This is known as the "male-female health-survival paradox"[8], and the sex variable can make a difference for health risks in males and females. To date, sex factors have attracted wide attention in the studies of human aging. In the discoveries of brain aging, sex bias has been well-recognized in the prevalence of certain brain aging-related diseases. For example, females with AD or other dementias exhibit a two-fold incidence compared with males[9]. Conversely, the prevalence of another progressive and age-related neurodegenerative disorder, Parkinson's disease (PD), is 1.5 times more common in men than women[10]. In addition, sex and gender can affect the risk factors and disease progression of aging-related diseases such as AD[11]. Thus, it is important to understand the sex difference in changes of normal brain aging, which should provide specific clues for understanding the sex-related mechanisms for age-related diseases and, in turn, may facilitate improved and personalized care during aging.

Here, we focus on reviewing the current literature reporting the sex difference in the functional changes (cognitive decline and vulnerability to neurodegenerative diseases), structural changes, and cellular hallmark changes of normal brain aging. To address this, we used the terms "sex difference", "brain aging", "cognitive aging", "brain structure", and keywords for cellular hallmarks of brain aging (mitochondria, oxidative stress, glia, ubiquitin-proteasome system, autophagy, DNA repair, stem cell exhaustion, and aberrant neuronal network activity) to search the literature in databases such as PubMed.

We first briefly review human data relating to the sex difference in cognitive decline and vulnerability to neurodegenerative diseases in the process of normal brain aging. Next, we discuss sex difference in potential anatomical changes underlying functional changes of brain aging with human evidence. Finally, we summarize the discoveries on sex difference in cellular hallmarks such as oxidative stress of brain aging from animal experiments and human data, which may offer clues for better therapeutics to cognitive decline in aging and neurodegenerative diseases.

SEXUAL DIFFERENCES IN VULNERABILITY OF MALES AND FEMALES TO COGNITIVE DECLINE IN AGING AND IN RELATED DISEASES

Sex difference of cognitive decline in normal brain aging

Cognitive function includes a variety of mental processes such as perception, attention, memory, decision making, and language comprehension. General sex differences in specific cognitive functions have been reported, with the most accepted findings being that men outperform women on spatial-based aspects, especially visual-spatial working memory tasks[12], while females excel in verbal memory and location memory tasks[13,14]. These differences seem to remain consistent from adolescents and young adults into older ages[15,16]. In line with previous reports, several studies have demonstrated that certain cognitive functions decline along with the normal process of the aging of the human brain[17,18]. Such studies monitor the trajectories of cognition change along with the process of aging in order to pick up any related changes of cognitive function that occur concurrently with stages of the aging process.

Longitudinal studies have been demonstrated as particularly useful and applicable in the study of both the difference of cognitive performance levels and the rates of cognitive change over time. In line with the results of the cross-sectional studies mentioned above, De Frias *et al.*[19] found that women performed better on episodic memory tasks and men had higher visuospatial ability, and this sex difference was stable across age groups (35-80 years) over a 10-year period. When detecting the cognition decline rate between females and males with aging, although a review published in 2013 which screened 13 longitudinal studies concluded that no sex differences were found in the rate of overall cognitive decline between the ages of 60-80 years[20], there are many other investigations that have shown sex differences existed in some specific cognitive tasks or in much older age (> 80) [Table 1]. Finkel *et al.*[21], for example, found men had a faster linear decline than women on a card rotation test from middle age (50). Another study conducted by Casaletto *et al.*[22] detected the age-related cognitive decline of 314 normal adults (average 69.3) and found that men tended to develop a declining episodic memory trajectory. Meanwhile, in recent longitudinal studies, McCarrey *et al.*[23] administered a series of memory and other cognitive tests to participants from the Baltimore Longitudinal Study of Aging to detect the cognitive change of females and males with age. They found men showed steeper rates of decline on measures of mental status, perceptuomotor speed and integration, and visuospatial ability, but no significantly differing declines on other cognitive abilities tested compared to women[23]. However, when analyzing increased numbers of people of the older age brackets, and after adjusting for age, education, and vascular factors, one study demonstrated that women showed a steeper decline of cognition than men after 80 years old[24]. Finkel *et al.*[21]'s study also demonstrated that women had a faster decline in information tests than men at ages beyond 65, with a much steeper decline after 80. However, McDowell *et al.*[25] showed a trend for a steeper decline in men when compared with women after 80 years. Because of the complexity of human studies, it is still difficult to have a consistent conclusion, and further studies are still warranted. Nevertheless, these findings seem to indicate that women tend to show more cognitive decline at later old age than men (> 80), particularly for some for specific cognition functions, while men may have a faster cognitive decline in earlier old age (50-65).

Limitations and difficulties of human research techniques may contribute to these disparities. Firstly, the backgrounds of individuals may represent differences in aspects such as education[24,26], lifestyle[27], physical activities[28], and weight[29], and these factors not only affect the baseline of individual cognitive function but also affect the rate of cognitive decline. This makes the study on the effect of the single factor of sex on the rate of cognitive decline difficult to isolate. Moreover, in longitudinal studies, subjects are incorporated into such studies from many different age groups, in which case a limited number of subjects will be of the same age, despite a large sample of total subjects being involved. Different cohorts also show different aspects of cognitive aging[30,31]. All these factors may cause discrepancies in results. To distinguish the true effect of sex

Table 1. Longitudinal studies for sex difference in normal cognitive decline

Ref.	Age (year)	Type of cognitive task	Decline rate
Finkel *et al.*[21]	> 65	Information test	F > M
	50-65	Card rotation test	M > F
Casaletto *et al.*[22]	47-99	The California verbal learning test	M > F
McCarrey *et al.*[23]	64.9-69.7	MMSE, fluent language production, digital symbol, card rotation	M > F
Proust-lima *et al.*[24]	> 80	Digit symbol substitution task	F > M
McDowell *et al.*[25]	> 65	Modified mini-mental state (3MS) cognitive Screening test	F > M (institution)
	> 80	Modified mini-mental state (3MS) cognitive Screening test	M > F

F: Female; M: male.

on cognitive decline with aging, larger sample sizes of confirmed similar backgrounds and the same ages should be involved, and the observation durations should be extended for these groups.

Sex differences in vulnerability to aging associated cognitive disorders

Brain aging is a natural process that results in a certain level of associated cognitive decline. However, as the brain ages, it is more susceptible to neurodegenerative diseases such as Alzheimer's disease (AD), Lewy body dementia (LBD), frontotemporal dementia (FTD), or Parkinson's disease (PD). These diseases usually occur in later life, worsening with subsequent aging. They often manifest with increasing age-related cognitive impairment, finally leading to dementia. Unlike the inconsistent results for the sex difference of cognition decline in normal brain aging, relatively consistent results have been demonstrated relating to females and males for some aspects of the vulnerabilities to these diseases [Table 2].

AD is the leading cause of dementia, which accounts for 60%-80% of dementia cases. Over the past 20 years, many studies have investigated sex differences in the risk, incidence, prevalence, or development of AD. For the analysis of risk, the Framingham Heart Study showed that, for a 65-year-old woman, the risk of AD over her remaining lifetime was 21.2%, while for a man, it was 11.6%. Correspondingly, the ratio of female to male risk for AD was noted as about 2:1[32]. For overall numbers, many epidemiological studies of varied global locations also highlighted a higher number of women than men with AD[33,34]. The prevailing explanation for such a difference is that women live longer than men on average, and that the incidences of AD correspond with increased age[32]. The argument could therefore be formulated that any apparent sex-based difference for this disease is simply due to the increased average longevity for women. However, upon a more specific analysis of prevalence, results seem to conflict with the lifespan explanation. Plassman *et al.*[35]'s Aging, Demographics, and Memory Study (ADAMS), including subjects aged 71 years and older from all regions of the USA, showed a higher prevalence of AD in women than in men of corresponding ages. In the 2015 World Alzheimer Report, Prince *et al.*[36] showed that, in East Asia, South Asia, the Caribbean, Western Europe, and Latin America, the prevalence of dementia for men was lower than for women, although no significant difference was noted for other regions. In addition, their review in 2016, summarizing several similar studies from Europe, indicates the same higher prevalence of AD in women[37]. As for the incidence of AD, the conclusion that there is no sex difference has been noted in some studies from the United States[38,39], while others have shown that women tended to have a higher incidence of AD[40]. These disparities may stem from regional and socioeconomic factors and/or cultural effects (such as diet and lifestyle). The 10/66 Dementia Research Group study of dementia in low- and middle-income countries found a higher incidence in women[41], and most studies on European[42,43] and Asian populations[44,45] have also observed a higher incidence in women at an older age. With regards to any sex differences related to the rate of cognitive decline beyond the diagnosis of AD, many studies show that the

Table 2. Sex difference of neurodegenerative diseases

Neurodegenerative diseases	Factors	Sex difference	Ref.
Alzheimer's disease (AD) The leading cause of dementia, which accounts for 60%-80% of dementia cases	Number	F > M	Chêne *et al.*[32] Hebert *et al.*[33] Alzheimer's Association 2021[34]
	Prevalence	F > M	Plassman *et al.*[35] Prince *et al.*[36] Prince *et al.*[37]
		No difference	Prince *et al.*[36] Rocca[152]
	Incidence	F > M	Fitzpatrick *et al.*[40] Prince *et al.*[41] Ruitenberg *et al.*[42] Rizzi *et al.*[43] Chen *et al.*[44] Yamada *et al.*[45]
		F = M (equal)	Tom *et al.*[38] Zahodne *et al.*[39]
	Cognitive decline rate	F > M	Hebert *et al.*[33] Holland *et al.*[46] Tifratene *et al.*[47] Lin *et al.*[48] Laws *et al.*[49] Gamberger *et al.*[50]
Lewy body dementia (LBD) The second most prevalent cause of neurodegenerative dementia	Number	M > F	Nelson *et al.*[54]
	Incidence	M > F	Savica *et al.*[55] Goodman *et al.*[56]
Frontotemporal dementia (FTD) The third most prevalent form of neurodegenerative dementias	Prevalence	M > F	Goodman *et al.*[56] Mercy *et al.*[58]
		F > M	Bernardi *et al.*[59] Ikeda *et al.*[60]
		No difference	Borroni *et al.*[61]
Parkinson's disease (PD) A movement disorder that can also lead to dementia	Prevalence	M > F	Pringsheim *et al.*[63] (worldwide) Hirsch *et al.*[64] Abbas *et al.*[65] GBD 2016 Neurology Collaborators[66]
		No difference	Pringsheim *et al.*[63] (Asia) Taylor *et al.*[67]
	Cognitive decline	M > F	Reekes *et al.*[71]

F: Female; M: male.

cognitive deterioration rate is faster in women than men in the progression of AD[33,46-50]. However, despite this faster progression of the disease in women, several studies have shown that men have an overall shorter lifespan beyond diagnosis with AD, as summarized by a recent review[51].

LBD is a neurodegenerative disease with abnormal *α-synuclein* accumulation (Lewy body proteins) in neurons, which can cause cognitive decline. LBD is the second most frequent form of neurodegenerative dementia. Reviews show the prevalence of LBD ranges 0.3%-24% in the general population and 3%-7% in the patients with dementia[52,53]. In accessing the registry autopsy series from the University of Kentucky Alzheimer's Disease Center and the National Alzheimer's Coordinating Center, researchers found that the number of male patients dying with Lewy body-associated pathologies was three times that of females[54]. Similarly, a study on the population of Olmsted County, Minnesota, showed that men had a higher incidence of LBD than women across the age spectrum[55]. A study on the prevalence of dementia subtypes in United States Medicare showed the same result[56]. FTD is considered the third most frequent form of

neurodegenerative dementia with more relatively young patients than other types of dementia (< 65)[57]. While many studies have demonstrated sex differences in the prevalence of this disorder, no clear consensus has been reached. Studies in the population of Cambridgeshire, UK, and a United States Medicare study both showed greater FTD prevalence in men than women[56,58]. However, many other studies failed to support these results[59-61]. The discrepancy here may be due to difficulties in the exact diagnosis of FTD, which presents with similar clinical symptoms to late onset psychiatric disorders and amyotrophic lateral sclerosis (ALS). Recently, Curtis's meta-analysis focusing on the sex difference of the prevalence of genetic mutations in FTD and ALS indicated a higher prevalence of progranulin (GRN)-muted FTD in female patients but no sex differences in chromosome 9 open reading frame 72 (C9orf72)- and microtubule-associated protein tau related FTD (MFTD), which should further help clarify the sex differences of prevalence of FTD[62]. Another neurogenerative disease is PD. PD is a movement disorder with bradykinesia, rigidity, tremor at rest, gait disturbance, and difficulty with speech. PD can also lead to dementia, and the proportion of patients with PD who are also diagnosed with Parkinson's disease dementia ranges from 10% to 15%. Studies are fairly consistent in demonstrating that there is a higher prevalence of PD presented among men than women from worldwide epidemiological data[63-66], especially in Western and South American populations[10,67-69]. However, there are reports showing the prevalence rates were almost equal between men and women in Asian populations[63,67]. Thus, there appears to be a difference between Asian and Western populations, which may stem from sex different behaviors such as smoking, methodologies, genetics, and ethnicity[65,70]. For the cognitive decline in PD, Reekes *et al.*[71] indicated that males with PD have significantly greater executive and processing speed impairments compared to women.

As mentioned above, the aging brain undergoes cognitive functional change and becomes increasingly susceptible to a number of cognitive diseases. Although no results have consistently or conclusively shown differences in cognitive decline rate between females and males, apparent sex differences have been shown to be involved in the cognitive performance and disease susceptibility of the elderly. In addition, females have been demonstrated as more susceptible to AD, and males are more vulnerable to LBD and PD. The underlying mechanism for such sex-based differences in brain aging behaviors and related diseases is a key area for further study.

BRAIN STRUCTURE AS A BASIS FOR SEX DIFFERENCES IN BRAIN AGING

To explain how function changes with aging, the most widely investigated aspect is the structural changes of the aging brain. With the advantages of noninvasive imaging techniques, researchers were able to study the aging brain in healthy living individuals. As many healthy volunteers were incorporated into such studies, this provided the opportunity to analyze the sex differences of the human brain anatomy relating to aging. Using magnetic resonance imaging, many researchers found more profound age-related decline in cortical grey matter volume in males than females[72-74]. However, there are many investigations that do not support the hypothesis that the effect of aging is accelerated in men and have failed to find age-by-sex interactions in adult and elderly populations[75-77]. When considering the sex differences of subcortical gray matter structure in the aging brain, conclusions are no more consistent [Table 3]. The subcortical structures studied include the basal ganglia (caudate, nucleus accumbens, putamen, and pallidum), thalamus, hippocampus, and amygdala. Among these, the hippocampus is the most studied, and some hippocampus studies have reported that females have larger volumes in the aging brain[78-80] while others have opposing results[81]. In old age, for thalamus, some studies found the male had a larger volume[72,80], while others opposed this[82]. When correcting for brain volume, Li *et al.*[78] found no significant sex difference in the relative volume of thalamus. Similarly, for the caudate, some found females had larger volume[83,84], while others found males had larger volume[78,80]. More consistently, the amygdala[78,85], pallidum[78,80], and putamen[78,80] have been invariably found to be larger in males.

Table 3. Sex difference of subcortical regions in the aging brain of cross-sectional studies

Subcortical regions	Ref.	Age (year)	Sex difference of volume in older age (> 45)	Sex difference of decline rate in older age (> 45)
Hippocampus	Li *et al.*[78]	19-70	F > M (relative volume)	M > F (relative volume)
	Nemeth *et al.*[79]	21-58	F > M	M > F
	Wang *et al.*[80]	19-86	F > M (> 70)	M > F
	Goto *et al.*[81]	41-77	M > F (absolute volume)	F > M (absolute volume)
Thalamus	Sullivan *et al.*[72]	20-85	M > F	Similar
	Li *et al.*[78]	19-70	Similar (relative volume)	Similar (relative volume)
	Wang *et al.*[80]	19-86	M > F	M > F
	Takahashi *et al.*[82]	20 to ≥ 80	F > M	M > F
Caudate nuclei	Li *et al.*[78]	19-70	M > F (relative volume)	F > M (relative volume)
	Wang *et al.*[80]	19-86	M > F	Similar
	Good *et al.*[83]	18-79	F > M	No test
	Luders *et al.*[84]	18-82	F > M	No test
Putamen	Li *et al.*[78]	19-70	M > F (relative volume)	F > M (relative volume)
	Wang *et al.*[80]	19-86	M > F	M > F (right)
Pallium	Li *et al.*[78]	19-70	M > F (relative volume)	F > M (relative volume)
	Wang *et al.*[80]	19-86	M > F	M > F (right)
Accumbens	Wang *et al.*[80]	19-86	Similar	Similar
Amygdala	Li *et al.*[78]	19-70	M > F (relative volume)	F > M (relative volume)
	Cheng *et al.*[85]	20-50	M > F	No test

F: Female; M: male.

Differences in these findings may be due to differences in the age range of subjects evaluated and methods used for analysis. The previous findings of brain structure changes in the aged brain of females and males are mainly based on cross-sectional studies, which only show the status at one specific time or with different status at different specific times. Longitudinal studies may be better suited to address the conflicts in cross-sectional studies. Over the last 15 years, increasing numbers of longitudinal studies have been performed to investigate the rate of brain change with aging. Among them, some studies have paid attention to the sex difference in aging[86-90]. Taki *et al.*[86]'s studies showed the annual percentage change in the grey matter ratio (APC_{GMR}) in the older female group was substantially lower than in the older male group, using such a longitudinal design running over a period of over six years in 381 healthy community-dwelling individuals. Jiang *et al.*[88] chose individuals aged 70-90 years as subjects. After a two-year follow-up, they found that women had thicker cortical regions but greater rates of cortical atrophy[88]. For the structural changes of cortex subregions, Pfefferbaum *et al.*[87] focused on the change of regional brain volume with aging in longitudinal studies. They found a more rapid increase of lateral ventricle volume and Sylvian fissures and more rapid decline of the centrum semiovale, anterior cingulate, parietal and precentral cortices, and thalamus in older men than older women, especially in those beyond 60 years of age[87]. Narvacan *et al.*[89] scanned a cohort of 55 subjects approximately three years apart. While finding that overall males had larger volumes than females for all subcortical structures, no sex differences in trajectories of change were detected[89]. Such differences may stem from longitudinal studies which can be limited by the age range of participants, sex distribution of the samples, or scanning intervals. In a recent study, Vinke *et al.*[90] used the Rotterdam study[91] to understand the different aspects in brain aging of middle- and old-aged males and females based on a large prospective population-based cohort study. Their analysis showed that an earlier acceleration of decrease for normal-appearing white matter volume, gray matter volume, total brain volume, hippocampus volume, and pallidum volume and increased cerebrospinal fluid volume had occurred in men compared with women. Meanwhile, men tended to have a higher prevalence of focal

lesions (microbleeds, lacunes, and cortical infarcts) compared with women. Although shorter time intervals and less time for scanning posed some limitations for the reliable representation of the longitudinal effect of those of older ages, this study, with the largest sample used for aging-related research, provides good background information for understanding the different changes in the female and male brain due to aging.

As for other studies dealing with functional age-related change in the human brain, limitations exist in both cross-sectional and longitudinal studies for investigating how the brain's structure changes with aging. Although no consistent results have been reached for such sex differences in brain structural changes, most studies have indicated that males have accelerated atrophy in the grey matter of the cortex. This may support some findings for the faster cognitive decline in males mentioned above. The noted differences in changes for different subcortical regions may help us to understand why females outperformed males in some specific tasks while males outperformed females in others. In their review, Nemeth *et al.*[79] demonstrated the possible functional consequences of sex difference of the subcortical grey matter, noting that their findings were relevant for dementia occurrence. However, the direct link among brain structures, cognition, and behavior is not currently clear and requires further investigation.

CELLULAR BASIS FOR SEX DIFFERENCES IN BRAIN AGING

Mattson and Arumugam[92] 2018 paper summarizes these findings well and organizes the main aspects of brain aging into nine hallmarks: (1) mitochondrial dysfunction; (2) oxidative damage; (3) impaired cellular "waste disposal" mechanisms (autophagy-lysosome and proteasome functionality); (4) impaired adaptive stress response signaling; (5) impaired DNA repair; (6) aberrant neuronal network activity; (7) dysregulated neuronal Ca^{2+} handling; (8) stem cell exhaustion; and (9) glia cell activation and inflammation. To date, several studies (mainly from animal experiments) have shown sex differences for these hallmarks, indicating possible cellular and molecular mechanisms for sex disparity of brain aging [Figure 1].

Sex difference in mitochondrial dysfunction and oxidative stress with brain aging

The mitochondrion is an important organelle in the cell, which plays crucial roles in ATP production, storage calcium ions, and the regulation of cellular proliferation[93]. Sex difference has been found in many aspects of brain mitochondrial function including morphology, pathways of biogenesis, autophagy, cell death, calcium, and redox homeostasis[94]. However, such results have been mainly based on investigations of the adult brain or injured brain. Investigations on sex differences of brain-based mitochondrial dysfunction related to the normal aging process are relatively sparse. However, sex factors of redox homeostasis have been directly studied relating to brain aging, where the mechanism of the balance of free radical production and antioxidants is required to maintain redox homeostasis.

Upon aging, brain neurons tend to suffer from oxidative damage by excessive generation of free radicals and reduced antioxidant defense. Animal studies showed that, in young adult rats, females exhibit lower release of cytochrome c and lower levels of mitochondrial hydrogen peroxide than males[95]. Moreover, in rats of similar ages, female brain mitochondria generated half the amount of peroxides and imposed dramatically less oxidative damage to mitochondrial DNA than those of males[96]. In contrast, Guevara *et al.*[97,98]'s studies on rat brains of different ages (6, 12, 18, and 24 months old) showed no significant sex-based differences for H_2O_2 production in any age class, despite H_2O_2 production being increased with age in both sexes.

For the antioxidant system, studies on rodents showed that young female brains have higher expression or activities of the antioxidant enzymes SOD and CAT[99-101]. However, upon brain aging, the factors of sex difference relating to oxidative stress become complicated. Although higher antioxidant defense occurred in young female rats, after ovariectomy, mitochondrial peroxide and glutathione (GSH) levels in females

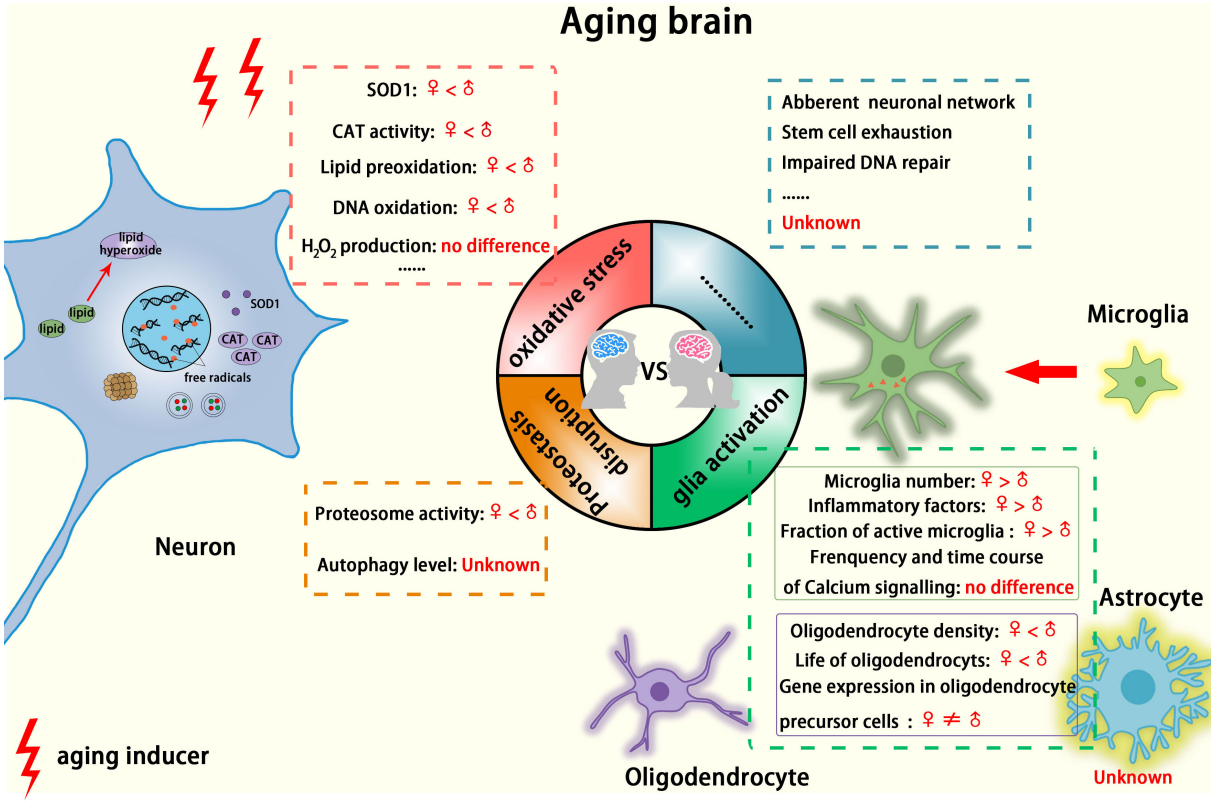

Figure 1. Sex differences implicated in cellular hallmarks of the aging brain. Shown in colored sections and dashed boxes, sex differences may exist in some hallmarks of brain aging.

became similar to those of males[96], indicating decreased antioxidant ability in menopausal female rats. Accordingly, other non-human studies also showed higher CAT activity in the aging (18-20 months) male mouse brain and higher SOD1 protein levels in the brain of aged (28-month-old) males[102]. Similar results were found in Human studies. Viña and Borrás[103] proposed that young females have a better ability to fight against oxidative stress with higher antioxidant levels prior to menopause. Mandal *et al.*[104] also found that young females (± 26 years old) have higher GSH levels than young men in the frontal and parietal cortex. Interestingly, the GSH levels decrease in the brains of older women (56 years old) compared with the younger women[104], indicating that the antioxidant ability changes with aging in women. In line with this, Rekkas *et al.*[105] found that oxidative stress in the brain increased rapidly in perimenopausal women. Bilateral oophorectomy-induced menopause was associated with increases in the GSH/GSSG ratio (increase in GSH, decrease in GSSG) and reduction in SOD and glutathione peroxidase (Gpx) mRNA expression[106], suggesting that women had decreased antioxidant ability after menopause.

When detecting oxidative damage in the brain, several studies in rodents have demonstrated males may exhibit greater oxidative damage, expressed as higher DNA oxidation[107] or higher levels of lipid peroxidation[107-109], than age-matched females over relatively young ages. This may be caused by lower free radical production and higher antioxidant defense in young females. However, other studies show no such sex differences[110,111] or higher oxidative damage in females[100,112]. These differences may stem from the different brain regions under examination or the different times of detection. Guevara *et al.*[97,98]'s studies on rats of different ages (6, 12, 18, and 24 months old) showed lesser oxidative damaged proteins and lipids in female brains, even in old age, an observation which they attributed in part to higher Gpx activity.

Uzun *et al.*[113]'s study also found that protein damages were greater in male brains of 24-month-old rats. However, in the brain of 6-month-old ovariectomy mice, females showed increased levels of lipid peroxidation compared to the sham group[114], indicating increased oxidative damage in postmenopausal females.

Overall, these findings on the sex difference in oxidative stress in the aging brain suggest that young females have a better ability to defend against oxidative stress, but these advantages may be lost upon aging, especially beyond menopause. As reviewed in Grimm *et al.*[115]'s paper, the change of redox state with aging is associated with variation in sex steroid levels. This finding may aid in the understanding of sex differences in vulnerability to neurodegenerative disease[115].

Sex differences in glia cell activation upon brain aging

Glial cells are distinguished from neurons in the brain and play important roles in brain function via facilitating crosstalk with neurons, maintaining the normal function of neurons, and defending against harmful stimuli. There are different types of glial cells in the brain, including astrocytes, microglia, and oligodendrocytes, each with distinct functions. Oligodendrocytes insulate axons and provide them with trophic support[116]; microglia are regarded as macrophages participating in local immunity[117]; and astrocytes provide biochemical support of neuronal activities by facilitating the appropriate glial surroundings in conjunction with microglia[117,118]. Various wide-ranging studies have demonstrated sex differences related to glial cells in physiological conditions or in response to pathological insults[119-121]. However, sex differences in aged glial cells remain relatively under-studied, and the discoveries mainly stem from animal experiments.

Sex differences in microglia are the most studied sex-related aspect in the process of brain aging, with previous studies quantifying changes of microglia in the aging brain[122,123]. Mouton *et al.*[123]'s study showed that, in both young and old mice, females had more astrocytes and microglia in DG and CA1 of the hippocampus than did age-matched male mice. In addition, with the development of sequencing technology, recent studies have paid increasing attention to the gene expression relationships for microglia in aging[124,125]. Mangold *et al.*[126] also detected the sex differences for microglial gene expression in the mouse hippocampus and cortex. They found that inflammatory genes were more highly expressed in microglia of older females than in corresponding older males[126]. Importantly, using single-cell RNA-sequencing analysis, Sala Frigerio *et al.*[127] found that aging or progressive amyloid-β accumulation accelerated the two main activated microglia states and that female mice progressed faster in this than males, which also converged with the pathway of sex differences relating to aging and AD. In addition, Kang *et al.*[128] mentioned in their article that tauopathy, amyloidosis, and aging had been shown to share a common APOE-driven transcriptional signature in microglia, which indicates that the increased expression of many of these transcripts of microglia in older mouse brains may be related to increased susceptibility to Alzheimer's disease in females. As regards to the functions of microglia in aging, one recent study on phagocytosis showed that aged female microglia had a greater ability for phagocytosis of neuronal debris, but they had lost their ability to adapt their phagocytic activity to inflammatory conditions[129]. Another study on microglial function, analyzing microglial Ca^{2+} signaling and process motility, suggested "faster aging" for microglia in female mice[130]. Taken together, the more active/faster aging microglia in older females may render them more vulnerable to some age-related neurodegenerative disease such as AD.

Relatively few studies have focused on astrocytes and oligodendrocytes in aging. Research on astrocytes has mainly been conducted in vitro via the detection of differences in the changes between the sexes under specific stimuli or in response to various pathological insults[131]. No specific analysis of the sex differences

relating to the aging process seems to have been conducted relating to astrocytes. For oligodendrocytes, Cerghet *et al.*[132,133] examined the sex difference of oligodendrocytes in the rat and mouse brain and found that the density of oligodendrocytes in the corpus callosum, fornix, and myelin proteins and myelin gene expression were all greater in males, and shorter life of oligodendrocytes was noted in females, a finding which did not simply represent younger mice and rats, but also held true for old mice. These differences for oligodendrocytes and myelin may be associated with the sex difference in the white matter volume in the adult and aging brain, which is discussed in "**BRAIN STRUCTURE AS A BASIS FOR SEX DIFFERENCES IN BRAIN AGING**". Oligodendrocyte precursor cells can generate new mature oligodendrocytes to defend against myelin impairment in the adult brain. Transcriptomic analyses have identified sex differences in oligodendrocyte precursor cells in the expression of genes encoding for proteins involved in the cell cycle, proliferation, maturation, and myelination, among other functions[134,135]. This difference renders older (12-month-old) female rats with greater abilities of remyelination than males after demyelination lesions[136].

Sex differences in proteasome degradation and autophagy upon brain aging

In most organisms, a balance in the protein system, which forms the basis for gene expression and protein synthesis and degradation, is important for the normal function of cells. Aging often shifts this balance with the subsequently altered gene expressions and protein synthesis and disrupted protein degradation, resulting in some notable pathological features[137]. In recent studies, many molecules have been demonstrated to be associated with brain aging including SFRS11[138], CD22[139], REST[140], and BAZ-2 and SET-6[141], the expressions of which change along with the aging process. However, no particular sex differences have been noted for these. Whether this is due to there not being any notable sex differences, or that this is an aspect yet to be properly examined, remains to be clarified.

Another important part of the protein system is protein degradation, which has been verified to be disrupted with brain aging[142]. In cells, two main protein degradation systems exist, namely the ubiquitin-proteasome system (UPS) and autophagy[143,144]. A few non-human data demonstrate that sex differences exist in these protein degradation systems of the aged brain. Ding and Keller[145]'s study showed that proteasome inhibition occurs with aging in the central nervous system, while Zeng *et al.*[146]'s study demonstrated that, in older (15-month-old) female mice and rats, catalytic activities of the proteasome are decreased in the cortex, striatum, cerebellum, globus pallidus, and substantia nigra with aging. In Jenkin *et al.*[147]'s study, investigators compared the activity of the proteasome in nine tissues of young (3-5-month-old) and middle-aged (10-15-month-old) female and male mice. They found young females showed no significant differences in their proteasome activity in the brain as compared to young males. However, from middle age onwards, males showed significant decreases in proteasome activity with subsequent aging, while no such change was noted for females[147]. One study on older fruit flies showed that basal activities of 20S proteasome had decreased in both female and male fruit-fly heads. However, in young female flies, the proteolytic capacity of the 20S proteasome could be increased via the induction of H_2O_2, but with this effect diminishing for older female flies. However, male flies showed no such age-related adaptation of the 20S proteasome[148]. This indicated the potential for an age-related sex difference for proteasome activity in its primed adaption from external stimuli. In another recent study, old (22-month-old) male rats showed impaired fear memory with impaired UPS activity (reduced phosphorylation of the Rpt6 proteasome subunit and accumulated K48 polyubiquitinated proteins) in the basolateral amygdala (BLA), while no behavioral change was noted for old females. In this study, such changes in the activity-driven markers of UPS activity occurred within the medial prefrontal cortex but not in the BLA of old females[149]. Such an observation of the sex differences of UPS activity in different brain regions of the aged brain aid in the understanding of sex differences related to cognitive decline with aging.

As for autophagy, while no direct articles have reported sex differences in the normal aging brain, multiple studies have demonstrated its role in AD[150]. Many investigations on other tissues (not brain tissue) have shown that females appear to have overall lower levels of autophagy, and ovariectomized animals show increased basal levels of autophagy in several cell types[151]. This highlights the changes in the nature and extent of autophagy after menopause in women. Although more research is needed, considering the results of Jenkin *et al.*[147]'s study, younger females show an overall higher proteostasis capacity, where there may be a particularly well-established balance in the two types of protein degradation systems, but these could then become disrupted upon aging, rendering elderly women more vulnerable to a number of associated diseases.

For the many other hallmarks of brain aging, few studies have paid attention to any potential sex differences. Although many of the above-noted differences in the cellular changes between the female and male aging brains are not fully understood, these discoveries will likely attract increasing numbers of researchers to consider the sex factor and to further illuminate the related cellular mechanisms. These will help link the hormonal effects with those that relate to cognition and behavior, in the process of aging.

CONCLUSION

Sex differences in the brain, as they function in developmental and adult stages, have been widely investigated. However, studies on the sex factors related to the aging brain are lagging behind and deserve increasing attention, particularly under the current situation of a rapidly aging global population. Although there are no consistent general conclusions related to sex differences on cognitive decline with aging, the differences of some aspects of cognitive performance in older adults and the increased vulnerability of females and males to various aspects of dementia are becoming fairly well established, especially for AD and PD where sex is regarded as a primary risk factor for these neurodegenerative diseases. Specifically, many studies have demonstrated that women have a higher prevalence of AD than age-matched men and exhibit faster cognitive decline beyond AD diagnosis. Conversely, almost all recent studies have demonstrated that men have a higher prevalence of PD. However, for treatment, the current interventions for dementia often fail to consider the sex factor. One possible reason may be that the underlying mechanisms of sex difference for the functional changes that occur during the process of brain aging are not yet fully clear and that present techniques and other socioeconomic factors render such factors difficult to examine in detail. However, it is clear that age-related cohorts need to be established and traced to provide information about how human aging-related phenotypes and molecular changes upon aging relate to sex, in order to guide future health improvements. Beyond human-based studies, to unpack the possible structural and cellular mechanisms for sex differences of the aging brain, the use of animal models should be increasingly established, with experiments designed that can incorporate the benefits of the model animal's simple genetic background. With a better understanding of the biological mechanism for the sex differences in brain aging, we can better understand the overall functional changes in the brain, elucidate how sex creates differences in disease risk, lay a stronger foundation for dealing with the newly emerging aspects of neurodegenerative disease, explore more directly the biomarkers for brain aging, and further promote personalized medicine that incorporates the factor of sex for improved and more individualized disease treatment.

DECLARATIONS

Authors' contributions

Made contributions to conception of this review article: Yang J, Ma H
Screened and gathered articles and wrote the abstract and introduction: Qu J

Wrote the other sections and made tables: Yang J

Availability of data and materials
Not applicable.

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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Ageing and Neurodegenerative Diseases

Review

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Influence of sleep disruption on protein accumulation in neurodegenerative diseases

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Abstract

Abnormal accumulation of disease proteins in the central nervous system is a neuropathological feature in neurodegenerative disorders. Recently, a growing body of evidence has supported a role of disruption of the sleep-wake cycle in disease development, pathological changes and abnormal protein accumulation in neurodegenerative diseases, especially in Alzheimer's disease and Parkinson's disease. Sleep deprivation promotes abnormal accumulation of disease proteins. Interestingly, amyloid-β (Aβ) has daily oscillations in human cerebral spinal fluid (CSF) and is cleared more in sleep. Both circadian genes and circadian hormones are associated with disease protein deposition. Recently, the glymphatic pathway and meningeal lymphatics have been shown to play a critical role in Aβ clearance, which is mediated by the aquaporin (AQP-4) water channel on astrocytes. The rate of the clearance of Aβ by the glymphatic pathway is different during the sleep/wake cycle. Most importantly, circadian rhythms facilitate glymphatic clearance of solutes and Aβ in the CSF and interstitial fluid in an AQP-4-dependent manner, which further provides evidence for the involvement of circadian rhythms in disease protein clearance.

Keywords: Neurodegenerative diseases, protein accumulation, glymphatic clearance, Alzheimer's disease, Parkinson's disease, circadian disruption

INTRODUCTION

The sleep-wake cycle is controlled by circadian rhythms. Circadian rhythms are biological processes that follow a daily cycle and respond to light and darkness in an organism's environment. Circadian rhythms are endogenously synchronized to the day/night cycle to form oscillations of 24 h[1]. The central pacemaker in mammals is the suprachiasmatic nucleus (SCN), which receives light signals from the retina through the retinohypothalamic pathway[2]. Ablation of the SCN in animals, aneurysms near the SCN, or pituitary tumors in patients disrupts daily rhythms[3-5]. Moreover, intrahypothalamic grafts of neonatal SCN tissue to SCN-ablated animals restore rest-activity rhythms[3]. Thus, the SCN plays a principal role in circadian rhythms.

The SCN clock entrains the peripheral clock, nearly all of the cells in the body, through autonomic innervation, body temperature, humoral signals, and feeding-related cues[6]. The rhythms of the cells from different organs are affected not only by the oscillation of hormones, the nervous system and temperature but also by local circadian oscillators[1]. Increasing lines of evidence demonstrate that dysfunction of circadian rhythms is associated with diseases in many systems and organs, including neurodegenerative diseases[7,8], cancer[9], hypertension[10], diabetes[11], and autoimmune diseases[12]. The circadian rhythms in mammals are generated internally but synchronized to the external environment (light and dark cycles by the Earth's 24 h rotation[13]. Sleep/wake cycles have a typical 24 h pattern, which is linked to circadian rhythms[13]. Sleep loss is a representative symptom of circadian rhythm disruption.

Aberrant accumulation of abnormal disease proteins is a common pathological feature of neurodegenerative diseases. The aggregation of proteins associated with neurodegenerative disease is present in patient brains in both familial and sporadic cases, such as the formation of extracellular plaques by β-amyloid (Aβ) and intracellular neurofibrillary tangles by tau in Alzheimer's disease (AD), intracellular Lewy bodies by α-synuclein (α-syn) in Parkinson's disease (PD), TDP-43 inclusions in amyotrophic lateral sclerosis and nuclear inclusions in Huntington disease[14-16]. Circadian rhythm abnormalities are frequently present in patients with neurodegenerative diseases, in which patients obviously show abnormalities in the sleep-wake cycle. Patients, especially with AD or PD, often decrease activities during the daytime and increase activities at night, showing changes in rest-activity patterns[7,8]. Increasing evidence suggests that circadian rhythms are associated with protein homeostasis, which functions in the clearance of abnormal proteins[17,18]. Disruption of circadian rhythms increases the accumulation of disease proteins, which promotes pathological changes in neurodegenerative diseases.

In this review, we discuss the associations of circadian rhythm disruption and protein homeostasis dysfunction in neurodegenerative diseases. We focused on the influence of protein aggregation and pathological changes by circadian rhythm abnormalities. We summarized the studies on circadian rhythm disruption in AD and PD and the protein homeostasis regulated by circadian rhythms. We also described the pathological significance of circadian rhythm disruption and the underlying mechanisms in neurodegenerative diseases.

CLINICAL ASSOCIATIONS BETWEEN SLEEP DISRUPTION AND NEURODEGENERATIVE DISEASES

Alterations in circadian rhythms can be presented by behavioral and physiological changes, including sleep-wake rhythms, body temperature, blood pressure and hormone levels. The sleep-wake cycle is often disrupted in patients with neurodegenerative diseases. The degeneration of neurons in neurodegenerative diseases is associated with or leads to circadian dysfunction and sleep disturbance, and it was previously believed that the disruption of circadian rhythms is a consequence of neurodegeneration. However, increasing evidence suggests that circadian dysfunction contributes to the formation of pathological changes, such as the accumulation of abnormal proteins and the progression of neurodegenerative diseases [Figure 1]. Importantly, sleep disorders are presented in many neurodegenerative diseases, including early AD, PD, dementia with Lewy bodies, frontotemporal dementia (FTD) and multiple system atrophy (MSA) patients[8,19-23], further suggesting that they play a role in disease development.

Alzheimer's disease

Sleep disturbance can occur at all stages in AD patients. AD patients lose a normal resting-activity pattern, showing an increase in daytime sleepiness. It has been reported that sleep fragmentation can increase the risk of AD and the rate of cognitive decline[24]. In a Swedish cohort, 214 Swedish adults aged 75 and over participated in a longitudinal study with 9 years of follow-up. All participants were dementia-free at baseline and the first follow-up (3 years later). After a total of 9 years of follow-up, participants with moderate or severe sleep problems showed an increased risk for developing dementia, particularly AD[25]. After adjusting for age, education and gender, moderate/severe sleep disruption causes a 2.5 times greater risk of all-cause dementia and a more than 3 times greater risk of AD[25]. In a prospective study, women without dementia were subjected to overnight polysomnography measurements to determine sleep status. After a 5-year follow-up, their cognitive status was evaluated. Women with sleep-disordered breathing have a high incidence of developing mild cognitive impairment or dementia, suggesting that sleep problems are associated with the development of dementia[26]. Shift workers are subjected to sleep deprivation, which causes circadian disruption. By analysis of a Danish nurse cohort (28,731 female nurses), shift work increased AD risk[27]. Moreover, sleep disorder is believed to occur at the preclinical stage of AD[28]. Using a tailored light treatment that maximally regulates the circadian system, AD patients show significant improvements in sleep, mood and behavior, further suggesting that there is an association between circadian system disruption and the symptoms in AD patients and that an entrainment of circadian rhythms benefits AD patients[29].

Parkinson's disease

It is estimated that nearly half of dopamine neurons in the substantia nigra are lost when patients start motor symptoms. Before motor symptoms, PD patients often demonstrate nonmotor symptoms, including mood disorders, pain, gastrointestinal dysfunction and sleep disorders. Sleep disturbance, including insomnia, excessive daytime somnolence (EDS), fragmented sleep and rapid eye movement sleep behavior disorder (RBD), is the most common nonmotor symptom, which appears in up to 90% of PD patients[30-32]. The changes in temporal sleep patterns, including insomnia, EDS and fragmented sleep, that are controlled by circadian rhythms suggest a disruption of the circadian system in PD. Characterized by polysomnography signals, sleep is divided into two states: rapid eye movement (REM) and nonrapid eye movement. Patients with PD at an early stage show increased sleep latency and decreased REM sleep as well as sleep efficiency[7]. In a study involving 3078 men free of PD and dementia, the risk of developing PD within 10 years was threefold higher in men with EDS than in those without EDS[33], suggesting that EDS increases the risk of PD and is a prodromal stage of PD. In a population-based prevalence study, RBD was identified as a symptom in the early phase of PD, which occurs in nearly 30% of newly diagnosed PD patients[34]. The prevalence of RBD in PD patients is estimated to be near 40%, showing that the risk for the

Figure 1. Interactions between sleep disturbance and neurodegenerative diseases. Sleep disturbance plays roles in neurodegenerative disease. It promotes the pathogenesis of Alzheimer's disease (AD) and Parkinson's disease (PD). Sleep disturbance is tightly associated with Aβ and tau deposition in AD and α-syn accumulation in PD. It also promotes the transmission of disease proteins, such as tau in AD and α-syn in PD. Furthermore, sleep disturbance is positively related to dementia in AD and excessive daytime somnolence (EDS) and rapid eye movement sleep behavior disorder (RBD) in PD. However, there are bidirectional relationships between sleep disturbance and neurodegenerative diseases. Neurodegeneration and pathogenesis in neurodegenerative diseases induce sleep disturbance, further leading to deterioration of the disease. Aβ: Amyloid-β; α-syn: α-synuclein.

conversion from idiopathic RBD to PD is extremely high. RBD is associated not only with the development of PD but also with a risk for dementia[20]. Importantly, among the sleep disorders in PD, RBD is mostly associated with the development of PD pathology. In an observational cohort study, patients with idiopathic RBD were followed up for 7 years and examined with dopamine transport imaging, transcranial sonography and olfactory testing[35]. With this cohort, 82% of patients with idiopathic RBD show neurodegenerative syndrome with an increased risk for developing PD and Lewy body dementia. In three patients with antemortem diagnosis of PD and Lewy body dementia, there were widespread Lewy bodies in the brains and α-syn aggregates in the peripheral autonomic nervous system, suggesting an association between RBD and synucleinopathy[35]. Interestingly, these three patients show Lewy pathology as well as a loss of neurons in the brainstem nuclei that regulate REM sleep atonia[35], which may also reflect a connection between synucleinopathy and REM sleep without atonia in PD.

Frontotemporal dementia

FTD includes a group of heterogeneous dementias with the frontal and temporal lobe atrophy in patients. Behavioral variant FTD (bvFTD) is a common form of FTD syndromes. In comparison to AD patients, FTD patients have more severe daytime somnolence and sleep disturbance[36]. The sleep disturbance appears earlier in FTD than in AD patients. Moreover, FTD patients have longer sleep onset and less total sleep time than AD patients[37]. In the prodromal symptoms, sleep disturbance occurs more frequent in bvFTD patients (40%) than in AD patients (12%)[38].

The hexanucleotide (G4C2) repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*) gene causes both FTD and amyotrophic lateral sclerosis (ALS)[39,40]. In a screen for *C9orf72* repeat expansion in 344 RBD patients, two of them have G4C2 expansion, suggesting a possible linkage between RBD and *C9orf72* mutation[41]. It is well known that dipeptide repeat proteins (DPRs) that are encoded by the expansion of *C9orf72* by non-ATG translation form inclusions in c9FTD/ALS. Interestingly, the abundant DPR inclusions are presented in pineal grand, as well as the supraoptic nucleus and paraventricular nucleus (PVN) that are related to the SCN, implying an association of sleep disruption and c9FTD/ALS[42].

Multiple system atrophy

The sleep disturbance occurs highly in MSA patients. RBD is a very common symptom in MSA patients. In a meta-analysis, the prevalence of RBD in MSA ranks from 25% to 100%[43]. In a cross-sectional study in which 165 MSA patients are engaged, sleep disorders are observed in most patients[44]. RBD occurs in 49.7% of patients, and the frequency of EDS is 27.3%[44]. Importantly, there is a positive correlation between sleep

disturbances and the severity of MSA[44]. Another study also shows that there is an association of RBD and MSA, with a frequency of RBD as high as 70.4% in MSA patients[45]. A cross-sectional study shows that the MSA patients with EDS have a higher score of Non-Motor Symptoms Scale and a higher apnea-hypopnea index as compared the MSA patients without EDS, suggesting that EDS in MSA patients is more associated with sleep-related breathing disorder and other the non-motor symptoms[46].

PATHOLOGICAL ACCUMULATION OF DISEASE PROTEINS IN ASSOCIATION WITH SLEEP DISRUPTION

The pathological hallmark is the presence of abnormal protein deposition in diseased brains in patients with neurodegenerative diseases. The formation of amyloid plaques by Aβ and intracellular neurofibrillary tangles by tau in AD or the formation of Lewy bodies by α-syn is a typical pathological process in AD or PD brains[47]. Soluble Aβ, tau or α-syn can form oligomers, protofibrils and fibrils, which accumulate and deposit in diseased brains (Soto and Pritzkow[48], 2018) [Figure 1].

Aβ

The homeostasis of Aβ, either accumulation or clearance, is important for AD pathology. Aβ is a small peptide that is released from amyloid precursor protein (APP) after APP is cleaved by β-secretase and subsequently cleaved by γ-secretase[49,50]. Mutations in APP or presenilin (catalytic subunit of γ-secretase) that cause early-onset familial AD increase the processing of APP, which promotes the generation of Aβ peptides[51,52]. The deposition of Aβ in AD brains and in APP transgenic mice is a typical pathological feature.

It has been reported that Aβ has daily oscillations[53]. The levels of soluble Aβ in brain interstitial fluid (ISF) in the hippocampus in wild-type or human APP transgenic mice have a pronounced diurnal rhythm[53]. Moreover, the fluctuation of Aβ also occurs in the cerebral spinal fluid (CSF) in humans[53]. In sleep-deprived mice, Aβ is increased in the ISF in the hippocampus in both wild-type and APP transgenic mice; however, Aβ is decreased in the ISF in the hippocampus in animals with more sleep[53]. In *APPswe/PS1δE9* mice, diurnal fluctuation of Aβ in the ISF and the hippocampus but not in the striatum was attenuated at 6 months of age. Diurnal fluctuation of Aβ in ISF in the striatum is decreased at 9 months of age when Aβ plaques appear in the striatum and more often in the hippocampus[54]. Animals also have significant disturbances in the sleep-wake cycle at 6 months of age[54]. Thus, the decrease in diurnal fluctuation of Aβ in the ISF occurs earlier in the hippocampus than in the striatum, and Aβ plaques appear earlier in the hippocampus than in the striatum, suggesting a link between Aβ pathological progression and daily oscillations. In addition, active immunolization with Aβ, which decreases Aβ deposition in *APPswe/PS1δE9* mice, restores the diurnal fluctuation of Aβ in the ISF and normal sleep-wake cycle[54], suggesting that Aβ deposition disrupts Aβ oscillations and the sleep-wake cycle.

In humans, the daily oscillation of Aβ in the CSF shows that Aβ increases during the day, reaches a peak in the evening, and then decreases overnight[53]. Loss of diurnal fluctuation of the CSF Aβ occurs in patients with presenilin mutation when Aβ deposition is detected by amyloid imaging with Pittsburgh Compound B[54]. An attenuation of diurnal fluctuation of the CSF Aβ also occurs in patients with presenilin mutation[54]. Most interestingly, using a radiotracer 18F-florbetaben that binds to Aβ, images from healthy individuals scanned by positron emission tomography show that even one night of sleep deprivation can increase Aβ in the hippocampus and thalamus, the regions vulnerable to damage in AD[55]. This study demonstrates the first evidence, in living humans, that sleep disturbance is directly associated with Aβ accumulation[55].

In animal models, *APPswe/PS1δE9* transgenic mice show changed sleep architecture compared with control mice. Moreover, sleep disturbance occurs at 4 months of age, but plaque deposition and tau phosphorylation typically occur at 6 months of age[56], suggesting that sleep changes occur earlier than AD pathology. APP transgenic mice also develop more Aβ plaques in the hippocampus after exposure to sleep deprivation[53,57]. Chronic sleep deprivation of 8 h per day for 2 months or chronic sleep deprivation of 20 h per day for 21 days increases Aβ plaques in the hippocampus in APP transgenic mice[53,57]. Sleep deprivation increases Aβ levels in the CSF in humans[53] and amyloid plaques in the hippocampus in APP transgenic mice[53,57], suggesting an impact of sleep disorders on Aβ accumulation. However, Aβ has a role in circadian rhythms in multiple transgenic mouse models with Aβ pathology. In *5×FAD* mice that model AD, the circadian rhythms are changed, evidenced by alterations in home cage activity and body temperature[58]. Thus, there is a bidirectional relationship between Aβ accumulation and sleep disorders[59].

The association of the accumulation of Aβ with sleep disturbance has been further verified in patients who accept sleep intervention or in animal models that are treated with drugs to improve sleep. In patients with obstructive sleep apnea, the Aβ levels in blood[60] and the Aβ deposition in brain[61] are increased. In AD patients with sleep-disordered breathing, a 6-month sleep intervention with a constitutive positive airway pressure ventilation decreases blood Aβ42/40 ratio in patients who are complete recovered from sleep disturbance[62]. Interestingly, there are no changes in blood Aβ42/40 ratio in those patients who have no improvement in sleep[62], suggesting an involvement of sleep in the regulation of Aβ levels. In animal, an administration of nobiletin, a natural compound that is able to enhance the amplitude of clock gene oscillation[63], improves clock gene expressions and decreases Aβ deposition in APP/PS1 mouse brains[64].

Tau

Tau is another important protein in association with AD. In the AD brain, tau is hyperphosphrylated and forms intracellular neurofibrillary tangles, a pathological hallmark of Aβ plaques. Tau pathology occurs in the early AD. Aggregates of tau first appear in the brainstem locus coeruleus (LC) and then spread to the transentorhinal and entorhinal regions and subsequently to the hippocampus[65]. The LC is strongly linked to wakefulness and sends norepinephrine-containing projections to the cortex, amygdala, hippocampus, cerebellum and spinal cord. In tau *P301S* transgenic mice expressing the human P301S tau mutation that is associated with parkinsonism linked to chromosome 17, chronic short sleep at the age of 2-3 months induces deterioration in behaviors and increases in tau oligomers with sustained increases in phosphorylated tau and neuronal loss in the LC and the amygdala[66]. The brain ISF tau in mice and the CSF tau in humans are also regulated by the sleep-wake cycle, although tau is known as an intracellular protein[18]. Tau in brain ISF in the hippocampus has diurnal oscillations similar to Aβ. In sleep-deprived mice, tau levels are 2-fold increased in brain ISF[18]. Interestingly, the increase in tau in brain ISF induced by sleep deprivation can be blocked by terodotoxin, a toxin that blocks voltage-dependent sodium channels, suggesting that neuronal activity is related to tau levels in brain ISF. In addition, sleep deprivation in humans increases CSF Aβ by 30%, while it increases CSF tau by over 50% in the same participants[18]. With chronic sleep deprivation, the administration of recombinant P301S human tau fibrils does not change tau seeding in the hippocampus; however, it increases tau spreading to the LC, a nucleus associated with wakefulness[18], suggesting that sleep disorders may induce tau spreading to other brain regions that are associated with the wake-sleep cycle, producing feedback effects on sleep.

α-syn

It has been reported that the severity of hypothalamic Lewy pathology is correlated with Braak stages[67]. Moreover, Lewy pathology is presented in the SCN with mild or moderate severity in most cases of PD, much fewer in pineal gland[67]. In multiple system atrophy, no Lewy pathology is present in the SCN or pineal gland[68], suggesting a tight association between circadian dysfunction and PD pathology. According

to the Braak hypothesis, α-syn pathology can spread from the peripheral autonomic nerve endings of the gastrointestinal tract to the brain[69]. In a study involving 602 patients with clinical assessment for RBD and neuropathological examination for Lewy-type α-synucleinopathy, Lewy-type α-synucleinopathy occurred in 79.2% of patients with RBD but only 39.5% of those without RBD[70]. In addition to the brain, α-syn pathology occurs in many organs, including the vagus nerve, gastrointestinal tract, adrenal gland and heart, in PD patients[71]. The presence of pathological α-syn in the gastrointestinal tract is identified in prodromal PD patients up to 20 years prior to diagnosis[72]. Moreover, the transmission of α-syn inclusions has been well identified in recent studies that show the development of α-syn pathology in the brain after injecting α-syn preformed fibrils into the duodenum[73,74]. Thus, peripheral tissues with α-syn pathology may reflect, at least partially, central pathology. In a study using biopsy samples of labial minor salivary glands, α-syn pathology occurred in labial minor salivary glands in 50% of patients with idiopathic RBD and 54% of patients with PD but only 3% of controls[75]. Moreover, the deposits of α-syn in the parotid gland in idiopathic RBD patients occur at the prodromal stage of PD[76]. Thus, studies suggest an association of α-syn deposition and RBD.

TDP-43

TDP-43 that is encoded by *TARDBP* is a major pathogenic protein in FTD and ALS[77]. Although the mutations in *TARDBP* only cover about 5% familial ALS cases and even less in FTD patients[78]. The TDP-43 pathology is presented in about 97% of ALS patients and 45 % FTD patients[79]. In ALS patients, the volume of the hypothalamus is decreased[80]. Moreover, the volume of the PVN in the hypothalamus is also decreased. Furthermore, TDP-43-positive inclusions are observed in the PVN, lateral hypothalamus and fornix[80]. TDP-43 inclusions are also presented in the reticular formation in the brainstem, which has an important role in the rhythmical cycle of sleep and wakefulness[81]. The presence of TDP-43 pathology in sleep-related brain regions and nuclei suggests a linkage between the abnormal accumulation of TDP-43 and the symptoms of sleep disorders in ALS and FTD patients.

FACTORS INVOLVED IN CIRCADIAN RHYTHM DISRUPTION IN NEURODEGENERATIVE DISEASES

Clock gene

The molecular basis of circadian rhythms is the oscillation of 24 h clock gene expression in the SCN[1]. The core clock gene products circadian locomoter output cycles kaput (CLOCK) and brain and muscle arnt-like 1 (BMAL1) form heterodimers that bind to E-boxes and drive the expression of peroid (PERs) and cryptochrome (CRYs). The expression of PERs and CRYs in turn represses CLOCK-BMAL1 activity, thus inhibiting their own expression, which forms a feedback loop that takes 24 h[1]. Thus, the molecules involved in the molecular clock are important for the maintenance of circadian rhythms.

It has been reported that the alteration of DNA methylation of BMAL1 in early AD patients causes changes in BMAL1 expression patterns in both amplitude and phase[82]. Knockout of Bmal1 disrupts Aβ oscillation in brain ISF in the hippocampus. Moreover, in tamoxifen-inducible global Bmal1 knockout mice with an APP transgenic background, Bmal1 knockout significantly increases hippocampal Aβ plaques 4 months after tamoxifen induction[17]. In AD patients, there is a disruption of circadian rhythms, typically showing sleep problems. Sleep deprivation also affects clock gene expression and the DNA binding patterns of BMAL1 and CLOCK heterodimers, which disturbs clock function. Three different polymorphisms of the CLOCK gene are associated with AD in the Chinese population[83,84]. Moreover, changes in expression patterns and decreases in expression levels of Bmal1 and Clock in senescent cells and aged rodent brains suggest a role of circadian genes in aging[84]. In *5×FAD* mice, the amplitude of BMAL1 and PER2 in a 24 h oscillation is greatly decreased[58]. Moreover, Aβ is able to induce BMAL1 degradation *in vitro*[58]. In

APP/PS1 mice, there is also a modest alteration of PER2 in the SCN, which is consistent with the findings in *5×FAD* mice[58,85]. In addition, at circadian time 4 (CT4, 4 h after the onset of activity of diurnal organisms, based on the free-running period of a rhythm), when microglia express higher levels of BMAL1 than at CT12, the clearance ability for fibrillary Aβ by microglia at CT4 is also higher than that at CT12 in *5×FAD* mice[86]. Pharmacological inhibition of REV-ERBs that is transactivated by BMAL1 and negative feedback on BMAL1 activity promote a microglial M2-like phenotype. Moreover, a constitutive deletion of Rev-erbα in *5×FAD* mice can repress amyloid plaque formation[86], further suggesting a role of the clock gene in protein homeostasis.

It has been reported that BMAL1 oscillation amplitude is decreased in PD patients and that BMAL1 mRNA levels are decreased in leukocytes in PD patients, suggesting that dysfunction of the clock gene Bmal1 may be associated with PD.

Melatonin

In the brain, circadian information in the SCN can be sent to different areas. Through multisynaptic projections, the SCN circadian information is integrated and sent to the superior cervical ganglia (SCG). The noradrenergic neurons in the SCG send projections to the pineal gland[87]. The pineal gland produces melatonin, the hormone that synchronizes and stabilizes circadian rhythms, which is important for the maintenance of the biological clock of the brain in the SCN. Melatonin is synthesized in the dark and shows oscillation at its level, which is controlled by the SCN [Figure 2]. The secretion of melatonin starts at early night and usually reaches a peak level at 3:00 to 4:00 am. With aging or neurodegenerative diseases, the circadian amplitude is decreased with desynchronization of physiological rhythms, leading to a decrease in melatonin levels[88]. Melatonin binds to melatonin receptor 1 (MT1) and MT2, which are G-protein coupled receptors and are highly expressed in neurons in the SCN, hippocampus, thalamus, vestibular nuclei and cerebral and cerebellar cortex[89]. In addition to the maintenance of circadian rhythms, melatonin has multiple functions, including antioxidative stress and regulating metabolism[88].

AD patients have lower melatonin levels than normal controls. The melatonin level is decreased in the CSF in early AD patients before clinical symptoms[90]. Meanwhile, the loss of neurons in the SCN further shows a correlation between AD and circadian rhythm dysfunction[91]. In patients with AD at the early stage, the initial evening secretion of melatonin is delayed and mildly decreased, suggesting that a circadian phase shift occurs in early AD patients[92]. Moreover, the risk variant rs12506228, which is located downstream of MTNR1A (MT1A-encoding gene), is associated with AD[93]. The rs12506228 variant leads to a decrease in MT1A expression and is associated with both clinical and pathological changes in AD patients[93], further suggesting a role of melatonin in AD.

It has been reported that melatonin has effects on Aβ deposits in AD. In *APP/PS1* mice, long-term melatonin treatment at ages starting from 2-2.5 months decreases Aβ deposits in the hippocampus and the entorhinal cortex when the animals are examined at the age of 7.5 months[94]. Meanwhile, there was a decrease in inflammatory cytokines in the hippocampus in *APP/PS1* mice treated with melatonin, suggesting a role of melatonin in anti-inflammation and decreasing Aβ accumulation[94]. It has also been reported that melatonin can induce lymphatic clearance of Aβ in *Tg2576* mice[95].

Using real-time PCR analysis, it has been identified that MT1 and MT2 are decreased in both the substantia nigra and the amygdala in PD patients compared to controls[96]. In PD patients, circulating melatonin is decreased in early PD patients compared with controls[7]. Moreover, plasma melatonin, both the amplitude of daily oscillation and the levels, is lower in PD patients than in controls[97]. In PD patients who accept

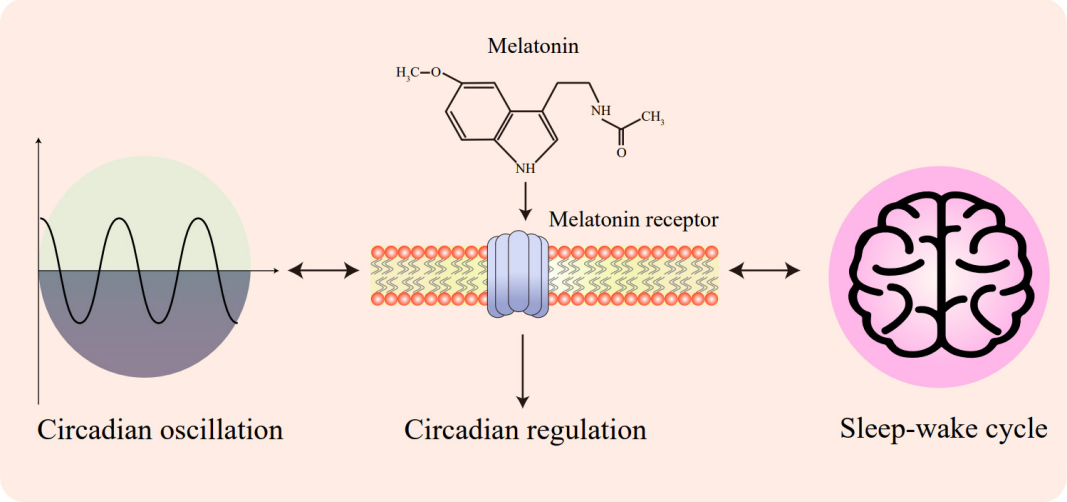

Figure 2. Impact of circadian genes and hormones on neurodegeneration and the glymphatic system. The secretion of the circadian hormone melatonin is controlled by circadian rhythm. In addition to functioning in circadian rhythm, melatonin has protective effects on neurons. In neurodegenerative diseases, the levels of melatonin are decreased.

melatonin treatment at a dose of 5 mg or a high dose of 50 mg 30 min before bedtime over 14 days, the nocturnal sleep that is evaluated with actigraphy is significantly improved in PD patients during melatonin treatment at both doses[98]. Interestingly, the treatment of PD patients with levodopa increases melatonin levels[99].

In a rotenone PD animal model[100] or in a 6-hydroxydopamine with or without pinealectomy animal model[101], melatonin protects DA neurons. Melatonin also improves rotenone-induced defects in behaviors, including grip strength and performance on rotarods[102]. In *MPTP* mice, melatonin protects DA neurons against MPTP-induced neurotoxicity, which may be mediated by antioxidant effects and neuroprotective effects of melatonin[103-105]. In *MPTP* mice that receive both melatonin and levodopa treatments, melatonin increases the therapeutic effects of levodopa in the improvement of MPTP-induced akinesia and catalepsy[105]. In a lentivirus-infected animal model that expresses A30P pathogenic α-syn, melatonin administration protects DA neurons against A30P elsyn-induced TH neuronal loss[106]. In amphetamine-treated 4-day-old postnatal rats, melatonin decreases amphetamine-induced α-syn accumulation in the substantia nigra, dorsal striatum, nucleus accumbens, and prefrontal cortex[107]. Thus, melatonin has multiple effects on circadian rhythm regulation, neuroprotection and protein clearance, which can be associated with the pathogenesis of neurodegenerative diseases [Figure 2]

GLYMPHATIC PATHWAY

Although the central nervous system is thought to anatomically lack lymphatic vessels for the removal of interstitial metabolic waste products, it has been recently discovered that glymphatic (glial-lymphatic)[108] and meningeal lymphatic vessels[109,110] execute functions to transport and drain brain wastes, such as the peripheral lymphatic system. The glymphatic system is a glial-dependent perivascular network that has lymphatic functions[111]. The CSF produced by the choroid plexuses flows into the brain along periarterial spaces surrounding cerebral arteries and arterioles, running in the same direction as blood flow, which is driven by arterial pulsation[112]. The CSF enters the brain parenchyma and mixes with ISF, which is facilitated by the aquaporin (AQP-4) water channel on the perivascular end-foot processes of astrocytes[113]. Mixtures of CSF and ISF with interstitial solutes outflow along the perivenous space and drain out of the brain via meningeal lymphatic vessels or along cranial and spinal nerves[113] [Figure 3]. The meningeal

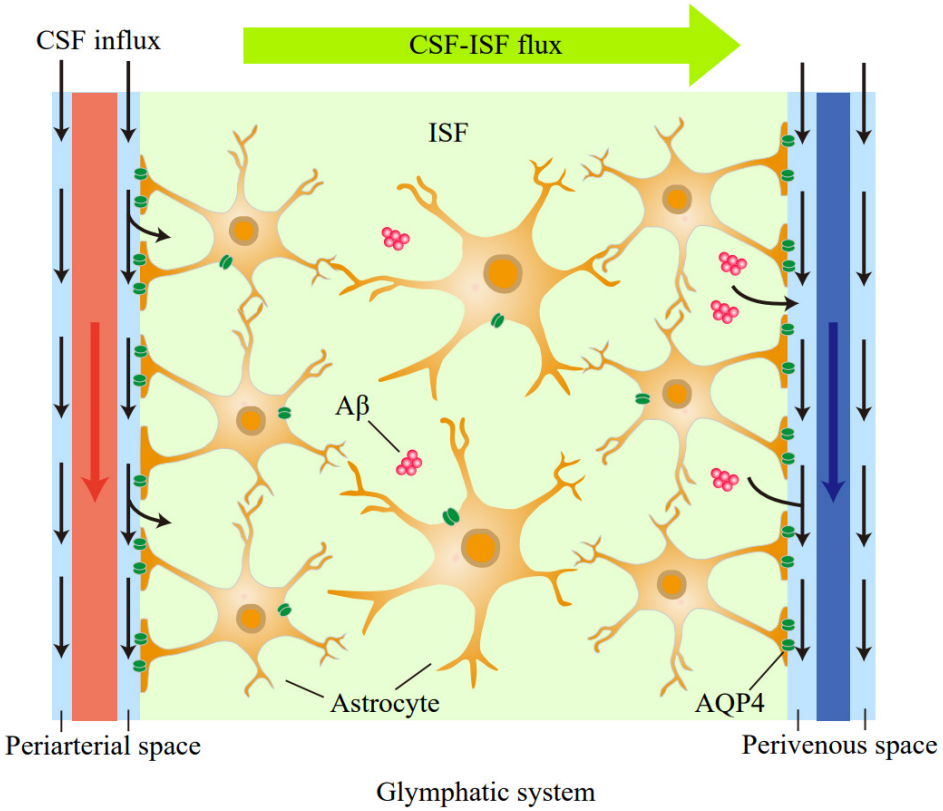

Figure 3. The glymphatic system in association with the clearance of neurodegenerative disease proteins. The glymphatic system is a perivascular network that has lymphatic functions. Driven by arterial pulsation, the CSF flows into the brain along periarterial spaces surrounding cerebral arteries and arterioles, running in the same direction as blood flow. The CSF enters the brain parenchyma mediated by the aquaporin (AQP-4) water channel on the perivascular end-foot processes of astrocytes. Mixtures of CSF and ISF with interstitial solutes outflow along the perivenous space and drain out of the brain, which can clear Aβ in the ISF. The glymphatic system depends on AQP-4 on astrocytes. Sleep enhances glymphatic system function to promote the clearance of Aβ and other disease proteins in the brain. CSF: Cerebral spinal fluid; ISF: interstitial fluid; Aβ: amyloid-β.

lymphatic vessels are located in the dura matter[109,110]. The metabolites in the CSF are cleared from the brain by fluid flow and finally drained to extracranial deep cervical lymph nodes[114]. Dysfunction of the glymphatic system may be a final common pathway to dementia[115]. Using gadobutrol as the CSF tracer with intrathecal administration, the glymphatic system assessed with magnetic resonance imaging demonstrates delayed glymphatic clearance of gadobutrol from the subarachnoid space, with an enhancement of the signal in the brain parenchyma, in idiopathic normal hydrocephalus, which is the first study in humans showing glymphatic clearance of a CSF tracer (solute)[116].

The glymphatic system is tightly associated with the clearance of neurodegenerative disease proteins, such as Aβ[108,113]. In PD patients, the glymphatic system is impaired, and in animals, blockage of meningeal lymphatic vessels increases α-syn preformed fibril-induced α-syn pathology[117]. Interestingly, the activity of the brain glymphatic system is controlled by circadian rhythms[118], which are related to the clearance of neurodegenerative disease proteins[119]. Glymphatic clearance is facilitated by AQP-4, which influences CSF influx into the brain parenchyma where it mixes with ISF[108]. In *Aqp4* (AQP-4 encoding gene) knockout mice, CSF tracer influx into the brain parenchyma is markedly reduced; however, the periarterial movement of the tracer is not significantly decreased[108]. Moreover, in *Aqp4* knockout mice, a 55% reduction in the clearance of Aβ occurs compared with wild-type mice when 125I-Aβ42 is infused into the striatum[108]. The interstitial space in sleeping mice is larger than that in awake mice[119], which increases the convective ISF

bulk flow in the brain parenchyma to increase glymphatic clearance of Aβ[119] [Figure 3]. In *APP/PS1* mice, Aβ also shows an impact on the glymphatic pathway, leading to dysfunction of the glymphatic pathway[120]. The inflow of Aβ40 into the brain and the clearance of Aβ40 by the glymphatic pathway in the brain are decreased in *APP/PS1* mice (Peng *et al.*[120], 2016). Disruption of meningeal lymphatic vessels in *5×FAD* mice also aggravates Aβ deposition in the meninges and Aβ accumulation in the brain parenchyma[121]. Most recently, it has been reported that there is a circadian rhythm in glymphatic influx, and loss of AQP-4 eliminates circadian CSF distribution, further suggesting a linkage between circadian rhythms and glymphatic clearance[118]. Interestingly, the localization of AQP-4 to the endfeet of astrocytes surrounding the vasculature has diurnal variation, showing an increase in AQP-4 polarization surrounding the vasculature during the day[118]. Correspondingly, glymphatic influx and the clearance of solutes are increased during the day. In addition, the influx that is indicated by a CSF tracer shows an increase during the rest phase compared with the active phase of animal behavior, further suggesting a role of circadian rhythms in glymphatic influx[118]. There are also some controversial studies. In *Aqp4* knockout mice and rats, no significant difference in tracer (Alexa 647-labeled ovalbumin) penetration into the striatal parenchyma from the paravascular space was observed[122]. The mechanisms for the clearance of waste by the glymphatic pathway are still largely unknown. Furthermore, the linkage between circadian rhythms and the glymphatic pathway is still being identified.

CONCLUSION

It is clear that there is a link between circadian disruption and neurodegenerative diseases. Sleep problems often start at the early stage in patients with neurodegenerative diseases. Clinical studies and animal models have revealed an association between the pathological progression of neurodegenerative diseases and circadian disruption. It has been well documented that sleep deprivation aggravates the deposition of neurodegenerative disease proteins in animal models. It is also known that sleep increases the glymphatic clearance of Aβ and tau in the CSF. However, our understanding of the role of circadian rhythms in protein homeostasis and disease development is very limited. There are still some key questions that need to be addressed: (1) whether circadian rhythms influence protein quality control systems, such as the ubiquitin-proteasomal pathway or autophagic pathway, facilitating disease protein degradation; (2) why RBD, within different sleep disturbances, is the highest risk factor associated with synucleinopathy, PD and dementia; (3) how the phenotypes of AQP-4 deficiency can be linked to the phenotypes of neurodegenerative diseases in animal models; and (4) whether there are different or the same mechanisms accounting for the interactions between abnormal accumulation of disease proteins and the dysfunction of circadian rhythms in patients with different neurodegenerative diseases. Further studies should address how circadian rhythm affects the clearance or accumulation of disease proteins and by which mechanism the abnormal proteins lead to dysfunction of circadian, which may guide the development of novel strategies for clinical treatment and drug targets for neurodegenerative diseases.

DECLARATIONS

Authors' contributions

Conceived the idea of this review article: Li J
Drafted the manuscript: Wang X
Revised the manuscript: Li J
Designed pictures: Wang R, Wang X
Completed the pictures: Wang R

Available of data and materials
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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
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Consent for publication
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AUTHOR INSTRUCTIONS

1. Submission Overview

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If an accepted manuscript was funded by National Institutes of Health (NIH), the authors may inform Editors of the NIH funding number. The Editors are able to deposit the paper to the NIH Manuscript Submission System on behalf of the authors.

2. Submission Preparation

2.1 Cover Letter

A cover letter is required to be submitted accompanying each manuscript. It should be concise and explain why the study is significant, why it fits the scope of the journal, and why it would be attractive to readers, etc.

Here is a guideline of a cover letter for authors’ consideration:

In the first paragraph: include the title and type (e.g., Original Article, Review, Case Report, etc.) of the manuscript, a brief on the background of the study, the question the author sought out to answer and why;

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2.2 Types of Manuscripts

There is no restriction on the length of manuscripts, number of figures, tables and references, provided that the manuscript is concise and comprehensive. The journal publishes Original Article, Review, Meta-Analysis, Case Report, Commentary, *etc.* For more details about paper type, please refer to the following table.

Manuscript Type	Definition	Abstract	Keywords	Main Text Structure
Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Review	A Review paper summarizes the literature on previous studies. It usually does not present any new information on a subject.	Unstructured abstract. No more than 250 words.	3-8 keywords	The main text may consist of several sections with unfixed section titles. We suggest that the author includes an "Introduction" section at the beginning, several sections with unfixed titles in the middle part, and a "Conclusion" section in the end.
Case Report	A Case Report details symptoms, signs, diagnosis, treatment, and follows up an individual patient. The goal of a Case Report is to make other researchers aware of the possibility that a specific phenomenon might occur.	Unstructured abstract. No more than 150 words.	3-8 keywords	The main text consists of three sections with fixed section titles: Introduction, Case Report, and Discussion.
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Systematic Review	A Systematic Review collects and critically analyzes multiple research studies, using methods selected before one or more research questions are formulated, and then finding and analyzing related studies and answering those questions in a structured methodology.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Technical Note	A Technical Note is a short article giving a brief description of a specific development, technique or procedure, or it may describe a modification of an existing technique, procedure or device applied in research.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Commentary	A Commentary is to provide comments on a newly published article or an alternative viewpoint on a certain topic.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Editorial	An Editorial is a short article describing news about the journal or opinions of senior editors or the publisher.	None required	None required	/
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2.3 Manuscript Structure

2.3.1 Front Matter

2.3.1.1 Title

The title of the manuscript should be concise, specific and relevant, with no more than 16 words if possible. When gene or protein names are included, the abbreviated name rather than full name should be used.

2.3.1.2 Authors and Affiliations

Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

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The abstract should be a single paragraph with word limitation and specific structure requirements (for more details please refer to Types of Manuscripts). It usually describes the main objective(s) of the study, explains how the study was done, including any model organisms used, without methodological detail, and summarizes the most important results and their significance. The abstract must be an objective representation of the study: it is not allowed to contain results which are not presented and substantiated in the manuscript or exaggerate the main conclusions. Citations should not be included in the abstract.

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Three to eight keywords should be provided, which are specific to the article, yet reasonably common within the subject discipline.

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2.3.2.1 Introduction

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Books	Sherlock S, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub; 1993. pp. 258-96.
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Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm. [Last accessed on 30 Oct 2017]
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