

Review

Emerging roles of oxidative stress in brain aging and Alzheimer's disease

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ABSTRACT

Reactive oxygen species (ROS) are metabolic byproducts that are necessary for physiological function but can be toxic at high levels. Levels of these oxidative stressors increase gradually throughout the lifespan, impairing mitochondrial function and damaging all parts of the body, particularly the central nervous system. Emerging evidence suggests that accumulated oxidative stress may be one of the key mechanisms causing cognitive aging and neurodegenerative diseases such as Alzheimer's disease (AD). Here, we synthesize the current literature on the effect of neuronal oxidative stress on mitochondrial dysfunction, DNA damage and epigenetic changes related to cognitive aging and AD. We further describe how oxidative stress therapeutics such as antioxidants, caloric restriction and physical activity can reduce oxidation and prevent cognitive decline in brain aging and AD. Of the currently available therapeutics, we propose that long term physical activity is the most promising avenue for improving cognitive health by reducing ROS while promoting the low levels required for optimal function.

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1. Introduction

In 1954, Denham Harman proposed that free radical reactions "may be involved in production of the aging changes associated with the environment, disease and an intrinsic aging process" (Harman, 1992). Time has confirmed that free radicals are heavily involved in the aging process, as well as diseases associated with advanced age such as Alzheimer's disease (AD) (Butterfield and

Abbreviations: ROS, reactive oxygen species; AD, Alzheimer's disease; mtDNA, mitochondrial DNA; SOD, superoxide dismutase; mCAT, mitochondrial catalase; MCI, mild cognitive impairment; AEBP1, adipocyte enhancer-binding protein 1; PCSK1/2, proprotein convertase 1 and 2; DRP1, dynamin-related protein 1; SSBs, single strand breaks; DSBs, double strand breaks; NMN, nicotinamide mononucleotide; BDNF, brain derived neurotrophic factor; IEG, immediate early gene; PKcs, protein kinase catalytic subunits; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; NAC, N acetyl-L-cysteine; SAMP8, senescence accelerated prone 8; CSF, cerebral spinal fluid; NQO1, NAD(P)H-quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; CRM, caloric restriction mimetic; p-AMPK, phospho-adenosine monophosphate protein kinase; GPX, glutathione peroxidase; AOPP, advanced oxidation protein products; GSSG, glutathione disulfide; GR, glutathione reductase; STZ, streptozotocin.

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ginning with the production of ROS as byproducts of mitochondrial respiration. We will then discuss the effects of these oxidative stressors on DNA through both direct and epigenetic manipulations. Lastly, we will examine current therapeutics for oxidative stress (antioxidants, caloric restriction mimetics and physical activity) and their ability to reduce oxidation in the aged and degenerated brain. We conclude that long term physical activity is an efficient means of reducing oxidative stress in the brain while maintaining physiologically necessary low levels of oxidation.

2. Oxidative stress is a byproduct of mitochondrial respiration

Mitochondria are the main hub of neuronal oxidative stress, as most free radicals are generated as byproducts of the mitochondrial electron transport chain. Superoxide is a byproduct of mitochondrial respiration which is produced from oxygen when there is an excess of ATP and electron transport is diminished (Sinha et al., 2013). Superoxide dismutase can then convert superoxide to hydrogen peroxide, which is further broken down into hydroxyl radicals and anions by the Fenton reaction (Singh et al., 2019). Mitochondrial ROS can lead to redox imbalance, neurotoxicity, genomic instability, pro-inflammatory gene transcription and cytokine release, such as IL-1, IL-6 and TNF alpha (Islam, 2017). In a positive feedback cycle, ROS species can damage and inactivate parts of the electron transport chain, leading to increased electron reduction of oxygen to superoxide (Islam, 2017; Sies et al., 2017; Sinha et al., 2013). Mitochondrial ROS are particularly damaging to mitochondrial DNA (mtDNA), which are not protected by histones and mutate at a higher rate. The frequent damage to mtDNA severely impairs the function of postmitotic neurons, making removal of damaged mitochondria via mitophagy critical (Islam, 2017; Lee et al., 2012). Mitophagy is the degradation of mitochondria due to signals such as starvation or oxidative stress. Oxidative stress can induce mitophagy by decreasing the mitochondrial membrane potential but can also impair mitophagy by interacting with its regulators. Parkin, a key mitophagy regulator involved in Parkinson's disease, can be S-nitrosylated by ROS, inhibiting it and preventing the removal of damaged mitochondria (Lee et al., 2012). The central role of mitochondria in ROS production has led to a working theory of aging that combines ROS, DNA damage and mitochondrial theories (Maynard et al., 2015). The theory posits that DNA damage activates kinases and PARP, which deplete NAD⁺ levels, a cofactor for many metabolic pathways and a donor in the production of ATP. A reduction in NAD⁺ increases the need for oxygen consumption and ATP production, causing mitochondria to couple in an attempt to meet high energy demands. Mitochondrial coupling increases membrane potential, decreases mitophagy, and elevates free radicals, the last of which causes further DNA damage (Fig. 1; Guillaumet-Adkins et al., 2017; Maynard et al., 2015). The close relationship between harmful ROS and mitochondria lends weight to the hypothesis that mitochondria play a critical role in aging and associated neurodegenerative diseases.

2.1. Mitochondrial oxidative stress and dysfunction increase in the aged brain

Mitochondrial dysfunction is prevalent in aging and improving mitochondrial function can have far reaching effects on systemic age-associated oxidative stress. In healthy aging, there are marked deficits in mitochondrial metabolism, specifically a reduction in the α subunit of the mitochondrial F1 ATP synthase, which couples oxidative phosphorylation to ATP synthesis. This leads to decreased ATP production, increased ROS production and increased DNA, protein and lipid oxidation (Grimm and Eckert, 2017; Lu et al., 2004; Mecocci et al., 2018). Mitochondrial impairment

leads not only to mtDNA damage but nuclear DNA damage as well, particularly in the promoter region of age-downregulated genes involved with vesicular function, synaptic plasticity and mitochondrial function (Lu et al., 2004). Antioxidant overexpression has been tested as a potential means of mitigating the deleterious effects of age but has proven ineffective in extending the lifespan except in superoxide dismutase (SOD) overexpressing flies (Pérez et al., 2009; Tower, 2000). However, overexpression of mitochondrial catalase (mCAT) significantly extends the mouse lifespan. Mitochondrial catalase overexpression provides additional benefits including an overall reduction in oxidation, preserved insulin signaling and delayed cardiac and cataract pathology (Dai et al., 2009; Pagliarunga et al., 2015; Schriener et al., 2005). DNA damage was similarly reduced in mCAT mice, both in aged skeletal muscle and mtDNA from asbestos exposed lungs (Campisi et al., 2019; Kim et al., 2016; Schriener et al., 2005). These findings suggest that mCAT mice might prevent DNA damage in other tissue types, and in nuclear as well as mitochondrial DNA. A focused increase in mitochondrial catalase effectively mitigates oxidative stress, indicating the critical role of mitochondria in regulating ROS with age.

2.2. Mitochondrial oxidative stress is a central cause of Alzheimer's disease

Dysfunctional mitochondria are also implicated in the pathogenesis of AD, as one of the first markers of AD is increased mtDNA oxidation (Grimm and Eckert, 2017; Mecocci et al., 2018). In fact, age-associated mitochondrial decline may be one of the first events in the pathogenesis of sporadic, late onset AD. The mitochondrial cascade hypothesis states that age-associated loss of mitochondrial function affects the expression and processing of APP, producing amyloid beta oligomers that accumulate into plaques in Alzheimer's disease (Swerdlow et al., 2014; Cheignon et al., 2018). Our lab was the first to show that the hydrophobic 25–35 region of amyloid beta leads to neuronal toxicity, and the Mattson group further demonstrated that it generates reactive oxygen species, demonstrating that amyloid beta is itself an effective source of oxidative stress (Hensley et al., 1994; Pike et al., 1992). It was later discovered that incubating neurons with amyloid beta 1–42 oligomers leads to lipid peroxidation as marked by protein bound 4-hydroxy-2-trans-nonenal (HNE) (Mark et al., 1997). The hydrophobic nature of amyloid beta allows it to reside in the lipid bilayer, and covalent bonding of HNE to neuronal proteins leads to cell death (Butterfield, 2020; Di Domenico et al., 2017; Pike et al., 1993). Amyloid beta induced-HNE further impairs glucose and glutamate transporters, decreases mitochondrial transmembrane potential and reduces the activity of the sodium-potassium ATPase (Keller and Mark et al., 1997; Keller and Pang et al., 1997). The oxidative stress caused by amyloid beta is likely due to the complexes it forms with redox active metals. Copper, zinc and iron all bind to amyloid beta, promoting its aggregation into plaques. Of these metals, copper forms the most stable bond, and this complex has been shown to generate superoxide and hydrogen peroxide (Cheignon et al., 2018; Reybier et al., 2016). The oxidative stress caused by metal-amyloid complexes lead to excitotoxicity, promotes membrane depolarization and impairs mitochondrial function.

Mitochondrial dysfunction in the AD brain is further exacerbated by impaired glucose metabolism, insulin production and mitophagy. Brain glucose metabolism is reduced prior to the onset of AD in patients with mild cognitive impairment (MCI), a pre-clinical stage of the disease (Tönnies and Trushina, 2017). The AD brain is further marked by an upregulation of the transcriptional repressor AEBP1 (adipocyte enhancer-binding protein 1), which down reg-

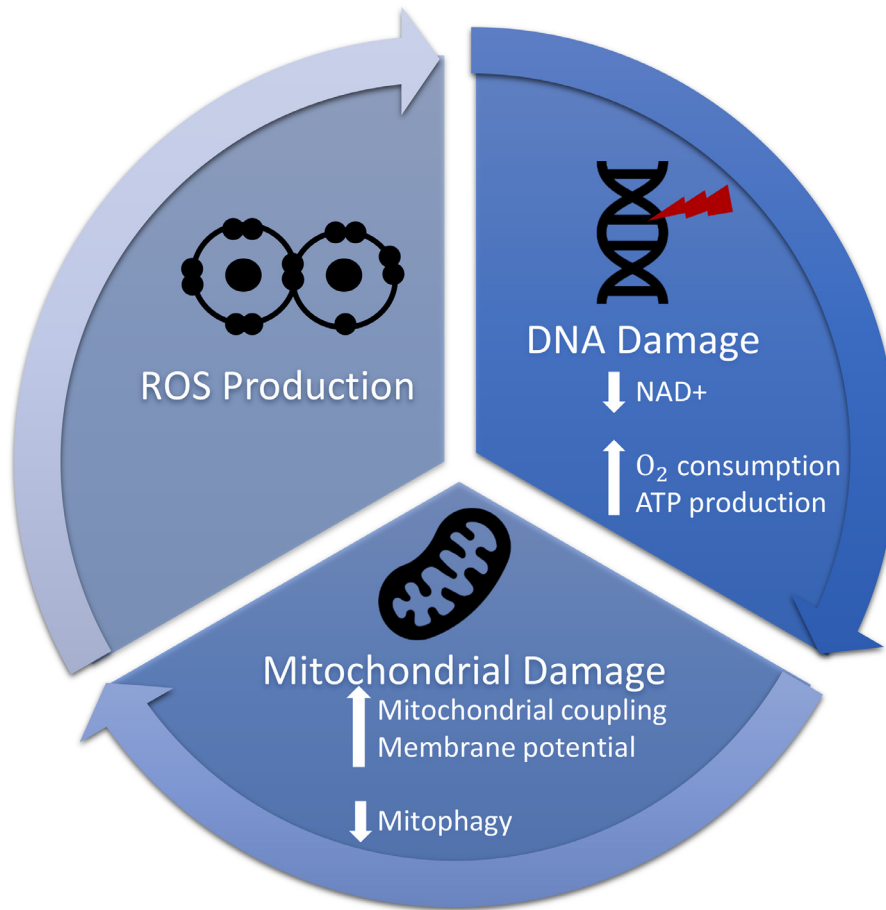


Fig. 1. Combined Theory of Aging. This theory combines the DNA damage, mitochondrial and free radical theories of aging. DNA damage activates kinases and PARP, which consume NAD⁺. The decline in NAD⁺ increases energy production needs, elevating oxygen consumption and ATP production. Compensatory mitochondrial coupling leads to increased membrane potential, a decline in mitophagy and higher levels of free radicals, which further elevate DNA damage.

ulates the insulin producing enzymes PCSK1 and PCSK2 (proprotein convertase 1 and 2). Downregulation of the insulin receptor MET causes further insulin resistance, leading to downstream mitochondrial dysfunction, synaptic dysfunction and cognitive impairments (Abolhassani et al., 2017). Mitophagy dynamics are also impaired in AD, as the mitochondrial fission protein DRP1 (dynamin-related protein 1) is S-nitrosylated and hyperactivated by high levels of amyloid beta peptide, leading to excessive mitochondrial breakdown and synaptic damage (Nakamura et al., 2010). Recent studies have shown that the damaging effects of mitochondrial dysfunction can be mitigated, attenuating the pathogenesis of AD. Overexpression of the mitophagy regulator parkin in 3xTg mice reduced amyloid beta accumulation and damaged mitochondria while restoring neurotransmitter synthesis (Khandelwal et al., 2011). In cultured fibroblasts from spontaneous AD patients, overexpression of PARK2 was able to reverse mitophagy impairments by increasing autophagic vacuoles, mitochondrial targeting and mitochondrial recycling. (Martín-Maestro et al., 2016). Thus targeting stressed and damaged mitochondria may be a key strategy for reducing oxidative stress in models of AD.

3. Types of DNA damage

A key marker of oxidative stress is DNA damage, which leads to apurinic and apyrimidinic DNA sites, oxidized purines and pyrimidines, and DNA breaks. These DNA breaks are either single strand

breaks (SSBs) or double strand breaks (DSBs), with DSBs being the more toxic of the two. SSBs are caused by the breakdown of the sugar phosphate backbone of DNA following ROS oxidation, while DSBs are longer lasting and can lead to changes in the transcription of gene promoters near break sites (Shanbhag et al., 2019; Thanan et al., 2014). DNA repair capacity is determined by levels of NAD⁺; while higher levels of NAD⁺ correlate with increased DNA repair, lower levels are indicative of ROS accumulation. NAD⁺ activates SIRT1, which decreases oxidative stress and resultant DNA damage by upregulating autophagy, mitophagy and protective histone methylation (Bosch-Presegué et al., 2011; Fang et al., 2017). NAD⁺ intermediates such as nicotinamide mononucleotide (NMN) were also found to increase SIRT1 activity, increasing NAD⁺ and MnSOD levels in turn (de Picciotto et al., 2016). While neuronal DNA is generally more vulnerable to damage than DNA in other cell types, mitochondrial DNA is particularly susceptible due to its proximity to the electron transport chain and its lack of protective histones (Mecocci et al., 2018).

3.1. Double strand breaks contribute to activity dependent gene expression

Double strand breaks are the most frequently quantified form of DNA damage due to their toxicity and the transcriptional changes they cause in nearby gene promoters (Shanbhag et al., 2019). The phosphorylation of H2AX (γ H2AX) is the first step in the DSB re-

pair process, and a widely used marker of DSB. H3K9me3, which our lab has shown leads to age-associated cognitive impairment and represses brain derived neurotrophic factor (BDNF), also assembles the DSB repair complex and reduces DNA damage (Ji et al., 2019; Snigdha et al., 2016). H3K9me3's seemingly contradictory roles have led us to hypothesize that H3K9me3 upregulation and resultant cognitive decline may be a compensatory mechanism for the increased DNA damage that occurs with age. Despite the toxicity of DSBs, they are also physiologically required for the expression of critical immediate early genes. Neuronal stimulation can also lead to DSBs (Suberbielle et al., 2013), particularly in the promoter regions of immediate early genes (IEGs) expressed by neuronal activity. DSB formation in IEG promoter regions is sufficient to induce their expression even in the absence of an external stimulus (Madabhushi et al., 2015). The DNA repair associated protein Gadd45 γ was further found to regulate expression of the IEGs Arc, cFOS, Npas4 and Cyr61 in the prefrontal cortex (Li et al., 2019). Overall, emerging evidence suggests that activity dependent DSB formation and repair is involved in neuronal early response gene expression.

3.2. DNA damage in the aged brain impairs cognition

DNA damage is elevated in both healthy aged and Alzheimer's diseased brains, and some theorize that unrepaired DNA damage is a root cause of the aging process (Maynard et al., 2015; Thanan et al., 2014). A foundational paper by Lu, et al. found that the promoters of many cortical genes are downregulated with age, beginning after age 40 and reaching peak levels after age 70. These downregulated genes are mainly involved in synaptic plasticity, vesicular transport and mitochondrial function, indicating that DNA damage directly impairs neuronal and synaptic health. Brain aging also leads to increased expression of genes that can compensate for age-associated deficits, including genes involved in protein folding (heat shock protein 70 and alpha crystallin), antioxidant defense (nonselenium glutathione peroxidase, paraoxonase and selenoprotein P) and metal ion homeostasis. Increased expression of the base-excision repair enzymes 8-oxoguanine DNA glycosylase and uracil DNA glycosylase are indicative of both neuroprotective changes in gene expression as well as increased single strand DNA damage. Lu further examined if promoters of downregulated genes have impaired DNA repair. Promoter reporter plasmids were damaged *in vitro* with H2O2 and transfected into SH-SY5Y cells. After damage with H2O2, reporters derived from promoters of age-downregulated genes had much lower activation rates than reporters from promoters of age-stable genes. This indicates that age-downregulated genes have reduced base excision repair, indicating an increase in single strand breaks. (Lu et al., 2004). Increased single strand breaks have also been observed in aged hippocampal pyramidal and granule cells as well as cerebellar granule cells, but not in cerebellar Purkinje cells (Rutten et al., 2007). While DNA damage is elevated with age in nuclear DNA, it is 10-fold higher in mitochondrial DNA from subjects 42 years and older, and 15-fold higher in subjects over 70 (Mecocci et al., 2018). An elevation in age-associated single strand breaks impairs the expression of genes involved in synaptic plasticity, learning and memory, but preferentially affects mitochondrial DNA involved in metabolism, energetics and neuron survival.

3.3. DNA repair is impaired in Alzheimer's disease

AD leads to an elevation in double as well as single stranded breaks, suggesting that dysfunctional DNA repair may be a root cause of AD (Thanan et al., 2014). As with normal aging, there are

deficits in the base excision repair pathway in AD (Lovell et al., 1999; Madabhushi et al., 2015). The DNA damage repair pathway is additionally impaired by reduced recruitment of 53BP1 to DSBs and a reduction in the levels of DSB repair proteins such as DNA PKcs (protein kinase catalytic subunits) and MRN complex proteins (Madabhushi et al., 2015). γ H2AX levels are elevated in the neurons and astrocytes of MCI and AD patients, as compared to age matched controls (Myung et al., 2008; Shanbhag et al., 2019). Similarly, the brains of hAPP mice have more neuronal DSBs than age matched controls before exploring a novel environment, and longer lasting increases in DSBs after exploration. In this study, DSBs were generated by physiological brain activity, but were further exacerbated by the amyloid beta elevation found in this transgenic AD model (Suberbielle et al., 2013). Our lab has previously found that high levels of nitrotyrosine and DNA damage precede tangle formation in the visual cortex of AD brains, suggesting that DNA damage occurs early in disease progression (Su et al., 1997). Along with high levels of oxidative stress and DNA damage, AD is also marked by deficiencies in DNA repair mechanisms. Cerebral spinal fluid from AD patients shows an increase in 8-OHdG in intact DNA, an oxidative damage marker, as well as a decrease in free 8-OHdG, a product of DNA repair (Lovell et al., 1999). Similarly, 3xTg mice crossed with mice that were haploinsufficient for PolB (a base excision repair polymerase) had more cognitive deficits, synapse loss and neuronal death, making the model more similar to human AD than the parent 3xTg model (Sykora et al., 2014). Taken together, these findings indicate that DNA repair mechanisms are impaired as oxidative stress is elevated in AD. The severity of DNA damage in the AD brain greatly surpasses that in the healthy aged brain, suggesting that a combination of DNA damage and impaired DNA repair mechanisms plays a critical role in the progression of the disease.

4. Oxidative stress generates epigenetic modifications

Recent studies have shown that oxidative stress directly affects epigenetic chromatin modifications that control how genes are expressed. These changes may drive physiological responses to oxidative stress and facilitate the progression of diseases, including neurodegeneration (Kreuz, 2016). Oxidative stress can directly reduce methylation of DNA by oxidizing DNA, increasing TET-mediated hydroxymethylation, and interfering with binding of DNA methyltransferases that produce the methyl donor S-adenosylmethionine (Chia et al., 2011; Thanan et al., 2014). ROS can also form oxidized DNA lesions by hydroxylating pyrimidines and 5-methylcytosine (5mC), which can interfere with 5-hydroxymethylcytosine (5hmC) epigenetic signals (Guillaumet-Adkins et al., 2017; Lewandowska and Bartoszek, 2011). Oxidative stress alters post translational histone modifications, which can change chromatin structure, gene expression, gene stability and replication. These effects are often indirect, as ROS impair metabolic efficiency, reducing levels of metabolites such as acetyl-CoA, Fe, NAD⁺ and ketoglutarate that are essential for histone-modifying enzymes (Guillaumet-Adkins et al., 2017). H2O2, the most common means of ROS induction *in vitro*, has been shown to increase H3K9me3, H3K4me3 and H3K27me3 while decreasing H3K9ac and H4K8ac in bronchial epithelial cells. Preincubation with ascorbate prevented this elevation, indicating that antioxidants can prevent ROS-induced epigenetic changes. The methylation effects of oxidation were transient and did not persist after H2O2 washout (Niu et al., 2015). In neuronal SH-SY5Y cells, H2O2 increased histone acetyltransferases and downregulated histone deacetylases, as well as hypomethylating APP and BACE1. H2O2 thus caused the upregulation of the APP and BACE1 gene promoters, leading to amyloid beta peptide overproduction (Gu et al.,

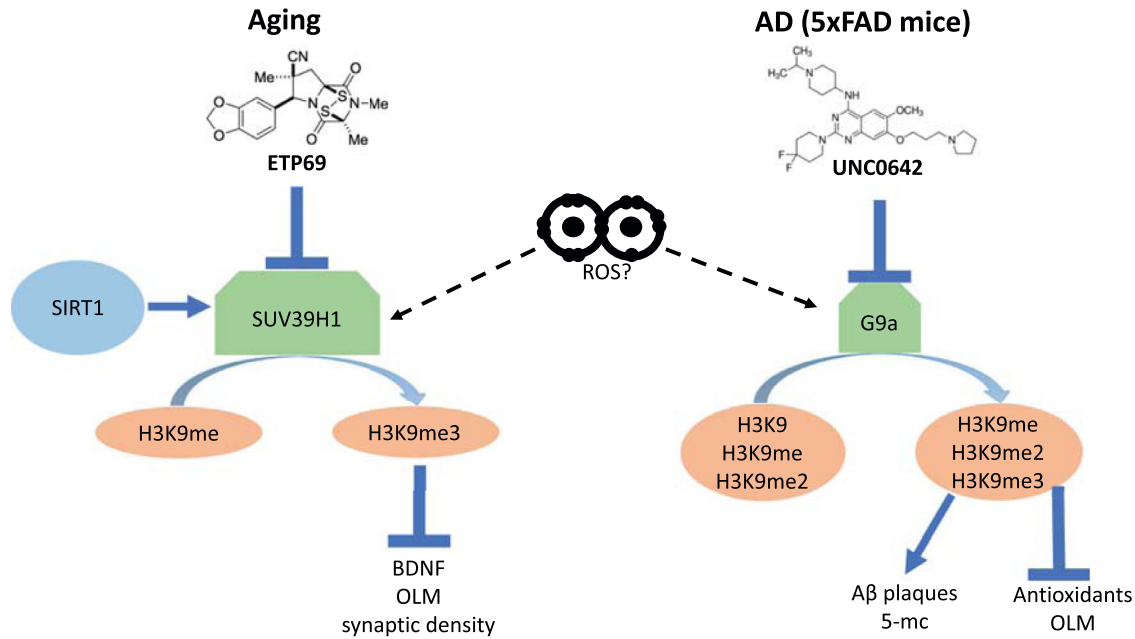


Fig. 2. Repressive Histone Methylation Leads to Cognitive Decline in Aging and AD. SIRT1 stabilizes SUV39H1, the histone methyltransferase that catalyzes H3K9me3 formation. The SUV39H1 inhibitor ETP69 reduces levels of H3K9me3, while increasing BDNF, synaptic density and the performance of aged mice in an object location memory (OLM) task. The G9a inhibitor UNC0642 has a similar effect in 5x FAD mice, reducing mono-, di- and trimethylated H3K9 along with amyloid beta plaques and 5-methylcytosine (5mc). Conversely, UNC0642 increases levels of antioxidants and improves OLM task performance. H3K9 methylation may underlie cognitive decline in both aging and AD, and the upregulation of SUV39H1 and G9a may be a compensatory response to oxidative stress.

2013). These findings suggest that oxidative stress generates transient epigenetic modifications which, among its wide-reaching effects, can accelerate amyloid beta pathology.

4.1. Oxidative stress upregulates H3K9 methylation

Our group has recently begun studying the epigenetics of aging, in which oxidative stress plays a principal role. We have shown that at least 1 specific repressive epigenetic mark, H3K9me3, increases with age in the hippocampus. We further reduced H3K9me3 by inhibiting its primary catalyzing enzyme, SUV39H1, with a specific inhibitor (ETP69) and found that this improved object location memory, increased synaptic density and elevated BDNF levels in aged mice (Snigdha et al., 2016). Work by the Vaquero group suggests that oxidative stress may be one cause of H3K9me3 elevation. They demonstrate that oxidative stress indirectly increases global H3K9me3 by upregulating SIRT1, which protects against oxidative stress by increasing mitophagy and autophagy (Fang et al., 2017). SIRT1 stabilizes the histone methyltransferase SUV39H1, and SIRT1's upregulation in turn leads to upregulation of SUV39H1 and its product H3K9me3 (Bosch-Presegué et al., 2011; Vaquero et al., 2007). This finding suggests that the increase in H3K9me3 we observed in our research may in fact be due to a protective SIRT1 upregulation caused by oxidative stress. A recent paper found that inhibition of the histone methyltransferase G9a is similarly protective in AD mice. The G9a inhibitor UNC0642 improved memory of objects and locations, reduced amyloid beta plaques, reduced 5-mc levels, and increased levels of antioxidants in 5XFAD mice (Griñán-Ferré et al., 2019). Unlike SUV39H1, G9a does not specifically catalyze 1 methylated form of H3K9 but can produce mono- di- or tri-methylated H3K9. Other studies using the UNC0642 inhibitor have led to a decline in both di- and trimethylated H3K9, so the specific source of the inhibitor's effects cannot be parsed out (Kim et al., 2017). Overall, there is emerging evidence that the methylation of H3K9 may be upregulated by oxidative stress, and that inhibiting this methylation may prevent

cognitive aging and reduce Alzheimer's pathology (Fig. 2). However, many questions remain about the different roles of methylated histones, and how oxidative stress regulates their expression.

5. Oxidative stress therapeutics for aging and Alzheimer's disease

5.1. Antioxidant treatment

Perhaps the most convincing evidence that oxidative stress is key to brain aging and AD is the plethora of therapeutics that target neuronal oxidative stress (Table 1). The most common means of reducing oxidative stress is antioxidant treatment, which in some cases has been effective in reducing age-associated neuronal impairments and markers of neurodegeneration. The combination of Vitamins C and E (ascorbic acid and α -tocopherol) has been shown to decrease lipid peroxidation in neuronal cells (Li et al., 2003). When 24 month old Wistar rats were fed N acetyl-l-cysteine (NAC), which increases glutathione levels in the brain, there was a coincident reduction in ROS and inflammation markers such as TNF alpha, IL-1 β and IL-6, as well as a protective upregulation in SIRT1 (Garg et al., 2018). 1 month of daily NAC and alpha-lipoic acid injections also improved the performance of SAMP8 (senescence accelerated prone 8) mice in learning and memory tasks (Farr et al., 2003). Our lab has similarly demonstrated that antioxidants are effective at improving performance in cognitive tasks. We found that supplementation with antioxidants and mitochondrial cofactors (Vitamins E and C, L-carnitine, alpha lipoic acid and 1% fruit and vegetable granules) improved the performance of aged beagles in a size and black and white discrimination task, but that this improvement was greater when coupled with behavioral enrichment (Milgram et al., 2005). In humans, Vitamin E consumption from food and supplements was associated with a lower rate of cognitive decline in a large-scale longitudinal study, although Vitamin C and carnitine consumption was not (Morris et al., 2002). Although the efficacy of different antioxidants varies across models, they re-

Table 1
Oxidative stress therapeutics and their effects on brain aging and Alzheimer's disease

Therapeutic	Effects on Brain Aging or AD	References	
Antioxidants 	Vitamin C and E	-Ascorbate helps cells retain Vitamin E and decreases peroxidation in SH SY5Y neuroblastoma -Vitamin E prevents A β toxicity, protein oxidation and ROS formation in primary rat hippocampal neurons -Vitamin E correlates with dementia incidence -400 IU/d vitamin E for 7–12 years had no effect on dementia incidence -2000 IU/d vitamin E for 5 years led to slower functional decline in mild to moderate AD patients -Increased dietary Vitamin E correlated with reduced cognitive decline in aged individuals, though Vitamin C and carnitine did not	Li, 2003 Yatin, 2000 Mecocci, 2018 Kryscio, 2017 Dysken, 2014 Morris, 2002
	NAC (N acetyl-L-cysteine)	-100mg/kg for 2 weeks increased TNF alpha, IL-1 β , IL-6, and SIRT1 in 24 month old Wistar rats -100 mg/kg for 4 weeks + 100mg/kg α -lipoic acid improved learning and memory in SAMP8 mice	Garg et al., 2018 Farr, 2003
	Antioxidant medley and mitochondrial cofactors	-Increased alpha-tocopherol acetate, L-carnitine, α -lipoic acid and ascorbic acid improved discrimination in aged beagles -800 IU/d vitamin E, 500 mg/d vitamin C and 900 mg/d α -lipoic acid reduced MMSE scores in acinical trial with aged individuals	Milgram et al., 2005 Galasko et al., 2012
	Mitochondria-targeted antioxidants (MitoQ and SS31) Selenium Polyphenols	25 nM prevented A β toxicity in primary neurons from Tg2576 mice and N2a neurons incubated with A β ; reduced H2O2 production in N2A neurons 200ug/d for 7–12 years had no effect on dementia incidence 8 months reduced homocysteine levels in AD patients	Manczak et al., 2010 Kryscio, 2017 Morillas-Ruiz et al., 2010
Caloric Restriction and Mimetics 	Flavonols Fisetin	Dietary flavonols significantly reduced the incidence of AD 15 mg/kg for 6 weeks upregulates SIRT1 and antioxidants while decreasing oxidants, mitochondrial membrane depolarization and apoptotic cell death in the aging rat brain	Holland et al., 2020 Singh, 2018
	Resveratrol	-Reduces oxidative stress in neuron cell cultures and mouse hippocampus -20 μ M in SH-SY5Y cells reduced ROS production, PS1, SIRT1, p53 and caspase 3, as well as levels of the amyloid beta producing APP and BACE -500–1000mg/d for 1 year reduced cognitive decline in mild to moderate AD patients, reduced MMP-9; however, treatment decreased brain volume, particularly in APOE4 carriers, and increased microglia and macrophages	Drygalski et al., 2018 Ko, 2015 Sawda, 2017
	Pinostilbene, a more bioavailable resveratrol analog Caloric restriction	0.1–10 μ M reduces oxidative stress in SH-SY5Y neuroblastoma cells -40% CR for 2 weeks reduced A β in APP mice -Intentional weight loss was associated with cognitive improvement in obese MCI patients	Chao et al., 2010 Patel et al., 2005 Horie, 2016
Long term physical activity 	15 weeks of moderate intensity treadmill running decreased ROS and increased PG1C1 alpha, p-AMPK, SOD1, SOD2 and GPx in 12 month old female Wistar rats 6 months of aerobic exercise decreased advanced oxidation protein products in the blood of elderly individuals Faster repair of radiation-induced DNA strand breaks after exhaustive exercise, protection from DNA strand breaks in lymphocytes 3 months of running wheel access decreased lipoperoxide, GSSG, GPX and GW while increasing CuZn-SOD in 12 month old female 3xTgAD mice. Exercised mice had improved spatial memory, reduced anxiety, A β and p-tau 4 weeks of treadmill running reduced oxidative stress, mitochondrial dysfunction, A β and p-tau in STZ rat model of AD 6 weeks of treadmill running alone increased H2O2 in the motor cortex, but reduced H2O2 and increased GPx in the presence of rotenone. In both rotenone and control rats, exercise reduced H2O2 in the spinal cord.	Marosi et al., 2012 Rytz et al., 2020 Moreno-Villanueva et al., 2019 García-Mesa et al., 2016 Lu et al., 2017 Melo et al., 2019	

main a powerful tool for reducing oxidative stress and improving cognition in the aged brain.

The success of antioxidants in reducing cognitive aging has spurred interest in their potential as AD therapeutics, although the results of these studies are conflicting. Vitamin E and mitochondrial targeted antioxidants such as mitoQ and ss31 reduced oxidative stress in cultured neurons and prevented amyloid beta toxicity

([Butterfield and Halliwell, 2019](#); [Manczak et al., 2010](#); [Yatin et al., 2000](#)). The association between antioxidants and AD in clinical studies is less clear. While high levels of Vitamin E in blood plasma generally correlate with lower risk of AD, the Conselice study found the prevalence of dementia was highest in those with higher concentrations of tocopherol ([Mecocci et al., 2018](#)). In another study supplementation with vitamin E, vitamin C and alpha-

lipid acid correlated with a reduction in Mini-Mental State Examination scores and did not change levels of CSF (cerebral spinal fluid) amyloid or tau (Galasko et al., 2012). The PREADVICE trial found that neither Vitamin E nor selenium prevented AD incidence when taken daily for 7–12 years (Kryscio et al., 2017), while the TEAM AD study found that daily alpha tocopherol slowed functional decline in AD patients (Dysken et al., 2014; Mecocci and Polidori, 2012). Another clinical trial with 100 AD patients found that 8 months of drinking a polyphenol beverage reduced levels of homocysteine, but had no other effects (Morillas-Ruiz et al., 2010). The mixed results of antioxidant-based clinical studies may be due to variations in dose, timing, antioxidant combinations, targets and diet. Most studies with Vitamin E, for instance, use much more than 400 IU/day, which can be toxic. Treatment with antioxidants should begin long before the onset of AD symptoms to be effective and should be consumed with Vitamin C or water-soluble electron acceptors to effectively remove ROS (Brewer, 2010; Jomova et al., 2010). Most antioxidant treatments are systemic and have poor target specificity for neuronal oxidative stressors. When diet is not also monitored, antioxidant treatment is not sufficient to counteract the effects of inflammatory and oxidant rich foods. Consuming antioxidants through food increases their absorption and physiological effectiveness, indicating that an antioxidant-rich diet can better manage oxidative stress than supplementation (Morris, 2016). A recent study showed that intake of flavonols in the form of leafy greens, fruits, beans and tea significantly reduced the incidence of Alzheimer's disease, supporting the increased therapeutic potential of diet over supplementation (Holland et al., 2020). At present, antioxidants alone are not a useful treatment for AD, but an antioxidant rich diet may more effectively mitigate ROS induced impairments.

5.2. Caloric restriction and mimetics

Caloric restriction is another means of reducing oxidative stress that has also been shown to improve neuronal health and may protect against neurodegeneration. Caloric restriction is a reliable means of increasing lifespan, decreasing ROS levels and stimulating hormesis, subtoxic levels of oxidative stressors that protect against more severe stressors (Birringer, 2011; Coskun and Busciglio, 2012). Hormetic conditions are normally present in the healthy brain and lead to increased production of antioxidants, DNA repair enzymes and antiapoptotic proteins (Fontana et al., 2010; Van Cauwenberghie et al., 2016). Sublethal superoxide has even been shown to enhance neuronal plasticity by increasing long term potentiation in the CA1 region and stimulating neuron outgrowth. Low levels of oxidative stress caused by caloric restriction also activate the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which upregulates protective enzymes such as heme oxygenase and NAD(P)H-quinone oxidoreductase 1 (NQO1) (Pall and Levine, 2015). These enzymes enable neurons to counteract more severe oxidative stressors by reducing free radicals (NQO1) and forming cytoprotective bilirubin (heme oxygenase 1) (Calabrese et al., 2010). Caloric restriction further increases levels of BDNF, which our lab and others have previously associated with ROS reduction in exercise models (Berchtold et al., 2002; Cotman and Berchtold, 2002; Neeper et al., 1995; Neeper et al., 1996; Van Cauwenberghie et al., 2016; Yang et al., 2014). However, long term caloric restriction is difficult to implement in humans, and the few available clinical studies are necessarily limited in duration and the extent of food restriction (Caristia et al., 2020). Caloric restriction mimetics (CRMs), such as resveratrol, offer similar benefits without reducing food intake (Van Cauwenberghie et al., 2016). CRMs are defined as synthetic or natural compounds that mimic the metabolic, hormonal and physiological effects of

caloric restriction. For instance, the CRM fisetin, a flavenoid that upregulates SIRT1, decreased oxidants, increased antioxidants, reduced mitochondrial membrane depolarization and reduced apoptotic cell death in the aging rat brain (Singh et al., 2018). Resveratrol has also been shown to reduce oxidative stress in both neuron cell cultures and the mouse hippocampus. One limitation of resveratrol is that it is not readily bioavailable (Drygalski et al., 2018). The resveratrol analog pinostilbene is methylated to make it more soluble, and similarly reduces ROS in neuronal models (Chao et al., 2010). These studies suggest that caloric restriction and CRMs can effectively reduce neuronal oxidative stress.

Although the effects of caloric restriction on age-related cognitive decline have not been thoroughly investigated, there is some evidence that caloric restriction can reduce neurodegeneration in AD. In vitro, 20 μ M of resveratrol in SH-SY5Y cells reduced ROS production, PS1, SIRT1, p53 and caspase 3, as well as levels of the amyloid beta producing APP and BACE (Ko et al., 2015). Dietary restriction also reduced amyloid beta deposition in APP transgenic mice (Patel et al., 2005). Caloric restriction has also been used to treat AD in several clinical trials. In a more recent trial, intentional weight loss in obese elderly MCI patients was associated with cognitive improvement. This association was strongest in younger seniors for memory and fluency, and in APOE4 carriers for executive function (Horie et al., 2016). In a study of the CRM resveratrol, 500–1000mg a day for 52 weeks was administered to patients with mild to moderate AD. Treatment reduced cognitive decline according to the Alzheimer's Disease Cooperative Study-Activities of Daily Living scale (ADCS-ADL), but led to a greater decline in brain volume, particularly in APOE4 carriers. Treatment further reduced neuroinflammation by reducing MMP-9, which regulates the permeability of the CNS, but elevated other inflammatory markers such as microglia and macrophages. While the results are contradictory, the increased inflammation may have been a beneficial adaptive immune response, and the decline in brain volume was not related to cognition (Sawda et al., 2017). In both studies, changes in oxidative stressors and their downstream effectors are not quantified, making the role of the oxidative pathway in the observed cognitive improvements unclear (Van Cauwenberghie et al., 2016). Further clinical studies involving caloric restriction should measure how markers of oxidative stress change in AD patients to explain potentially conflicting changes in inflammation, neurodegeneration and cognition.

5.3. Physical activity

Physical activity is a proven means of improving learning and memory by increasing levels of BDNF (Berchtold et al., 2002; Cotman and Berchtold, 2002; Neeper et al., 1995; Neeper et al., 1996;). Similar to caloric restriction, exercise promotes hormesis and Nrf2 induction, as it protects against oxidative damage through a BDNF mediated pathway while increasing free radicals (Pall and Levine, 2015). Exercise induced BDNF activates CREB, which upregulates APE1, involved in the base excision repair pathway. Through increased BDNF, physical activity protects neurons from oxidative DNA damage by enhancing DNA repair (Yang et al., 2014). At the same time, contracting muscles produce free radicals to promote the exercise training response, including activating PGC1 alpha, involved in exercise induced BDNF elevation. Endurance exercise also increases the amount of antioxidant enzymes in cardiac and skeletal muscles (Powers et al., 2016). Physical activity thus signals the brain to compensate for exercise induced ROS by producing additional neuroprotective factors.

The length of an exercise program is the determining factor in its efficacy as an oxidative stress therapeutic. While short term exercise tends to increase ROS, long term exercise increases an-

tioxidant enzymes, reducing overall oxidative stress (Belviranh and Gökbel, 2006). When 12-month-old female Wistar rats were exercised for 15 weeks on a treadmill at moderate intensity, hippocampal levels of ROS decreased while levels of PGC1 alpha, phospho-adenosine monophosphate protein kinase (p-AMPK), SOD1, SOD2 and glutathione peroxidase (GPX) increased (Marosi et al., 2012). In a clinical study, long term exercise decreased advanced oxidation protein products (AOPP) in the blood of older adults who engaged in aerobic exercise 3 times a week for 6 months (Rytz et al., 2020). Exercise has even been shown to accelerate DNA repair. A recent study of sedentary and active male volunteers found that while all participants had radiation induced DNA damage, trained individuals had more rapid repair of radiation-induced DNA strand breaks after exhaustive exercise. In addition, exhaustive exercise only produced DNA strand breaks in the lymphocytes of sedentary individuals (Moreno-Villanueva et al., 2019).

Long term exercise was also shown to reduce oxidative stress in models of AD. When 12-month-old 3xTgAD female mice had access to a running wheel for 3 months, levels of lipoperoxide, glutathione disulfide (GSSG), GPX and glutathione reductase (GR) were reduced to control levels while the antioxidant CuZn-SOD was increased. A network analysis showed that this reduction in oxidative stress was central to other behavioral and pathological changes, such as improved spatial memory, reduced amyloid beta and p-tau, and reduced anxiety (García-Mesa et al., 2016). Similar findings were reported in a streptozotocin (STZ) induced rat model of AD that were exercised on a treadmill for 4 weeks. The rats displayed a reduction in oxidative stress, mitochondrial dysfunction, amyloid beta and p-tau (Lu et al., 2017). These studies both demonstrate that the reduction in neurodegeneration observed with exercise is at least partially due to a reduction in oxidative stress. A recent paper suggests that long term exercise does not intrinsically reduce oxidative stress, but rather makes the brain more resilient to other stressors. 8 to 9-month-old Lewis rats were treated with rotenone, a pesticide that inhibits complex 1 of the mitochondrial respiratory chain to mimic sporadic neurodegeneration. By itself, 6 weeks of exercise increased hydrogen peroxide in the motor cortex of these rats. However, in the presence of rotenone, exercise reduced hydrogen peroxide and increased the activity of the antioxidant GPX. Additionally, prior exercise training reduced hydrogen peroxide levels in the spinal cords of both DMSO and rotenone exposed rats (Melo et al., 2019). In the presence of age, toxins, or neurodegeneration, long term exercise reduces oxidative stress and helps restore homeostatic conditions in the damaged brain.

6. Conclusion

Oxidative stress plays a key role in the progression of brain aging and Alzheimer's disease. Mitochondria produce free radicals as a byproduct of respiration and are dysfunctional in both aging and AD. The mitochondrial cascade hypothesis posits that damaged mitochondria increase the production of amyloid beta, which is itself a toxic oxidative stressor. Mitochondrial antioxidants in aged mice reduce oxidative stress and DNA damage, while restoring mitophagy in AD models can minimize pathology. Free radicals lead to DNA damage, the majority of which occurs in unprotected, histone free mitochondrial DNA. The severe elevation in double strand breaks in the AD brain far exceeds the damage in the aged brain, and dysfunctional DNA repair further contributes to disease progression. Oxidative stress can even manipulate the epigenome and has wide reaching effects ranging from repression of cognitive genes by H3K9me3 to hypomethylation and activation of the APP promoter. While oxidative stress may not be the only cause of mental decline or neurodegeneration, it clearly contributes to the dysfunctional pathways associated with brain aging.

Emerging therapeutics targeting oxidative stress vary in their ability to improve cognition and reduce ROS levels. Antioxidant treatments have mixed effects on cognition in both aged and AD patients, and dosage, timing, antioxidant combinations and diet must be carefully considered to produce consistent benefits. Caloric restriction mimetics such as resveratrol have been shown to reduce neuronal oxidative stress in rodent and neuronal models, and even reduced cognitive decline in studies of MCI and AD patients; however, the clinical effects of mimetics on brain aging have not been extensively studied. Long term caloric restriction is challenging for most people and its effects on cognitive aging have not been well investigated. Clinical trials are necessarily limited in duration and extent of food restriction, and studies using CRMs have mixed results. Compared to caloric restriction, long term exercise is a more practical means of inducing BDNF and lifelong hormetic conditions.

It is promising that clinical studies on long term exercise are becoming more prevalent; however, there remains a need for rigorous, large scale exercise intervention trials for both normal cognitive aging and Alzheimer's disease. Most clinical trials have relatively small and homogenous sample populations and examine the effects of exercise over a limited time. Studies should aim to increase the size, diversity, and length of their exercise studies, as in studies of lifelong exercisers compared to sedentary older adults. It is also critical that clinical trials measure not only markers of cognition, but also markers of oxidative stress to further clarify their role in exercise-induced cognitive improvements. While much work remains, long term exercise has thus far been proven to reduce oxidative stress, restore antioxidant activity, reduce pathological markers, and increase the brain's resilience to toxins. Of the currently available therapies, long term physical activity shows the greatest potential to reduce oxidative stress while promoting the homeostatic levels of stressors needed for optimal brain function.

Disclosure statement

The authors have no conflicts of interest to disclose.

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