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REVIEW PAPER

Reductive Reprogramming: A Not-So-Radical Hypothesis of Neurodegeneration Linking Redox Perturbations to Neuroinflammation and Excitotoxicity

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Abstract

Free radical-mediated oxidative stress, neuroinflammation, and excitotoxicity have long been considered insults relevant to the progression of Alzheimer's disease and other aging-related neurodegenerative disorders (NDD). Among these phenomena, the significance of oxidative stress and, more generally, redox perturbations, for NDD remain ill-defined and unsubstantiated. Here, I argue that (i) free radical-mediated oxidations of biomolecules can be dissociated from the progression of NDD, (ii) oxidative stress fails as a descriptor of cellular redox states under conditions relevant to disease, and (iii) aberrant upregulation of compensatory reducing activities in neural cells, resulting in reductive shifts in thiol-based redox potentials, may be an overlooked and paradoxical contributor to disease progression. In particular, I summarize evidence which supports the view that reductive shifts in the extracellular space can occur in response to oxidant and inflammatory signals and that these have the potential to reduce putative regulatory disulfide bonds in exofacial domains of the *N*-methyl-D-aspartate receptor, leading potentially to aberrant increases in neuronal excitability and, if sustained, excitotoxicity. The novel reductive reprogramming hypothesis of neurodegeneration presented here provides an alternative view of redox perturbations in NDD and links these to both neuroinflammation and excitotoxicity.

Keywords Excitotoxicity · Neurodegenerative disease · Neuroinflammation · Oxidative stress · Protein thiols · Redox signaling · Reductive stress

Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
BACE1	β-Secretase 1
DTT	Dithiothreitol
Grx	Glutaredoxin
GSH	Reduced glutathione
GSSG	Oxidized glutathione
IL-β	Interleukin 1-β
LPS	Lipopolysaccharide
NDD	Neurodegenerative disease
NMDA	*N*-methyl-D-aspartate
PD	Parkinson's disease
Prx	Peroxiredoxin
RNOS	Reactive nitrogen oxide species
ROS	Reactive oxygen species
SOD-1	Superoxide dismutase-1
TNFalpha	Tumor necrosis factor-α
Trx	Thioredoxin
TrxR	Thioredoxin reductase
Txnip	Thioredoxin-interacting protein

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Introduction

The biochemical pathways which detour healthy brain aging and set the course for Alzheimer's disease (AD) and other aging-related neurodegenerative disorders (NDD), such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), remain enigmatic. Shared lifestyle and environmental risk factors for NDD (Chin-Chan et al. 2015; Vyas et al. 2016; Cruz-Haces et al. 2017; McKenzie et al. 2017), together with overlapping molecular and clinical hallmarks of these conditions (Armstrong et al. 2005), support the likelihood that common neuropathological phenomena such as oxidative stress (Sonnen et al. 2008), neuroinflammation

(Stephenson et al. 2018), and excitotoxicity (Lewerenz and Maher 2015) may contribute to the development of diverse NDD. Among these, the pathways by which oxidative stress and, more generally, redox imbalances may contribute to NDD are the least well understood.

The brain consumes an inordinate amount of oxygen by mass and has long been considered vulnerable to oxidative stress (Cobley et al. 2018), a term originally defined as an imbalance in the prooxidant and antioxidant status of cells in favor of the former (Sies 2015). Oxidative stress may occur in tissues from increased levels of activated oxygen metabolites known collectively as reactive oxygen (ROS) and reactive nitrogen oxide (RNOS) species. Traditionally, oxidative challenges have generally been considered to promote functional impairment of cells and tissues by damaging proteins and other biomolecules via one-electron (i.e., free radical) pathways (Sonnen et al. 2008). Indeed, free radical-mediated oxidative "damage" has been the focus of prominent and longstanding theories of Alzheimer's disease (AD) (Volicer and Crino 1990; Smith and Perry 1995; Markesbery 1997) as well as other aging-related neurodegenerative disorders (NDD) such as Parkinson's disease (PD) (Olanow 1990) and amyotrophic lateral sclerosis (ALS) (Bergeron 1995). After three decades, these theories remain a major stimulus of research although they continue to be generally vaguely defined, focused largely on nonspecific actions of oxidants, and unsubstantiated. Alternative, more precisely defined, redox hypotheses of NDD are warranted.

Here, I make three arguments, all backed by substantial published work and in keeping with advancements in redox biology, which should facilitate debate and discussion about the roles of redox perturbations in NDD. First, free radical-mediated oxidations of biomolecules can be dissociated from the progression of NDD. Second, oxidative stress fails as a descriptor of complex cellular redox chemistry under conditions relevant to disease. Third, aberrant upregulation of compensatory reducing activities in neural cells, in response to oxidant and/or inflammatory signals and resulting in reductive shifts in intracellular and/or extracellular compartments, may be an unrecognized and somewhat paradoxical contributor to disease progression. The novel *reductive reprogramming hypothesis* of neurodegeneration presented here provides an alternative view of redox perturbations in NDD and links these to both neuroinflammation and excitotoxicity.

Evidence for Oxidative Stress in the Brain in NDD

ROS and RNOS can be distinguished, broadly, as (i) nonradicals (e.g., hydrogen peroxide, peroxynitrite, nitrosonium ion), (ii) selectively reactive free radicals (i.e., superoxide, nitric oxide), and (iii) highly reactive free radicals (e.g., hydroxyl radical, nitrogen dioxide) (Jones 2008). Nonradical oxidants tend to be electrophilic and to oxidize thiols on proteins and low molecular weight substances (e.g., glutathione) by reversible, two-electron, pathways. In contrast, reactive free radicals can indiscriminately oxidize proteins, lipids, and nucleic acids by irreversible, one-electron, routes.

Much of the support for oxidative stress theories of NDD has come from postmortem studies showing that disease-relevant tissues (i.e., brain regions but also the spinal cord in ALS) from individuals with AD (Subbarao et al. 1990; Mecocci et al. 1994; Palmer and Burns 1994; Lovell et al. 1995; Sayre et al. 1997; Smith et al. 1997b; Gabbita et al. 1998; Marcus et al. 1998; Markesbery and Lovell 1998; Pratico et al. 1998; Nourooz-Zadeh et al. 1999), PD (Dexter et al. 1989; Alam et al. 1997), and ALS (Ferrante et al. 1997; Bowling et al. 1993) contained higher levels of free radical-initiated oxidations of lipids, proteins, and nucleic acids compared to tissues from age-matched controls. Indeed, early oxidative stress theories of NDD were free radical damage-centered and essentially represented extensions of the free radical theory of aging put forth by Harman in 1956 (Harman 1956). Simply stated, the basic premise of these theories is that the accrual of oxidative damage should eventually result in impairment of tissue functions and give way to disease. Free radical generation in the brain in NDD may be catalyzed, in part, by redox-active transition metals, which tend to accumulate at neuronal lesions associated with long-lived protein aggregates (Hirsch et al. 1991; Smith et al. 1997a). Of particular interest in research related to PD, such transition metals can also promote oxidations of dopamine to electrophilic quinones potentially capable of covalently modifying proteins and producing neurotoxicity in vitro and in vivo (Hastings et al. 1996; Monzani et al. 2018).

More limited reports that levels of reduced (GSH) glutathione, or ratios of GSSH to oxidized (GSSG) glutathione, were lower in relevant brain regions in AD (Mandal et al. 2015), PD (Sian et al. 1994), or ALS (Weiduschat et al. 2014), combined with findings that activities of some antioxidant enzymes can be upregulated (Martins et al. 1986; Lovell et al. 1995, 2000; Palmer 1999; Russell et al. 1999; Aksenov and Markesbery 2001; Dunn et al. 2014), suggesting compensatory responses to oxidative stress, have provided broader support for the relevance of oxidative challenges to NDD.

Free Radical Oxidations Can Be Dissociated from the Progression of NDD

Oxidative damage as a cause of NDD remains unproven. Indeed, the notion that oxidative damage is a primary cause of disease progression is challenged by the inconsistencies,

uncertainties, and contradictions summarized below and highlighted in Table 1.

The fundamental premise and widely accepted view that more oxidations of biomolecules exist in nervous tissues from individuals with NDD than in tissues from control subjects have not always held. Thus, results of a number of studies show, at least for AD, that neurodegeneration can proceed in the *absence* of differences, from controls, in free radical oxidations (Jeandel et al. 1989; Te Koppele et al. 1996; McIntosh et al. 1997; Smith et al. 1991; Lyras et al. 1997) in relevant brain regions. Moreover, the results of one pivotal study demonstrated that oxidative stress, determined by levels of the RNA-derived 8-hydroxyguanosine and of nitrotyrosine, *decreased*, rather than increased, with the progression of AD (Nunomura et al. 2001). On a related point, it is important to emphasize that increased levels of oxidized lipids (Noda et al. 1982), proteins (Smith et al. 1991), and nucleic acids (Mecocci et al. 1993), and increased oxidations of glutathione (Rebrin et al. 2011), have all been reported in the brains of humans and model organisms during apparent normal aging, in other words, in the absence of disease. Thus, while aging is considered the major risk factor for NDD, the biochemical pathways that derail healthy brain aging and set the course for NDD remain obscure.

Even if free radical oxidations in NDD brains were consistently different from controls, either quantitatively or qualitatively, it is difficult to envision how such differences would produce neural functional impairment as the extents of free radical-oxidized biomolecules are often exceedingly small when expressed as a fraction of the total population of molecules (Sohal and Orr 2012). For example, in one study, levels of the oxidized nucleotide 8-hydroxy-2′-deoxyguanosine in both AD and control brains were estimated to be 2 molecules per 10^5 molecules of 2′-deoxyguanosines, thus representing about 0.02% of the total population of 2′-deoxyguanosine (Te Koppele et al. 1996). In addition, the probabilities of oxidations of amino acid side chains in proteins damaging the few active-site amino acid residues most critical for protein functions should be quite low if these oxidations occur relatively indiscriminately as can be expected for nonenzymatic reactions involving highly reactive free radicals. Indeed, the results of one study demonstrated that the mitochondrial respiratory activity of the brain cortex of Fischer 344 rats was unaffected with age despite increases in levels of free radical-mediated modifications of mitochondrial proteins, giving rise to protein carbonyls, nitrotyrosines, and 4-hydroxynonenal adducts (Gilmer et al. 2010).

Perhaps most detrimental to oxidative damage-centered theories of NDD have been findings that antioxidants, including the free radical-scavenging antioxidants vitamin E (tocopherols) and vitamin C (ascorbate), generally do not lower the incidence or slow the progression of AD (Mecocci and Polidori 2012; Persson et al. 2014), PD (Filograna et al.

Table 1 Findings which (i) argue against the importance of oxidative damage as a driver of NDD and (ii) support the relevance of redox reprogramming

Observation	References
Dissociation of NDD from free radical oxidations in the brain	
No differences in oxidations observed between NDD and controls	Jeandel et al. (1989) Te Koppele et al. (1996) McIntosh et al. (1997) Smith et al. (1991) Lyras et al. (1997)
Oxidations decreased with progression of AD	Nunomura et al. (2001)
Oxidations increased with aging in the absence of overt disease	Noda et al. (1982) Smith et al. (1991) Mecocci et al. (1993) Rebrin et al. (2011)
Free radical-scavenging antioxidants provide little or no protection	Mecocci and Polidori (2012) Persson et al. (2014) Filograna et al. (2016) Hughes et al. (2016)
Tocopherol/ascorbate/lipoic acid lowered free radical oxidations but accelerated cognitive decline	Galasko et al. (2012)
Evidence for redox reprogramming in NDD	
Increased G6PDH in AD	Martins et al. (1986) Palmer (1999) Russell et al. (1999)
Increased G6PDH in PD	Dunn et al. (2014)
Increased TrxR in AD	Lovell et al. (2000)
Increased GSH/GSSG in AD	Adams et al. (1991)
Increased neuronal thiols in AD	Russell et al. (1999)

2016; Hughes et al. 2016), or ALS (Orrell et al. 2008). Particularly undermining to the oxidative stress theory of AD are the results of one clinical trial showing that a combination of tocopherol, ascorbate and lipoic acid effectively lowered central nervous system oxidative damage, as evidenced by lower levels the lipid peroxidation product isoprostane F2 in the cerebrospinal fluid, but *accelerated* cognitive decline (Galasko et al. 2012).

Protein Thiol-Based Redox Homeostasis and Signaling: Alternatives to Free Radical Damage

Reversible, two-electron, oxidations of catalytic and putative regulatory cysteine thiols on potentially numerous proteins by nitric oxide (Gould et al. 2013) and by hydrogen peroxide (Garcia-Santamarina et al. 2014), the most stable products of which are disulfide bonds, have emerged as the basis for "redox signaling". While the full scope of redox signaling in tissues, in vivo, remains to be established, protein thiol oxidation-based paradigms offer alternatives to free radical-centered perspectives with which to consider the impacts of nitro-oxidative stresses on cellular and tissue health (Jones 2008; Sohal and Orr 2012).

Nitric oxide metabolism gives way to nitrosonium ion (NO^+) donors such as N_2O_3 which can nitrosylate protein thiols, a process termed S-nitrosylation (Gould et al. 2013). S-nitrosylation results in a formal oxidation of protein thiols and is considered to be the major pathway by which nitric oxide signaling occurs. While some protein nitrosothiols may be long-lived, many convert readily to disulfide bonds (Wolhuter et al. 2018). Hydrogen peroxide can effect cellular changes by promoting formation of disulfide bonds by direct or peroxidase-catalyzed oxidations of protein thiols, the relative importance of which remains to be established (Stocker et al. 2018). Operationally, disulfide bonds involving proteins can be distinguished as (i) protein disulfides, bridged by vicinal (defined here as closely spaced) thiols within the same or associated polypeptide chains, and (ii) mixed disulfides linking proteins and low-molecular-weight thiols such as glutathione (i.e., protein S-glutathionylation).

Reduction of reversibly oxidized forms of protein sulfur is achieved by the enzymatic transfer of reducing equivalents from NADPH and GSH. Protein disulfides (Arner and Holmgren 2000) and protein nitrosothiols (Benhar et al. 2009) are reduced largely by thioredoxins (Trx), the reduced forms of which are regenerated by NADPH-dependent thioredoxin reductases (TrxR). Multiple isoforms of Trx and TrxR are expressed in cells in a compartment-specific fashion (Lu and Holmgren 2014). S-glutathionylated proteins are reduced mainly by GSH-dependent glutaredoxin (Grx) (Shelton et al. 2005). The TrxR/Trx system also functions in concert with methionine sulfoxide reductases to reduce sulfur oxidized to sulfoxides on protein methionine residues (Lu and Holmgren 2014). The significance of protein methionine oxidations for cell signaling and disease is not known.

We have provided evidence that dithiol-disulfide transitions may regulate the activities of brain proteins including triosephosphate isomerase (Foley et al. 2010), protein phosphatases (Foley et al. 2004, 2007, 2011; Foley and Kintner2005), SNAP-25 (Foley et al. 2012), and glyceraldehyde-3-phsophate dehydrogenase (Foley et al. 2016). Moreover, we have demonstrated that a fraction of total proteins from brain contain vicinal thiol pairs that can transition between dithiol and disulfide forms in response to apparent physiological perturbations of tissue redox in vivo (Foley et al. 2014, 2016). The importance of protein vicinal thiols as redox-sensitive motifs is supported by our findings (Foley et al. 2014) and earlier studies by others (Beer et al. 2004; Hansen et al. 2009; Adimora et al. 2010) demonstrating that oxidative stress in tissues and cells may result more readily in the oxidative crosslinking of protein vicinal thols than in formation of mixed disulfides between proteins and glutathione.

In principle, deregulation of protein thiol redox, resulting in aberrant oxidations or excessive reductions, may impair protein stability, redox signaling, and neurotransmission. Indeed, excessive formation of protein disulfides (Cumming and Schubert 2005) and protein nitrosothiols (Nakamura et al. 2015) have been hypothesized to contribute to neurodegeneration although studies of nitro-oxidative thiol modifications occurring in NDD and control brains, in vivo, are very limited. The possibility that over-activation of compensatory reductive pathways, leading to increased Trx-mediated reductions of oxidized protein sulfur, may produce adverse changes in neuronal function is detailed below.

Oxidative Stress Fails as a Descriptor of Cellular Redox States Under Disease Conditions

The complexities of thiol redox chemistry are certain to defy attempts to describe the impacts of metabolic and disease-associated perturbations globally using one-size-fits-all descriptors such as "oxidative stress". Thus, cells maintain multiple thiol-based redox couples which, although kinetically linked, are held at distinct, compartment-specific, redox potentials that can be affected differently in response to cellular insults (Banerjee 2012; Go and Jones 2008; Kemp et al. 2008). For example, although crosstalk between the Trx and glutathione systems occurs (Casagrande et al. 2002; Du et al. 2013), and these systems can serve as back-ups to each other (Tan et al. 2010; Du et al. 2012; Peskin et al. 2016), ratios of reduced to oxidized Trx or glutathione can be modified preferentially during cellular differentiation

(Nkabyo et al. 2002) and following exposures of cells to epidermal growth factor (Halvey et al. 2005) and metal ions (Hansen et al. 2006).

Neural cells, especially astrocytes, display a high capacity to adapt to elevated ROS/RNOS by upregulating the activities of reductive (i.e., antioxidant) systems by both post-translational and transcriptional routes (Garcia-Nogales et al. 2003; Gavillet et al. 2008; Baxter and Hardingham 2016). The net effects of ROS/RNOS on any cellular redox couple will depend on both the rates of oxidation of the reduced form of this couple and the rates of reduction of the oxidized form. Critically, the latter will be a function of the robustness of the upregulated reductive responses to ROS/RNOS. If these are sufficiently large or sustained, they may, in principle, give rise to excessive decreases in oxidations of a particular redox couple, resulting in a reductive shift in the corresponding redox potential. Thus, it would not be outside the bounds of redox biology for one or more redox couples to be unaffected, or to even become more reduced, while others become more oxidized in response to a metabolic or disease-associated stressor. Indeed, exposures of astrocytes to the AD-associated β-amyloid peptide were found to promote oxidation of the intracellular GSH/GSSG couple while, simultaneously, triggering a reduction of the extracellular cysteine/cysteine couple (Garg et al. 2011).

That protein thiol reductive stresses can occur under pathological conditions, also associated with free radical-mediated oxidations of biomolecules, is supported by in vitro findings that exposures of cells to (i) high glucose (Wadham et al. 2007), (ii) the inflammatory mediator tumor necrosis factor-α (TNFα) (Hoffman et al. 2001), (iii) oxidized low-density lipoprotein (Hoffman et al. 2001), and (iv) cellular expression of superoxide dismutase-1 (SOD-1) containing a familial ALS-linked mutation (Schonhoff et al. 2006) can trigger widespread denitrosylation of protein thiols. We have recently reported that prolonged stress, achieved by restraint in a rat model, and also associated with increased neural release of TNFα (Madrigal et al. 2002), can lead to widespread denitrosylation in the brain compared to milder stress (Foley et al. 2019). In related preliminary experiments, we have found that prolonged restraint stress decreased also the level of protein disulfides (Foley et al., unpublished observation). Together, these observations are consistent with a possible stress-induced upregulation of the protein sulfur-reducing activities of Trx.

Compensatory Reductive Reprogramming and Relevance to NDD

Neural cells respond to elevated oxidants (Garcia-Nogales et al. 2003; Baxter and Hardingham 2016) and to pro-inflammatory signals, such as the cytokines TNFα and interleukin-1β (IL-1β) (Gavillet et al. 2008) and lipopolysaccharide (LPS) (Garcia-Nogales et al. 1999), by upregulating the pentose phosphate pathway (PPP), considered the major source of NADPH. NADPH is necessary for the regeneration of GSH from GSSG and for the regeneration of reduced Trx. In addition, neural cells can also respond to oxidant stress by increasing the expression of γ-glutamylcysteine synthetase, which catalyzes the rate-limiting step in GSH biosynthesis (Baxter et al. 2015; Baxter and Hardingham 2016). Moreover, astrocytes increase both the production and the release, into the extracellular space, of GSH in response to both oxidants (Baxter and Hardingham 2016) and the pro-inflammatory cytokines (Iwata-Ichikawa et al. 1999; Gavillet et al. 2008; Stelle et al. 2013; He et al. 2015; Chowdhury et al. 2018). Increased astrocytic release of GSH following combined exposure to TNFα and interleukin-1β was associated with a marked TNFα-induced increase in ROS and RNOS suggesting an adaptive response to potential oxidant stress (Gavillet et al. 2008). Following enzymatic hydrolysis of extracellular GSH, the resulting free cysteine can be taken up by neurons to further support GSH biosynthesis (Dringen 2000; Banerjee 2012; Baxter and Hardingham 2016). This reductive reprogramming, resulting in increased availability of reducing equivalents, is likely necessary, at least in part, to remove excess hydrogen peroxide and to maintain the sulfur atoms of cysteine and methionine residues in the largely reduced states necessary to support the native structures and functions of neuronal proteins (Fig. 1).

As outlined below and in Table 1, the results of limited studies suggest that reductive pathways may be activated in NDD brains. Specifically, glucose-6-phosphate dehydrogenase, the enzyme that catalyzes the rate-limiting step of the PPP, is upregulated in AD (Martins et al. 1986; Palmer 1999; Russell et al. 1999) and PD (Dunn et al. 2014) brains compared to age-matched controls. The presumed increase in PPP-derived NADPH production is expected to increase the rates of regeneration of GSH and reduced Trx. In addition, the activity of TrxR is higher in AD brains (Lovell et al. 2000). Further evidence in support of a reductive reprogramming in AD, specifically, comes from findings of one study that the ratios of GSH/GSSG in multiple brain regions were higher in AD brains compared to controls (Adams et al. 1991). This is the only study of which I am aware that determined the ratios of GSH to GSSG in AD brains. In keeping with the results of this study, another report noted that levels of total reduced thiols were greater in pyramidal neurons from AD brain when compared to those from age-matched controls (Russell et al. 1999). Still another found that, while protein thiols were modestly lower in hippocampus from AD brain, total thiol content was no different from controls (Aksenov and Markesbery 2001). The basis for lower protein thiol content was not examined; it could result from oxidation, decreased expression of thiol-rich proteins, or occlusion of thiols following protein

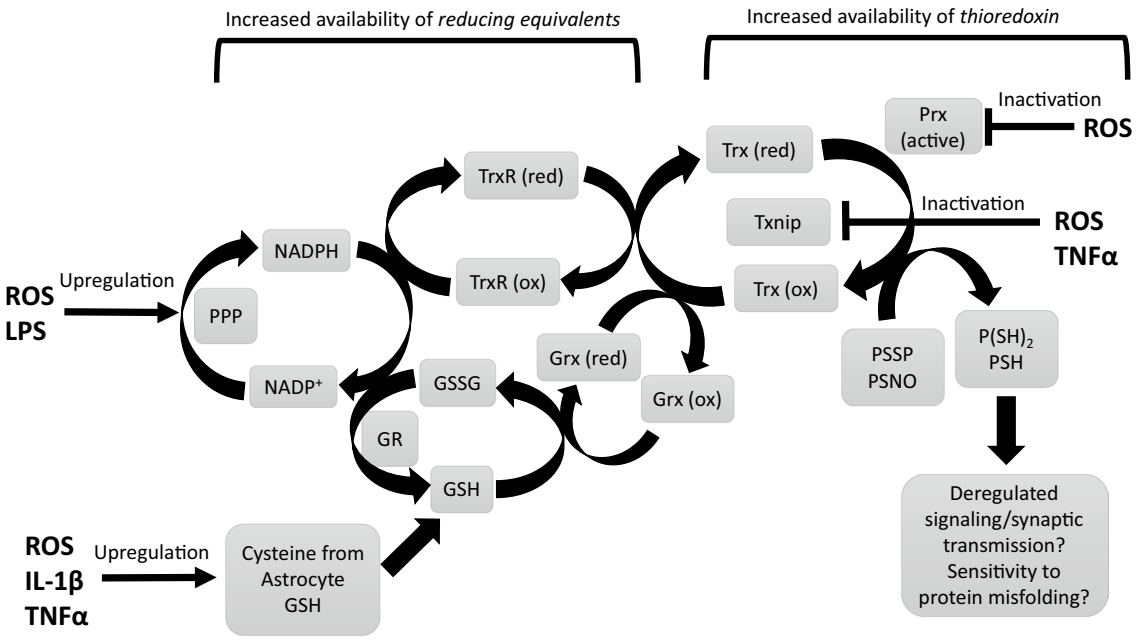

Fig. 1 Pathways facilitating reductive reprogramming under conditions relevant to NDD. ROS and pro-inflammatory signals (LPS, IL-1β, TNFα) can promote increases in the availability of reducing equivalents by upregulating the activity of the PPP and/or the synthesis of GSH (Garcia-Nogales et al. 1999, 2003; Gavillet et al. 2008; Baxter and Hardingham 2016). In addition, high levels of ROS can make available more Trx for reductions of reversibly oxidized protein sulfur, including disulfides (PSSP) and nitrosothiols (PSNO) by over-oxidizing the catalytic thiols of two-cysteine Prxs (Yang et al. 2002), which compete with oxidized sulfur atoms on other proteins for reducing equivalents from Trx. Trx may also be activated by dissociation from Txnip following oxidation by ROS (Hwang et al. 2014) and/or TNFα-induced degradation (Kelleher et al. 2014). The increased availability of both reducing equivalents and Trx would allow the reduction and, possibly, over-reduction of the small fraction of intracellular proteins forming structural or regulatory disulfides as well as nitrosothiols. Higher levels of GSH and cysteine in the extracellular space following by ROS, IL-1β, and/or TNFα-triggered upregulation of the biosynthesis and export of GSH by astrocytes (Iwata-Ichikawa et al. 1999; Gavillet et al. 2008; Stelle et al. 2013; He et al. 2015; Baxter and Hardingham 2016; Chowdhury et al. 2018) can promote a reductive shift in the extracellular space (see Fig. 2). Reductive protein thiol stress may occur at the expense of GSH as GSH consumption may be elevated by both increased pressures on GSH peroxidases, in the face of inactivated Prxs, and by the GSH/Grx-mediated backup of TrxR (Du et al. 2012; Peskin et al. 2016)

aggregation. As a whole, these findings argue that upregulation of thiol-reducing capacities in NDD brains may be sufficient to offset any increases in thiol oxidants that may also occur and, under some circumstances, may result in greater reductions of oxidized thiols than is evident in the controls.

In addition to the availability of reducing equivalents in the form of NADPH, the availability of Trx for reductions of oxidized protein sulfur is likely to be an important factor but one that has not been widely considered. The major oxidized thiol-containing substrates of the reducing activity of Trx are the highly expressed two-cysteine subtypes of peroxiredoxin (Prx) peroxidases (Lu and Holmgren 2014), the most abundant of which in neurons is Prx-2 (Hattori and Oikawa 2007). The active-sites of two-cysteine Prxs contain an unusually reactive peroxidatic cysteine and a resolving cysteine which cycle between dithiol and disulfide forms (Wood et al. 2003). The high activities of these Trx-dependent enzymes under conditions of increased hydrogen peroxide would be expected to limit the availability of Trx for reductions of oxidized sulfur atoms on other proteins (Day et al. 2012). In addition, Trx is subject to inhibition in cells by a disulfide-linked association with Trx-interacting protein (Txnip) (Hwang et al. 2014). Thus, access of oxidized sulfur on non-Prx proteins to reducing equivalents from Trx may be limited by competition by abundant oxidized Prxs and by sequestration of Trx by Txnip.

Total levels of Trx have been reported to be either lower (Lovell et al. 2000) or unchanged (Cumming et al. 2007; Gil-Bea et al. 2012) in AD brains compared to controls. Differences in the availability of Trx for reductions of oxidized protein sulfur in the brain during the progression of NDD are unknown but may be inferred. Thus, over-oxidation of the catalytic thiols of two-cysteine Prxs to sulfinic/sulfonic acids inactivates these peroxidases (Yang et al. 2002) and has been suggested to be necessary, in part, to make available Trx for protein sulfur reductions (Day et al. 2012). In this light, it may be important that Prx-2 displayed a more acidic isoelectric point, consistent with over-oxidation of the catalytic thiols, in AD brains compared to controls (Cumming et al. 2007).

The Trx-inhibitory activity or expression level of Txnip can be suppressed by multiple mechanisms under conditions relevant to NDD, potentially activating the protein sulfur-reducing activities of Trx. Specifically, hydrogen peroxide can promote the dissociation of Trx from Txnip possibly by cleaving, via further oxidation, the disulfide bond linking the two proteins (Hwang et al. 2014). In addition, the inflammatory cytokine TNFalpha can activate Trx by stimulating the ubiquitination and proteosomal degradation of Txnip (Kelleher et al. 2014). Furthermore, increases in neuronal activity, which may occur in early and presymptomatic stages of NDD (Stargadt et al. 2015; Verma et al. 2018), both enhance ROS production (Hongpaisan et al. 2004; Brennan et al. 2009; Baxter et al. 2015) and lower the expression of Txnip (Papadia et al. 2008).

Relevance of Reductive Reprogramming to Neurodegenerative Pathology

Upregulation of reducing activities in the brain in NDD has generally been viewed as wholly compensatory and neuroprotective and has been cited as further support for oxidative stress theories of NDD. However, such changes may not only maintain reduced protein sulfur but, if excessive or prolonged, have the potential to adversely affect redox-sensitive signaling, synaptic activity, and proteostasis (Fig. 1). Thus, while most protein thiols in reducing intracellular compartments, such as the cytosol and the mitochondrial matrix, are considered to be largely reduced, exceptions are emerging. Proteins containing oxidized but reducible forms of thiols in these spaces may confer sensitivity of cells to reductive stresses. For example, reduction of intramolecular disulfide bonds, or preventing disulfide bond formation, can promote the misfolding of SOD-1 (Khan et al. 2017) and tau (Walker et al. 2012) implicated in ALS and AD, respectively. In addition, activation of inflammation-linked nuclear factor kappaB signaling is facilitated by reduction of basal levels of S-nitrosylation of this transcription factor and of multiple of its regulators (Marshall et al. 2004). Notably, constitutive S-nitrosylation of β-secretase-1 (BACE1), the enzyme which catalyzes the β-cleavage of amyloid precursor protein to AD-associated β-amyloid peptides, was found to be markedly decreased in AD brains (Kwak et al. 2011). This denitrosylation was suggested by the authors to contribute to the activation of BACE1 in AD.

The Extracellular Space May Be Especially Sensitive to Reductive Reprogramming

Redox states of thiols on proteins in the extracellular space are buffered primarily by the cysteine/cystine couple and, to a lesser extent, by the GSH/GSSG couple operating in this space (Go and Jones 2008; Banerjee 2012). These redox couples are highly sensitive to perturbations in intracellular redox states and metabolism, which can influence, via available transport systems, the extracellular levels of cystine, cysteine, and GSH (Banerjee 2012). In the brain, these extracellular redox buffers are also affected by levels of glutamate, the major excitatory neurotransmitter, and are controlled largely by astrocytes (Banerjee 2012). Thus, astrocytes release GSH into the synaptic space, where it is hydrolyzed to release cysteine, in response to oxidative stress (Baxter and Hardingham 2016) and pro-inflammatory cytokines (Gavillet et al. 2008; Stelle et al. 2013; Chowdhury et al. 2018) (Fig. 2). In addition, engagement of astrocytes by infiltrating T cells, expected to occur during brain injury and neuroinflammation, triggers an extracellular reductive shift stemming from stimulation, by T cell-derived glutamate, of the release of GSH and cysteine by astrocytes (Garg et al. 2008). Moreover, exposure of astrocytes to the AD-associated β-amyloid peptide promoted a modest intracellular oxidative shift in the GSH/GSSG couple while generating a larger extracellular reductive shift in the cysteine/cystine couple (Garg et al. 2011). These studies establish that the extracellular space can undergo reductive shifts, even under conditions promoting oxidations of intracellular compartments, in response to NDD-associated insults.

Disulfide bonds are much more prevalent in extracellular proteins, including exofacial domains of integral membrane proteins, than they are in intracellular proteins (Fass 2012). These protein disulfides are potential sites of reduction upon development of more reducing redox potentials in the extracellular space; reduction of these may couple reductive shifts to changes in cellular metabolism and viability. Importantly, 4- and 8- fold reductions of oxidized thiols on the surface of dendritic cells and T cells, respectively, occur in co-cultures of these immune cells designed to model T cell activation following GSH and cysteine-mediated reductive shifts in the extracellular space (Yan et al. 2009; Banerjee 2012). These findings demonstrate that marked reductions of exofacial protein disulfides can, indeed, occur following physiologically-relevant reductive shifts in the extracellular space. Moreover, the extracellular reductive shift of − 30 mV induced by exposure of astrocytes to Aβ is predicted to promote as much as a 10-fold shift in the ratios of disulfides to dithiols in exofacial proteins (Garg et al. 2011).

The identities of the neural proteins containing exofacial and GSH and/or cysteine-sensitive disulfide bonds, the functional significance of reductions of these disulfides, and the pathways that may confer speed and specificity on such reductions remain to be established.

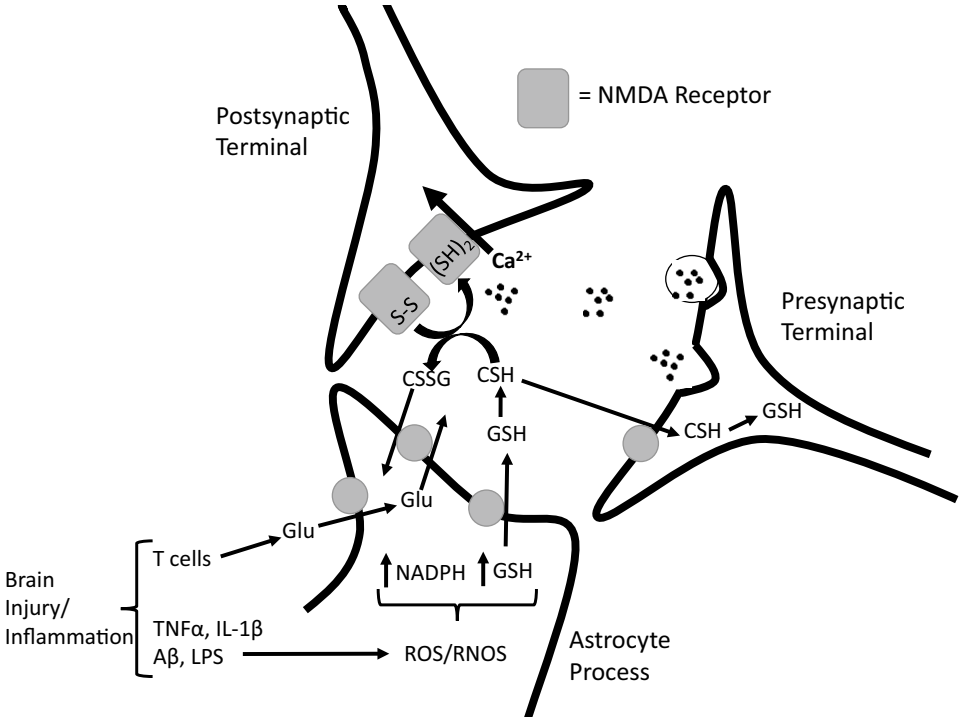

Fig. 2 Reductive shifts in the extracellular space triggered by disease-related insults. Diverse brain injury and inflammation-related factors including oxidants (Iwata-Ichikawa et al. 1999; Baxter and Hardingham 2016), pro-inflammatory cytokines (TNFα and IL-β) (Gavillet et al. 2008; Stelle et al. 2013; He et al. 2015; Chowdhury et al. 2018), infiltrating T cells (Garg et al. 2008), AD-associated Aβ (Garg et al. 2011), and lipopolysaccharide (LPS) (Garcia-Nogales et al. 1999) can trigger upregulation of the PPP and GSH biosynthesis and release, by astrocytes, of GSH. Extracellular GSH provides cysteine (CSH) as a precursor for the biosynthesis of GSH by neurons (Dringen 2000) and can promote reductive shifts at least in the extracellular CSH/CSSC redox potentials (Garg et al. 2008, 2011; Banerjee 2012) which are predicted to promote reductions of exofacial disulfide bonds on synaptic membrane proteins. Infiltrating T cells may drive a reductive shift in the CSH/CSSG couple, at least in part, by releasing glutamate (Glu), the uptake of which by astrocytes stimulates removal of extracellular cystine (CSSG) via the X_C^- transporter (Garg et al. 2008). Reduction of an exofacial and putative regulatory disulfide bond(s) on the NMDA receptor is linked to potentiation of NMDA receptor activity in vitro (Aizenman et al. 1989; Janaky et al. 1993; Levy et al. 1990; Mathisen et al. 1996; Regan and Guo 1999; Reynolds et al. 1990; Sucher et al. 1990; Sullivan et al. 1994; Tang and Aizenman 1993a, b), which can lead to excitotoxic neuron death

The NMDA Receptor is an Example of a Synaptic Membrane Protein Containing Exofacial and Redox-Sensitive Disulfide Bonds with Immediate Relevance to NDD

Aberrant excitatory synaptic transmission, mediated by overactivation of Ca^{2+}-permeable, glutamate-gated, ion channels, has long been suspected to contribute to the progression of multiple NDD (Lewerenz and Maher 2015). While controlled increases in neuronal activity can be neuroprotective, prolonged activation, particularly involving Ca^{2+} permeability through the *N*-methyl-D-aspartate (NMDA) subtype of glutamate-gated ion channels, can lead to synaptic impairment and, in the extreme, to excitotoxic neuronal death (Lewerenz and Maher 2015). Furthermore, hyperexcitability is emerging as a common feature of early and presymptomatic stages of NDD and as a possible contributor to the neurodegenerative process (Stargadt et al. 2015; Verma et al. 2018).

Reducing agents able to break disulfide bonds, including dithiothreitol (DTT), 2-mercaptoethanol, cysteine, and GSH, are well established to potentiate activation of the NMDA receptor by glutamate or NMDA and can produce excitotoxic neuron death, in vitro, by increasing NMDA receptor-gated Ca^{2+} conductance (Aizenman et al. 1989; Janaky et al. 1993; Levy et al. 1990; Mathisen et al. 1996; Regan and Guo 1999; Reynolds et al. 1990; Sucher et al. 1990; Sullivan et al. 1994; Tang and Aizenman 1993a, b). Potentiation of NMDA receptor activity by reducing agents has been attributed to reduction of at least one disulfide bond in the exofacial N-terminal domain of NMDA receptor subunits (Sullivan et al. 1994). Notably, other ion channels have also been found to be sensitive to reducing agents (Ruppersberg et al. 1991; Evans and Bielefeldt 2000; Nelson et al. 2007) although the structural basis for redox modulation of these proteins is less clear. These findings raise the possibility that aberrant reductive shifts in the extracellular synaptic space, following oxidant (Baxter and Hardingham

2016) and inflammation (Stelle et al. 2013; Chowdhury et al. 2018)-stimulated export of GSH from astrocytes, might contribute to the development of hyperexcitability and excitotoxicity in NDD (Fig. 2). Notably, high levels of extracellular cysteine can also activate the NMDA receptor by direct, redox-independent, binding (Olney et al. 1990).

Protection by Thiol Oxidants Supports a Role for Aberrant Reductive Reprogramming in Neurodegeneration

The role of reductive shifts in protein thiol redox states in neurodegeneration, proposed here, remains to be investigated. Nevertheless, findings that compounds able to oxidize protein thiols can be neuroprotective are consistent with this hypothesis. The most prominent examples of such substances are ebselen and lipoic acid (Fig. 3). It is important to point out that these redox-active compounds are often touted as antioxidants although they are, in fact, administered in their oxidized forms. Antioxidant activities of ebselen and lipoic acid appear to require reduction by reductase enzymes at the expense of cellular reducing equivalents (Packer and Cadenas 2011; Ren et al. 2018) which, by definition, is an oxidative stress. More to the point, as described below, the neuroprotective effects of these substances may involve, at least in part, the introduction of disulfide bonds in proteins.

Ebselen is a seleno-organic compound that contains an oxidized and thiol-reactive selenium atom in a Se-N bond (Ren et al. 2018). It can oxidize thiols on proteins and low-molecular-weight substances either stoichiometrically or catalytically, the latter owing to a peroxidase-like activity

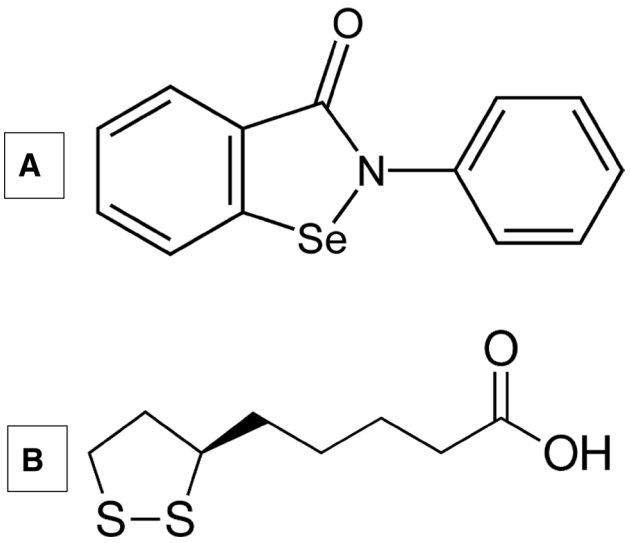

Fig. 3 The structures of ebselen (*A*) and lipoic acid (*B*)

of the compound. Thus, ebselen has been shown to rapidly oxidize the catalytic vicinal thiols of Trx (Zhao et al. 2002). In addition, this substance was recently reported to promote formation of the native disulfide bond in unfolded SOD1 (Capper et al. 2018), an pro-oxidant activity which might be beneficial in some forms of ALS. With regards to neuronal hyperexcitability and excitotoxicity, ebselen was demonstrated to reverse the potentiation of NMDA-induced currents by DTT in Chinese hamster ovary cells expressing NMDA receptor subunits (Herin et al. 2001) and it protected against NMDA- and glutamate-triggered excitotoxic neuronal death, in vitro (Herin et al. 2001; Porciuncula et al. 2001), presumably by oxidizing the redox-sensitive thiols of the receptor (Herin et al. 2001). In contrast to ebselen, the antioxidants Trolox (a water-soluble analog of vitamin E), GSH-methylester, and *N*-acetylcysteine provided no protection. At the organismal level, ebselen was reported to inhibit neuropathology and clinically relevant symptoms of neurodegeneration in transgenic AD mice (Xie et al. 2017) and in a primate model of PD (Moussaoui et al. 2000).

Lipoic acid is an oxidized cofactor of mitochondrial dehydrogenases (Packer and Cadenas 2011). It contains vicinal sulfur atoms linked by a disulfide bond and can induce disulfide bond generation in proteins, including SOD1, by thiol-disulfide exchange (Donelly et al. 2018). Like ebselen, lipoic acid reversed the potentiation of NMDA-evoked responses produced by DTT also apparently by oxidizing the redox modulatory thiols of the NMDA receptor (Tang and Aizenman 1993a). In contrast, dihydrolipoic acid, the reduced and therefore the supposed antioxidant form of lipoic acid, potentiated NMDA responses similar to DTT. Remarkably, small-scale administration of lipoic acid to humans with AD has appeared to greatly slow or halt disease progression (Hager et al. 2001, 2007; Fava et al. 2013). The results of another study also cited above, however, found that a combination of lipoic acid with tocopherol and ascorbate, the latter able to reduce disulfides on low molecular weight substances (Giustarini et al. 2008), hastened cognitive decline in AD patients compared to age-matched controls (Galasko et al. 2012). It is tempting to speculate that a reduction, by ascorbate, of lipoic acid to dihydrolipoic acid in the latter study may have contributed to these disparate results.

Summary

Despite thirty years of research, free radical-centered oxidative stress theories of NDD remain vague and unproven and the roles of redox perturbations in NDD continue to be uncertain. Backed by the existing literature and advancing tenets of redox biology, I argue here that (i)

free radical-mediated oxidative stress can be dissociated from the progression of NDD, (ii) oxidative stress can be a very misleading descriptor of cellular redox states under disease conditions, and (iii) reductive reprogramming, triggered by oxidant and/or inflammatory signals, may be a previously unrecognized contributor to NDD. In particular, astrocytic export of GSH to the extracellular synaptic space may promote reduction of regulatory disulfide bonds on the NMDA receptor, resulting in enhancement of glutamate-gated Ca^{2+} influx which, if sustained, may produce excitotoxicity. The possible involvement of excessive reductions of oxidized protein thiols in neurodegenerative pathways is supported by neuroprotective actions of thiol oxidants.

Compliance with Ethical Standards

Conflict of interest The author declares that he has no conflicts of interest.

Ethical Approval This article is a review and, as such, does not contain any newly reported studies with human participants or animals performed by the author.

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