Review article

13 reasons why the brain is susceptible to oxidative stress

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**ABSTRACT**

The human brain consumes 20% of the total basal oxygen (O<sub>2</sub>) budget to support ATP intensive neuronal activity. Without sufficient O<sub>2</sub> to support ATP demands, neuronal activity fails, such that, even transient ischemia is neurodegenerative. While the essentiality of O<sub>2</sub> to brain function is clear, how oxidative stress causes neurodegeneration is ambiguous. Ambiguity exists because many of the reasons why the brain is susceptible to oxidative stress remain obscure. Many are erroneously understood as the deleterious result of adventitious O<sub>2</sub> derived free radicals and non-radicals species generation. To understand how many reasons underpin oxidative stress, one must first re-cast free radical and non-radical species in a positive light because their deliberate generation enables the brain to achieve critical functions (e.g. synaptic plasticity) through redox signalling (i.e. positive functionality). Using free radicals and non-radical derivatives to signal sensitises the brain to oxidative stress when redox signalling goes awry (i.e. negative functionality). To advance mechanistic understanding, we rationalise 13 reasons why the brain is susceptible to oxidative stress. Key reasons include inter alia unsaturated lipid enrichment, mitochondria, calcium, glutamate, modest antioxidant defence, redox active transition metals and neurotransmitter auto-oxidation. We review RNA oxidation as an underappreciated cause of oxidative stress. The complex interplay between each reason dictates neuronal susceptibility to oxidative stress in a dynamic context and neural identity dependent manner. Our discourse sets the stage for investigators to interrogate the biochemical basis of oxidative stress in the brain in health and disease.

1. The brain and oxygen: locked in a lethal dance to the death

Despite weighing a mere ~1400 g the human brain voraciously consumes ~20% of the total basal oxygen (O<sub>2</sub>) budget to power its ~86 billion neurons and their unfathomably complex connectome spanning trillions of synapses [1–3]—abetted by ~250–300 billion glia [4,5]. The brain must “breathe” to think—even transient ischemia heralds mass neurodegeneration [6]. Depriving the brain of O<sub>2</sub> for just 30 min in ischemic stroke exacts a devastating toll: every minute ~1.9 million neurons and ~14 million synapses perish [6]. Neurons and their synapses perish because without sufficient O<sub>2</sub>, mitochondria are unable to reduce O<sub>2</sub> to H<sub>2</sub>O to support ATP synthesis [7]. Yet, perversely, at least *prima facia*, the brain carefully regulates O<sub>2</sub> use. For the simple biochemical reason that ground state molecular O<sub>2</sub> is a di-radical and, therefore, a potentially toxic mutagenic gas. Fortuitously, the potential oxidising power of O<sub>2</sub> is constrained by a chemical quirk: because the two lone electrons spin in parallel O<sub>2</sub> can only accept one electron at a time [8,9].

If spin restriction limits its reactivity, why is O<sub>2</sub> considered toxic? The answer lies in its ability to give rise to free radical and non-radicals, notably superoxide anion (O<sub>2</sub>−), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl (·OH) (their biochemistry is reviewed in [8,10,11]). Such species are usually considered to constitute the “dark side” of O<sub>2</sub> biochemistry—the unavoidable cost of using O<sub>2</sub> to respire [12]. It has long been assumed that their adventitious and unwanted generation sensitises the brain to “oxidative stress”. Indeed, oxidative stress is intimately tied to neurodegeneration [13,14]. However, the simple dichotomy that O<sub>2</sub> is good and its reactive progeny (e.g. O<sub>2</sub>−) are bad, fails to explain why and how the brain is susceptible to oxidative stress because it is incorrect. To understand why and how the brain is susceptible to oxidative stress, one must abandon the dogma that O<sub>2</sub> derived free radicals and non-radicals are just deleterious metabolic by-products and consider their nuances. For example, nestled within the brains sensitivity to hypoxia, resides an extraordinary molecular detail: mitochondrial O<sub>2</sub>− signals beneficial adaptive responses [7]. Far from being an exception, such redox signalling is pervasive [15,16]. Oxidative stress can arise when redox signalling goes awry (i.e. the “Janus” face of redox signalling). Redox nuances mean the brains susceptibility to oxidative...
stress is seldom rationalised, which hinders attempts to disambiguate the complex relationship between oxidative stress and neurodegeneration. To achieve mechanistic understanding, we biochemically rationalise 13 reasons why the brain is susceptible to oxidative stress. To do so, we draw on the seminal work of Barry Halliwell and John Gutteridge [17–19].

1.1. Redox signalling: reactive species play useful biological roles

A singular and indeed often overlooked reason why the brain is susceptible to oxidative stress is because reactive species play useful biological roles [19,20]. Two exemplars serve to illustrate the point. First, Chang’s group [21] have shown that NADPH oxidase 2 (NOX2) derived O2•− and H2O2 regulate adult hippocampal progenitor cell growth via PI3K/Akt signalling. Their findings reveal a beneficial, homeostatic role for NOX2 derived O2•−/H2O2 in the maintenance of essential neural progenitors [21]. The expression of NOX2, a dedicated O2•−-producing enzyme [22,23], alone hints at an essential role for redox signalling. A related corollary is that NOX isoforms regulate hippocampal long term potentiation (LTP)—important for learning and memory [24]. Deleting NOX2 causes cognitive impairment in mice [25]. Second, Vriz’s group, have identified beneficial roles for NOX derived H2O2 in axonal pathfinding and regeneration [26,27]. Axonal pathfinding wires the developing brain [28], in part, via secreted chemotactant and chemo-repellent cues that ensure correct target innervation. Pharmacologically inhibiting NOX2 mediated O2•−/H2O2 generation retards retinal ganglion cell axon outgrowth in vivo in larval zebrafish, placing H2O2 as an endogenous chemotactant [26].

1.2. Calcium

Action potentials cause dramatic calcium (Ca2+) fluxes in presynaptic terminals, raising [Ca2+]i by ~four orders of magnitude (from 0.01 to ~100 µM [29]). Ca2+ transients trigger neurotransmitter vesicle exocytosis [29]. Consequently, activity dependent Ca2+ transients control bidirectional synaptic plasticity [30]. Bidirectional synaptic plasticity is fundamental to brain function—being required for learning and memory to give just one prominent example [31–33]. The brains reliance on Ca2+ signalling [34] can cause oxidative stress: the nature of which is variable and context dependent owing to the complex relationship between Ca2+ and the intracellular redox environment [19]. The interested reader is referred elsewhere for a comprehensive review of Ca2+/ redox interplay [35], our discourse is confined to three points. First, Ca2+ transients stimulate neuronal nitric oxide synthase (nNOS) mediated nitric oxide (NO) synthesis [36], provided sufficient O2 and NADPH are available for NO synthesis [37]. Residually elevated intracellular [Ca2+]i may, therefore, increase NO−, which can inhibit mitochondrial respiration by binding to cytochrome c oxidase (COX) [38]. NO− reacts at a diffusion controlled rate with O2•− to yield peroxynitrite (ONOO−) [39]. ONOO− can lead to carbon dioxide (CO2) and hydrogen dioxide (H2O2) radical generation secondary to reaction with carbon dioxide (CO2) to yield peroxonitroxocarbonic acid [40]. CO2− and NO− may contribute to neurodegeneration—for example, by nitrating heat shock protein 90 to induce apoptosis in amyotrophic lateral sclerosis (ALS) [41]. A related corollary: Ca2+ can increase phospholipase A2 activity [34]. Phospholipase A2 isosforms de-esterify membrane phospholipids—which can promote enzymatic (i.e. via LOX [42]) and non-enzymatic peroxidation of bi-allylic unsaturated lipids [43].

Second, intracellular Ca2+ release—important for synaptic plasti-

city [44]—is redox regulated [45,46]. For example, Hajnoczy’s group [47] show that mitochondrial H2O2 nanodomains regulate Ca2+ transients. Ca2+ transients induce endoplasmic reticulum (ER) mitochondria contacts, termed ER associated mitochondria membranes (MAM [48,49]), leading to mitochondrial Ca2+ uptake. Mitochondrial Ca2+ uptake amplifies ER Ca2+ release by inducing potassium uptake to thereby increase matrix volume and compress the MIS to concentrate matrix H2O2 at the MAM [47]. These authors suggest H2O2 induces ER Ca2+ release via the IP3 receptor, consistent with its redox regulation via cysteine oxidation [50]. Because the MAM regulates a host of mitochondrial functions (e.g. transport and biogenesis [48]) one can easily envisage how dysregulated inter-organelle communication can cause aberrant local Ca2+/H2O2 signalling associated oxidative stress [45]. To be sure, dysregulated MAM signalling is linked to neurodegeneration in AD and ALS [51]. For example, Stoica et al. [52] show that mutant TD43—a pathological trigger in ALS and frontotemporal dementia [53]—reduces MAM contacts and thereby disrupts Ca2+ homeostasis. (Figs. 1–6)

A third related point of interplay: mitochondrial Ca2+ overload opens the mitochondrial permeability transition pore (mPTP) [54], mPTP opening induces O2•−/H2O2 efflux and abolishes ATP synthesis [55–57]. Transient mPTP opening enables mitochondria to re-set matrix Ca2+ [54,58], and is, perhaps, permissive for redox signalling by enabling O2•−/H2O2 to exit mitochondria to evade matrix metabolism [59] (a phenomenon that may be linked to mitochondrial contractions [60,61]). Prolonged mPTP opening heralds necroptosis [62]. In addition, Ca2+ overload can regulate intrinsic apoptosis. Importantly, necroptosis and apoptosis are linked to neurodegeneration [63,64]. Because mitochondrial Ca2+ uptake supports ATP synthesis [65–67], decreased mitochondrial (Ca2+) may cause oxidative stress by...
increasing [NADH] and concomitant O$_2^-$ generation at the complex I [68,69]. Cytochrome c could also use H$_2$O$_2$ to oxidise cardioliopin, an essential inner membrane phospholipid, to trigger intrinsic apoptosis [70,71]. Unsurprisingly the brain expends considerable ATP to maintain intracellular Ca$^{2+}$ homeostasis and (2) neurodegenerative diseases are usually associated with disrupted Ca$^{2+}$ homeostasis.

### 1.3. Glutamate

Excessive glutamate uptake (e.g. by N-methyl-D-aspartate receptors (NMDARs)) causes excitotoxicity [72,73] secondary to aberrant Ca$^{2+}$ signalling—for example, leading to sustained calpain signalling [74]. Glutamate excitotoxicity leads to Ca$^{2+}$ overload linked mitochondrial [O$_2^-$/H$_2$O$_2$] release associated cell death, typically via apoptosis and necrosis [17,75,76]. Ca$^{2+}$ influx can activate nNOS: opening up the possibility that NO$^-$/H$_2$O$_2$ release associated mitochondrial O$_2^-$ generation is neurodegenerative whereas NOX2 linked synaptic NMDA receptor linked O$_2^-$ generation is protective [83]. Beyond receptors, glutamate can cause excitotoxicity by inhibiting the system Xc$^-$ transporter [84]—which exchanges intracellular glutamate for extracellular cystine [85]. Intracellular cystine is reduced to cysteine, which can be used by glutamate cysteine ligase for de novo glutathione (GSH) synthesis [86]. Inhibiting cystine uptake causes oxidative stress by depleting intracellular [GSH] protective [83]. Instead of propounding the somewhat prosaic view that O$_2^-$/H$_2$O$_2$ is obligate, toxic by-products of mitochondrial respiration that cause oxidative damage, we interpret neuronal susceptibility to mitochondrial oxidative stress from a signalling perspective [111]. How mitochondria produce O$_2^-$/H$_2$O$_2$ (see Murphy [68] for a comprehensive review) places them as sentinels of organelle health [112]. Their mitochondrial biogenesis [111,122,123]. Dysfunctional mitochondria can be neurodegenerative. Failing to terminate mitochondrial O$_2^-$ generation by clonal expanding their number because O$_2^-$ sensitises neurons to MG toxicity: 250 µM MG is sufficient to saturate neuronal Mg metabolism whereas astrocytic metabolism remains intact at 2 mM [96]. With the caveat that “free” [MG] is typically 2–4 µM [100]. Notwithstanding, Mg is reactive—50,000 fold more so than glucose—and readily forms Schiff bases to glycate proteins, RNA and DNA [100]. In particular, protein glycation underlies the formation of advanced glycation products (AGE), which can cause oxidative stress by stimulating inflammation via their receptor, impairing protein and mitochondrial function [100–102]. AGEs can arise in absence of high glycolytic rates because lipid peroxidation can yield MG [101]. In sum, the brain is susceptible to glucose induced oxidative stress [97].

### 1.5. Mitochondria

Disproportionate O$_2^-$ uptake supports oxidative phosphorylation to help fuel the brains extraordinary ATP demand [3]. Neurons spend ATP to maintain ion gradients and support synaptic activity [103,104]. The sheer energetic costs of synaptic activity are exemplified by neurotransmitter loaded vesicle release alone consuming 1.64 × 10$^5$ ATP per second per vesicle [104,105]. Meeting neuronal ATP demands requires mitochondria, particularly synaptic mitochondria [106] owing to limited ATP diffusion. Neurons are especially reliant on mitochondria because they constitutively degrade PFK to limit glycolysis [95]—although glycosomes can temporarily support synaptic ATP synthesis [107]. Beyond oxidative phosphorylation, mitochondria are essential signalling hubs regulating a veritable plethora of essential processes, from Ca$^{2+}$ homeostasis, Fe-S cluster synthesis to cell fate [55,108,109]. Neuronal mitochondria are a quintessential double-edged sword: endowing neurons with ATP and signalosomes while imparting intrinsic neurodegenerative vulnerability to their dysfunction [110].

Instead of propounding the somewhat prosaic view that O$_2^-$/H$_2$O$_2$ are obligate, toxic by-products of mitochondrial respiration that cause oxidative damage, we interpret neuronal susceptibility to mitochondrial oxidative stress from a signalling perspective [111]. How mitochondria produce O$_2^-$/H$_2$O$_2$ (see Murphy [68] for a comprehensive review) places them as sentinels of organelle health [112]. Their delibereate generation is intimately tied to adaptive redox signalling [113]. Hypoxia signalling is a cogent example. Mitochondria sense hypoxia (i.e. 0.3–3% O$_2$) by generating complex I and complex III derived O$_2^-$/H$_2$O$_2$ to activate hypoxia inducible factor one alpha (HIF1-α) via degrading propyl hydroxylase. HIF1-α initiates adaptive transcriptional responses [114–118]. Because mitochondrial O$_2^-$/H$_2$O$_2$ production at a given site reflects: [O$_2$] (reduced site) and the kinetics of the reaction [68], hypoxia reduces complex I and III to trigger O$_2^-$ generation (which may be abetted by reduced COX activity to increase local O$_2$ availability). HIF1-α transcribes NDUFAL2, an alternate complex I subunit, to suppress O$_2^-$ generation to conclude hypoxia signalling [119]. Aberrant redox signalling can be neurodegenerative. Failing to terminate mitochondrial O$_2^-$ generation could initiate redox regulated intrinsic apoptosis [120,121]. In addition, misassembled respiratory chains owing to mi-to-nuclear mismatch could induce the signal (i.e. O$_2^-$/H$_2$O$_2$) without the cue (i.e. hypoxia), leading to mal-adaptive responses [111]. If mutant mitochondria accumulate, they may cause dysfunction by clonal expanding their number because O$_2^-$/H$_2$O$_2$ regulate mitochondrial biogenesis [111,122,123].
1.6. Endogenous neurotransmitter metabolism generates hydrogen peroxide

Endogenous amine based neurotransmitter (e.g. dopamine) metabolism generates mitochondrial H$_2$O$_2$ via monoamine oxidase enzymes. Monoamine oxidase A (MOA-A) and B (MOA-B) catalyse a deamination reaction: amine + O$_2$ + H$_2$O $\rightarrow$ aldehyde + H$_2$O$_2$ + NH$_3$. While both enzymes metabolise dopamine, tyramine, tryptamine and noradrenaline, MOA-A preferentially metabolises 5-hydroxytryptamine whereas MOA-B prefers 2-phenylethylamine [124,125]. During the catalytic cycle, amine oxidation to imine reduces a prosthetic flavin moiety, which reacts with O$_2$ to yield H$_2$O$_2$ [126,127]. Once the flavin is reduced, the rate of O$_2$ binding controls H$_2$O$_2$ generation, with the implication that [O$_2$] influences enzyme activity. The affinity of each isoform for O$_2$ is 10 and 240 µM for MOA-A and MOA-B, respectively [127]. Under O$_2$ saturated conditions, their capacity to produce H$_2$O$_2$ is considerable—Cadenas and colleagues [128] showed that tryamine deamination increases H$_2$O$_2$ levels by approximately 1 nmol/kg$^{-1}$/min$^{-1}$ in brain mitochondria. Axiomatically, the presence of a H$_2$O$_2$ generating enzyme together with neuronal activity induced substrate flux can cause oxidative stress [17,18].

![Diagram](image)

**Fig. 2. Mitochondrial redox signalling.** A) In normoxia, mitochondrial O$_2^-$/H$_2$O$_2$ release is depicted as being low, based on the assumption that mitochondria are generating ATP. Electron flux through CI and CIII is depicted with minimal O$_2^-_i$ generation. PD uses O$_2$ to hydroxylate HIF1-α before pVHL degrades HIF1-α. B) In hypoxia, reduced CI and CIII generate O$_2^-$/H$_2$O$_2$. O$_2^-$/H$_2$O$_2$ inactivate PD, possible by liberating active site Fe$^{3+}$. PD inhibition enables HIF1-α to enter the nucleus to transcribe hypoxia associated gene programs. C) Aberrant redox signalling. A mis-assembled CI owing to mito-nuclear mismatch is depicted (i.e. grey box over CI). CI mismatch diverts electrons to O$_2$ to generate O$_2^-$. Various signalling abnormalities may ensue including biogenesis, DNA damage responses and apoptosis owing to persistent mitochondrial O$_2^-_i$ generation are graphically depicted.
fashion [132–134], which may link MOA/B derived H2O2 and neuronal cell death. Unsurprisingly, aberrant MOA-A/B activity has been linked to ageing [135] and related neurodegenerative disorders, notably Alzheimer’s disease (AD) and Parkinson’s disease (PD) [136,137]. Spurring interest in the use of synthetic MOA-A/B inhibitors to treat neurodegeneration and indeed mood disorders [124,125,138].

An underappreciated aspect of MOA/B biology is by restricting O2− induced neurotransmitter oxidation and subsequent redox cycling, they may limit H2O2 generation. For example, dopamine oxidation can yield multiple H2O2 molecules [139] whereas stoichiometric MOA-A/B metabolism produces a single H2O2 molecule. With the caveat that certain aldehydes products (e.g. 4-dihydroxyphenylacetaldehyde) can redox cycle [140,141]. A related corollary is that by helping to terminate neuronal activity MOA isoforms may protect against excitotoxicity. MOA-A/B may also protect the brain from exogenous xenobiotics. Notwithstanding, electrophilic aldehydes can conjugate macromolecules to cause damage [142]. For example, 3,4-dihydroxyphenylacetaldehyde, a dopamine metabolite readily conjugates proteins and is toxic to neurons [143]—a rise from 2–3 to 6 µM is sufficient to cause cell death [144]. MOA isoform activity must, therefore, be counter-balanced with aldehyde dehydrogenase (ADH) activity to prevent toxicity. Because ADH2 [145] is localised to the mitochondrial matrix the MIS may be unable to remove aldehydes enzymatically, which would favour macromolecule conjugates—especially if electrical charge occludes passive diffusion. Perhaps, electrophilic aldehydes, as opposed to H2O2, underlie MOA induced oxidative stress. While speculative, H2O2 signalling may inform the nucleus that aldehydes are being formed. ADH inhibition contributes to PD [146]—which underscores the importance of counter-balancing MOA activity. In sum, MOA isoforms can cause oxidative stress in the brain.

1.7. Neurotransmitters can auto-oxidise

In their seminal works, Cohen and Heikkal [147,148] showed that dopamine reacts with O2 to generate a dopamine semiquinone radical, which can then react with another O2 to generate O2− and a dopamine quinone. While the initial rate of semiquinone radical formation is often slow [19], it can be accelerated by redox active transition metals [19], it can be accelerated by redox active transition metals that averts redox cycling [151]. Dopamine oxidation products can also redox cycle [152]. For example, 6-hydroxydopamine can be reduced to a semiquinone radical which reacts with O2 to yield O2−, in turn, O2− reacts with another 6-hydroxydopamine to regenerate the semiquinone radical and H2O2. A situation that leads to further O2− generation [139] and .OH detected in the semi-quinone radical. The semi-quinone radical then reacts with O2 to generate O2−.

In 1994, Soo Goo Rhee’s group identified the PRDX family as ubiquitous cytosolic peroxidases [171]. PRDX isoforms are reduced by TRX, oxidised TRX is, in turn, reduced by thioredoxin reductase at the mitochondrial isoform [154]. In sum, PRDX isoforms are reduced by thioredoxin reductase at the mitochondrial isoform [154]. A functional PRRX-TRDX system may enable neurons to predominantly fuels mitochondrial 
PAR T I V ELYH I G H T N E U R O N S A S T H E Y P R E F E R E N T I A L I N T H E M I T O CHONDRIAL ISOFORM [154]. Insu sum e, G S H l i n k e d e n z y m a t i c s y s t e m s a r e modest in neurons.

In 1994, Soo Goo Rhee’s group identified the PRDX family as ubiquitous cytosine dependent H2O2 peroxidases [171]. PRDX isoforms are reduced by TRX, oxidised TRX is, in turn, reduced by thioredoxin reductase at the expense of NADPH [172–176]. Their discovery has important implications for neuronal H2O2 metabolism because neurons express PRDX-TRXD isoforms [162,177]. A functional PRRX-TRXD system may enable neurons to metabolise H2O2—particularly when it is considered that PRDX isoforms are abundant and distributed throughout the cell [171,175,178]. We are unaware of any report to the effect that PRDX-TRXD activity is comparatively high in neurons as they preferentially funnel glucose into the NADPH generating PPP [95]. However, enzymes (e.g. NOS) use NADPH to generate NO and .OH detected in the semi-quinone radical [179,180], so we are unable to assume NADPH exclusively fuels “antioxidants” [181]. However, PRDX6 prefers GSH as a reductant [182] so comparatively low GSH may limit its activity. It is remiss to consider PRDX-TRXD as “only” H2O2 “neutralisers” because compelling biochemical evidence suggests PRDX-TRXD transduce redox signals [161,172,183–187]. As Flohé et al. [188] elegantly enunciate nature is unlikely to have evolved over ten peroxidases just to remove H2O2. Transition metals may react with H2O2 to yield indiscriminately reactive .OH.

1.8. Modest endogenous antioxidant defence

As reviewed by Halliwell [17,18], modest endogenous antioxidant defence sensitises the brain to oxidative stress. That is, comparatively low endogenous antioxidant defence relative to many tissues (e.g. liver) makes the brain susceptible to disrupted redox homeostasis. While low catalase content—neurons possess 50 times lower catalase content compared to hepatocytes [162]—is a frequently cited exemplar [163], the relative importance of catalase to steady-state H2O2 removal is questionable. Aside from catalase being largely restricted to peroxisomes, its mechanism of action is not well understood [164].—which may restrict its activity at nanomolar H2O2 [165]. GSH, however, provides a cogent example. Cytosolic GSH is ~50% lower in neurons compared with other cells (e.g. ~5 mM in neurons compared with 10–11 mM in hepatocytes). Low cytosolic GSH reflects, in part, a reduced capacity for GSH synthesis owing to low γ-GCL content—a corollary of minimal Nrf-2 content and activity [81,166]. Comparatively, low cytosolic GSH may restrict GSH/GPx activity [130], which may explain neuronal sensitivity to ferropotosis [167]. Low GSH may also limit the ability of neurons to metabolise electrophiles, particularly electrophilic aldehydes. From the discussion so far, it would seem the apparent defect relates to H2O2 metabolism [18,19] because SOD isoform content and activity is normative (i.e. no defect in O2− metabolism). Intact O2− (i.e. SOD) activity is essential because neurons are unable to survive genetic deletion of MnSOD [168–170], the mitochondrial isof orm [154]. In sum, GSH linked enzymatic systems are modest in neurons.
redox signals. If so, such a state of affairs is perilous, if PRDX isozymes become over-oxidised when \([\text{H}_2\text{O}_2]\) rises to high nanomolar levels that seem to herald cell death [45,180]. Particularly, given the modest capacity of neuronal GSH linked enzyme systems [81,166], PRDX-TR Dix provide a means to remove, as well as, harness \(\text{H}_2\text{O}_2\) for cell signalling but the possibility remains that beyond a critical threshold elevated \([\text{H}_2\text{O}_2]\) easily short circuits this system to cause oxidative stress.

1.9. Microglia

Microglia are specialised, resident immune cells [189,190] that perpetually scan their local niche for homeostatic threats [191,192]. Microglia deploy extended processes to survey synapse health by monitoring neuronal activity [193]. By monitoring neuronal activity, microglia play an important role in removing unhealthy cells, neuronal wiring during development and activity dependent synaptic plasticity [194–197]. The ground breaking work of Bernard Babior [198], showed that active immune cells produce \(\text{O}_2^-\) via NOX isozymes (principally NOX2 [23]). The role of \(\text{O}_2^-\) in bacterial killing was one of the first examples of a biologically useful role for free radicals [199]. It is unsurprising, therefore, that microglia generate \(\text{O}_2^-\) and related reactive progeny during phagocytosis [200]. However, one should note that because \(\text{O}_2^-\) production depends on \(\text{O}_2\), microglia activity will be extremely sensitive to local \(\text{O}_2\) bioavailability—their \(\text{O}_2\) use may even be one way to remove synapses by consuming \(\text{O}_2\) to power \(\text{O}_2\)-dependent reactions [200]. EvenDepartment of Pharmacology, University of Otago, Dunedin, New Zealand.

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14 The redox status of the brain—such as the incorporation of \(\text{O}_2\) into neurotransmitter production [201]—might also contribute to oxidative stress via the generation of \(\text{O}^-\) and \(\text{H}_2\text{O}_2\). For example, neuronal activity redistributes the loosely chelated \(\text{Cu}^+\) pool from the soma to dendrites, which regulates spontaneous neuronal activity [227,229,230]. Moreover, Cu is an essential co-factor for enzymes [212], prominent examples being COX and copper zinc SOD (CuZnSOD) [231,232,233]. Neuronal 

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enzyme is regenerated using GSH, which is, in turn, regenerated using NADPH dependent glutathione reductase [130,131,188]. Ferroptosis [90,252] explains why genetically deleting GPX4 is embryonically lethal [253,254] because GPX4 regulates ferroptosis by removing ROOH [223,255]—gain and loss of GPX activity is sufficient to disable and activate ferroptosis [256], respectively. Modest [GSH] may render neurons particularly susceptible to ferroptosis confirmed by the observation that conditionally deleting GPX4 is lethal to neurons [257–259]. Consistent with lipid peroxidation contributing to the pathogenesis of neurodegenerative diseases (e.g. AD [209,211]).

1.12. The brain uses NOS and NOX for signalling

The brain harnesses nNOS and NOX isoforms to achieve essential functions. First, nNOS uses O2, NADPH and L-arginine to catalytically synthesise NO. [37]. The affinity of nNOS for O2 is 300 µM, mean brain O2 [2] is ~20 µM, which may limit NO synthesis [260]. NO regulates essential physiological processes, including LTP [261,262], axon growth [263] and pruning [264]. As discussed, NO can underlie oxidative stress—especially when O2 [2] is co-generated. nNOS biochemistry means that O2 [2] and NO can be spatially co-generated making [ONO] generation likely [265]. Co-fluxes occur when nNOS is uncoupled. Uncoupling typically arises when essential co-factors (e.g. tetrahydrobiopterin) become oxidised or unbound. Second, NOX isoforms use prosthetic redox groups to oxidise NADPH to reduce O2 to O2 [2•] [22,23]. NOX isoforms are important in the brain (reviewed in [266]) to support microglia and LTP to give just two examples [24]. Because NOX isoform mediated O2 [•] generation is far from adventitious being regulated at several levels [22,23,267], NOX isoform associated oxidative stress likely stems from the unwanted and continued presence of activating cues coupled to a sustained supply of NADPH and O2 to support enzyme activity. Such a scenario may manifest in neuronal inflammation [266] wherein cytokines provoke and sustain microglia NOX2 associated O2 [•] generation [265].

1.13. RNA oxidation

RNA oxidation is a seldom appreciated reason why the brain is susceptible to oxidative stress [268]. Beyond essential messenger RNA, the brain heavily relies on non-coding RNAs, particularly long non-coding RNAs and microRNAs (reviewed in [269–271]). From a biochemical perspective, RNA is equally susceptible to oxidation as DNA, undergoing analogous reactions [272]. For example, 8-oxo-guanine is a principal outcome of both DNA and RNA oxidation [273]. Owing to its single-stranded nature, RNA is also vulnerable to oxidation and indeed alkylation [274] at Watson-Crick interfaces. RNA also lacks protective histones and nuclear compartment in axons and synapses. Although local protein synthesis is essential to synaptic function [275,276], the possibility that RNA oxidation perturbs local protein synthesis is unexplored. Oxidised RNA associated coding errors stall ribosomal protein synthesis [277], which can if left uncorrected cause truncated, mutated and mis-folded proteins [278]. The spatial positioning of mRNA close to mitochondria and the temporal dynamics of RNA oxidation (order of seconds) compared with translation (order of hours) make RNA oxidation likely—especially in neurons with divalent redox active transition metals present to catalyse Fenton chemistry [279–281]. The mandate to consider mRNA oxidation as a cause of oxidative stress associated neurodegeneration is strengthened by the observation that oxidised CuZnSOD mRNA is an early pre-clinical feature of ALS [282]. As a number of excellent reviews [268,272] surmise further work is required to understand oxidised RNA recognition, turnover and repair [283]. Only with a better understanding of each process can one appreciate the neuronal susceptibility to RNA oxidation because [oxidised RNA] is a function of formation and removal over time.

2. Perspectives

We wish to propose an overarching perspective for interpreting why redox signalling leads to oxidative stress in the brain. The ultimate price of using redox signalling to inform brain function is innate susceptibility to oxidative stress when signals go awry—as seems to be the case in disease. Few neuroscientists would deny the central importance of neuronal activity. Based on how mitochondrial produce O2 [•] rather than H2O2 [68], neuronal activity should divergently regulate mitochondrial O2 [•]/H2O2 generation. At an active synapse, ATP demands—provided they can be met—should reduce net mitochondrial O2 [•]/H2O2 generation. Whereas at an inactive synapse, low ATP demands and a reduced respiratory chain should favour mitochondrial O2 [•]/H2O2 generation, potentially placing mitochondrial O2 [•]/H2O2 release triggers long-term depotentiation (LTD) and even synapse pruning—especially if the same pathway is used reiteratively [284]. Mitochondrial apoptosis regulates LTD and pruning [285–287]. Mitochondrial inactivity associated O2 [•]/H2O2 release may induce local sub-lethal intrinsic apoptosis to induce LTD and pruning. Perhaps, redox regulated apoptosis enables the developing brain to prune...
synapses—the essential prelude to a complex connectome and mandatory requirement for continued sculpting in adulthood [284]. Placing mitochondria with their hands on the proverbial shears renders the brain vulnerable to unwanted synapse loss. If mitochondria are unable to meet ATP demands or if O₂ is limiting resultant O₂⁻/H₂O₂ release may recapitulate the “pruning” signal to cause unwanted synapse loss. Biological precedent exists: unwanted reactivation of developmental pruning signalling contributes to synapse loss in AD [207].

In biochemically rationalising 13 reasons why the brain is susceptible to oxidative stress, we deliberately adopted a global view focusing on “neurons” as a collective for the purposes of a general primer. Apt parallels between the monolithic umbrella terms neurons and reactive species exist [20]. Reactive species subsumes chemically heterogeneous species, that can differ in their rate of reaction with a given substance by orders of magnitude (e.g. for guanine .OH reacts at a diffusion controlled rate whereas O₂⁻ leaves guanine unscathed owing to low reactivity). Analogous to reactive species, neurons are heterogeneous being ill-served by global monikers because they can widely differ in many key parameters, including function, location, connectivity, myelination and axon length. Neuronal heterogeneity informs differential susceptibility to oxidative stress both within a neuron (i.e soma vs. synapse), subdomain (i.e synaptic mitochondria vs. synaptic membranes) and between neuronal populations. Dopaminergic neurons in the substantia nigra pars compacta exemplify differential vulnerability: they experience residual (i.e. without additional homeostatic perturbation) oxidative stress because an L-type Ca²⁺ channel defined mitochondrial O₂⁻/H₂O₂ axis controls their autonomous pace-making capacity [288]. Teetering on the edge of an oxidative breakpoint, even minor unchecked shifts in the intracellular redox environment—perhaps related to dopamine metabolism [160]—seem sufficient to herald their demise.

Neuronal sensitivity to oxidative stress oscillates. Just as steady state [O₂] reflects its dynamic rate of generation and removal in a given compartment [68], a myriad of interconnected factors dynamically set neuronal sensitivity to oxidative stress over time. We briefly consider Fe²⁺ mediated ROOH reduction to ROO₂⁻. As a topical example relevant to ferroptosis [223]. The second order bimolecular elementary reaction is informed by the rate constant, [ROOH]₀ and [Fe²⁺]₀. Reactant availability at a given time governs the probability of ROO₂⁻ generation—with GPX4 catalysed ROOH metabolism and ferritin mediated Fe²⁺ chelation being prominent examples. If a xenobiotic conjugates GSH [19] to abrogate local [GSH] to compromise GPX4 activity, ROO₂⁻ generation may be favoured. That the “history” of the neuron influences susceptibility to a redox challenge adds complexity. For example, synaptic activity associated sub-lethal redox challenges herald co-ordinated transcellular neuronal-glial adaptive responses that increase neuronal [GSH] (reviewed in [81]). In our example, an adapted neuron is better able to buffer the xenobiotic mediated GSH conjugation to abrogate ROO₂⁻ generation to thereby raise the peroxidised lipid load required for ferroptosis [89]. As a cautionary note, adaption requires frequent stimulus because [GSH] is transcription dependent at multiple levels. An intriguing parallel with the exercise physiology axiom “use it or lose it” emerges: activity dependent beneficial adaptive redox responses persist with continued activity but progressively decay with inactivity.

From a translational perspective, the sheer complexity of neuronal redox homeostasis helps rationalise the failure of nutritional antioxidants to treat neurodegenerative diseases [289]. Bioavailability concerns aside, their failure relates to kinetic and spatial constraints (reviewed in [181,290–293]). The probability of any one compound possessing sufficient biochemical versatility to significantly modify each reason simultaneously is low. Above all, the failure of nutritional antioxidants reinforces their inherent biochemical strictures—being insufficient evidence to dismiss a causative role for oxidative stress. Much brain redox homeostasis in health and disease remains opaque. Only when basic research unmasks the mechanistic details can one rationally design redox active therapeutics for neurodegenerative diseases.

3. Conclusion

A complex interconnected myriad of reasons render the brain susceptible to oxidative stress; just 13 (many more exist [17,18]) reasons include unsaturated lipid enrichment, glucose, mitochondria, calcium, glutamate, modest antioxidant defence, redox active transition metals, neurotransmitter auto-oxidation and RNA oxidation. The brain is susceptible to oxidative stress because it harness chemically diverse redox-active species to perform heterogeneous signalling functions. From using lipid radicals to trigger ferroptosis when lipid signalling fails, NO to fine-tune synaptic plasticity or mitochondrial O₂⁻/H₂O₂ to signal hypoxia. The balance between species specific useful and harmful biochemistry is a fine one, which, in the brain, means the relationship is bittersweet: exquisite redox signalling functionality easily gives rise to oxidative stress when electrons go awry.
Fig. 6. 13 reasons why the brain is susceptible to oxidative stress. (1) Redox signalling. Depicts HO2 induced activation of a signalling protein via sulphenic acid (SOH) formation. (2) Calcium. Depicts mitochondrial Ca2+ overload induced O2.-/H2O2 generation. (3) Glutamate. Depicts glutamate induced Ca2+ release inducing nNOS mediated NO generation and mitochondrial O2.-/H2O2 generation, leading to ONOO- and excitotoxicity. (4) Glucose. Depicts protein inactivation via AGE formation. (5) Mitochondria. Depicts mitochondrial O2.- generation at CI and CIII. (6) Metabolism. Depicts MOA isoform catalysed H2O2 generation. (7) Neurotransmitter oxidation. Depicts redox active transition metal (Mn+) catalysed dopamine auto-oxidation to a semi-quinone radical. (8) Modest antioxidant defence. Depicts constrained GPX4 activity owing to low (GSH). (9) Microglia. Depicts NOX2 mediated O2.- generation within an end-foot process. (10) Redox active transition metals. Depicts Mn+ catalysed ROO- and ·OH generation. (11) Lipid peroxidation. Depicts ROS and ROI. within a neuronal cell membrane. (12) NOX/NOX expression. Depicts nNOS mediated NO generation. (13) RNA oxidation. Depicts ·OH mediated RNA oxidation (guanine is shown as an example).

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Conflict of interest

The authors declare that no conflicts of interest exist.

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