Review

Johannes Ebding, Fiorella Mazzone, Stefan Kins*, Jan Pielage* and Tanja Maritzen*

How neurons cope with oxidative stress

https://doi.org/10.1515/hsz-2024-0146
Received November 26, 2024; accepted January 31, 2025;
published online February 25, 2025

Abstract: Neurons are highly dependent on mitochondrial respiration for energy, rendering them vulnerable to oxidative stress. Reactive oxygen species (ROS), by-products of oxidative phosphorylation, can damage lipids, proteins, and DNA, potentially triggering cell death pathways. This review explores the neuronal vulnerability to ROS, highlighting metabolic adaptations and antioxidant systems that mitigate oxidative damage. Balancing metabolic needs and oxidative stress defenses is critical for neurons, as disruptions are implicated in neurodegenerative diseases. Neurons uniquely modulate metabolic pathways, favoring glycolysis over oxidative phosphorylation in cell bodies, to minimize harmful ROS production. Key antioxidants, including superoxide dismutases and glutathione peroxidases, play crucial roles in neuronal protection, as evident from genetic studies linking deficiencies to neurodegeneration. Notably, neurons have the ability to adapt to oxidative conditions in compartment-specific manners and also utilize ROS as a signaling molecule to promote adaptive synaptic plasticity. Future research should aim to elucidate differential ROS signaling and antioxidant responses across neuronal compartments for improved therapeutic strategies.

Keywords: antioxidants; ferroptosis; metabolic adaptations; reactive oxygen species (ROS); redox; structural plasticity

***Corresponding authors: Stefan Kins**, Department for Human Biology, RPTU University Kaiserslautern-Landau, Erwin-Schrödinger-Straße 13, D-67663 Kaiserslautern, Germany, E-mail: kins@rptu.de; and **Jan Pielage**, Department for Neurobiology and Zoology, RPTU University Kaiserslautern-Landau, Erwin-Schrödinger-Straße 13, D-67663 Kaiserslautern, Germany, E-mail: pielage@rptu.de; and **Tanja Maritzen**, Department for Nanophysiology, RPTU University Kaiserslautern-Landau, Paul-Ehrlich-Straße 23, D-67663 Kaiserslautern, Germany, E-mail: maritzen@rptu.de. https://orcid.org/0000-0003-4993-0340 (T. Maritzen)
Johannes Ebding, Department for Neurobiology and Zoology, RPTU University Kaiserslautern-Landau, Erwin-Schrödinger-Straße 13, D-67663 Kaiserslautern, Germany
Fiorella Mazzone, Department for Nanophysiology, RPTU University Kaiserslautern-Landau, Paul-Ehrlich-Straße 23, D-67663 Kaiserslautern, Germany

1 Introduction

Eukaryotic cells rely to a large degree on mitochondrial respiration to produce energy for powering cellular life. In this process, which is also called oxidative phosphorylation, reducing equivalents such as NADH are oxidized, and their electrons are passed through a number of protein complexes forming together the electron transport chain. This leads on the one hand to the generation of an electrochemical proton gradient across the inner mitochondrial membrane to drive ATP production, and on the other hand, to the reduction of molecular oxygen to water. A by-product of this process is the generation of reactive oxygen species (ROS), which cause oxidative stress by exerting toxic effects towards a range of cellular molecules. Therefore, all eukaryotic cells have to deal with ROS to prevent cellular damage.

The term ROS encompasses a range of highly reactive partially reduced forms of oxygen such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$). Within the mitochondrial electron transport chain the main ROS production sites are Complex I (NADH ubiquinone oxidoreductase), the entry point for electrons from NADH, and Complex III (cytochrome C oxidase) which relays electrons from ubiquinone to cytochrome C (Di Meo et al. 2016; Quinlan et al. 2013). Due to univalent oxidation of electron carriers superoxide can be generated (Turrens and Boveris 1980), which the mitochondrial dismutase SOD can turn into hydrogen peroxide which can be further converted into hydroxyl radicals via the Fenton reaction (Di Meo et al. 2016).

However, mitochondria are not the only sites of ROS production. ROS can also be generated by cytosolic enzymes, especially in pathological contexts. For example, the enzyme xanthine dehydrogenase normally catalyzes the oxidation of hypoxanthine via xanthine to uric acid using $NADP^+$ as electron acceptor. In pathological conditions, the enzyme can be converted into its oxidase form which produces superoxide radicals during hypoxanthine oxidation by transfering electrons to molecular oxygen (Di Meo et al. 2016; McCord et al. 1985). Xanthine oxidase is also found in peroxisomes (Angermuller et al. 1987) which harbor in addition an inducible nitric oxide synthase which can produce NO (Stolz et al. 2002). Furthermore, metabolic reactions in the ER, for example involving cytochromes P450 for detoxification of xenobiotics, can also contribute to ROS production

(Di Meo et al. 2016). In addition, functionally impaired lysosomes can also turn into sites of ROS production (see section "Lysosomal storage and oxidative stress"). At the plasma membrane ROS can arise when arachidonic acid, released from phospholipids by phospholipase A_2, is converted by membrane-associated enzymes such as lipoxygenase and cyclooxygenase into signaling molecules including prostaglandins and leukotrienes (Cho et al. 2011; Di Meo et al. 2016). In addition, plasma membrane resident NADPH oxidases (NOXs) produce superoxide. In contrast to the other mentioned enzymes, for NOXs it is their main function to generate ROS which serve as signaling molecules in neurons (see section "ROS as a plasticity signal").

1.1 Consequences of excessive oxidative stress

The cellular targets of ROS are very diverse. Firstly, the double bonds present in unsaturated fatty acids such as linoleic or arachidonic acid are very vulnerable to attacks by radicals which lead to lipid peroxidation. This initial reaction produces highly reactive lipid peroxy radicals which can cause further damage to nearby lipids. In addition, this chain reaction triggers the generation of breakdown products such as 4-hydroxy-2,3-nonenal (HNE), malondialdehyde and F2-isoprostanes (Barnham et al. 2004). Secondly, proteins can be altered in several ways by oxidative stress. For example, proteins can be modified by breakdown products like HNE, which can crosslink to cysteine, lysine or histidine residues, they can become S-glutathionylated by oxidized glutathione, or they can be carbonylated. Proteins containing labile iron-sulfur clusters such as the mitochondrial aconitase can also lose their iron. Finally, all four bases within DNA can be damaged by oxidative stress via hydroxylation (Gabbita et al. 1998).

The extent of oxidative stress in cells can be measured directly by determining ROS levels via various fluorescent probes (e.g. CellROX (Tian et al. 2021) or CM-H_2DCFDA (Holzerova et al. 2016)), or indirectly either by analyzing the levels of peroxidized lipids (e.g. measured via Liperfluo (Tian et al. 2021) or C11-BODIPY (Tian et al. 2021)), the levels of by-products of lipid peroxidation (e.g. HNE (Smith et al. 1998; Wei et al. 2023)), the level of altered proteins (e.g. measured as protein carbonyl levels (Ferrante et al. 1997)), the amount of damaged DNA (e.g. measured via the deoxyguanosine oxidation product OH^8dG (Ferrante et al. 1997)) or by analzying the presence of DNA damage response proteins like gH2ax (Junghans et al. 2022; Wei et al. 2023)).

In agreement with the diverse cellular targets of ROS, cellular function and thus cell survival will be compromised at different levels upon oxidative stress exposure. If the damage to lipids cannot be repaired, lipid peroxidation will trigger the ferroptosis cell death program (Stockwell et al. 2017). In addition, breakdown products such as HNE have been reported to initiate apoptosis by activating the kinases c-Jun aminoterminal kinase (JNK) and mitogen activated protein kinase (MAPK) p38 (Tamagno et al. 2003). Damaged DNA can induce the DNA damage response which can lead to apoptosis if the damage exceeds the cell's repair capabilities. Altered proteins can either lose their functionality or be differentially regulated. For striatal neurons, for example, it has been shown that the S-glutathionylation of the channel TRPC5 causes its activation and thereby an increased Ca^{2+} influx which leads to striatal neuron death (Hong et al. 2015). Therefore, it is essential for all cells to counteract ROS with antioxidant programs.

1.2 Neuronal vulnerability to oxidative stress

If all cells are vulnerable to ROS, why is it of special interest to study how neurons cope with oxidative stress? In fact, the brain is especially vulnerable to oxidative damage, and there are several reasons for this: First of all, neurons have a higher energy demand than most other cells in order to fulfill their various functions such as activating ion transport proteins to maintain the membrane potential, triggering action potentials, releasing neurotransmitters and recycling synaptic vesicles. This high energy demand is reflected by the fact that the brain, despite constituting only about 2 % of our body weight, accounts for 20 % of the overall oxygen consumption. This increased metabolic activity is accompanied by an elevated production of ROS. Secondly, neurons often harbor high levels of redox-active metals, especially iron, which not only supplies iron-sulfur clusters for mitochondrial respiration, but is also involved in myelin synthesis, and neurotransmitter metabolism (Ward et al. 2014). As components of metallo-enzymes redox-active metals are exploited for activating O_2, however, in an unbound state they can also act outside of these metabolic contexts and contribute to ROS production. An example is the Fenton reaction which leads to the decomposition of H_2O_2 generating highly reactive hydroxyl radicals (Jomova et al. 2012). Thirdly, neuronal membranes are rich in poly-unsaturated fatty acids that contain double bonds, which are easily damaged by ROS. Finally, the brain has a very limited potential for regeneration since neurons are very long-lived cell types making oxidative damage more prone to accumulate, and dead cells are not easily replaced. Interestingly, neurons do not only differ from other cell types in their

degree of vulnerability to oxidative stress, but also among themselves. For instance, dopaminergic neurons are more sensitive to oxidative stress than cortical neurons (Wei et al. 2023).

1.3 Oxidative stress in neurodegenerative diseases

The importance of a well maintained redox homeostasis for neuronal life is also underlined by the fact that numerous neuronal diseases are associated with an increase of oxidative stress markers, including Parkinson's disease (Yoritaka 1996), Alzheimer's disease (Subbarao et al. 1990), Huntington's disease (Sorolla et al. 2008), Amyotrophic lateral sclerosis (Cunha-Oliveira et al. 2020; Ferrante et al. 1997), epilepsy (Kalita et al. 2019), ataxias (Lupoli et al. 2018) and traumatic brain injury (Varma 2003).

In Alzheimer's disease, for example, there is increasing evidence suggesting that brain tissue of patients shows elevated ROS levels throughout the disease progression (Gella and Durany 2009). Altered mitochondria are believed to play a significant role in ROS generation in Alzheimer's disease, and dysregulation of the electron transport chain increases the level of aggregated Aβ and neurofibrillary tangles, which in turn further stimulate ROS production. Subsequently, neuronal function is likely disrupted due to reduced ATP production as a result of damaged mitochondria and damage to DNA, proteins and lipids (Dhapola et al. 2024). In addition, several molecular pathways involving the proteins RCAN, CREB, Nrf2 and PP2A have been reported to contribute to the oxidative stress-induced progression of Alzheimer's disease (Dhapola et al. 2024). These signaling pathways are altered under stress conditions and affect downstream pathways leading to the progression of the disease. Interestingly, some individuals are able to maintain their cognitive abilities despite the presence of significant Alzheimer's disease associated neuropathological changes. These resilient individuals showed lower levels of oxidative DNA damage, but the exact cellular and molecular mechanisms are not yet understood (de Vries et al. 2024).

While the relationship between elevated oxidative stress and neurodegenerative diseases remains unclear regarding the question whether ROS are cause or consequence, studies show that overexpression of antioxidant enzymes such as superoxide dismutase (SOD) or catalase can ameliorate symptoms in animal models of neurodegenerative diseases, and antioxidant therapies are currently being tested to combat diverse neurological disorders (Patel 2016). However, in Parkinson's disease, despite high ROS levels and oxidative stress being considered as major disease factors (Dias et al. 2013), antioxidants have failed to demonstrate symptomatic benefits in clinical trials (Duarte-Jurado et al. 2021, Gogna et al. 2024), leaving ROS's role as an initial trigger in neurodegenerative diseases unresolved.

1.4 Metabolic adaptation

Since neurons are more vulnerable to oxidative stress, which mechanisms do they employ to avoid excessive ROS production? For a long time, it was assumed that the high energy needs of neurons would necessarily require high levels of mitochondrial respiration throughout the neuron accompanied by a high generation of ROS. However, we know now that neurons exhibit specific metabolic adaptations for mitigating the ROS burden. For maximized energy production, glucose is normally metabolized by glycolysis leading to the generation of pyruvate which is shuttled into the tricarboxylic acid (TCA) cycle to generate reducing equivalents for oxidative phosphorylation. Yet, glucose can also be fed into the pentose phosphate pathway, producing not only precursors for the synthesis of nucleotides, but also maintaining the activity of the antioxidant enzyme glutathione peroxidase (GPx) (Ben-Yoseph et al. 1996) (Figure 1). For the detoxification of H_2O_2 glutathione peroxidase requires reduced glutathione which is oxidized in the process. Reduced glutathione is then regenerated by glutathione reductase, consuming NADPH supplied by enzymes of the pentose phosphate pathway. To keep glycolysis at submaximal levels for promoting the pentose phosphate pathway with its antioxidant benefits, neurons downregulate the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (Pfkfb3) which acts as a positive regulator of glycolysis. The importance of this metabolic adaptation becomes apparent upon artificial upregulation of Pfkfb3 in neurons which leads to increased oxidative stress and apoptotic death (Herrero-Mendez et al. 2009).

A recent study uncovered another neuron-specific metabolic adaptation for decreasing the ROS burden. By measuring metabolic flux separately in neuronal somata and nerve terminals, Wei et al. realized that somata in comparison to synapses carry out less oxidative phosphorylation and more aerobic glycolysis (Wei et al. 2023). Like many cancer cells neurons can metabolize glucose to lactate despite the availability of oxygen for oxidative phosphorylation, a phenomenon known as Warburg effect. While this entails a much less efficient ATP production (2 ATP instead of 36 ATP per molecule of glucose), it has the advantage of a lower ROS generation. Wei et al. could trace the difference in the utilization of oxidative phosphorylation between somata and terminals back to a higher level of the glycolytic enzyme

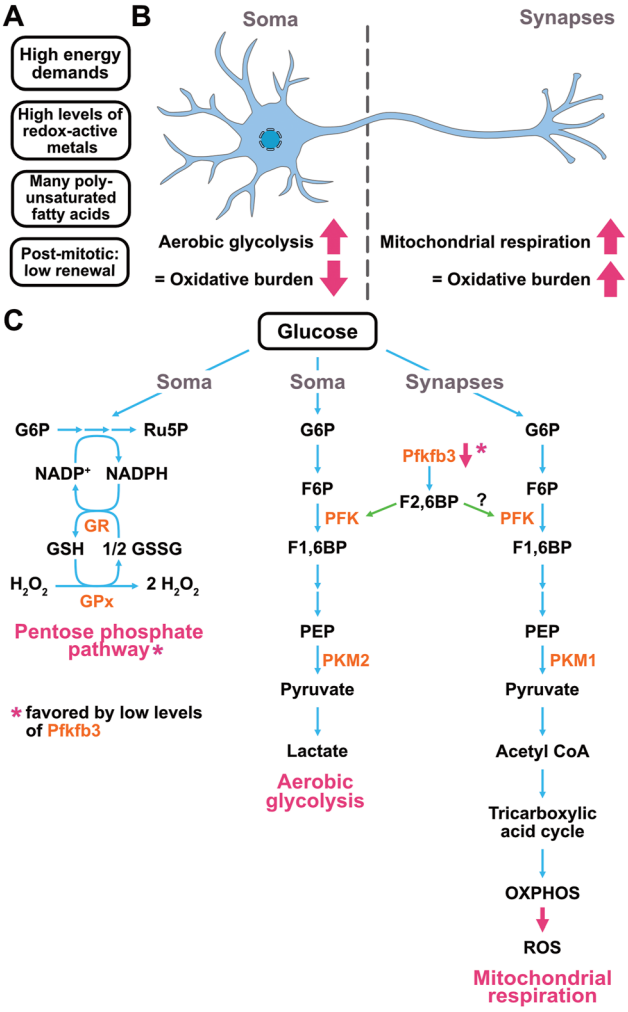

Figure 1: Metabolic adaptations of neurons to oxidative stress. (A) Factors causing increased vulnerability of neurons to oxidative stress. (B) Neuronal compartments differ in their preferred energy production pathways. Aerobic glycolysis is favored in neuronal somata keeping the oxidative burden low, while mitochondrial respiration is favored at synapses for efficient energy production at the expense of a higher oxidative burden. (C) Depiction of three metabolic pathways that are differentially regulated across neuronal compartments: PKM2 expression in neuronal somata favors aerobic glycolysis, while PKM1 expression at synapses promotes mitochondrial respiration. At the same time a lower expression of Pfkfb3 in neurons than in astrocytes facilitates the entry of part of the glucose pool into the pentose phosphate pathway with its antioxidant benefits. For more details please refer to the text. F6P, fructose 6-phosphate; F1,6BP, fructose 1,6-bisphosphate; F2,6BP, fructose 2,6-bisphosphate; G6P, glucose 6-phosphate; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; OXPHOS, oxidative phosphorylation; PEP, phosphoenolpyruvate; PFK, phosphofructokinase 1; Pfkfb3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PKM1, pyruvate kinase M1; PKM2, pyruvate kinase M2.

pyruvate kinase 2 (Pkm2) in somata. Pkm2 is one of the two major pyruvate kinase isoforms in the brain. While Pkm1 favors the entry of pyruvate into the TCA cycle, Pkm2 promotes the conversion of pyruvate to lactate (Christofk et al. 2008; Israelsen and Vander Heiden 2015) (Figure 1). Consequently, elevated Pkm2 levels in somata lead to increased aerobic glycolysis. The relevance of this adaptation could be shown in a Pkm2 knockout (KO) mouse model where the loss of Pkm2 resulted in a switch back to oxidative phosphorylation in neuronal somata resulting in increased oxidative damage and the death of dopaminergic neurons (Wei et al. 2023).

Thus, neuronal compartments differ in their preferred energy production pathway. The use of the more efficient oxidative phosphorylation in synapses likely helps to fulfill the high local energy need for neurotransmission, meanwhile exposing axon terminals to higher oxidative stress levels. However, the increased oxidative damage might be easier to handle by synapses than by cell bodies due to the possibility to exploit synaptic structural plasticity pathways to restore terminals. The increased utilization of aerobic glycolysis in the long-lived somata will protect the bulk of the biosynthetic machinery and the cellular genome from lethal oxidative damage. At the same time, the higher expression of Pkm2 in the somata does not only promote aerobic glycolysis over mitochondrial respiration, but its lower overall pyruvate kinase activity (Board et al. 1990) compared to Pkm1 also allows some of the glucose to enter into the pentose phosphate pathway, thereby further strengthening the antioxidant protection of neuronal somata (Wei et al. 2023) (Figure 1).

Nevertheless, the overall level of aerobic glycolysis has to be well regulated since excessive glycolysis will cause the production of the toxic by-product methylglyoxal. Therefore, the neuronal metabolism seems to be delicately balanced to fulfill the dual neuronal need of high synaptic energy supply and long-time cell survival.

1.5 Antioxidant systems

In addition to metabolic adaptations, neurons have several antioxidant systems in place to cope with the remaining ROS production (Figure 2). A genome-wide CRISPRi screen designed to identify essential neuronal genes emphasized the importance of these systems for neuronal survival (Tian et al. 2021). Among the top 10 hits of this screen, which evaluated the survival of human iPSC-derived neurons upon CRISPRi-mediated knockdown, were the genes for the superoxide dismutases SOD1 and SOD2 (Tian et al. 2021). These enzymes convert free superoxide radicals to molecular oxygen and H_2O_2 which can then be detoxified by glutathione peroxidase or catalase. Interestingly, these genes are

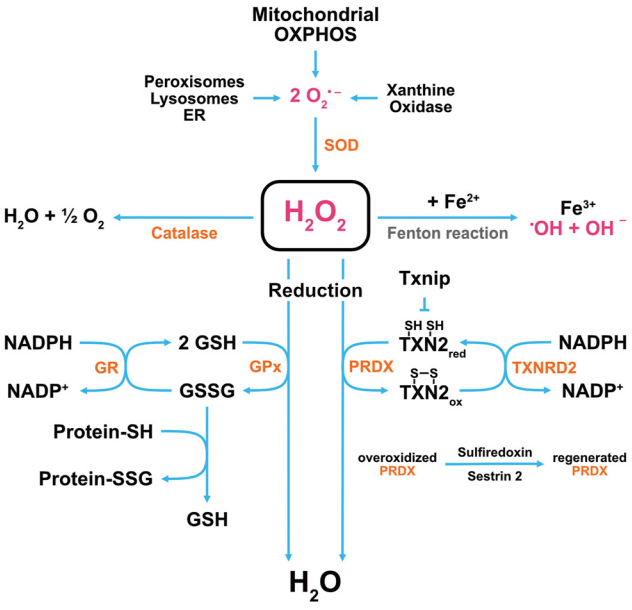

Figure 2: Overview of antioxidant systems. Superoxide dismutases (SOD) convert free superoxide radicals (O_2•-), that are produced by reactions within organelles such as the mitochondrial oxidative phosphorylation (OXPHOS) and by cytoplasmic enzymes such as xanthine oxidase, to hydrogen peroxide (H_2O_2). In the presence of free iron, the Fenton reaction can occur causing the decomposition of H_2O_2 to highly reactive hydroxyl radicals. Catalases can reduce the burden of reactive oxygen species (ROS) by converting H_2O_2 to water and oxygen. In neurons, mitochondria express only low levels of catalases, thus, two alternative detoxification systems are utilized: (1) the glutathione (GSH)/glutathione peroxidase (GPx) system reduces H_2O_2 to H_2O, a process in which two monomeric GSH molecules are oxidized and form one glutathione disulfide (GSSG). GSSG is then converted by the glutathione reductase (GR) back to GSH, consuming NADPH in the process. Thiol groups of proteins (Protein-SH) can also be S-glutathionylated (Protein-SSG) by GSSG. (2) In the thioredoxin (TXN2)/peroxiredoxin (PRDX) system, PRDX reduces H_2O_2 to H_2O accompanied by the oxidation of TXN2, which can by reverted by the thioredoxin reductase 2 (TXNRD2) by using NADPH. PRDX that became overoxidized in this process can be regenerated by reduction through sulfiredoxin and sestrin 2.

not essential for survival in all cell types, supporting the idea that neurons are especially vulnerable to oxidative stress.

To further dissect the factors that protect neurons against oxidative stress, the Kampmann lab designed another genome-wide CRISPRi screen which evaluated neuronal survival under mild oxidative stress (Tian et al. 2021). This mild stress was created by omitting the antioxidants that are commonly contained in neuronal culture medium. Among the identified candidates was the selenoprotein glutathione peroxidase 4 (GPX4) which counteracts oxidative damage to lipids by reducing peroxidized lipids and thereby preventing ferroptosis. Other candidates were factors required for selenocysteine incorporation into selenoproteins such as PSTK, SEPHS2 and SEPSECS. There are 25 human selenoproteins of which many function as antioxidants with the selenocysteine residue catalyzing redox reactions (Zhang et al. 2020). Prominent examples are the already mentioned glutathione peroxidases which detoxify H_2O_2 and repair lipid peroxides, but also thioredoxins discussed below (Zhang et al. 2020). Therefore, defects in the machinery for selenoprotein biosynthesis severely compromise the neuronal ability to cope with oxidative stress. In line with this, a recent preprint demonstrated the importance of a tRNA modifying enzyme, ALKHB8, which promotes selenoprotein synthesis, for redox homeostasis in Drosophila neurons (Madhwani et al. 2024).

Earlier work in mice has also highlighted the importance of antioxidant systems. Consistent with the identification of SOD1 and SOD2 as essential genes in the CRISPRi screen (Tian et al. 2021), SOD2 KO mice showed a severely reduced viability and exhibited neuronal loss (Lebovitz 1996). The murine KO of the small redox protein thioredoxin 2 (TXN2, TRX2) impaired viability even more leading to exencephaly and early embryonic death (Nonn et al. 2003). Investigations of the mutations underlying early-onset neurodegeneration have revealed that thioredoxin 2 is also a crucial component of the neuronal antioxidant defense in humans (Holzerova et al. 2016). Thioredoxin 2, which displays its highest expression levels in the brain (Rybnikova et al. 2000), localizes to mitochondria and constitutes together with peroxiredoxin (PRDX) a part of the mitochondrial H_2O_2 detoxification system. In fact, mitochondria are not only a site of ROS production, but also an important site for ROS removal (Drechsel and Patel 2010). Regarding the employed detoxification systems, brain mitochondria differ from liver or heart mitochondria in expressing little, if any, catalase, an enzyme converting H_2O_2 to water and oxygen (Sinet et al. 1980). Instead, brain mitochondria preferentially utilize two peroxidase systems, the glutathione/glutathione peroxidase system and the thioredoxin/peroxiredoxin system with the latter contributing the larger share (Drechsel and Patel 2010). Peroxiredoxin acts similarly to glutathione peroxidase in reducing H_2O_2. The concomitant oxidation of peroxiredoxin is reverted by an oxidation of thioredoxin 2 which in turn can be reduced via thioredoxin reductase 2 (TXNRD2) which utilizes NADPH for this step such as glutathione reductase. In addition to reducing peroxiredoxin, thioredoxin can also restore thiol groups in other proteins that were damaged by oxidative stress. Holzerova et al. identified a homozygous stop mutation in thioredoxin 2 in a patient suffering from an early-onset neurodegeneration causing severe cerebellar atrophy. Using patient-derived fibroblasts they confirmed the loss of thioredoxin 2 and

demonstrated increased ROS levels. Consistent with the notion that the elevated ROS levels are causative for the disease, application of the antioxidant Idebenone (a synthetic analog of coenzyme Q10) ameliorated patient symptoms during a four months follow up (Holzerova et al. 2016).

Moreover, the mitochondrially localized isoform of peroxiredoxin, PRDX3, has been shown to be crucial for the health of the human brain. Recessive mutations in PRDX3 were identified in patients suffering from cerebellar atrophy and ataxia, and PRDX3 knockdown experiments showed increased H_2O_2 levels and decreased cell viability (Rebelo et al. 2021). Another oxidative stress related protein for which loss-of-function mutations have been associated with cerebellar atrophy in mice and humans is OXR1 (Wang et al. 2019). OXR1 stands for oxidation resistance 1, a name reflecting its ability to promote cellular resistance to oxidative stress in *E. coli* (Volkert and Crowley 2020) as well as in mouse cerebellar granule cells (Oliver et al. 2011). In contrast to thioredoxin 2 and peroxiredoxin 3, the molecular mode of action of OXR1 is still only partially understood. It has been shown that OXR1 can react with H_2O_2 through the oxidation of reactive cysteine residues (Oliver et al. 2011). However, newer results render it more likely that OXR1 exerts its beneficial effects by preserving retromer function, a complex necessary for the recycling of proteins and lipids from endosomes to the trans-Golgi network (TGN) or the cell membrane, and thus maintaining endolysosomal function (Wilson et al. 2024). As we will discuss below, impairments of lysosomal degradation are an important trigger of oxidative stress.

It is intriguing and still an open question why the loss of thioredoxin 2, peroxiredoxin 3 and OXR1 have the most pronounced effects on the cerebellum, while in other circumstances striatal neurons exhibited, at least compared to cortical neurons, a higher vulnerability to oxidative stress (Tian et al. 2021). There is still much to learn about the differences in the antioxidant defenses employed by different types of neurons. In addition to the enzymatic systems that we have discussed, there are also non-catalytic antioxidants present such as vitamin E whose production might also vary in a neuron-type specific manner.

1.6 Lysosomal storage and oxidative stress in neurons

As non-dividing cells, neurons are also more sensitive than other cells to impairments in the breakdown of lysosomal material. Defects in lysosomal hydrolases which catalyze the degradation of diverse molecules that are delivered to lysosomes lead to the accumulation of lysosomal storage material and the further impairment of lysosomal function (Parenti et al. 2021). The loss of the lysosomal protein prosaposin (PSAP) which is associated with lysosomal storage diseases, has recently been shown to cause increased levels of ROS, iron and lipid peroxidation specifically in neurons, thereby compromising neuronal health in line with earlier observations in Drosophila (Hindle et al. 2017). Prosaposin is a precursor of saposins A-D which activate glycosphingolipid degradation by lysosomal hydrolases. In their absence glycosphingolipids accumulate in lysosomes leading to the appearance of aggregated lysosomal storage material, also known as lipofuscin, on electron microscopic images. Iron derived from the lysosomal degradation of iron-containing proteins accumulates within this lipofuscin and is believed to cause ROS generation via the Fenton reaction (Brunk et al. 1992) leading, for example, to lipid peroxidation. The accumulation of oxidized lipids will further impair lysosomal function and in the end might also trigger cell death. In fact, in absence of antioxidants in the neuronal medium, PSAP KO neurons showed high levels of lipid peroxidation which finally induced ferroptosis of the KO neurons (Tian et al. 2021). Thus, efficient lysosomal function is also crucial to protect neurons from oxidative stress.

1.7 Adaptation of antioxidant pathways

The generation of ROS is a normal event in the life of every mammalian cell, and, as we have discussed, there are multiple systems in place to detoxify the highly reactive molecules. Therefore, ROS only turn into dangerous oxidative stress when there is an imbalance between oxidants and antioxidant defenses, leading to a disruption of redox homeostasis and subsequent macromolecular damage. To prevent this, cells generally have stress response pathways which allow their adaptation to adverse changes, and these pathways exist also for oxidative stress in neurons. Only when the adaptive responses are saturated, does ROS becomes a threat to survival. These adaptive responses regulate on the one hand components of the antioxidant systems (e.g. overoxidized peroxiredoxin which can be reduced by sulfiredoxin and sestrin 2) and on the other hand trigger repair processes that can reverse oxidative damage to DNA (e.g. via base excision repair), lipids (e.g. via GPX4) and proteins (e.g. via thiol repair by methionine sulfoxide reductase) (Patel 2016). Even though we do not have a full picture of these neuronal oxidative stress responses yet, some pathways have been elucidated. One of them is the mechanism which tailors the antioxidant system to the level

of neuronal activity. It is easy to envision that highly active neurons with their greater energy demand will need elevated antioxidant defenses. In line with this assumption, synaptic activity has been shown to alter the regulation of components of the antioxidant system (Papadia et al. 2008). The neuroprotective effect of synaptic activity was traced back to trans-synaptic NMDAR activation (Papadia et al. 2005, 2008) leading to an upregulation of the thioredoxin-peroxiredoxin system (Papadia et al. 2008). In one branch of this pathway, PI3K and Akt become activated triggering the nuclear export of the transcription factor FOXO (forkhead box O) which positively regulates the transcription of Txnip, a thioredoxin inhibitor. Consequently, less Txnip is transcribed leading to an increased thioredoxin activity and thus better regeneration of oxidized peroxiredoxin. In a second branch the transcription factor AP-1 is activated leading to an upregulation of sulfiredoxin causing a more efficient reduction of overoxidized peroxiredoxin. Thirdly, the transcription factor C/EBPb (CCAAT enhancer binding protein) is transcriptionally upregulated inducing an elevated transcription of sestrin 2, elevating the cellular capacity to reduce overoxidized peroxiredoxin even further (Papadia et al. 2008). Based on these findings Papadia et al. raised the alluring question of whether boosting brain activity might prolong brain health by protecting against neurodegeneration via the upregulation of antioxidant defenses.

However, not only synaptic activity triggers the adaptation of the antioxidant system, also ROS themselves serve as feedback signal indicating that increased antioxidant capacities are needed. A critical ROS sensor is DJ-1 (also known by its gene name PARK7), one of the disease genes underlying familial Parkinson's disease (Hijioka 2017). DJ-1 defective neurons are more sensitive to oxidative stress (Martinat et al. 2004). DJ-1 contains redox-active cysteines whose oxidation state determines DJ-1 activity thus turning it into a redox sensor. Among its many functions, DJ-1 can activate the Nrf2 (nuclear factor erythroid 2-related factor 2)-dependent antioxidant responsive element (ARE) pathway (Im et al. 2012) which plays a critical role in redox homeostasis. Upon Nrf2 activation, a range of antioxidant genes, including glutathione, thioredoxin, thioredoxin reductase and peroxiredoxin, are upregulated strengthening the antioxidant defenses of the cell. It is important to note, however, that in mammalian forebrain neurons NRF2 is only expressed at low levels (Bell et al. 2011, 2015, Shih et al. 2003). Here, alternative pathways including the transcription factor AP-1, utilizing Ca^{2+} levels as a readout of increased electrical activity, can induce transcription of a similar set of antioxidant response genes as NRF2 (see below; reviewed in detail in (Qiu et al. 2020)).

1.8 ROS as a plasticity signal

As highlighted above, ROS do not only act as cell-damaging agents but can activate cellular signaling systems to upregulate antioxidant capacities of the cell. In addition, ROS signaling can induce structural adaptations in neurons both under pathological and non-pathological conditions. In a Drosophila model of neurodegenerative lysosomal storage disease, it has been demonstrated that elevated levels of ROS lead to an activation of the JNK/AP-1 pathway resulting in excessive synaptic overgrowth at the larval neuromuscular junction (Milton et al. 2011). Interestingly, elevated neuronal activity caused by excitotoxicity results in similar morphological adaptations. These phenotypes can be mimicked by application of oxidants or by increasing the endogenous activity of the JNK/AP-1 pathway. Conversely, the expression of antioxidant enzymes decreases the morphological changes (Milton et al. 2011; Oswald et al. 2018; Peng et al. 2019). Consistently, increasing JNK/AP-1 activity decreased ROS levels and rendered flies resistant to chemically induced oxidative stress while decreased JNK/AP-1 activity increased ROS levels. This pathway is conserved in mammalian cortical neurons with JNK/AP-1, activated by chemically induced ROS, resulting in a compensatory expression of the antioxidant protein SRXN-1 (Ugbode et al. 2020). In contrast, activity-induced elevations in ROS trigger the ERK pathway to achieve a similar upregulation of SRXN-1 (Papadia et al. 2008) which localizes to dendrites and synapses, sites within neurons which are particularly sensitive to ROS (Ugbode et al. 2020).

In non-pathological conditions, ROS has the potential to serve as a synaptic plasticity signal which induces adaptive modifications in synaptic and dendritic structure in response to increased neuronal activity. At the Drosophila presynaptic neuromuscular terminal, the Parkinson's Disease-linked redox sensor DJ-1b (PARK7) regulates the balance between PTEN, a phosphoinositide phosphatase, and phosphoinositide 3-kinase (PI3K) activity to induce an increase in synaptic arborization and compensatory reductions in the dendritic arbor of motoneurons (Dhawan et al. 2021; Oswald et al. 2018). These studies identified mitochondria as a source of regulatory ROS. However, it has been demonstrated in Drosophila motoneurons that the NADPH oxidases Nox and Duox, located in the plasma membrane of the presynaptic terminal (Nox and Duox) and dendrites (Duox), can generate ROS in the extracellular space. Import of ROS via the two aquaporins Bip and Drip likely contributes to structural plasticity changes and has the potential to integrate ROS signals from neighboring neurons (Dhawan et al. 2021; Sobrido-Camean et al. 2022) (Figure 3).

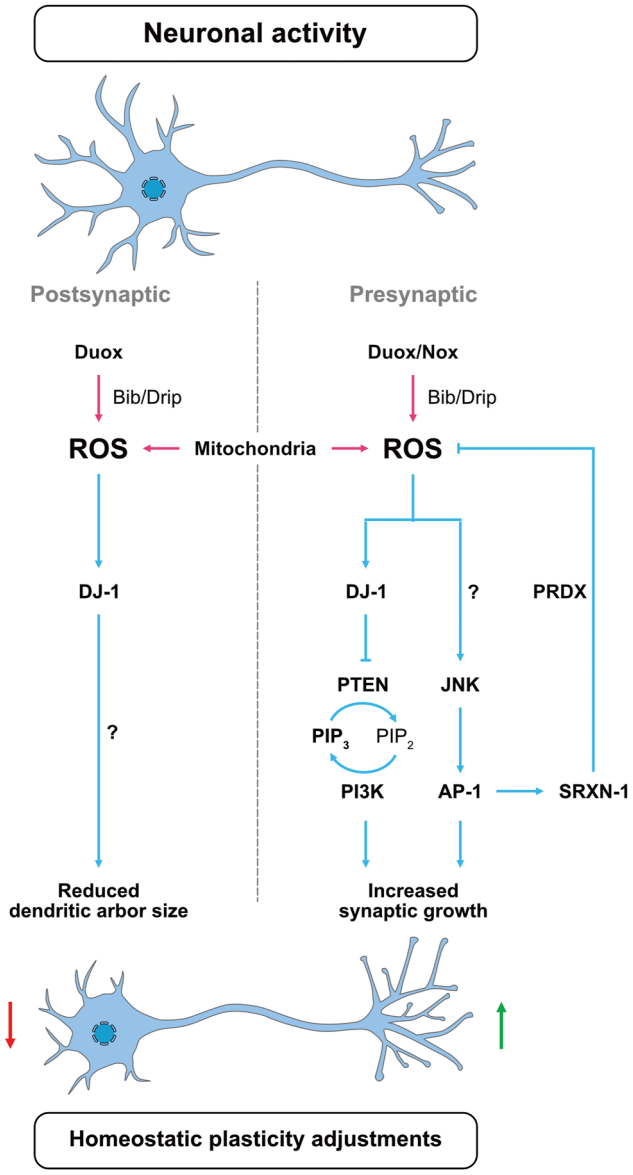

Figure 3: ROS as a signaling molecule in structural synaptic plasticity. During neuronal activity, ROS is generated either intracellularly by mitochondria or extracellularly by the NADPH oxidases Duox (postsynaptic) or Duox and Nox (presynaptic). Extracellular ROS, generated by the same neuron or adjacent neurons, can be shuttled into the synaptic terminals by the aquaporins Bib and Drip. DJ-1 acts as a redox sensor and can detect elevated ROS in the neuron. At the postsynapse, oxidation of DJ-1 by ROS was shown to activate downstream pathways resulting in reduced dendritic arbor sizes. At the presynapse, DJ-1 was shown to inhibit the phosphatase PTEN, thereby favoring PIP_3 production by phosphoinositide 3-kinase (PI3K). The c-Jun N-terminal kinase (JNK) responds to increased ROS levels by activating the transcription factor activator protein 1 (AP-1). Both pathways lead to a compensatory increase of presynaptic growth and active zone number. In addition, AP-1 activity regulates the expression of the antioxidant gene sulfiredoxin-1 (SRXN-1), a protein that can reduce overoxidized peroxiredoxin (PRDX), which in turn reduces ROS.

2 Outlook

Taken together, it has become clear that neuronal cells are able to cope with ROS using elaborate metabolic adaptations and cellular signaling systems that operate differentially within dendrites, somata and presynaptic terminals. It will be of high interest to further disentangle the different sources and signaling pathways of ROS that enable survival of neurons during stress conditions and to elucidate structural and functional adaptations maintaining neuronal function in the face of continuous ROS production over the lifetime of the organism.

Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
Research funding: This research was supported by the Deutsche Forschungsgemeinschaft (DFG; GRK2737 STRESSistance, project A1 to T.M. and project B3 to J.P.) and the Forschungsinitiative Rheinland-Pfalz BioComp (to T.M., S.K., J.P.).
Data availability: Not applicable.

References

Angermuller, S., Bruder, G., Volkl, A., Wesch, H., and Fahimi, H.D. (1987). Localization of xanthine oxidase in crystalline cores of peroxisomes. A cytochemical and biochemical study. *Eur. J. Cell Biol*. 45: 137–144.

Barnham, K.J., Masters, C.L., and Bush, A.I. (2004). Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discov.* 3: 205–214.

Bell, K.F., AL-Mubarak, B., Martel, M.A., Mckay, S., Wheelan, N., Hasel, P., Markus, N.M., Baxter, P., Deighton, R.F., Serio, A., et al. (2015). Neuronal development is promoted by weakened intrinsic antioxidant defences due to epigenetic repression of Nrf2. *Nat. Commun*. 6: 7066.

Bell, K.F., Fowler, J.H., AL-Mubarak, B., Horsburgh, K., and Hardingham, G.E. (2011). Activation of Nrf2-regulated glutathione pathway genes by ischemic preconditioning. *Oxid. Med. Cell Longev.* 2011: 689524.

Ben-Yoseph, O., Boxer, P.A., and Ross, B.D. (1996). Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cerebrocortical cultures from oxidative stress. *J. Neurochem*. 66: 2329–2337.

Board, M., Humm, S., and Newsholme, E.A. (1990). Maximum activities of key enzymes of glycolysis, glutaminolysis, pentose phosphate pathway and tricarboxylic acid cycle in normal, neoplastic and suppressed cells. *Biochem. J*. 265: 503–509.

Brunk, U.T., Jones, C.B., and Sohal, R.S. (1992). A novel hypothesis of lipofuscinogenesis and cellular aging based on interactions between oxidative stress and autophagocytosis. *Mutat. Res*. 275: 395–403.

Cho, K.J., Seo, J.M., and Kim, J.H. (2011). Bioactive lipoxygenase metabolites stimulation of NADPH oxidases and reactive oxygen species. *Mol. Cells* 32: 1–5.

Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L., and Cantley, L.C. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 452: 230–233.

Cunha-Oliveira, T., Montezinho, L., Mendes, C., Firuzi, O., Saso, L., Oliveira, P.J., and Silva, F.S.G. (2020). Oxidative stress in amyotrophic lateral sclerosis: pathophysiology and opportunities for pharmacological intervention. *Oxid. Med. Cell. Longev.* 2020: 5021694.

DE Vries, L.E., Huitinga, I., Kessels, H.W., Swaab, D.F., and Verhaagen, J. (2024). The concept of resilience to Alzheimer's disease: current definitions and cellular and molecular mechanisms. *Mol. Neurodegener.* 19: 33.

Dhapola, R., Beura, S.K., Sharma, P., Singh, S.K., and Harikrishnareddy, D. (2024). Oxidative stress in Alzheimer's disease: current knowledge of signaling pathways and therapeutics. *Mol. Biol. Rep*. 51: 48.

Dhawan, S., Myers, P., Bailey, D.M.D., Ostrovsky, A.D., Evers, J.F., and Landgraf, M. (2021). Reactive oxygen species mediate activity-regulated dendritic plasticity through NADPH oxidase and aquaporin regulation. *Front. Cell. Neurosci.* 15: 641802.

DI Meo, S., Reed, T.T., Venditti, P., and Victor, V.M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxid. Med. Cell Longev.* 2016: 1245049.

Dias, V., Junn, E., and Mouradian, M.M. (2013). The role of oxidative stress in Parkinson's disease. *J. Parkinsons Dis.* 3: 461–491.

Drechsel, D.A. and Patel, M. (2010). Respiration-dependent H_2O_2 removal in brain mitochondria via the thioredoxin/peroxiredoxin system. *J. Biol. Chem*. 285: 27850–27858.

Duarte-Jurado, A.P., Gopar-Cuevas, Y., Saucedo-Cardenas, O., Loera-Arias, M.J., Montes-de-Oca-Luna, R., Garcia-Garcia, A., and Rodriguez-Rocha, H. (2021). Antioxidant therapeutics in Parkinson's disease: current challenges and opportunities. *Antioxidants (Basel)* 10: 453.

Ferrante, R.J., Browne, S.E., Shinobu, L.A., Bowling, A.C., Baik, M.J., Macgarvey, U., Kowall, N.W., Brown, R.H., Jr., and Beal, M.F. (1997). Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J. Neurochem*. 69: 2064–2074.

Gabbita, S.P., Lovell, M.A., and Markesbery, W.R. (1998). Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem*. 71: 2034–2040.

Gella, A. and Durany, N. (2009). Oxidative stress in Alzheimer disease. *Cell Adh. Migr*. 3: 88–93.

Gogna, T., Housden, B.E., and Houldsworth, A. (2024). Exploring the role of reactive oxygen species in the pathogenesis and pathophysiology of Alzheimer's and Parkinson's disease and the efficacy of antioxidant treatment. *Antioxidants (Basel)* 13: 1138.

Herrero-Mendez, A., Almeida, A., Fernandez, E., Maestre, C., Moncada, S., and Bolanos, J.P. (2009). The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat. Cell Biol*. 11: 747–752.

Hijioka, M., Inden, M., Yanagisawa, D., and Kitamura, Y. (2017). DJ-1/PARK7: a new therapeutic target for neurodegenerative disorders. *Biol. Pharm. Bull.* 40: 548–552.

Hindle, S.J., Hebbar, S., Schwudke, D., Elliott, C.J.H., and Sweeney, S.T. (2017). A saposin deficiency model in Drosophila: lysosomal storage, progressive neurodegeneration and sensory physiological decline. *Neurobiol. Dis.* 98: 77–87.

Holzerova, E., Danhauser, K., Haack, T.B., Kremer, L.S., Melcher, M., Ingold, I., Kobayashi, S., Terrile, C., Wolf, P., Schaper, J., et al. (2016). Human thioredoxin 2 deficiency impairs mitochondrial redox homeostasis and causes early-onset neurodegeneration. *Brain* 139: 346–354.

Hong, C., Seo, H., Kwak, M., Jeon, J., Jang, J., Jeong, E.M., Myeong, J., Hwang, Y.J., Ha, K., Kang, M.J., et al. (2015). Increased TRPC5 glutathionylation contributes to striatal neuron loss in Huntington's disease. *Brain* 138: 3030–3047.

Im, J.Y., Lee, K.W., Woo, J.M., Junn, E., and Mouradian, M.M. (2012). DJ-1 induces thioredoxin 1 expression through the Nrf2 pathway. *Hum. Mol. Genet*. 21: 3013–3024.

Israelsen, W.J. and Vander Heiden, M.G. (2015). Pyruvate kinase: function, regulation and role in cancer. *Semin. Cell Dev. Biol.* 43: 43–51.

Jomova, K., Baros, S., and Valko, M. (2012). Redox active metal-induced oxidative stress in biological systems. *Transition Met. Chem.* 37: 127–134.

Junghans, M., John, F., Cihankaya, H., Schliebs, D., Winklhofer, K.F., Bader, V., Matschke, J., Theiss, C., and Matschke, V. (2022). ROS scavengers decrease γH2ax spots in motor neuronal nuclei of ALS model mice in vitro. *Front. Cell Neurosci.* 16: 963169.

Kalita, J., Misra, U.K., Singh, L.S., and Tiwari, A. (2019). Oxidative stress in status epilepticus: a clinical-radiological correlation. *Brain Res*. 1704: 85–93.

Lebovitz, R.M., Zhang, H., Vogel, H., Cartwright, J., Jr., Dionne, L., Lu, N., Huang, S., and Matzuk, M.M. (1996). Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 592: 718–727.

Lupoli, F., Vannocci, T., Longo, G., Niccolai, N., and Pastore, A. (2018). The role of oxidative stress in Friedreich's ataxia. *FEBS Lett*. 592: 718–727.

Madhwani, K.R., Sayied, S., Ogata, C.H., Hogan, C.A., Lentini, J.M., Mallik, M., Dumouchel, J.L., Storkebaum, E., Fu, D., and O'Connor-Giles, K.M. (2024). tRNA modification enzyme-dependent redox homeostasis regulates synapse formation and memory. *Proc. Natl. Acad. Sci. U. S. A*. 121: e2317864121.

Martinat, C., Shendelman, S., Jonason, A., Leete, T., Beal, M.F., Yang, L., Floss, T., and Abeliovich, A. (2004). Sensitivity to oxidative stress in DJ-1-deficient dopamine neurons: an ES-derived cell model of primary Parkinsonism. *PLoS Biol*. 2: e327.

Mccord, J.M., Roy, R.S., and Schaffer, S.W. (1985). Free radicals and myocardial ischemia. The role of xanthine oxidase. *Adv. Myocardiol* 5: 183–189.

Milton, V.J., Jarrett, H.E., Gowers, K., Chalak, S., Briggs, L., Robinson, I.M., and Sweeney, S.T. (2011). Oxidative stress induces overgrowth of the Drosophila neuromuscular junction. *Proc. Natl. Acad. Sci. U. S. A*. 108: 17521–17526.

Nonn, L., Williams, R.R., Erickson, R.P., and Powis, G. (2003). The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. *Mol. Cell. Biol*. 23: 916–922.

Oliver, P.L., Finelli, M.J., Edwards, B., Bitoun, E., Butts, D.L., Becker, E.B., Cheeseman, M.T., Davies, B., and Davies, K.E. (2011). Oxr1 is essential for protection against oxidative stress-induced neurodegeneration. *PLoS Genet*. 7: e1002338.

Oswald, M.C., Brooks, P.S., Zwart, M.F., Mukherjee, A., West, R.J., Giachello, C.N., Morarach, K., Baines, R.A., Sweeney, S.T., and Landgraf, M. (2018). Reactive oxygen species regulate activity-dependent neuronal plasticity in Drosophila. *Elife* 7: e39393.

Papadia, S., Soriano, F.X., Leveille, F., Martel, M.A., Dakin, K.A., Hansen, H.H., Kaindl, A., Sifringer, M., Fowler, J., Stefovska, V., et al. (2008). Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat. Neurosci.* 11: 476–487.

Papadia, S., Stevenson, P., Hardingham, N.R., Bading, H., and Hardingham, G.E. (2005). Nuclear Ca^{2+} and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. *J. Neurosci.* 25: 4279–4287.

Parenti, G., Medina, D.L., and Ballabio, A. (2021). The rapidly evolving view of lysosomal storage diseases. *EMBO Mol. Med.* 13: e12836.

Patel, M. (2016). Targeting oxidative stress in central nervous system disorders. *Trends Pharmacol. Sci*. 37: 768–778.

Peng, J.J., Lin, S.H., Liu, Y.T., Lin, H.C., Li, T.N., and Yao, C.K. (2019). A circuit-dependent ROS feedback loop mediates glutamate excitotoxicity to sculpt the Drosophila motor system. *eLife* 8: e47372.

Qiu, J., Dando, O., Febery, J.A., Fowler, J.H., Chandran, S., and Hardingham, G.E. (2020). Neuronal activity and its role in controlling antioxidant genes. *Int. J. Mol. Sci*. 21: 1933.

Quinlan, C.L., Perevoshchikova, I.V., Hey-Mogensen, M., Orr, A.L., and Brand, M.D. (2013). Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. *Redox Biol*. 1: 304–312.

Rebelo, A.P., Eidhof, I., Cintra, V.P., Guillot-Noel, L., Pereira, C.V., Timmann, D., Traschutz, A., Schols, L., Coarelli, G., Durr, A., et al. (2021). Biallelic loss-of-function variations in PRDX3 cause cerebellar ataxia. *Brain* 144: 1467–1481.

Rybnikova, E., Damdimopoulos, A.E., Gustafsson, J.A., Spyrou, G., and Pelto-Huikko, M. (2000). Expression of novel antioxidant thioredoxin-2 in the rat brain. *Eur. J. Neurosci.* 12: 1669–1678.

Shih, A.Y., Johnson, D.A., Wong, G., Kraft, A.D., Jiang, L., Erb, H., Johnson, J.A., and Murphy, T.H. (2003). Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *J. Neurosci.* 23: 3394–3406.

Sinet, P.M., Heikkila, R.E., and Cohen, G. (1980). Hydrogen peroxide production by rat brain *in vivo*. *J. Neurochem*. 34: 1421–1428.

Smith, R.G., Henry, Y.K., Mattson, M.P., and Appel, S.H. (1998). Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Ann. Neurol.* 44: 696–699.

Sobrido-Camean, D., Oswald, M.C.W., Bailey, D.M.D., Mukherjee, A., and Landgraf, M. (2022). Activity-regulated growth of motoneurons at the neuromuscular junction is mediated by NADPH oxidases. *Front. Cell Neurosci.* 16: 1106593.

Sorolla, M.A., Reverter-Branchat, G., Tamarit, J., Ferrer, I., Ros, J., and Cabiscol, E. (2008). Proteomic and oxidative stress analysis in human brain samples of Huntington disease. *Free Radic. Biol. Med.* 45: 667–678.

Stockwell, B.R., Friedmann Angeli, J.P., Bayir, H., Bush, A.I., Conrad, M., Dixon, S.J., Fulda, S., Gascon, S., Hatzios, S.K., Kagan, V.E., et al. (2017). Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 171: 273–285.

Stolz, D.B., Zamora, R., Vodovotz, Y., Loughran, P.A., Billiar, T.R., Kim, Y.M., Simmons, R.L., and Watkins, S.C. (2002). Peroxisomal localization of inducible nitric oxide synthase in hepatocytes. *Hepatology* 36: 81–93.

Subbarao, K.V., Richardson, J.S., and Ang, L.C. (1990). Autopsy samples of Alzheimer's cortex show increased peroxidation *in vitro*. *J. Neurochem*. 55: 342–345.

Tamagno, E., Robino, G., Obbili, A., Bardini, P., Aragno, M., Parola, M., and Danni, O. (2003). H2O2 and 4-hydroxynonenal mediate amyloid β-induced neuronal apoptosis by activating JNKs and p38MAPK. *Exp. Neurol*. 180: 144–155.

Tian, R., Abarientos, A., Hong, J., Hashemi, S.H., Yan, R., Drager, N., Leng, K., Nalls, M.A., Singleton, A.B., Xu, K., et al. (2021). Genome-wide CRISPRi/a screens in human neurons link lysosomal failure to ferroptosis. *Nat. Neurosci.* 24: 1020–1034.

Turrens, J.F. and Boveris, A. (1980). Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem. J*. 191: 421–427.

Ugbode, C., Garnham, N., Fort-Aznar, L., Evans, G.J.O., Chawla, S., and Sweeney, S.T. (2020). JNK signalling regulates antioxidant responses in neurons. *Redox Biol*. 37: 101712.

Varma, S. (2003). F2-isoprostane and neuron-specific enolase in cerebrospinal fluid after severe traumatic brain injury in infants and children. *J. Neurotrauma*. 20: 781–786.

Volkert, M.R. and Crowley, D.J. (2020). Preventing neurodegeneration by controlling oxidative stress: the role of OXR1. *Front. Neurosci.* 14: 611904.

Wang, J., Rousseau, J., Kim, E., Ehresmann, S., Cheng, Y.T., Duraine, L., Zuo, Z., Park, Y.J., Li-Kroeger, D., Bi, W., et al. (2019). Loss of oxidation resistance 1, OXR1, is associated with an autosomal-recessive neurological disease with cerebellar atrophy and lysosomal dysfunction. *Am. J. Hum. Genet*. 105: 1237–1253.

Ward, R.J., Zucca, F.A., Duyn, J.H., Crichton, R.R., and Zecca, L. (2014). The role of iron in brain ageing and neurodegenerative disorders. *Lancet Neurol.* 13: 1045–1060.

Wei, Y., Miao, Q., Zhang, Q., Mao, S., Li, M., Xu, X., Xia, X., Wei, K., Fan, Y., Zheng, X., et al. (2023). Aerobic glycolysis is the predominant means of glucose metabolism in neuronal somata, which protects against oxidative damage. *Nat. Neurosci*. 26: 2081–2089.

Wilson, K.A., Bar, S., Dammer, E.B., Carrera, E.M., Hodge, B.A., Hilsabeck, T.A.U., Bons, J., Brownridge, G.W., III, Beck, J.N., Rose, J., et al. (2024). OXR1 maintains the retromer to delay brain aging under dietary restriction. *Nat. Commun*. 15: 467.

Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M., Stadtman, E.R., and Mizuno, Y. (1996). Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. P*roc. Natl. Acad. Sci. U. S. A.* 93: 2696–2701.

Zhang, Y., Roh, Y.J., Han, S.J., Park, I., Lee, H.M., Ok, Y.S., Lee, B.C., and Lee, S.R. (2020). Role of selenoproteins in redox regulation of signaling and the antioxidant system: a review. *Antioxidants (Basel)* 9: 383.