



Noxious Iron–Calcium Connections in Neurodegeneration

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Iron and calcium share the common feature of being essential for normal neuronal function. Iron is required for mitochondrial function, synaptic plasticity, and the development of cognitive functions whereas cellular calcium signals mediate neurotransmitter exocytosis, axonal growth and synaptic plasticity, and control the expression of genes involved in learning and memory processes. Recent studies have revealed that cellular iron stimulates calcium signaling, leading to downstream activation of kinase cascades engaged in synaptic plasticity. The relationship between calcium and iron is Janus-faced, however. While under physiological conditions iron-mediated reactive oxygen species generation boosts normal calcium-dependent signaling pathways, excessive iron levels promote oxidative stress leading to the upsurge of unrestrained calcium signals that damage mitochondrial function, among other downstream targets. Similarly, increases in mitochondrial calcium to non-physiological levels result in mitochondrial dysfunction and a predicted loss of iron homeostasis. Hence, if uncontrolled, the iron/calcium self-feeding cycle becomes deleterious to neuronal function, leading eventually to neuronal death. Here, we review the multiple cell-damaging responses generated by the unregulated iron/calcium self-feeding cycle, such as excitotoxicity, free radical-mediated lipid peroxidation, and the oxidative modification of crucial components of iron and calcium homeostasis/signaling: the iron transporter DMT1, plasma membrane, and intracellular calcium channels and pumps. We discuss also how iron-induced dysregulation of mitochondrial calcium contributes to the generation of neurodegenerative conditions, including Alzheimer's disease (AD) and Parkinson's disease (PD).

Keywords: neurodegenerative diseases, reactive oxygen species, mitochondria, HIF-1, Nrf-2, inflammation, ferroptosis

INTRODUCTION

Iron and calcium ions are both essential for maintaining normal brain function. Iron is required for oxidative phosphorylation, Krebs cycle, iron–sulfur cluster and heme synthesis, synaptic plasticity, and the development of cognitive functions (Oexle et al., 1999; Bolton et al., 2000; Hidalgo and Núñez, 2007; Tong and Rouault, 2007; Zorov et al., 2007; Huang et al., 2011; Muñoz et al., 2011; Murray-Kolb, 2013; Mena et al., 2015; Requejo-Aguilar and Bolanos, 2016), while increases in cytoplasmic calcium concentration known as calcium signals mediate the secretion

of neurotransmitters, synaptic plasticity, and axonal growth (Berridge, 1998). Moreover, nuclear calcium signals command the expression of neuronal genes involved in learning and memory processes (Bading, 2013).

Recent studies have revealed that cellular iron stimulates neuronal calcium signaling, leading to downstream activation of kinase cascades engaged in synaptic plasticity, a neuronal response associated to memory and learning (Hidalgo and Núñez, 2007; Hidalgo et al., 2007; Muñoz et al., 2011). The relationship between calcium and iron is Janus-faced, however. Dysregulation of iron levels induces calcium dyshomeostasis and abnormal calcium signaling, whereas increased calcium levels enhance redox-active iron levels; neurodegenerative conditions exhibit both types of dysregulation. Thus, current evidence indicates that increased intracellular calcium and aberrant calcium signaling occur in neurodegenerative disorders that include Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) (reviewed in Pchitskaya et al., 2018). Similarly, disruption of neuronal iron homeostasis linked to iron accumulation is a common feature in a plethora of neurodegenerative diseases, which included and other parkinsonisms (Lee et al., 2013), AD (Bulk et al., 2018), Friedrich's ataxia, pantothenate kinase-associated neurodegeneration (Swaiman, 1991), and other neuronal pathologies that entail brain iron accumulation (Wiethoff and Houlden, 2017).

Here, we will review the evidence that points to a rarely acknowledged relationship between iron homeostasis and calcium homeostasis and signaling, in which dysregulation of one of these processes results in dysregulation of the other, generating a vicious cycle that ends up in neuronal death. The multiple cell-damaging responses generated by the unregulated iron/calcium self-feeding cycle, such as excitotoxicity, free radical-mediated lipid peroxidation, and the oxidative modification of crucial components of iron and calcium homeostasis and signaling, including the iron transporter DMT1, plasma membrane and intracellular calcium channels and pumps will be presented. This review article concludes with a discussion of how dysregulation of calcium homeostasis and signaling contributes to the generation of neurodegenerative diseases with an iron accumulation component, focusing in particular on AD and PD.

IRON HOMEOSTASIS IN NEURONAL CELLS

Through the Fenton reaction, redox-active iron is a net producer of the hydroxyl radical, the most reactive radical species in nature (Davies, 2005). In a reductive environment, such as the intracellular milieu, through redox cycling redox-active iron promotes the production of hydroxyl radicals at the expense of O₂ and GSH consumption (Núñez et al., 2012). Consequently, under physiological conditions, redox-active iron levels that conform the labile iron pool are very tightly regulated to a cytosolic concentration of 0.5–1.5 μM, which comprises <5% of total intracellular iron (Cabantchik, 2014). In mammals, cellular iron levels are maintained by transcriptional, translational, and

vesicular flux mechanisms (Núñez, 2010). The iron regulatory protein (IRPs: IRP1 and IRP2)/iron-responsive element (IRE) is the best-characterized system that regulates at the translation level the expression of iron homeostasis proteins. These proteins include the transferrin receptor 1 (TfR1, involved in iron uptake), the divalent metal transporter 1 (DMT1, involved in iron uptake), ferroportin 1 (FPN1, involved in iron efflux from cells), and ferritin (involved in iron storage) (Wilkinson and Pantopoulos, 2014). The IRP/IRE is a plentiful-oriented system, since it is activated under low iron conditions to ensure an adequate supply of iron for cellular processes. Of note, the IRE–IRP regulatory system is not only regulated by cellular iron status but it is also regulated by reactive oxygen species (ROS), whereby cells elicit a defense mechanism against iron toxicity and iron-catalyzed oxidative stress (Ray et al., 2012).

The iron uptake protein DMT1 (SLC11A2) is a crucial component of cell iron homeostasis. *SLC11A2* transcription generates four alternatively spliced mRNAs that differ at their 5'-untranslated region (coding for the DMT1 isoforms 1A and 1B) and at its 3'-untranslated region (coding for isoforms +IRE and -IRE) (Garrick et al., 2006). Thus, the expression of the 1A and 1B isoforms of DMT1 is subjected to differential transcriptional regulation.

THE CRUCIAL RELATIONSHIP BETWEEN IRON AND THE HYPOXIA-INDUCIBLE TRANSCRIPTION FACTOR (HIF)

At the systemic level, the hypoxia-inducible transcription factor (HIF) transcription factor family coordinates the cellular response to low oxygen levels by regulating the expression of a large array of target genes during hypoxia, which results in adaptive changes in the hematopoietic, cardiovascular, and respiratory systems (Smith et al., 2008; Mole et al., 2009). The HIF-1 is kept at basal levels by HIF prolyl hydroxylase domain (PHD) enzymes; prolyl-hydroxylation of HIF-1 via PHD signals for its degradation via the ubiquitin-proteasome system (Bruick and McKnight, 2001; Myllyharju, 2013; Yeh et al., 2017). The PHD enzymes are both oxygen- and iron-dependent; thus, hypoxia and iron chelation results in decreased PHD activity and increased HIF-1α activity (Hewitson et al., 2003; Nandal et al., 2011; Flagg et al., 2012).

In recent years, a series of iron chelating agents that exert neuroprotective effects have been developed (Núñez and Chana-Cuevas, 2018). In particular, M30, which is an 8-hydroxyquinoline-based iron chelator developed by the group of Moussa Youdim at Technion-Israel Institute of Technology (Weinreb et al., 2016), stabilizes HIF-1α, most probably by inactivating HIF-1α PHD. In the brain, HIF-1α stabilization by M30 leads to the expression of a broad number of neuroprotective-adaptive mechanisms and pro-survival signaling pathways (Kupershmidt et al., 2011). Real-time RT-PCR revealed that M30 differentially induces the expression of a variety of cellular components, including vascular

endothelial growth factor, erythropoietin, enolase-1, TfR1, heme oxygenase-1, inducible nitric oxide synthase (iNOS), glucose transporter 1, brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor, and the antioxidant enzymes catalase, superoxide dismutase-1, and glutathione peroxidase (Kupersmidt et al., 2009). Further reports have supported the role of iron chelators in inducing neuronal survival pathways (Mechlovich et al., 2014; Guo et al., 2015, 2016; Xiao et al., 2015). It follows that the capacity of iron chelators to induce HIF-1 α -mediated neuroprotection adds to the recognized neuroprotective effects of iron chelators, through their ability to prevent hydroxyl radical production via the Fenton reaction. A tempering note comes from the report that treatment of human skin cells with the iron chelator *N*-(2-hydroxybenzyl)-*L*-serine (HBSer) does not induce HIF-1 α activation, as opposed to desferrioxamine (DFO) and salicylaldehyde isonicotinoyl hydrazone (SIH) used as positive controls (Creighton-Gutteridge and Tyrrell, 2002). The authors conjectured that the lack of HIF-1 α activation by HBSer might be related to its lower affinity for iron as compared to DFO and SIH. Of relevance to the theme of this review, however, is the fact that the transcription factor HIF-1 α activates the expression of several genes associated with iron homeostasis (Lee and Andersen, 2006), which in non-excitabile cells results in an increase in cellular iron content (Qian et al., 2011).

INCREASED REACTIVE OXYGEN/NITROGEN SPECIES GENERATION INDUCES IRON DYSHOMEOSTASIS

A significant number of studies have shown that physiological levels of ROS and reactive nitrogen species (RNS) act as signaling molecules in a variety of biological responses (Sen, 2001; Ray et al., 2012; Asimwe et al., 2016; Lourenco et al., 2017; Moldogazieva et al., 2018; Nemes et al., 2018). The brain is an organ highly susceptible to oxidative stress (Cobley et al., 2018). Hence, neuronal cells have to maintain physiological levels of ROS and RNS to avoid oxidative or nitrosative stress, which arises when excessive ROS/RNS production overcomes the cellular antioxidant systems, which by affecting the redox environment favors excitotoxicity. Henceforth, we will use the broad term oxidative stress to refer to both oxidative and nitrosative stress. Oxidative stress perturbs the function of many cellular processes; it causes glutathione deficiency (Wu et al., 2004) and promotes cell death by inducing mitochondrial depolarization, membrane lipid peroxidation, and DNA damage, among other harmful effects. To counteract the damaging effects of excess ROS/RNS (Duncan and Heales, 2005; Halliwell, 2006; Moncada and Bolaños, 2006), cells make use of enzymatic and non-enzymatic defense mechanisms, some of which engage calcium signals (Gordeeva et al., 2003). Depending on the extent and persistence of the cellular damage, oxidative stress may play a key role as a causative agent of neurodegenerative disease emergence and or progression.

Iron uptake into primary hippocampal neurons stimulates ROS generation (Muñoz et al., 2011), and decreases the redox potential established by the intracellular levels of oxidized and reduced glutathione in neuroblastoma cells (Núñez et al., 2004). The relationship between increased redox-active iron and increased oxidative stress is well accepted (Núñez et al., 2012). Similarly, in cellular models convincing evidence points to a link between increased oxidative tone and iron accumulation. Initial observations indicated that hydrogen peroxide activates IRP1 (Martins et al., 1995; Pantopoulos and Hentze, 1995; Hanson and Leibold, 1999; Caltagirone et al., 2001; Mueller et al., 2001), and that this activation results in increased cellular iron uptake mediated by increased translation of TfR1 and decreased ferritin synthesis (Caltagirone et al., 2001; Sureda et al., 2005). A subsequent study reported that, besides activation of IRP1, exposure of SH-SY5Y cells to H₂O₂ results in the degradation of the exo-transporter FPN1, which coupled to a decrease in ferritin translation leads to a time-dependent increase in the cellular labile iron pool (Dev et al., 2015), with the ensuing generation of oxidative damage through the production of hydroxyl radicals by the Fenton reaction. In animal models, evidence linking increased ROS as a cause, and iron homeostasis loss as an effect, is still lacking. An early report indicated that vitamin A deficiency results in increased hepcidin mRNA expression and increased iron spleen concentrations, accompanied by increased protein oxidation. These results suggest that vitamin A protects the liver against protein oxidation by maintaining iron homeostasis (Arruda et al., 2009). In a mouse model of experimentally induced hemolysis, excess heme increases intracellular ROS production, which signals for the induction of the unique iron exporter ferroportin, resulting in iron export from macrophages (Jeney et al., 2002; Marro et al., 2010). Of note, decreasing ROS levels by treatment with the antioxidant *N*-acetylcysteine prevents ferroportin induction and normalizes intracellular iron levels in a mice model of experimentally induced hemolysis (Tangudu et al., 2018). In a separate study using the APP^{swe}/PS1 Δ E9 AD mice model, administration for 3 months of antioxidants of plant origin decreased iron levels and malondialdehyde content and increased antioxidant defenses (Zhang et al., 2018). Overall, these results are consistent with the notion that oxidative stress dysregulates iron homeostasis both in cellular and animal models.

Nitric oxide also modifies IRP1 activity; it increases IRP1 binding to IRE and the increase IRE binding activity of IRP1 is coincident with inhibited ferritin synthesis and increased TfR1 mRNA levels (Phillips et al., 1996). Nitric oxide activates IRP1 directly by destabilizing the 4Fe-4S cluster of IRP1 (Drapier et al., 1993; Soum and Drapier, 2003), and may activate IRP1 indirectly by mobilizing intracellular Fe, thus decreasing the intracellular labile iron pool and activating IRP1 (Pantopoulos et al., 1996; Wardrop et al., 2000).

Altogether, these studies point to a robust association between increased ROS, nitric oxide, and increased levels of redox-active iron, which in turn mediate oxidative damage to lipids, protein, and nucleic acids. Yet, a recent study showed that in cultured macrophages, the labile iron pool apparently attenuates peroxynitrite-dependent damage (Damasceno et al., 2018). Whether this protective effect of the labile iron pool is

translatable to other cell types and to neurons in particular, remains to be established.

IRON DYSHOMEOSTASIS IN NEURODEGENERATION

Increased iron levels and the associated ROS generation are important initiators and mediators of cell death (Dixon and Stockwell, 2014), whereas iron dyshomeostasis has a key role in neurodegeneration. Mutations of genes encoding proteins involved in iron homeostasis are associated with degeneration of central nervous system (CNS) cells (Ponka, 2004; Zecca et al., 2004), while oxidative stress induces cell injury by disrupting cellular iron balance (Carocci et al., 2018; Kerins and Ooi, 2018), thus leading to a vicious circle. Both AD and PD entail increased brain iron content and oxidative stress (Crichton et al., 2011; Ward et al., 2014; Tonnie and Trushina, 2017; Guo et al., 2018), with the implied threat of hydroxyl radical-induced neuronal damage. Of note, binding of Cu or Fe to A β peptides generates H₂O₂ (Huang et al., 1999), which may induce neuronal oxidative stress during AD progression (Cristovao et al., 2016). Moreover, iron overload increases neuronal amyloid- β production and enhances cognitive impairment in a transgenic mice model of AD (Becerril-Ortega et al., 2014) while accumulating evidence suggests that impaired iron homeostasis is an early event in AD progression (Peters et al., 2015).

Parkinson's disease is a progressive neurodegenerative disease characterized by rigidity, tremor, and slowness of movement as well as by a wide range of debilitating non-motor symptoms. A key feature of PD is the selective loss of the dopaminergic neurons of the substantia nigra; ample evidence shows iron accumulation in these neurons (Jellinger, 1999; Zecca et al., 2004; Berg and Youdim, 2006; Muñoz et al., 2016; An et al., 2018). The observation that pharmacological agents with iron chelation capacity prevent neuronal death in AD or PD experimental models (recently reviewed in Núñez and Chana-Cuevas, 2018) highlights the pivotal role of iron as a mediator of neuronal death in AD and PD.

In addition, spinal neurons from ALS patients display increased iron and calcium levels (Kasarskis et al., 1995). The success of iron chelation therapy in ALS mouse models sustains the central role of iron in ALS pathogenesis (Jeong et al., 2009; Kupersmidt et al., 2009). Alterations of proteins involved in iron metabolism, inhibition of anterograde axonal transport leading to iron accumulation in ventral motor neurons, and increased mitochondrial iron load in neurons and glia have been proposed as pathogenic mechanisms to explain the abnormal iron accumulation displayed by the neurons and glia of ALS mice (Jeong et al., 2009).

IRON DYSHOMEOSTASIS IN INFLAMMATION

Inflammation, a condition often found in neurodegenerative environments, is yet another factor that induces iron

accumulation (reviewed in Urrutia et al., 2014). Both the p50 and the p65 subunits of the transcription factor NF- κ B bind to a NF- κ B-responsive element present in the 5' untranslated region of the DMT1-1B promoter, inducing the expression of the 1B isoforms of DMT1 (Paradkar and Roth, 2006a,b). The transcription factor NF- κ B is often activated by inflammatory signals (Rothwell and Luheshi, 2000; Hanke and Kielian, 2011; Shih et al., 2015). Accordingly, the possible association between inflammation and iron accumulation has been recently explored (Pelizzoni et al., 2013; Urrutia et al., 2013, 2014; McCarthy et al., 2018). To this purpose, the effects of lipopolysaccharide (LPS) and the pro-inflammatory cytokines TNF- α and IL-6 on mRNA and protein levels of DMT1, FPN1, and hepcidin were investigated in astrocytes, microglia, and neurons isolated from rat brain. A causal association between inflammation and iron accumulation was reported, since in addition to increasing neuronal iron content, treatment with LPS, TNF- α , or IL-6 increases DMT1 expression and protein levels in neurons (Urrutia et al., 2013). Similar results were reported in studies of ventral mesencephalic neurons in primary culture, in which treatment with TNF- α or IL-1 β induces an increment in DMT1 and TfR1 protein levels, together with a reduction of FPN1 levels (Wang et al., 2013). Considering that NF- κ B activation is downstream of the TNF- α , IL-1, and LPS signaling pathways, these results are consistent with a circuit in which inflammatory stimuli induce DMT1 expression and iron accumulation via NF- κ B activation and hepcidin expression.

An additional link between inflammation and increased oxidative stress resides in the observation that inflammatory stimuli activate the NADPH oxidase (NOX) enzyme, the main regulated source of cellular ROS generation (Green et al., 2001; Urrutia et al., 2014; Sorce et al., 2017). In particular, pro-inflammatory cytokines activate the phagocytic NOX2 enzyme, which is highly expressed in microglia (Sorce and Krause, 2009; Lim et al., 2013; Barrett et al., 2017). In turn, NOX2 activation generates an oxidative extracellular environment that increases ROS levels in neighboring neurons (Schiavone et al., 2009; Gao et al., 2012; Surace and Block, 2012; Zhang et al., 2014).

Of note, NOX activation has also been reported in experimental models of PD and AD. In particular treatment with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a prodrug to the Parkinsonian neurotoxin 1-methyl-4-phenylpyridinium (MPP⁺), results in increased synthesis of the pro-inflammatory cytokine IL-1 β , and increased membrane translocation of the cytosolic NOX sub-unit p67^{phox}, which is prevented by minocycline, a tetracycline derivative that exerts multiple anti-inflammatory effects (Wu et al., 2002). In addition, iron supplementation to midbrain neuron-glia cell cultures causes the selective death of dopaminergic neurons. This death is mediated by microglial NOX2 since cells derived from NOX2^{-/-} mice do not present iron-induced neuronal death (Zhang et al., 2014). Similarly, brain tissue from AD patients reveals an elevation in NOX activity coupled to membrane translocation of cytosolic NOX2 subunits and increased NOX1 and NOX3 mRNA transcripts (Shimohama et al., 2000; Ansari and Scheff, 2011). Significantly, the authors found an inverse correlation between

postmortem NOX activity and ante-mortem cognitive status (Ansari and Scheff, 2011). Altogether, these results indicate that inflammatory stimuli generate a neuronal phenotype of increased iron influx and increased NOX-produced ROS, with the consequent risk of oxidative damage observed in neurodegenerative diseases.

ROLE OF IRON IN EXCITOTOXICITY

Earlier reports indicate that iron plays a role in excitotoxicity, a death process mediated by high levels of intracellular calcium caused by excessive activity of excitatory neurotransmitters. Seminal observations by Cheah and collaborators described a signaling cascade in which stimulation of *N*-methyl-D-aspartate (NMDA) receptors activates the small GTPase Dexas1 via S-nitrosylation, mediated by activation of neuronal nitric oxide synthase (nNOS). Activated Dexas binds to the peripheral benzodiazepine receptor-associated protein PAP7, which in turn binds to the iron transporter DMT1 (Cheah et al., 2006). Most importantly, treatment with neurotoxic concentrations of NMDA elicits a major increase in iron uptake, resulting in three- to fivefold increase of hydroxyl radical levels and the death of more than 90% of cells, whereas co-treatment with NMDA and a cell-permeant iron chelator largely blocked NMDA-induced cell death. Overall, these results indicate that glutamate-NMDA excitotoxicity elicits DMT1 activation and iron-mediated cell death (Cheah et al., 2006). Although the role of calcium was not explored, the authors hypothesized initial calcium entrance through the NMDA receptor may activate nNOS, thus initiating this neurodegenerative process. As discussed below, crosstalk between NMDA receptor-mediated calcium influx and iron is likely to have a relevant role in neurodegenerative diseases such as AD and PD.

NEURONAL CALCIUM HOMEOSTASIS AND SIGNALING

Calcium is a universal second messenger that regulates numerous cellular processes over a wide temporal range (Berridge et al., 2003). In neurons, the free calcium concentration at rest lies in the 70–100 nM range (Clapham, 2007), whereas extracellular calcium concentration is ~1.5 mM. This large concentration gradient, combined with resting potential values of 70–90 mV (negative inside), generate a significant electrochemical gradient that favors calcium entry. Calcium homeostasis and signaling are vital for cellular function and survival; thus, neuronal cells possess powerful systems to maintain calcium homeostatic and signaling systems (Berridge, 1998; Brini et al., 2014), which regulate the neuronal processes underlying synaptic plasticity and complex brain cognitive functions, such as information processing, learning, and memory. Calcium is stored also in intracellular organelles such as the endoplasmic reticulum (ER), where its free concentration lies in the 0.5 mM range. The main systems in charge of maintaining calcium homeostasis are two plasma membrane calcium (PMCA) transporters, the

PMCA pump and the sodium/calcium exchanger (NCX), plus the sarco/ER Ca^{2+} -ATPase (SERCA) pump that maintains the steep concentration gradient between the ER and the cytoplasm (Brini et al., 2014). These transporters utilize different mechanisms and display a range of calcium affinities, transport rates, and capacities to ensure the proper managing of the variety of intracellular increases in free calcium concentration, known as calcium signals, generated by the diverse stimuli neuronal cells receive.

Neuronal activity generates calcium signals that via sequential activation of calcium-dependent signaling cascades and transcription factors promote the expression of genes underlying dendritic development, synaptic plasticity, and neuronal survival (Brini et al., 2014). In response to neuronal stimulation, calcium influx through plasma membrane pathways, including agonist-operated, store-operated, or voltage-gated calcium channels (VGCCs), generates neuronal calcium signals. In addition, the release of calcium from intracellular stores such as the ER also contributes to neuronal calcium signal generation (Brini et al., 2014). All neuronal compartments crucial for neurotransmission have the two types of calcium release channels: the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) and the ryanodine receptor (RyR) channels (Berridge, 1998). These large channels have complex gating and regulation and mediate a cellular process known as calcium-induced calcium release (CICR), whereby calcium promotes its own release from intracellular calcium stores (Berridge, 1998). This CICR mechanism likely contributes to propagate calcium signals that reach distant targets such as the nucleus (Berridge, 1998), where calcium promotes short or long-lasting changes in neuronal function and structure (Bading, 2013).

ROS LEVELS INFLUENCE CALCIUM HOMEOSTASIS AND SIGNALING

Significant evidence gathered in the last years indicates that increased cellular ROS levels modify the function of key proteins engaged in calcium homeostasis and signaling (Gordeeva et al., 2003; Hidalgo and Donoso, 2008; Goriach et al., 2015). Redox-dependent regulation of components that maintain neuronal Ca^{2+} homeostasis may influence the direction and/or the efficiency of Ca^{2+} -signaling pathways. Interactions among ROS and calcium-dependent signaling pathways are bidirectional; while ROS regulate cellular calcium signaling, calcium signals are essential for ROS production because a number of ROS-generating and antioxidant systems of living cells are calcium-dependent (Gordeeva et al., 2003).

Reversible modifications of proteins via ROS-promoted oxidation of their amino acids modify the properties of neuronal proteins engaged in signal transduction, such as protein kinases, protein phosphatases, and transcription factors (Wang et al., 2012). An increase in cellular ROS levels results in the inhibition of NMDA receptor function and in decreased activity of both the PMCA and the SERCA calcium pumps, while it enhances the activity of IP_3R and RyR calcium channels (Hidalgo and Donoso, 2008; Joseph et al., 2018). Consequently, ROS generation in neuronal cells increases cellular calcium levels by promoting

calcium release mediated by IP₃R and RyR channels and by inhibiting both the PMCA and the SERCA pumps: the ROS-mediated increase in calcium levels promotes changes in several calcium-dependent neuronal pathways (Yermolaieva et al., 2000; Riquelme et al., 2011). Depending on the magnitude of the calcium increase, activation of kinases or phosphatases implicated in synaptic plasticity process will occur (reviewed in Hidalgo and Arias-Cavieres, 2016). In addition, activation of the large-conductance Ca²⁺-activated K⁺ (BKCa) by oxidation of SH cysteine residues, by decreasing neuronal excitability reduces the vulnerability of hippocampal neurons to hypoxia (Hepp et al., 2005).

DEFECTIVE CALCIUM HOMEOSTASIS AND SIGNALING IN NEURODEGENERATION

A link between excessive intracellular calcium accumulation and neuronal death is by now firmly established (Demuro et al., 2010; Stefani et al., 2012; Fukui, 2016). Neuronal cells are particularly sensitive to calcium-mediated cytotoxic damage and the failure of neurons to maintain Ca²⁺ homeostasis is a common feature of aged-linked neurodegenerative pathologies (Berrocal et al., 2015; Brini et al., 2017; Pchitskaya et al., 2018; Strehler and Thayer, 2018). Calcium dyshomeostasis (Pchitskaya et al., 2018) and altered neuronal calcium signaling (Chan et al., 2009; Overk et al., 2015) have been reported in a number of disease conditions, including AD, PD, and ALS (for recent reviews, see Franco-Iborra et al., 2018; Popugaeva et al., 2018; Post et al., 2018; Sirabella et al., 2018). Likewise, defective calcium signaling in glia and autophagy-related pathways has been implicated in neurodegenerative disease (Mustaly-Kalimi et al., 2018). Accruing evidence implicates decreased levels and functional decline of PMCA isoforms in neurodegeneration (Hajieva et al., 2018; Strehler and Thayer, 2018). In addition, in many pathological conditions, disruptions of ER Ca²⁺ signaling disrupt neuronal function and promote neuronal cell death (Okubo et al., 2018). To date, however, the detailed mechanisms of how neuronal Ca²⁺ homeostasis and signaling are perturbed in neurodegenerative diseases are not well understood.

The most common neurodegenerative disorder and the leading cause of dementia in the elderly is AD, whereas PD is the most prevalent age-associated movement disorder that entails early and selective degeneration of dopaminergic neurons. The pathological hallmarks of AD are neuronal loss and the presence of amyloid plaques and neurofibrillary tangles in the brain of affected individuals. Yet, before the emergence of plaques and/or neurofibrillary tangles other defects, including Ca²⁺ and iron dyshomeostasis, mitochondrial dysfunction, increased contacts between the ER and the mitochondria, neuroinflammation, and alterations in lipid metabolism have been reported (Mattson, 2010; Area-Gomez and Schon, 2017; Lane et al., 2018). How these early defects relate to the emergence and progression of AD remains to be established. Of note, a recent report by the Alzheimer's Association Calcium Hypothesis

Workgroup [AACHW] (2017) analyzed how defective calcium signaling may be an early upstream event in AD progression, and how alterations of the many cellular components that control calcium hemostasis and signaling leads to the decline in the function of more vulnerable neuronal cells. In particular, these more vulnerable neuronal cells present early synaptic dysfunction, upregulation of some calcium signaling related genes and significant decreases in the expression of genes related to synaptic neurotransmission, neurotrophic factor activity, mitochondrial metabolism, and energy production, among others (Alzheimer's Association Calcium Hypothesis Workgroup [AACHW], 2017).

In PD, dopaminergic neurons of the substantia nigra are especially vulnerable to Ca²⁺ dyshomeostasis, because their autonomous pace-making activity, through which these neurons generate action potentials in the absence of synaptic input (Guzman et al., 2009). This particular property of dopaminergic neurons imposes a high pressure on adequate handling of activity-generated Ca²⁺ signals, presumably mediated by the significant calcium influx through plasma membrane L-type calcium channels that markedly surpasses the calcium influx displayed by other neurons (Surmeier et al., 2010).

Dopaminergic neurons are highly vulnerable to oxidants and mitochondrial inhibition compared to other neuronal types (Sherer et al., 2003; Halliwell, 2006; Burbulla et al., 2017). Thus, brain permeant toxins such as rotenone, an inhibitor of NADH dehydrogenase, exclusively affect dopaminergic neurons (Sherer et al., 2003; Cannon et al., 2009). This special vulnerability is likely to produce the mitochondrial Ca²⁺ imbalance that occurs in PD (Ludtmann and Abramov, 2018). As discussed below, iron-induced ROS increases result in calcium signal generation, which may contribute to the basal calcium burden of dopaminergic neurons.

Several studies have reported defective Ca²⁺ signaling in other neurodegenerative conditions such as ALS (Siklos et al., 1998; Grosskreutz et al., 2010; Jaiswal, 2013; Muhling et al., 2014) and multiple sclerosis (MS; Gonsette, 2008). The death of motor neuron is a characteristic feature of ALS. Excitotoxicity, which involves glutamate and calcium overload, or cell death caused by high levels of intracellular calcium caused by excessive activity of excitatory neurotransmitters, may contribute to ALS pathology. Calcium dysregulation is also present in MS, where the associated disability results from neuronal and axonal loss initiated by microglia activation and mediated by oxidative stress, excitotoxicity, calcium dysregulation, and mitochondrial dysfunction, leading to proteolytic enzyme production and apoptosis (Gonsette, 2008).

Additionally, calcium dysregulation has been associated with neuroinflammation, a CNS response that comprises biochemical and cellular responses to injury, infection, or neurodegenerative diseases (DiSabato et al., 2016). Neuroinflammation markers increase in some neurodegenerative conditions; microglia, the innate CNS immune cells, play key roles as mediators of these neuroinflammatory responses. Of note, Ca²⁺ and neuroinflammatory signaling mechanisms exhibit extensive crosstalk and bidirectional interactions. As an example, chronic brain inflammation leads to a feedback reduction in NMDA

receptors caused by excess synaptic glutamate activity during microglial activation (Rosi et al., 2004).

Despite the abundant literature that points to defective calcium homeostasis and signaling in neurodegeneration, scarce information is available in the literature regarding the protective effects of calcium modulators in preventing these defects. Yet, all recent reports on this subject highlight the fact that this is an urgent task. The non-competitive NMDA receptor antagonist Memantine, which reduces excitotoxicity by inhibiting neuronal calcium entry (Bezprozvanny and Mattson, 2008), has been the most common drug for treating moderate to severe AD; nevertheless, Memantine provides only limited benefits for AD patients. A recent report showed that immunotherapy with a murine analog of the anti-A β antibody Aducanumab restores calcium homeostasis in a transgenic AD mice model (Kastanenka et al., 2016), whereas a recent review addresses the evidence linking neurodegeneration to RyR dysfunction and describes novel therapeutic approaches to target abnormal RyR function (Kushnir et al., 2018). There are no reports, however, on the efficacy of these strategies for treating AD patients.

THE HIF-1-CALCIUM RELATIONSHIP

In SH-SY5Y neuroblastoma cells, chelation of calcium by 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) induces HIF-1 α protein accumulation and nuclear localization, whereas the cytosolic calcium elevation produced by inhibition of the SERCA pump attenuates these effects (Berchner-Pfannschmidt et al., 2004). Further experiments indicated that calcium chelation attenuates the interaction of HIF-1 α with the von-Hippel-Lindau protein, thus decreasing the proteasomal degradation of HIF-1 α (Berchner-Pfannschmidt et al., 2004). Control experiments indicated that BAPTA did not complex intracellular iron ions, thus the above effects were caused by calcium chelation and not by stimulation of iron-activated PHD enzymes (Berchner-Pfannschmidt et al., 2004). Subsequent studies showed that intermittent hypoxia causes HIF-1 α accumulation through a mechanism that involves increased cell calcium and increased generation of ROS by NOX, which induces a complex signaling pathway that results in increased HIF-1 α synthesis and decreased hydroxylase-dependent HIF-1 α degradation (Yuan et al., 2008).

Although brain levels of HIF-1 α are considered neuroprotective (Kietzmann et al., 2001; Siddiq et al., 2005; Guo et al., 2016; Lee et al., 2017; Merelli et al., 2018), under certain conditions, HIF-1 α acts as a pro-apoptotic factor (Piret et al., 2002; Zhang et al., 2007; Merelli et al., 2018). For example, the anesthetic isoflurane induces apoptotic neurodegeneration in a process mediated by HIF-1 α , since knockdown of HIF-1 α expression attenuates isoflurane-induced neurotoxicity (Jiang et al., 2012). In addition, isoflurane induces a significant elevation of cytoplasmic calcium levels in cultured neurons. Based on previous observations, showing that calcium mediates HIF-1 α expression (Yuan et al., 2005; Riganti et al., 2009), the authors hypothesized that the high calcium levels induced by isoflurane

promote HIF-1 α -mediated apoptotic neurodegeneration. Studies discerning the calcium concentrations and other cell physiological condition, which elicit each of the above responses, should shed light on these apparent contradictory responses.

Overall, the emerging picture from the above results is consistent with the idea that low calcium concentrations stabilize HIF-1 α protein levels by decreasing its proteasomal degradation. The effect of calcium concentrations above homeostatic levels is not clear and may depend on cell type: high calcium levels may induce a protective response consisting in increased HIF-1 α synthesis coupled to decreased degradation or may mediate HIF-1 α degradation.

IRON MODIFIES NEURONAL CALCIUM HOMEOSTASIS AND CALCIUM SIGNALING PATHWAYS

Iron overload increases intracellular calcium levels, which in turn activate calcineurin (Lee et al., 2016), a calcium-dependent phosphatase which has a key role in synaptic plasticity and memory processes (Baumgartel and Mansuy, 2012). Increases in cytoplasmic calcium levels via the NMDA receptor stimulate the neuronal NOS enzyme (Ghosh and Salerno, 2003) and generate superoxide anion in hippocampal neurons (Brennan et al., 2009), while in cortical neurons, this calcium increase induces a signaling cascade that enhances superoxide generation through NOX activation (Girouard et al., 2009).

Both synaptic plasticity and memory processes require activity-dependent hippocampal ROS generation (Bindokas et al., 1996; Kahlert et al., 2005; Kishida and Klann, 2007). In primary hippocampal neurons, the ROS increase induced by iron promotes calcium release from the ER mediated by redox-sensitive RyR channels; the resulting increase in intracellular calcium levels link NMDA receptor stimulation to ERK1/2 activation and nuclear translocation (Muñoz, 2012). Since several reports indicate that CREB-dependent transcription of synaptic plasticity related genes entails ERK1/2-mediated long-term CREB phosphorylation (Wu et al., 2001; Lynch, 2004; Thomas and Haganir, 2004). Accordingly, these findings indicate that iron-induced, RyR-mediated, calcium release contributes to this activity-dependent neuronal response.

In summary, the current evidence suggests that crosstalk between ROS and calcium plays an essential role in many pathophysiological conditions including neurodegenerative diseases such as PD and AD. Here we complement this idea by adding the component of iron overload, and the ensuing oxidative stress, as a significant factor in promoting calcium dysregulation in neurodegeneration.

THE SYNERGISM BETWEEN IRON AND CALCIUM IN LIPID PEROXIDATION

A link between iron-mediated lipid peroxidation and calcium dyshomeostasis was reported more than 30 years ago. Peroxidation of rat brain synaptosomes by Fe²⁺ and

H_2O_2 generates rapid and large uptake of Ca^{2+} by isolated synaptosomes; Fe^{2+} also enhances Ca^{2+} uptake by spinal cord neurons in culture, an effect that is coupled to lipid peroxidation and the release of arachidonic acid from cells, in a process prevented by the iron chelator DFO (Braugher et al., 1985). The authors concluded that Fe^{2+} and Ca^{2+} act synergistically to produce lipid peroxidation and damage to neuronal membranes (Braugher et al., 1985). A further report proposed that this is not a direct effect of calcium but the result of independently initiated processes of lipid peroxidation and Ca^{2+} translocation, which interact subsequently in a synergistic manner (Bors et al., 1990).

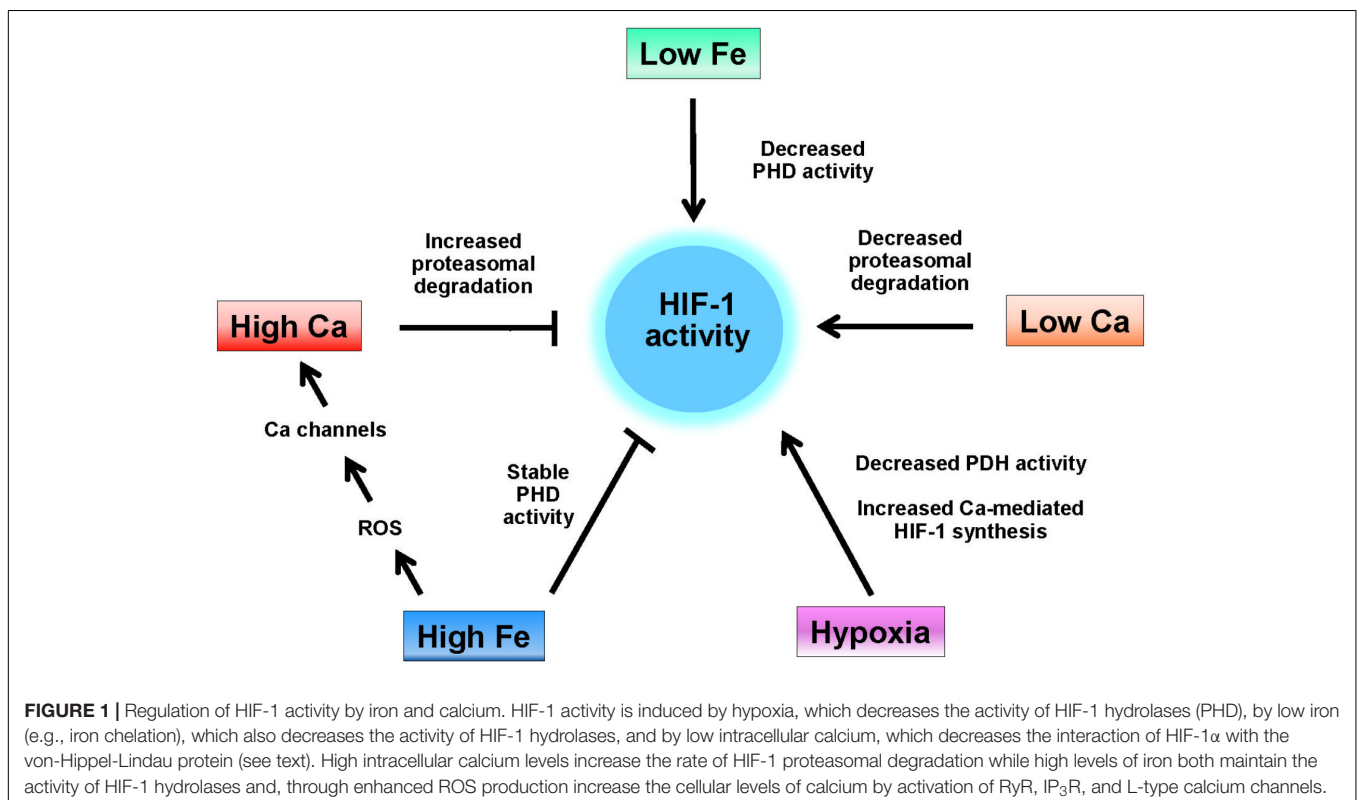
The relationship between iron-induced lipid peroxidation and dysregulation of Ca^{2+} -ATPase is long-dated (Pelizzoni et al., 2008; Mata and Sepulveda, 2010; Zaidi, 2010). Plasma membrane lipid peroxidation initiated by the Fenton reaction results in increased calcium content, derived from the inhibition of the plasma membrane and ER Ca^{2+} -ATPases (Pereira et al., 1996). This inhibitory effect is shared by other radical species, in particular by peroxynitrite (Zaidi and Michaelis, 1999). Oxidants also inhibit SERCA activity while promoting calcium release from the ER (Gordeeva et al., 2003; Hidalgo and Donoso, 2008). Arguably, the observed upsurge in intracellular Ca^{2+} induced by iron-mediated lipid peroxidation could be compounded by an oxidant-induced loss of PMCA and SERCA activity, which combined with ROS-mediated activation of IP_3R/RyR channels would result in further and deleterious increases in the intracellular Ca^{2+} concentration.

IRON, CALCIUM, AND HIF-1 ACTIVITY

Considering the overlapping effects of iron and calcium on HIF-1 activity detailed above, a Fe/Ca bipartisan regulation of HIF-1 can be hypothesized (Figure 1). In neurodegenerative diseases with an iron accumulation component, decreased HIF-1 activity is predicted by a mechanism that involves the maintenance of maximal HIF-1 hydrolase activities and the increase of intracellular calcium mediated by dysregulation of redox-sensitive calcium homeostatic and signaling pathways. In neurons, decreased HIF-1 activity results in decreased neuroprotective, neuroregenerative, and antioxidant responses associated to HIF-1 activity (see above).

CALCIUM-INDUCED IRON DYSHOMEOSTASIS: POSSIBLE ROLE IN NEURODEGENERATION

Excessive intracellular calcium levels, such as those associated with neurodegenerative conditions, are likely to produce high H_2O_2 levels via NOX2 and to enhance mitochondrial calcium uptake and ROS production. These two ROS sources, NOX2, and mitochondria should affect jointly iron homeostasis by increasing the cellular labile iron pool, as occurs in SH-SY5Y cells exposed to H_2O_2 (Dev et al., 2015). These findings raise the possibility that conditions that increase postsynaptic calcium levels following NMDA receptor activation, which promote H_2O_2 generation in neocortical neurons via a cascade engaging the NOS and NOX2



enzymes (Girouard et al., 2009), may lead to an upsurge of the labile iron pool.

The role of voltage-gated Ca^{2+} channels as mediators of iron transport into neuronal cells is well established (Gaasch et al., 2007; Lockman et al., 2012). Another link between iron and calcium during neurodegeneration was provided by the observation that the hippocampal and nigral neuron loss induced by intra-cerebroventricular FeCl_3 injection in rats is largely prevented by pre-treatment with nifedipine, a blocker of L-type VGCCs (Bostanci and Bagirici, 2013). The authors concluded that these channels mediate the neurotoxic effects of extracellular iron by inducing either calcium and/or iron uptake. The question remains as to whether the protective effect of nifedipine is due to decreased iron influx, decreased calcium influx, or to a calcium-mediated process leading to neurodegeneration downstream of iron-induced oxidative stress. Other studies have also provided evidence of iron influx through NMDA receptors or voltage-operated calcium channels in primary hippocampal neurons (Pelizzoni et al., 2011) and the dopaminergic MES23.5 cell line (Wang et al., 2017).

THE MITOCHONDRION MELTING POT: CALCIUM, IRON, AND APOPTOTIC CELL DEATH

Mitochondria, by taking up and releasing calcium, act as important regulators of cellular calcium levels. The large mitochondrial membrane potential difference (negative inside) provides the driving force for calcium entry through the mitochondrial calcium uniporter (MCU), a component of the inner mitochondrial membrane; in physiological conditions, calcium antiporters rapidly extrude calcium from the mitochondria and restore the basal calcium levels (Giorgi et al., 2018). Mitochondrial calcium concentration modulates ATP production and mitochondrial metabolism, whereas mitochondrial calcium overload induced by deregulated intracellular calcium levels promotes apoptotic cell death, necrosis, and autophagy (Giorgi et al., 2012; Rimessi et al., 2013; Marchi et al., 2018). In pathological conditions, the MCU has been implicated in excitotoxicity, iron overload, inflammation, and oxidative stress-induced mitochondrial dysfunction and cell death (Liao et al., 2017). The ROS generated by mitochondrial enzymes or the electron transport chain presumably regulate diverse cellular physiological pathways, whereas dysregulated ROS generation may contribute to the development of human diseases (Angelova and Abramov, 2017).

Neurodegenerative disorders such as AD, PD, and ALS display dysregulation of calcium homeostasis and mitochondrial dysfunction; in addition, excitotoxicity leads to mitochondrial calcium overload and promotes necrotic or apoptotic-like excitotoxic cell death (Hansson Petersen et al., 2008; Rosales-Corral et al., 2012; Bose and Beal, 2016; Gibson and Thakkar, 2017; Giorgi et al., 2018). An increase in mitochondrial calcium is of particular importance in dopaminergic neurons, which are constantly exposed to calcium influx (Surmeier et al., 2011). The interaction between calcium overload and excessive ROS

production (Chinopoulos and Adam-Vizi, 2006) causes further damage to the electron transport chain and promotes oxidative stress, which induces the oxidation of mitochondrial lipids, proteins, and DNA leading to cytotoxicity (Muravchick and Levy, 2006). The vicious cycle between calcium overload and oxidative stress favors the sustained opening of the mitochondrial permeability transition pore (mPTP), leading to the collapse of the mitochondrial membrane potential and mitochondrial swelling, which in turn promote the apoptotic response (Di Lisa and Bernardi, 2009). Opening of the mPTP has a central role in the pathogenesis of neurodegenerative disorders (Vila and Przedborski, 2003; Perier et al., 2005).

Excessive iron levels promote oxidative stress leading to the upsurge of unrestrained calcium signals that damage mitochondrial function. Iron accumulation in the cytoplasm induces excessive iron influx into mitochondria through mitoferrin1/2 (Paradkar et al., 2009), which causes mitochondrial dysfunction by increased ROS production leading to oxidative damage. Hence, if uncontrolled, this calcium/iron self-feeding cycle becomes deleterious to mitochondrial and neuronal function, leading eventually to neuronal death. In this regard, it is of note that, by preserving mitochondrial calcium homeostasis, the iron-binding protein lactoferrin protects vulnerable dopamine neurons from degeneration (Rousseau et al., 2013).

In primary hippocampal neurons, iron-induced stimulation of redox-sensitive RyR-mediated calcium release causes significant mitochondrial fragmentation (Sanmartin et al., 2014). Iron overload induces oxidative stress in the ER earlier than in the mitochondria, thereby increasing ER stress and calcium levels, and consequently causing mitochondrial fragmentation and neuronal cell death (Lee et al., 2016). Mitochondrial fragmentation presumably contributes to the impairment of neuronal function produced by iron overload, since inhibition of mitochondrial division protects neuronal cells from excitotoxicity (Ruiz et al., 2018). Moreover, iron overload of HT-22 hippocampal neuron cells increases intracellular calcium levels, causes mitochondrial fragmentation via dephosphorylation of Drp1, and increases apoptotic neuronal death; of note, calcium chelation and inhibition of calcineurin prevented mitochondrial fragmentation and cell death (Lee et al., 2016). Based on these findings, the authors suggested that calcium-mediated calcineurin signaling, by regulating mitochondrial dynamics, has a key role in iron-induced neurotoxicity.

In summary, mitochondrial calcium overload combined with excessive iron-mediated ROS generation and mPTP opening compose a cell death-inducing sequence implicated in neuronal dysfunction and neurodegeneration.

OXYTOSIS/FERROPTOSIS A POSSIBLE MEETING POINT FOR CALCIUM AND IRON-INDUCED CELL DEATH

Oxytosis was first described as a glutamate-induced, calcium-dependent form of cell death (Murphy et al., 1988). Initial studies on the molecular mechanisms of oxytosis pointed to a decrease

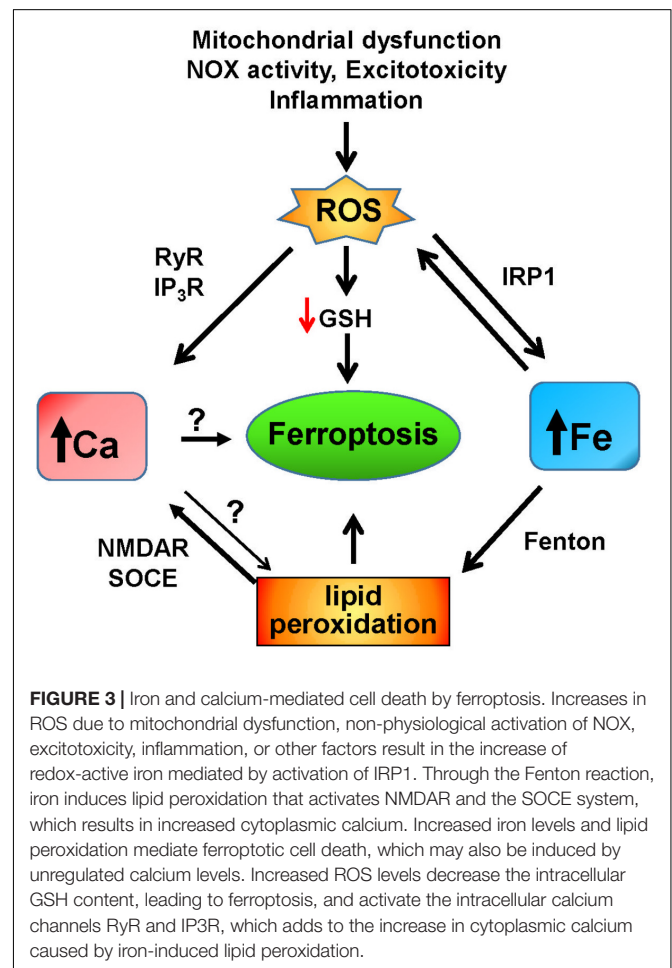
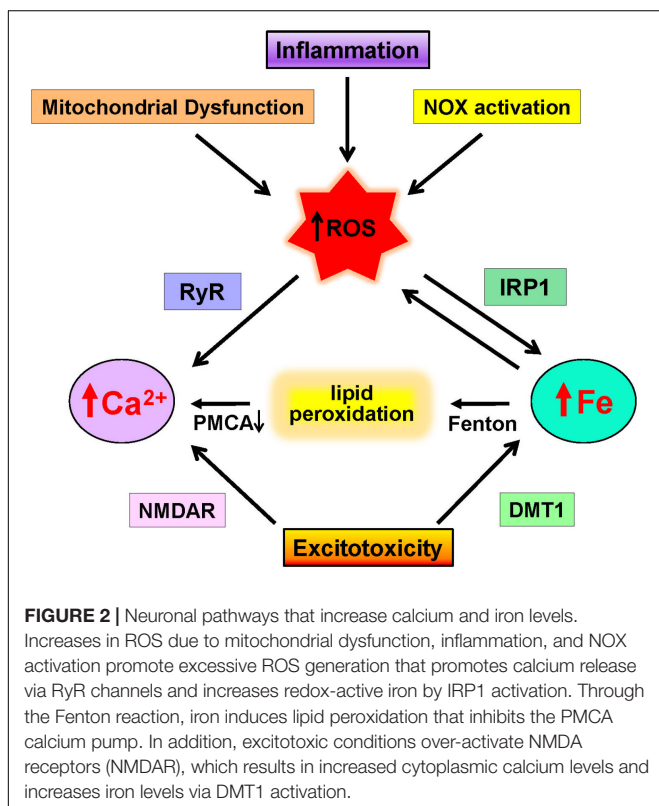
in intracellular glutathione levels, enhanced mitochondrial ROS production, lipid peroxidation, and massive calcium influx as causes of death (Miyamoto et al., 1989). The mechanistic link between glutamate exposure and glutathione depletion was the inhibition of the cysteine/glutamate antiporter xc⁻ by glutamate, which deprived cells of cystine, a precursor in glutamate synthesis (Murphy et al., 1989). As glutathione levels decrease, ROS levels increase exponentially (Tan et al., 1998), which gives rise to the activation of signaling pathways that culminate in large influx of calcium and cell death (Maher et al., 2018). Thus, the increased calcium influx is an essential mediator of the cell death program, as cells do not die in calcium-free medium.

Ferroptosis is a form of regulated cell death that occurs because of lethal lipid peroxidation. Ferroptotic cell death is blocked by iron chelators, lipophilic antioxidants, and by agents that block propagation of lipid peroxidation (Stockwell et al., 2017; Fricker et al., 2018). Ferroptosis is induced by inhibition of the cystine-glutamate antiporter xc⁻ or by inhibition of glutathione peroxidase 4 (Gpx4). Induction of ferroptosis by erastin (an inhibitor of the antiporter xc⁻) results in cell death, which is morphologically characterized by mitochondrial shrinkage, with no overt signs of apoptosis or necrosis (Dixon et al., 2012). Ferroptotic death derives from depletion of cell glutathione, increased oxidative stress, and iron-mediated lipid peroxidation to lethal levels (Stockwell et al., 2017). Inhibition of Gpx4 also causes ferroptosis (Yang et al., 2014). Gpx4 is a lipid peroxidase that directly reduces phospholipid hydroperoxides at the expense of reduced glutathione. In mice, conditional

deletion in forebrain neurons of Gpx4 resulted in hippocampal neurodegeneration and behavior dysfunction, associated with markers of ferroptosis such as elevated lipid peroxidation, ERK1/2 activation, and neuroinflammation (Hambright et al., 2017). Given the large similarity in their cell death mechanisms, the current view is that oxytosis and ferroptosis are similar forms of cell death (Fricker et al., 2018; Lewerenz et al., 2018; Maher et al., 2018).

Although a lethal influx of calcium is an intrinsic feature of oxytosis, the participation of calcium in ferroptosis is still poorly defined. Initial work by Dixon et al. (2012) revealed that calcium chelators do not inhibit erastin-induced HT-1080 cell death. Nevertheless, as mentioned above, an increase in cellular ROS levels enhances the activity of IP₃R and RyR calcium release channels and decreases the activity of both the PMCA and the SERCA calcium pumps. These effects increase cellular calcium levels by promoting calcium release from the ER while inhibiting its removal by the calcium pumps.

Lipid peroxidation-derived modification of some calcium channels could also be intermediates in ferroptotic death (Maher et al., 2018). In particular, 4-hydroxynonenal, a product of hydroxyl radical-mediated lipid peroxidation, induces the opening of NMDA receptor and VGCCs (Lu et al., 2002). Indeed,



the store-operated calcium entry (SOCE) system, which activates calcium influx channels in the plasma membrane upon the release of intracellular calcium from the ER participates in the neuronal death induced by MPP+, a Parkinsonian toxin that inhibits mitochondria electron transport chain complex I. Inhibition of the SOCE response decreased apoptotic cell death, reduced ROS production, and decreased lipid peroxidation induced by MPP+ (Li et al., 2013). In the same vein, a recent report shows that the pharmacological or transcriptional inhibition of SOCE proteins protects against oxidative glutamate toxicity and against MPP+ injury (Maher et al., 2018).

Overall, these results point to a possible participation of calcium in ferroptosis, a participation driven by raises in cytoplasmic calcium because of oxidative- and lipid peroxidation-derived modifications of the calcium homeostasis machinery (Figure 3). This participation may be specific to cells with weak antioxidant defenses, which could allow for the oxidative activation of calcium channels.

PROTECTION OF IRON OVERLOAD BY NRF2; THE CALCIUM CONNECTION

Under oxidative stress conditions, the transcription factor known as nuclear factor (erythroid-derived 2)-like 2 (Nrf2) directs the expression of a large array of cytoprotective genes (Tonelli et al., 2018). In particular, Nrf2 protects cells from the injurious effects of iron overload by regulating the expression of genes involved

in iron storage and iron export (Chorley et al., 2012; Kerins and Ooi, 2018). These findings raise the possibility that Nrf2 may counteract the oxidative stress induced in neuronal cells by iron overload. Thus, Nrf2 may restore the intracellular labile iron pool by inducing the expression of the iron exporter FPN1 and ferritin, which oxidizes Fe^{2+} to Fe^{3+} and stores it within its structure making it unavailable for the Fenton reaction (Orino et al., 2001). In addition, intracellular iron levels act as a link between HIF-1 α and Nrf2: Nrf2-induced ferritin upregulation activates HIF-1 α by making iron unavailable to HIF prolyl-hydroxylase (Siegert et al., 2015), whereas Nrf2 inhibition results in decreased activation of HIF-1 α by hypoxia (Kim et al., 2011; Ji et al., 2014).

Recent studies point to a connection between ROS, cellular calcium signals, and Nrf2. The neurotrophin BDNF, besides inducing complex neuronal signaling cascades underlying synaptic plasticity (Bolton et al., 2000), also induces neuronal antioxidant responses through BDNF-induced Nrf2 nuclear translocation (Bouvier et al., 2017; Bruna et al., 2018). In this regard, a recent study showed that BDNF-mediated activation of Nrf2 in astrocytes, which is under circadian control, protects dopaminergic neurons from ferroptosis (Ishii et al., 2018). Of note, in primary hippocampal neurons, ROS and RyR-mediated calcium signals are required for BDNF-induced nuclear translocation of Nrf2 (Bruna et al., 2018). It remains to be established if iron overload in hippocampal neurons engages BDNF and RyR-mediated calcium signals to promote Nrf2-induced expression of protective genes.

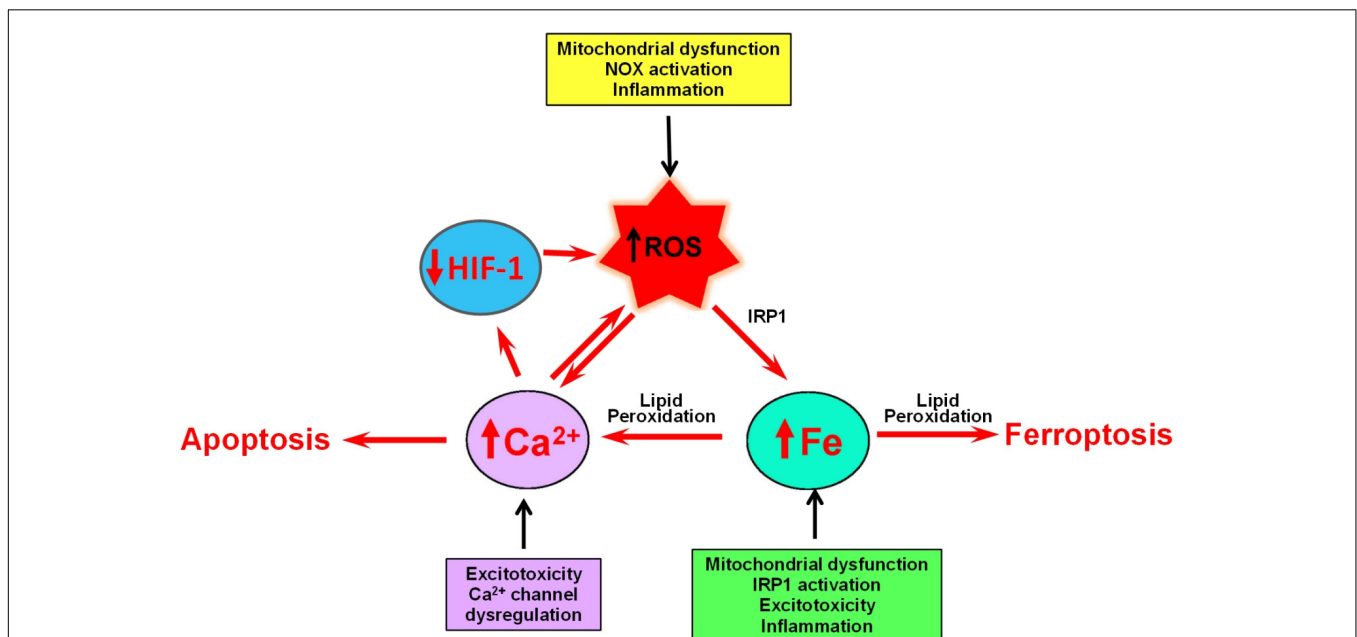


FIGURE 4 | The self-feeding cycle ROS–Fe–Ca. Mitochondrial dysfunction promotes in increased ROS and activation IRP1, which results in increased iron accumulation. Excess iron promotes lipid peroxidation by hydroxyl radicals derived from the Fenton reaction. Lipid peroxidation modifies the activity of a variety of proteins involved in calcium homeostasis that results in massive calcium influx. Increased cytoplasmic calcium levels result in increased mitochondrial calcium and accentuated mitochondrial dysfunction, oxidative stress, and damage. If uncontrolled, this ROS–iron–calcium self-feeding cycle becomes deleterious to mitochondrial and neuronal function, leading eventually to apoptotic or ferroptotic cell death. In yet another aspect of this cycle, high intracellular calcium levels increase the rate of HIF-1 proteasome degradation, which results in increased ROS levels, product of decreased expression of antioxidant proteins.

CONCLUSION

This article reviews the little acknowledged relationship between iron and calcium in neurodegeneration. The main premise of this article is that increased ROS production generated by iron accumulation profoundly influences calcium homeostasis and signaling (Figure 4).

The influence of increased ROS is clearly shown in the process of oxytosis, in which lipid peroxidation initiated by Fenton-derived hydroxyl radicals results in the modification of a variety of proteins involved in calcium homeostasis that brings to calcium upsurges and cell death. Increased cytoplasmic calcium levels caused by iron dysregulation result in increased mitochondrial calcium and accentuated oxidative stress and damage. If uncontrolled, this calcium/iron self-feeding cycle becomes deleterious to mitochondrial and neuronal function, leading eventually to neuronal death. In yet another aspect of neurodegeneration, high intracellular calcium levels increase the rate of HIF-1 proteasomal degradation while high levels of iron both maintain the activity of HIF-1 hydrolases and, through oxidative stress

increase the cellular levels of calcium by activation of ER and PMCA channels. The main conclusion we draw from this review is that dysregulated iron or calcium levels promote deleterious crosstalk between iron and calcium that can result in neuronal dysfunction and death. To our knowledge, this is the first attempt in literature to systemize this important relationship.

AUTHOR CONTRIBUTIONS

MN and CH wrote this article.

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REFERENCES

- Alzheimer's Association Calcium Hypothesis Workgroup [AACHW] (2017). Calcium hypothesis of Alzheimer's disease and brain aging: a framework for integrating new evidence into a comprehensive theory of pathogenesis. *Alzheimers Dement.* 13, 178.e17–182.e17. doi: 10.1016/j.jalz.2016.12.006
- An, H., Zeng, X., Niu, T., Li, G., Yang, J., Zheng, L., et al. (2018). Quantifying iron deposition within the substantia nigra of Parkinson's disease by quantitative susceptibility mapping. *J. Neurol. Sci.* 386, 46–52. doi: 10.1016/j.jns.2018.01.008
- Angelova, P. R., and Abramov, A. Y. (2017). Alpha-synuclein and beta-amyloid - different targets, same players: calcium, free radicals and mitochondria in the mechanism of neurodegeneration. *Biochem. Biophys. Res. Commun.* 483, 1110–1115. doi: 10.1016/j.bbrc.2016.07.103
- Ansari, M. A., and Scheff, S. W. (2011). NADPH-oxidase activation and cognition in Alzheimer disease progression. *Free Radic. Biol. Med.* 51, 171–178. doi: 10.1016/j.freeradbiomed.2011.03.025
- Area-Gomez, E., and Schon, E. A. (2017). On the pathogenesis of Alzheimer's Disease: the MAM hypothesis. *FASEB J.* 31, 864–867. doi: 10.1096/fj.201601309
- Arruda, S. F., Siqueira, E. M., and De Valencia, F. F. (2009). Vitamin A deficiency increases hepcidin expression and oxidative stress in rat. *Nutrition* 25, 472–478. doi: 10.1016/j.nut.2008.11.030
- Aasiwe, N., Yeo, S. G., Kim, M. S., Jung, J., and Jeong, N. Y. (2016). Nitric oxide: exploring the contextual link with alzheimer's disease. *Oxid. Med. Cell. Longev.* 2016:7205747. doi: 10.1155/2016/7205747
- Bading, H. (2013). Nuclear calcium signalling in the regulation of brain function. *Nat. Rev. Neurosci.* 14, 593–608. doi: 10.1038/nrn3531
- Barrett, J. P., Henry, R. J., Villapol, S., Stoica, B. A., Kumar, A., Burns, M. P., et al. (2017). NOX2 deficiency alters macrophage phenotype through an IL-10/STAT3 dependent mechanism: implications for traumatic brain injury. *J. Neuroinflammation* 14:65. doi: 10.1186/s12974-017-0843-4
- Baumgartel, K., and Mansuy, I. M. (2012). Neural functions of calcineurin in synaptic plasticity and memory. *Learn. Mem.* 19, 375–384. doi: 10.1101/lm.027201.112
- Becerril-Ortega, J., Bordji, K., Freret, T., Rush, T., and Buisson, A. (2014). Iron overload accelerates neuronal amyloid-beta production and cognitive impairment in transgenic mice model of Alzheimer's disease. *Neurobiol. Aging* 35, 2288–2301. doi: 10.1016/j.neurobiolaging.2014.04.019
- Berchner-Pfannschmidt, U., Petrat, F., Doege, K., Trinidad, B., Freitag, P., Metzén, E., et al. (2004). Chelation of cellular calcium modulates hypoxia-inducible gene expression through activation of hypoxia-inducible factor-1alpha. *J. Biol. Chem.* 279, 44976–44986. doi: 10.1074/jbc.M313995200
- Berg, D., and Youdim, M. B. (2006). Role of iron in neurodegenerative disorders. *Top. Magn. Reson. Imaging* 17, 5–17. doi: 10.1097/01.rmr.0000245461.90406.ad
- Berridge, M. J. (1998). Neuronal calcium signaling. *Neuron* 21, 13–26. doi: 10.1016/S0896-6273(00)80510-3
- Berridge, M. J., Bootman, M. D. and Roderick, H. L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* 4, 517–529. doi: 10.1038/nrm1155
- Berrocal, M., Corbacho, I., Vazquez-Hernandez, M., Avila, J., Sepulveda, M. R., and Mata, A. M. (2015). Inhibition of PMCA activity by tau as a function of aging and Alzheimer's neuropathology. *Biochim. Biophys. Acta* 1852, 1465–1476. doi: 10.1016/j.bbdis.2015.04.007
- Bezprozvanny, I., and Mattson, M. P. (2008). Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.* 31, 454–463. doi: 10.1016/j.tins.2008.06.005
- Bindokas, V. P., Jordan, J., Lee, C. C., and Miller, R. J. (1996). Superoxide production in rat hippocampal neurons: selective imaging with hydroethidine. *J. Neurosci.* 16, 1324–1336. doi: 10.1523/JNEUROSCI.16-04-01324.1996
- Bolton, M. M., Pittman, A. J., and Lo, D. C. (2000). Brain-derived neurotrophic factor differentially regulates excitatory and inhibitory synaptic transmission in hippocampal cultures. *J. Neurosci.* 20, 3221–3232. doi: 10.1523/JNEUROSCI.20-09-03221.2000
- Bors, W., Buettner, G. R., Michel, C., and Saran, M. (1990). Calcium in lipid peroxidation: does calcium interact with superoxide? *Arch. Biochem. Biophys.* 278, 269–272. doi: 10.1016/0003-9861(90)90258-Z
- Bose, A., and Beal, M. F. (2016). Mitochondrial dysfunction in Parkinson's disease. *J. Neurochem.* 139(Suppl. 1), 216–231. doi: 10.1111/jnc.13731
- Bostanci, M. O., and Bagirci, F. (2013). Blocking of L-type calcium channels protects hippocampal and nigral neurons against iron neurotoxicity. The role of L-type calcium channels in iron-induced neurotoxicity. *Int. J. Neurosci.* 123, 876–882. doi: 10.3109/00207454.2013.813510
- Bouvier, E., Brouillard, F., Molet, J., Claverie, D., Cabungcal, J. H., Cresto, N., et al. (2017). Nrf2-dependent persistent oxidative stress results in stress-induced vulnerability to depression. *Mol. Psychiatry* 22, 1701–1713. doi: 10.1038/mp.2016.211
- Braugher, J. M., Duncan, L. A., and Chase, R. L. (1985). Interaction of lipid peroxidation and calcium in the pathogenesis of neuronal injury. *Cent. Nerv. Syst. Trauma* 2, 269–283. doi: 10.1089/cns.1985.2.269
- Brennan, A. M., Suh, S. W., Won, S. J., Narasimhan, P., Kauppinen, T. M., Lee, H., et al. (2009). NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. *Nat. Neurosci.* 12, 857–863. doi: 10.1038/nn.2334

- Brini, M., Cali, T., Ottolini, D., and Carafoli, E. (2014). Neuronal calcium signaling: function and dysfunction. *Cell. Mol. Life Sci.* 71, 2787–2814. doi: 10.1007/s00018-013-1550-7
- Brini, M., Leanza, L., and Szabo, I. (2017). Lipid-mediated modulation of intracellular ion channels and redox state: physiopathological implications. *Antioxid. Redox Signal.* doi: 10.1089/ars.2017.7215 [Epub ahead of print].
- Bruick, R. K., and McKnight, S. L. (2001). A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 294, 1337–1340. doi: 10.1126/science.1066373
- Bruna, B., Lobos, P., Herrera-Molina, R., Hidalgo, C., Paula-Lima, A., and Adasme, T. (2018). The signaling pathways underlying BDNF-induced Nrf2 hippocampal nuclear translocation involve ROS, RyR-Mediated Ca(2+) signals, ERK and PI3K. *Biochem. Biophys. Res. Commun.* 505, 201–207. doi: 10.1016/j.bbrc.2018.09.080
- Bulk, M., Van Der Weerd, L., Breimer, W., Lebedev, N., Webb, A., Goeman, J. J., et al. (2018). Quantitative comparison of different iron forms in the temporal cortex of Alzheimer patients and control subjects. *Sci. Rep.* 8:6898. doi: 10.1038/s41598-018-25021-7
- Burbulla, L. F., Song, P., Mazzulli, J. R., Zampese, E., Wong, Y. C., Jeon, S., et al. (2017). Dopamine oxidation mediates mitochondrial and lysosomal dysfunction in Parkinson's disease. *Science* 357, 1255–1261. doi: 10.1126/science.aam9080
- Cabantchik, Z. I. (2014). Labile iron in cells and body fluids: physiology, pathology, and pharmacology. *Front. Pharmacol.* 5:45. doi: 10.3389/fphar.2014.00045
- Caltagirone, A., Weiss, G., and Pantopoulos, K. (2001). Modulation of cellular iron metabolism by hydrogen peroxide. Effects of H₂O₂ on the expression and function of iron-responsive element-containing mRNAs in B6 fibroblasts. *J. Biol. Chem.* 276, 19738–19745. doi: 10.1074/jbc.M100245200
- Cannon, J. R., Tapias, V., Na, H. M., Honick, A. S., Drolet, R. E., and Greenamyre, J. T. (2009). A highly reproducible rotenone model of Parkinson's disease. *Neurobiol. Dis.* 34, 279–290. doi: 10.1016/j.nbd.2009.01.016
- Carocci, A., Catalano, A., Sinicropi, M. S., and Genchi, G. (2018). Oxidative stress and neurodegeneration: the involvement of iron. *Biometals* 31, 715–735. doi: 10.1007/s10534-018-0126-2
- Chan, C. S., Gertler, T. S., and Surmeier, D. J. (2009). Calcium homeostasis, selective vulnerability and Parkinson's disease. *Trends Neurosci.* 32, 249–256. doi: 10.1016/j.tins.2009.01.006
- Cheah, J. H., Kim, S. F., Hester, L. D., Clancy, K. W., Patterson, S. E. III, Papadopoulos, V., et al. (2006). NMDA receptor-nitric oxide transmission mediates neuronal iron homeostasis via the GTPase Dexas1. *Neuron* 51, 431–440. doi: 10.1016/j.neuron.2006.07.011
- Chinopoulos, C., and Adam-Vizi, V. (2006). Calcium, mitochondria and oxidative stress in neuronal pathology. Novel aspects of an enduring theme. *FEBS J.* 273, 433–450. doi: 10.1111/j.1742-4658.2005.05103.x
- Chorley, B. N., Campbell, M. R., Wang, X., Karaca, M., Sambandan, D., Bangura, F., et al. (2012). Identification of novel NRF2-regulated genes by ChIP-Seq: influence on retinoid X receptor alpha. *Nucleic Acids Res.* 40, 7416–7429. doi: 10.1093/nar/gks409
- Clapham, D. E. (2007). Calcium signaling. *Cell* 131, 1047–1058. doi: 10.1016/j.cell.2007.11.028
- Cobley, J. N., Fiorello, M. L., and Bailey, D. M. (2018). 13 reasons why the brain is susceptible to oxidative stress. *Redox Biol.* 15, 490–503. doi: 10.1016/j.redox.2018.01.008
- Creighton-Gutteridge, M., and Tyrrell, R. M. (2002). A novel iron chelator that does not induce HIF-1 activity. *Free Radic. Biol. Med.* 33, 356–363. doi: 10.1016/S0891-5849(02)00884-5
- Crichton, R. R., Dexter, D. T., and Ward, R. J. (2011). Brain iron metabolism and its perturbation in neurological diseases. *J. Neural Transm.* 118, 301–314. doi: 10.1007/s00702-010-0470-z
- Cristovao, J. S., Santos, R., and Gomes, C. M. (2016). Metals and neuronal metal binding proteins implicated in Alzheimer's Disease. *Oxid. Med. Cell. Longev.* 2016:9812178. doi: 10.1155/2016/9812178
- Damasceno, F. C., Condeles, A. L., Lopes, A. K. B., Facci, R. R., Linares, E., Truzzi, D. R., et al. (2018). The labile iron pool attenuates peroxynitrite-dependent damage and can no longer be considered solely a pro-oxidative cellular iron source. *J. Biol. Chem.* 293, 8530–8542. doi: 10.1074/jbc.RA117.000883
- Davies, M. J. (2005). The oxidative environment and protein damage. *Biochim. Biophys. Acta* 1703, 93–109. doi: 10.1016/j.bbapap.2004.08.007
- Demuro, A., Parker, I., and Stutzmann, G. E. (2010). Calcium signaling and amyloid toxicity in Alzheimer disease. *J. Biol. Chem.* 285, 12463–12468. doi: 10.1074/jbc.R109.080895
- Dev, S., Kumari, S., Singh, N., Kumar Bal, S., Seth, P., and Mukhopadhyay, C. K. (2015). Role of extracellular hydrogen peroxide in regulation of iron homeostasis genes in neuronal cells: implication in iron accumulation. *Free Radic. Biol. Med.* 86, 78–89. doi: 10.1016/j.freeradbiomed.2015.05.025
- Di Lisa, F., and Bernardi, P. (2009). A CaPful of mechanisms regulating the mitochondrial permeability transition. *J. Mol. Cell Cardiol.* 46, 775–780. doi: 10.1016/j.yjmcc.2009.03.006
- DiSabato, D. J., Quan, N., and Godbout, J. P. (2016). Neuroinflammation: the devil is in the details. *J. Neurochem.* 139(Suppl. 2), 136–153. doi: 10.1111/jnc.13607
- Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149, 1060–1072. doi: 10.1016/j.cell.2012.03.042
- Dixon, S. J., and Stockwell, B. R. (2014). The role of iron and reactive oxygen species in cell death. *Nat. Chem. Biol.* 10, 9–17. doi: 10.1038/nchembio.1416
- Drapier, J. C., Hirling, H., Wietzerbin, J., Kaldy, P., and Kuhn, L. C. (1993). Biosynthesis of nitric oxide activates iron regulatory factor in macrophages. *EMBO J.* 12, 3643–3649. doi: 10.1002/j.1460-2075.1993.tb06038.x
- Duncan, A. J., and Heales, S. J. (2005). Nitric oxide and neurological disorders. *Mol. Aspects Med.* 26, 67–96. doi: 10.1016/j.mam.2004.09.004
- Flagg, S. C., Martin, C. B., Taabazuing, C. Y., Holmes, B. E., and Knapp, M. J. (2012). Screening chelating inhibitors of HIF-prolyl hydroxylase domain 2 (PHD2) and factor inhibiting HIF (FIH). *J. Inorg. Biochem.* 113, 25–30. doi: 10.1016/j.jinorgbio.2012.03.002
- Franco-Iborra, S., Vila, M., and Perier, C. (2018). Mitochondrial quality control in neurodegenerative diseases: focus on parkinson's disease and huntington's disease. *Front. Neurosci.* 12:342. doi: 10.3389/fnins.2018.00342
- Fricker, M., Tolkovsky, A. M., Borutaite, V., Coleman, M., and Brown, G. C. (2018). Neuronal cell death. *Physiol. Rev.* 98, 813–880. doi: 10.1152/physrev.00011.2017
- Fukui, K. (2016). Reactive oxygen species induce neurite degeneration before induction of cell death. *J. Clin. Biochem. Nutr.* 59, 155–159. doi: 10.3164/jcbn.16-34
- Gaasch, J. A., Geldenhuys, W. J., Lockman, P. R., Allen, D. D., and Van Der Schyf, C. J. (2007). Voltage-gated calcium channels provide an alternate route for iron uptake in neuronal cell cultures. *Neurochem. Res.* 32, 1686–1693. doi: 10.1007/s11064-007-9313-1
- Gao, H. M., Zhou, H., and Hong, J. S. (2012). NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. *Trends Pharmacol. Sci.* 33, 295–303. doi: 10.1016/j.tips.2012.03.008
- Garrick, M. D., Singleton, S. T., Vargas, F., Kuo, H. C., Zhao, L., Knopfel, M., et al. (2006). DMT1: which metals does it transport? *Biol. Res.* 39, 79–85. doi: 10.4067/S0716-97602006000100009
- Ghosh, D. K., and Salerno, J. C. (2003). Nitric oxide synthases: domain structure and alignment in enzyme function and control. *Front. Biosci.* 8, D193–D209. doi: 10.2741/959
- Gibson, G. E., and Thakkar, A. (2017). Interactions of mitochondria/metabolism and calcium regulation in alzheimer's disease: a calcinist point of view. *Neurochem. Res.* 42, 1636–1648. doi: 10.1007/s11064-017-2182-3
- Giorgi, C., Baldassari, F., Bononi, A., Bonora, M., De Marchi, E., Marchi, S., et al. (2012). Mitochondrial Ca(2+) and apoptosis. *Cell Calcium* 52, 36–43. doi: 10.1016/j.ceca.2012.02.008
- Giorgi, C., Marchi, S., and Pinton, P. (2018). The machineries, regulation and cellular functions of mitochondrial calcium. *Nat. Rev. Mol. Cell Biol.* 19, 713–730. doi: 10.1038/s41580-018-0052-8
- Girouard, H., Wang, G., Gallo, E. F., Anrather, J., Zhou, P., Pickel, V. M., et al. (2009). NMDA receptor activation increases free radical production through nitric oxide and NOX2. *J. Neurosci.* 29, 2545–2552. doi: 10.1523/JNEUROSCI.0133-09.2009
- Gonsette, R. E. (2008). Neurodegeneration in multiple sclerosis: the role of oxidative stress and excitotoxicity. *J. Neurol. Sci.* 274, 48–53. doi: 10.1016/j.jns.2008.06.029
- Gordeeva, A. V., Zvyagilskaya, R. A., and Labas, Y. A. (2003). Cross-talk between reactive oxygen species and calcium in living cells. *Biochemistry* 68, 1077–1080.

- Gorlach, A., Bertram, K., Hudecova, S., and Krizanova, O. (2015). Calcium and ROS: a mutual interplay. *Redox Biol.* 6, 260–271. doi: 10.1016/j.redox.2015.08.010
- Green, S. P., Cairns, B., Rae, J., Errett-Baroncini, C., Hongo, J. A., Erickson, R. W., et al. (2001). Induction of gp91-phox, a component of the phagocyte NADPH oxidase, in microglial cells during central nervous system inflammation. *J. Cereb. Blood Flow Metab.* 21, 374–384. doi: 10.1097/00004647-200104000-00006
- Grosskreutz, J., Van Den Bosch, L., and Keller, B. U. (2010). Calcium dysregulation in amyotrophic lateral sclerosis. *Cell Calcium* 47, 165–174. doi: 10.1016/j.ceca.2009.12.002
- Guo, C., Hao, L. J., Yang, Z. H., Chai, R., Zhang, S., Gu, Y., et al. (2016). Deferoxamine-mediated up-regulation of HIF-1 α prevents dopaminergic neuronal death via the activation of MAPK family proteins in MPTP-treated mice. *Exp. Neurol.* 280, 13–23. doi: 10.1016/j.expneurol.2016.03.016
- Guo, C., Zhang, Y. X., Wang, T., Zhong, M. L., Yang, Z. H., Hao, L. J., et al. (2015). Intranasal deferoxamine attenuates synapse loss via up-regulating the P38/HIF-1 α pathway on the brain of APP/PS1 transgenic mice. *Front. Aging Neurosci.* 7:104. doi: 10.3389/fnagi.2015.00104
- Guo, J. D., Zhao, X., Li, Y., Li, G. R., and Liu, X. L. (2018). Damage to dopaminergic neurons by oxidative stress in Parkinson's disease (Review). *Int. J. Mol. Med.* 41, 1817–1825. doi: 10.3892/ijmm.2018.3406
- Guzman, J. N., Sanchez-Padilla, J., Chan, C. S., and Surmeier, D. J. (2009). Robust pacemaking in substantia nigra dopaminergic neurons. *J. Neurosci.* 29, 11011–11019. doi: 10.1523/JNEUROSCI.2519-09.2009
- Hajjeva, P., Baeken, M. W., and Moosmann, B. (2018). The role of plasma membrane calcium ATPases (PMCA) in neurodegenerative disorders. *Neurosci. Lett.* 663, 29–38. doi: 10.1016/j.neulet.2017.09.033
- Halliwel, B. (2006). Oxidative stress and neurodegeneration: where are we now? *J. Neurochem.* 97, 1634–1658. doi: 10.1111/j.1471-4159.2006.03907.x
- Hambright, W. S., Fonseca, R. S., Chen, L., Na, R., and Ran, Q. (2017). Ablation of ferroptosis regulator glutathione peroxidase 4 in forebrain neurons promotes cognitive impairment and neurodegeneration. *Redox Biol.* 12, 8–17. doi: 10.1016/j.redox.2017.01.021
- Hanke, M. L., and Kielian, T. (2011). Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. *Clin. Sci.* 121, 367–387. doi: 10.1042/CS20110164
- Hanson, E. S., and Leibold, E. A. (1999). Regulation of the iron regulatory proteins by reactive nitrogen and oxygen species. *Gene Expr.* 7, 367–376.
- Hansson Petersen, C. A., Alikhani, N., Behbahani, H., Wiehager, B., Pavlov, P. F., Alafuzoff, I., et al. (2008). The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13145–13150. doi: 10.1073/pnas.0806192105
- Hepp, S., Gerich, F. J., and Muller, M. (2005). Sulfhydryl oxidation reduces hippocampal susceptibility to hypoxia-induced spreading depression by activating BK channels. *J. Neurophysiol.* 94, 1091–1103. doi: 10.1152/jn.00291.2005
- Hewitson, K. S., McNeill, L. A., Elkins, J. M., and Schofield, C. J. (2003). The role of iron and 2-oxoglutarate oxygenases in signalling. *Biochem. Soc. Trans.* 31, 510–515. doi: 10.1042/bst0310510
- Hidalgo, C., and Arias-Cavieres, A. (2016). Calcium, reactive oxygen species, and synaptic plasticity. *Physiology* 31, 201–215. doi: 10.1152/physiol.00038.2015
- Hidalgo, C., Carrasco, M. A., Muñoz, P., and Núñez, M. T. (2007). A role for reactive oxygen/nitrogen species and iron on neuronal synaptic plasticity. *Antioxid. Redox Signal.* 9, 245–255. doi: 10.1089/ars.2007.9.245
- Hidalgo, C., and Donoso, P. (2008). Crosstalk between calcium and redox signaling: from molecular mechanisms to health implications. *Antioxid. Redox Signal.* 10, 1275–1312. doi: 10.1089/ars.2007.1886
- Hidalgo, C., and Núñez, M. T. (2007). Calcium, iron and neuronal function. *IUBMB Life* 59, 280–285. doi: 10.1080/15216540701222906
- Huang, M. L., Lane, D. J., and Richardson, D. R. (2011). Mitochondrial mayhem: the mitochondrion as a modulator of iron metabolism and its role in disease. *Antioxid. Redox Signal.* 15, 3003–3019. doi: 10.1089/ars.2011.3921
- Huang, X., Atwood, C. S., Hartshorn, M. A., Multhaup, G., Goldstein, L. E., Scarpa, R. C., et al. (1999). The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. *Biochemistry* 38, 7609–7616. doi: 10.1021/bi990438f
- Ishii, T., Warabi, E., and Mann, G. E. (2018). Circadian control of BDNF-mediated Nrf2 activation in astrocytes protects dopaminergic neurons from ferroptosis. *Free Radic. Biol. Med.* doi: 10.1016/j.freeradbiomed.2018.09.002 [Epub ahead of print].
- Jaiswal, M. K. (2013). Calcium, mitochondria, and the pathogenesis of ALS: the good, the bad, and the ugly. *Front. Cell. Neurosci.* 7:199. doi: 10.3389/fncel.2013.00199
- Jellinger, K. A. (1999). The role of iron in neurodegeneration: prospects for pharmacotherapy of Parkinson's disease. *Drugs Aging* 14, 115–140. doi: 10.2165/00002512-199914020-00004
- Jeney, V., Balla, J., Yachie, A., Varga, Z., Vercellotti, G. M., Eaton, J. W., et al. (2002). Pro-oxidant and cytotoxic effects of circulating heme. *Blood* 100, 879–887. doi: 10.1182/blood.V100.3.879
- Jeong, S. Y., Rathore, K. I., Schulz, K., Ponka, P., Arosio, P., and David, S. (2009). Dysregulation of iron homeostasis in the CNS contributes to disease progression in a mouse model of amyotrophic lateral sclerosis. *J. Neurosci.* 29, 610–619. doi: 10.1523/JNEUROSCI.5443-08.2009
- Ji, X., Wang, H., Zhu, J., Zhu, L., Pan, H., Li, W., et al. (2014). Knockdown of Nrf2 suppresses glioblastoma angiogenesis by inhibiting hypoxia-induced activation of HIF-1 α . *Int. J. Cancer* 135, 574–584. doi: 10.1002/ijc.28699
- Jiang, L., Bechtel, M. D., Galeva, N. A., Williams, T. D., Michaelis, E. K., and Michaelis, M. L. (2012). Decreases in plasma membrane Ca(2+)-ATPase in brain synaptic membrane rafts from aged rats. *J. Neurochem.* 123, 689–699. doi: 10.1111/j.1471-4159.2012.07918.x
- Joseph, S. K., Young, M. P., Alzayady, K., Yule, D. I., Ali, M., Booth, D. M., et al. (2018). Redox regulation of type-I inositol triphosphate receptors in intact mammalian cells. *J. Biol. Chem.* 293, 17464–17476. doi: 10.1074/jbc.RA118.005624
- Kahlert, S., Zundorf, G., and Reiser, G. (2005). Glutamate-mediated influx of extracellular Ca²⁺ is coupled with reactive oxygen species generation in cultured hippocampal neurons but not in astrocytes. *J. Neurosci. Res.* 79, 262–271. doi: 10.1002/jnr.20322
- Kasarskis, E. J., Tandon, L., Lovell, M. A., and Ehmann, W. D. (1995). Aluminum, calcium, and iron in the spinal cord of patients with sporadic amyotrophic lateral sclerosis using laser microprobe mass spectroscopy: a preliminary study. *J. Neurol. Sci.* 130, 203–208. doi: 10.1016/0022-510X(95)00037-3
- Kastanenka, K. V., Bussiere, T., Shakerdige, N., Qian, F., Weinreb, P. H., Rhodes, K., et al. (2016). Immunotherapy with aducanumab restores calcium homeostasis in Tg2576 Mice. *J. Neurosci.* 36, 12549–12558. doi: 10.1523/JNEUROSCI.2080-16.2016
- Kerins, M. J., and Ooi, A. (2018). The roles of NRF2 in modulating cellular iron homeostasis. *Antioxid. Redox Signal.* 29, 1756–1773. doi: 10.1089/ars.2017.7176
- Kietzmann, T., Knabe, W., and Schmidt-Kastner, R. (2001). Hypoxia and hypoxia-inducible factor modulated gene expression in brain: involvement in neuroprotection and cell death. *Eur. Arch. Psychiatry Clin. Neurosci.* 251, 170–178. doi: 10.1007/s004060170037
- Kim, T. H., Hur, E. G., Kang, S. J., Kim, J. A., Thapa, D., Lee, Y. M., et al. (2011). NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1 α . *Cancer Res.* 71, 2260–2275. doi: 10.1158/0008-5472.CAN-10-3007
- Kishida, K. T., and Klann, E. (2007). Sources and targets of reactive oxygen species in synaptic plasticity and memory. *Antioxid. Redox Signal.* 9, 233–244. doi: 10.1089/ars.2007.9.233
- Kupersmidt, L., Weinreb, O., Amit, T., Mandel, S., Bar-Am, O., and Youdim, M. B. (2011). Novel molecular targets of the neuroprotective/neurorescue multimodal iron chelating drug M30 in the mouse brain. *Neuroscience* 189, 345–358. doi: 10.1016/j.neuroscience.2011.03.040
- Kupersmidt, L., Weinreb, O., Amit, T., Mandel, S., Carri, M. T., and Youdim, M. B. (2009). Neuroprotective and neurotogenic activities of novel multimodal iron-chelating drugs in motor-neuron-like NSC-34 cells and transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J.* 23, 3766–3779. doi: 10.1096/fj.09-130047
- Kushnir, A., Wajsberg, B., and Marks, A. R. (2018). Ryanodine receptor dysfunction in human disorders. *Biochim. Biophys. Acta Mol. Cell. Res.* doi: 10.1016/j.bbamcr.2018.07.011 [Epub ahead of print].

- Lane, D. J. R., Ayton, S., and Bush, A. I. (2018). Iron and Alzheimer's disease: an update on emerging mechanisms. *J. Alzheimers Dis.* 64, S379–S395. doi: 10.3233/JAD-179944
- Lee, D. G., Park, J., Lee, H. S., Lee, S. R., and Lee, D. S. (2016). Iron overload-induced calcium signals modulate mitochondrial fragmentation in HT-22 hippocampal neuron cells. *Toxicology* 365, 17–24. doi: 10.1016/j.tox.2016.07.022
- Lee, D. W., and Andersen, J. K. (2006). Role of HIF-1 in iron regulation: potential therapeutic strategy for neurodegenerative disorders. *Curr. Mol. Med.* 6, 883–893. doi: 10.2174/156652406779010849
- Lee, J. C., Tae, H. J., Kim, I. H., Cho, J. H., Lee, T. K., Park, J. H., et al. (2017). Roles of HIF-1 α , VEGF, and NF- κ B in ischemic preconditioning-mediated neuroprotection of hippocampal CA1 pyramidal neurons against a subsequent transient cerebral ischemia. *Mol. Neurobiol.* 54, 6984–6998. doi: 10.1007/s12035-016-0219-2
- Lee, J. H., Han, Y. H., Kang, B. M., Mun, C. W., Lee, S. J., and Baik, S. K. (2013). Quantitative assessment of subcortical atrophy and iron content in progressive supranuclear palsy and parkinsonian variant of multiple system atrophy. *J. Neurol.* 260, 2094–2101. doi: 10.1007/s00415-013-6951-x
- Lewerenz, J., Ates, G., Methner, A., Conrad, M., and Maher, P. (2018). Oxytosis/ferroptosis-(Re-) emerging roles for oxidative stress-dependent non-apoptotic cell death in diseases of the central nervous system. *Front. Neurosci.* 12:214. doi: 10.3389/fnins.2018.00214
- Li, X., Chen, W., Zhang, L., Liu, W. B., and Fei, Z. (2013). Inhibition of store-operated calcium entry attenuates MPP(+)-induced oxidative stress via preservation of mitochondrial function in PC12 cells: involvement of Homer1a. *PLoS One* 8:e83638. doi: 10.1371/journal.pone.0083638
- Liao, Y., Dong, Y., and Cheng, J. (2017). The function of the mitochondrial calcium uniporter in neurodegenerative disorders. *Int. J. Mol. Sci.* 18:E248. doi: 10.3390/ijms18020248
- Lim, H., Kim, D., and Lee, S. J. (2013). Toll-like receptor 2 mediates peripheral nerve injury-induced NADPH oxidase 2 expression in spinal cord microglia. *J. Biol. Chem.* 288, 7572–7579. doi: 10.1074/jbc.M112.414904
- Lockman, J. A., Geldenhuys, W. J., Bohn, K. A., Desilva, S. F., Allen, D. D., and Van Der Schyf, C. J. (2012). Differential effect of nimodipine in attenuating iron-induced toxicity in brain- and blood-brain barrier-associated cell types. *Neurochem. Res.* 37, 134–142. doi: 10.1007/s11064-011-0591-2
- Lourenco, C. F., Ledo, A., Barbosa, R. M., and Laranjinha, J. (2017). Neurovascular-neuroenergetic coupling axis in the brain: master regulation by nitric oxide and consequences in aging and neurodegeneration. *Free Radic. Biol. Med.* 108, 668–682. doi: 10.1016/j.freeradbiomed.2017.04.026
- Lu, C., Chan, S. L., Fu, W., and Mattson, M. P. (2002). The lipid peroxidation product 4-hydroxynonenal facilitates opening of voltage-dependent Ca²⁺ channels in neurons by increasing protein tyrosine phosphorylation. *J. Biol. Chem.* 277, 24368–24375. doi: 10.1074/jbc.M201924200
- Ludtmann, M. H. R., and Abramov, A. Y. (2018). Mitochondrial calcium imbalance in Parkinson's disease. *Neurosci. Lett.* 663, 86–90. doi: 10.1016/j.neulet.2017.08.044
- Lynch, M. A. (2004). Long-term potentiation and memory. *Physiol. Rev.* 84, 87–136. doi: 10.1152/physrev.00014.2003
- Maher, P., Van Leyen, K., Dey, P. N., Honrath, B., Dolga, A., and Methner, A. (2018). The role of Ca(2+) in cell death caused by oxidative glutamate toxicity and ferroptosis. *Cell Calcium* 70, 47–55. doi: 10.1016/j.ceca.2017.05.007
- Marchi, S., Patergnani, S., Missiroli, S., Morciano, G., Rimessi, A., Wieckowski, M. R., et al. (2018). Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. *Cell Calcium* 69, 62–72. doi: 10.1016/j.ceca.2017.05.003
- Marro, S., Chiabrando, D., Messina, E., Stolte, J., Turco, E., Tolosano, E., et al. (2010). Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter. *Haematologica* 95, 1261–1268. doi: 10.3324/haematol.2009.020123
- Martins, E. A., Robalinho, R. L., and Meneghini, R. (1995). Oxidative stress induces activation of a cytosolic protein responsible for control of iron uptake. *Arch. Biochem. Biophys.* 316, 128–134. doi: 10.1006/abbi.1995.1019
- Mata, A. M., and Sepulveda, M. R. (2010). Plasma membrane Ca-ATPases in the nervous system during development and ageing. *World J. Biol. Chem.* 1, 229–234. doi: 10.4331/wjbc.v1.i7.229
- Mattson, M. P. (2010). ER calcium and Alzheimer's disease: in a state of flux. *Sci. Signal.* 3:e10. doi: 10.1126/scisignal.3114pe10
- McCarthy, R. C., Sosa, J. C., Gardeck, A. M., Baez, A. S., Lee, C. H., and Wessling-Resnick, M. (2018). Inflammation-induced iron transport and metabolism by brain microglia. *J. Biol. Chem.* 293, 7853–7863. doi: 10.1074/jbc.RA118.001949
- Mechlovich, D., Amit, T., Bar-Am, O., Mandel, S., Youdim, M. B., and Weinreb, O. (2014). The novel multi-target iron chelator, M30 modulates HIF-1 α -related glycolytic genes and insulin signaling pathway in the frontal cortex of APP/PS1 Alzheimer's disease mice. *Curr. Alzheimer Res.* 11, 119–127. doi: 10.2174/1567205010666131212112529
- Mena, N. P., Urrutia, P. J., Lourido, F., Carrasco, C. M., and Núñez, M. T. (2015). Mitochondrial iron homeostasis and its dysfunctions in neurodegenerative disorders. *Mitochondrion* 21, 92–105. doi: 10.1016/j.mito.2015.02.001
- Merelli, A., Rodriguez, J. C. G., Folch, J., Regueiro, M. R., Camins, A., and Lazarowski, A. (2018). Understanding the role of hypoxia inducible factor during neurodegeneration for new therapeutics opportunities. *Curr. Neuropharmacol.* 16, 1484–1498. doi: 10.2174/1570159X16666180110130253
- Miyamoto, M., Murphy, T. H., Schnaar, R. L., and Coyle, J. T. (1989). Antioxidants protect against glutamate-induced cytotoxicity in a neuronal cell line. *J. Pharmacol. Exp. Ther.* 250, 1132–1140.
- Moldogazieva, N. T., Lutsenko, S. V., and Terentiev, A. A. (2018). Reactive oxygen and nitrogen species-induced protein modifications: implication in carcinogenesis and anticancer therapy. *Cancer Res.* 78, 6040–6047. doi: 10.1158/0008-5472.CAN-18-0980
- Mole, D. R., Blancher, C., Copley, R. R., Pollard, P. J., Gleadle, J. M., Ragoussis, J., et al. (2009). Genome-wide association of hypoxia-inducible factor (HIF)-1 α and HIF-2 α DNA binding with expression profiling of hypoxia-inducible transcripts. *J. Biol. Chem.* 284, 16767–16775. doi: 10.1074/jbc.M901790200
- Moncada, S., and Bolaños, J. P. (2006). Nitric oxide, cell bioenergetics and neurodegeneration. *J. Neurochem.* 97, 1676–1689. doi: 10.1111/j.1471-4159.2006.03988.x
- Mueller, S., Pantopoulos, K., Hubner, C. A., Stremmel, W., and Hentze, M. W. (2001). IRP1 activation by extracellular oxidative stress in the perfused rat liver. *J. Biol. Chem.* 276, 23192–23196. doi: 10.1074/jbc.M100654200
- Muhling, T., Duda, J., Weishaupt, J. H., Ludolph, A. C., and Liss, B. (2014). Elevated mRNA-levels of distinct mitochondrial and plasma membrane Ca(2+) transporters in individual hypoglossal motor neurons of endstage SOD1 transgenic mice. *Front. Cell. Neurosci.* 8:353. doi: 10.3389/fncel.2014.00353
- Muñoz, P. (2012). Iron-mediated redox modulation in neural plasticity. *Commun. Integr. Biol.* 5, 166–168. doi: 10.4161/cib.18710
- Muñoz, P., Humeres, A., Elgueta, C., Kirkwood, A., Hidalgo, C., and Núñez, M. T. (2011). Iron mediates N-methyl-D-aspartate receptor-dependent stimulation of calcium-induced pathways and hippocampal synaptic plasticity. *J. Biol. Chem.* 286, 13382–13392. doi: 10.1074/jbc.M110.213785
- Muñoz, Y., Carrasco, C. M., Campos, J. D., Aguirre, P., and Núñez, M. T. (2016). Parkinson's Disease: the mitochondria-iron link. *Parkinsons Dis.* 2016:7049108. doi: 10.1155/2016/7049108
- Muravchick, S., and Levy, R. J. (2006). Clinical implications of mitochondrial dysfunction. *Anesthesiology* 105, 819–837. doi: 10.1097/0000542-200610000-00029
- Murphy, T. H., Malouf, A. T., Sastre, A., Schnaar, R. L., and Coyle, J. T. (1988). Calcium-dependent glutamate cytotoxicity in a neuronal cell line. *Brain Res.* 444, 325–332. doi: 10.1016/0006-8993(88)90941-9
- Murphy, T. H., Miyamoto, M., Sastre, A., Schnaar, R. L., and Coyle, J. T. (1989). Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron* 2, 1547–1558. doi: 10.1016/0896-6273(89)90043-3
- Murray-Kolb, L. E. (2013). Iron and brain functions. *Curr. Opin. Clin. Nutr. Metab. Care* 16, 703–707. doi: 10.1097/MCO.0b013e3283653ef8
- Mustaly-Kalimi, S., Littlefield, A. M., and Stutzmann, G. E. (2018). Calcium signaling deficits in glia and autophagic pathways contributing to neurodegenerative disease. *Antioxid. Redox Signal.* 29, 1158–1175. doi: 10.1089/ars.2017.7266
- Myllyharju, J. (2013). Prolyl 4-hydroxylases, master regulators of the hypoxia response. *Acta Physiol.* 208, 148–165. doi: 10.1111/apha.12096

- Nandal, A., Ruiz, J. C., Subramanian, P., Ghimire-Rijal, S., Sinnamon, R. A., Stemmler, T. L., et al. (2011). Activation of the HIF prolyl hydroxylase by the iron chaperones PCBP1 and PCBP2. *Cell Metab.* 14, 647–657. doi: 10.1016/j.cmet.2011.08.015
- Nemes, R., Koltai, E., Taylor, A. W., Suzuki, K., Gyori, F., and Radak, Z. (2018). Reactive oxygen and nitrogen species regulate key metabolic, anabolic, and catabolic pathways in skeletal muscle. *Antioxidants* 7:E85. doi: 10.3390/antiox7070085
- Núñez, M. T. (2010). Regulatory mechanisms of intestinal iron absorption-uncovering of a fast-response mechanism based on DMT1 and ferroportin endocytosis. *Biofactors* 36, 88–97. doi: 10.1002/biof.84
- Núñez, M. T., and Chana-Cuevas, P. (2018). New perspectives in iron chelation therapy for the treatment of neurodegenerative diseases. *Pharmaceuticals* 11:E109. doi: 10.3390/ph11040109
- Núñez, M. T., Gallardo, V., Muñoz, P., Tapia, V., Esparza, A., Salazar, J., et al. (2004). Progressive iron accumulation induces a biphasic change in the glutathione content of neuroblastoma cells. *Free Radic. Biol. Med.* 37, 953–960. doi: 10.1016/j.freeradbiomed.2004.06.005
- Núñez, M. T., Urrutia, P., Mena, N., Aguirre, P., Tapia, V., and Salazar, J. (2012). Iron toxicity in neurodegeneration. *Biometals* 25, 761–776. doi: 10.1007/s10534-012-9523-0
- Oexle, H., Gnaiger, E., and Weiss, G. (1999). Iron-dependent changes in cellular energy metabolism: influence on citric acid cycle and oxidative phosphorylation. *Biochim. Biophys. Acta* 1413, 99–107. doi: 10.1016/S0005-2728(99)00088-2
- Okubo, Y., Mikami, Y., Kanemaru, K., and Iino, M. (2018). Role of endoplasmic reticulum-mediated Ca(2+) signaling in neuronal cell death. *Antioxid. Redox Signal.* 29, 1147–1157. doi: 10.1089/ars.2018.7498
- Orino, K., Lehman, L., Tsuji, Y., Ayaki, H., Torti, S. V., and Torti, F. M. (2001). Ferritin and the response to oxidative stress. *Biochem. J.* 357, 241–247. doi: 10.1042/bj3570241
- Overk, C. R., Rockenstein, E., Florio, J., Cheng, Q., and Masliah, E. (2015). Differential calcium alterations in animal models of neurodegenerative disease: reversal by FK506. *Neuroscience* 310, 549–560. doi: 10.1016/j.neuroscience.2015.08.068
- Pantopoulos, K., and Hentze, M. W. (1995). Rapid responses to oxidative stress mediated by iron regulatory protein. *EMBO J.* 14, 2917–2924. doi: 10.1002/j.1460-2075.1995.tb07291.x
- Pantopoulos, K., Weiss, G., and Hentze, M. W. (1996). Nitric oxide and oxidative stress (H₂O₂) control mammalian iron metabolism by different pathways. *Mol. Cell. Biol.* 16, 3781–3788. doi: 10.1128/MCB.16.7.3781
- Paradkar, P. N., and Roth, J. A. (2006a). Nitric oxide transcriptionally down-regulates specific isoforms of divalent metal transporter (DMT1) via NF-kappaB. *J. Neurochem.* 96, 1768–1777.
- Paradkar, P. N., and Roth, J. A. (2006b). Post-translational and transcriptional regulation of DMT1 during P19 embryonic carcinoma cell differentiation by retinoic acid. *Biochem. J.* 394, 173–183.
- Paradkar, P. N., Zumbrennen, K. B., Paw, B. H., Ward, D. M., and Kaplan, J. (2009). Regulation of mitochondrial iron import through differential turnover of mitoferrin 1 and mitoferrin 2. *Mol. Cell. Biol.* 29, 1007–1016. doi: 10.1128/MCB.01685-08
- Pchitskaya, E., Popugaeva, E., and Bezprozvanny, I. (2018). Calcium signaling and molecular mechanisms underlying neurodegenerative diseases. *Cell Calcium* 70, 87–94. doi: 10.1016/j.ceca.2017.06.008
- Pelizzoni, I., Macco, R., Morini, M. F., Zacchetti, D., Grohovaz, F., and Codazzi, F. (2011). Iron handling in hippocampal neurons: activity-dependent iron entry and mitochondria-mediated neurotoxicity. *Aging Cell* 10, 172–183. doi: 10.1111/j.1474-9726.2010.00652.x
- Pelizzoni, I., Macco, R., Zacchetti, D., Grohovaz, F., and Codazzi, F. (2008). Iron and calcium in the central nervous system: a close relationship in health and sickness. *Biochem. Soc. Trans.* 36, 1309–1312. doi: 10.1042/BST0361309
- Pelizzoni, I., Zacchetti, D., Campanella, A., Grohovaz, F., and Codazzi, F. (2013). Iron uptake in quiescent and inflammation-activated astrocytes: a potentially neuroprotective control of iron burden. *Biochim. Biophys. Acta* 1832, 1326–1333. doi: 10.1016/j.bbdis.2013.04.007
- Pereira, C., Ferreira, C., Carvalho, C., and Oliveira, C. (1996). Contribution of plasma membrane and endoplasmic reticulum Ca(2+)-ATPases to the synaptosomal [Ca²⁺]_i increase during oxidative stress. *Brain Res.* 713, 269–277. doi: 10.1016/0006-8993(95)01554-X
- Perier, C., Tieu, K., Guegan, C., Caspersen, C., Jackson-Lewis, V., Carelli, V., et al. (2005). Complex I deficiency primes Bax-dependent neuronal apoptosis through mitochondrial oxidative damage. *Proc. Natl. Acad. Sci. U.S.A.* 102, 19126–19131. doi: 10.1073/pnas.0508215102
- Peters, D. G., Connor, J. R., and Meadowcroft, M. D. (2015). The relationship between iron dyshomeostasis and amyloidogenesis in Alzheimer's disease: two sides of the same coin. *Neurobiol. Dis.* 81, 49–65. doi: 10.1016/j.nbd.2015.08.007
- Phillips, J. D., Kinikini, D. V., Yu, Y., Guo, B., and Leibold, E. A. (1996). Differential regulation of IRP1 and IRP2 by nitric oxide in rat hepatoma cells. *Blood* 87, 2983–2992.
- Piret, J. P., Mottet, D., Raes, M., and Michiels, C. (2002). Is HIF-1alpha a pro- or an anti-apoptotic protein? *Biochem. Pharmacol.* 64, 889–892.
- Ponka, P. (2004). Hereditary causes of disturbed iron homeostasis in the central nervous system. *Ann. N. Y. Acad. Sci.* 1012, 267–281. doi: 10.1196/annals.1306.022
- Popugaeva, E., Pchitskaya, E., and Bezprozvanny, I. (2018). Dysregulation of intracellular calcium signaling in alzheimer's disease. *Antioxid. Redox Signal.* 29, 1176–1188. doi: 10.1089/ars.2018.7506
- Post, M. R., Lieberman, O. J., and Mosharof, E. V. (2018). Can interactions between alpha-synuclein, dopamine and calcium explain selective neurodegeneration in Parkinson's disease? *Front. Neurosci.* 12:161. doi: 10.3389/fnins.2018.00161
- Qian, Z. M., Wu, X. M., Fan, M., Yang, L., Du, F., Yung, W. H., et al. (2011). Divalent metal transporter 1 is a hypoxia-inducible gene. *J. Cell Physiol.* 226, 1596–1603. doi: 10.1002/jcp.22485.
- Ray, P. D., Huang, B. W., and Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell. Signal.* 24, 981–990. doi: 10.1016/j.cellsig.2012.01.008
- Requejo-Aguilar, R., and Bolanos, J. P. (2016). Mitochondrial control of cell bioenergetics in Parkinson's disease. *Free Radic. Biol. Med.* 100, 123–137. doi: 10.1016/j.freeradbiomed.2016.04.012
- Riganti, C., Doublier, S., Viariso, D., Miraglia, E., Pescarmona, G., Ghigo, D., et al. (2009). Artemisinin induces doxorubicin resistance in human colon cancer cells via calcium-dependent activation of HIF-1alpha and P-glycoprotein overexpression. *Br. J. Pharmacol.* 156, 1054–1066. doi: 10.1111/j.1476-5381.2009.01117.x
- Rimessi, A., Bonora, M., Marchi, S., Patergnani, S., Marobbio, C. M., Lasorsa, F. M., et al. (2013). Perturbed mitochondrial Ca²⁺ signals as causes or consequences of mitophagy induction. *Autophagy* 9, 1677–1686. doi: 10.4161/auto.24795
- Riquelme, D., Alvarez, A., Leal, N., Adasme, T., Espinoza, I., Valdes, J. A., et al. (2011). High-frequency field stimulation of primary neurons enhances ryanodine receptor-mediated Ca²⁺ release and generates hydrogen peroxide, which jointly stimulate NF-kappaB activity. *Antioxid. Redox Signal.* 14, 1245–1259. doi: 10.1089/ars.2010.3238
- Rosales-Corral, S., Acuna-Castroviejo, D., Tan, D. X., Lopez-Armas, G., Cruz-Ramos, J., Muñoz, R., et al. (2012). Accumulation of exogenous amyloid-beta peptide in hippocampal mitochondria causes their dysfunction: a protective role for melatonin. *Oxid. Med. Cell. Longev.* 2012:843649. doi: 10.1155/2012/843649
- Rosi, S., Ramirez-Amaya, V., Hauss-Wegrzyniak, B., and Wenk, G. L. (2004). Chronic brain inflammation leads to a decline in hippocampal NMDA-R1 receptors. *J. Neuroinflammation* 1:12. doi: 10.1186/1742-2094-1-12
- Rothwell, N. J., and Luheshi, G. N. (2000). Interleukin 1 in the brain: biology, pathology and therapeutic target. *Trends Neurosci.* 23, 618–625. doi: 10.1016/S0166-2236(00)01661-1
- Rousseau, E., Michel, P. P., and Hirsch, E. C. (2013). The iron-binding protein lactoferrin protects vulnerable dopamine neurons from degeneration by preserving mitochondrial calcium homeostasis. *Mol. Pharmacol.* 84, 888–898. doi: 10.1124/mol.113.087965
- Ruiz, A., Alberdi, E., and Matute, C. (2018). Mitochondrial division inhibitor 1 (mdivi-1) protects neurons against excitotoxicity through the modulation of mitochondrial function and intracellular Ca(2+) signaling. *Front. Mol. Neurosci.* 11:3. doi: 10.3389/fnmol.2018.00003
- Sanmartin, C. D., Paula-Lima, A. C., Garcia, A., Barattini, P., Hartel, S., Núñez, M. T., et al. (2014). Ryanodine receptor-mediated Ca(2+) release underlies iron-induced mitochondrial fission and stimulates mitochondrial Ca(2+)

- uptake in primary hippocampal neurons. *Front. Mol. Neurosci.* 7:13. doi: 10.3389/fnmol.2014.00013
- Schiavone, S., Sorce, S., Dubois-Dauphin, M., Jaquet, V., Colaianna, M., Zotti, M., et al. (2009). Involvement of NOX2 in the development of behavioral and pathologic alterations in isolated rats. *Biol. Psychiatry* 66, 384–392. doi: 10.1016/j.biopsych.2009.04.033
- Sen, C. K. (2001). Antioxidants in exercise nutrition. *Sports Med.* 31, 891–908. doi: 10.2165/00007256-200131130-00001
- Sherer, T. B., Betarbet, R., Testa, C. M., Seo, B. B., Richardson, J. R., Kim, J. H., et al. (2003). Mechanism of toxicity in rotenone models of Parkinson's disease. *J. Neurosci.* 23, 10756–10764. doi: 10.1523/JNEUROSCI.23-34-10756.2003
- Shih, R. H., Wang, C. Y., and Yang, C. M. (2015). NF-kappaB signaling pathways in neurological inflammation: a mini review. *Front. Mol. Neurosci.* 8:77. doi: 10.3389/fnmol.2015.00077
- Shimohama, S., Tanino, H., Kawakami, N., Okamura, N., Kodama, H., Yamaguchi, T., et al. (2000). Activation of NADPH oxidase in Alzheimer's disease brains. *Biochem. Biophys. Res. Commun.* 273, 5–9. doi: 10.1006/bbrc.2000.2897
- Siddiq, A., Ayoub, I. A., Chavez, J. C., Aminova, L., Shah, S., Lamanna, J. C., et al. (2005). Hypoxia-inducible factor prolyl 4-hydroxylase inhibition. A target for neuroprotection in the central nervous system. *J. Biol. Chem.* 280, 41732–41743. doi: 10.1074/jbc.M504963200
- Siebert, I., Schodel, J., Nairz, M., Schatz, V., Dettmer, K., Dick, C., et al. (2015). Ferritin-Mediated iron sequestration stabilizes hypoxia-inducible factor-1alpha upon LPS activation in the presence of ample oxygen. *Cell Rep.* 13, 2048–2055. doi: 10.1016/j.celrep.2015.11.005
- Siklos, L., Engelhardt, J. I., Alexianu, M. E., Gurney, M. E., Siddique, T., and Appel, S. H. (1998). Intracellular calcium parallels motoneuron degeneration in SOD-1 mutant mice. *J. Neuropathol. Exp. Neurol.* 57, 571–587. doi: 10.1097/00005072-199806000-00005
- Sirabella, R., Valsecchi, V., Anzilotti, S., Cuomo, O., Vinciguerra, A., Cepparulo, P., et al. (2018). Ionic homeostasis maintenance in ALS: focus on new therapeutic targets. *Front. Neurosci.* 12:510. doi: 10.3389/fnins.2018.00510
- Smith, T. G., Robbins, P. A., and Ratcliffe, P. J. (2008). The human side of hypoxia-inducible factor. *Br. J. Haematol.* 141, 325–334. doi: 10.1111/j.1365-2141.2008.07029.x
- Sorce, S., and Krause, K. H. (2009). NOX enzymes in the central nervous system: from signaling to disease. *Antioxid. Redox Signal.* 11, 2481–2504. doi: 10.1089/ARS.2009.2578
- Sorce, S., Stocker, R., Seredenina, T., Holmdahl, R., Aguzzi, A., Chio, A., et al. (2017). NADPH oxidases as drug targets and biomarkers in neurodegenerative diseases: what is the evidence? *Free Radic. Biol. Med.* 112, 387–396. doi: 10.1016/j.freeradbiomed.2017.08.006
- Soum, E., and Drapier, J. C. (2003). Nitric oxide and peroxynitrite promote complete disruption of the [4Fe-4S] cluster of recombinant human iron regulatory protein 1. *J. Biol. Inorg. Chem.* 8, 226–232. doi: 10.1007/s00775-002-0412-9
- Stefani, I. C., Wright, D., Polizzi, K. M., and Kontoravdi, C. (2012). The role of ER stress-induced apoptosis in neurodegeneration. *Curr. Alzheimers Res.* 9, 373–387. doi: 10.2174/156720512800107618
- Stockwell, B. R., Friedmann Angeli, J. P., Bayir, H., Bush, A. I., Conrad, M., Dixon, S. J., et al. (2017). Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 171, 273–285. doi: 10.1016/j.cell.2017.09.021
- Strehler, E. E., and Thayer, S. A. (2018). Evidence for a role of plasma membrane calcium pumps in neurodegenerative disease: recent developments. *Neurosci. Lett.* 663, 39–47. doi: 10.1016/j.neulet.2017.08.035
- Surace, M. J., and Block, M. L. (2012). Targeting microglia-mediated neurotoxicity: the potential of NOX2 inhibitors. *Cell. Mol. Life Sci.* 69, 2409–2427. doi: 10.1007/s00018-012-1015-4
- Sureda, A., Hebling, U., Pons, A., and Mueller, S. (2005). Extracellular H2O2 and not superoxide determines the compartment-specific activation of transferrin receptor by iron regulatory protein 1. *Free Radic. Res.* 39, 817–824. doi: 10.1080/10715760500164045
- Surmeier, D. J., Guzman, J. N., and Sanchez-Padilla, J. (2010). Calcium, cellular aging, and selective neuronal vulnerability in Parkinson's disease. *Cell Calcium* 47, 175–182. doi: 10.1016/j.ceca.2009.12.003
- Surmeier, D. J., Guzman, J. N., Sanchez-Padilla, J., and Goldberg, J. A. (2011). The origins of oxidant stress in Parkinson's disease and therapeutic strategies. *Antioxid. Redox Signal.* 14, 1289–1301. doi: 10.1089/ars.2010.3521
- Swaiman, K. F. (1991). Hallervorden-Spatz syndrome and brain iron metabolism. *Arch. Neurol.* 48, 1285–1293. doi: 10.1001/archneur.1991.00530240091029
- Tan, S., Sagara, Y., Liu, Y., Maher, P., and Schubert, D. (1998). The regulation of reactive oxygen species production during programmed cell death. *J. Cell. Biol.* 141, 1423–1432. doi: 10.1083/jcb.141.6.1423
- Tanguido, N. K., Alan, B., Vinchi, F., Worle, K., Lai, D., Vettorazzi, S., et al. (2018). Scavenging reactive oxygen species production normalizes ferroportin expression and ameliorates cellular and systemic iron disbalances in hemolytic mouse model. *Antioxid. Redox Signal.* 29, 484–499. doi: 10.1089/ars.2017.7089
- Thomas, G. M., and Haganir, R. L. (2004). MAPK cascade signalling and synaptic plasticity. *Nat. Rev. Neurosci.* 5, 173–183. doi: 10.1038/nrn1346
- Tonelli, C., Chio, I. I. C., and Tuveson, D. A. (2018). Transcriptional regulation by Nrf2. *Antioxid. Redox Signal.* 29, 1727–1745. doi: 10.1089/ars.2017.7342
- Tong, W. H., and Rouault, T. A. (2007). Metabolic regulation of citrate and iron by aconitases: role of iron-sulfur cluster biogenesis. *Biomaterials* 20, 549–564. doi: 10.1007/s10534-006-9047-6
- Tonnies, E., and Trushina, E. (2017). Oxidative stress, synaptic dysfunction, and Alzheimer's Disease. *J. Alzheimers Dis.* 57, 1105–1121. doi: 10.3233/JAD-161088
- Urrutia, P., Aguirre, P., Esparza, A., Tapia, V., Mena, N. P., Arredondo, M., et al. (2013). Inflammation alters the expression of DMT1, FPN1 and hepcidin, and it causes iron accumulation in central nervous system cells. *J. Neurochem.* 126, 541–549. doi: 10.1111/jnc.12244
- Urrutia, P. J., Mena, N. P., and Núñez, M. T. (2014). The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. *Front. Pharmacol.* 5:38. doi: 10.3389/fphar.2014.00038
- Vila, M., and Przedborski, S. (2003). Targeting programmed cell death in neurodegenerative diseases. *Nat. Rev. Neurosci.* 4, 365–375. doi: 10.1038/nrn1100
- Wang, J., Song, N., Jiang, H., Wang, J., and Xie, J. (2013). Pro-inflammatory cytokines modulate iron regulatory protein 1 expression and iron transportation through reactive oxygen/nitrogen species production in ventral mesencephalic neurons. *Biochim. Biophys. Acta* 1832, 618–625. doi: 10.1016/j.bbadis.2013.01.021
- Wang, Q. M., Xu, Y. Y., Liu, S., and Ma, Z. G. (2017). Isradipine attenuates MPTP-induced dopamine neuron degeneration by inhibiting up-regulation of L-type calcium channels and iron accumulation in the substantia nigra of mice. *Oncotarget* 8, 47284–47295. doi: 10.18632/oncotarget.17618
- Wang, Y., Yang, J., and Yi, J. (2012). Redox sensing by proteins: oxidative modifications on cysteines and the consequent events. *Antioxid. Redox Signal.* 16, 649–657. doi: 10.1089/ars.2011.4313
- 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. doi: 10.1016/S1474-4422(14)70117-6
- Wardrop, S. L., Watts, R. N., and Richardson, D. R. (2000). Nitrogen monoxide activates iron regulatory protein 1 RNA-binding activity by two possible mechanisms: effect on the [4Fe-4S] cluster and iron mobilization from cells. *Biochemistry* 39, 2748–2758. doi: 10.1021/bi991099t
- Weinreb, O., Amit, T., Bar-Am, O., and Youdim, M. B. (2016). Neuroprotective effects of multifaceted hybrid agents targeting MAO, cholinesterase, iron and beta-amyloid in ageing and Alzheimer's disease. *Br. J. Pharmacol.* 173, 2080–2094. doi: 10.1111/bph.13318
- Wiethoff, S., and Houlden, H. (2017). Neurodegeneration with brain iron accumulation. *Handb. Clin. Neurol.* 145, 157–166. doi: 10.1016/B978-0-12-802395-2.00011-0
- Wilkinson, N., and Pantopoulos, K. (2014). The IRP/IRE system in vivo: insights from mouse models. *Front. Pharmacol.* 5:176. doi: 10.3389/fphar.2014.00176
- Wu, D. C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., et al. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J. Neurosci.* 22, 1763–1771. doi: 10.1523/JNEUROSCI.22-05-01763.2002

- Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R., and Turner, N. D. (2004). Glutathione metabolism and its implications for health. *J. Nutr.* 134, 489–492. doi: 10.1093/jn/134.3.489
- Wu, G. Y., Deisseroth, K., and Tsien, R. W. (2001). Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proc. Natl. Acad. Sci. U.S.A.* 98, 2808–2813. doi: 10.1073/pnas.051634198
- Xiao, J., Lv, Y., Lin, B., Tipoe, G. L., Youdim, M. B., Xing, F., et al. (2015). A novel antioxidant multitarget iron chelator M30 protects hepatocytes against ethanol-induced injury. *Oxid. Med. Cell. Longev.* 2015:607271. doi: 10.1155/2015/607271
- Yang, W. S., Sriramaratnam, R., Welsch, M. E., Shimada, K., Skouta, R., Viswanathan, V. S., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156, 317–331. doi: 10.1016/j.cell.2013.12.010
- Yeh, T. L., Leissing, T. M., Abboud, M. I., Thinnis, C. C., Atasoylu, O., Holt-Martyn, J. P., et al. (2017). Molecular and cellular mechanisms of HIF prolyl hydroxylase inhibitors in clinical trials. *Chem. Sci.* 8, 7651–7668. doi: 10.1039/c7sc02103h
- Yermolaieva, O., Brot, N., Weissbach, H., Heinemann, S. H., and Hoshi, T. (2000). Reactive oxygen species and nitric oxide mediate plasticity of neuronal calcium signaling. *Proc. Natl. Acad. Sci. U.S.A.* 97, 448–453. doi: 10.1073/pnas.97.1.448
- Yuan, G., Nanduri, J., Bhasker, C. R., Semenza, G. L., and Prabhakar, N. R. (2005). Ca²⁺/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. *J. Biol. Chem.* 280, 4321–4328. doi: 10.1074/jbc.M407706200
- Yuan, G., Nanduri, J., Khan, S., Semenza, G. L., and Prabhakar, N. R. (2008). Induction of HIF-1 α expression by intermittent hypoxia: involvement of NADPH oxidase, Ca²⁺ signaling, prolyl hydroxylases, and mTOR. *J. Cell. Physiol.* 217, 674–685. doi: 10.1002/jcp.21537
- Zaidi, A. (2010). Plasma membrane Ca-ATPases: targets of oxidative stress in brain aging and neurodegeneration. *World J. Biol. Chem.* 1, 271–280. doi: 10.4331/wjbc.v1.i9.271
- Zaidi, A., and Michaelis, M. L. (1999). Effects of reactive oxygen species on brain synaptic plasma membrane Ca(2+)-ATPase. *Free Radic. Biol. Med.* 27, 810–821. doi: 10.1016/S0891-5849(99)00128-8
- Zecca, L., Youdim, M. B., Riederer, P., Connor, J. R., and Crichton, R. R. (2004). Iron, brain ageing and neurodegenerative disorders. *Nat. Rev. Neurosci.* 5, 863–873. doi: 10.1038/nrn1537
- Zhang, W., Yan, Z. F., Gao, J. H., Sun, L., Huang, X. Y., Liu, Z., et al. (2014). Role and mechanism of microglial activation in iron-induced selective and progressive dopaminergic neurodegeneration. *Mol. Neurobiol.* 49, 1153–1165. doi: 10.1007/s12035-013-8586-4
- Zhang, X., Zhou, K., Wang, R., Cui, J., Lipton, S. A., Liao, F. F., et al. (2007). Hypoxia-inducible factor 1 α (HIF-1 α)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. *J. Biol. Chem.* 282, 10873–10880. doi: 10.1074/jbc.M608856200
- Zhang, Y., Kong, W. N., and Chai, X. Q. (2018). Compound of icariin, astragalus, and puerarin mitigates iron overload in the cerebral cortex of Alzheimer's disease mice. *Neural Regen. Res.* 13, 731–736. doi: 10.4103/1673-5374.230302
- Zorov, D. B., Isaev, N. K., Plotnikov, E. Y., Zorova, L. D., Stelmashook, E. V., Vasileva, A. K., et al. (2007). The mitochondrion as janus bifrons. *Biochemistry* 46, 1115–1126.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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