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THE ROLE OF OXIDATIVE STRESS IN ANXIETY DISORDER: CAUSE OR CONSEQUENCE?

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Abstract

Anxiety disorders are the most common mental illness in the US affecting 18% of the population. The cause(s) of anxiety disorders is/are not completely clear, and research in the neurobiology of anxiety at the molecular level is still rather limited. Although mounting clinical and pre-clinical evidence now indicates that oxidative stress may be a major component of anxiety pathology, whether oxidative stress is the cause or consequence remains elusive. Studies conducted over the past few years suggest that anxiety disorders may be characterized by lowered antioxidant defenses and increased oxidative damage to proteins, lipids and nucleic acids. In particular, oxidative modifications to proteins have actually been proposed as a potential factor in the onset and progression of several psychiatric disorders, including anxiety and depressive disorders. Oxidized proteins are normally degraded by the Proteasome proteolytic complex in the cell cytoplasm, nucleus, and endoplasmic reticulum. The Lon protease performs a similar protective function inside mitochondria. Impairment of the Proteasome and/or the Lon protease results in the accumulation of toxic oxidized proteins in the brain, which can cause severe neuronal trauma. Recent evidence points to possible proteolytic dysfunction and accumulation of damaged, oxidized proteins as factors that may determine the appearance and severity of psychotic symptoms in mood disorders. Thus, critical interactions between oxidative stress, Proteasome, and the Lon protease may provide keys to the molecular mechanisms involved in emotional regulation, and may also be of great help in designing and screening novel anxiolytics and antidepressants.

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Keywords

Anxiety Disorder; Psychiatric Disorders; Antioxidants; Nrf2; Oxidative Stress; Proteasome; Lon protease; Inflammation

Introduction

1. How Oxidative Stress May be Associated with the Genesis of Anxiety

Traditionally, oxidative stress is defined as an imbalance between pro-oxidants and antioxidants, in favor of a pro-oxidant state [1, 2]. It is very clear that high concentrations of oxidants can damage cell components, including proteins, lipids and nucleic acids [3]. Such damage leads to severe physiological distress with impairment of normal cell functions, or even cell death [1] (Fig. 1). The brain represents only 2% of total body weight for human beings, but accounts for around 20% of our total oxygen consumption. Despite the high reliance on oxygen metabolism, the Central Nervous System (CNS) is especially vulnerable to oxidative stress due to several factors: 1) The limited regenerative capacity of neural cells, because adult neurons are post-mitotic cells that do not replicate [4]; 2) Intrinsic metabolic and structural characteristics of neurons make them more sensitive to oxidation compared to cells in other organs [5]; 3) Neuronal membranes are rich in polyunsaturated fatty acids, that make the brain more vulnerable to lipid oxidation[6]; 4) Several neurotransmitters, such as dopamine, norepinephrine, and serotonin easily undergo autoxidation [7]; 5) Brain microglia when activated inappropriately, can produce massive amounts of reactive oxygen and nitrogen species [8, 9]; and 6) CNS antioxidant defenses are relatively modest in comparison with those of other tissues. In particular, the CNS contains rather low levels of both glutathione peroxidase and catalase [5, 10].

Current evidence connects oxidative stress to several psychiatric disorders, including anxiety and major depression, although the mechanism(s) and pathway(s) involved are not fully understood[11–13] (Fig. 2). Glutamate excitotoxicity is a major effector that causes oxidative stress in the CNS, and autoxidation of neurotransmitters can also generate reactive oxygen species such as the superoxide anion radical ($O_2^{\bullet -}$)[14]. Therefore, oxidative stress could be a primary cause of neuropsychiatric disorders in some instances, or merely a downstream consequence in other cases.

Several studies have reported that inhibition of the Proteasome may play a causal role in the neurotoxicity associated with oxidative stress, under conditions that may even be sufficient to induce neural death[15]. The Proteasome is the major proteolytic enzyme responsible for maintaining protein homeostasis (proteostasis) in the cell cytoplasm, nucleus, and endoplasmic reticulum, and plays a particularly important role in dealing with oxidatively damaged proteins [16–22] This functional understanding of the relationship between oxidative stress and anxiety disorder may pave the way for the discovery of novel targets for the treatment of neuropsychiatric disorders [23, 24].

In this review we examine some of the recent discoveries that link oxidative stress with anxiety disorder, particularly focusing on abnormalities at the molecular, mitochondrial, and

immunological levels that may be associated with the onset and progression of neuropathology of anxiety, and how the Proteasome may be essential to understanding the neurobiology of anxiety disorder.

1.1. Oxidative Stress and the CNS -—Oxidative stress is an inevitable result of life in an oxygen-rich environment [25] Generation of reactive oxygen species is an aspect of aerobic life and the origin of a complex antioxidant system, synthesized by all known aerobic organisms [26] Since oxidative damage still occurs, however, aerobic organisms have also evolved complex damage removal and repair systems, [27, 28] as well as complex mechanisms to transiently elevate their defense and repair 'armories' via processes such as adaptive homeostasis [29–31].

1.1.1 Source of Free Radicals -: In normal mitochondria, oxygen is reduced to water by the cytochrome c oxidase complex (Complex IV) in four consecutive one-electron steps, because molecular oxygen possesses a triplet state configuration (Fig. 3). Release of partially reduced oxygen intermediates does not occur during this process because of the high binding affinity of cytochrome c [27]. However, some 1–2% of the molecular oxygen consumed during normal physiological respiration is converted into $O_2^{\bullet-}$ by electron 'leakage' to oxygen at other sites in the mitochondrial electron transport chain, such as complexes I and III [7, 27]. The $O_2^{\bullet-}$ may also be formed by other cellular sources, such as xanthine oxidase, membrane-bound NADPH oxidases, cytochrome P450 in the endoplasmic reticulum, and flavin oxidases inside peroxisomes [32].

Intracellular superoxide dismutase (SOD) in its two forms (Cu-Zn-SOD localized in the cytosol and Mn-SOD localized in the mitochondrial matrix) is responsible for the dismutation of $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2) [27]. The H_2O_2 generated by Mn-SOD can be removed by glutathione peroxidase in the mitochondria or, if it diffuses into the cytosol, H_2O_2 can be removed by cytosolic antioxidants, such as catalase (CAT), and peroxiredoxins (Prx), that act in conjunction with SOD. Although it is often cited as an important cellular antioxidant enzyme, catalase in mostly found within peroxisomes; therefore, although catalase is certainly an important antioxidant enzyme for peroxisomes, its antioxidant value to other parts of the cell is less clear. [33]

Under conditions in which mitochondrial $O_2^{\bullet-}$ generation is significantly increased, however, or when antioxidant systems are depleted, H_2O_2 may accumulate and lead to a state of mitochondrial oxidative stress. In this situation, H_2O_2 may react with mitochondrial Fe^{2+}, resulting in the formation of the highly oxidizing hydroxyl radical (HO$\bullet$), via the Fenton reaction[7]. This can cause severe damage to cell structures including membrane phospholipids, proteins, and both nuclear and mitochondrial DNA, and also cause inactivation of membrane receptors, enzymes and ion channels [34]. Under such conditions, changes may occur in neurotransmitter systems and brain activity, leading to a series of cognitive and behavioral alterations commonly observed in several psychiatric disorders [35, 36]. However, the identification of microenvironments affected by oxidative stress and specifically associated with discrete mental disorders has proven to be a challenging task. *In vivo* molecular imaging techniques, combined with positron emission tomography (PET) have been successfully used to elucidate neuropathological brain changes and their relation

to schizophrenia [37]. These techniques may also help to elucidate pathophysiological brain changes associated with specific anxiety disorders in the future.

1.2. Neurobiology of Anxiety Disorder and Oxidative Stress ––Normal levels of anxiety can have a great adaptive value, developing the alert signal that triggers behavioral, physiological, and cognitive changes, that allow us to deal with novel situations or threats [38]. Nevertheless, the persistence of a high alert state, without corresponding risk circumstances, may cause an individual to develop maladaptive responses to real stress, with the manifestation of syndromes such as generalized anxiety disorder, panic disorder, agoraphobia, other phobias, and social anxiety disorders [39]. Similarly, the ability of the body to maintain homeostasis in response to stressors, allostasis, has great benefit, however when severity or frequency of stress is too great the response systems become overloaded, which leads to pathophysiology or allostatic load/overload [40]. This illustrates the necessity of health-promoting behaviors and practices that can help maintain allostasis over the long term to avoid allostatic load.

1.2.1 Allostatic Load/Overload leads to Neuropathology –: When experiencing an approaching threat, or in anticipation of a threatening experience, the hypothalamic-pituitary-adrenal (HPA) axis stimulates a response mediated by glucocorticoids (also known as stress hormones) to increase the organism's fitness to cope with the threat [41, 42]. As a protective acute-phase response system this is highly effective and efficient in maintaining allostasis. However, the long-term maintenance of such a defensive state requires an additional physiological cost that can overburden the system and lead to allostatic load/overload. Increasing evidence indicates that hypersecretion of glucocorticoids and dysregulation of glucocorticoid receptor function is involved in the pathogenesis of anxiety disorders [43]. Postmortem studies have revealed that oxidative damage to limbic structures is responsible for modulation of anxiety behavior [44]. For example, the HPA axis is activated during stress responses, and can induce significant hippocampal cellular oxidation [45]. In parallel, clinical evidence shows that patients with panic disorder and obsessive–compulsive disorder, have elevated levels of oxidized compounds in their peripheral blood, red blood cells, mononuclear cells, urine and cerebrospinal fluid [46]. Elevated glucocorticoid levels are associated with an increase in oxidant production and therefore elevated oxidative damage [47], and should, therefore, be considered a possible mediator between oxidative stress and anxiety disorders.

Glucocorticoids may also directly affect mitochondrial metabolism and may regulate mitochondrial bioenergetics in rat liver mitochondria [48, 49]. Recent studies have revealed that glucocorticoids modulate mitochondrial calcium homeostasis and the generation of oxidants. [50]. Translocation of glucocorticoids into mitochondria via the glucocorticoid receptor can modulate mitochondrial gene expression [51, 52]. Although the mechanism of this regulation remains unclear, recent studies indicate that release of cytochrome C and calcium from mitochondria is altered when rat brain cells are treated with corticosterone. Regulation of mitochondrial function by corticosterone appears to correlate with neuroprotection; that is, treatment with low doses of corticosterone had a neuroprotective effect, whereas treatment with high doses of corticosterone was toxic to cortical neurons

[53, 54]. These results may ultimately contribute to a better understanding of the mechanisms by which glucocorticoids and stress regulate cellular plasticity and maintain allostasis [40], and to the future development of improved therapeutics.

1.2.2. Animal Models link Oxidative Stress and Neuropathology –: Animal studies have been quite useful in clarifying the role of oxidative stress in anxiety-like behaviors (e.g. model mammal systems such as mice and rats) [55–59]. Hovatta et al. (2010), were the first to demonstrate a link between expression of antioxidant defense system genes in the brain, and anxiety-like behaviors in six different mice strains [13]. Berry et al. (2008) showed that deletion of the p66Shc gene, responsible for the regulation of certain reactive oxygen species, results in lower levels of oxidative stress, and reduced anxiety-like behavior in mice evaluated with the elevated plus-maze test [60]. Desrumaux et al. (2010) demonstrated that decreased vitamin E levels and increased levels of central oxidative stress markers, such as cholesterol oxides and cellular peroxides results in anxiogenic behavior in the mice [61].

Several studies have reported a role for oxidative stress in anxiety-like-behaviors in rodents [62–65]. For example, Souza et al (2007) showed that a highly palatable diet (enriched with sucrose) increases protein oxidation in the frontal cortex and appeared to induce anxiety-like behavior in rats [66]. Furthermore, inhibition of GSH synthesis by administration of L-buthionine-(S,R) sulfoximine directly into the mouse hippocampus induced an anxiety-like behavior [67]. In sum, several studies in animal models suggest that genetic or pharmacologic alterations of the redox balance produce behavioral changes related to anxiety disorders. Treatment with antioxidants appears to prevent many of these effects [68–71].

Finally, the evidence presented above might situate glucocorticoid and mitochondrial oxidative interactions as the "*Rosetta* stone" with which to translate stress hormone effects into psychiatric disorders, mediated by the oxidation of key elements at neurons, astrocytes, or even glial cells. Since many anxiety disorders are an inappropriate cognitive response to a non-threatening environment (though there are notable exceptions), the mechanisms that contribute to the physiopathology of anxiety disorder are important to clarify fully.

2. Oxidative Stress, Antioxidants and Selective Serotonin Reuptake Inhibitors (SSRIs)

2.1 Neurotransmitters and SSRIs ––Predominantly, studies about anxiety disorder have been focused on the regulatory systems, including gamma-aminobutyric (GABA) acidergic and serotoninergic systems [72, 73]. GABA is the principal inhibitory neurotransmitter of the CNS, and is crucial for maintaining homeostasis by counterbalancing the neuronal excitability that characterizes anxiety disorder [74].

When first introduced benzodiazepines, which are selective agonists for the gamma-aminobutyric acid–A receptor (GABA–A), were initially considered to be first-line treatments for anxiety because of their tolerability and rapid mode of action. However, benzodiazepines carry the risk of dependence, sedation and tolerance [75]. *A Posteriori* evidence from numerous preclinical and clinical studies, suggesting that dysfunction in serotonergic neurotransmission could have a role in the pathophysiology of anxiety disorder, culminated in the classical serotonin hypothesis of anxiety [73]. Subsequently, studies with agents that targeted particular molecular systems, such as the selective serotonin reuptake

inhibitors and the serotonin and noradrenaline reuptake inhibitors, constituted another important step as treatments for anxiety disorder [76]. Despite advantages to the introduction of the monoaminergic modulator, the treatment needs several weeks before a therapeutic effect can be observed and the efficacy and duration of relief have not actually improved in most cases [77].

Given the enormous contribution of anxiety disorders to the burden of human disease, it is key to optimize their prevention and treatment. At the moment, more focused development of medications with selective mechanisms of action, followed by rigorous clinical trials to quantify their efficacy and safety are sorely needed [78]. A number of studies have suggested that an imbalance between oxidative stress and antioxidant defenses may be associated with the development of neuropsychiatric disorders, such anxiety and depression [79–82].

2.2 Antioxidant Effects on Pathophysiology ––—Oxidative stress is increased in anxiety disorder, and some have suggested that antioxidant therapy may be useful as a treatment, alongside classical medications [83]. Various forms of antioxidants, such as Vitamins E and C, creatine, and CoQ10 have been tested for their neuroprotective potential [83]. Vitamin E deficiency can affect the mitochondrial permeability transition pore and lead to dopaminergic neurotoxicity[84]. On the other hand, administering vitamin E reportedly ameliorated oxidative stress induced by iron accumulation in the mouse brain [85]. A neuroprotective role for CoQ10 was also seen in cultured human dopaminergic neurons where iron-induced cellular damage, mediated by pro oxidants, was attenuated[86]. Similarly, vitamin C ameliorated energy depletion and apoptosis, caused by glutamate-induced excitation, in the hippocampus of developing rat brains [87]. Dietary creatine supplementation was able increase brain creatine concentrations in Huntington's disease transgenic mice to wildtype levels and resulted in neuroprotective effects [88]. Antioxidant therapies appear to be receiving increasing attention in clinical neurology, and a number of large randomized controlled trials have been initiated.

Interestingly, antioxidant effects of conventional antidepressants have also been reported in several studies [89, 90]. For example, studies have shown that modulation of serotonin may also affect the levels of oxidants in the brain [91]. Battal et al[91] suggested that modulation of serotonin levels by fluoxetine, a selective serotonin reuptake inhibitor, decreases the levels of oxidative stress and is also accompanied by a reduction in anxiety levels as measured by the elevated plus-maze test. The same study also reported that fluoxetine caused an increase in antioxidant enzyme capacity, as measured by increased catalase and glutathione-S-transferase activities in rat hippocampus [91]. Further work indicated that chronic administration of fluoxetine was capable of significantly decreasing oxidative damage in the cerebral cortex and hippocampus of the stressed animals [92]. A similar effect has been observed following administration of several antioxidants used in clinical trials of neurodegenerative diseases [93, 94], and psychiatric disorders such as depression and anxiety disorders [95–97]. A recent systematic review performed by Cipriani et al., revealed that there is variability in the efficacy of antidepressants that may be rooted in the different mechanisms of action[98]. How antioxidant effects factor into drug efficacy would be an interesting research question that would greatly inform the clinical administration of these antidepressents.

The mechanism(s) of action of antioxidants at the CNS is/are not well elucidated, however, one popular hypothesis suggests that antioxidants may exert their antidepressant effects similarly to conventional antidepressants, by increasing the availability of serotonin and noradrenaline in the synaptic cleft [99, 100]. It has also been noted that some polyphenols have antioxidant properties that may underlie the anxiolytic-like effects that several of them produce in in rodents [101]. Thus, there is significant evidence to suggest the relevance of pharmacological interventions focusing on cellular oxidation as a promising strategy for auxiliary, or possibly even primary, treatment of anxiety disorder.

3. Oxidative stress, Inflammation and Microglia

Several clinical studies have reported that neuroinflammation plays a role in the pathogenesis of neurological disorders, such as anxiety, depression and neurodegenerative diseases [102]. Microglia are macrophages that represent the primary immune cells of the CNS, and they play an important role in initiating and mediating neuroinflammation [103]. Furthermore, microglia are involved in the modulation of various neurological functions, such as immunological, neurochemical, neuroendocrine, and behavioral activities [104]. In fact, it has been demonstrated that microglia participate in neurogenesis [105], neuronal transmission [106], and neuronal plasticity [107].

Abnormal activation of microglia may produce high levels of inflammatory molecules such as tumor necrosis factor-α, interleukin-1β, and Nuclear Factor Kappa B (NF-κB) [108]. The release of proinflammatory cytokines can contribute to the development of neuropsychiatric disorders, such as depression and anxiety disorder [109], although the cellular mechanisms responsible for initiating these processes during the stress response remain poorly understood. An important and detrimental consequence of increased cytokine production is the increased generation of reactive oxygen species (that can cause tissue damage and even cell death) such as $O_2^{\bullet-}$ whose main source in microglia is NADPH oxidase[110]. The NADPH oxidase complex is also a major source of intracellular pro-oxidant generation in both macrophages and neutrophils. Neutrophils are involved in host-defense responses by oxidation of crucial cellular signaling proteins. The pro-oxidants act as both signaling molecules and mediators of inflammation [111]. The increased microglial cell numbers associated with neuroinflammatory states have recently been found to depend on changes induced by different psychogenic stressors including increased glucocorticoids levels [112]. Several researchers have suggested that an acute stress situation could cause the high levels of inflammation seen in anxiety, so it is possible that dysregulation in cytokine signaling could lead to anxiety disorder and cognitive dysfunction [113–115].

The results of pharmacological strategies to suppress abnormal microglial activity support the involvement of these cells in the development of disease-induced neuroinflammation and behavioral alterations [116]. For example, Fluoxetine, a selective serotonin reuptake inhibitor, affords robust neuroprotection in the post-ischemic brain through its anti-inflammatory effect [117]. Recently, fluoxetine has demonstrated several beneficial effects on ischemic stroke patients [118] as well as several animal models of stroke [119]. Similarly, Propranolol, a β-adrenergic receptor antagonist was capable of attenuating anxiety-like behaviors induced by brain proinflammatory profile (infiltration of peripheral macrophages into the brain and

microglial activation) [120]. These results suggest that modulation of microglial proinflammatory profiles could be responsible for anxiolytic effects.

4. Interactions Between Nrf2, Proteasome, and Anxiety Disorder.

As discussed above, the mechanisms involved in changing microglial activity from beneficial to chronic detrimental neuroinflammation are not always clear. However, it is known that there is an increase in oxidant production and several studies have shown that the nuclear factor erythroid 2 related factor 2 (Nrf2), guardian of redox homeostasis, has an essential role in modulating macrophage activation in response to neuroinflammation [121–123]. Innamorato et al., showed that Nrf2 knockout mice were hypersensitive to the neuroinflammation induced by lipopolysaccharides (LPS) [124]. The chronic *intraperitoneal* administration of LPS induced the increase of several markers of inflammation, such as IL-6 and TNF-alpha [121].

4.1. Nrf2 and Neuropathology ——

Nrf2 plays a key role in neuronal resistance to oxidative stress and glutamate-induced excitotoxicity [125]. Various studies show that Nrf2 has neuroprotective effects against oxidative damage injury following cerebral ischemia/reperfusion in rats [126, 127]. Furthermore, Nrf2 deficiency may affect the psychological behavior and neurotransmitter systems in mice, such as reduced mobility in swimming tests, possibly by increasing dopaminergic and serotonergic neurotransmitters[128]. The pre-activation of Nrf2 by electrophilic agents protects cells, partially through enhanced H_2O_2 scavenging by the glutathione/glutathione peroxidase system, and the detoxification of reactive quinones by NAD(P)H:quinone oxidoreductase 1 [129]. Still, excessive extracellular dopamine itself can be an endogenous signal to activate Nrf2-dependent neuroprotective pathways [129]. Studies with primary cell cultures have also revealed that excessive dopamine release can act as an endogenous Nrf2-inducing signal [130].

Recently, a clinical study reported increased activation of Nrf2 in peripheral blood mononuclear cells of patients with depression, which indicates a pro-oxidative state [131]. In fact, the chronic fluoxetine treatment suggested that fluoxetine-induced neuroprotection may operate via an unexpected mechanism involving 5-HT (serotonin receptor) and a serotonin transporter blockade with Nrf2 signaling. However, the contribution to CNS function remains to be elucidated [132]. It is actually not clear which pathway connects Nrf2 regulation and brain damage in anxiety disorder. Considering the regulation of antioxidant defenses through the Nrf2 pathway, this factor has emerged as a promising approach for neuroprotection, and it is possible that Nrf2 may also play an important role in the regulation of brain inflammation via interactions with NF-κB. Nrf2 clearly has vital functions in various physiological and pathological stresses, and it has been implicated as a causative factor in the pathophysiology of many psychiatric disorders, including anxiety disorder [133].

4.2 Nrf2 and Proteasome Activation –

As previously mentioned, Nrf2 is an important component of the responses to oxidative stress by binding to electrophile responsive element, also called antioxidant response element (EpRE/ARE), sites on numerous target genes. Such binding promotes the synthesis of several antioxidant enzymes, as well as enzymes that are responsible for the repair and/or

removal of oxidized cell components that allow restoration of normal cell function [134] (Fig. 1). One such enzyme is the Proteasome proteolytic complex that is comprised of multiple protein subunits, encoded by multiple different genes. Proteasomes are crucial proteolytic enzymes in eukaryotes that are the primary guardians of proteostasis in the cell cytoplasm, nucleus, and endoplasmic reticulum.[135] The 26S Proteasome recognizes and degrades poly-ubiquitinylated proteins (ubiquitin-tagged for degradation by a series of ubiquitin E1 activating, E2 conjugating, and E3 ligating enzymes) in a process that requires multiple steps of ATP hydrolysis. The 26S Proteasome, along with ubiquitin and the E1, E2, and E3 enzymes are collectively referred to as the Ubiquitin-Proteasome System (or UPS). During periods of homeostasis, cells primarily rely on the 26S Proteasome to maintain proteostasis. The 26S Proteasome is formed from the addition of a 19S subunit to each of the α rings of the 20S Proteasome, in an ATP-dependent manner[136]. Under oxidative stress conditions, however, the 26S Proteasome is transiently 'dismantled or disasembeled' by Ecm29 and HSP70 [137–139] and the resulting 20S Proteasome (+/− 11S activator) and the Immunoproteasome (+/− 11S activator) are primarily responsible for degrading oxidatively damaged proteins in a process that does not utilize ATP or ubiquitin [16–22].

Under homeostatic conditions, Nrf2 is under tight regulation by the UPS [18, 140]. In the absence of stress, Nrf2 is bound to Keap1which also has a Cul3 E3 ubiquitin ligase attached to the complex. Thus, under normal, non-stressful, conditions, the Keep1-Cul3 complex promotes the ubiquitinylation of Nrf2 and its consequent degradation by the 26S Proteasome, thus keeping the cellular levels of Nrf2 very low. When oxidative stress occurs, however, and the 26S Proteasome is disassembled by Ecm29 and HSP70, Nrf2 can no longer be degraded and its levels rapidly rise [137–139]. Nrf2 also undergoes phosphorylation at multiple sites [141] and is then able to translocate into the cell nucleus where it binds to EpRE sequences (also called ARE sequences) of target genes to activate their increased transcription[142].

4.3 Proteasome Dysfunction and Neuropathology –

Dysfunction of Proteasomes, or other components of the UPS, may be related to deficits in the clearance of misfolded cytoplasmic, nuclear, and ER proteins, leading to intracellular protein aggregation, cytotoxicity, and cell death [143, 144]; such dysfunction(s) is/are also associated with several neurological diseases, including Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease, and Huntington's disease [145]. The Proteasome has been shown to lose its effectiveness and responsiveness under chronic, repeated, or sever stress regimes [30, 146]. Similarly, dysfunction of the Lon protease in mitochondria (which protects mitochondrial integrity by selectively removing oxidized intramitochondrial proteins) has been associated with a number of neurological disorders [147–150]. Recently, Gragnoli et al., [151] reported that PSMD9, a protein subunit of the 26S Proteasome complex, could potentially contribute to generalized anxiety disorder, but the underlying cause(s) of these changes is/are not known. However PSMD9 does seem to be associated with a significant clinical response to desipramine, a drug used for generalized anxiety treatment [151].

Proteasome dysfunction occurs with aging. With age, the activity and inducibility of the Protease decreases [152]. With the loss of Proteasome activity, negative oxidative outcomes likely become more prevalent and may magnify the negative outcome of anxiety disorders. Similarly, anxiety is also associated with accelerated aging (CITATION). The age-related dysfunction of the Proteasome potentially contributes to the neuropathology associated anxiety dependent accelerated aging.

A number of animal studies have shown increased levels of protein degradation in limbic structures, such as the frontal cortex and hippocampus, in anxiety-like rat models [57, 153–155] and it is well known that oxidative stress can cause significant protein modification and damage. Better understanding of the relationships between protein damage and protein turnover in anxiety disorders may help to provide new therapeutic targets for the development of future drugs.

5. Conclusions

Although there is evidence supporting the involvement of oxidative stress in the pathophysiology of anxiety disorders, the discussion is still open as to whether oxidative stress is the cause or consequence of anxiety. However, the relationship between oxidative stress and anxiety disorders seem more evident in disorders associated with inflammation. In these cases, dysregulation of physiological pathways results in oxidative stress that leads to neuroinflammation and the subsequent manifestation of an anxiety disorder. Further evidence of this link is the fact that effective anxiolytics, such as Fluoxetine decrease inflammation to exert their antianxiety effects. Factors such as neurodevelopment, epigenetic modulation, the neuroendocrine system, the immune system, and the effects of exposure to oxidants, appear to compose the repertoire of elements involved in neuropsychiatric pathogenesis. Animal models of anxiety have produced bidirectional data, and provided important clues to help understand the potential molecular mechanisms associated with, or directly involved in, anxiety-related disorders. In particular, the Nrf2–Proteasome-signaling pathway may provide valuable insights for therapeutic interventions capable of restoring Nrf2-mediated redox homeostasis. Nevertheless, research in neurobiology of anxiety at a molecular level suggests antioxidant therapy for anxiety disorders in humans may be premature, while other reports in the literature suggest a more optimistic potential for antioxidant therapy. The combined use of antioxidants and classic anxiolytics may actually be promising for anxiety-like disorders, but further research is clearly needed to test and validate these new therapies.

Whether oxidative stress is the cause or consequence of anxiety disorders is complex and likely depends on a complex interaction between the specific anxiety disorder, environment, and individual physiology. However, recent focus on Nrf2 and the Proteasome offer promising new avenues for maintaining allostasis and avoiding allostatic overload associated with anxiety disorders. In contrast to antioxidant therapies that limit oxidative load on biological systems, Nrf2-Proteasome focused therapies would aim to maintain the body’s innate defense systems. Clinical applications focused on reinforcing the body’s existing oxidative defense mechanisms, such as Nrf2 and the Proteasome, in conjunction with existing therapies may increase the efficacy of treatments for anxiety disorders.

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7. List of abbreviations:

ALS	Amyotrophic Lateral Sclerosis
CAT	catalase
CNS	Central Nervous System
Cu-Zn-SOD	cytosol mitochondrial superoxide dismutase
EpRE/ARE	electrophile responsive element/antioxidant response element
GABA	gamma-aminobutyric acid
GABAergic	gamma-aminobutyric acidergic
H2O2	peroxide hydrogen
HPA	hypothalamic-pituitary-adrenal
MAOI	monoamine oxidase inhibitors
Mn-SOD	matrix of mitochondrial superoxide dismutase
NF-κB	Nuclear Factor Kappa B
Nrf2	factor erythroid 2 related factor
O2	anion superoxide
Prx	peroxiredoxins
PET	positron emission tomography
SOD	superoxide dismutase
TCAs	tricyclic antidepressants
TNFα	tumor necrosis factor-α
UPS	Ubiquitin-Proteasome System
LPS	lipopolysaccharides
XO	xanthine oxidase

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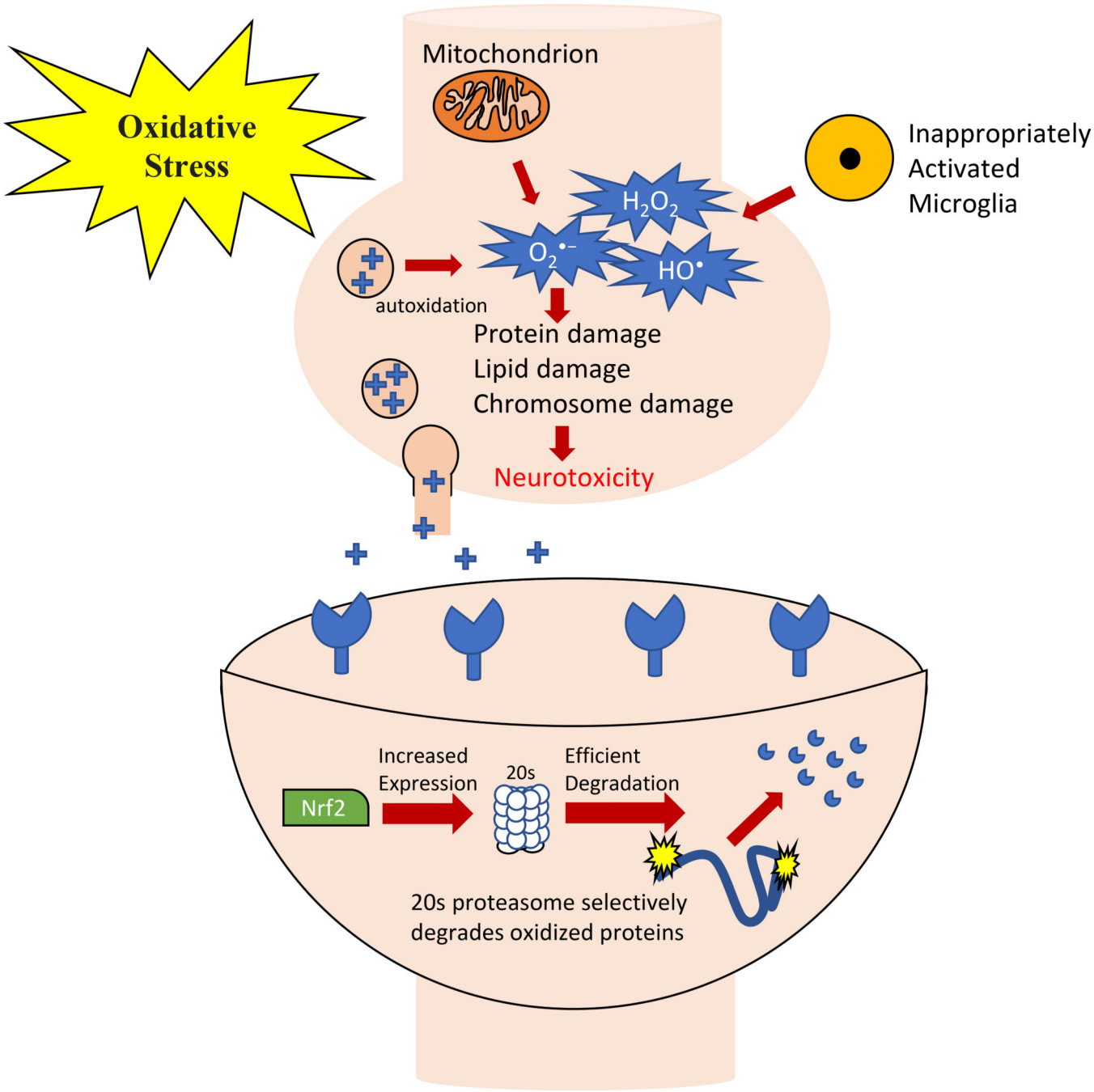

Figure 1. Neurotoxic effects of oxidative stress.

Neurotoxicity may occur through an elevation of superoxide anion ($O_2^{\bullet-}$) by mitochondria, by autoxidation of neurotransmitters such as Dopamine (DA), or by inappropriately activated microglia, any or all of which may result in increased H_2O_2 levels, increased formation of the highly reactive hydroxyl radical ($HO^{\bullet}$) and superoxide ($O_2^{\bullet-}$). Phospholipids and proteins, sugars, RNA and DNA are all susceptible to damage by $HO^{\bullet}$ as are cell membranes, and both nuclear and mitochondrial chromosomes. These factors closely link hypotheses involving mitochondrial dysfunction, neuro-inflammation, oxidative

stress, and the essential role of Nrf2 in protein degradation, through activation of the 20S Proteasome that selectively degrades oxidized proteins, and modulating macrophage activation in response to neuro-inflammation.

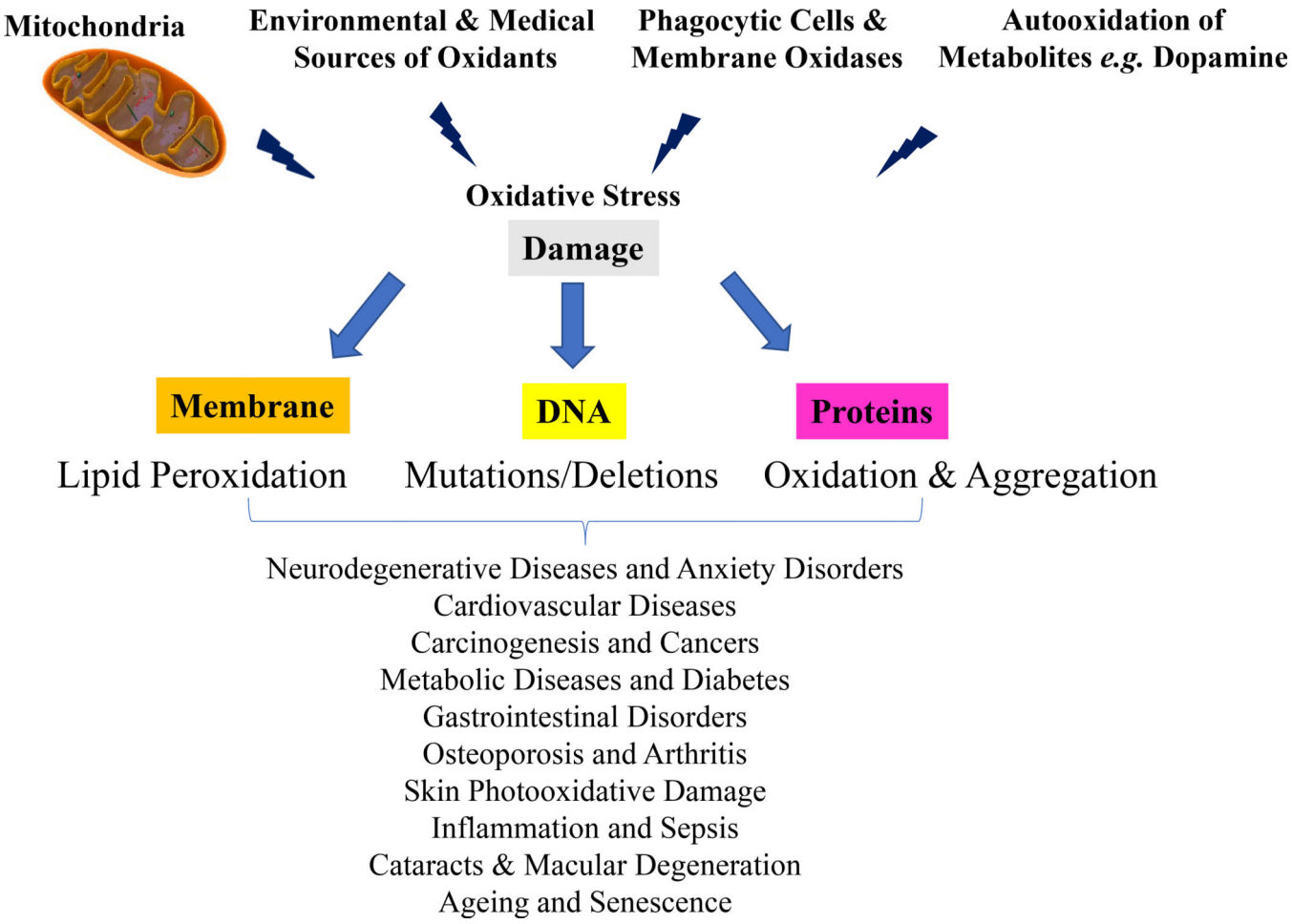

Figure 2. Damaging Effects of Oxidative Stress on Cell Structures and its Relation to Disease initiation/Progression, Ageing, and Senescence.

Oxidative stress arising (largely) from $O_2^{\bullet -}$ and H_2O_2 generated by mitochondria; by environmental and medical sources; by phagocytes such as astrocytes, glia, neutrophils, macrophages, monocytes, etc.; and by autoxidation of metabolites such as dopamine can damage cell structures. Phospholipids, both soluble and membrane-bound proteins, and nuclear and mitochondrial DNA, are easily damaged by oxidation which can lead to subsequent cellular malfunction, tissue dysfunction, and even organ failure. Ultimately, such molecular damage is thought to contribute to the initiation and/or progression of many age-related disorders and diseases that are among the major causes of morbidity and mortality in ageing populations (some of the major ones are shown at the bottom of the figure), and in the very processes of aging and senescence.

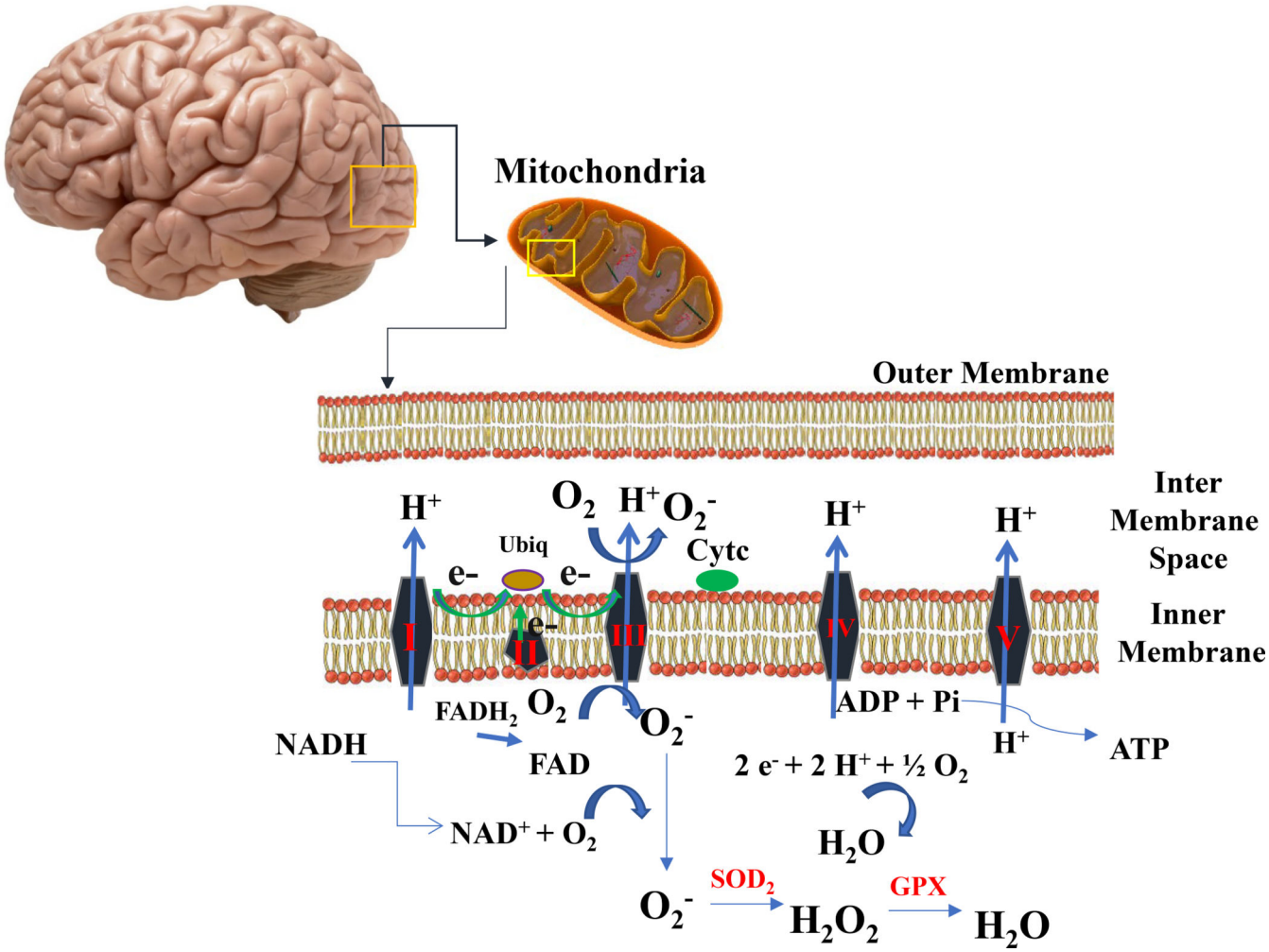

Figure 3. Formation and Neutralization of Reactive Oxygen Species in the Mitochondrial Electron Transport Chain (ETS).

The Krebs cycle is a series of enzymatic reactions that provide electrons (from pyruvate via acetyl CoA) to the ETS in the form of NADH and $FADH_2$. These electrons then undergo vectorial transport along the ETS, generating an electrochemical energy gradient by which ADP can be phosphorylated to ATP at complex V. In order to maintain electron flow (and ATP generation) electrons must ultimately be 'removed' from the ETS, and this is accomplished at Complex IV (cytochrome oxidase) where the electrons reduce oxygen to water in four consecutive (but concerted) one-electron steps. Although the whole process is really rather efficient, some 1–2% of the molecular oxygen consumed during normal physiological respiration is reduced in one-electron side reactions (mostly at Complex 's I and III) into the superoxide anion radical, $O_2^{\bullet -}$ (also commonly just called 'superoxide'). The $O_2^{\bullet -}$ so generated is almost immediately dismutated to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) and, H_2O_2 can then be removed by the enzyme, glutathione peroxidase (GPX).