

Chapter 2

Why Is the Nervous System Vulnerable to Oxidative Stress?

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Abstract The nervous system is especially vulnerable to reactive oxygen species (ROS)-mediated injury for the following reasons. (1) High oxygen consumption of the brain for high energy needs, that is, high O_2 consumption, results in excessive ROS produced. (2) Neuronal membranes are rich in polyunsaturated fatty acids (PUFA), which are particularly vulnerable to free radical attack. (3) The ratio of membrane surface area to cytoplasmic volume is high. (4) Specialized neuronal conduction and synaptic transmission activity depend on efficient membrane function. (5) Extended axonal morphology is prone to peripheral injury. (6) Neuronal anatomic network is vulnerable to disruptions. (7) The excitotoxic glutamate is the major effector that causes oxidative stress (OS). (8) The high Ca^{2+} traffic across neuronal membranes and interference of ion transport increase intracellular Ca^{2+} , often leading to OS. (9) Auto-oxidation of neurotransmitters can generate O_2 and quinones that reduce glutathione. (10) Iron is formed throughout the brain, and brain damage readily releases iron ions capable of catalyzing free radical reactions. (11) Antioxidant defense mechanisms are modest, in particular, low levels of catalase, glutathione peroxidase, and vitamin E. (12) ROS directly downregulate proteins of tight junctions and indirectly activate matrix metalloproteinases (MMP) that contribute to open the blood–brain barrier (BBB). (13) Activated microglia produce ROS and cytokines in a perpetual process. (14) Cytochrome P450 produces ROS. (15) Loss of trophic support can activate NADPH oxidase, which increases ROS. (16) The presence of hemoglobin within the neural tissues secondary to spontaneous, iatrogenic, or traumatic causes is neurotoxic. Heme and iron are released and promote ROS. (17) Neuronal mitochondria generate O_2 . (18) The interaction of NO with superoxide can be implicated also in neuronal degeneration. (19) Neuronal cells are nonreplicating and thus are sensitive to ROS. In comparison with other organs, the neuronal network may be especially

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vulnerable to ROS-mediated injury because of the following anatomic, physiological, and biochemical properties of the brain.

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1 High O₂ Consumption [1, 2]

The brain, which accounts for only 2% of body weight, consumes 20% of the total oxygen inspired. The brain processes much O₂ per unit tissue mass. The discrepancy is even more striking in young children who have much smaller bodies but without proportionally smaller brains. A major reason for the high O₂ uptake by the brain is the vast amounts of adenosine triphosphate (ATP) needed for its normal activity (4×10^{12} ATP molecules every minute). The large amount of ATP is necessary to maintain neuronal intracellular ion homeostasis in face of all the openings and closings of ion channels associated with propagation of action potentials and neurosecretion. The heavy reliance of the brain on oxidative phosphorylation makes the brain vulnerable to interruptions of O₂ supply. Similarly, inhibitors of mitochondrial ATP synthesis readily cause neuronal death. Examples include cyanide 3-nitropropionic acid (an inhibitor of mitochondrial succinate dehydrogenase) and rotenone (an inhibitor of complex I). Because approximately 5% of oxygen consumed by cells is estimated to be reduced to reactive oxygen species (ROS), a relatively higher amount of ROS may be generated in the brain as compared to other tissues that use less oxygen.

2 Neuronal Membranes Rich in PUFA [3, 4]

Neuronal membrane lipids are rich in polyunsaturated fatty acids (PUFA) side chains, especially those of eicosapentanoic (C_{20:5}) and docosahexanoic (C_{22:6}) acids. PUFAs are particularly vulnerable to free radical attack because of the double bonds, within the membrane allowing easy removal of hydrogen ions by ROS such as OH[•]. Homogenization of brain tissue causes rapid lipid peroxidation, which can be largely inhibited by ion-chelating agents such as desferrioxamine. In addition, products of lipid peroxidation can injure the brain. 4-Hydroxy-nonenal, which is one of those products, is especially cytotoxic to neurons, increasing Ca²⁺ levels, inactivating glutamate transporters, and damaging neurofilament proteins. It can also inactivate α -ketoglutarate dehydrogenase (α -KGDH), a key enzyme of the

tricarboxylic acid cycle. 4-Oxo-2-nonenal may be equally or more toxic, and isoprostanes (IPs) may act as vasoconstrictive agents in brain and can damage developing oligodendrocytes in premature babies. Other products of the IP pathway may also be neurotoxic by damaging the proteasome, for example.

- 3 High Ratio of Membrane Surface Area to Cytoplasmic Volume**
- 4 Specialized Neuronal Conduction and Synaptic Transmission Activity Depend on Efficient Membrane Function**
- 5 Extended Axonal Morphology Is Prone to Peripheral Injury**
- 6 Neuronal Anatomic Network Is Vulnerable to Disruption**
- 7 Oxidative Stress and Excitotoxic Amino Acids [5, 6]**

Although multiple factors can precipitate intracellular oxidative stress (OS), the neurotransmitter glutamate is the major effector of this process in the brain, primarily through activation of its ionotropic receptors. Brain extracellular concentrations of glutamate are normally low ($< \mu\text{M}$). Neuronal death or collapse of normal ion gradients (e.g., owing to severe energy depletion) in neurons cause massive glutamate release. Glutamate and related excitatory amino acids account for most of the excitatory synaptic activity in the mammalian central nervous system (CNS) and are released by as many as 40% of all synapses.

The *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxasol-propionic acid (AMPA), and kainic acid (KA) ionotropic receptors can be distinguished by their pharmacologic and electrophysiological properties. The binding to neuronal receptors leads to excessive and prolonged increases in $\ddot{\text{O}}_2$ and NO formation and intracellular Ca^{2+} . Neurons treated with excess glutamate or other excitatory toxins swell rapidly and die, usually by necrosis. OS can damage neurons and promote the release of excitatory amino acids, generating a vicious cycle of events. Several ROS are able to decrease glutamate uptake by glial cells and to inactivate glutamine synthetase, preventing conversion of glutamate to glutamine. This enzyme is inactivated in Alzheimer's disease. The early drop in cellular glutathione (GSH) levels observed together with oxidative glutamate toxicity is very similar to the changes seen in vivo within neurons responding to both acute and chronic injury.

8 High Ca^{2+} Traffic Across Neuronal Membranes [7, 8]

The high Ca^{2+} traffic across neuronal membranes interferes with ion transport (e.g., by disruption of energy metabolism) and can produce rapid rises in intracellular free Ca^{2+} , often leading to OS. Increase in intracellular free Ca^{2+} activates neuronal nitric oxide synthase (nNOS), phospholipase A_2 , and calpain.

9 Autooxidation of Neurotransmitters [9–11]

Several neurotransmitters can be autooxidized. Dopamine, its precursor L-DOPA, serotonin, and norepinephrine can react with O_2 to generate not only O_2 but also quinones/semiquinones that can deplete reduced glutathione (GSH) and bind to protein sulfhydryl (SH) groups. Oxidation can be catalyzed by transition metal ions, but when $\bar{\text{O}}_2$ is in excess, it can react with norepinephrine, dopamine, and serotonin to initiate their oxidation, which then continues with production of more ROS, quinones, etc. Monoamine oxidase (MAO) exists in two forms, MAO-A and -B. MAO-A preferentially oxidizes hydroxylated amines such as serotonin and noradrenalin and is found primarily in catecholaminergic neurons. MAO-B preferentially oxidizes nonhydroxylated amines and is located in serotonergic neurons. Both oxidases are present in glial cells and are able to oxidize dopamine.

10 Iron [12, 13]

Iron is found throughout the brain. Important iron-containing proteins include cytochromes, ferritin, aconitases, mitochondrial non-heme iron proteins, cytochrome P450, and iron is found in both tyrosine and tryptophan hydroxylase enzymes, which catalyze the first steps in the synthesis of dopamine and serotonin, respectively. Several brain areas (e.g., substantia nigra, caudate nucleus, putamen, and globus pallidus) have high iron content. Ferritin contains a major portion of the healthy brain iron, and smaller amounts are found in hemosiderine. The iron content of the brain is low at birth and rapidly increases during early life to reach the adult level at the age of 30 years. If insufficient dietary iron is present in infants and children, it may cause impairment of brain function. Transferrin delivers most of the required iron across the blood–brain barrier (BBB), utilizing receptors located at the brain microvasculature. However, when the brain is damaged, it readily releases iron (and copper) ions in forms capable of catalyzing such free radical reactions as $\text{OH}^\bullet$ formation from H_2O_2 , lipid peroxidation, and autooxidation of neurotransmitters. Injection of iron salts into the brains of animals can produce epileptic seizures, dopamine depletion, and death of neurons accompanied by lipid peroxidation, oxidative damage, and $\text{OH}^\bullet$ generation. Catalytic iron released by brain damage

can persist because the cerebrospinal fluid (CSF), which surrounds the brain and spinal cord, has little or no iron-binding capacity. Total iron values in CSF from normal humans range, according to various reports, from 0.29 to 1.11 μM . The transferrin content is 0.24 μM . Because 1 mol of transferrin binds 2 mol of iron ions, these data suggest that CSF transferrin is often at, or close to, its iron saturation.

11 Antioxidant Defenses are Modest [14, 15]

Brain antioxidant defenses are modest. In particular, catalase levels are low in most brain regions; levels are higher in hypothalamus and substantia nigra than in the cortex or cerebellum. Brain catalase is located in small peroxisomes (micro-peroxisomes), and its activity in rat or mouse brain is rapidly inhibited if aminotriazole is administered to the animals. This agent inhibits only catalase of complex I, confirming that the brain generates H_2O_2 in vivo and that at least some of it reaches catalase. The catalase probably cannot handle H_2O_2 generated in other subcellular compartments. The brain contains less catalase, glutathione peroxidase (GPx), and vitamin E as compared to liver. Ascorbic acid, which can act as an antioxidant as well as a pro-oxidant, is present at elevated levels in both white and gray matter. Transport systems exist in the choroid plexus and neurons that serve to concentrate ascorbic acid into brain cells and the CSF. Ascorbic acid acts as a pro-oxidant when the free iron of the brain increases as a result of intracerebral hemorrhage. The developing brain may be particularly susceptible to free radical injury during ischemia because of a relative developmental deficiency in its antioxidant enzymes. The activity of those enzymes has not yet been determined in the cerebral cortex of developing humans, although expression levels and cellular localization have been reported in the cortex, basal ganglia, and brainstem nuclei by immunohistochemical methods. The premature infant has low circulating levels of glutathione (GSH) and relative inability to sequester iron because of low transferrin levels. It is well known that glial cells are more resistant to OS than neurons, probably the result of transcriptional upregulation of glutathione synthesis.

12 The Blood–Brain Barrier [16, 17]

The brain needs a barrier that can selectively and efficiently prevent harmful molecules from entering the brain from the blood, thus enabling the rigorous control of the brain microenvironment that is necessary for complex neuronal signaling. ROS, both directly (e.g., by downregulating the synthesis of proteins involved in tight junctions between cells) and/or by activation of matrix metalloproteinases (MMP), can contribute to “open up” the BBB, allowing the entry of neurotoxins and inflammatory cells. The adult brain neurons are protected by

the BBB, and the blood–axon barrier acts similarly in the peripheral nervous system (PNS). In the immature nervous system, these barriers are not fully formed, and thus it is more vulnerable. In elderly people, the combination of accumulating vascular damage with associated breakdown of those barriers may render them sensitive to exposure to ROS and toxins. Taken together, oxidative stress emerges as a common underlying cause of BBB dysfunction.

13 Microglia and ROS [18, 19]

The microglia are resident macrophage-type cells that arise from monocytes entering the brain during embryonic development. Normally, they help clear cellular debris (including apoptotic cells) and are “alert” to threats to neurons. However, microglia can become activated to produce $\dot{O}_2$, H_2O_2 , and cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor- α (TNF- α). Subsequently such cytokines can cause microglia to generate more ROS and to produce iNOS (induced NO synthase) and hence excess of $NO^\bullet$ (nitric oxide). Cytokines can additionally be produced by activated astrocytes, and the latter may again respond to cytokines by iNOS induction. Thus, microglia and astrocytes are major mediators in brain inflammation.

14 Cytochrome P450 [20, 21]

Cytochrome P450 (CYPs), such as CYP46 that metabolizes cholesterol, CYP2D6, and CYP2E1 are present in several brain regions. Because CYP2E1 leaks electrons readily during its catalytic cycle, it produces more ROS than most other CYPs. Thus, it is another possible source of oxidative stress, although its magnitude may be small because brain CYPs levels are low compared to their levels in the liver. However, CYP2E1 metabolizes ethanol, acetone, halothane, and organic solvents such as CCl_4 and $CHCl_3$ and may be inducible in human brain by ethanol and smoking. Thus, it could contribute to solvent neurotoxicity.

15 Loss of Trophic Support [22, 23]

Loss of trophic support can lead to oxidative stress and apoptosis in neurons, in part by excess activation of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase (NOX). The latter were first detected in phagocytes, but are now known to be widely distributed in animal tissues, seemingly producing $\dot{O}_2$ for defense and for signaling purposes. Neuronal NOX enzymes may promote necessary apoptosis

during development of the nervous system, but if trophic support is lost in the developing brain, they are overactivated, leading to neuronal death.

16 Hemoglobin Is Neurotoxic [24–27]

Hemoglobin (Hb) is neurotoxic. This protein is normally safely transported in erythrocytes, which are rich in antioxidant enzymes. However, isolated Hb is degraded when exposed to excess H_2O_2 , with release of pro-oxidant iron ions from the heme ring. Heme can also be released and is a powerful promoter of lipid peroxidation. In addition, Hb reacts with H_2O_2 and other peroxides to form oxidizing species capable of stimulating lipid peroxidation. Hb also binds NO avidly, producing vasoconstriction.

17 Mitochondria [28]

Neuronal mitochondria generate $\bar{\text{O}}_2$, mostly from complex I. The levels of 8-hydroxydeoxyguanosine (8-OHdG) mutations and deletions increase in brain mitochondrial DNA with aging.

18 Nitric Oxide

NO, also referred to as endothelium-derived relaxing factor because of its production in the endothelial cells, is also formed in neurons by the enzyme nitric oxide synthase (NOS), which is widely distributed in the brain and is activated by calmodulin. The interaction of NO with superoxide radicals is believed to be implicated not only in the normal metabolism of the neuron but also in its degradation.

19 Neurons are Nonreplicating [29]

Neurons are generally postmitotic cells, and thus the brain is sensitive to loss of function if neurons die. Neurons are very active and are highly susceptible to a reduction in the supply of oxygen and glucose. The insidious reduction in the number of neurons and their synaptic connections eventually compromises virtually all CNS functions. Fortunately, many parts of the brain have considerable redundancy and plasticity. Neurons in culture are prone to injury, often leading to necrosis or apoptosis if treated with toxins that interfere with energy metabolism,

or exposed to ROS, or if neurotrophic factors are withdrawn from the culture medium.

In this chapter, 19 reasons were mentioned why the nervous system is especially vulnerable to ROS-mediated injury. It was suggested that the main effects of ROS on cells are through their actions on signaling pathways rather than by causing nonspecific damage. I suggested that with aging these pathways deteriorate, causing accumulation of higher concentrations of ROS in amounts beyond the capacity of the brain's antioxidant system to handle. This deterioration results in age-associated neurodegenerative disorders such as stroke and CNS trauma, as well as Parkinson's disease, Alzheimer's disease, and other neurodegenerative disorders of aging.

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