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1. Introduction

Around 100 billion neurons in the human nervous system orchestrate an exceptionally wide range of motor, sensory, regulatory, behavioural, and executive functions. Such diverse functional output is the product of different molecular events occurring in nervous cells and particularly, neurons. Morphologically, central nervous system (CNS) neurons differ in size, number and complexity of dendrites, number of synaptic connections, length of axons and distance across which synaptic connections are established, extent of axonal myelination, and other cellular characteristics. Neuronal diversity is also amplified by the inclusion of chemical specificity on the basis of the neurotransmitters, which they use for chemical transmission or neuromodulation. This great diversity among neuronal populations is a strong indication that although all neurons contain the same genetic code in their genome, each neuronal population has their own gene expression profile. While the diversity of neuronal structures and functions are well documented, what is less appreciated is the diverse response of neurons to stresses and adverse factors during aging or as a result of neurodegenerative diseases. Furthermore, to add complexity to an already heterogeneous landscape, non-neuronal populations, often described as a ‘supporting matrix’ are recently being recognized as active, information-rich, cellular counterpart in CNS function.

In this scenario of cellular diversity emerges the concept of selective neuronal vulnerability (SNV). SNV is described as the differential sensitivity of neuronal populations in the nervous system to stresses that cause cell damage or death and can lead to neurodegeneration [1,2]. The fact that specific regions of the nervous system exhibit differential vulnerabilities to aging and various neurodegenerative diseases is a reflection of
both the specificity in the aetiology of each disease and of the heterogeneity in neuronal (and non-neuronal) responses to cell-damaging processes associated with each of the diseases [3]. The appearance of SNV is not limited to cross-regional differences in the nervous system, as within a single e.g. brain region, such as the hippocampus or the entorhinal cortex, where SNV is manifested as internal, sub-regional differences in relative sensitivities to stress and disease [2,3]. Among these cell-damaging processes one could count inflammatory, proteotoxicity, vascular and many other pathophysiological processes, including an excess of lipid peroxidation (see later).

Oxidative stress (OS) is involved in the basic mechanisms of nervous system aging; whilst an excessive oxidation has been invoked as an etiopathogenic or physiopathologic mechanism for neurodegeneration. Oxidative stress, the result of an imbalance between production of free radicals and the enzymatic or non-enzymatic detoxification of these highly reactive species, is detrimental to cells because free radicals chemically modify lipids, proteins, and nucleic acids. So, it is very important to define the vulnerability of the different neuronal populations in terms of susceptibility to oxidative stress in physiological conditions in order to extent this knowledge to improve our understanding of how this particular form of cell vulnerability causes selective neuronal losses in nervous system, as well as reveal potential molecular and cellular mechanisms that bring about relative resistance or sensitivity of neurons to stresses.

2. Mitochondrial free radical generation and membrane fatty acid composition in the CNS

2.1. Mitochondrial free radical production

Chemical reactions in cells of the nervous system are under strict enzyme control and conform to a tightly regulated metabolic program in order to minimize unnecessary side reactions. Nevertheless, apparently uncontrolled and potentially deleterious reactions occur, even under physiological conditions. Reactive oxygen species (ROS) express a variety of molecules and free radicals (chemical species with one unpaired electron) physiologically generated from the metabolism of molecular oxygen [4]. They are extremely reactive and have damaging effects. Superoxide anion, the product of a one-electron reduction of oxygen, is the precursor of most ROS and a mediator in oxidative chain reactions [4] (Figure 1). The character of radical is not circumscribed to oxygen containing species, as nitrogen, chloride and sulphide containing molecules could also play a significant role. Globally, in cells of the CNS the major sites of physiological ROS generation are the complex I and III of the mitochondrial electron transport chain, which contains several redox centers (flavins, iron–sulphur clusters, and ubisemiquinone) capable of transferring one electron to oxygen to form superoxide anion [5,6]. Oxidative damage is a broad term used to cover the attack upon biological molecules by free radicals. ROS attack/damage all cellular constituents [6], but especially biological membranes.
Region Specific Vulnerability to Lipid Peroxidation in the Human Central Nervous System

2.2. Membrane fatty acid composition in neural cells

All living organisms have lipid membranes. Biological membranes are dynamic structures that generally consist of bilayers of amphipathic molecules held together by non-covalent bonds [7]. Phospholipids, consisting of a hydrophilic head group with attached hydrophobic acyl chains, are the predominant membrane lipids and are, from a topographic point of view, asymmetrically distributed across the bilayer. The variation in head groups and aliphatic chains allows the existence of a huge range of different phospholipid species [8,9]. The acyl chains are either saturated, monounsaturated or polyunsaturated hydrocarbon chains that normally vary from 14 to 22 carbons in length (Figure 2), with an average chain length strictly maintained around 18 carbon atoms, and a relative distribution between saturated and unsaturated fatty acids of 40:60 (SFA and UFA, respectively) [10]. Polyunsaturated fatty acids (PUFAs) are essential components of cellular membranes that strongly affect their fluidity, flexibility and selective permeability, as well as many cellular and physiological processes [11].

Long-chain polyunsaturated fatty acids are highly enriched in the nervous system. Docosahexaenoic acid (DHA; 22:6n-3; 4,7,10,13,16,19-C22:6), in particular, is the most abundant PUFA in the brain and is concentrated in aminophospholipids of cell membranes. Numerous studies have indicated that this concentration of DHA in the nervous system is essential for optimal neuronal functions. Although the underlying mechanisms of its essential function are still not clearly understood, emerging evidence suggests that unique metabolism of DHA in relation to its incorporation into neuronal membrane phospholipids plays an important role.

Accretion of DHA in the CNS actively occurs during the developmental period, primarily relying on circulating plasma DHA derived from diet or from biosynthesis in the liver [12]. However, local biosynthesis of DHA also occurs in the brain, providing an alternative source of DHA for its accumulation in the brain [13]. It is well established that DHA can be biosynthesized from α-linolenic acid (18:3n-3; 9,12,15-C18:3), a shorter chain n-3 fatty acid precursor, through chain elongation and desaturation processes [14] (Figure 3). Linolenic
Figure 2. Fatty acid nomenclature. As an example, the structure of linoleic acid (C18:2n-6) is given.
Figure 3. Long chain and very long-chain fatty acid biosynthesis in mammals. The long chain saturated fatty acids and unsaturated fatty acids of the n-10, n-7 and n-9 series can be synthesized from palmitic acid (C16:0) produced by the fatty acid synthase (FAS). Long-chain fatty acids of the n-6 and n-3 series can only be synthesized from precursors obtained from dietary precursors (DIET). Elovl, elongation of very long chain fatty acids (fatty acid elongase); Fads, fatty acid desaturases.

DHA synthesis in astrocytes is negatively influenced by the availability of preformed DHA [18] and thus may represent a quantitatively minor source for the neural DHA accretion when the circulating DHA supply is adequate. Incorporation of circulating DHA across the blood brain barrier appears to be an important route for maintaining adequate levels of DHA in the brain. In agreement with this notion, it has been shown that constant basal turnover of esterified DHA in the brain with unesterified DHA in plasma occurs at an estimated rate of 2–8% per day in adult rats [19]. Generally, it is difficult to deplete DHA from the neural membranes of adult mammals even with a DHA low diet, presumably because of preferential uptake of DHA into the brain to support the basal turnover. In the case of insufficient supply of n-3 fatty acids during development, the loss of DHA does occur but is compensated with DPAn-6 through reciprocal replacement, suggesting a requirement of very long-chain, highly unsaturated fatty acids in neural membranes.

Whether brain DHA is derived from the circulating plasma pool or biosynthesized locally, in the astrocytes, which are situated in close contact with neurons, appear to play an important role in supplying DHA to neurons. DHA can be released readily from astroglial membranes under basal and stimulated conditions, and supplied to neurons. Despite its
high abundance in neuronal membranes, DHA is not easily released but is tenaciously retained in the neuronal membranes under the conditions in which AA can be released. Considering the fact that astroglia support neurons by providing neurotrophic factors, DHA supplied by astroglia may also be trophic. Indeed, DHA has been shown to promote neuronal survival [20] and differentiation [21] in both transformed and primary neuronal cells in culture.

3. Membrane unsaturation and lipoxidation-derived molecular damage

The susceptibility of membrane phospholipids to oxidative demise is related to two inherent traits, the physico-chemical properties of the membrane bilayer and the intrinsic chemical reactivity of the fatty acids composing the membrane [10,22]. The first property is related to the fact that oxygen and free radicals are more soluble in the fluid lipid bilayer than in the aqueous solution. Thus, membranes contain an interior organic phase in which the oxygen may tend to concentrate. Therefore, these differences in solubility are important when considering the availability of oxygen/free radicals for chemical reactions inside living systems: organic regions may contain more free radicals than aqueous regions [8] and, consequently, membrane lipids become primary targets of oxidative damage. The second property is related to the fact that PUFA residues of phospholipids are extremely sensitive to oxidation. Every membrane phospholipid contains an unsaturated fatty acid residue esterified to the 2-hydroxyl group of its glycerol moiety. Many of these are polyunsaturated and the presence of a methylene group between two double bonds renders the fatty acid more sensitive to ROS-induced damage. Therefore, the sensitivity of these molecules to oxidation increase exponentially as a function of the number of double bonds per fatty acid molecule [22,23]. Consequently, the high concentration of PUFAs in phospholipids not only makes them prime targets for reaction with oxidizing agents but also enables them to participate in long free radical chain reactions. Reactive free radicals can pull off hydrogen atoms from PUFA side chains. A hydrogen atom (H•) has only one electron. This hydrogen is bonded to a carbon in the fatty acid backbone by a covalent bond. Hence, the carbon from which H• is abstracted now has an unpaired electron (i.e. it is a free radical). PUFA side chains (two or more double bonds) are more sensitive to attack by radicals than are SFAs (no double bonds) or monounsaturated fatty acids (MUFA, one double bond) side chains. When C• radicals are generated in the hydrophobic interior of membranes, their most likely fate is combination with oxygen dissolved in the membrane. The resulting peroxyl radical is highly reactive: it can attack membrane proteins and oxidize adjacent PUFA side chains. So, the reaction is repeated and the whole process continues in a free radical chain reaction, generating lipid hydroperoxides [4]. Lipid hydroperoxides are more hydrophilic than unperoxidized fatty acid side chains. They try to migrate to the membrane surface to interact with water, thus disrupting the membrane structure, altering fluidity and other functional properties and making the membrane leaky.

Lipid peroxidation generates hydroperoxides as well as endoperoxides, which undergo fragmentation to produce a broad range of reactive intermediates called reactive carbonyl species (RCS) (Figure 4) with three to nine carbons in length, the most reactive being α,β-
unsaturated aldehydes [4-hydroxy-trans-2-nonenal (HNE) and acrolein], di-aldehydes [malondialdehyde (MDA) and glyoxal], and keto-aldehydes [4-oxo-trans-2-nonenal (ONE) and isoketals] [24]. 2-Hydroxyheptanal (2-HH) and 4-hydroxyhexenal (4-HHE) are aldehydic product of lipid peroxidation of PUFA-n-6. Additionally, a number of other short chain aldehydes are produced during lipid peroxidation through poorly understood mechanisms. These carbonyl compounds, ubiquitously generated in biological systems, have unique properties contrasted with free radicals. Thus, compared with reactive oxygen and nitrogen species, reactive aldehydes have a much longer half-life (i.e., minutes to hours instead of microseconds to nanoseconds for most free radicals). Further, the non-charged structure of aldehydes allows them to migrate relatively ease through hydrophobic membranes and hydrophilic cytosolic media, thereby extending the migration distance far from the production site. Based on these features alone, these carbonyl compounds can be more destructive than ROS and may have far-reaching damaging effects on target sites within or outside membranes. For the same reason, their long half-life allows them to subserve as second-messenger, signalling for important cellular responses in a mostly unknown fashion.

Carbonyl compounds react with nucleophilic groups in macromolecules like proteins, DNA, and aminophospholipids, among other, resulting in their chemical, nonenzymatic, and irreversible modification and formation of a variety of adducts and crosslinks collectively named Advanced Lipoxidation Endproducts (ALEs) [10,25,26] (Figure 5). Thus, by reacting with nucleophilic sites in proteins (belonging basically to Cys, Lys, Arg, and His residues), carbonyl compounds generate ALE adducts such as MDA-Lys, HNE-Lys, FDP-Lys, carboxymethyl-lysine (CML) and S-carboxymethyl-cysteine; and the crosslinks glyoxal-lysine dimer (GOLD), and methylglyoxal-lysine dimer (MOLD), among several others. The accumulation of MDA adducts on proteins is also involved in the formation of lipofuscin (a nondegradable intralysosomal fluorescent pigment formed through lipoxidative reactions). Lipid peroxidation-derived endproducts can also react at the exocyclic amino groups of deoxyguanosine, deoxyadenosine, and deoxycytosine to form various alkylated products. Guanine is, however, the most commonly modified DNA base because of its high nucleophilicity. Some common enals that cause DNA damage, analogously to proteins, are

Figure 4. General structures of principal lipoxidative reactive carbonyl species detected in biological systems.
Figure 5. Reactive carbonyl species react with nucleophilic groups in macromolecules (A, proteins; B, DNA) resulting in their chemical, nonenzymatic, and irreversible modification and formation of a variety of adducts and crosslinks collectively named Advanced Lipoxidation Endproducts (ALEs).
MDA, HNE, and acrolein, among others. Thus, the most common adducts arising from enals are exocyclic adducts such as etheno adducts, and MDA-deoxyguanosine (M1dG). Finally, the amino group of aminophospholipids can also react with carbonyl compounds and initiate some of the reactions occurring in proteins and DNA, leading to the formation of adducts like MDA-phosphatidylethanolamine, and carboxymethyl-phosphatidylethanolamine [10].

4. ALEs: Molecular and cellular effects

Reactive carbonyl species (RCS) generated during lipid peroxidation reactions exhibit a wide range of molecular and biological effects, ranging from protein, DNA, and phospholipid damage to signaling pathway activation and/or alteration. The detailed mechanisms of ‘toxicity’ are, however, mostly unknown.

4.1. Molecular damage

Lipoxidation reactions lead to structural and functional changes in proteins [25-27] such as i) alterations in physico-chemical properties (conformation, charge, hydrophobicity, elasticity, solubility, and electrophoretic mobility, among others); ii) decrease/inhibition in enzyme activity and growth factors; iii) alteration of protein degradation; iv) alteration in traffic and processing of proteins; and v) formation of intra- and inter-molecular protein cross-links and aggregates.

DNA lipoxidative damage is present in the genome of healthy humans and other animal species at biologically significant levels similar or even higher that oxidation markers sensu stricto. DNA damage is mutagenic, carcinogenic, and have powerful effects on signal transduction pathways [28].

Finally, the amino group of aminophospholipids can also react with carbonyl compounds and initiate some of the reactions occurring in proteins [29]. Biological processes involving aminophospholipids could be potentially affected by this process. Among these processes, it may be highlighted i) asymmetrical distribution of aminophospholipids in cellular and different subcellular membranes; ii) translocation between and lateral diffusion in the membrane; iii) membrane physical properties; iv) biosynthesis and turnover of membrane phospholipids; and v) activity of membrane-bound proteins that require aminophospholipids for their function.

4.2. Cellular adaptive responses

The peroxidation of the PUFA chains of phospholipids generates a complex mixture of carbonyl compounds. Initially, these aldehydes were believed to produce only “cytotoxic” effects associated with oxidative stress (by inducing molecular damage and damaging cellular responses based on inflammatory responses, changes in gene expression and apoptosis) [10], but as depicted above, evidence is increasing in the sense that these
compounds can also have specific signaling roles inducing adaptive responses driven to decrease oxidative damage and improve antioxidant defences.

Thus, available studies support the notion that superoxide radical produced by the mitochondrial electron transport chain can cause mild uncoupling of mitochondria by activating the membrane proton conductance by uncoupling proteins (UCPs). Insight into the molecular mechanism by which superoxide radical activates UCPs comes from the finding that the lipid peroxidation product 4-HNE and its homologs induce uncoupling of mitochondria through UCPs and also through the adenine nucleotide translocase [30]. This and other observations support a model in which endogenous superoxide production generates carbon-centred radicals that initiate lipid peroxidation, producing alkenals like 4-HNE that activate UCPs and adenine nucleotide translocase. So, UCPs respond to overproduction of matrix superoxide by catalyzing mild uncoupling, which lower proton motive force and would decrease superoxide production by the electron transport chain. This negative feedback loop will protect cells from ROS-induced damage and might represent the ancestral function of all UCPs.

In addition, RCS can also activate the ‘antioxidant response’ likely to prevent their accumulation to toxic levels [31]. This signaling cascade culminates in the nuclear translocation of and transactivation by the transcription factor Nrf2, the master regulator of the response [32]. Nrf2 activity is repressed by an inhibitory binding protein, Keap1. Keap1 retains Nrf2 in the cytosol, closely associated with the actin cytoskeleton, and promotes proteasomal degradation of Nrf2 through Cullin3-dependent polyubiquitination. Following exposure to RCS, Keap1 can be directly modified on several cysteine residues, and this modification can promote release of Nrf2. Nrf2 contains a C-terminal basic leucine zipper structure that facilitates dimerization and DNA binding, specifically to the antioxidant response element (ARE). The binding of Nrf2 to the ARE stimulates transcription of downstream cytoprotective genes [32].

5. The selective neuronal vulnerability

The idea that oxygen radicals, especially those of mitochondrial origin, are causally related to the basic aging process is increasingly receiving support from several independent sources [reviewed in 6,33]. Accordingly, the mitochondrial oxygen radical theory of aging apparently fulfills the main characteristics of this natural process: reactive oxygen species (ROS) are endogenously produced at mitochondria under normal physiological conditions, they are produced continuously throughout life (and can thus lead to progressive aging changes), and their deleterious effects on macromolecules may inflict irreversible damage during aging in post-mitotic tissues. The detrimental effects of aging are best observed in postmitotic tissues because cells that are irreversibly damaged or lost cannot be replaced by mitosis of intact ones. Nervous system is considered a postmitotic tissue, and therefore highly susceptible to aging. As this process is involved as a risk factor in most neurodegenerative diseases, and oxidative modifications play a key role in aging, it is often accepted that these diseases should have increased oxidative damage.
In this context, and from an inter-organ comparative approach, two main properties emerge as characteristics that render nervous system as especially sensible to oxidative modification: i) the % free radical leak, and ii) the membrane unsaturation. The % free radical leak (%FRL) refers the fraction (%) of electrons out of sequence which reduce oxygen to oxygen radicals (instead of reducing oxygen to water at cytochrome oxidase) in the mitochondrial respiratory chain. Since two electrons are needed to reduce one molecule of oxygen to H$_2$O$_2$, whereas four electrons are needed to reduce one molecule of oxygen to water, the free radical leak is easily calculated by dividing the rate of ROS production by 2 times the rate of oxygen consumption, the result being multiplied by 100. Results show that the higher %FRL corresponds to brain, suggesting that mitochondria are more inefficient in this tissue than in other organs (Tables 1 and 2, and Figure 6).

### Table 1. Rates of mitochondrial oxygen consumption (nmoles of O$_2$/min/mg protein) of different organs from male adult rats.

| Substrate            | Brain     | Heart     | Kidney    | Liver     | Skeletal muscle |
|----------------------|-----------|-----------|-----------|-----------|-----------------|
| Pyruvate/malate      | State 4   | 11.2 ± 1.7| 28.4 ± 4.7| 7.8 ± 1.0 | 22 ± 3          |
|                      | State 3   | 23.1 ± 3.0| 72.2 ± 5.4| 19.5 ± 2.4| 127 ± 18        |
| Glutamate/malate     | State 4   | 11.5 ± 2.1| -         | 23.2 ± 4.2| 9.4 ± 0.8       |
|                      | State 3   | 23.6 ± 4.1| -         | 88.1 ± 13.4| 72.1 ± 5.3     |
| Succinate/rotenone   | State 4   | 15.8 ± 1.6| 85.8 ± 9.4| 55.6 ± 11.6| 26.2 ± 2.0     |
|                      | State 3   | 24.0 ± 2.0| 112. ± 5.3| 148.1 ± 27.0| 100.9 ± 9.1   |

Values are mean ± SEM from 8 different animals. State 4, oxygen consumption in the absence of ADP; State 3, oxygen consumption in the presence of ADP. Data from references: 34-36 and unpublished results.

### Table 2. Rates of mitochondrial H$_2$O$_2$ production (nanomoles H$_2$O$_2$/min/mg protein) of different organs from male adult rats.

| Substrate            | Brain     | Heart     | Kidney    | Liver     | Skeletal muscle |
|----------------------|-----------|-----------|-----------|-----------|-----------------|
| Pyr/mal              | 0.2 ± 0.04| 0.24 ± 0.04| -         | 0.06 ± 0.01| 0.084 ± 0.020  |
| Pyr/mal + Rot        | 1.05 ± 0.13| 1.49 ± 0.12| -         | 0.38 ± 0.04| 0.73 ± 0.14     |
| Glut/mal             | 0.17 ± 0.04| -         | 0.075 ± 0.05| 0.16 ± 0.02| -              |
| Glut/mal + Rot       | 0.72 ± 0.10| -         | 0.66 ± 0.12| 0.46 ± 0.04| -              |
| Succ + Rot           | 0.26 ± 0.05| 0.51 ± 0.05| 0.10 ± 0.04| 0.32 ± 0.04| 0.31 ± 0.06    |
| Succ                 | 1.72 ± 0.17| 0.71 ± 0.12| 0.52 ± 0.08| 1.00 ± 0.18| 1.57±0.31      |
| Succ + AA            | 2.67 ± 0.31| -         | 5.97 ± 0.45| 3.00 ± 0.41| 2.57±0.30      |

Values are mean ± SEM from 8 different animals. Pyr/mal = pyruvate/malate; Glu/mal = glutamate/malate; Succ, succinate; Rot, rotenone; AA, antimycin A. Data from references: 34-36 and unpublished results.
As mentioned above, the susceptibility of biological membranes to oxidative alterations is related to two inherent traits, the physico-chemical properties of the lipid bilayer and the chemical reactivity of the fatty acids which make up the membrane. Table 3 shows the mitochondrial fatty acid composition (mol%) of different organs. Data clearly indicate that average chain length is maintained around 18 carbon atoms and that the % saturated:unsaturated follows the ratio around 40:60. By contrast, the more relevant
differences are droved to the distribution of the different types of PUFAs. Thus, brain is characterized by the presence of the higher content of monounsaturated fatty acids, as well as PUFAn-3, and particularly, the docosaheaxaenoic acid (22:6n-3) compared to the other postmitotic organs (Table 3). This higher PUFAs content which are more susceptible to oxidative damage leads to a higher steady-state level of lipoxidation-derived molecular damage at least at mitochondrial level (Figure 7).

|       | Brain         | Heart        | Kidney       | Liver         | Skeletal muscle |
|-------|---------------|--------------|--------------|---------------|----------------|
| 14:0  | 0.14±0.03     | 1.17±0.03    | 0.36±0.03    | 0.18±0.01     | 0.82±0.14      |
| 16:0  | 10.55±0.46    | 22.68±0.60   | 10.00±0.38   | 17.31±0.44    | 20.39±0.45     |
| 16:1n-7| 0.28±0.02    | 0.91±0.04    | 0.48±0.09    | 1.11±0.07     | 0.67±0.05      |
| 18:0  | 18.12±0.39    | 26.49±0.34   | 12.67±0.56   | 17.19±0.24    | 18.34±0.73     |
| 18:1n-9| 24.78±0.31   | 13.23±0.39   | 8.97±0.44    | 9.09±0.17     | 8.44±0.98      |
| 18:2n-6| 1.55±0.07    | 9.27±0.42    | 14.10±0.42   | 19.27±0.66    | 17.73±1.23     |
| 18:3n-3| 0.12±0.03    | 0.38±0.01    | 0.47±0.11    | 0.18±0.04     | 0.80±0.12      |
| 18:4n-6| 2.38±0.08    | 0.11±0.01    | 4.67±0.40    | 20:0          |                |
| 20:0  | 1.05±0.07     | 0.20±0.01    | 0.51±0.07    | 20:1n-9       |                |
| 20:1n-9| 4.12±0.18    | 0.19±0.02    | 0.69±0.09    | 20:2n-6       |                |
| 20:2n-6| 0.52±0.05    | 0.97±0.14    | 0.92±0.06    | 0.35±0.01     |                |
| 20:3n-6| 0.10±0.01    | 0.79±0.06    | 0.38±0.03    | 0.27±0.02     |                |
| 20:4n-6| 12.74±0.13   | 15.69±0.45   | 36.32±1.21   | 20:5n-3       |                |
| 20:5n-3| 0.14±0.01    | 0.15±0.01    | 0.40±0.07    | 0.35±0.02     | 1.66±0.44      |
| 22:0  | 1.57±0.26     | 0.21±0.02    | 2.24±0.16    | 22:4n-6       |                |
| 22:4n-6| 4.19±0.16    | 0.73±0.06    | 0.46±0.02    | 0.16±0.008    | 1.54±0.35      |
| 22:5n-6| 0.68±0.03    | 1.40±0.22    | 0.46±0.04    | 0.28±0.04     | 1.99±0.23      |
| 22:5n-3| 2.00±0.16    | 0.84±0.04    | 1.56±0.11    | 0.79±0.04     | 2.95±0.23      |
| 22:6n-3| 12.81±0.33   | 4.28±0.26    | 1.81±0.19    | 22:6n-3       |                |
| 24:0  | 0.14±0.04     | 0.25±0.04    | 0.27±0.04    | 24:5n-3       |                |
| 24:5n-3| 0.26±0.07    | 0.93±0.22    | 24:6n-3      | 1.65±0.16     | 1.24±0.28      |
| ACL   | 19.12±0.05    | 18.15±0.03   | 18.97±0.03   | 18.49±0.01    | 18.54±0.02     |
| SFA   | 31.61±0.52    | 51.02±0.71   | 26.07±0.62   | 34.69±0.50    | 39.56±1.04     |
| UFA   | 68.38±0.52    | 48.97±0.71   | 73.92±0.62   | 65.30±0.50    | 60.43±1.04     |
| MUFA  | 29.19±0.32    | 14.34±0.44   | 10.15±0.51   | 10.20±0.18    | 9.11±1.01      |
| PUFAn-6| 39.19±0.73   | 34.63±0.94   | 63.77±1.07   | 55.09±0.56    | 51.32±1.41     |
| PUFAn-3| 22.18±0.20   | 28.97±0.92   | 57.33±1.16   | 46.97±0.95    | 36.91±1.18     |
| PUFAn-3| 17.00±0.57   | 5.66±0.25    | 6.43±0.32    | 8.12±0.41     | 14.41±0.88     |

Values: mean±SEM. N x group: 8. ACL, average chain length; SFA, saturated fatty acids; UFA, unsaturated fatty acids; PUFAn-6/n-3, polyunsaturated fatty acids n-6 or n-3 series; MUFA, monounsaturated fatty acids. Data from references: 34-36 and unpublished results.

Table 3. Mitochondrial fatty acid composition (mol%) of different organs from male adult rats.
Compared to other postmitotic organs, brain seems to be the tissue more susceptible to oxidative damage. However, are there cross-regional differences in the nervous system? Available data seems to indicate that this is the case. Thus, Table 4 reflects the fatty acid composition in seven different regions from human nervous system samples. The data are conclusive: despite to maintain a stable average chain length (around 18 carbon atoms) and a ratio saturated:unsaturated practically identical (40:60), there is a cross-regional difference with respect to the type of PUFA distribution affecting very specially to both monounsaturated and polyunsaturated fatty acids that seems to be inversely related. In other words, the higher the presence of monounsaturated fatty acids for a given region, the lower the PUFA content. The meaning of this differential distribution remains to be elucidated, but it is evident that determines a differential susceptibility to oxidative damage.

|        | Frontal Cortex | Occipital Cortex | Hippocampus | Amygdala | Substantia Nigra | Medulla Oblongata | Spinal Cord |
|--------|----------------|------------------|-------------|-----------|------------------|-------------------|------------|
| 14:0   | 0.51±0.04      | 0.54±0.05        | 0.42±0.05   | 0.56±0.05 | 0.54±0.04        | 0.50±0.04        | 0.48±0.05  |
| 16:0   | 21.13±0.51     | 20.30±1.70       | 19.54±0.27  | 20.69±0.67 | 12.68±0.39       | 13.57±0.38       | 14.75±0.25  |
| 16:1n-7| 0.98±0.12      | 0.76±0.19        | 0.89±0.10   | 1.48±0.12  | 1.68±0.18        | 1.21±0.03        | 0.94±0.06  |
| 18:0   | 21.35±1.23     | 19.23±0.41       | 21.89±0.20  | 20.19±0.67 | 24.61±0.27       | 25.64±0.61       | 22.07±1.72  |
| 18:1n-9| 23.78±1.51     | 28.57±1.16       | 26.12±0.50  | 28.40±0.77 | 33.57±0.19       | 30.13±0.87       | 33.51±1.43  |
| 18:2n-6| 0.77±0.12      | 0.57±0.07        | 0.59±0.04   | 0.53±0.09  | 0.33±0.06        | 0.56±0.01        | 0.96±0.61  |
| 18:3n-3| 0.14±0.01      | 0.21±0.04        | 0.23±0.02   | 0.08±0.06  | 0.17±0.003       | 0.23±0.01        | 0.34±0.05  |
| 20:0   | 1.25±0.23      | 2.40±0.52        | 1.44±0.09   | 1.71±0.12  | 4.95±0.12        | 5.58±0.23        | 7.62±0.53  |
| 20:1   | 0.19±0.04      | 0.32±0.05        | 0.37±0.01   | 0.28±0.02  | 0.22±0.02        | 0.45±0.02        | 0.75±0.09  |
| 20:2n-6| 0.23±0.01      | 0.22±0.01        | 0.23±0.02   | 0.20±0.02  | 0.23±0.03        | 0.31±0.06        | 0.28±0.01  |
| 20:3n-6| 0.63±0.07      | 0.64±0.04        | 0.90±0.10   | 0.60±0.08  | 0.61±0.02        | 0.99±0.03        | 0.87±0.16  |
| 20:4n-6| 8.28±0.19      | 6.07±0.35        | 8.05±0.24   | 7.42±0.42  | 3.70±0.29        | 3.95±0.33        | 3.13±0.09  |
| 22:4n-6| 4.77±0.14      | 5.06±0.69        | 6.24±0.20   | 4.79±0.27  | 4.32±0.15        | 4.07±0.08        | 3.16±0.17  |
| 22:5n-6| 0.67±0.09      | 0.59±0.09        | 0.92±0.08   | 0.94±0.11  | 0.12±0.007       | 0.40±0.02        | 0.24±0.04  |
| 22:5n-3| 0.26±0.03      | 0.19±0.02        | 0.33±0.02   | 0.13±0.04  | 0.06±0.006       | 0.09±0.01        | 0.38±0.14  |
| 22:6n-3| 13.68±0.17     | 13.71±0.71       | 10.97±0.31  | 9.99±0.58  | 9.74±0.28        | 9.28±0.45        | 8.84±0.71  |
| 24:0   | 0.38±0.06      | 0.31±0.05        | 0.45±0.04   | 0.47±0.03  | 0.64±0.06        | 1.19±0.22        | 1.00±0.08  |
| 24:1n-9| 0.93±0.23      | 0.21±0.03        | 0.33±0.04   | 0.46±0.32  | 1.73±0.18        | 1.75±0.11        | 0.59±0.10  |

Values: mean±SEM. N x group: 3-9. ACL, average chain length; SFA, saturated fatty acids; UFA, unsaturated fatty acids; PUFA n-6/n-3, polyunsaturated fatty acids n-6 or n-3 series; MUFA, monounsaturated fatty acids. (*) Unpublished results.

**Table 4.** Fatty acid composition (mol%) in the human nervous system: a cross-regional comparative approach 1.
This means that saturated and monounsaturated fatty acyl chains (SFA and MUFA) are essentially resistant to peroxidation while polyunsaturates (PUFA) are damaged. Furthermore the greater the degree of polyunsaturation of PUFA the more prone it is to peroxidative damage. Indeed Holman [23] empirically determined (by measurement of oxygen consumption) the relative susceptibilities the different acyl chains (see Figure 8): Docosahexaenoic acid (DHA), the highly polyunsaturated omega-3 PUFA with six double bonds is extremely susceptible to peroxidative attack and is eight-times more prone to peroxidation than linoleic acid (LA) which has only two double bonds. DHA is 320-times more susceptible to peroxidation than the monounsaturated oleic acid (OA) [22,23].

![Figure 8. The relative susceptibilities of selected unsaturated fatty acids to peroxidation. Data are from [23], and all were empirically determined as rates of oxygen consumption. They are expressed relative to the rate for linoleic acid (18:2n-6) which is arbitrarily given a value of 1.](image)

Combining the relative susceptibilities of different fatty acids with the fatty acid composition of membrane lipids it is possible to calculate a peroxidizability index\(^1\) (a measure of the susceptibility to peroxidation) for any particular membrane. The peroxidation index of a membrane is not the same as its unsaturation index (sometimes also called its “double bond index”) which is a measure of the density of double bonds in the membrane. For example, a membrane bilayer consisting solely of MUFA will have an unsaturation index of 100 and a peroxidation index of 2.5, while a membrane bilayer consisting of 95% SFA and 5% DHA will have an unsaturation index of 30 and a peroxidation index of 40. This means that although the 5% DHA-containing membrane has only 30% the density of double bonds of the monounsaturated bilayer, it is 16-times more susceptible to peroxidative damage. In this context, data clearly show the existence of very important cross-regional differences in peroxidizability index in human central nervous system (Figure 9).

\[\text{Peroxidizability Index (PI)} = 0.025 \times (\% \text{ monoenoics}) + 1 \times (\% \text{ dienoics}) + 2 \times (\% \text{ trienoics}) + 4 \times (\% \text{ tetraenoics}) + 6 \times (\% \text{ pentaenoics}) + 8 \times (\% \text{ hexaenoics}), \quad \text{while} \quad \text{Unsaturation index (UI)} = 1 \times (\% \text{ monoenoics}) + 2 \times (\% \text{ dienoics}) + 3 \times (\% \text{ trienoics}) + 4 \times (\% \text{ tetraenoics}) + 5 \times (\% \text{ pentaenoics}) + 6 \times (\% \text{ hexaenoics})\]

\(^1\) (Peroxidizability Index (PI) = 0.025 x (% monoenoics) + 1 x (% dienoics) + 2 x (% trienoics) + 4 x (% tetraenoics) + 6 x (% pentaenoics) + 8 x (% hexaenoics), while Unsaturation index (UI) = 1 x (% monoenoics) + 2 x (% dienoics) + 3 x (% trienoics) + 4 x (% tetraenoics) + 5 x (% pentaenoics) + 6 x (% hexaenoics))
The different cross-regionally PIs observed are due to changes in the type of unsaturated fatty acid that participates in membrane composition. So, there is a systematic redistribution between the types of PUFAs present from highly unsaturated fatty acids to the less unsaturated that is region-specific. Surprisingly, the change shows a gradient that follows the cranio-caudal axis considering the structural organization of the CNS. The mechanism(s) responsible for the cross-regional-related differences in fatty acid profile can be related, in principle, to the fatty acid desaturation pathway, and the deacylation-reacylation cycle. The available delta-5 and delta-6 estimated desaturase activities indicate that they are several folds higher in frontal cortex than in spinal cord (Figure 10). The mechanism underlying to the membrane unsaturation regulation could explain the differences in membrane fatty acid composition and, in turn, the peroxidizability index, and suggest a regulatory mechanism region-specific that is expressed differentially in a cranio-caudal axis likely associated to the development process and even the evolution of the central nervous system.

Figure 9. Cross-regional differences in the peroxidizability index in the human central nervous system. FC, frontal cortex; OC, occipital cortex; HC, hippocampus; AM, amygdala; SN, substantia nigra; MO, medulla oblongata; SC, spinal cord.

Figure 10. Delta-5 and delta-6 desaturase activities in different regions of the human central nervous system. FC, frontal cortex; OC, occipital cortex; HC, hippocampus; AM, amygdala; SN, substantia nigra; MO, medulla oblongata; SC, spinal cord.
In summary, membrane unsaturation is a key characteristic able to define the selective neuronal vulnerability. In this context, it is plausible to postulate that membrane unsaturation could be a main determinant factor in determining differences in the rate of aging for different regions of the CNS, and in the occurrence of neurodegenerative disorders [e.g., Alzheimer’s disease (AD), Parkinson’s disease (PD), or amyotrophic lateral sclerosis (ALS), among others] during the sixth, seventh and eighth decades of life. Interestingly, this property is also causally related to the aging process and the lifespan of animal species [6].

6. Conclusions and perspectives

A major goal of research into aging is to extend ‘healthspan’ by identifying approaches for delaying or preventing age-related diseases. The fact that many individuals maintain a well-functioning nervous system and continue productive lives through their seventies, eighties and even nineties is encouraging. The implication is that if the cellular and molecular mechanisms that determine whether nervous systems adapt positively or develop a disease during aging can be identified, then disease processes can be averted. In this regard, oxidative and metabolic stress and impaired cellular stress adaptation, are mechanisms of aging that render neurons vulnerable to degeneration. On this background of age-related endangerment, genetic and environmental factors likely determine whether a disease process develops. These include causal mutations, more subtle genetic risk factors and environmental factors, including aspects of diet and lifestyle. Because of the cellular and molecular complexity of the nervous system, and the signalling mechanisms that influence neuronal plasticity and survival, the basis of SNV remains elusive. However, available evidence from both an inter-organ comparative approach and cross-regional differences seems to confirm this idea, highlighting membrane unsaturation as a key trait associated with selective neuronal vulnerability. Interestingly, this property is also causally related to the aging process and the lifespan of animal species [6,10], and it is apparently operative in multiple neurodegenerative disorders [3,37]. Currently, most efforts to prevent and treat neurodegenerative disorders are focusing on diet, on lifestyle modification, and on drugs that target disease processes [3,6,33]. Although data on humans is still limited, the emerging evidence that dietary restriction (along with exercise and cognitive stimulation) can bolster neuroprotective mechanisms suggests that diet and lifestyle changes could reduce the risk of neurodegenerative disorders [6,26,33]. Therefore, it seems likely that extension of neural healthspan is possible for most individuals.

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