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Review article

Neurovascular-neuroenergetic coupling axis in the brain: master regulation by nitric oxide and consequences in aging and neurodegeneration

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ABSTRACT

The strict energetic demands of the brain require that nutrient supply and usage be fine-tuned in accordance with the specific temporal and spatial patterns of ever-changing levels of neuronal activity. This is achieved by adjusting local cerebral blood flow (CBF) as a function of activity level – neurovascular coupling – and by changing how energy substrates are metabolized and shuttled amongst astrocytes and neurons – neuroenergetic coupling. Both activity-dependent increase of CBF and O_2 and glucose utilization by active neural cells are inextricably linked, establishing a functional metabolic axis in the brain, the neurovascular-neuroenergetic coupling axis. *This axis incorporates and links previously independent processes that need to be coordinated in the normal brain.* We here review evidence supporting the role of neuronal-derived nitric oxide ($^{\bullet}NO$) as the master regulator of this axis. Nitric oxide is produced in tight association with glutamatergic activation and, diffusing several cell diameters, may interact with different molecular targets within each cell type. Hemeproteins such as soluble guanylate cyclase, cytochrome c oxidase and hemoglobin, with which $^{\bullet}NO$ reacts at relatively fast rates, are but a few of the key in determinants of the regulatory role of $^{\bullet}NO$ in the neurovascular-neuroenergetic coupling axis. Accordingly, critical literature supporting this concept is discussed. Moreover, in view of the controversy regarding the regulation of catabolism of different neural cells, we further discuss key aspects of the pathways through which $^{\bullet}NO$ specifically up-regulates glycolysis in astrocytes, supporting lactate shuttling to neurons for oxidative breakdown. From a biomedical viewpoint, derailment of neurovascular-neuroenergetic axis is precociously linked to aberrant brain aging, cognitive impairment and neurodegeneration. Thus, we summarize current knowledge of how both neurovascular and neuroenergetic coupling are compromised in aging, traumatic brain injury, epilepsy and age-associated neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, suggesting that a shift in cellular redox balance may contribute to divert $^{\bullet}NO$ bioactivity from regulation to dysfunction.

1. Introduction

The brain is like no other organ in the sense that it is critically dependent on continuous and well-regulated blood flow to support its structural and functional integrity. It is a complex and functionally heterogeneous organ that, despite representing only 2% of total body mass, consumes more than 20% of the metabolic substrates (O_2 and glucose) and receives nearly 15% of the cardiac output [1,2]. Ironically, the brain has very limited energy reserves, which implies that neurons must extract O_2 and glucose from the blood on an as-needed basis to support ongoing neural function, and thus, the blood supply must be dynamically regulated with fine temporal and regional precision to meet the physiological demands imposed by neural activation [3]. The first evidence for an intrinsic regulation of CBF dates from the nineteen century. In a seminal work, Roy and Sherrington performed a series of experiments that allowed them to conclude that “chemical products of cerebral metabolism contained in the lymph which bathes the walls of the arterioles of the brain can cause variations of the caliber of the cerebral vessel” and proposed that the brain has an “intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations in functional activity” [4]. This hypothesis was validated by decades of intensive research and yet, due to the intrinsic cellular and molecular complexity of the process, as well as the difficulty to study it in vivo on a dynamic basis, the underlying mechanisms of neurovascular coupling have still to be fully elucidated. Nonetheless, it is generally agreed that 1) neurovascular coupling arises

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from the concerted communication between neuronal and vascular cells, with the likely involvement of other cell types (e.g. astrocytes and pericytes); 2) it is intimately associated with glutamatergic transmission and likely triggered by synaptically-released glutamate; 3) it may result from the cooperation of diverse vasoactive molecules and/or pathways; and 4) the mechanism may differ amongst brain regions as a reflection of their diverse neuroanatomic and functional properties [5].

An equally important concept is that of neurometabolic coupling, or in other words, the activity-dependent regulation of glucose and O_2 utilization by neural cells. As with neurovascular coupling, the intricate interplay between astrocyte and neuron energetic metabolism is intimately associated with glutamatergic signaling. Activation of glutamate receptors is intimately associated with the synthesis of nitric oxide (•NO), an intercellular signaling molecule that can interact with multiple targets, thus modulating physiological pathways including regulation of CBF and utilization of O_2 and glucose. This suggests that •NO may have a pivotal role in establishing a neurovascular-neuroenergetic coupling axis.

Derailment of this axis, in particular the functional implications in terms of decreased CBF, has been linked to cognitive dysfunction, conversion from a normal or mild cognitive impairment state to dementia diagnosis and age-related functional alterations. Considering, as argued here on the basis of available evidence, the master regulatory role of •NO in neurovascular-neuroenergetic coupling axis, changes in the bioavailability of •NO impact on the functionality of this very axis. Along these lines, the reaction of •NO with superoxide radical (yielding peroxynitrite and limiting •NO bioavailability) is a likely event that leads to limited signaling from neurons to blood vessels.

2. The role of nitric oxide in neurovascular coupling

2.1. What makes nitric oxide distinctive in conveying information from neurons to vessels?

Although not without controversy, several lines of evidence support the notion that •NO is an important player in neurovascular coupling. Soon after the recognition of •NO as an intercellular diffusible messenger, Gally and collaborators proposed that •NO might regulate CBF associated to neuronal activity [6]. Indeed •NO is well suited to mediate the intimate communication between neural cells and vessels. **Firstly**, it is a potent vasodilator that has been implicated in the regulation of vascular tone in a large number of tissues [7]. While •NO can promote vasodilation through alternative pathways [8,9], classically it involves the activation of soluble guanylate cyclase (sGC) in smooth muscle cells with subsequent activation of cGMP-dependent protein kinases. The later promotes the dephosphorylation of myosin light chain which, ultimately, results in vasorelaxation [10].

Secondly, in the brain, •NO signaling is intimately associated with glutamatergic transmission. The synthesis of •NO involves the stimulation of ionotropic glutamate receptors, particularly the NMDA-type, to which nNOS is physically coupled: the binding of glutamate to NMDAr results in an influx of Ca^{2+} that upon binding calmodulin, and providing that substrates and co-factors are available, activates the nNOS, thus leading to •NO production [11].

Thirdly, •NO possesses unique physicochemical properties that are of the outmost relevance for intercellular communication: it is a hydrophobic and highly diffusible molecule. The ability to diffuse in the tissue and reach molecular targets such as sGC at an effective concentration is key to the biological activity of •NO. We have determined the coefficient of diffusion of exogenously applied •NO in the cortex in vivo to be $D_{NO} = 2.2 \pm 0.13 \times 10^{-5}\ cm^2\ s^{-1}$, with a first order decay rate $\lambda = 1.09 \pm 0.21\ s^{-1}$. Interestingly, the experimental data and mathematical modeling used strongly suggest that cellular membranes may act as facilitating pathways for •NO diffusion, as opposed to diffusion through the protein-crowded cytosol, thus increasing the distance that •NO can diffuse [12]. This hypothesis is corroborated not only by the increased partition of •NO in lipid phases as opposed to water phases in liposomes as well as by observation that O_2 diffusion, a diatomic molecule similar to •NO, is also increased by lipids [13]. In hippocampal slices, the activation of multiple nNOS-containing neurons within a 50 μm radius results in a diffusional spread of •NO close to 400 μm [14]. The diffusional field of •NO is expectedly lower under in vivo conditions where operant blood flow promotes the scavenging of •NO [15]. In either case, evidence supports that •NO produced in a finite volume by multiple sources diffuses in the brain tissue and integrates the activity of multiple cells (neurons, astrocytes, endothelial cells, etc) without the requirement of physical connection to the •NO producing cell [16]. The concept of •NO volume transmission in the brain was shown by Steinert *et al.* by combining mathematical modeling and •NO detection in brainstem slices using the fluorescent indicator DAR-4 M alongside Ca^{2+} imaging (Fura-2). The authors showed that •NO is produced upon NMDAr activation and that it's actions extend beyond the producing cell, affecting neurons that are not activated [17]. Combined with the multiplicity of molecular targets with which •NO can interact, this feature of volume transmission endows •NO with the ability to modulate and integrate different pathways in neurovascular coupling.. In line with this, •NO can contribute to upstream vasodilation by regulating gap junctional intercellular communication [8].

Fourthly, there is a favorable anatomic location of neuronal sources of •NO with respect to small blood vessels. The mean distance between arterioles and NADPH-diaphorase-stained nerve fibers along the longitudinal axis of pyramidal layer neurons in the *CA1* region of hippocampus ranges from ~70–150 μm [18]. In the somatosensory cortex the neuritis of NOS-positive interneurons were detected in intimate contact with local blood vessels, representing 28% of the interneurons located within 50 μm of the blood vessel in the layer I-III [19]. This anatomical proximity, allied with the highly diffusible character of •NO, forwards the hypothesis of •NO produced by neurons being able to diffuse up to blood vessels to promote vasodilation.

Finally, considering that erythrocyte-mediated scavenging of NO constitutes the most relevant pathway for its inactivation in the brain [15], one can envisage that the signaling conveyed by •NO and translated into increased CBF (and thus an increase in the circulating erythrocytes) may shape the •NO underlying signaling pathway in a self-regulated manner.

2.2. Experimental evidence for the implication of nitric oxide in the neurovascular coupling

More than twenty years have passed since the proposal of •NO as the “missing link” in the neurons to blood vessels communication [20], but while the involvement of •NO seems to be ascertained, the complete understanding of its role in neurovascular coupling has still to be achieved.

It is thus critical to integrate the evidence obtained from different experimental designs used so far, namely in vivo, *ex vivo* and in vitro studies, pharmacological approaches and direct measurements of the molecular entities involved, dynamic and real-time or static measurements. In Table 1 we have compiled some studies addressing the involvement of •NO in the regulation of CBF coupled to neuronal activation. The first experimental evidence was published in the early 90 s on basis of the observation that the competitive antagonism of NOS was able to attenuate the cerebrovascular response elicited by different experimental paradigms of neuronal activation [21–26]. Later studies using more selective inhibitors, namely 7-nitroindazole, suggested that specifically neuronal-derived •NO was implicated in this process [27–33]. Concurrently, similar pharmacological approaches resulted in contradictory observations supporting the lack of •NO participation in neurovascular coupling [24,25,34,35]. To note, these later observations were made either in conscious restrained animals, a stressful

Table 1
Compilation of studies addressing the involvement of ˙NO in neurovascular coupling.

Indirect evidence

Pharmacological approaches

Animal Model	Preparation	Brain Region	Experimental paradigm	NOS Inhibitor	Method	Observation	Reference
Wistar Rats	In vivo, anesthetized	Cerebral cortex, (S1HL)	Sciatic nerve stimulation	L-NAME	HC	↓ CBF	[21]
Wistar Rats	In vivo, anesthetized	Cerebral cortex (S1BF)	Vibrissae stimulation	L-NNA	LDF	↓ CBF	[22]
New Zealand Rabbits	In vivo, anesthetized	Cerebral cortex	Topical NMDA application	L-NNA	MV	↓ vessel diameter	[23]
Wistar Rats	In vivo, awake	Cerebral cortex, (S1BF)	Vibrissae stimulation	L-NNA	AR	No effect	[24]
Wistar Rats	In vivo, anesthetized	Cerebellum	Afferent electrical stimulation	L-NNA	LDF	↓ CBF	[25]
Sprague Dawley Rats	In vivo, awake	Cerebral cortex (S1BF)	Vibrissae stimulation	L-NAME	AR	No effect	[242]
Newborn Pigs	In vivo, anesthetized	Cerebral cortex	Glutamate and NMDA application	L-NNA	MV	↓ vessel diameter	[26]
New Zealand Rabbits	In vivo, anesthetized	Cerebral cortex	Topical NMDA application	7-NI	MV	↓ vessel diameter	[27]
Sprague Dawley Rats	In vivo, anesthetized	Cerebellum	Afferent electrical stimulation	L-NNA	LDF	↓ CBF	[243]
Sprague Dawley Rats	In vivo, anesthetized	Cerebellum	Glutamate application	7-NI	LDF	↓ CBF	[30]
Sprague Dawley Rats	In vivo, anesthetized	Cerebral cortex	Systemic NMDA application	L-NNA	LDF	↓ CBF	[244]
Sprague Dawley Rats	In vivo, anesthetized	Cerebral cortex (S1BF)	Vibrissae stimulation	7-NI	AR/LDF	↓ CBF	[245]
Sprague Dawley Rats	In vivo, anesthetized	Cerebellum	Afferent electrical stimulation	L-NNA,7-NI	LDF	↓ CBF	[246]
Sprague Dawley Rats	In vivo, anesthetized	Cerebral cortex	Glutamate and NMDA application	7-NI	LDF	↓ CBF	[31]
Sprague Dawley Rats	In vivo, awake	Cerebral cortex (S1BF)	Vibrissae stimulation	L-NA	AR	No effect	[34]
Wistar Rats	In vivo, anesthetized	Cerebral cortex (S1BF)	Vibrissae stimulation	L-NNA	LDF	↓ CBF	[50]
Sprague Dawley Rats	In vivo, anesthetized	Cerebral cortex (S1BF)	Vibrissae stimulation	7-NI	LDF	↓ CBF	[213]
Wistar Rats	Brain slices	Cerebral cortex	Afferent electrical stimulation	L-NAME	MV	No effect	[35]
Wistar Rats	In vivo, anesthetized	Cerebral cortex (S1FL)	Forepaw Stimulation	L-NNA	LDF	↓ CBF	[247]
Wistar Rats	In vivo, anesthetized	Cerebral cortex (S1BF)	Vibrissae stimulation	L-NNA,7-NI	LDF	↓ CBF	[248]
Sprague Dawley Rats	In vivo, anesthetized	Cerebral cortex	Afferent electrical stimulation	7-NI	LDF	↓ CBF	[32]
Wistar Rats	Brain slices	Hypothalamus	Hyperosmotic stimulation	L-NAME +cPTIO, 7-NI	MV	↓ vessel diameter	[249]

GENETIC APPROACHES

Animal Model	Preparation	Brain Region	Experimental paradigm	Method	Observation	Reference
nNOS -/- mice	In vivo, anesthetized	Cerebral cortex (S1BF)	Vibrissae stimulation	LDF	No effect	[40]
nNOS -/- mice	In vivo, anesthetized	Cerebellar cortex	Electrical stimulation and glutamate application	LDF	↓ CBF	[41]
nNOS -/- mice	In vivo, anesthetized	Cerebral cortex (S1FL)	Forepaw Stimulation	MV	↓ CBF	[39]

DIRECT EVIDENCE

Animal Model	Preparation	Brain Region	Experimental paradigm	Method	Pharmacology	Reference
Wistar Rats	In vivo, anesthetized	Cerebral cortex (S1FL)	Forepaw Stimulation	LDF and ˙NO microelectrodes	No	[45]
Wistar Rats	Brain slices	Cerebellum	NMDA application	MV and ˙NO microelectrodes	Yes	[46]
Wistar Rats	In vivo, anesthetized	Hippocampus	Glutamate application	LDF and ˙NO microelectrodes	Yes	[33]
Wistar Rats	Brain slices	Cerebellum	Afferent electrical stimulation	MV and DAF-FM imaging	Yes	[47]

Abreviations: S1HL – primary somatosensory cortex, hindlimb region, S1FL - primary somatosensory cortex, forelimb region, S1BF - primary somatosensory cortex, barrel field, L-NAME - L-N^G-Nitroarginine methyl ester, L-NNA - L-N^G-Nitroarginine;, 7-NI – 7-nitroindazole, cPTIO- 2-(4-Carboxyphenyl) – 4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, HC – hydrogen clearance, AR - autoradiography, LDF – laser Doppler flowmetry, MV – Microscopic direct visualization, BOLD – blood oxygenation level-dependent, nNOS -/- - knockout mice for neuronal isoform of NOS.

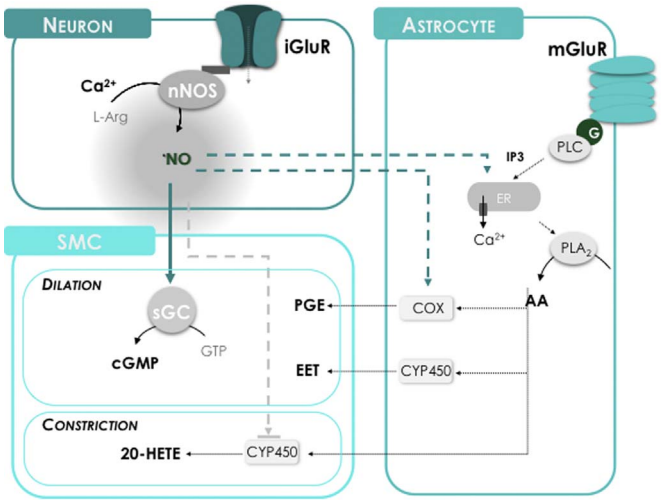

Fig. 1. Schematic representation of the signaling pathways by which •NO may impact over neurovascular coupling. In neurons, synaptically released glutamate activates ionotropic glutamate receptors (iGluR), allowing the influx of Ca^{2+} and activating neuronal nitric oxide synthase (nNOS). The •NO produced may directly promote vasorelaxation and increase in the cerebral blood flow by activating sGC in smooth muscle cells (SMC) of arterioles and/or in the pericytes surrounding capillaries. Indirectly, •NO may promotes vasodilation by modulating the signaling pathways involving arachidonic acid metabolites (dashed lines). In astrocytes, activation of metabotropic glutamate receptors (mGluR), by raising $[Ca^{2+}]_i$ activates phospholipase A_2 (PLA_2) which promotes the release of AA from membranes. •NO can either stimulate the activity of cyclooxygenase (COX), favoring the synthesis of prostaglandins (PGE) (blue dashed line), and inhibit the activity of cytochrome P450 hydroxylase, limiting the production of the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE) (grey dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

condition that may challenge the interpretation of results due to its putative effect over cerebrovascular regulation [36], or in brain slices, a preparation devoid of functional vascular tone [37]. A complementary strategy used to address the involvement of •NO in neurovascular coupling took advantage of genetically modified animals. However, the use of nNOS knockout mice generated by disruption of exon 2 of the NOS1 gene [38] also resulted in disparate conclusions [39–41].

Overall, these controversial results imply that alternative and more direct approaches are required for a clearer understanding of the role played by such an elusive free radical as •NO in neurovascular coupling. One approach we have used in an attempt to better understand the mechanisms underlying the role of neuronal derived NO in neurovascular coupling has been to directly measure the activity-dependent changes in •NO using modified carbon fiber microelectrodes [42] simultaneously with CFB in vivo in the anesthetized rat brain [33,43,44].

A significant advance has been achieved via direct measurement of •NO dynamics in vivo with selective microelectrodes during neurovascular coupling. Overcoming the technological challenge associated to the real time dynamic measure of •NO, Buerk and collaborators made a relevant contribution by firstly providing evidence for the temporal correlation of the •NO dynamics and CBF changes in the rat somatosensory cortex upon forepaw electrical stimulation, establishing that increases in •NO proceeded that of CBF. However, the authors failed to address the interdependency of the two events [45]. Following this line, and by complementing the simultaneous in vivo measurement of •NO dynamics and CBF with pharmacological approaches, we pinpointed •NO derived from nNOS as the direct mediator of neurovascular coupling in the hippocampus. Data obtained supports the canonical •NO/cGMP-dependent pathway for vasodilation [33]. Experiments in cerebellar slices *ex vivo* also support that •NO from neuronal origin is required for vasodilation induced by NMDAr activation [46,47].

2.3. Nitric oxide in neurovascular coupling: mediator or modulator?

Despite the putative involvement of several molecular messengers in the process of neurovascular coupling (*eg.* arachidonic acid and its derivatives, K^+, adenosine, lactate, among other vasoactive and metabolic factors), evidence globally supports that, upon glutamatergic stimulation, •NO acts as a key player of the coupling in several neuronal networks [5]. The studies focusing on the role of •NO in neurovascular coupling have established that •NO can act either as a direct mediator of the process or as a modulator of other operative pathways. This appears to be determined, at least partially, by differences among brain regions, the specificities of the neuronal networks (neurochemicals released) and their activation (e.g. single versus population neuronal stimulation). This is not surprising considering that while nNOS is scattered through different brain areas, there is a heterogeneous distribution of its expression and activity [48]. A pseudo-quantitative analysis of nNOS expression revealed a higher density in the hippocampus compared to the cerebral cortex, which was translated into higher •NO fluxes upon glutamatergic activation in the former region [48,49]. In the hippocampus and cerebellum, major evidence supports that •NO plays a direct role, via volume signaling, conveying the information from neurons to blood vessels, thus inducing dilation via activation of sGC in smooth muscle cells of parenchymal arterioles (or in pericytes surrounding capillaries). Conversely, in the cerebral cortex, and although direct dynamic measurements in vivo have yet to be systematically undertaken to establish volume signaling properties, •NO apparently may also act as a modulator of other pathways, mainly centered in astrocytes as intermediaries bridging neuronal activity and hemodynamic response [5,50].

2.3.1. Astrocytic pathway

Several lines of research support the notion that astrocytes act as intermediaries bridging neuronal activity and hemodynamic changes. Astrocytes are located between neurons and blood vessels and form an extensive interconnected network mediated by gap junctions that is ideally suited to propagate information upstream along the vascular tree [5]. The proposed astrocytic-dependent pathways rely on the activation of metabotropic glutamate receptors, namely mGluR1 and mGluR5, and the increase of $[Ca^{2+}]_i$ via inositol 1,4,5-trisphosphate (IP3). The increase in $[Ca^{2+}]_i$ activates phospholipase A2, leading to the release of arachidonic acid, which, depending on the downstream pathway, can be metabolized to different vasoactive compounds [35,51,52]. Notably, arachidonic acid is metabolized by heme-containing enzymes, the activity of which can be modulated by •NO (Fig. 1). There is evidence indicating that •NO may favor vasodilation by inhibiting cytochrome P450 (CYP) ω-hydroxylase activity [9,53] or upregulating cyclooxygenase (COX) activity [54]. Also, •NO may potentiate ryanodine receptor activation and thus the increase in $[Ca^{2+}]_i$ [55].

However, the role of astrocytes in neurovascular coupling through the mGluR-dependent pathway has been recently contested, mainly due to the observation of downregulated expression of mGluR5 in adult animals [56] and unaltered neurovascular coupling in mice devoid of IP_3 type 2 receptors [57]. An alternative pathway by which astrocytes can regulate vasodilation is via ATP release to the extracellular space. Both ATP and adenosine (formed from the hydrolysis of ATP) are suggested to activate purinergic receptors, either in smooth muscle cells or in endothelial cells, and promote vasodilation (reviewed by [58]). In the somatosensory cortex, it was recently substantiated that neurovascular coupling is regulated by the activation of P2Y1 in endothelial cells with subsequent production of •NO by the endothelial isoform of NOS (eNOS) [59]. ATP is also suggested to induce •NO production in hippocampal neurons independently of glutamate signaling [60]. Conversely, •NO may modulate the purinergic signaling, for instance, by potentiating the adenosine release [61,62].

2.3.2. Arterioles versus capillaries: different pathways for different targets

The classical view of the regulation of CBF presumes that arterioles, more precisely smooth muscle cells, are the sole effectors of vasodilation. This view has been challenged by evidence supporting that CBF can be controlled at the capillary level by pericytes, contractile cells that wrap themselves around the small vessels (both capillary and venules) [63]. Attwell and collaborators demonstrated that dilation of cortical capillaries, arising from pericytes contractility, preceded arteriolar dilation during somatosensory stimulation [64]. More recently, the same group forwarded the hypothesis that different signaling pathways at the capillary and arteriolar level may drive neurovascular coupling. More specifically, regulation of CBF in the capillary bed is suggested to be mediated by pericytes, via mGluR-independent astrocytic signaling pathways, while at the arteriolar level, it is suggested to occur via neuronal mechanisms involving the $^{\bullet}$NO-NMDAR signaling pathway [65].

2.4. Other sources of nitric oxide

Although the main focus of this review is on $^{\bullet}$NO derived from neurons, one cannot disregard the involvement of $^{\bullet}$NO produced by other sources in the regulation of CBF and in neurovascular coupling. In the brain, $^{\bullet}$NO can also be produced enzymatically by other members of the NOS family: the endothelial isoform of NOS (eNOS), constitutively expressed mainly in the endothelial cells, and inducible NOS (iNOS), expressed upon demand in multiple cell types, including glial cells, in response to inflammatory stimuli [66]. In the somatosensory cortex, neurovascular coupling was recently shown to be regulated by the activation of P2Y1 in endothelial cells with subsequent production of $^{\bullet}$NO by eNOS [59]. Also, iNOS expression in glia is suggested to contribute towards the regulation of vascular tone [67].

Another pathway for $^{\bullet}$NO production has been proposed in connection with the reduction of nitrite to $^{\bullet}$NO, either with or without enzymatic intervention [68]. Non-enzymatically. $^{\bullet}$NO can be generated by univalent reduction of nitrite under combined acidic and reducing conditions (reviewed in [69]). Growing body of evidence supports the notion that $^{\bullet}$NO generated via nitrate-nitrite-$^{\bullet}$NO pathway plays a relevant role in the regulation of CBF in response to neuronal activation [70–72].

3. Beyond neurovascular coupling towards a master regulation of neurometabolism: nitric oxide as modulator of brain neuroenergetics

3.1. Neuroenergetic coupling – maximizing energetic efficiency of neuronal activity

The change of CBF in response to neuronal activity is, as stated above, critical to assure adequate delivery of metabolic substrates such as glucose and O_2 to meet the activity level and energy demands of neurons. This brings us to the concepts of neurometabolism and neuroenergetic coupling.

The energetic requirements of the brain can largely be attributed to the energetic cost of glutamatergic neurotransmission as there is a 9:1 ratio between excitatory and inhibitory cells in the brain and the large majority of chemical synapses release glutamate [73]. Glutamatergic neurotransmission encompasses several energy demanding processes such as generation and propagation of action potentials, reposition of ionic gradients, vesicles exo- and endocytosis, uptake and recycling of glutamate and Ca^{2+} handling, to state a few [74]. Because the cost of synaptic transmission is high, the brain has evolved to maximize the ratio of information transmitted as a function of energy cost as opposed to maximizing coding capacity, that is, the number of distinct patterns that may be represented while minimizing redundancy [75]. In other words, although neuronal cells or populations may have the potential for higher coding capacity (increased firing frequency), the requirement of energetic efficiency imposes optimal upper and lower limits [75]. Considering a firing rate of 4 Hz and a probability of vesicle release of $p = 0.25$, the energetic cost of synaptic transmission has been calculated to be roughly 1.64×10^5 ATP/s or 24000 ATP molecules per bit of information transmitted [76]. It is likely that the supply of energy may impose a limit on neuronal activity under normal conditions, highlighting the need for strict adjustment between energetic demand and supply.

On average, almost all of adult brain ATP results from the complete oxidation of glucose, with glycolysis followed by oxidative phosphorylation resulting in a glucose:O_2 ratio of 1:6. As such, mitochondria provide 93% of all ATP, while 7% are supplied by glycolysis (reviewed in [76]).

Traditional studies on both brain function and neurometabolism have focused mainly on task-evoked responses and although fruitful, such approaches ignore the more realistic possibility that brain functions are mainly intrinsic and ongoing. Interestingly, energy consumption on the face of increased brain activity such as that imposed by a perceptual task during a functional imaging experiment is small [77,78] and may amount only to a 5% increase from baseline level [79]. Further observations from positron emission tomography have revealed that task-induced increase in neuronal activity is accompanied by a 51% increase in CBF that, although accompanied by an increase in glucose use of 50%, is not met by a proportional increase in O_2 use (5%), suggesting a predominance in glycolytic breakdown of glucose as opposed to complete oxidation [80,81]. However, stimulation of neuronal activity produces not only an increase in O_2 consumption observed both in *ex vivo* slice experiments and in vivo [82–84] but also a decrease in intracellular NADH [85,86], suggesting that ATP generation at the synaptic level is dependent on oxidative phosphorylation [87].

3.2. Aerobic glycolysis – a role for lactate

How may one reconcile these apparently contradictory observations? It has been suggested that task-induced increase neuronal activity, while producing an increase in CBF to atone for increased energetic demand, might not necessarily result in an increase in the rate of oxidative phosphorylation. In fact, many have proposed that both under resting state and in task challenges, “aerobic glycolysis” is an important metabolic pathway for energy (ATP) production.

Brain aerobic glycolysis is the process by which glucose is metabolized to lactate and in excess to the requirement of the system's oxidative phosphorylation, despite the presence of adequate levels of O_2. Although lactate resulting from glycolysis was for many years considered a metabolic dead-end, seminal work by Pellerin and Magistretti showed that lactate is preferentially transported from astrocytes to neurons, where it can be converted to pyruvate to fuel the tricarboxylic acid (TCA) cycle [88–90]. The dichotomized metabolism of the brain consolidates the symbiotic relationship between neurons and astrocytes and is supported by what has been coined the reverse Warburg effect [91].

Stimulation of brain tissue produces changes in how both astrocytes and neurons produce ATP. Reports of differential metabolic characteristics between the two cell types go as far back as 1963, when Hamberger and Hayden observed that neurons responded to stimulation by increasing oxidative capacity, while glial cells rather showed an increase in the glycolytic pathway under the same situations [92]. In light of their observations, the authors went as far as to propose a metabolic cooperation between the two main cell types of the nervous system. These initial observations were later corroborated by studies using primary cell cultures revealing that the higher glycolytic rate in astrocytes compared to neurons is accompanied by lactate release [93]. Two-photon microscopy has allowed researchers to further investigate this metabolic cooperation in more complex biological preparations, such as hippocampal slices in which both connectivity and intercellular

trafficking remain functional. One such study revealed a biphasic shift in NADH fluorescence upon electrical stimulation: an early decrease phase associated with increase oxidative metabolism [85,86] and lactate use [94] in neurons and a late-phase increase in NADH signal apparently associated not only to increased oxidative metabolism in neurons [86] but also to increased glycolytic rate in astrocytes [95]. Also, despite the higher energetic expenditure of neurons compared to astrocytes [74,96], glucose transport and metabolism studied in slices from the hippocampus and cerebellum appear to be faster in both glia and astrocytes as compared to neurons [97], an observation that has been corroborated in in vivo for the rat somatosensory cortex both under resting and activated conditions [98].

The fact that neurons use lactate derived from astrocytic aerobic glycolysis may at first sight seem absurd, as neurons express glucose transporters and are infamously dependent on glucose supply for adequate function. However, glucose metabolism supports other important cellular functions beyond neuroenergetics. Because the blood brain barrier blocks the passage of circulating neuroactive compound such as glutamate into the brain, neurons rely on glucose as a carbon source to support neurotransmitter synthesis, as well as amino acids, other monosaccharides and carbohydrates implicated in neurotransmission [99–102]. Glycolytic-derived glyceraldehyde-3-phosphate is diverted to intermediates of the pentose phosphate pathway, which also guarantees adequate NADPH production to sustain not only biosynthetic reactions but also support defense against oxidative stress, as discusses below. A significant amount of evidence has been collected throughout the years showing that lactate is indeed an adequate substrate for neurons: normal synaptic function is maintained in hippocampal slices for hours with lactate as a sole substrate [103].

3.3. Mechanisms underlying the regulatory role of neuronal-derived nitric oxide

The regulation of the metabolic axis comprising neurovascular and neuroenergetic coupling may share key elements, such as $^{\bullet}NO$, O_2 and even lactate. The local concentration of O_2 in the brain tissue can influence the synthesis of messengers involved in regulation of CBF, namely enzymes using O_2 as a substrate such as nNOS and those associated with arachidonic acid metabolism, which in turn $^{\bullet}NO$ is suggested to modulate. On the other hand, lactate resulting from aerobic glycolysis in astrocytes and released to the extracellular space appears to reduce the clearance of the vasodilator PGE_2 by the astrocyte prostaglandin transporter [104]. The unequivocal link between stimulated activity and both vascular and metabolic coupling is glutamate neurotransmission and the signaling linked to receptor activity. In particular, glutamate uptake at the astrocytes appears to produce a sodium-dependent signal that couples glutamatergic transmission to increased glucose utilization by astrocytes [90]. A tight coupling between Na^+-dependent glutamate uptake and glucose utilization has been further confirmed in isolated astrocytes [105] and also in vivo [106,107].

To date, little has been forwarded to explain the predominant glycolytic metabolic profile of astrocytes, which allows the shuttling of lactate to neurons as opposed to complete oxidation of glucose. Considering the tight coupling between neurons, astrocytes and vasculature supporting the neurovascular unit, as discussed above, it is reasonable to consider the occurrence of an intercellular signal regulating the metabolic characteristic of each cell type as a function of activity status, and one clear candidate is $^{\bullet}NO$.

Nitric oxide is a quintessential messenger molecule in the brain and can actually be synthetized by any one of the 3 cell types comprising the neurovascular unit: neurons, astrocytes and endothelial cells [108]. As discussed in detail above, $^{\bullet}NO$ can regulate vascular tone, but it is interesting to note that once produced upon neuronal activation $^{\bullet}NO$ can also regulate energy metabolism in the brain. In an elegant series of assays, Almeida and collaborators were able to show that $^{\bullet}NO$ blocks oxidative phosphorylation in both astrocytes and neurons, but only in astrocytes does it enhance glycolysis. While neurons exposed to $^{\bullet}NO$ show continuous depletion of ATP and loss of mitochondrial membrane potential ($\Delta\psi_m$), astrocytes exposed to the same conditions show lesser and contained ATP depletion, hyperpolarization of $\Delta\psi_m$ and increased lactate production. The authors suggest that glycolytically produced ATP hydrolyzed at the F_1F_0-ATPase supports maintenance of $\Delta\psi_m$ [109].

Inhibition of oxidative phosphorylation is a well-recognized result of the biological activity of $^{\bullet}NO$ [110]. The chemical similarity between the two hydrophobic gases $^{\bullet}NO$ and O_2 supports the competitive inhibition of CcO, the terminal complex of the mitochondrial electron transporter chain. Nitric oxide binds to the fully reduced heme-copper active site [111,112] and increases the K_m for O_2. Alternatively, $^{\bullet}NO$ can also react with the oxidized complex at the Cu^{2+} through an uncompetitive mechanism, in which reversal to an active state implies the oxidation of $^{\bullet}NO$ to NO_2^- [113]. Interestingly, this reversible inhibition occurs for the $^{\bullet}NO$ concentration range observed upon activation of glutamatergic neurotransmission [14,82]. Inhibition of CcO by $^{\bullet}NO$ is more significant for lower concentrations of O_2 or, alternatively, when enzyme activity increases (as in the case of increased energetic demand) [114]. One interesting observation supporting the role of $^{\bullet}NO$-mediated inhibition of oxidative phosphorylation as a key regulatory element of neuroenergetic coupling is the finding that CcO, nNOS and the NMDAr co-localize, at least in the rat brain [115].

While inhibition of oxidative phosphorylation by $^{\bullet}NO$ is quite straightforward, understanding the selective upregulation of glycolysis in astrocytes is not. Treatment with equal levels of exogenously applied $^{\bullet}NO$ has been shown to decrease fructose-6-phosphate and increase fructose-1,6-biphosphate (the substrate and product, respectively, of phosphofructokinase 1, PFK1) in astrocytes, but not neurons. Furthermore, although PFK1 expression level is similar in both cell types, its activity is four-fold higher in astrocytes [116]. The authors have subsequently demonstrated that astrocytes (but not neurons) exposed to $^{\bullet}NO$ produce a metabolite capable of fully activating PFK1 in both cell types – fructose-2,6-bisphosphate ($F2,6P_2$), a potent allosteric modulator of PFK1, produced and degraded by the bidirectional enzyme 6-phosphofructo 2-kinase/fructose-2,6-bisphosphatase 3 (PFK2).

Of the four isoforms of PFK2 (PFK2.1–4), PFK2.3 shows the highest kinase/phosphatase activity ratio, which is to say, this is the isoform for which highest levels of $F2,6P_2$ are obtained [117,118]. As compared to astrocytes, neurons express low levels of the enzyme PFK2.3 [119]. Functional PFK2.3 in astrocytes is essential for the observance of increased PFK1 activity induced by exposure to $^{\bullet}NO$ [116]. The activation of glycolysis in astrocytes promoted by $^{\bullet}NO$ is cGMP-independent, but requires inhibition of mitochondrial oxidative phosphorylation [116]. Inhibition of oxidative phosphorylation by $^{\bullet}NO$ decreases ATP levels in the astrocytes by 25% [109] and can increase the AMP: ATP ratio up to five-fold [120], leading to the activation of AMP-activated kinase (AMPK), thus increasing PFK2 activity and up-regulating glycolysis is astrocytes. Lack of PFK2.3 in neurons renders such a mechanism inaccessible.

Although most of these studies were conducted in cell cultures, they all clearly point towards a strong impact of $^{\bullet}NO$ on neural energetic metabolism. But the role of $^{\bullet}NO$ in regulating neuroenergetic coupling may not end here. Nitric oxide can up-regulate glucose transport into astrocytes and neurons. In HEK-293T cells $^{\bullet}NO$ enhances translocation of glucose transporters GLUT1 and GLUT3 to the cell membrane by a mechanism that also requires phosphorylation by AMPK [120]. Furthermore, it was also observed that $^{\bullet}NO$ mediates the increase in glucose uptake in astrocytes [121,122] and even in skeletal muscle cells [122].

But how is it that neurons have a low glycolytic rate? Lactate entering the neuron is converted to pyruvate, shifting the redox balance towards a more reduced state (NAD^+/NADH decreases), inhibiting the

glycolytic pathway at glyceraldehyde-3-phosphate dehydrogenase (GAPDH) due to NAD^+ limitation [123,124]. This redox switch allows neurons to dedicate glucose usage for biosynthesis purposes and the pentose phosphate pathway while using astrocyte-derived lactate converted into pyruvate to fuel the TCA cycle [125]. Interesting enough, the mechanism of inhibition of GAPDH can be critically determined by the redox environment of the cellular milieu: while $^{\bullet}NO$ reversibly inhibits enzyme activity via an oxidative pathway (with enzyme activity recovered by GSH); *S*-nitrosothiols such as CysNO irreversibly inhibit GAPDH activity via *S*-nitrosation followed by NADPH attachment [126]. Irreversible inhibition of GAPDH can also result from nitration mediated by peroxynitrite [127], a potent oxidant resulting from the diffusion-limited reaction between $^{\bullet}NO$ and $O_2^{\bullet-}$ [128]. *S*-nitrosothiols have been recognized as important signaling molecules and many of the cGMP-independent $^{\bullet}NO$ signaling pathways have been attributed to S-nitrosation [129]. However, it is also important to refer that both *S*-nitroso compounds and peroxynitrite may result from the reaction of $^{\bullet}NO$ under situations of altered redox balance of the cellular milieu [130] and their reactivity has been associated with pathophysiological processes rather than physiological regulatory pathways [131,132]. A summarized representation of the above-described $^{\bullet}NO$-mediated regulation of neurometabolic coupling is presented in Fig. 2.

In investigating the putative role of $^{\bullet}NO$ signaling in regulating metabolism an initial report comparing 52 brain regions of eNOS and nNOS knockout mice with those of wild-type mice found no relevant differences in the cerebral metabolic rate for glucose [133].

One major limitation to gaining fuller insight to the mechanisms of neurometabolic axis has been the lack of in vivo studies. Understanding the flow direction of metabolites such as lactate, glucose, $^{\bullet}NO$ and O_2 in a complex system is undoubtedly challenging, more so in considering that only a multi-modal approach can address such complex issues – measuring one single parameter is insufficient. Understanding the coupling of the neurometabolic axis requires the ability to simultaneously measure fluxes in metabolites (glucose, O_2, lactate), neurotransmitters (glutamate), neuromodulators ($^{\bullet}NO$) and neuronal activity levels with high spatial and temporal resolutions.

3.4. Other hypothesis

Although the focus of the present review is the putative role $^{\bullet}NO$ might play in regulating neurometabolic coupling, it would be unwise to not mention the concurring hypothesis that can be found in the literature and that are not without merit.

The previous section highlights the hypothesis presented initially by Pellerin and Magistretti in 1994 the astrocyte-neuron lactate shuttle (ANLS), according to which glycolytic breakdown of glucose to pyruvate supports the energetic requirements of astrocytes during neuronal activity. This net production of 2 ATP per glucose is sufficient to account for the ATP usage associated with glutamate neurotransmission at the astrocyte: 1 ATP used by the Na^+/K^+/ATPase to extrude Na^+ entering the Na^+/glutamate co-transporter during uptake to the

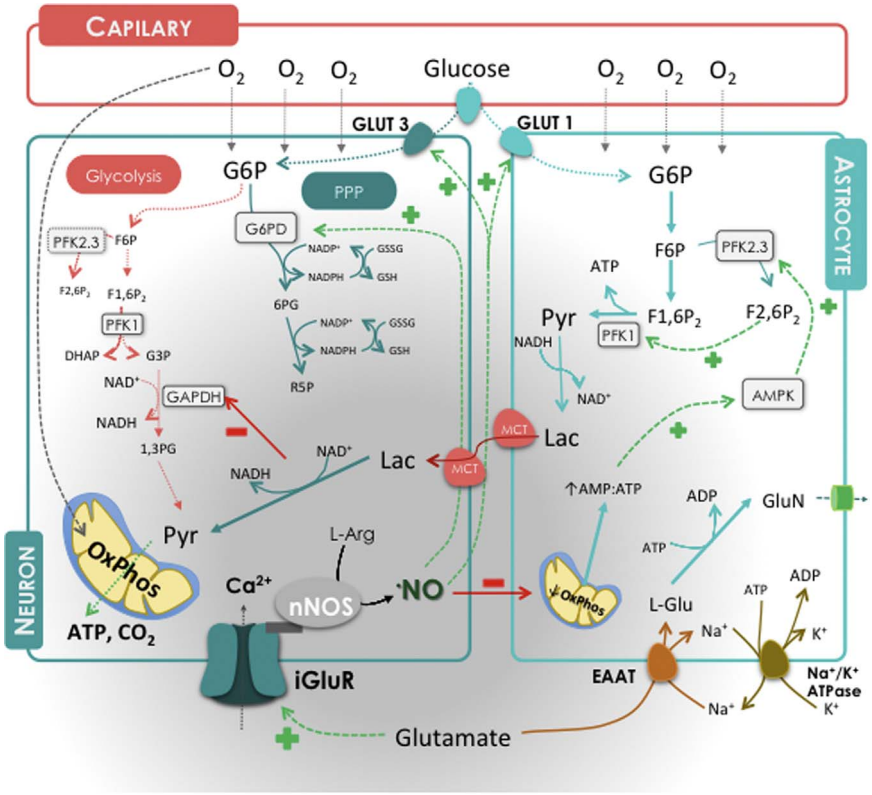

Fig. 2. Summarized representation of the signaling pathways by which $^{\bullet}NO$ may regulate neurometabolic coupling. Nitric oxide produced upon activation of ionotropic glutamate receptors (iGluR) in neurons can diffuse and inhibit mitochondrial respiration in astrocytes by competing with O_2 for binding to CcO. The increase in AMP: ATP leads to activation of AMP-dependent kinase (AMPK), thus upregulating the activity of phosphofrutokinase 2.3 (PFK2.3) and increasing the levels of fructose-2,6-bis-phosphate ($F2,6P_2$), an allosteric activator of PFK1. This concerted pathway leads to upregulation of glycolysis in astrocytes to meet the increased energy demand imposed by active glutamate uptake by excitatory amino acid transporters (EAATs) and glutamate recycling (conversion to glutamine to be shuttle to neurons), as well as reposition of ionic gradients by the Na^+/K^+/ATPase. Pyruvate (Pyr) resulting from glycolysis is converted to lactate (Lac) and shuttled to neurons via monocarboxylate transporters (MCT). Once in the neurons, lactate is converted to pyruvate at the expense of NADH oxidation. The increase in NAD^+/NADH leads to downregulation of neuronal glycolysis by decreasing the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Pyruvate enters the TCA cycle and powers neuronal oxidative phosphorylation (OxPhos). Lack of PFK2.3 activity in neurons hinders $F2,6P_2$ and PFK1 upregulation. As opposed to glycolytic breakdown, glucose is diverted to the pentose phosphate pathway (PPP) to support both biosynthesis and maintenance of an adequate pool of reduced glutathione (GSH). Nitric oxide can also increase glucose uptake in both cell types by increasing glucose transporters (GLUT) density at the cell membrane. 6PG 6-Phosphogluconolactone; R5P – Ribulose-5-Phosphate. The stoichiometry of the reactions was omitted for the sake of graphical clarity.

astrocyte and a second ATP used to convert glutamate to glutamine (reviewed in [134]).

Cerdán and collaborators proposed an alternative role for lactate shuttling from astrocytes to neurons – rather than serving the purpose of substrate shuttling, it rather serves the purpose of shuttling redox equivalents. Once in the neuron, lactate is converted back to pyruvate with a net production of 1 NADH *per* lactate. Pyruvate is then shuttled back to astrocytes and reconverted to lactate at the expense of 1 NADH *per* pyruvate. The purpose of this redox shuttle is to up-regulate glycolysis in astrocytes while supplying NADH for neuronal oxidative phosphorylation [125].

Based on cellular concentrations of glucose and lactate as well as the kinetic properties of their respective transporters, an alternative transport-metabolism model – the neuron-to-astrocyte lactate shuttle – has also been proposed. Accordingly, increased brain activity produces a disproportional increase in both CBF and cerebral metabolic rate for glucose which is not met by an equivalent increase in the cerebral metabolic rate for O_2, resulting in increased lactate concentration in neurons which diffuses to the extracellular space to be uptaken by astrocytes. Astrocytic lactate is discharged to circulation, signaling for cerebral blood regulation (reviewed in [99]).

4. Impairment of neurovascular and neuroenergetic coupling under pathological conditions

From the notions discussed above it is not surprising that the failure of tight regulation of blood supply and energy utilization as a function of neuronal activity leads to neuronal dysfunction, impaired cognition and, ultimately, to neurodegeneration [1,135,136]. Indeed, several lines of evidence support that both the regulation of CBF and metabolism are perturbed during non-pathological brain aging and under neuropathological conditions that have been associated with deregulation of $^{\bullet}$NO-signaling pathways, as discussed in the next sections.

4.1. Brain aging

The “normal”, “physiological” or “non-pathological” aging of the brain relates to the deterioration of brain function that occurs progressively with advancing age dissociated from other age-related pathological conditions (e.g. neurodegenerative disorders, cardiovascular diseases). It is a multifactorial process conventionally characterized by stereotypical structural alterations (eg. atrophy of the hippocampus and amygdala, white matter abnormalities) and often associated to some frailty in cognitive functions [137]. It should be stressed that the aged brain is highly prone to neurodegenerative disorders, with which it shares several features. As such, the boundary between “physiological” and “pathological” aging is not categorical.

The derailment in the axis of neurovascular and neuroenergetic coupling is accepted as an invariant pathophysiological feature extensively associated with the decline of cognitive function in aging. Data collected from several clinical studies demonstrate a negative correlation between resting CBF and the age of healthy subjects [138–141]. In addition, the change in CBF coupled to neuronal activation is reported to be impaired during non-pathological aging [141–143]. Brain aging is also intimately associated to significant alterations in energy metabolism, both related to glucose transport and energy-transducing capacity (reviewed in [144]). In line with the putative involvement of $^{\bullet}$NO in aging, there is evidence of changes in NOS activity and protein expression with advancing age. While a major relevance is attributed to iNOS [145], several lines of evidence also support the alterations in the constitutive nNOS [146–148].

4.2. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive and irreversible cognitive decline linked to neuronal loss in brain areas associated to memory processing, namely the hippocampus and cerebral cortex. Classically, research has identified neuropathological features of the disease in the form of accumulated damaged proteins, namely amyloid β peptide (Aβ) and neurofibrilary tangles, but early dysfunctional pathways have remained elusive [149]. Data collected from several studies demonstrate impairment in the hemodynamic response coupled to neuronal activation in several experimental models of AD [150–152] and AD patients [153–155]. Coherently, by simultaneously measuring $^{\bullet}$NO and CBF in the hippocampus of a rodent model of AD, we recently observed that the increase in hemodynamic response elicited by glutamatergic activation was diminished both in aging and AD, in spite of $^{\bullet}$NO production remaining functional [43].

During the past decades, intensive research has shown that brain energy metabolism is impaired in AD. In particular, a consistent reduction of cerebral glucose consumption (hypometabolism) has been observed in the brains of AD patients using positron emission tomography [156–159]. Consistent with the decline in brain energy metabolism, using high resolution respirometry to evaluate oxidative phosphorylation in hippocampal slices, we have also observed a decline in all respiratory parameters in both aged wild-type and 3xTg-AD mice. Most relevant is the observation of a decrease in mitochondrial sparing capacity – the ability of mitochondria to up-regulate oxidative phosphorylation turnover to adjust for higher energetic demand [160]. Others have shown that, brain mitochondria isolated from 3xTg-AD show decreased respiratory efficiency as well as decreased expression and activity of pyruvate dehydrogenase and CcO accompanied by an increase in glycolytic activity determined in primary neuronal cultures from 3xTgAD [161].

Several lines of evidence suggest that all NOS isoforms operate as mediators in AD, but the picture for the role of $^{\bullet}$NO is still to be defined [162]. It is likely that the changes in $^{\bullet}$NO signaling occurs differently dependent on the state of disease progression, brain region and pathways involved. A recent report suggests that $^{\bullet}$NO signaling in an early stage of AD is recruited and upregulated as a compensatory mechanism to boost synaptic transmission and plasticity [163]. Furthermore, along age progression, a different profile in the alteration $^{\bullet}$NO signaling was observed in animal models of AD depending on the subregion of the hippocampus evaluated (CA1 versus dentate gyrus) [43,160].

4.3. Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the elderly population disease. It is clinically characterized by parkinsonism (resting tremor, bradykinesia, rigidity, and postural instability) and histopathological changes include progressive loss of neurons in the *substantia nigra pars compacta* accompanied by the presence of Lewy bodies and Lewy neuritis (for review see [164]).

The selective sensitivity of dopaminergic neurons led researchers to focus on the dopamine molecule itself, however, $^{\bullet}$NO is reported to play a major role in the pathogenesis of PD. Evidence of altered $^{\bullet}$NO bioactivity is afforded by the observation of increased nitration and oxidation products in *post mortem* brains of patients with PD [165], namely nitration of α-synuclein [166]; detection of *S*-nitrosation of key proteins in the context of PD such as parkin [167], PTEN-induced putative kinase 1 (PINK1) [168]; or that the inhibition of both nNOS and iNOS activities produces resistance to MPTP induced PD-like phenotype in non-human primates [169].

The relevance of mitochondrial dysfunction in PD became evident as a result of efforts to produce animal models of the disease, as both MPTP and rotenone, two inhibitors of complex I of the mitochondrial chain, are used to induce PD-like phenotype in rodents and non-human primates, namely Lewy body-type intracellular inclusions and degeneration of nigrostriatal dopaminergic neurons [170]. Dopamine meta-

bolism (with production of reactive derivatives such as free radicals, quinones and 6-hydroxydopamine), and changes in the profile of •NO signaling may cross paths and the chemical interaction between •NO and dopamine as well as that of their derivatives produce a myriad of neurotoxic species [171] that can negatively impact mitochondrial function. For instance, DOPAC (3,4-dihydroxyphenylacetic acid), formed via the activities of monoamine oxidase and aldehyde, both located at the mitochondria [172,173], reacts with •NO to produced potentially harmful compounds, such as the nitroxyl anion, *o*-semiquinone radicals and *o*-quinones [174]. In line with this, we have shown that an •NO-derived product of the DOPAC-NO reaction (most likely the nitroxyl radical) inhibits mitochondrial respiration at the level of CcO [175]. In PC12 cell, co-treatment with DOPAC and •NO also induces mitochondrial dependent apoptosis through a pathway that is independent of caspase activation [176], which has also been described to occur in PD models [177,178].

Also, a recent work has shown that maintaining "optimal" concentrations of •NO is important in the maintenance of mitophagy, the regulated authophagy of dysfunctional mitochondria [179]. Removal of damaged mitochondria appears to be controlled by PINK1 and parkin [180] and parkin recruitment to mitochondrial, a key step in mitophagy, depends on PINK1 interaction with nNOS and maintenance of a low or "optimal" concentration of NO.

As in the case of AD, neuronal degeneration and bioenergetic derailment in PD are accompanied by cerebrovascular dysfunction and alteration in cerebral metabolism. Decreases in CBF values are observed in basal ganglia, hippocampus, prefrontal cortex and parietal white matter PD patients when compares to healthy subjects and correlate negatively with motor and cognitive dysfunction and depression (depending on the region) [181]. While some authors have shown evidence of bipolar pattern of abnormal regional metabolism with bilateral hypermetabolism in sub-cortical regions (putamen, thalamus, cerebellum, pons and sensorimotor cortex) and hypometabolism in cortical regions [182], others have reported global decrease of CMRglu in PD patients [183], arguing that cortical hypermetabolism may be an artifact introduced by global mean normalization of the data. Contrary to AD, there appears to be no dysfunction of neurovascular coupling NVC in PD [184].

4.4. Epilepsy

Epilepsy is the most prevalent non-communicable neurological disorder in humans, affecting as many as 70 million people worldwide [185]. Epilepsy is defined as a disease in which either recurrent unprovoked seizures, a heightened tendency for unprovoked recurring seizures are observed or when an epilepsy syndrome is diagnosed [186]. Seizures, which are paroxysmal and caused by excessive neuronal activity, are accompanied by profound neurometabolic and neurovascular alterations. The significant increase in neuronal firing produces transient hypoxia due to increased energetic demand, followed by prolonged increase in CBF and hyperemia. As seizures progress, hyperemia declines while metabolic rate remains high, leading to depletion of cerebral energy sources and evolution on intracellular acidosis. Furthermore, seizures can cause disruption of the blood-brain barrier, acute loss of cerebral auto-regulation and delayed impairment in neurovascular coupling [187].

Not far after the report that •NO was produced upon NMDAr mediated local vasodilatation [23] the same group showed that in pentylenetetrazole-induced seizures in rats, prolonged dilation of cerebral arterioles was also mediated by •NO [188]. Using a similar strategy (unspecific NOS inhibition with L-arginine analogous), the same was confirmed in other rodent models of epilepsy/seizure induced by kainate [189] and bicuculline [190]. The implication of neuronal derived •NO in the CBF response during seizures was later confirmed in kainate treated rats in which the selective nNOS inhibitor 7-NI blocked the hyperemic response in freely moving animals [191].

Not surprisingly, the role and origin of •NO in regulating local CBF response during seizure activity has, in more recent years, been revealed to be more complex, as studies using $nNOS^{-/-}$ mice suggest a role of neuronal •NO only in the cerebrovascular response it the surround but not the focus of seizures [192], substantiating an important role for endothelial derived •NO [193].

In line with what was discussed above in Section 3.2, one may conceive that during seizure activity, the increased energetic demands imposed by increased glutamate released and neuronal hyperexcitability may be met by a combination of increased astrocytic glycolysis and extrusion of lactate for neuronal uptake to fuel oxidative phosphorylation. In line with this, epilepsy is accompanied by changes in the expression pattern of monocarboxylate transporters in astrocytes, facilitating the exchange of lactate within the brain parenchyma as opposed to extrusion to circulation [194]. Extracellular lactate concentration is reported to increase as much as 90% during seizures in humans [195] and around 400% in pilocarpine-treated rats [196].

4.5. Traumatic brain injury

Traumatic brain injury (TBI) is a pathological condition generally arising from an external force hitting the brain (e.g. during a fall, penetration of a projectile) with consequent alterations of neural function. The severest cases are fatal or result in long-term disability for the surviving individuals. There is growing evidence that spreading depolarization (SD), slow propagating waves of depolarization of neural cells reported to occur in 50–70% of TBI patents, critically contributes to the pathogenesis of secondary neuronal injury in TBI, exacerbating the mismatch between blood supply and metabolic demand and thereby worsening clinical outcome [197,198]. Coherently, a prevalent impaired of neurovascular coupling has been demonstrated in SD events monitored in surgical TBI patients, characterized by a transient hypoperfusion instead of hyperemia, [199]. Furthermore, TBI is accompanied by relevant metabolic disturbances, with the magnitude and profile of these changes being correlated with worse behavioral and cognitive outcomes [200]. It was recently suggested that the deregulation of glucose metabolism results not only from the impairment in the neurovascular and neurometabolic coupling, but also from enzymatic and genetic modifications of metabolic pathways, particularly in severe TBI [201].

A major relevance has been attributed to •NO in TBI based on consistent observations regarding changes in NOS activity and/or expression in the different stages of TBI [202]. For instance, in rodents, the activity of constitutive NOS is increased as early as 5 min after injury and decreased in the following days [203]. It was suggested that decreased basal levels of •NO after TBI are associated to a lower threshold for SD deleterious events [204]. Furthermore, evidence also supports the upregulation of iNOS for several days after TBI [202]. It is argued that •NO is implicated in the abnormalities in CBF occurring after TBI [205] and increase in iNOS expression, has been reported in days following injury [206].

4.6. Oxidative stress as a common denominator: a shift in nitric oxide bioactivity

Where does this all come together? As discussed above, aging, neurodegenerative disorders, epilepsy and TBI have in common several neuropathological features, such as deregulation of CBF, altered metabolism and dysfunctional •NO-signaling pathways. Furthermore, there is evidence supporting the interconnection of these conditions. Advanced age is major risk factor for neurodegenerative disorders, such are AD and PD as well as epilepsy. Also, TBI is associated to the genesis of some forms of epilepsy [207] and is also considered a risk factor for age-related neurodegenerative disorders [208]. But what is the common denominator for these causalities?

Converging evidence highlights changes in the redox balance of the

cellular milieu towards an oxidative stress as a molecular basis for neurodegeneration in brain aging and neuropathological conditions (reviewed in [209,210]) In this context, a particular role has been attributed to its impact in neurovascular and neuroenergetic dysfunction. Amongst the multiple potential sources of reactive oxygen and nitrogen species (RONS) in the brain, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family and mitochondria have been pinpointed as the most likely culprits [211–213]. The activation of NADPH oxidase, which results in the production of large amounts of superoxide radical ($O_2^{-\bullet}$), is reported to occur in non-pathologic aging and more so in the context of AD [214,215], as well as in consequence of cerebral hypoperfusion [216] and as a result of seizure activity [217,218]. In turn, genetic ablation of NOX2, one isoform of the catalytic subunit of NADPH oxidase, is able to counteract the oxidative stress and neurotoxicity in PD [219] and TBI,[220] and also the cerebrovascular dysfunction in aging [215] and AD [221]. Mitochondria are another important source of $O_2^{-\bullet}$, generated as by-product of the oxidative phosphorylation system. While mitochondria have an efficient antioxidant defense system to cope with the $O_2^{-\bullet}$ - the mitochondrial isoform of superoxide dismutase (MnSOD) - there is evidence supporting a dysregulation of this system, among others in AD [222,223], epilepsy [224] and TBI [225].

One of the mechanism by which $O_2^{-\bullet}$ negatively impact many physiological conditions, including neurovascular and neuroenergetic regulation, relates with its diffusion-controlled reaction of $^{\bullet}NO$ [226]. This reaction not only yields a strong nitrating and oxidant species, peroxynitrite ($ONOO^-$) but also inevitably decreases the bioavailability of $^{\bullet}NO$, thus hampering $^{\bullet}NO$-mediated pathways, such is vasodilation [227]. Accordingly, SOD mimetics have shown to abrogate the attenuation of whisker stimulation CBF response observed in aged [215] and AD mice [221]. Other molecules endowed with reducing/antioxidant ability have also demonstrated potential to rescue dysfunctional neurovascular coupling in animal models of aging [228] and AD [229]. In addition to decreasing $^{\bullet}NO$ bioavailability, the reaction with $O_2^{-\bullet}$ leading to the formation of $ONOO^-$, can modify the structure and alter the biological activity of lipids, proteins and nucleic acids via nitration and oxidation reactions. One of the potential toxic pathways for $ONOO^-$ (in spite of the very low yields) involves the nitration of proteins, mainly at tyrosine residues, yielding 3-nitrotyrosine [226,230]. This modification is associated to altered function of several proteins likely due to the ability of tyrosine to be phosphorylated at the level of the 4′ hydroxyl group [231]. In this regard, a large body of research supports marked increase in 3-nitrotyrosine in the brain associated with aging [232,233], AD [234], PD [235], epilepsy [236,237] and TBI [238,239]. Common entries in proteomic lists of nitrated proteins observed in some of these pathological conditions include proteins that play crucial rules in energy production, including relevant glycolytic enzymes, such are GAPDH, phosphoglycerate mutase (PGM) and α-enolase (ENO-1), the mitochondrial enzyme ATP synthase and voltage-dependent anion channel (VDAC) located in the outer mitochondrial membrane [240,241]. It is expected that the modification of these proteins, usually associated to a decrease in their enzymatic activity, contributes to the alterations in brain energetic regulation, and consequently to neurodegeneration.

5. Conclusion

Several lines of evidence support the role of $^{\bullet}NO$ as a master regulator of the complex interdependent mechanisms that guarantee the fine-tuning between neuronal activity, energetic demand and vascular response. The localized burst of $^{\bullet}NO$ produced by nNOS upon neuronal glutamatergic stimulation can diffuse a few hundreds of microns reaching nearby blood vessels and, via increase of CBF, augmenting the local bioavailability of energetic substrates (O_2 and glucose). Neuronal derived $^{\bullet}NO$ can, concurrently, regulate local O_2 utilization by CcO in the mitochondrial respiratory chain, as well as modulate glucose utilization by up-regulating glycolysis in astrocytes. This later mechanism allows increased production of lactate to be shuttled to neurons to fuel oxidative phosphorylation and ATP production. In view of the central relevance of the neurovascular-neuroenergetic coupling axis, an increasing amount of evidence suggests that altered cerebral perfusion and energy metabolism may play primordial roles in several neuropathological conditions. These changes are reported to occur along with modifications of $^{\bullet}NO$-signaling pathways. Altered redox (micro)environment is pin-pointed as a major culprit decreasing $^{\bullet}NO$ bioavailability and subverting $^{\bullet}NO$ bioactivity in the regulation of neurovascular and neuroenergetic coupling towards pathological pathways, such is nitroxidative post-translational modification of proteins.

In sum, *the neurovascular-neuroenergetic axis –under the coordinated regulation by neuronal nitric oxide– is critical for brain homeostasis and impairment of this coupling axis leads to cellular and CBF dysfunction inherent in brain aging and neuropathological disorders.*

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