Antioxidants and Redox Signaling

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# Title: Oxygen sensing in neurodegenerative diseases: current mechanisms, implication of transcriptional response and pharmacological modulation

Running title: Oxygen sensing and neurodegenerative diseases

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**ABSTRACT**

***Significance:*** *Oxygen sensing is the fundamental process through which organisms respond to changes in oxygen levels. Complex networks exist allowing the maintenance of oxygen levels through the perception, capture, binding, transport and delivery of molecular oxygen. The brain extreme sensitivity to oxygen balance makes the dysregulation of related processes crucial players in the pathogenesis of neurodegenerative diseases. Here we wish to review the most relevant advances in oxygen sensing in relation to Alzheimer's Disease, Parkinson's Disease and Amyotrophic Lateral Sclerosis.*

***Recent Advances:*** *Over the years, it has been clarified that most neurodegenerative diseases share common pathways, a great number of which are in relation to oxygen imbalance. These include hypoxia, hyperoxia, ROS production, metabolism of metals, protein misfolding and neuroinflammation.*

***Critical Issues:*** *There is still a gap in knowledge concerning how oxygen sensing plays a role in the above indicated neurodegenerations. Specifically, oxygen concentrations are perceived in body sites which are not limited to the brain, but primarily reside in other organs. Moreover, the mechanisms of oxygen sensing, gene expression and signal transduction seem to correlate with neurodegeneration but many aspects are mechanistically still unexplained.*

***Future Directions:*** *Future studies should focus on the precise characterization of oxygen levels disruption and oxygen sensing mechanisms in neurodegenerative diseases. Moreover, advances need to be made also concerning the techniques used to assess oxygen sensing dysfunctions in these diseases. There is also the need to develop innovative therapies targeting this precise mechanism rather than its secondary effects, as early intervention is necessary.*

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## 1. Introduction

The capacity to perceive and respond to changes in oxygen ($O_2$) levels is fundamental for the survival of both prokaryotic and eukaryotic organisms. As the name suggests, $O_2$ sensing is defined as the ability to "sense" and, consequently, "respond" to changes in $O_2$ levels. In an organism, this is a key mechanism necessary to maintain cellular and tissue homeostasis (Giaccia et al., 2004). Changes in cellular $O_2$ availability, secondary to environmental challenges or diseases, stimulate a vastitude of adaptive responses that can be rapid (seconds) or more prolonged (weeks to months) (Wilson et al., 2020). The molecules and mechanisms involved in these versatile $O_2$ sensing signaling pathways are fundamental to the pathogenesis of highly prevalent medical conditions, among which are respiratory depression, hypertension, tumor progression, neurodegeneration and inflammation (Liao and Zhang, 2020; Semenza, 2014; Sieck, 2004).

In 1931, Otto Warburg was awarded the Nobel Prize in Medicine for identifying cytochrome aa3 (cytochrome oxidase) as the carbon monoxide (CO)-sensitive respiratory enzyme (Otto, 2016). Since then, many discoveries have been made elucidating the mechanisms through which organisms can perceive and adapt to $O_2$ levels but many questions on this topic remain predominantly unanswered (Liao and Zhang, 2020). What is currently known is that complex networks exist which allow to maintain $O_2$ homeostasis at tissue level, through the capture, binding, transport and delivery of molecular $O_2$ (Giaccia et al., 2004). Specifically, the alterations in $O_2$ levels can be perceived by "$O_2$ sensing organs" with a specific localization in the body and presenting molecular entities, with specific electrophysiological properties that enable $O_2$-dependent modulation of cell excitability and intracellular transduction mechanisms. These can then lead to a specific regulation of gene expression and cellular adaptations to $O_2$ imbalance (Wilson et al., 2020). In this review, we are presenting a brief overview of mechanisms pertaining $O_2$ sensing both in the periphery and in the central nervous system (CNS). We will also discuss $O_2$ sensing intracellular mechanisms in both a physiological state and in neurodegenerative diseases (NDs). Moreover, in a specific section we aimed to assess what molecular signatures have been identified to be associated to '$O_2$ sensing' and NDs and current advances/limitations in techniques and therapeutic strategies for investigating $O_2$

imbalance in NDs. These are a heterogeneous class of disorders, typically characterized by the progressive degeneration of the structure and function of the CNS or peripheral nervous system. The investigation of $O_2$ sensing mechanisms appears to be relevant in numerous NDs, and in this review we focus on three amongst the most studied diseases, namely Parkinson's Disease (PD), Alzheimer's Disease (AD) and Amyotrophic Lateral Sclerosis (ALS).

## 2. Overview of $O_2$ sensing mechanisms: whole-body response to changes in $O_2$ levels

All living organisms can perceive changes in the partial pressure of $O_2$ ($pO_2$) and are thus able to trigger a compensatory response and avoid systemic damage. Specifically, the human body is a highly aerobic organism, with the necessity to match oxygen supply at tissue level to the metabolic demand. In order to ensure this, there are numerous organs defined as "O2 sensing", which can detect changes in $O_2$ and thus elicit specific responses. These will be reviewed in detail in the next section and include the carotid bodies (CB), the PreBötzinger Complex (preBötC) in the CNS, the pulmonary and cardiovascular system and the kidneys (Fig. 1).

### 2.1 Acute neurological $O_2$ sensing: role of the carotid bodies

The primary $O_2$ sensing mechanisms present in the human body relies on the detection of changes in $pO_2$. Small arterial changes of $pO_2$ are primarily detected in the CB, an organ made of glomus cells, the main $O_2$ sensing cells, and supporting cells, both surrounded by a network of thin vessels (López-Barneo et al., 2016). Glomus cells are electrically excitable and present $O_2$-sensitive potassium (K+) channels in their membranes (Pardal and López-Barneo, 2002). The two CBs are situated bilaterally at the bifurcation of the common carotid artery. This anatomical structure favors the detection of changes in the arterial blood composition before the stimulus reaches the brain, which is highly dependent on $O_2$ and glucose (Teppema and Dahan, 2010). The blood supply to the CB thus originates mostly from the carotid artery, which supplies the highest blood flow per tissue weight in the whole body. A low $pO_2$, also known as hypoxia, leads to the inhibition of $K^+$ channels in the plasma membrane of glomus cells, with the activation of cardiorespiratory reflexes through calcium ($Ca^{2+}$) entry, depolarization and neurotransmitters release (López-Barneo et al., 1988; Pardal and López-Barneo, 2002). In turn, the CB activates the respiratory

centre in the brainstem to induce adaptive ventilatory responses. Intrastriatal grafting of the CB was performed in parkinsonian rats, an *in vivo* model obtained treating the animals with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Toledo-Aral et al., 2003; Villadiego et al., 2005). The rationale behind CB grafting relies on the fact that CB glomus cells are highly dopaminergic and express the glial cell line derived neurotrophic factor (GDNF) (Mínguez-Castellanos et al., 2007; Toledo-Aral et al., 2003; Villadiego et al., 2005). In CB transplanted parkinsonian rats, GDNF was still produced with an increased glomus cells survival rate after transplantation and a neurotrophic recovery of the treated animals. This kind of approach was used in other murine and primate models of PD obtaining promising results (Espejo et al., 1998; Hao et al., 2002; Luquin et al., 1999; Shukla et al., 2004). With this preclinical evidence, a phase I-II clinical study was performed to assess feasibility, long term safety, clinical and neurochemical effects of CB auto transplantation in PD patients (Mínguez-Castellanos et al., 2007). Blind tests highlighted a clinical amelioration in PD outcomes in 10 out of 12 patients, with a mean improvement of 23% after 6 months (Mínguez-Castellanos et al., 2007). Interestingly, the β-amyloid precursor protein cleaving enzyme 1 (BACE1) (Vassar et al., 1999), was recently found expressed in the rat CB, with a reversible reduced expression following cyclic intermittent hypoxia (Li et al., 2020a). BACE1 is able to generate the β-amyloid (Aβ) peptide, a crucial initiator of AD pathogenesis.

This evidence suggests that CB may play a role in NDs, but the exact molecular dysregulation of this organ in the diseases is yet to be defined. These aspects are an interesting and understudied research topic and still need to be eviscerated with further studies.

### 2.2. $O_2$ sensing in the brain

The brain is extremely sensitive to $O_2$ balance, as it is entirely aerobic (Bailey, 2019). Indeed, in humans 20-25% of resting metabolic rate, meaning the energy needed when at rest, is reserved for brain functioning (Bailey, 2019). This is necessary in order to support the high rate of ATP formation and consumption, which allows the maintenance of ionic equilibria and neurotransmitters uptake, both necessary processes for synaptic transmission (Bailey, 2019). Even so, the brain has limited $O_2$ reserves, and if blood supply

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were to be interrupted it would be able to sustain cerebral metabolism for 1 second only, subsequently resulting in neurodegeneration (Bailey, 2019; Leithner and Royl, 2014). Indeed, neurons require a constant supply of $O_2$ along with a removal of carbon dioxide ($CO_2$) and other metabolites (Gourine and Funk, 2017). For this reason, the oxygenation of the brain is strictly monitored by the CBs. Even so, there are more aspects that need to be considered when thinking about the strict connection between the CNS and its associated NDs and $O_2$ intake and metabolism. Indeed, the inspiratory rhythm of breathing is generated at the level of a medullary structure in the brainstem called the preBötC (SheikhBahaei, 2020; Smith et al., 1991). The activity of this region is strictly regulated by inputs from other brain regions which include functional inputs such as volitional, physiological and emotional inputs, along with direct projections from neurons throughout the brains (Yang et al., 2020). Specifically, excitatory and inhibitory preBötC neurons receive projections from neurons in the breathing central pattern generator (bCPG), including the contralateral preBötC, the Bötzinger Complex, the nucleus of the solitary tract, the parafacial region, and the parabrachial nuclei (Yang et al., 2020). In neurodegenerative diseases affecting the brainstem such as multiple system atrophy (MSA), preBötC neurons were reduced suggesting that the central respiratory network primarily contributes to breathing disorders in MSA (Schwarzacher et al., 2011). Moreover, the 6-hydroxydopamine hydrochloride (6-OHDA) rodent model of PD presents with a reduced respiratory frequency and NK1r-immunoreactivity in the preBötC, indicating that this decrease is an important contributor to the development of breathing abnormalities in PD (Oliveira et al., 2021).

Along with the CBs, there is now mounting evidence highlighting the existence of central respiratory $O_2$ chemo-sensors (Uchiyama et al., 2020). Interestingly, astrocytes have been found to rapidly respond to moderate hypoxia via the sensor cation channel transient receptor potential (TRP) A1 (Uchiyama et al., 2020). These appear to specifically respond to a decrease of $pO_2$ as they do not respond to hyperoxia, carbon dioxide and oxidants molecules (Uchiyama et al., 2020). Other evidences also highlight how changes in neuronal-glial interactions can contribute to the hypoxic ventilatory response, the “coping” mechanism that the brain utilizes to respond to a decrease of $pO_2$ (Angelova et al., 2015; Rajani et al., 2018; Sheikhbahaei et al., 2018). Studies also highlight how astrocytes can

respond to decreases in $_PO_2$ with an elevation in intracellular $Ca^{2+}$, and interestingly this "sensor" is in the mitochondria, key organelle in $O_2$ metabolism (Angelova et al., 2015). Astrocytes-neurons interactions and mitochondria are relevant mechanisms in the pathogenesis of NDs (Mulica et al., 2021).

Specific brain areas affected in NDs can also play a role in oxygen sensing mechanisms. Indeed, the hippocampus, primarily implicated in AD, is extremely vulnerable to hypoxic insults, and an impaired HIF-$\alpha$ signaling in this area may contribute to age-associated cognitive decline (Snyder et al., 2022). Moreover, blood flow, blood oxygenation and neurovascular coupling were found to be decreased in the hippocampus compared to the neocortex, and features of the hippocampal vasculature may restrict oxygen availability thus explaining its sensitivity to damage in AD, where the brain's energy supply results also decreased (Shaw et al., 2021). PD loss of dopaminergic neurons in the substantia nigra (SN) is a primary hallmark of the disease, and this area is extremely vulnerable to oxidative stress (Trist et al., 2019). Interestingly, even though this is surely true, and hypoxia plays a critical role in the pathogenesis of the disease, a novel computational model of SN cells highlights how hypoglycemia plays an even more crucial role in leading to ATP deficits (Muddapu and Chakravarthy, 2021).

### 2.3 Other mechanisms of $O_2$ sensing: implications for peripheral organs in NDs

Several organs can lead to changes in peripheral and central $O_2$ levels, with direct consequences in NDs. Peripheral organs include the kidneys, the cardiovascular circuitry, and the pulmonary system, each presenting a specific response to changes in $O_2$ levels (Table 1). First of all, the kidney is sensitive to falls in the $pO_2$, and in a hypoxic condition it can trigger reflex adjustments acting as $O_2$ sensor, increasing perfusion pressure chronically (Patinha et al., 2017). A condition of hypoxia impairs hydrogen sulphide metabolism and increases its concentration, leading to vasodilation and stimulation of chemoreceptor afferent neurons, especially in the renal medulla (Bełtowski, 2010). The kidney metabolism is also relevant in NDs, as kidney injury was found to be a risk factor for the development of both PD (Lin et al., 2016) and AD (Zhang et al., 2020). Moreover, the Receptor for Advanced Glycation End products (RAGE), critical for chronic kidney disease progression, also mediates the transport of pathophysiologically relevant concentrations

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of Aβ into the CNS. RAGE has been found to be involved in both AD and hypertension, inducing plaque formation, Aβ deposition around blood vessels and cognitive impairment (Carnevale et al., 2012). The RAGE pathway is tightly connected to the renin/angiotensin/aldosterone axis which regulates systemic blood pressure, and it also has a role in oxidative stress (Gugliucci and Menini, 2014; Pickering et al., 2019).

Other peripheral organs relevant for $O_2$ sensing are the lungs, as it was also found that lack of $O_2$ induces selective pulmonary vasoconstriction in order to redirect blood flow to the most ventilated areas of the lung, promoting vascular angiogenesis and vasodilation in the brain (Wang et al., 2001). The genetic relationship between Chronic Obstructive Pulmonary diseases (COPD), lung function and AD was recently investigated without any specific evidence of association (Higbee et al., 2021). Acute respiratory distress syndrome (ARDS), a syndrome characterized by severe hypoxia requiring intensive hospitalization, may result in long term (at least 2 years) neurocognitive morbidity and decreased quality of life (Hopkins et al., 2005). Obstructive sleep apnea syndrome (OSAS), an example of pathological intermittent hypoxia, can also be associated with mild cognitive impairment. Proteomic data suggest that OSAS and AD share biomarkers which include insulin, angiopoietin-1, and IL1B, indicating also the possibility of a shared pathogenesis between these diseases (Lal et al., 2019). Ongoing studies are also investigating the relationship between COVID19 hypoxic condition and consequent neurological impairment, which, in the most severe cases, may resemble AD, or, as it has been suggested, predispose to AD future development (Almeria et al., 2020; Heneka et al., 2020).

Lastly, the cardiovascular response to hypoxia, like other stress situations is peripherally mediated through chromaffin cells in the adrenal medulla. CB chemoreflex via increased sympathetic activity regulates the ensuing transcriptional regulation of pro- and anti-oxidant enzymes contributing to oxidative stress in adrenal medulla (Kumar et al., 2015). Excessive afferent signalling from the CBs may lead to the development of pathological conditions such as hypertension (Patinha et al., 2017), a risk factor for NDs (Bergantin, 2019). Treatment-resistant hypertension has been shown to impact on blood brain barrier integrity, inducing changes in $O_2$ delivery and altered neural signaling homeostasis (Katsi et al., 2020).

In conclusion, many districts of the body are implicated in sensing changes in $O_2$ levels. These include the CBs, the brain, the pulmonary, cardiovascular and renal system, which can also work cooperatively to avoid the induction of disruptive mechanisms. Alterations in these districts present some correlations with NDs, but this aspect is currently understudied and would need further investigation.

## 3. Cellular response to imbalance in $O_2$ levels and its correlation with NDs

We so far presented the mechanisms of $O_2$ sensing at the "whole-body" level, attempting to eviscerate how our organism can respond to changes in $O_2$ levels and how this is connected to NDs. It is now worth focusing on the intracellular responses to $O_2$ as a signaling molecule, in normal physiological conditions and in conditions of reduced (hypoxia) $O_2$ levels (Fig. 2).

### 3.1 Membrane-associated mechanisms of $O_2$ sensing

The first question that needs to be answered is how $O_2$ can enter the cell, and as we previously mentioned the cell membrane of $O_2$-sensing cells contains $O_2$-sensitive ion channels, specifically K+ ion channels (López-Barneo et al., 1988). Following the first reported evidence, at least three families of $O_2$-sensitive K+ channels have now been identified (Prabhakar and Peers, 2014; Vjotosh, 2020). Specifically, these channels are able to rapidly respond to a reduction in $O_2$ concentration. Although, to our knowledge, there is currently no direct association between these specific channels and NDs, many evidences connect K+ ion channels to $O_2$ levels imbalance and to the pathogenesis of these diseases. For example, the K(+) channels encoded by the Kv1.3 subtype of the voltage-dependent Kv1 gene family, inactivated following an hypoxic signaling (Conforti et al., 2003) (Fig. 2), have been found highly expressed by activated and plaque-associated microglia in AD post-mortem brains (Rangaraju et al., 2015). The selective inhibition of this channel through selective blockades with the small molecule PAP-1 leads to a reduced neuroinflammation, decreased cerebral amyloid load, enhanced hippocampal neuronal plasticity and improved behavioral deficits in murine models of the disease (Maezawa et al., 2018). Along with Kv1.3, the inhibition of the cation channel TRPV1 with 5-iodo-resiniferatoxin (I-RTX) leads to a reduction in microglial reactive oxygen species (ROS) production following acute stimulation of microglial cells with fibrillar or soluble amyloid

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fragments (Schilling and Eder, 2011). Modulation of TRPV1 with the agonist capsaicin in an experimental model of PD also leads to a positive effect on the survival of dopaminergic neurons in the SN (Park et al., 2012). Furthermore, K+ channels can be modified by oxidizing agents, and recent evidence has shown that they undergo an age-dependent oxidation, impairing neuronal functions (Cai and Sesti, 2009; Sesti, 2016).

Even if the importance of K+ channels in $O_2$ sensing has been clarified, there is still a lot that needs to be discovered about this mechanism (Vjotosh, 2020) and how it is related to NDs pathogenesis.

### 3.2. Cytoplasmic $O_2$ sensors: The role of HIF-1

Changes in $O_2$ levels typically converge to the activation of a specific transcriptional response aimed at counterbalancing this dysregulation. It is well known that the central elements of the cytoplasmic $O_2$ sensor pool are the Hypoxia-Inducible Factors (HIFs) (Liu et al., 2020). The human genome encodes three different HIF subtypes: HIF-1, HIF-2 and HIF-3, which are heterodimers composed of a functional α subunit and a stably expressed β subunit (Dengler et al., 2014). Specifically, HIF-1α is the main transcription factor involved in $O_2$ sensing as it targets genes which encode for proteins that increase $O_2$ delivery and mediate adaptive responses to $O_2$ deprivation (Lee et al., 2019; Semenza, 2010, 2000; Vjotosh, 2020). At normal $O_2$ levels, HIF-1$\alpha$ is hydroxylated by $O_2$-dependent prolyl-4-hydroxylases (PHDs) that enhance its binding with Von Hippel Lindau protein (VHL). The newly formed complex can be recognized by the E3 ubiquitin ligase complex, and thus ultimately leads to HIF degradation by the proteasome (Sharp and Bernaudin, 2004). Moreover, HIF-1$\alpha$ subunits are also hydroxylated by factors inhibiting HIFs (FIHs), which inhibits the binding of HIF with co-activators p300/CREB-binding protein. Under hypoxic conditions, the activity of PHDs and FIHs are suppressed, and HIF-1α subunits translocate into the nucleus to bind with HIF-1β (Sharp and Bernaudin, 2004). The heterodimeric HIF-1α: HIF-1β transcription factor complex activates the hypoxia-responsive elements (HREs) in HIF target genes to modulate their transcriptional upregulation. This activates transcription of inducible nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF) and erythropoietin (EPO), which increases $O_2$ availability by promoting erythropoiesis and angiogenesis, and inducing glucose transporter 1 (GLUT1), glycolytic

enzymes and many other HIF target genes that are involved in glucose transport and metabolism (Lee et al., 2019; Sharp and Bernaudin, 2004) (Fig. 2). Furthermore, the translation of HIF-1α mRNA into protein is subjected to regulation by the PI3K/Akt/mTOR and PI3K/Akt/FRAP signaling pathways. Among the cellular processes activated in response to $O_2$ imbalance, a number of pathways are independent of HIF, such as the nuclear factor-κB (NF-κB) pathway. Indeed, according to early studies, IκBα is phosphorylated during hypoxia, allowing the degradation of IκBα and the activation of NF-κB1 (Singh and Singh, 2020).

Since the brain is a great energy consumer it is particularly susceptible to $O_2$ imbalance and hypoxia. Consequently, the decrease of $O_2$ levels can contribute to brain damage by inducing cell death and NDs. Hypoxia may influence many pathological aspects of AD including amyloid β metabolism, tau phosphorylation, autophagy, neuroinflammation, oxidative stress, endoplasmic reticulum stress, and mitochondrial and synaptic dysfunction, which may collectively result in neurodegeneration (Zhang et al., 2019). The activation of HIF-1 through the repression of PHDs can provide neuroprotection, ameliorate the outcomes, or prevent the pathogenesis in these pathological conditions. The beneficial effects of HIF-1 arise mainly from the increased expression of HIF-1 target genes, which combat oxidative stress, improve blood $O_2$ and glucose supply, promote glucose metabolism, regulate iron homeostasis, activate the synthesis of dopamine, and block cell death signal pathways (Merelli et al., 2018; Zhang et al., 2011). The HIF-1 activation may be a potent strategy to ameliorate the outcomes of AD. An association between decreased HIF-1 levels and an increase in tau protein and neurofilaments presence has been reported (Mitroshina et al., 2021). These processes cause lead to a decreased presence of a panel of genes, including HIF-1, known for their role in maintaining the viability and synaptic transmission of nerve cells (Mitroshina et al., 2021). Specifically, M30, one of the HIF-1α activator, increases the HIF-1α protein expression and its target genes VEGF and EPO. Moreover, M30 also attenuates tau phosphorylation and protects neurons against Aβ (Snell et al., 2014). Furthermore, deferoxamine (DFO), another HIF-1 inducer, has been used in a clinical trial in AD showing a slowed cognitive decline, highlighting how an increased activity of HIF-1 can prevent neuron death and

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improve AD symptoms (Zhang et al., 2011). There is a high number of studies highlighting the neuroprotective role of HIF-1α and its subsequent signaling pathway, and for this reason a novel therapeutic strategy in NDs worth investigating is that aimed at the stabilization of HIF-1 (Merelli et al., 2018). Specifically, pharmacological activation of HIF-1 might be used in therapy thanks to its neuroprotective effect. The increase in HIF-1 activity, along with that of his targets genes has been shown to slow down the cognitive decline present in AD patients along with the progression of the disease (Iyalomhe et al., 2017). Furthermore, lactoferrin administration leads to ERK signaling pathway transduction, activating ADAM10 through the HIF-1α pathway (Mechlovich et al., 2014). This results in a non-amyloidogenic processing of APP, which ultimately leads to improved results in spatial learning tests and cognitive outcomes assessment (Mechlovich et al., 2014). The most direct linkage between HIF-1 and PD is the tyrosine hydroxylase activity (TH), the rate-limiting enzyme in the synthesis of dopamine in dopaminergic neurons, also considered to be a hypoxia response element (Schnell et al., 2003). As widely explained, hypoxia and DFO activate HIF-1, which, in turn, has been seen to increase TH expression in rat brains. Meanwhile, the knockdown of HIF-1$\alpha$ in mice caused the decrease of TH expression in the SN (Milosevic et al., 2007). Recent studies have shown that HIF-1 has a fundamental role in both the differentiation and survival of dopaminergic neurons, and for this reason a reduction in HIF-1 could play a crucial role in PD pathogenesis. Subsequently, increasing the expression of HIF-1α could represent an innovative therapeutic approach for PD-affected patients (Mehrabani et al., 2020). These beneficial effects on dopaminergic neurons are found in both in *in vitro* and *in vivo* PD models, and they seem to be induced by HIF-1 complex activation in relation to the expression of EPO and VEGF (Strowitzki et al., 2019). The administration of EPO appears to result in long-term synaptic plasticity, as well as an antioxidant effect, and a reduction in the inflammatory responses, thus highlighting the important role of HIF-mediated regulation of EPO in PD therapy (Thompson et al., 2020). Moreover, recent studies have also demonstrated that the stabilization of HIF-1 may protect dopaminergic neurons through the alteration in iron homeostasis and defense against oxidative stress and mitochondrial dysfunction. Specifically, the inhibition of PHDs activities with 3,4-dihydroxybenzoate (DHB) results in HIF-1$\alpha$ protein stabilization and thus leads to the increase of HIF-1 target genes

expression, such as ferroportin and HO-1, in the SN (Zhang et al., 2011). In addition, HIF-1 can directly influence the expression of Leucine-rich repeat kinase 2 (LRRK2), involved in the pathogenesis of autosomal dominant PD, whereas hypoxia can trigger beta-synuclein accumulation (Bae et al., 2018). The proteins that lead to HIF-1 degradation can be inhibited, thus allowing for a modulation of its subsequent signaling and improving the neurons protection from oxidative stress. Several studies highlighted how it is possible to inhibit HIF-specific prolyl hydroxylases with interfering RNA or low-molecular-weight inhibitors (Aimé et al., 2020; Li et al., 2018; Mehrabani et al., 2020). Specifically, prolyl hydroxylase allows the activation of TH, and this leads to an enhancement in dopamine synthesis and release. Moreover, in in vivo models, the treatment with PHD inhibitors leads to a reduction in the loss of TH-positive neurons in the SN, attenuating behavioral deficits in murine models of the disease. The inhibition of HIF PHD can also lead to an amelioration in mitochondrial functions (Zhang et al., 2018). A decreased expression of EPO and VEGF, which results in tissue hypoxia, is also characteristic of ALS pathogenesis. Thus, the lack of glucose and $O_2$ caused by hypoxia can lead to motor neurons death and to the occurrence of ALS. HIF-1α is highly expressed before the onset of clinical ALS symptoms but its expression appears to be reduced in later stages of the pathology (Nomura et al., 2019). The altered expression of HIF-1α leads to a subsequent dysregulation of its downstream signaling pathway, which as it is implicated in the anti-hypoxic response, can worsen the motor neuron degeneration observed in ALS (Nagara et al., 2013). Many studies in SOD1G93A animal model highlighted hypoxia as the major cause of motor neuron death (Tankersley et al., 2007). The SOD1G93A model is an in vivo murine model of genetically manipulated mice harboring the pathogenetic G93A mutation in the SOD1 gene, causative of familial ALS (Marcuzzo et al., 2011; Rey et al., 2021a). Indeed, SOD1 is transcriptionally regulated in response to oxidative stress (Dell'Orco et al., 2016), and the activation of the HIF-1-VEGF pathway can induce angiogenesis and increase blood supply to motor neurons (Tankersley et al., 2007). Moreover, recent studies highlighted a negative correlation between VEGF levels in neurons and the severity of hypoxia in ALS patients, indicating a deregulation of VEGF in ALS and suggesting that an impaired HIF-1-VEGF pathway may contribute to the pathogenesis of ALS (Wang et al., 2007). The overexpression of VEGF in SOD1G93A mutant mice delays the degeneration of

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motor neurons and neuronal death and prolongs the survival of ALS mice (Wang et al., 2007). Furthermore, numerous studies performed in in vitro and in vivo models of ALS highlight a neuroprotective effect of HIF activation. Indeed, the activation of HIF1-1α pathway of action in an in vivo model of ALS, lead to a reduction in the hypoxic damage, ultimately resulting in neuroprotective and anti-inflammatory effects, with a subsequent reduction in motor neuron degeneration (Nomura et al., 2019). Researchers also showed that inhibiting PHD in an in vitro model of ALS can lead to the activation of HIF1-1α also in astrocytes, and this in turn leads to the expression of VEGF and GLUT, with an increased number of surviving neurons (Wiesner et al., 2013). Even so, it is important to note that there is some controversial evidence, as the reduction of HIF-1α expression using an analog of prostacyclin named ONO-1301-MS was found to improve behavioral outcomes and survival in an in vivo model of the disease (Tada et al., 2019). Furthermore, a common shared pathway observed in the three NDs is the decrease of HIF-1α levels, and an understudied, worth investigating, part of research is represented by its potential use as biomarker and it would thus be worthy to keep a look-out for studies analyzing HIF-1α expression in peripheral tissues.

### 3.3. Consequences of $O_2$ imbalance: implications for NDs

Another important aspect to consider within the context of $O_2$ sensing is how the cell can "compensate" a disbalance in $O_2$ levels and whether this is related to NDs pathogenesis. To this end, it is worth mentioning what is the role of HIF-1 activators, which are strictly correlated with a response to hypoxia (Bell et al., 2005). Amongst them, the mitochondrial electron transport chain surely plays a role, as it is required to regulate PHDs activity and thus HIF-1 signaling (Bell et al., 2005). Interestingly, amongst the complex I inhibitors that prevent hypoxic stabilization of HIF-1 there are MPTP and rotenone, both PD-causing neurotoxins (Agani et al., 2000; Bell et al., 2005). These toxins, along with many others, can lead to the presence of deficits in the activity of the mitochondrial electron transport complex, reduce movement of mitochondria, increase the mitochondrial permeability transition, increase generation of ROS, and the activity of nitric oxide synthase in the mitochondria. Complex I activity results impaired not only in the SN but also in skeletal muscles, platelets and leukocytes of PD patients (Monzio Compagnoni et al., 2020).

Indeed, studies suggest that mitochondrial dysfunctions may occur early in PD pathogenesis and moreover these are present in both sporadic and familial forms of PD (Malpartida et al., 2021).

The mitochondria is also relevant in HIF-1 hypoxic stabilization as the electron transport chain can increase the production of ROS during hypoxia, stabilizing the transcription factor (Brunelle et al., 2005; Chandel et al., 1998). This is also strictly related to the pathogenesis of NDs, as the role of oxidative stress has been well documented in the pathogenesis of AD and the first possible mechanisms concern the relation of ROS production with Aβ plaques. Similarly, many evidence demonstrated that PD patients display increased levels of oxidized lipids, proteins, and DNA, along with reduced levels of glutathione in the SN (Nakabeppu et al., 2007). Specifically, data collected from early-stage PD patients show that oxidative stress is a robust feature of initial disease stages, occurring prior to significant neuron loss (Ferrer et al., 2011). Lastly, evidence for ROS implication in ALS arose from multiple pathological studies which reported data of increased oxidative stress in ALS postmortem tissues compared to control samples (Islam, 2017). Specifically, markers for lipid oxidation were detected in spinal cord from sporadic ALS patients but were absent in controls (Shibata et al., 2001).

There are other consequences of $O_2$ disbalance that can then impact on NDs pathogenesis (Fig. 3), and amongst them there is iron metabolism, as iron is a key component of hemoglobin. Indeed, following a condition of hypoxia there is also an increased demand for iron, in order to limit the damage to the neuronal system. On one hand, intraerythrocytic hemoglobin, increased by HIF-induced EPO production, may protect neurons against hypoxia and hyperoxia (van der Kooij et al., 2008). On the other hand, extracellular free hemoglobin and its degradation products (such as heme and free iron) may trigger inflammatory immune and oxidative stress, and interact with pathological processes such as the Aβ deposition in AD (Atamna, 2006). Moreover, the increase in ROS production can lead at body level to the dysregulation of the inflammatory response. Indeed, many studies demonstrate the role of neuroinflammation to be essential in neurodegenerative processes in all three NDs considered in this review (Carelli et al., 2018; Hirsch and Hunot, 2009; Kinney et al., 2018; Liu and Wang, 2017). Moreover, it is relevant

to point out that the activation of HIF-1α may lead to the upregulation of pro-inflammatory cytokines and macrophage migration inhibitory factors, thus indicating that the rescue of HIF-1α needs to be well balanced in order to avoid excessive neuroinflammation (Basile et al., 2020)

Lastly, oxidative stress and ROS production can lead to impairment in processes such as calcium signaling, protein misfolding and synaptic dysfunction, all crucial players in NDs (Merelli et al., 2018) (Yeung et al., 2021). Nrf2 is a master regulator in oxidative stress, as it is implicated in the Nrf2-ARE pathway, an intrinsic mechanism of defense against oxidative stress (Buendia et al., 2016). Compelling evidence suggests that oxidative stress increases the damage in NDs due to an increased production of oxidative species and the failure of antioxidant defenses. Nrf2 is able to activate the phase II antioxidant response declines with aging, thus contributing to an exacerbated status of oxidative stress. Therefore, the activation of the Nrf2–EpRE pathway has been pointed as a key target for the development of new drugs for NDs (Buendia et al., 2016).

This evidence highlights how changes in $O_2$ levels can perturbate the cells in different areas. Specifically, these changes can be perceived primarily through the activation of $O_2$-sensitive K+ ion channels, which then lead to an intracellular cascade through molecules associated to them termed as "$O_2$-susceptible" (HO-2, NADPH, CSE, GC, cGMP, and PKG). There are also other cytoplasmic $O_2$ sensors, of which HIF-1 is the most relevant for its transcriptional signature aimed at counteracting hypoxia. HIF-1 can be activated by other intracellular organelles and mechanisms, such as mitochondria and ROS production, and these are tightly bound to NDs pathogenesis.

## 4. Transcriptional dysregulation of $O_2$ sensing in NDs

There is currently consistent amount of evidence highlighting the role of transcriptional dysregulation in NDs, and the impact that this has on their specific pathogenesis (Garofalo et al., 2020; La Cognata et al., 2021). Indeed, more and more studies are now aimed at highlighting changes in the gene expression signature in disease-affected patients, with the hope to identify novel disease players and possible biomarkers (112). Pathway analysis of differentially expressed genes allows the identification of those targets specifically involved in a certain process, and for this reason with this review we aim to identify also


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the transcriptional signature responsible of $O_2$ sensing dysregulation in three NDs considered here (i.e., AD, PD and ALS). Thus, the public RNA-Seq data stored on GEO Datasets were interrogated following the workflow reported in panel A of Fig. 4 to search for dysregulated genes involved in $O_2$ imbalance in previously published experimental dataset (Butovsky et al., 2015; Simchovitz et al., 2020; Xicoy et al., 2020; Yang et al., 2021). The detailed analysis relative to datasets processing and quality is reported in Supplementary Table 1.

The approach returned 104 dysregulated genes for PD, 53 for AD and 187 for ALS. Among these, the analysis highlighted genes associated with HIF-1 and changes in $O_2$ levels as reported in Table 2. Interestingly, among the genes associated to HIF-1 and $O_2$ imbalance none of them were found dysregulated in ALS.

Adra2b emerged as upregulated in PD. This is a G protein-coupled receptor that regulates neurotransmitter release from sympathetic nerves and from adrenergic neurons in the CNS (Wang et al., 2002). Previous studies have demonstrated that Adra2b levels are increased in hypoxic hepatic stellate cells even if its upregulation occurred independently of HIF-1α (Copple et al., 2011). Angpt2 emerged as upregulated in PD and it has been correlated with HIF-1 as it is transcriptionally activated with other angiogenic genes and receptors by HIF-1 expression during hypoxia (Zimna and Kurpisz, 2015). Moreover, Cxcr4, found upregulated in PD, has been linked to hypoxia and HIF-1 activation as it was observed that hypoxia increased Cxcr4 expression through HIF-1α activation in human monocytes, macrophages, endothelial cells and cancer cells, allowing the identification of a Hyp–HIF-1α–CXCR4 pathway that controls cell migration and localization and with a relevance in the pathogenesis of different human diseases (Schioppa et al., 2003). Gbe1 also emerged as deregulated in PD and it has been associated with hypoxia, as recent studies demonstrated that it is transcriptionally regulated by HIF-1α and that it affects tumor progression (Li et al., 2020b). Lastly, Cox2 and Tph2 emerged as deregulated in AD whereas no significant genes involved in $O_2$ sensing emerged in ALS. A condition of hypoxia leads to a TNF-α-induced regulation of Cox2 in osteoblast whereas the hypoxia-induced impairment of Tph2 and serotonergic functions can be mediated by NOS, involving the

generation of free radicals and decreasing the antioxidant status (Rahman and Thomas, 2014; Xing et al., 2015) (Table 2).

The pathways analysis of the dysregulated genes previously described allowed to extract those related to alterations due to $O_2$ imbalance. When considering NDs, there is often a common signature in the diseases as many pathways are shared amongst the conditions (please see section 3), but a selective neurodegenerative pathogenetic mechanism is present which leads to the degeneration of specific cell-types in each disease. In support of this, it is interesting to note that alterations in $O_2$ levels cause the dysregulation of metabolic processes. Moreover, the pathways analysis highlights how $O_2$ imbalance alters not only processes involved in metabolism and signal transduction, but also disease-related such as “GABA receptor activation”, “NOTCH signaling”, and “Dopamine receptors” (Fig. 4B-D; Supplementary Table 2).

Furthermore, it is interesting to observe that most pathways linked to $O_2$ imbalance are specific for each disease (Fig. 5A), whilst three of them are in common between PD and ALS (Extracellular matrix organization, Neutrophil degranulation, Metabolism of carbohydrates) and five between PD and AD (Voltage gated K+ channels, G alpha (q) signaling events, Neuronal System, GPCR ligand binding, Signal Transduction) (Fig. 5A). This is even more remarked when considering the gene-signature responsible for the pathways’ dysregulation (Fig. 5B). Indeed, no dysregulated gene is shared between the 3 NDs, whilst DSP (encoding for Desmoplakin) is the only one shared amongst PD and ALS (Fig. 5B-C).

RNA-sequencing analyses provide researchers with an extremely high amount of information, but there is often a lack of subsequent validation or data integration. The data hereby presented provides a first inspection of the genes and pathways pertaining $O_2$ sensing mechanisms in the three considered NDs (AD, PD and ALS), with the aim to identify selective regulators for each disease which can then be assessed functionally (please see section 5) or even prove to be new pharmacological targets (please see section 6). These preliminary results shed a light on the role of oxygen sensing in NDs, but also indicate an strong need for further studies to correlate these mechanisms with NDs pathogenesis.



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## 5. Current methodologies to investigate $O_2$ imbalance

To gain further insights on $O_2$ sensing it is necessary to discuss the possible approaches through which this can be investigated in NDs, and indeed experimental techniques have been developed over the years to assess the level of oxygenation in cells and tissues (Silva and Oliveira, 2018). These techniques are depicted in Fig. 6, and they can be subdivided in direct $O_2$ evaluation where microelectrodes and Seahorse technique are highlighted; fluorescence approaches, with particular attention to innovative techniques as Fluorescence Lifetime Imaging Microscopy and MitoTracker and finally magnetic resonance approaches where the two most exploited techniques are pointed out.

### 5.1 Direct oxygenation evaluation

Microelectrodes are the most common method to measure directly $O_2$ consumption as they represent the gold standard for tissue oximetry (Springett and Swartz, 2007). They consist in an ultrafine tip of biopotential electrodes which can be inserted directly into biological cultures (Springett and Swartz, 2007). The $O_2$ tension is measured in a wide surface, and it is particularly used in neurophysiological studies.

In the recent years, extracellular flux (XF) analysis is becoming a gold standard method for the assessment of bioenergetics in adherent cells in vitro and in vivo tissues (Salabei et al., 2014). Furthermore, mitochondrial activity can also be assessed in vitro in real time using Seahorse XF and this new set up is more suitable with primary neurons (Lejri et al., 2019; Rey et al., 2022). Seahorse XF Analyzers measure $O_2$ consumption rate (OCR) and extracellular acidification rate (ECAR) of live cells in label-free conditions, evaluating cellular functions such as mitochondrial respiration and glycolysis. Sonntag et al. exploited this innovative technique to investigate bioenergetic profiles in late-onset AD. Here, fibroblasts from patients exhibited a peculiar redox potential and an impaired mitochondrial metabolic potential, associated with reduced nicotinamide adenine dinucleotide metabolism. Indeed, the $O_2$ consumption rate, the extracellular acidification rate and proton production rate were increased in patients' fibroblast respect to controls (Sonntag et al., 2017). Microglia activation metabolic profiles was tested in primary microglia obtained from murine brain using the Glycolysis Stress Test and Mito Stress Test

Kits using the Seahorse XFe96 Analyser, demonstrating that higher levels of glucose transporter 1 (GLUT1) were expressed in microglia (Wang et al., 2019).

Another interesting oxygen evaluation method *in vitro* is high-resolution respirometry (HRR) to analyze mitochondrial respiratory pathways (Burtscher et al., 2015; Connolly et al., 2018; Djafarzadeh and Jakob, 2017). In particular, this technique was also exploited in NDs studies. This technique can be applied to measure respiration in a wide range of cell types and also provides information on mitochondrial quality and integrity. A challenge is to understand why mitochondrial fails in particular brain regions under specific pathological conditions. Risiglione et al. deeply investigate oxygen consumption in differentiated neuroblastoma cells exposed to the neurotoxin MPP+ and they highlighted the presence of mitochondrial damages at the inner membrane level (Risiglione et al., 2020).

### 5.2 Fluorescence techniques

Miniaturized optical sensors have been tested and optimized (Grist et al., 2010). These have as strongpoint the lack of contamination, the fact that they do not require a direct physical contact between the sensor and the optical detector, and, moreover, they do not consume $O_2$ (Papkovsky et al., 2000). They include fluorescence resonance energy transfer (FRET) and two-photon imaging using luminescent quenching. Moreover, these methods are non-invasive and suitable for sequential monitoring. Nowadays, sensor technologies and advances in fiber optics improve the measurement of dissolved $O_2$ using stable phosphorescent dyes, such as ruthenium chloride, whose quenching is proportional to the surrounding $O_2$ level (Zeitouni et al., 2015). In 2021, Shin et al. studied an oxide-sensitive fluorogenic molecular probes, benzenesulfonylated resorufin derivatives (BSRs), newly developed for optical bioimaging of oxidative events in neurodegenerative processes, in particular for AD. The researchers demonstrated by immunofluorescence imaging the capability of this new probe to detect intracellular $O_2$ in vitro in inflammatory and microglia cells and in animal models upon treatment with an oxidative stimulus (Aβ) or the byproduct of oxidative stress (4-hydroxynonenal, HNE) (Shin et al., 2021).

The sensitivity of fluorescence oxygen optodes can be tuned to specific $_PO_2$ values, resulting in a higher resolution (Ndubuizu and LaManna, 2007). Indeed, Lubbers et al.,



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used pyrene butyric acid to design a specific optode for the in vivo measurements of oxygen tension (Lübbers and Opitz, 1975; Opitz and Lübbers, 1987). Furthermore, Nguyen et al. set a specific method based on functional near-infrared spectroscopy with bundled-optode for detection of the changes of oxy-hemoglobin (HbO) and deoxy- hemoglobin (HbR) concentrations to analyze brain activity with higher spatial resolution (Nguyen and Hong, 2016).

In the recent years fluorescence lifetime imaging microscopy (FLIM) has become increasingly relevant (Perottoni et al., 2021). Using this advanced imaging technique and an independent $O_2$ sensor, it is possible to evaluate and measure changes in fluorescence dyes lifetime with corresponding changes in $O_2$ level, specifically in NDs (Sanchez et al., 2018). In this context, Pokusa et al., provide data on localization of intracellular changes of NADH associated with PD finding a co-localization of Thioflavine fluorescence signal with those of mitochondria and NADH. Furthermore, these evidences corresponded to the accumulation of α-synuclein and of NADH in rotenone treated cells (Pokusa and Kráľová Trančíková, 2018). Moreover, interesting research published by Gomez-Virgilio et al. investigated the translational potential of analyzing patient-derived olfactory neural precursors non-invasively isolated through NADH FLIM to reveal AD-related oxidative stress. This innovative technique permits to discriminate between the contribution of the cytoplasm and mitochondria (Gómez-Virgilio et al., 2021). Another interesting approach to assess in vitro mitochondrial damage is the MitoTracker probe. MitoTracker is chemically reactive, linking to thiol groups in the mitochondria and the analysis can be performed alternatively on both fixed samples and alive cells (Chazotte, 2011; Rey et al., 2022, 2021b). This approach was adopted to investigate mitochondrial dysfunction and mitophagy defects in PD patients with heterozygous GBA mutations, finding mitochondrial and autophagy deficits in brain tissues (Li et al., 2019). Moreover, a recent study by Sabogal-Guáqueta analyzed with the above-mentioned technique the role of linalool on glutamate-induced mitochondrial oxidative stress in AD (Sabogal-Guáqueta et al., 2019).

### 5.3 Magnetic Resonance Techniques

The above-described techniques are widely used in in vitro NDs model but are not exploited in pre-clinical studies. In this context, another innovative method to quantify the


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$O_2$ consumption of cells is the Electron Paramagnetic Resonance (EPR) oximetry, widely used for mitochondria and submitochondrial particles (Hyodo et al., 2010). This method is extremely useful for detecting free radicals and ROS (He et al., 2014). ROS are reactive and they also have limited half-lives in biological environments. It is thus difficult to directly measure these species but the new rapid-scan EPR methods can improve the sensitivity for these samples (Suzen et al., 2017).

Interesting research by Manabe et al. evaluated EPR combined with a mitochondria-targeted redox-sensitive nitroxide probe to elucidate the etiology of AD. With this technique they demonstrated that an increased oxidative stress was observed in brain mitochondria of transgenic mouse model of AD (Manabe et al., 2019). Moreover, with resonance technique, it is possible to evaluate $O_2$ consumption also in vivo in preclinical animal model of AD under non-invasive conditions that could be a potential key for early diagnosis and monitoring the progression of NDs. Here, researchers observed that mitochondrial dysfunction and oxidative stress in early onset of AD and increased ROS levels associated with defects of mitochondrial and cognitive dysfunction (Fang et al., 2016). A novel approach is based on Deuterium Magnetic Resonance (DMR): this non-invasive technique allows the detection of stable deuterated compounds in vivo and therefore does not decay during biologic processing (Hartmann et al., 2021). This technique was exploited by Vilaplana et al., to investigate the neuroinflammatory mechanisms in early stages of AD and in vivo patterns of neuroinflammation, proteinopathies and brain function in aging (Vilaplana et al., 2020).

### 5.4 Clinical frontiers on $O_2$ sensing

In patients with NDs different parameters are often evaluated, but to our knowledge $O_2$ sensing is still understudied. In Israel in 2014-2017, on a small group of patients with PD subjected to subthalamic deep brain stimulator surgery, the brain $O_2$ levels were measured with a non-invasive near infrared spectroscopy device, with results yet to be published (NCT02278406).

In conclusion, several in vitro and in vivo studies exploit systems for $O_2$ detection. In particular, magnetic resonance techniques, widely used in preclinical research, are also

used in different phases of clinical trials, allowing direct on $O_2$ sensing evaluations also on patients.

## 6. Pharmacological targeting of $O_2$ imbalance in NDs

For years many researchers have been trying to develop new pharmacological procedures aimed at modulating $O_2$ sensing mechanisms in the brain, specifically targeting $O_2$-related pathways (Ferrara and Adamis, 2016; Li et al., 2018; Scheuermann et al., 2009). However, only few successes have been reported, especially concerning NDs (Fig. 7).

### 6.1 HIF-1 modulation as potential therapeutic target in NDs

Even if specific drugs modulating $O_2$ sensing for NDs are not commercially available, in the last decade of basic and clinical research a number of regulating responses (e.g., HIF) have been found in cells exposed to hypoxia which have a relevant role in $O_2$ metabolism. These processes have proven to be highly important for neurodevelopment, neuronal survival and neurodegeneration (Schmidt-Kastner et al., 2006). It was first believed that as HIF acts as DNA-binding transcription factor it wouldn't be druggable. As a consequence, for many years researchers only tried to intervene downstream against components which are under the control of HIF, such as VEGF (Rey et al., 2022). This approach was successful in oncology and ophthalmology where it allowed to develop many monoclonal antibodies (e.g., Ranibizumab, Bevacizumab) and aptamers (e.g., Pegaptanib) (Ferrara and Adamis, 2016). On the other hand, it has been demonstrated, in preclinical experimental models, that VEGF administration inhibits loss of dopaminergic neurons (Kumar et al., 2022) whilst its antagonism leads to the reduction of synaptic functions and plasticity (Sharma et al., 2019). Indeed, it has been discovered that the use of VEGF-inhibitors may be linked to PD-like events, dementia, or variants of these diseases (Sultana et al., 2020). Therefore, these approaches do not represent appropriate treatments for NDs. Experimental and clinical evidence has demonstrated that regulating HIF-1 might ameliorate the cellular and tissue damage in the NDs. Thus, it would be interesting to consider HIF-1 inducers as potential strategies for NDs. Specifically, iron chelators such as DFO and M30, provide neuroprotection by inhibiting the activation of PHDs. DFO prevents formation of a catalytically active center in the PHDs, thus enabling dopamine synthesis and secretion in

PD and slowed cognitive decline in AD as emerged in clinical trials. Moreover, M30, which up-regulates HIF-1 expression, protects NSC-34 motor neuron cells from oxidative damage in vitro and significantly delays the onset of ALS in SOD1G93A mutant mice and simultaneously attenuates tau phosphorylation and protects cortical neurons against Aβ toxicity in AD experimental models (Zhang et al., 2011). These new findings suggest HIF-1 as a potential medicinal target for the neurodegenerative diseases

In 2009, Scheuermann et al. published a landmark study in which they identified a druggable pocket in HIF-2α as well as a compound that could bind to this site (Scheuermann et al., 2009). This led to the discovery of HIF-2 antagonists, such as Roxadustat (FG-4592), a small molecule for the treatment of anemia which has been recently approved by EMA and currently under revision from FDA. FG-4592 acts as an antagonist reversibly inhibiting the activity of PHD in normoxia. When PHD is inhibited, all the three subtypes (1, 2 and 3) which compose HIF-α subunit are not hydroxylated and degraded by the proteasome. Therefore, more stable HIF-α enters the nucleus and forms heterodimers with HIF-2β (Haase, 2017, 2013), which activates target gene expression, including EPO, VEGF and GLUT1 (Semenza and Wang, 1992; Warnecke et al., 2004). The effects of FG-4502 has been investigated also in PD experimental models. Specifically, FG-4592 is able to exert protective effects in the in vivo MPTP-induced PD model reducing both the loss of TH-positive neurons in the SN and the subsequent behavioral alterations in both *in vitro* and *in vivo* experiments (Li et al., 2018). These evidences suggest that this mechanism of action may lead to neuroprotective effects on PD patients. The role of dysbiosis and its effect on HIF has been investigated in AD experimental models, as oral bacteriotherapy appears to be a promising preventive and therapeutic strategy through the remodeling of gut microbiota. Indeed, this strategy appears to delay the onset and progression of AD through a reduction of neuroinflammation and protein aggregation. Specifically, chronic supplementation with SLAB51 enhances the expression of HIF-1α and decreases the levels of PHD2 in the brain. Moreover, it successfully counteracts the increase of iNOS cerebral expression along with the nitric oxide plasma levels in AD mice, highlighting another mechanism through which SLAB51 can exert its neuroprotective and anti-inflammatory effects (Bonfili et al., 2021). The implication for dysbiosis and gut

microbiota highlights how the environment and nutritional dysregulation could impact on the oxygen sensing process, and indeed antioxidants molecules and nutritional supplements could be used, in combination, to address oxygen disfunctions in NDs.

Furthermore, given the importance of HIF-1, it would be interesting to evaluate potential strategies that envisage its production or availability in patients with NDs. To this end, Xue et al. proposed a rational drug design of HIF-1α/VHL inhibitors. Specifically, they developed an effective strategy to identify and design new inhibitors for protein-protein interaction targets. Through alanine scanning, site-directed mutagenesis, and molecular dynamics simulations they observed that the interactions between Y565 and H110 played a key role in the binding of VHL/HIF-1α. Based on the interactions, they synthetized 8 derivates of VH032, 16a-h, by introducing various groups bounded to H110, that exhibited higher binding affinity to VHL and markable or modest improvement in stabilization of HIF-1α or HIF-1α-OH in HeLa cells (Xue et al., 2022).

In conclusion, novel drugs for NDs could be highly promising candidates in the treatment of these disorders, but still much work is needed to discover new potential biological targets.

### 6.2 Targeting of $O_2$ imbalance: consequences for the amelioration of NDs symptoms

Since therapies that directly target $O_2$ sensing are limited, conventional drugs still remain the first-line treatment for NDs. Sometimes, these approaches include molecules that act against oxidative damage or its consequences, such as antioxidants or EPO itself (Ehrenreich et al., 2007; Moussa et al., 2017; Wüstenberg et al., 2011). Antioxidants are exogenous or endogenous molecules which can act against oxidative stress neutralizing ROS and other kinds of free radicals. These molecules are contained in numerous foods we consume, including flavonoids and phenolic compounds, lipoic acid (thioctic acid), ubiquinone and idebenone, β-carotene and vitamin C (Chen et al., 2012).

Even if there is no FDA-approved antioxidant therapy for NDs yet, several clinical trials produced promising results in animal models of AD (Rajasekar et al., 2013; Sancheti et al., 2014) and in PD patients (Fahn, 1991). These trials include the use of the vitamin E (alpha tocopherol) and vitamin C as strong antioxidant agents. This has been investigated to

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partially restore cognitive functions in individuals with early PD (Fahn, 1991) and in patients with mild to moderate AD (Dysken et al., 2014; Sano et al., 1997). Unconclusive results were also obtained when considering clinical trials with the polyphenolic compound curcumin, a molecule with antioxidant and anti-inflammatory effects (Ringman et al., 2012). Curcumin has indeed proved beneficial in multiple NDs models and it has been suggested that the improvement of drug bioavailability could be effective in AD (Gagliardi et al., 2020, 2018; Ringman et al., 2012). Besides antioxidants, the research of molecules that act on mitochondria represents an innovative approach aimed at mitigating local ROS production or at reducing their induced damage (Brieger et al., 2012). These compounds include EPO, a cytokine induced by hypoxia expressed in the brain that has been demonstrated to exert many fundamental effects such as neuroprotection and neuroregeneration (Brines and Cerami, 2005; Carelli et al., 2018; Digicaylioglu et al., 1995; Rey et al., 2021b, 2019), neurodevelopment (Victor et al., 2021), neuroplasticity (Brines and Cerami, 2005), when stimulated by mild local hypoxia (Wakhloo et al., 2020) or when administered as recombinant human (rh)EPO in different in vitro and in vivo pre-clinical experimental models (Fernando et al., 2018; Maurice et al., 2013; Rey et al., 2021b, 2019)(147, 186, 189, 190). The neuroprotective effects of rhEPO have been demonstrated also in two clinical trials in PD affected patients (Jang et al., 2014; Pedroso et al., 2012).

Moreover, EPO and its receptor (EPOR) were found in catecholaminergic glomus cells type I of CB (Soliz et al., 2005) where it has been shown to regulate the also the activity of carotid sinus. Research highlights that systemic EPO can activate the CB chemosensory activity after a hypoxic and hypercapnic stimulation (Andrade et al., 2018). Recent findings also suggest a dual effect of EPO in CSN in mice, as it stimulates the CSN hypoxic response at low concentration (<0.5 IU/ml) whilst it inhibits hypoxic and hypercapnic CSN activation at higher concentrations (>1 IU/ml) following an increase in NO production by type I cells (Arias-Reyes et al., 2021). In conclusion, divergent results have been achieved during EPO and antioxidants research on NDs, and many aspects regarding their role in the CNS remain elusive and need to be elucidated.

Non-pharmacological treatments and lifestyle interventions, which include exercise and caloric restriction, are gaining increasing attention due to their overall beneficial effect on

$O_2$ imbalance, health and life span (Mendiola-Precoma et al., 2016). Specifically, grounded on a population-based perspective, the Alzheimer's Association has identified regular physical exercise as one of the strategies to reduce the risk of cognitive decline and the development of dementia (Baumgart et al., 2015). Indeed, regular physical activity was associated with reduced oxidative stress, increased antioxidant capacity and anti-inflammatory effects (Baumgart et al., 2015). To sum up, the molecular mechanisms implicated in the beneficial effects of exercise are not fully understood. Therefore, a better understanding of lifestyle modifications is needed to develop integrated strategies effective in counteraction the evolution of neurodegenerations.

## 7. Concluding remarks

$O_2$ sensing mechanisms in the brain are crucial to maintain tissue homeostasis and organ functionality. Even so, these mechanisms do not strictly occur in the brain, but rather are the result of the cooperation amongst different organs, which primarily include the CB, preBötC, the cardiovascular, renal and pulmonary systems. It is thus of course necessary to consider the whole-body regulation of $O_2$ sensing and its implication in NDs, but there is also a need to identify the cellular responses to these changes. Specifically, even if more and more evidence is mounting each year concerning the physiology of $O_2$ sensing, the number of researches correlating these evidences with the three NDs considered in this review article, AD, PD and ALS, is still limited. On the contrary, literature evidence primarily focuses on the dysfunctions induced by these processes, which include the production of ROS, mitochondria's health, protein misfolding and neuroinflammation, all pathways characteristic of NDs. These pathways are strongly altered also when considering the transcriptional deregulation present in AD, PD and ALS. There is thus a crucial need to investigate $O_2$ sensing mechanisms, and to identify novel strategies for detection of these altered pathways and their correlation with specific NDs. Moreover, therapeutic approaches currently primarily focus on the "correction" of the above-mentioned secondary effects of the dysfunction rather than the O2 sensing pathway itself. In our opinion, novel approaches targeting this aspect would be of fundamental relevance.

## Authors' Contributions

F.R. Conceptualization, Writing – original draft, Writing – review & editing, Investigation; L.M. Formal Analysis, Investigation, Writing – original draft, Writing – review & editing; E.M. Data curation, Writing – original draft; GC: Investigation, Writing – original draft, Writing – review & editing; S.O. B.B Investigation, Writing – original draft; M.T.R., C.C., S.C., G.Z Funding acquisition, Writing – review & editing; E.E., I.P. Project administration, Writing – review & editing; S.C. Conceptualization, Writing – original draft, Writing – review & editing,Project administration

## Authorship Confirmation Statement

All authors have contributed to this study and read, edited (where needed), and approved the article as submitted.

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**Abbreviations Used:**

6-OHDA = 6-hydroxydopamine hydrochloride

AD = Alzheimer's Disease

ALS = Amyotrophic Lateral Sclerosis

ARDS = acute respiratory distress syndrome

BACE1 = β-amyloid precursor protein cleaving enzyme 1

bCPG = breathing central pattern generator

BSRs = benzenesulfonylated resorufin derivatives

$Ca^{2+}$ = calcium

CB = carotid bodies

cGMP = cyclin guanosine monophosphate

CNS = central nervous system

CO = carbon monoxide

$CO_2$ = carbon dioxide

COPD = chronic obstructive pulmonary diseases

CSE = cystathionine-γ-lyase

DFO = deferoxamine

DHB = 3,4-dihydroxybenzoate

DMR = deuterium magnetic resonance

ECAR = extracellular acidification rate

EPO = erythropoietin

EPOR = erythropoietin receptor

EPR = electron paramagnetic resonance

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FG-4592 = Roxadustat

FIHs = factors inhibiting hypoxia-inducible factors

FLIM = fluorescence lifetime imaging microscopy

FRET = fluorescence resonance energy transfer

GC = guanine-cytosine content

GDNF = glial cell line derived neurotrophic factor

GLUT1 = glucose transporter 1

HbO = oxy-hemoglobin

HbR = deoxy-hemoglobin

HIFs = hypoxia-inducible factors

HNE = 4-hydroxynonenal

HO-2 = heme oxygenase 2

HREs = hypoxia-responsive elements

HRR = high-resolution respirometry

I-RTX = 5-iodo-resiniferatoxin

iNOS = inducible nitric oxide synthase

$K^+$ = potassium

LRRK2 = leucine-rich repeat kinase 2

MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MSA = multiple system atrophy

NADPH = nicotinamide adenine dinucleotide phosphate

NDs = neurodegenerative diseases

NF-κB = nuclear factor-κB

$O_2$ = oxygen

OCR = oxygen consumption rate

OSAS = obstructive sleep apnea syndrome

PD = Parkinson's Disease

PHD = prolyl hydroxylase domain

PHDs = $O_2$-dependent prolyl-4-hydroxylases

PKG = protein kinase G

pO2 = partial pressure of oxygen

preBötC = PreBötzinger Complex

RAGE = receptor for advanced glycation end products

ROS = reactive oxygen species

SN = substantia nigra

TRP = transient receptor potential

VEGF = vascular endothelial growth factor

VHL = Von Hippel Lindau protein

XF = extracellular flux

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**Table 1: Contributions of peripheric organs to oxygen (O2) sensing and their implications in Neurodegenerative Diseases (NDs).**

| Organ | Mechanism | Implication in NDs | Bibliography |
|---|---|---|---|
| **Kidney** | It can act as $O_2$ sensor during hypoxia, increasing perfusion pressure chronically; Erythropoietin production. | Kidney injury is a risk factor of PD and AD. Receptor for Advanced Glycation End products mediates the transport of Aβ into the CNS. | (Carnevale et al., 2012; Lin et al., 2016; Zhang et al., 2020) |
| **Pulmonary System** | Lack of O2 induces selective pulmonary vasoconstriction | Acute respiratory distress syndrome and obstructive sleep apnea syndrome can result in cognitive impairment. COVID19 hypoxic condition can lead to an AD-like phenotype. | (Almeria et al., 2020; Heneka et al., 2020; Hopkins et al., 2005; Lal et al., 2019) |
| **Cardiovascular System** | Increased sympathetic activity regulates the ensuing transcriptional regulation of pro- and anti-oxidant enzymes which contributes to oxidative stress in the adrenal medulla | Excessive afferent signaling from the CBs may lead to the development of pathological conditions such as hypertension, a risk factor for NDs. | (Bergantin, 2019) |



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This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

**Table 2: Dysregulated genes associated to HIF-1 and changes in oxygen levels***.

| Gene name | AD | PD | Function | Bibliography |
|---|---|---|---|---|
| **Adra2b** | // | ↑ | G protein-coupled receptor involved in neurotransmission | (Copple et al., 2011) |
| **Angpt2** | // | ↑ | Angiopoietin family of growth factors, antagonist of angiopoietin 1. It is implicated in the direct control of inflammation-related signaling pathways. | (Zimna and Kurpisz, 2015) |
| **Cxcr4** | // | ↑ | CXC chemokine receptor specific for stromal cell-derived factor 1. It acts with the CD4 protein to support HIV entry into cells and is also highly expressed in breast cancer cells. | (Schioppa et al., 2003) |
| **Gbe1** | // | ↓ | Glycogen branching enzyme that catalyzes the transfer of alpha-1,4-linked glucosyl units from the outer end of a glycogen chain to an alpha-1,6 position on the same or a neighboring glycogen chain. | (Li et al., 2020) |
| **Cox2** | ↓ | // | Also known as cyclooxygenase, it is the key enzyme in prostaglandin biosynthesis, and acts both as a dioxygenase and as a peroxidase. | (Xing et al., 2015, p. 2) |
| **Tph2** | ↓ | // | The encoded protein catalyzes the first and rate limiting step in the biosynthesis of serotonin, an important hormone and neurotransmitter. | (Rahman and Thomas, 2014) |

*Amongst all the deregulated genes found for the three specific NDs, 6 were related to HIF activation. The table reports the gene name, the ND where it emerged and the specific dysregulation in term of up (↑) or downregulation (↓), the gene function and the reference of source where the finding is reported. // means no available information in the specific ND.

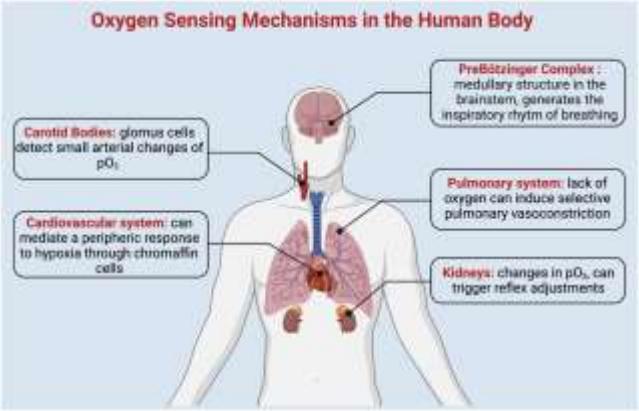


**FIG. 1**. **The human body is a complex system which largely depends on oxygen ($O_2$) for its correct functioning.** To this end, numerous organs are able to sense changes in $O_2$ partial pressure ($pO_2$) and elicit responses to avoid systemic damage. These include the carotid bodies, the main organ with this function, made up of glomus ($O_2$-sensing) cells, the preBötzinger Complex, a medullary structure in the brainstem able to generate the inspiratory rhythm of breathing, the pulmonary, the cardiovascular and the renal systems, which can induce peripheric responses to these changes. Created with Biorender.com



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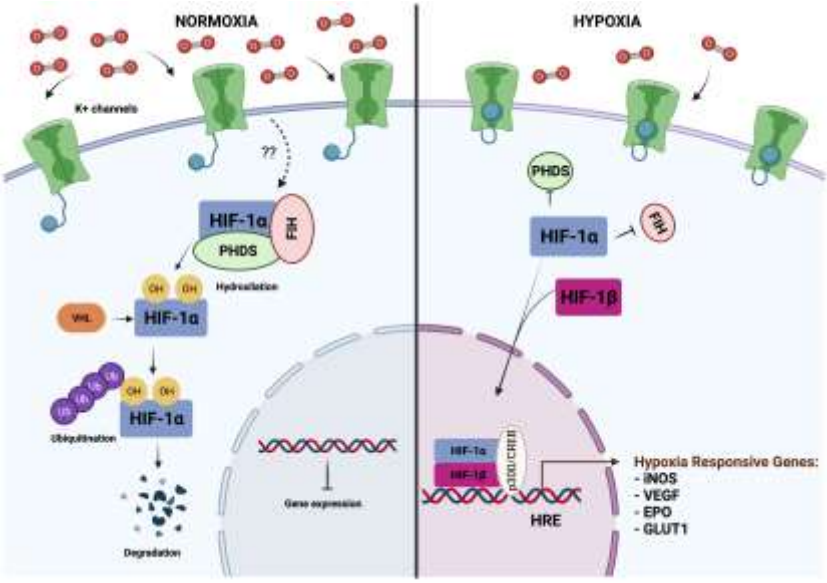


**FIG. 2: Overview of oxygen ($O_2$) sensing at cellular level.** The image depicts the two scenarios to which cells respond. On the left, at a normal $O_2$ concentration, the potassium (K+) $O_2$ sensitive channels are active. Hypoxia-Inducible Factor 1 (HIF-1)$\alpha$ is thus hydroxylated by $O_2$-dependent prolyl-4-hydroxylases (PHDs) that enhance its binding with Von Hippel Lindau protein (VHL), and by factors inhibiting HIFs (FIHs), inhibiting the binding of HIF with co-activators p300/CREB-binding protein. The newly formed complex acts as substrate recognition component of the E3 ubiquitin ligase complex, which leads to proteasomal degradation of HIF-1$\alpha$. Under hypoxic conditions, the $O_2$-sensitive K+ channels are inactive. The activity of PHDs and FIHs are suppressed, and HIF-1α subunits translocate into the nucleus to bind with HIF-1β. The heterodimeric HIF-1α: HIF-1β transcription factor complex activates the hypoxia-responsive elements (HREs) in HIF target genes to modulate their transcriptional upregulation. This activates transcription of inducible nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF), erythropoietin (EPO), glucose transporter 1 (GLUT1), glycolytic enzymes and many other HIF target genes that are involved in glucose transport and metabolism. Created with Biorender.com

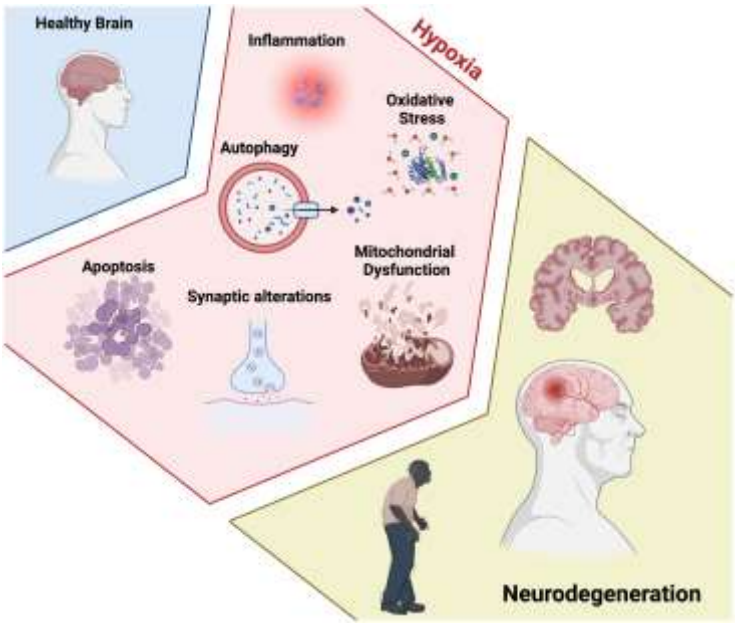


**FIG. 3: A condition of hypoxia can lead to intracellular damages resulting in neurodegeneration**. A condition of constant reduction in oxygen levels, specifically hypoxia, can lead to numerous intracellular perturbations. These include oxidative stress, mitochondrial dysfunctions, autophagy, synaptic alterations, inflammation and ultimately, cell death. The concomitance of these effects leads to neurodegeneration, and these pathways are often common players in neurodegenerative diseases. Created with Biorender.com



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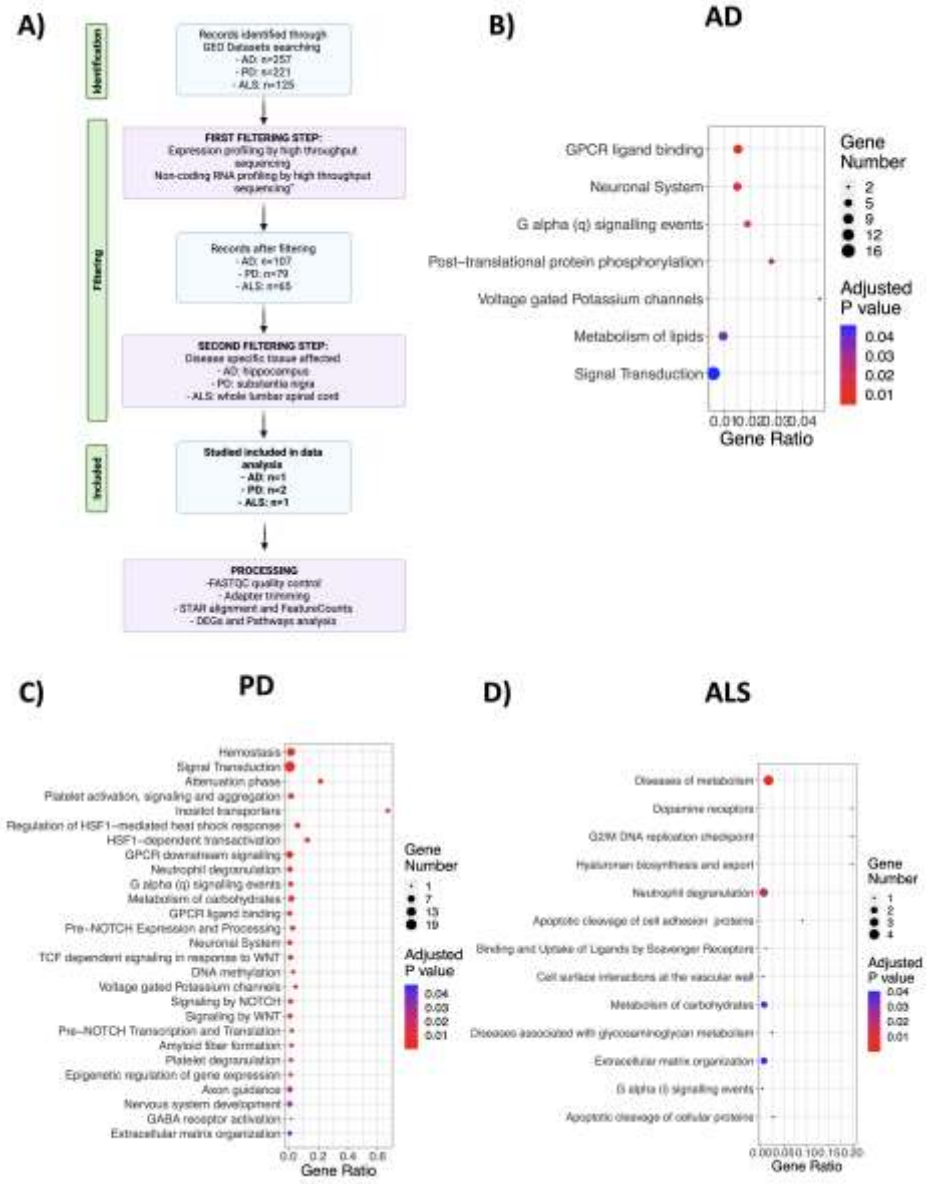


**FIG. 4: Processing of RNA-Seq datasets for AD, PD and ALS.** (A) Workflow of processing pipeline: The GEO Datasets was interrogated with the terms “Alzheimer’s Disease” (AD), “Parkinson’s Disease” (PD) and “Amyotrophic Lateral Sclerosis” (ALS), filtering for “homo sapiens” and expression studies (micro-arrays, high-throughput screening and genome tilting arrays). The results were subsequently filtered for high throughput studies and disease-specific affected tissues (e.g. hippocampus for AD, Substantia Nigra for PD and whole lumbar spinal cord for ALS) obtaining a final number of 1 AD dataset, 2 PD datasets and 1 ALS datasets. The datasets were re-processed to obtain comparable and homogeneous data. Specifically, quality of individual sequences was evaluated using FastQC software before and after overrepresented sequences removal with the Cutadapt

software. Reads were computed using the STAR software using Gencode Release 38 (GRCh38). Reads abundance was inspected with FeatureCounts whereas DEGs analysis was performed through DESeq2 R package. Made with Biorender.com. The dotplots report the pathways pertaining oxygen-sensing mechanisms in AD (B), PD (C), and ALS (D). Gene enrichment analysis was then performed using g:Profiler, ranking terms according to their fold change and using a Bonferroni-Hochberg FDR of 0.05 as threshold and the R software was used to generate dotplot graphs (ggplot2 library). The y-axis represents the name of the pathway, the x-axis represents the gene ratio, dot size represents the number of different genes, and the color indicates the adjusted p-value

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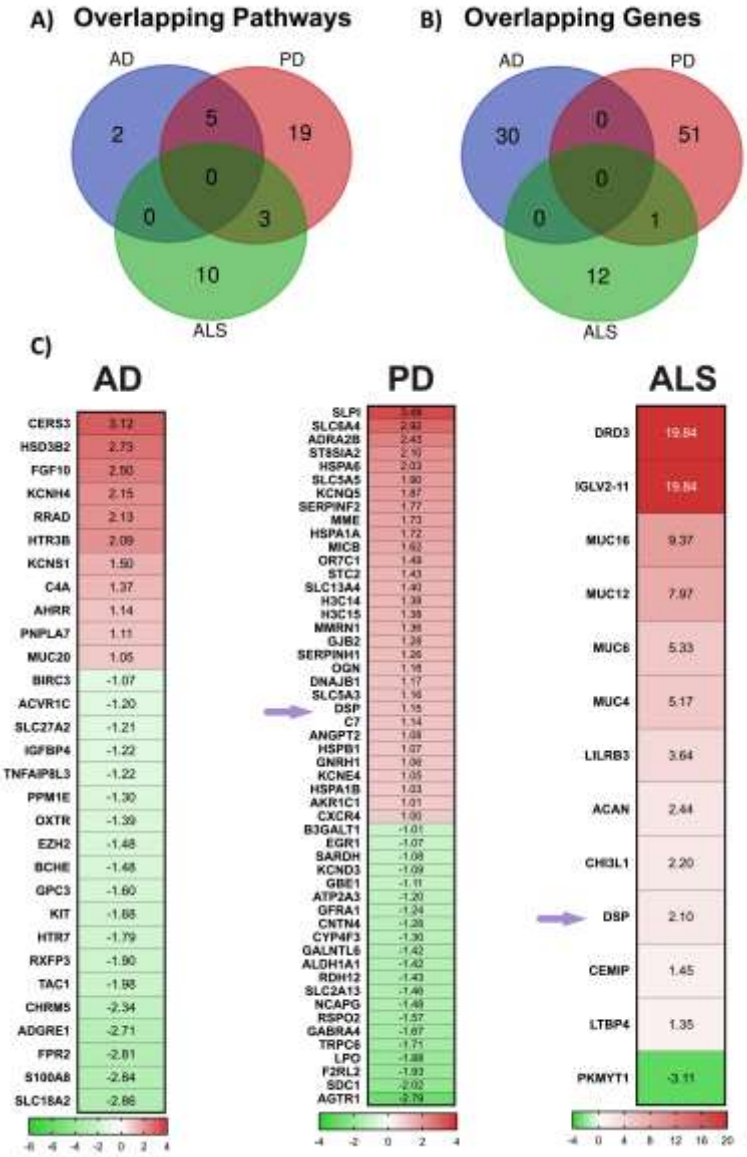


**FIG. 5: The oxygen-related signature is divergent in the three investigated NDs.** (A) The Venn diagram displays how many pathways obtained with Reactome filtered for their relevance with oxygen-sensing mechanisms are shared amongst conditions (http://bioinformatics.psb.ugent.be/webtools/Venn/, last accessed on 04 October 2021). (B) Genes pertaining oxygen-sensing were extrapolated from the respective pathways. The Venn diagram displays how many of these genes are shared amongst conditions (http://bioinformatics.psb.ugent.be/webtools/Venn/, last accessed on 04 October 2021). (C) Heatmap of Differentially Expressed RNAs (DE RNAs) related to oxygen sensing in AD, PD and ALS. The violet arrow indicates the only term shared amongst PD and ALS.

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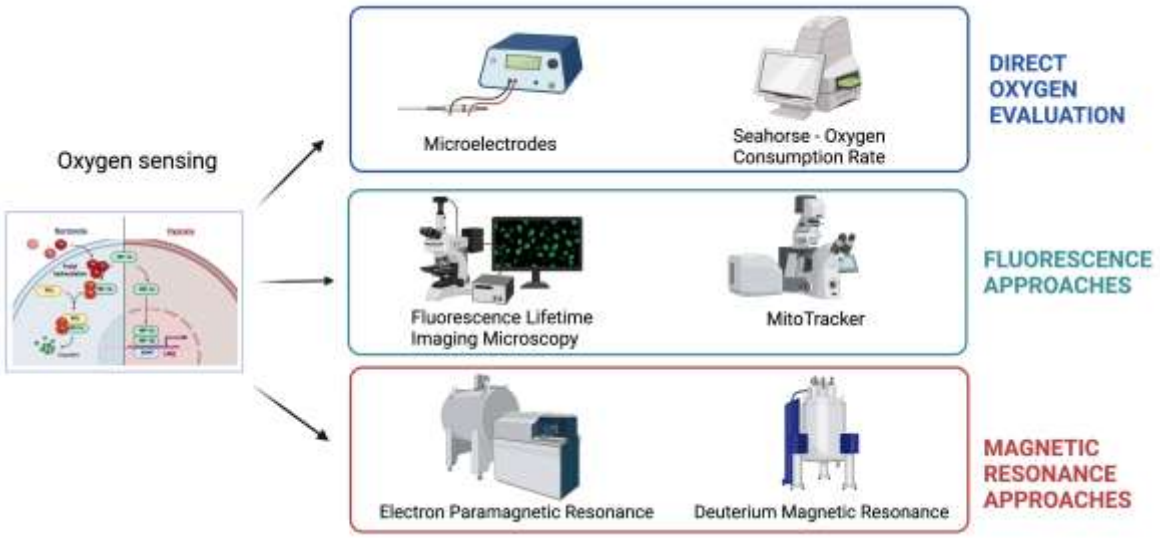


**FIG. 6: Overview of techniques and approaches used to assess $O_2$ imbalance.** These techniques can be subdivided in direct oxygen evaluation, where microelectrodes, Seahorse technique and high resolution respirometry are highlighted; fluorescence approaches, with particular attention to innovative techniques as oxygen optodes, Fluorescence Lifetime Imaging Microscopy and MitoTracker and finally magnetic resonance approaches, with the two most exploited techniques being electron paramagnetic resonance and deuterium magnetic resonance. Created with Biorender.com

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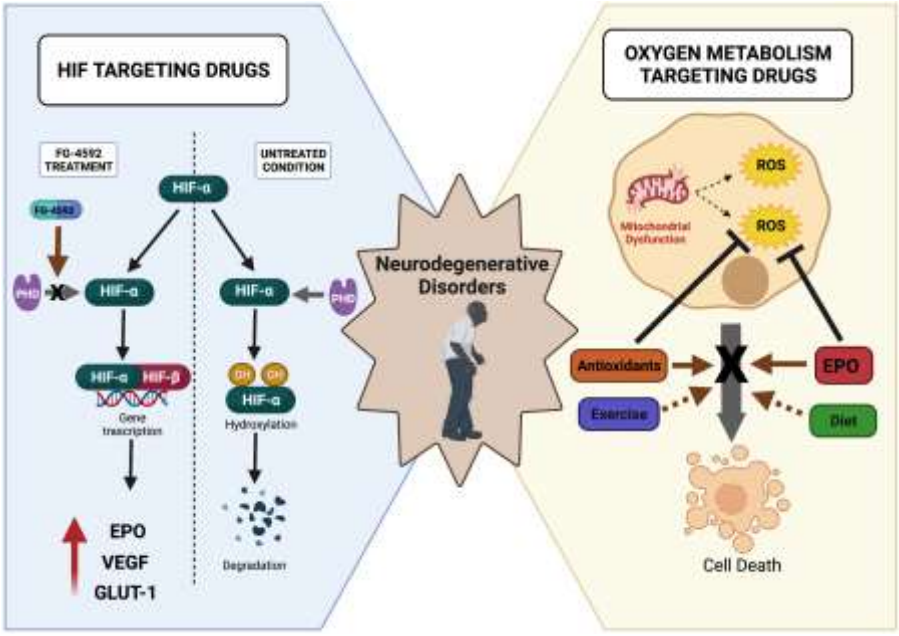


**FIG. 7: Overview of possible innovative treatments for oxygen imbalance or its metabolism.** These approaches can be classified into two main groups: Hypoxia Inducible Factor (HIF) targeting drugs (left) and oxygen metabolism targeting drugs (right). As the name suggests, the first group acts on HIF pathway, specifically on the activity of prolyl hydroxylase domain protein (PHD). These drugs include Roxadustat (FG-4592) which inhibits the hydroxylation (OH) and degradation of HIF-α subunit leading to transcription of gene targets. On the other hand, oxygen metabolism targeting drugs have broad mechanisms of action which include decrease of Radical Oxygen Species (ROS) production and reduction of cellular senescence. This group includes drugs such as Erythropoietin (EPO), antioxidant nutrients and non- pharmaceutical approaches (e.g. diet and physical exercise) whose molecular effects are still to be fully discovered.