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► **To cite this version:**

Yuri Zilberter, Irina Popova, Misha Zilberter. Unifying mechanism behind the onset of acquired epilepsy. *Trends in Pharmacological Sciences*, 2022, 43 (2), pp.87-96. 10.1016/j.tips.2021.11.009 . hal-04100844

HAL Id: hal-04100844

<https://amu.hal.science/hal-04100844>

Submitted on 22 Jul 2024

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Unifying mechanism behind the onset of acquired epilepsy

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Abstract

Acquired epilepsy can result from a number of brain insults and neurological diseases with wide etiological diversity sharing one common outcome of brain epileptiform activity. This implies that despite their disparity, all these initiating pathologies affect the same fundamental brain functions underlying network excitability. Identifying such mechanisms and their availability as therapeutic targets would help develop an effective strategy for epileptogenesis prevention. In this opinion, we propose that the vicious cycle of NADPH oxidase-mediated oxidative stress and glucose hypometabolism is the underlying cause of acquired epilepsy, as available data reveal a critical role for both pathologies in epileptogenesis and the process of seizure initiation. Altogether, here we present a novel view on the mechanisms behind the onset of acquired epilepsy and identify therapeutic targets for potential clinical applications.

Keywords

Acquired epilepsy, Alzheimer's disease, oxidative stress, glucose hypometabolism, NADPH oxidase, ketogenic diet

Acquired epilepsy and available treatments.

A variety of brain insults can trigger the process of **epileptogenesis** and result in **acquired epilepsy (AE)**, as discussed in detail in recent comprehensive reviews [1,2]. It is generally assumed that following the initial brain insult and the resulting epileptogenesis, there is a latent period lasting from days to years before AE is established; it is precisely this delay that may present the most opportunities for intervention with preventive treatment. However, today there is no clinical treatment capable of preventing AE development in patients at risk [2].

Notably, risk factors for AE epileptogenesis can differ drastically in their etiology, varying from acute brain injury to neurodegenerative diseases. Nevertheless, all of them result in brain hyperactivity, suggesting that these pathologies primarily affect similar fundamental brain functions directly linked to network excitability modulation. We suggest that the common trait unifying the outcomes of various risk factors is the vicious cycle of oxidative stress and glucose hypometabolism that results in epileptogenesis and AE. In this opinion, we will focus on the evidence supporting our theory.

Critical events in AE initiation.

Patients who are at relatively high risk for AE include individuals with brain injury, intracerebral hemorrhage, brain tumors, status epilepticus, and neurodegenerative diseases [3], e.g. Alzheimer's disease (AD) [4]. Importantly, most if not all major risk factors for acquired epilepsy are associated with oxidative stress which is defined as an imbalance between the cellular production of reactive oxygen species (**ROS**) and the cellular antioxidant system's ability to neutralize them. This is well documented, for instance for traumatic brain injury [5], ischemic stroke [6], status epilepticus [7,8] and major neurodegenerative diseases [9] such as AD [10].

Arising from different brain insults, AEs also share one common early stage brain biomarker -- glucose hypometabolism. Clinical tests using **FDG-PET** imaging established decreased glucose utilization during quiescent (interictal) periods as a reliable biomarker for human epilepsy [11]. Mirroring this observation, multiple studies in rodent epilepsy models show that reduced glucose metabolism is predictive of epilepsy initiation [12]. Glucose is the major brain fuel, providing energy for cells, but it is also critical for many other critical brain functions such as oxidative stress management, biosynthesis of neurotransmitters and neuromodulators, among others [13]. Therefore, any dysfunction in glucose utilization is bound to result in pathological consequences. However, can

glucose hypometabolism be a trigger for brain hyperactivity, i.e. can it actually initiate epileptogenesis? Our *in vivo* experiments suggest that this is indeed the case. We showed directly that chronic partial inhibition of brain glycolysis by **2-deoxy-D-glycose (2-DG)** intracerebroventricular injections in rats led to the initiation of paroxysmal brain activity and, following a latent period of 3-4 weeks, to tonic-clonic seizures in some animals [14,15].

Thus, AE is associated with glucose hypometabolism which triggers network hyperactivity while oxidative stress, linked with most AE risk factors, has been shown to result from seizures [16,17]. Moreover, all these essential AE pathologies are interconnected: we showed previously that H₂O₂ application inhibits glucose utilization [18] and that epileptiform activity is associated with rapid H₂O₂ release [18,19], resulting in a pronounced decrease in glucose consumption [18]. In addition, we found that **beta-amyloid (A β ₁₋₄₂)** induces acute oxidative stress (see also [20]) which strongly reduces network glucose utilization and results in long-lasting network hyperactivity underlying AD-associated AE [21].

In summary, based on available data we posit that oxidative stress is a direct cause of reduced glucose utilization that leads to network hyperactivity and seizures, while seizures in turn exacerbate oxidative stress and subsequently reduce glucose utilization -- thus creating a vicious reciprocal feedback loop of epileptogenesis.

The dominant role of NADPH oxidase in oxidative stress.

Since we suggest oxidative stress to be the principal trigger of epileptogenesis, it is important to focus on the dominant sources of ROS overproduction in brain cells. Under normal conditions, mitochondria are the primary source of ROS, producing about 90% of cellular ROS as a by-product of oxidative phosphorylation [22–24]. However, mitochondria also possess a very efficient antioxidant system consisting of several detoxifying enzymes that scavenge ROS as soon as they are generated [23,25–27]. Therefore, in the context of oxidative stress, the physiological emission of ROS from mitochondria into cytosol is negligible [25] and may serve a signaling function instead [28]. Moreover, due to their powerful scavenging potentials, mitochondria are capable of neutralizing cytoplasmic ROS and actually serve as a ROS sink [25,26].

Pathological overproduction of ROS, no matter the source, may lead to mitochondrial impairment and result in increased ROS release into the cytoplasm. However, in *ex-vivo* experiments mitochondria were shown not to be the main source for ROS accumulation during seizures [8,29]. We also did not observe any reductions in oxygen consumption following epileptiform network

hyperactivity [18,30,31], suggesting normal mitochondrial functioning. Therefore, despite the high rates of ROS generation, mitochondria are unlikely to dominate oxidative stress early in AE pathogenesis.

Among the other known sources of cellular ROS production, one principal player in oxidative stress during neurological pathologies is **NADPH oxidase (NOX)** - the only known enzyme with the primary function of generating ROS [32]. Under "resting conditions" NOX is normally dormant but when activated by specific stimulation, the cytosolic subunits translocate to the membrane and associate to the functioning complex [32] that transports an electron from cytosolic NADPH to reduce oxygen to O_2^- . There are seven isoforms of NOX with NOX1, NOX2 and NOX4 expressed in multiple brain regions including the cerebral cortex, hippocampus, cerebellum, hypothalamus, midbrain and striatum [33], with NOX2 as the dominant form expressed by microglia, neurons, and astrocytes.

NOX has recently emerged as a major source of oxidative stress in a number of neurological diseases including epilepsy and AD. We demonstrated *ex-vivo* that NOX activation is a trigger of **ictal events** similar to human seizures during temporal lobe epilepsy [19], and *in vivo* that inhibition of NOX abolished hyperactivity in several murine seizure models [19].

Combining all available results, we conclude that in early AE, NOX is likely the molecule most responsible for oxidative stress leading to network hyperactivity and epileptogenesis (Fig. 1).

Reciprocal feedback of NOX-induced oxidative stress and glucose hypometabolism in AD and associated network hyperactivity.

AE is a well-established early co-morbidity of AD [34], with studies reporting unprovoked seizures in as high as 64% of AD patients [35] (See Box 1). Similar to AE, one earliest clinical marker for AD is glucose hypometabolism [36,37] associated with oxidative stress [38] [linked to A \$\beta\$ -induced NOX activation](#) [9,39].

Our recent study [31] has tied the multiple toxic A β effects into one cohesive causal chain showing in mice that A β induced oxidative stress, diminished brain glucose utilization, and resulted in consequent long-lasting network hyperactivity along with behavioral changes. Crucially, all pathologies were prevented by NOX2 inhibition. Two separate studies on A β -overexpressing AD model mice lacking NOX2 showed a significant attenuation of cognitive impairment, cerebrovascular dysfunctions, and tau hyperphosphorylation [40,41]. Taken together, our and others'

studies indicate that AE and AD share fundamental pathological mechanisms related to network excitability that are initiated by NOX-mediated oxidative stress and consequent glucose hypometabolism (Fig. 1).

The role of glucose in antioxidant defense.

ROS are inevitably generated as a by-product of multiple metabolic reactions during brain activity and to provide a proper balance between ROS generation and neutralization (cellular redox state) and to thus avoid oxidative stress, brain cells employ several antioxidant systems. The most powerful of these systems is the cytoplasmic one that uses **the glucose pentose-phosphate-pathway (PPP)** [42]. Importantly, the cellular redox state is also controlled by specific gene transcription factors (e.g. **Nrf2**) which regulate more than 200 genes including those containing an **antioxidant response element (ARE)** in their promoter which are responsible for the induction of enzymes involved in antioxidant defense [43]. Moderate oxidative stress, e.g. as the result of seizures, activates the Nrf2-ARE signaling pathway resulting in up-regulation of antioxidant and detoxifying enzymes which may alleviate oxidative damage. The increased Nrf2 mRNA levels have been observed in human hippocampal tissue obtained from treatment-resistant patients with temporal lobe epilepsy [44]. Artificial activation of the Nrf2-ARE signaling pathway resulted in a clear anti-epileptogenic effect following kainate-induced **status epilepticus** in rats [45]. Moreover, activation of Nrf2 strongly decreased ROS accumulation from NOX and mitochondria [46,47].

Counteracting oxidative stress by Nrf2-ARE signaling activation is mostly based on the increased expression of enzymes involved in pentose-phosphate-pathway which is exclusively based on glucose metabolism. One major function of pentose-phosphate-pathway is the generation of reducing equivalents (co-enzymes) in the form of NADPH required for neutralization of H₂O₂ by the glutathione system [13,42,48]. The PPP-based antioxidant defense is the fastest known adaptive response of brain cells to acute oxidative stress: glutathione system activation occurs in seconds while the onset of transcriptional responses takes hours [42,49]. PPP requires glucose-6-phosphate (**Glc-6-P**) availability; under physiological "resting" conditions the NADPH/NADP⁺ ratio is very high and Glc-6-P dehydrogenase is inhibited by NADPH, thus preventing Glc-6-P from entering the PPP. The cytoplasmic ROS regulation is mainly controlled by peroxiredoxins and glutathione peroxidases, with PPP estimated to account for 3-7% of overall brain glucose utilization in adult animals [13,50,51] and for about 7% in healthy humans [52]. In the healthy brain, NOX expression is maintained at low levels with low H₂O₂ steady-state estimated at 1-10 nM intracellularly (depending on the cell type) and about 100 pM in the cytoplasm [50,51]. Importantly, during oxidative stress, the PPP flux is amplified, rerouting the metabolic flow of Glc-6-P and enhancing the antioxidant

efficiency of the system [13,42,49] that can reach up to 30% of glucose utilization. Therefore, PPP has a large reserve capacity for upregulation, ensuring tight control of ROS levels even during physiological NOX activation.

Importantly, NADPH can be used by NOX for ROS production [48]; active NOX2 generates $O_2^{\cdot-}$ with **K_m** values for NADPH of 40–45 μ M [32]. However, NOX2 activation requires the assembly of six cytoplasmic subunits, and while NADPH is obligatory it alone is not sufficient for activation. Meanwhile, the basal cytosolic **NAD⁺** (NADP⁺ precursor) levels are in the 200-500 μ M range [53] while NADPH levels are about 100 μ M [54], so if the activation factor prompts NOX assembly there are always sufficient basal NADPH levels for it to function. Therefore, NOX activity should be unaffected by fluctuations in NADPH because its **K_m** values are far below the cytosolic NADPH levels. However, during oxidative stress the activated glutathione system "consumes" NADPH, thus limiting its availability for activated NOX. The outcome of the opposition of the two systems - NOX producing ROS and pentose-phosphate-pathway neutralizing them - thus depends on their relative efficiency.

In AE-related pathologies, cellular antioxidant defense fails to cope with ROS accumulation from excessive NOX activity and intracellular H_2O_2 levels may exceed 100 nM [50]. The resulting oxidative stress disrupts glucose utilization, leading to antioxidant defense deficiency and thus closing the harmful feedback loop with multiple pathological consequences for brain function. Unfortunately, attempts of using exogenous antioxidants to counteract this pathological oxidative stress proved to be ineffective (see Box 2).

Potential mechanisms behind ROS-induced hypometabolism and subsequent network hyperactivity.

ROS can suppress glycolysis by inhibiting multiple glycolytic enzymes including pyruvate kinase, phosphofructokinase, and glyceraldehyde-3-phosphate-dehydrogenase [55]. The exact molecular and cellular mechanisms of resulting hypometabolism-induced hyperexcitability are yet to be fully elucidated. However, we have shown previously that 2-DG [15] as well as $A\beta$ [21,30] induced significant depolarization of both the neuronal resting membrane potential and the reversal potential of GABA-mediated currents (which controls the efficacy of synaptic inhibition). Both parameters are **ATP**-dependent and regulate neuronal excitability, making them primary mechanisms that link brain hypometabolism with network hyperactivity [56].

Ketogenic diet is antiepileptic thanks to its reinforcement of glucose metabolism and antioxidant properties.

Glucose is the exclusive neurometabolite possessing a number of unique functions where it cannot be replaced by other metabolic substrates [57]. Therefore, a common notion that inhibition of glycolysis may be antiepileptic (e.g., [58]) is unjustified, especially considering the fact of glucose hypometabolism prevalence in epilepsy. Such proposition of glycolysis inhibition as means of treatment is typically based on the hypothesized action of the **ketogenic diet (KD)**. The antiepileptic efficacy of the KD is indisputable. The high-fat, low-carbohydrate KD is associated with the production of ketones (e.g. **β -hydroxybutyrate (BHB)**) in the liver which can substitute glucose as mitochondrial fuel. KD has been in use for almost a century for epilepsy treatment, sometimes being the only effective option in drug-resistant epilepsies [59]. For reasons yet unclear, the 2-DG actions have frequently been compared to those of KD, despite at least one obvious fundamental difference: in contrast to 2-DG, KD does not induce energy deprivation. It has been presumed that similar to 2-DG effects, brain glycolysis is inhibited during KD. However, this assumption is simply not supported by experimental data (discussed in more detail in [57]). In short, we posit that ketones are capable of partially replacing glucose as mitochondrial fuel and thus spare glucose for its other exclusive functions, which is especially important in pathology when glucose metabolism is disrupted. *In vivo* data from a recent experimental study [60] supports our hypothesis, concluding that by replacing glucose as a mitochondrial fuel, BHB spared glucose for its other important functions.

In case of epilepsy, one such function critical in pathogenesis is the antioxidant defense. Restoring the efficacy of impaired pentose-phosphate-pathway at early stages of AE may have profound benefits (see Fig. 2). In the last few years, researchers focused on possible ways of promoting endogenous antioxidant systems to counteract epileptogenesis and seizures [45,61]. KD can also activate the Nrf2-ARE signaling pathway [62] and therefore, KD exerts a dual effect on glucose metabolism: a) ketones replace glucose in mitochondrial utilization, thus sparing glucose for other functions; b) KD enhances the efficacy of pentose-phosphate-pathway antioxidant defense via activation of the Nrf2-ARE signaling pathway. In addition, ketone metabolism requires fewer NAD⁺ for mitochondrial ATP generation, so KD provides more NAD⁺ for NADP⁺ production [63].

Despite all the data, one common agreement on diverse beneficial effects of KD is that a fundamental mechanism of action remains elusive. We suggest it is the KD's effect on the interplay of oxidative stress and glucose metabolism that represents such a mechanism. Recently, the KD effect on NAD⁺ availability was also suggested as a candidate (reviewed in [63]), and we agree that

this may be an effect on a substantial part of a more general function of glucose metabolism. Meanwhile, a number of inferred molecular effects of the KD have also been reported (e.g., reviewed in [59]). However, it is surprising indeed that such an evident feature of KD as glucose sparing during pathological glucose hypometabolism has never been considered as the major beneficial mechanism of the diet despite the critical role of glucose in a number of fundamental cell functions.

Notably, in their anti-epileptic action, the effects of KD are in many respects similar to those resulting from pyruvate supplementation [18,64]. We showed previously that full substitution of glucose with pyruvate caused a metabolic collapse in the neuronal network -- despite the sufficient mitochondrial fuel provided by pyruvate -- a pathology induced by acute oxidative stress [65]. However, at normal extracellular glucose concentrations, when glucose utilization was impaired due to oxidative stress induced either by seizures or A β , addition of pyruvate resulted in a significant and rapid (within a few minutes) normalization of glucose utilization [18,30]. Moreover, *in vivo*, pyruvate administration showed a strong antiepileptic effect in three different chronic AE models ([64]; see also [66]). Analyzing the pyruvate effect mechanisms ([18,64], we emphasized the capacity of pyruvate to directly scavenge H₂O₂ as well as pyruvate's potential to prevent overactivation of **Poly [ADP-ribose] polymerase 1 (PARP-1)** ([67]; see their Fig. 1). PARP-1, a molecule implicated in the regulation of several DNA repair processes, consumes NAD⁺ in order to function [68]; its overactivation is mainly caused by oxidative stress [68,69] and was found to be associated with NAD⁺ depletion in different epilepsy models [63]. The transformation of extra pyruvate to lactate is associated with oxidation of NADP to NAD⁺, thus increasing NAD⁺ amount available for NADP⁺ generation, akin to the KD effect. Finally, pyruvate's ability to replace glucose as mitochondrial fuel is unequivocal. These properties of pyruvate presumably play a major role in its antiepileptic efficacy and seem to mirror the features of KD, supporting our hypothesis that reinforcement of glucose metabolism is the major pathway of KD's antiepileptic effects.

Concluding Remarks and final perspectives

A variety of brain insults, often possessing radically different etiologies, result in a similar outcome of brain hyperactivity defined as acquired epilepsy. Considering the fundamental brain functions unifying the pro-epileptic effects of all the risk factors, we conclude that epileptogenesis is likely underlain by a positive feedback loop of oxidative stress and glucose hypometabolism. Of critical importance in this cycle is the dominant role of NOX in pathological oxidative stress as well as its involvement in glycolysis inhibition and seizure generation. Based on ours and others' experimental data we propose that a combinatory treatment merging NOX inhibition and enhancement of endogenous antioxidant defense may provide a long-awaited way to treat AE. Analysis also suggests

that the primary mechanism behind the antiepileptic action of the KD is the boosting of glucose metabolism. Encouraging results supporting this rationale have been reported recently [45] where inhibition of NOX coupled with Nrf2 pathway activation showed a strong antiepileptic effect. More studies are certainly needed to test a combinatory treatment adequate for medical applications, which would include clinically relevant NOX inhibitors together with Nrf2 pathway activators and glucose metabolism promoters (e.g., pyruvate or ketogenic diet). Such a regime must first be tested chronically *in vivo* using several accepted models of AE. Hopefully, these investigations will be performed in the near future and will help advance strategies of effective AE prevention and treatment.

Funding

This work was supported by grant R01AG061150 to MZ from the National Institutes of Health and RSF grant #20-65-46035 to IP.

[The authors declare no conflicts of interest.](#)

Text Box 1

The role of NOX in AE and AD.

NOX has emerged recently as a major source of oxidative stress in various neurological diseases including epilepsy [5,7,29] and AD. One study on drug-resistant patients revealed the presence of activated NOX in their surgically resected tissue [7]. Following brain trauma, NOX is a major ROS source contributing to the pathophysiology [5] and mediates trauma-induced loss of inhibitory interneurons in neocortex and hippocampus (which alone can prompt epileptogenesis). In rats following kainate treatment, inhibition of NOX in parallel with the enhancement of cellular antioxidant defense via activation of Nrf2 completely prevented epileptogenesis [45].

AE shares a lot of common symptoms and pathologies with AD [70]. Moreover, drug-resistant temporal lobe epilepsy patients are up to 10x more likely to develop AD [71] and display increased A β ₁₋₄₂ levels and hyperphosphorylated tau [70]. One of the earliest clinical markers for AD that is shared with AE and is often detected concurrently with A β positivity is glucose hypometabolism. It

occurs in **MCI patients**, has been detected in AD patients almost two decades prior to the onset of clinical symptoms, and has been proven to be an accurate predictive marker for AD development [36,37]. Importantly, brain hypometabolism in AD is always associated with oxidative stress [38]. Studies including post-mortem analyses of AD patients' cerebral cortices have shown that oxidative stress resulting from NOX2 activation plays a significant role in the development of AD [9,33]. In humans, there was a robust correlation between NOX activity and the individual's cognitive status such that as the enzyme activity increased, cognitive performance decreased [72]. More specifically, A β was reported to induce brain oxidative stress [38] largely via activation of NOX [9,39], and correlation between the levels of A β and NOX2 activity has been reported in multiple studies of AD patients [73]. Data suggest that blocking the initial A β -induced NOX2 activation in AD can potentially prevent all the resulting pathologies shared with AE, from glucose hypometabolism to network hyperactivity, highlighting the critical role of NOX-mediated oxidative stress in AE.

Text Box 2

Exogenous antioxidants are unlikely to prevent oxidative damage from oxidative stress.

Given the critical role of oxidative stress in various neurological diseases, treatment strategies using exogenous antioxidants would make sense. However, so far clinical trials on antioxidants for MCI and AD have been disappointing [20,74,75], and clinical data on antioxidants in epilepsy are not available [7]. In animal experiments, despite the acknowledged importance of redox signaling in various brain functions, the majority of up-to-date studies used fluorescent probes for ROS detection. Such methods lack sufficient temporal resolution and therefore, with a few exceptions, the dynamic, real-time measurements of ROS generation during network activity in the brain slices or *in vivo* have been lacking. We recently used the amperometric technique [76] for measuring extracellular H₂O₂ during network activation both in hippocampal slices and *in vivo* [18,19], and found that the dynamic release of ROS from activated cells is rapid, reaching peak levels in less than 2s. Notably, we tested the effects of a potent antioxidant Tempol [77,78] and apocynin on extracellular H₂O₂ levels. Tempol is significantly more effective than most other frequently used antioxidants and is far more effective than vitamins [77]; apocynin is a strong antioxidant and also inhibits O₂⁻ release by NOX in the presence of myeloperoxidase [79]. Nevertheless, even at high concentrations (4 mM) and applied directly onto brain slices, Tempol did not reduce the amplitude of network activation-induced H₂O₂ transient by any more than 20%. At the same time, both apocynin (0.5 mM) and Tempol failed to buffer rapid H₂O₂ release during epileptic seizures (see Malkov et al. Suppl. Figure 1 [18]). These results provide a reasonable explanation as to why the antioxidant treatments failed in multiple clinical trials [75]: following oral or systemic administration, the exogenous antioxidants are highly unlikely to reach brain levels sufficient for effective inhibition of ROS generated during intense

network activity. Therefore, any effective treatment of brain oxidative stress should not attempt to scavenge ROS but should target the sources of ROS production instead.

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Legends to figures

Figure 1. Relationship between oxidative stress, glucose hypometabolism, and seizures in AE.

A. Schematic representing basic outcome of oxidative stress on brain metabolism, summarizing data from three studies. We have previously shown that simulating acute oxidative stress by direct application of H₂O₂ results in a robust decrease in glucose consumption and glycolysis, paralleled by an increase in oxidative phosphorylation (presumably as compensation). Same outcomes were observed after exposure to AD-associated toxic peptide amyloid-beta (A β ₁₋₄₂) as well as following induced seizures and/or network hyperactivity. The fact that three fundamentally different experimental paradigms resulted in identical outcomes suggests that they share the same underlying mechanism of action. Importantly, NOX blockade completely prevented the hypometabolism resulting from A β or seizures, highlighting a central role of this enzyme in pathogenesis.

B. The vicious cycle of multiple reciprocal oxidative stress - glucose hypometabolism - seizures feedback loops initiated by NOX overactivation.

Figure created with [Biorender.com](https://biorender.com).

Figure 2. Glucose functions are critical in preventing epileptogenesis.

Glucose enters the brain cell via glucose transporters (GLUT) and enters the glycolytic pathway to produce pyruvate which is converted to acetyl coenzyme A (Acetyl-CoA) as mitochondrial fuel. The pentose-phosphate pathway metabolizes Glc-6-P produced in glycolysis and utilizes the glutathione

pathway to mitigate oxidative stress (see details in [13]). NADH oxidase (NOX), a major contributor to oxidative stress, can be activated in neurons (and potentially astrocytes) by NMDA receptors as well as by amyloid- β ($A\beta_{1-42}$). Reactive oxygen species (ROS) depress glycolysis via inhibition of **glyceraldehyde 3-phosphate-dehydrogenase** (GAPDH) and via overactivation of Poly [ADP-ribose] polymerase 1 (PARP-1) (discussed in [55,56]).

Nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response elements (ARE) signaling regulates the expression of antioxidant proteins. Exogenous pyruvate and ketones (e.g. β -hydroxybutyrate, BHB) enter the cell via monocarboxylate transporters (MCT). Both substrates can substitute glucose for mitochondria fueling, and in addition, in pathological conditions pyruvate restores depressed glycolysis and prevents overactivation of PARP-1, while the ketogenic diet activates the Nrf2 pathway.

Glossary

2-DG: 2-deoxy-D-glucose is a glucose analog that competitively inhibits glucose phosphorylation by hexokinase. Metabolism of DG is limited and it is not further metabolized beyond the DG-6-P step by enzymes in the glycolytic pathway.

Acquired epilepsy: In acquired epilepsies, spontaneous seizures begin after injury to a normal brain as a consequence of trauma, stroke, infection, status epilepticus, or neurological diseases.

ATP: Adenosine triphosphate, a universal source of energy for all biochemical processes.

A β : Amyloid-beta peptide, the main component of the amyloid plaques in tissues of patients with Alzheimer's disease.

BHB: β -hydroxybutyrate, is a ketone body that can be used as an energy source by the brain.

FDG-PET: Positron emission tomography (PET) with the tracer [^{18}F]-fluorodeoxyglucose (FDG) as a marker for the tissue uptake of glucose.

GA3P&GAPDH: Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) is an enzyme that catalyzes glyceraldehyde-3-phosphate (GA3P) oxidation using NAD $^{+}$. GAPDH is an important target of oxidative stress and is inhibited by H $_2$ O $_2$.

Glucose pentose-phosphate-pathway: PPP, a metabolic pathway parallel to glycolysis, metabolizes Glc-6-P and generates NADPH required for antioxidant defense. It also produces ribose 5-phosphate, a precursor for the synthesis of nucleotides.

Epileptogenesis: epileptogenesis is the gradual process by which a normal brain develops epilepsy.

Ictal: ictal is defined as the period of a seizure and interictal refers to the period between seizures.

Km values: Km is the Michaelis-Menten constant, the concentration of substrate providing half of enzyme maximal activity.

KD: ketogenic diet, a high-fat/low-carbohydrate diet that in medicine is used mainly to treat refractory epilepsy in children.

MCI patients: Mild cognitive impairment (MCI) is an early stage of memory loss or other cognitive ability loss (such as language or visual/spatial perception) in individuals who maintain the ability to independently perform most activities of daily living.

NAD⁺: Nicotinamide adenine dinucleotide is a coenzyme central to metabolism.

NADPH: Nicotinamide adenine dinucleotide phosphate, a cofactor used in anabolic reactions.

NOX: NADPH oxidase, a transmembrane enzyme that is currently the only enzyme known to produce ROS as its sole function that are mostly used by phagocytes for the "host defense" (e.g., microbial killing) in organisms.

Nrf2 & ARE: The transcription factor Nrf2 (nuclear factor E2-related factor 2) targets cellular defense genes containing antioxidant response elements (ARE). Activation of these protective genes in response to Nrf2 [signaling](#) enables the cell to maintain redox balance and to remove damaged proteins under conditions of oxidative.

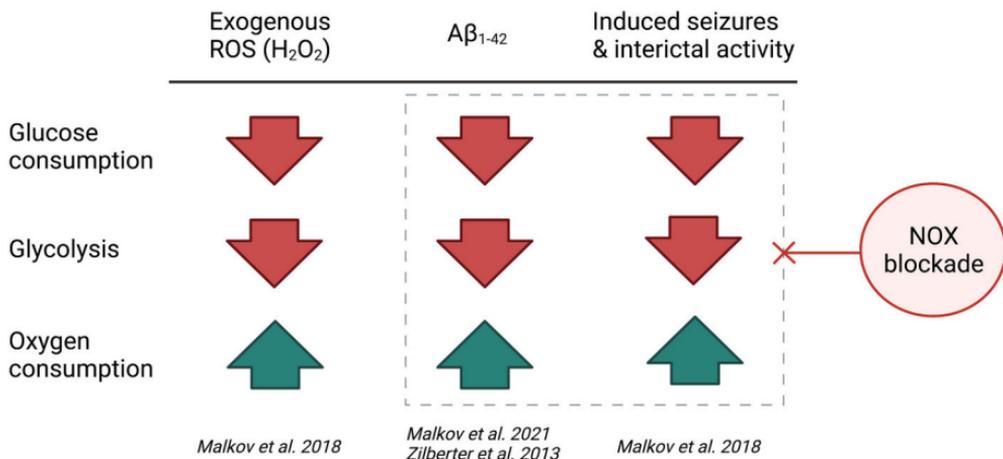
PARP-1: Poly(ADP-ribose) polymerase-1, a nuclear enzyme utilizes NAD to synthesize poly(ADP-ribose) for repairing DNA breaks following various injuries. As PARP-1 utilizes NAD⁺ to form poly(ADP-ribose) polymers, extensive PARP-1 activation may result in energy failure because of NAD⁺ depletion.

ROS: Reactive oxygen species, highly reactive chemical molecules such as superoxide anion (O²⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (HO^{*}), consist of radical and non-radical oxygen species formed by the partial reduction of oxygen.

Status epilepticus: Status epilepticus is a single seizure lasting more than 5 minutes or two or more seizures within a five-minute period without the person returning to normal between them.

A

Experimental paradigm



B

