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Linking early-life NMDAR hypofunction and oxidative stress in schizophrenia pathogenesis — Source link

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**Linking early-life NMDAR hypofunction and oxidative stress in schizophrenia
pathogenesis**

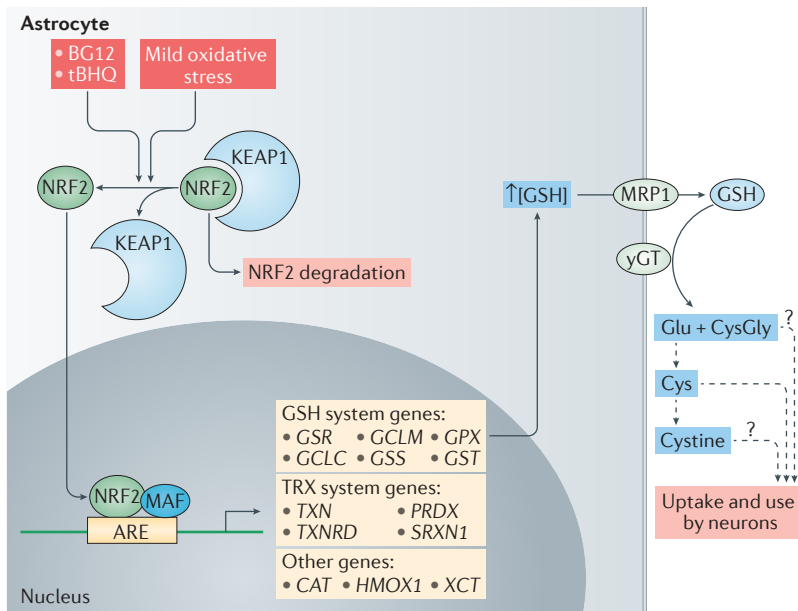
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Figure 5



Abstract | Molecular, genetic and pathological evidence suggests that deficits in GABAergic parvalbumin-positive interneurons contribute to schizophrenia pathophysiology through alterations in the brain's excitation-inhibition balance that result in impaired behaviour and cognition. Although the factors that trigger these deficits are diverse, there is increasing evidence that they converge on a common pathological hub that involves NMDA receptor hypofunction and oxidative stress. These factors have been separately linked to schizophrenia pathogenesis, but evidence now suggests they are mechanistically interdependent and contribute to common schizophrenia-associated pathology.

Schizophrenia is a psychiatric disorder that affects 1% of the population, with typical onset at late adolescence and early adulthood. Current treatments, which primarily induce D(2) dopamine receptor (DRD2) blockade, are most effective for the positive symptoms of schizophrenia (such as hallucinations or delusions) but have little effect on the negative symptoms (such as flattening affect or social withdrawal) or cognitive deficits (such as impaired memory, attention, and executive functions) and have major side effects.

The development of more effective schizophrenia treatments requires a better understanding of disease aetiology and the underlying mechanisms of what is a multi-factorial disorder. Both human epidemiological and animal model studies point to genetic and environmental factors that affect critical periods in early and adolescent brain development and that take place in advance of symptoms ¹. Pathologically, many diverse causes of schizophrenia and schizophrenia-like behaviours appear to converge on a similar deficit: the aberrant function of fast-spiking parvalbumin-positive interneurons (PVI) ², which leads to altered excitation-inhibition balance ³. Together with myelination defects ⁴, this may account for the emergence of schizophrenia pathophysiology and symptoms in early adulthood. The relatively long period over which adverse genetic and environmental factors integrate to cause schizophrenia, coupled with the existence of a clear prodromal phase, points to a therapeutic opportunity that mechanistic insight into schizophrenia causation may allow clinicians to exploit.

In this Perspective article, we summarize recent research that suggests that diverse causes of schizophrenia converge on a pathological hub that is centred on two interdependent factors: brain redox imbalance and NMDA receptor (NMDAR) hypofunction. We describe evidence that this pathological hub, when activated during development, might lead to some of the key hallmarks of schizophrenia, including defects in PVI and in the

Figure 3

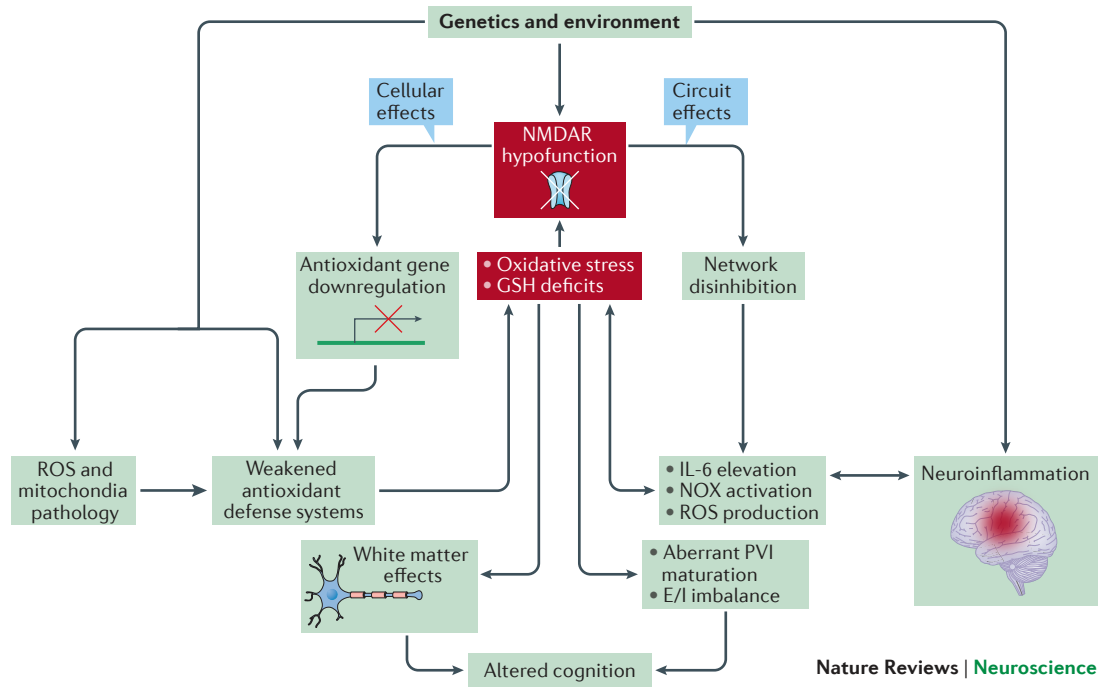
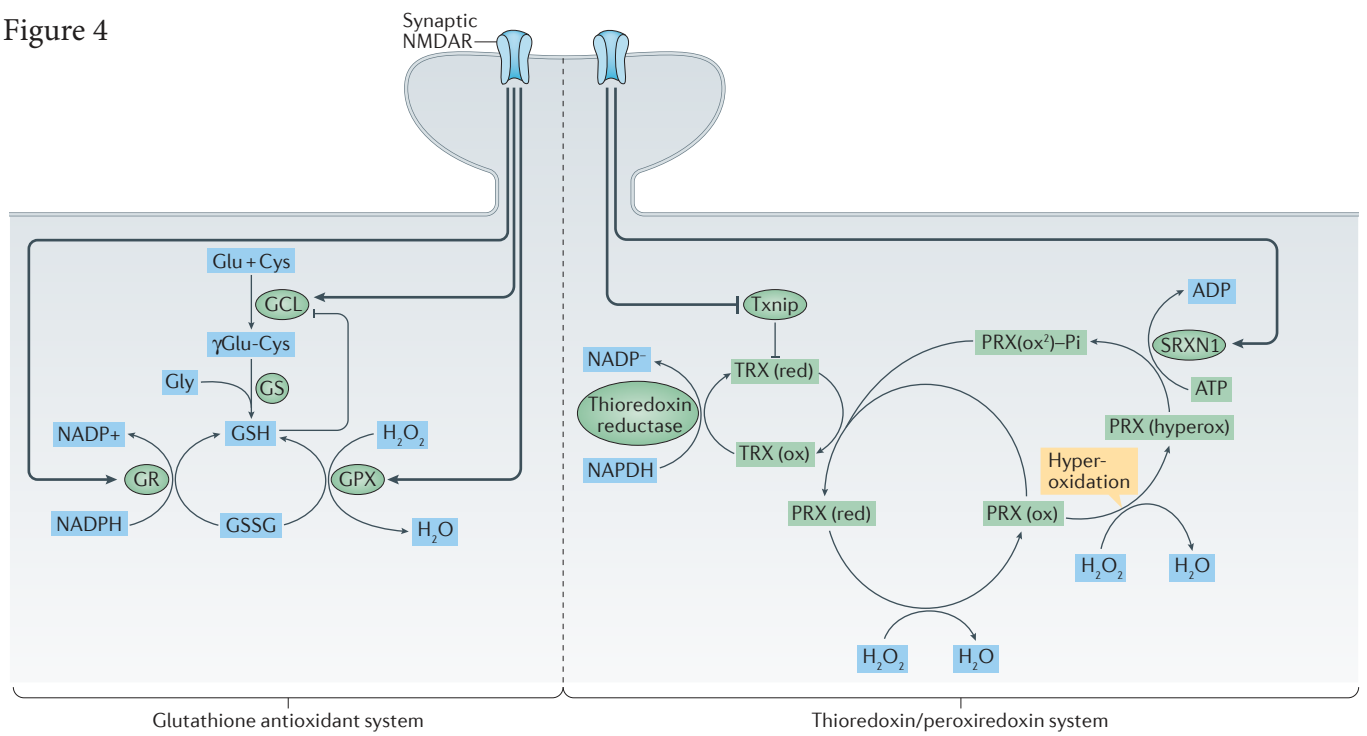


Figure 4



function of their associated networks, as well as high frequency neuronal synchrony^{2, 5}. We also discuss the clinical studies that will be required to prove or disprove this hypothesis and to determine which therapeutic strategies may prove effective in targeting this pathological hub both before and after diagnosis.

NMDAR dysfunction in schizophrenia

NMDARs (N-methyl-D-aspartate (NMDA) receptors) are glutamate-gated cation-passing channels that play a key role in the CNS. They are permeant to Ca^{2+} , which mediates many of the consequences of NMDAR activity, including synaptic modification, learning and memory, activity-dependent development, and neuroprotective/homeostatic signaling⁶⁻⁸.

Human studies suggest NMDAR hypofunction

The hypothesis that NMDAR hypofunction might be a pathogenic trigger for schizophrenia⁹,¹⁰ arose from observations that administration of the dissociative anesthetics phencyclidine and ketamine to healthy subjects mimicked the primary symptoms of schizophrenia^{11, 12} coupled with the finding that these compounds are NMDAR antagonists¹³. Recently, autoimmune diseases associated with anti-NMDAR antibodies were reported to be associated with severe psychosis¹⁴. Subsequent behavioral, neurophysiological and functional imaging studies have supported the NMDAR hypofunction hypothesis of schizophrenia, showing that indicators of sensory dysfunction in patients with schizophrenia — such as mismatch negativity (MMN) and changes in auditory and visual event-related potentials — can be mimicked by treating otherwise healthy individuals with NMDAR antagonists^{12, 15}. Post-mortem studies of the brain of patients with schizophrenia have revealed lower levels of expression of the obligate NMDAR subunit GRIN1 (glutamate receptor ionotropic, NMDA 1), increases in the expression of the endogenous NMDAR antagonist kynurenate, and a reduction in levels of the NMDAR co-agonist D-serine and the enzyme that catalyses its production, D-serine racemase^{10, 16-18}. An NMDAR single-photon emission computed tomography (SPECT) study pointed to lower NMDAR activity in unmedicated patients with schizophrenia¹⁹, although this finding awaits confirmation with newer, **more specific** probes²⁰. Proton magnetic resonance spectroscopy (MRS studies) revealed hyperglutamatergic activity in patients with schizophrenia and groups at high risk of developing schizophrenia, which may be attributable to NMDAR hypofunction²⁰.

Genetic evidence also points to disturbed NMDAR signaling in schizophrenia. A recent genome-wide association study (GWAS) implicated serine racemase (*SRR*) and *GRIN2A* as risk genes for schizophrenia²¹ as well as other genes functionally up- and downstream of NMDAR activity^{21, 22}. Exome sequencing has revealed schizophrenia-linked de novo mutations in *GRIN2A* and *GRIN2B* [Tarabeux, 2011 #3386; Myers, 2011 #3486] which

Figure 1

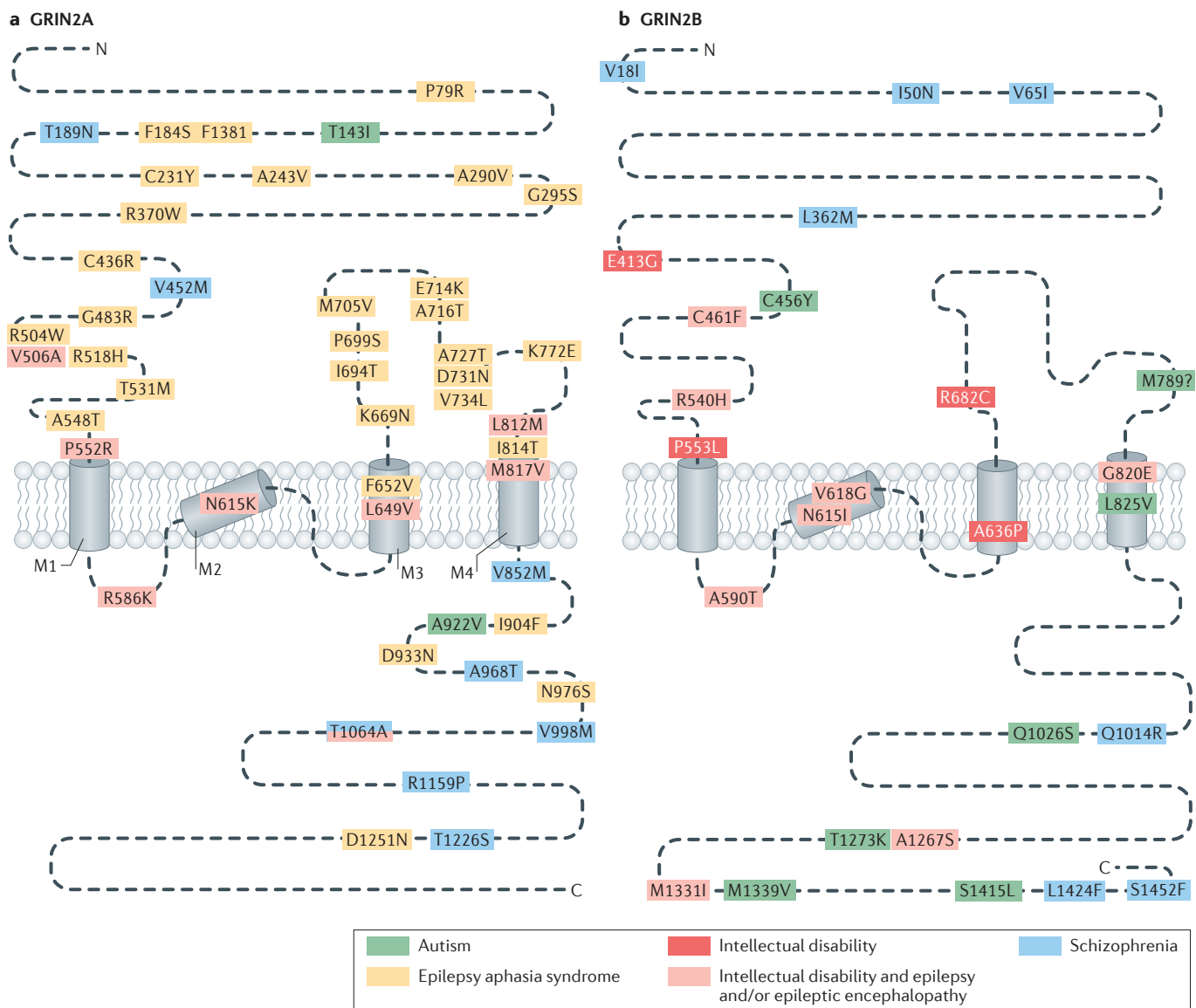
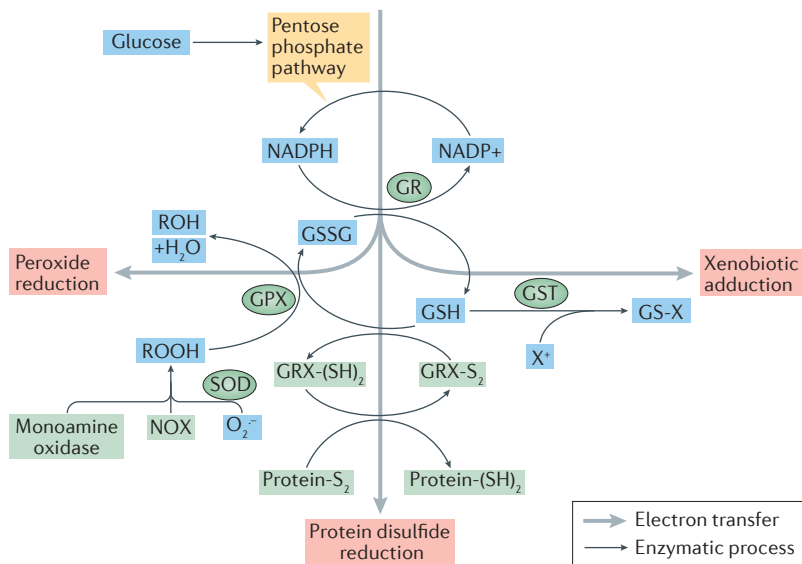


Figure 2



is consistent with a growing number of NMDAR subunit mutations associated with neurodevelopmental disorders (Figure 1).

NMDAR hypofunction in animal models

As in humans, treatment of adult rodents with NMDAR antagonists triggers acute schizophrenia-related behaviours, including deficits in attention and/ or vigilance, learning and memory and sensory gating, broadly mimicking the symptoms of patients with schizophrenia^{10, 15, 23, 24}. Genetically-modified mouse models of NMDA hypofunction — including the *Grin1* hypomorphic mouse²⁵ and the *Srr* knockout mouse²⁶ — have also revealed schizophrenia-relevant phenotypes and neurochemical deficits.

Specific vulnerability to NMDAR hypofunction

The existence of prenatal risk factors for schizophrenia, such as maternal infections, nutritional deficiency, obstetrical complications and stress, suggests that there is a developmental window of sensitivity in which a transient early insult leads to long-lasting developmental perturbations¹. In supporting of this notion, NMDAR antagonism during an equivalent period in rodents (first 2 post-natal weeks) induces long-lasting behavioural and cognitive disturbances that are relevant to the schizophrenia phenotype and which extend into adolescence and adulthood^{10, 27}. Moreover, severe NMDAR blockade induces forebrain apoptosis in rats, but only when administered between the first and third postnatal week²⁸. A milder antagonism regime caused long-lasting behavioural changes and a specific long-lasting decrease in cortical PVI numbers²⁹, mimicking a key pathological feature of schizophrenia².

Genetic models also support the notion of the developmental sensitivity of PVIs to NMDAR hypofunction. Early post-natal, but not adolescent, deletion of *Grin1* in mouse forebrain interneurons reduced their expression of parvalbumin and glutamate decarboxylase 1 (GAD1, also known as GAD67), and caused cortical disinhibition and asynchrony^{30, 31}. This interneuron-specific deletion was sufficient to induce schizophrenia-relevant phenotypes after adolescence, including novelty-induced hyperlocomotion, deficits in mating and nest-building and anxiety-like behaviors³⁰, as well as spatial memory impairment³¹. The behavioural deficits induced by interneuronal *Grin1* deletion are exacerbated by social isolation stress³⁰, which models aspects of known environmental risks for schizophrenia, including childhood maltreatment and psychosocial adverse events¹.

Thus, studies suggest that aberrant NMDAR function during development particularly affects PVIs and that these effects are sufficient to induce long-lasting delayed behavioural and cognitive abnormalities in the adult.

Redox imbalance in schizophrenia

Oxidative stress, is defined as an imbalance between levels of prooxidants and antioxidants and is known to result in macromolecular damage and the activation of redox-sensitive signals. The maintenance of redox balance in the brain is challenging due to its high lipid content and metabolic rate, as well as the non-regenerative nature of CNS neurons³²⁻³⁴. During development, even subtle perturbations to redox balance can affect the signaling pathways and processes involved in neurogenesis and neuronal differentiation³⁵.

The antioxidant systems employed to neutralise reactive oxygen species (ROS) and reverse oxidative damage in the brain have been reviewed comprehensively elsewhere^{32, 34}. These systems function to maintain redox balance by supplying reducing equivalents (electrons) to electrophilic xenobiotics, ROS and proteins. As described below, the antioxidant glutathione (GSH) has special relevance to schizophrenia pathophysiology (Figure 2).

Human studies of redox imbalance

Evidence for oxidative stress has been found in patients with schizophrenia, including increased lipid and protein oxidation in blood, cerebrospinal fluid (CSF) and post-mortem tissue^{36, 37}, as well as altered levels of plasma antioxidants (such as vitamins C and E, catalase, GSH and GSH-peroxidase)^{36, 38} and CSF superoxide dismutase-1 levels³⁹. Proteomic postmortem studies also point to the activation of oxidative stress responses in schizophrenia⁴⁰. In particular, GSH system deficits have been linked to schizophrenia pathophysiology in several ways. GSH levels have been shown to be lower in chronic schizophrenia, as measured in the CSF and post-mortem brain, and by MRS-based in vivo imaging^{41, 42} and the GSH system enzymes glutathione peroxidase (GPX) and glutathione reductase (GR) are also dysregulated³⁶. Importantly, low cortical GSH levels have been found to correlate with more severe negative symptoms in patients with schizophrenia⁴³.

Genetic evidence also points to a role for GSH system deficits in schizophrenia etiopathogeny. An allelic variant of the *GCLC* gene, which encodes the catalytic subunit of GSH biosynthetic enzyme glutamate-cysteine ligase (GCL) is associated with schizophrenia. This polymorphism is associated with reduced induction of GSH, GCL activity, and *GCLC* expression in schizophrenia patient-derived fibroblasts⁴⁴. Evidence that copy number variants and polymorphisms within glutathione-s-transferase (GST) genes may be susceptibility factors for schizophrenia has also been revealed⁴⁵.

Animal studies of redox imbalance

Animal studies suggest that brain GSH deficits and oxidative stress are sufficient to induce schizophrenia-like behaviour. Mice deficient in the GCL regulatory subunit *Gclm* have

reduced brain GSH levels and exhibit schizophrenia-relevant behavioural and cognitive deficits, including altered stress responses, amphetamine responses, social behaviour, prepulse inhibition (PPI) and learning ^{46, 47}. Pharmacological depletion of brain GSH using a GCL inhibitor induces similar sensory and cognitive disturbances ^{48, 49}.

Investigations of the biological roles of known schizophrenia risk genes such as dystrobrevin binding protein 1 (*DTNBP1*, *dysbindin-1*), proline dehydrogenase 1 (*PRODH*), neuregulin 1 (*NRG1*), D-amino acid oxidase activator (*DAOA*, also known as *G72*) and disrupted in schizophrenia 1 (*DISC1*) also suggest that oxidative stress may be involved in schizophrenia etiopathogeny. For example, DTNBP1 forms a complex that interacts with peroxiredoxins 1 and 2 ⁵⁰, key CNS antioxidant proteins ³³, and DTNBP1 is itself degraded by oxidative stress ⁵¹. PRODH is a mitochondrial flavoenzyme that metabolizes proline and is essential for the antioxidant effects of proline ⁵². NRG1–ERBB4 signalling is involved in ROS-induced neuronal differentiation ⁵³. Proteomic analysis of the brains of mice transgenic for the primate specific *G72* gene revealed perturbations associated with oxidative stress, mitochondrial dysfunction and white matter deficits ⁵⁴. Oxidative stress is also observed in a mouse expressing a dominant negative form of DISC1 ⁵⁵. The causal relevance of oxidative stress was further strengthened by the demonstration that juvenile antioxidant treatment using N-Acetyl cysteine (NAC) prevents oxidative stress in a developmental rat model of schizophrenia (neonatal ventral hippocampal lesion (NVHL)) and, in doing so, inhibits the emergence of morphological, electrophysiological and behavioural deficits ⁵⁶. Cognitive deficits in the *G72* transgenic mouse were also rescued by NAC therapy ⁵⁷.

Specific vulnerability to oxidative stress

PVIs are highly sensitive to redox status and ROS signalling. For example, neuron-specific reduction of the levels of selenoproteins (a group of proteins that include several antioxidants) selectively impaired PVI development ⁵⁸, which was also observed in the prefrontal and ventral hippocampal regions in *Gclm*-deficient mice ^{46, 59, 60}. Targeted deletion of *Gclc* in PVIs triggers oxidative stress, impairs their development and causes a delay and prolongation in the critical period of cortical plasticity resulting in a failure to stabilise cortical circuits ⁶¹. Moreover, adult mice in which *Gclc* was deleted specifically in PVIs exhibit impaired contralateral bias index following monocular deprivation, indicative of long-lasting defects ⁶¹.

The vulnerability of PVI function to oxidative stress is high early in development, resulting in permanent consequences for the adult: in *Gclm*-deficient mice, oxidative challenges at juvenile and peripubertal ages, but not in the adult, lead to long-lasting PVI impairments in the prefrontal cortex (PFC) ^{59, 60}. Moreover, transient pharmacological depletion of brain GSH early in development (between postnatal days 5 and 16) leads to PVI

abnormalities in the adult anterior cingulate cortex as well as cognitive and olfactory discrimination deficits ⁴⁹. Transient developmental disruption of DISC1 signaling using an inducible and reversible transgenic model results in defects in plasticity in the adult cortex ⁶². Given that disrupting DISC1 signalling induces oxidative stress and reduces PVI immunoreactivity ^{55,63}, it is tempting to speculate that oxidative stress is a contributor to these observed defects. Evidence causally linking oxidative stress to PVI deficits in other schizophrenia models also exists: NAC treatment prevents PVI abnormalities in the NVHL model ⁵⁶ and blocking superoxide overproduction by NADPH oxidase (NOX) prevents the PVI impairment induced by social isolation ⁶⁴.

Mechanistically, PVI deficits after oxidative stress could be due to loss of ensheathing perineuronal nets (PNN)-networks of extracellular matrix, which play a protective role against oxidative stress and yet are vulnerable themselves to ROS ⁶⁰. Moreover, the high intrinsic spiking rate of PVIs, with its attendant metabolic demands and ROS production, may place particular demands on their antioxidant defences. One outstanding issue is the mechanism by which oxidative stress results in the long-lasting reduction of GAD and PV expression and whether this reflects the activation of specific ROS-activated signals such as dopamine metabolites, or is simply the result of a global defect in development due to non-specific oxidative damage. It is also important to note that white matter deficits are another schizophrenia-relevant pathology influenced by oxidative stress in development ⁶⁵. A correlation exists between PFC GSH levels and white matter integrity in the cingulum bundle in both control and early psychosis patients, which may reflect the role of GSH in controlling oligodendrocyte progenitor proliferation and differentiation ⁶⁶.

To conclude, several schizophrenia models provide evidence of oxidative stress that preferentially induces PVI deficits. Moreover, development stage- or PVI-specific oxidative stress is sufficient to induce long-lasting behavioural and cognitive abnormalities in the adult.

Making the link

There are clear similarities between the impact of developmental NMDAR hypofunction and that of oxidative stress on the adult rodent: both cause a selective impairment in PVI function and similar behavioural and cognitive disturbances. Indeed, increasing evidence suggests that NMDAR hypofunction and redox imbalance may be reciprocally linked (Figure 3).

The NMDAR is regulated by redox state: both GRIN1 and GRIN2A possess pairs of redox-sensitive cysteine residues whose disulfide bond formation decreases NMDAR currents ^{67, 68}, while an overlapping group of cysteine residues are subject to inhibitory S-nitrosylation which facilitates disulfide bond formation ⁶⁷. Redox regulation is particularly strong for GRIN2A-containing NMDARs, where a region of the N-terminus is sufficient to mediate the potentiation of currents by reducing agents such as Dithiothreitol or GSH ^{68, 69}.

Of note, even transient GSH deficits are sufficient to induce NMDAR hypofunction⁷⁰. Recently it has been shown that changes in intracellular redox status can also modulate NMDAR activity in a manner that is relevant to age-dependent cognitive decline^{71, 72}. Age-associated shifts in intracellular redox state to a pro-oxidizing environment have been linked to reduced NMDAR activity via the redox regulation of calcium/ calmodulin-dependent protein kinase type II (CaMKII), and can be rescued by intracellular GSH⁷². Thus, the redox balance of the brain, acting in no small part via GSH, controls NMDAR activity.

Critically, there is also a reciprocal relationship: NMDAR hypofunction itself leads to cortical oxidative stress and GSH deficits^{73, 74}. At the cellular level, synaptic NMDAR activity enhances the capacity of both the glutathione system and thioredoxin/peroxiredoxin system (another important antioxidant pathway in the brain⁷⁵) through the transcriptional control of several key antioxidant genes^{73, 76, 77} (Figure. 4). Of note, the GSH deficit induced by NMDAR blockade in the developing mouse is associated with transcriptional downregulation of *Gclc* and reduced GCL activity, which contributes to the deleterious effects observed *in vivo*⁷⁷. Moreover, GABAergic interneuron-specific deletion of *Grin1* has been shown to lead to increased ROS levels which are exacerbated by post-weaning social isolation, in association with down-regulation of peroxisome proliferative activated receptor- γ , coactivator 1 α (*PPARGC1A*, also known as *PGC-1 α*), a regulator of mitochondrial energy metabolism and antioxidant defences⁷⁸. The functional benefits of the coupling of synaptic NMDAR activity to the control of antioxidant defences are still a matter of speculation, although it may be an adaptive mechanism to tune neuronal antioxidant defences to the elevated needs of an electrically and metabolically active neuron. Thus, NMDAR hypofunction may uncouple synaptic activity from the regulation of antioxidant systems.

NMDAR hypofunction also contributes to oxidative stress through its circuit-level effects. NMDAR antagonism likely induces a hyperglutamatergic state by reducing the activity of cortical interneurons, which particularly rely on NMDARs for their excitatory drive early in development^{29, 79}. Moreover, and as a result, PVI function itself might be permanently impaired as a result of relatively mild transient NMDAR hypofunction in the developing mouse that does not induce neuronal death^{29, 80}. The mechanism underlying this proposed loss of PVI phenotype involves cortical disinhibition leading to neuronal IL-6 production and consequent activation of NADPH oxidase, which generates H₂O₂²⁹. One can envisage that both circuit and cellular-level effects could combine to exacerbate oxidative stress (Figure 3).

The weaker intrinsic antioxidant defences of neurons as compared to other brain cells such as astrocytes³⁴ may underly their vulnerability to oxidative stress, especially during development. Indeed, they express little nuclear factor erythroid 2-related factor 2 (NFE2L2, also known as NRF2), a transcription factor and master regulator of antioxidant defence

genes^{81, 82} (Figure 5). NFE2L2 is epigenetically repressed in cortical neurons early in development and what little NFE2L2 exists is highly unstable, leading to reduced intrinsic antioxidant defences^{81, 83}. Weakened defences appear to be necessary to activate redox-sensitive neuronal maturation pathways⁸¹. However, this deprives neurons of a useful adaptive response, since NFE2L2-dependent gene expression is activated by mild oxidative stress^{82, 84, 85}. Interestingly, many of the antioxidant genes transcriptionally controlled by synaptic activity in neurons are known NFE2L2 target genes, but are induced via NFE2L2-independent routes (e.g. via activator protein 1 (AP-1) family and activating transcription factor 4 (ATF4)), suggesting that activity-dependent signaling may act as a substitute for an absent NFE2L2 pathway^{73, 86, 87}. Moreover, a recent study has shown that persistent activation of astrocytic NMDARs can activate NFE2L2-dependent gene expression, raising the possibility that synaptic activity can control the NFE2L2 pathway in astrocytes⁸³.

Another consequence of relatively weak intrinsic antioxidant defences is that neurons rely on astrocytes to provide extrinsic support⁸². In response to oxidative stress, astrocytes increase GSH production (via NFE2L2-dependent mechanisms) and release. The released GSH is broken down and used for neuronal GSH production (Figure 5). The vulnerability of cortical neurons to oxidative stress and NMDAR hypofunction early in development could be due to the relatively low number of astrocytes at this stage, coupled with the high metabolic demands of rapid synaptogenesis. Regardless, the interdependence of NMDAR hypofunction and oxidative stress in development means that different genetic and environmental factors can potentially converge on a similar pathological outcome underlying schizophrenia-like phenotypes (Figure 3).

Interactions with neuroinflammation

Considerable evidence implicates neuroinflammation in the etiology of schizophrenia⁸⁸. Epidemiological evidence points to maternal infection being a risk factor in schizophrenia¹ and maternal infection in mice is sufficient to induce long-term PPI and social behavioural changes in the offspring, changes that are attributable to inflammation⁸⁹. Inflammation is also likely to be an aggravating factor in the effects of NMDAR hypofunction and oxidative stress: prenatal immune challenge by the viral mimetic Poly:I:C causes a long-lasting sensitivity of the offspring to peri-pubertal stress and NMDAR antagonists⁹⁰. Furthermore, PVI deficits in the DISC1-dominant negative transgenic mouse are exacerbated by a neonatal Poly:I:C challenge⁹¹.

The deleterious consequences of neuroinflammation may in any case be mediated in part by oxidative stress, and so be mechanistically coupled to the oxidative stress-NMDAR hypofunction hub of schizophrenia etiology (Figure 3). Activated innate immune cells both produce and are activated by ROS: antioxidant therapy with NAC reduces the pathological

and behavioural consequences of maternal LPS treatment and reduces foetal pro-inflammatory cytokine production^{92, 93}. At the molecular level, the antagonism between antioxidant-promoting NFE2L2 and inflammatory gene-regulating NF-κB may control the balance between oxidative stress and inflammation⁹⁴: disruption of NFE2L2 induces NF-κB activation, probably by modifying NF-κB inhibitor α (NFKB1A, also known as IκBα) degradation and NF-κB binding to DNA^{95, 96}. Inversely, NF-κB increases oxidative stress by inhibiting NFE2L2 transcription through repression of its creb binding protein (CBP) binding and by inducing ARE hypoacetylation⁹⁷. Altogether, inflammation and oxidative stress are closely interactin and potentiate each other.

Future prospects

In the coming years, there is hope for the further illumination of schizophrenia etiology, and the refining of ideas regarding potential treatments and therapeutic windows. Further human data, including patient-based imaging will be important in establishing the role of oxidative stress and NMDAR hypofunction (plus any links between them) in schizophrenia. In particular, longitudinal prospective studies of high risk groups will be informative in defining features both pre- and post-diagnosis, contrasting with individuals who do not progress to schizophrenia. For example, it will be of interest to learn the trajectory of glutamatergic state, NMDAR function (using positron emission tomography (PET) ligands) and GSH levels (by MRS) and how they link both to clinical symptoms (progression to schizophrenia or not) as well as other metrics such as gamma synchrony (by electroencephalography (EEG)) and white matter imaging (such as diffusion tensor imaging (DTI)). Moreover, the reverse translation of these and other studies will enable preclinical researchers to focus on the key pathological triggers and core deficits in schizophrenia, which is apposite since there is no single ideal mouse model of schizophrenia.

Similar methods will also prove valuable for assessing the efficacy of therapeutic interventions, including those aimed at boosting NMDAR function, which have focussed on the co-agonist (glycine or D-serine) site as a potential therapeutic target. Indeed, the utility of D-serine as a mono- or adjunct therapy for schizophrenia in the prodromal phase remains an active area of clinical research²². A recent trial with clinically high risk patients (scored high on the Scale of Prodromal Symptoms (SOPS)) showed that D-serine induced an improvement in negative symptoms, a promising result that needs to be confirmed in a larger cohort⁹⁸. Unfortunately, a glycine transporter antagonist recently failed in Phase III trials; however, it is important to note that which of glycine or D-serine act on the NMDAR may depend on activation conditions⁹⁹ and modulating them may therefore not give equivalent results. Being able to monitor NMDAR function with specific PET ligands may provide information on the likely efficacy of these and other treatments that directly or indirectly

influence NMDAR function, and allow therapies to be tuned to avoid NMDAR-mediated neurotoxicity. Indeed co-administration of antioxidants may also help to mitigate such adverse effects.

Antioxidant therapies have the potential to reduce oxidative stress and in doing so reduce NMDAR hypofunction¹⁰⁰ and inflammation. NAC, which upon deacetylation provides cells with the limiting amino acid for GSH biosynthesis, is a well-tolerated compound and the focus of several published and ongoing clinical studies. Published studies have revealed beneficial effects in schizophrenia patients as an add-on to maintenance medication¹⁰⁰⁻¹⁰³ and ongoing studies are following up their results and including additional metrics of efficacy such as brain GSH imaging in order to reflect the BBB permeability analyses of NAC that have been performed in rodents¹⁰⁴. The therapeutic window for antioxidant therapy remains an open question. For example, intervention shortly after, or even before diagnosis, may rescue or limit PVI deficits that are irreversible in the patient with chronic schizophrenia. The potential for juvenile NAC treatment to prevent the emergence of electrophysiological and behavioural deficits in a rodent developmental model of schizophrenia⁵⁶ suggests that prophylactic treatment for high-risk groups may be effective, particularly with a drug or dietary supplement with a good safety profile like NAC or polyunsaturated fatty acids such as omega 3s¹⁰⁵.

Nevertheless, NAC may not represent an optimal antioxidant therapy, since its principal modus operandus — the supply of increased cysteine for GSH biosynthesis — is of limited help unless the brain can use it to produce, recycle and utilise GSH. A more holistic approach to rebalancing the brain's redox status may be to target the NFE2L2 pathway, which regulates dozens (if not hundreds) of genes controlling antioxidant and detoxification processes (Figure 5). Several NFE2L2-activating compounds have efficacy in preclinical models of neurodegenerative disease⁸² and one, sulforaphane, has shown some promise in a preliminary open-label trial for SZ¹⁰⁶. Moreover, BG-12 (also known as Tecfidera or dimethyl fumarate) is licensed for the relapsing remitting phase of multiple sclerosis and has cytoprotective and anti-inflammatory actions attributable to NFE2L2 activation¹⁰⁷. This may therefore be a promising candidate for schizophrenia antioxidant therapy. It is important to note that even though NFE2L2 is only weakly activatable in neurons^{81, 83}, NFE2L2 activators promote neuronal resistance to oxidative stress via non cell-autonomous mechanisms involving NFE2L2 activation in astrocytes, including human astrocytes^{82, 108}

To conclude, multiple strands of evidence indicate that NMDAR hypofunction and oxidative imbalance are a pathological hub in schizophrenia etiology upon which several genetic and environmental influences converge. The coming years will tell us whether this extra knowledge can be converted to effective symptomatic, preventive or disease-modifying therapies.

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Figure Legends

Figure 1. Human *GRIN2* mutations associated with neurodevelopmental and psychiatric disorders. Schematic showing the locations of *GRIN2A* (glutamate receptor ionotropic NMDA 2a) and *GRIN2B* heterozygous missense mutations identified in people with neurodevelopmental disorders. The extreme extracellular N-terminus of these subunits contains allosteric modulation sites. The region between the N-terminus and the M1 domain, plus the extracellular loop between the M3 and M4 domains encode the glutamate-binding domain. The M2 domain features many sidechains that point towards the receptor channel pore and dictate ion permeability. Finally, the long cytoplasmic C-terminal domain is involved in receptor targeting and coupling to downstream signaling complexes. It is important to note that schizophrenia-associated mutations tend to be located at the N- and C-termini, away from the ligand binding and pore-forming regions. Mutations in the ligand binding and pore regions are predicted to be more fundamentally disruptive and tend to be more associated with childhood-onset intellectual disability and global delay (and often with epilepsy), as are nonsense or frameshift mutations in any domain ¹⁰⁹. This suggests that schizophrenia-associated NMDA receptor hypofunction may be either milder or more temporally or spatially restricted than that caused by genome-level subunit ablation. Figure based on data in references¹¹⁰⁻¹¹⁸. [Au: correct? Are there any other references that should be cited here?] [Au: in these images does each dash in the extracellular domain represent an amino acid or are they approximations of the size of each region?].

Figure 2. Key functions of the glutathione antioxidant system. Reduced glutathione (GSH) facilitates the transfer of reducing equivalents (species which transfer the equivalent of one electron) via NADPH generated by the pentose phosphate pathway of glucose metabolism, to a range of end points (red arrows). This transfer facilitates the reduction of cellular peroxides, catalysed by glutathione peroxidases (GPX), as well as the reduction of protein thiol groups, catalysed by glutaredoxins (Grx). GSH plays a key role in the neutralization of electrophilic xenobiotic compounds, becoming conjugated to them in a reaction catalysed by glutathione-s-transferases (Gst). Glutathione reductase (Gr) catalyses the reduction of oxidized glutathione (GSSG) back to GSH, using NADPH as a cofactor. Also shown are two sources of cellular peroxide: superoxide dismutation by SOD, monoamine oxidase metabolism of dopamine, and NADPH oxidase (NOX). Collectively, the GSH and GSH system enzymes play a key role in cellular redox homeostasis. X⁺: electrophilic xenobiotic; GS-X: GSH conjugated to xenobiotic; Grx-S₂ (oxidized) and Grx-(SH)₂ (reduced) glutaredoxin; ROOH: organic peroxide; ROH: organic hydroxide.

Figure 3. Reciprocal links between NMDA receptor (NMDAR) hypofunction and oxidative stress. NMDAR hypofunction triggers both cellular effects (the downregulation of antioxidant genes) and circuit level disinhibition of cortical networks that leads to reactive oxygen species (ROS) generation through activation of NADPH oxidase (NOX). Both of these events have the capacity to lead to oxidative stress and glutathione (GSH) depletion, which in turn can further repress NMDAR activity. The consequences of oxidative stress and GSH deficits during development can include impaired development and maturation of parvalbumin-expressing interneurons (PVIs, leading to excitation/inhibition (E/I) imbalance) and white matter abnormalities, which collectively may lead to altered cognition, behaviour and sensory processing in schizophrenia. Key external factors may also influence this pathological hub: neuroinflammation can act both up- and down-stream of ROS and inflammatory cytokine (such as interleukin-6 (IL6)) production. Mitochondrial dysfunction can also be both up- and down-stream of oxidative stress. Moreover, environmental and genetic factors have the capacity to influence NMDAR function, antioxidant defences, and inflammation.

Figure 4. Synaptic activity boosts the capacity of neuronal antioxidant systems. Schematic shows the changes in antioxidant system gene expression induced by synaptic activity. Transcriptionally induced genes are targeted by red arrows, repressed genes by red lines ended in flat bars. The capacity of neurons to produce, utilize, and recycle glutathione (GSH) is enhanced by synaptic activity. Activity-dependent induction of *GCLC*, which encodes the catalytic subunit of GCL (glutamate-cysteine ligase), increases the capacity of the rate-limiting step in GSH biosynthesis, whereas the induction of glutathione reductase GR boosts recycling capacity (reducing oxidized GSSG back to GSH). Transcriptional induction of glutathione peroxidase (GPX) activity enhances the ability of the GSH system to reduce cellular peroxides; however, as a result of increased GR and GCL activity, neuronal levels of GSH can be sustained. There are also activity-dependent changes to genes in the thioredoxin and/ or peroxiredoxin system. Peroxiredoxins (PRX) catalyse the reduction of peroxides, and become oxidized in the process, upon which they are themselves reduced by thioredoxin (TRX). Under conditions of excessive peroxide, they can become hyperoxidized, and rely on sulfiredoxin (SRXN1) to catalyse the formation of a phospho-ester moiety that can be reduced by thioredoxin. Synaptic activity induces expression of *Srxn1*, and represses expression of *Txnip*, an inhibitor of thioredoxin. (red)=reduced; (ox)=oxidized; (ox²)=hyperoxidized.

Figure 5. The astrocytic NFE2L2 pathway boosts the brain's antioxidant defences.

Under normal conditions, Nuclear factor erythroid 2-related factor 2 (NFE2L2) is targeted for ubiquitin-mediated degradation by KEAP1 (Kelch-Like ECH-Associated Protein 1). Both oxidative stress and certain small molecules (often electrophiles (**e.g. tBHQ and BG12**)) interfere with this degradation, enabling NFE2L2 to accumulate, translocate to the nucleus and drive the transcription of genes whose promoters contain antioxidant response elements (AREs). ARE-containing genes comprise antioxidant genes, and related phase II detoxification and/ or xenobiotic conjugation genes (such as glutathione S-transferases) and include key components of the glutathione (GSH) and thioredoxin (TRX) systems. NFE2L2 activation boosts GSH levels in astrocytes. In response to oxidative stress, this GSH is released through the multidrug resistance protein (MRP1) and broken down to the Cys-Gly dipeptide by γ -glutamyl transpeptidase (γ GT). Cys-Gly, cysteine, cystine or a combination thereof are taken up by neurons and used for GSH biosynthesis. By increasing the supply of GSH precursors to neurons, the action of astrocytic NFE2L2 is additive and complementary to the effects of synaptic NMDA receptor activity in boosting GSH biosynthetic capacity⁷⁷ (see Figure. 3).