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

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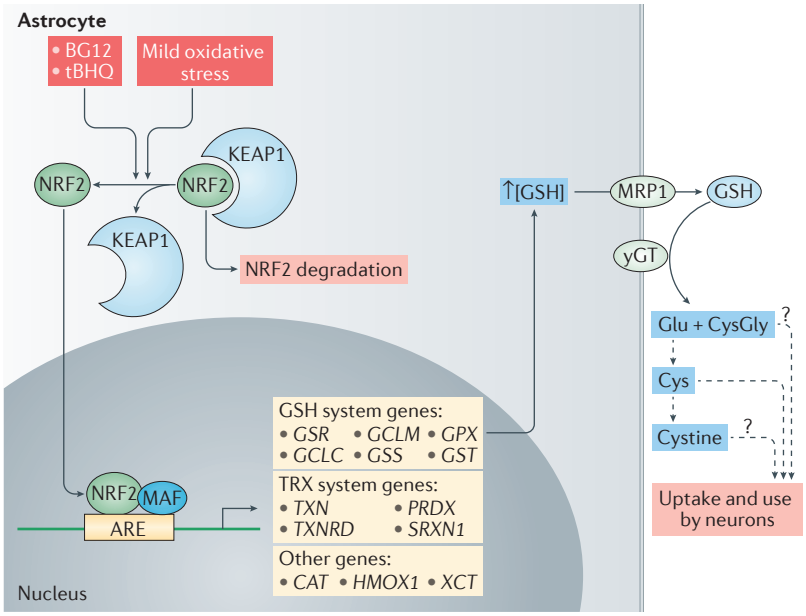
Figure 5



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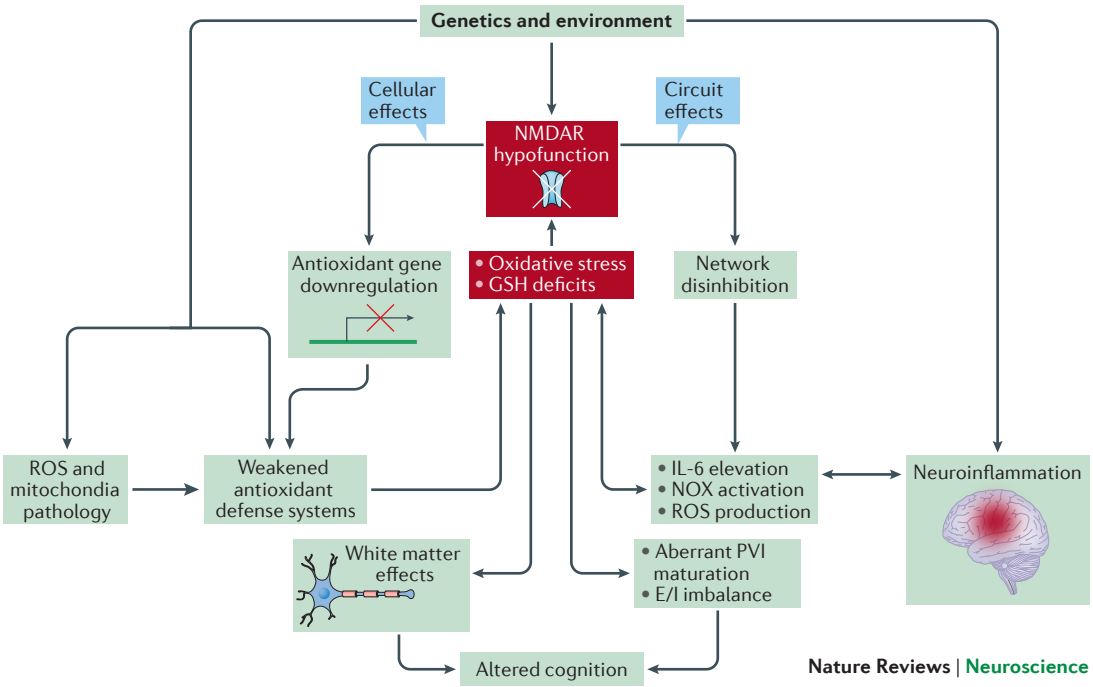
Abstract| Molecular, genetic and pathological evidence suggests that deficits in GABAergic parvalbumin-positive interneurons contribute to schizophrenia pathophysiology through alterations in the brain's excitation-inhibition balance that result in impaired behaviour and cognition. Although the factors 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 (PVIs) [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 schilzophrenia, including defects in PVIs and in the

Figure 3



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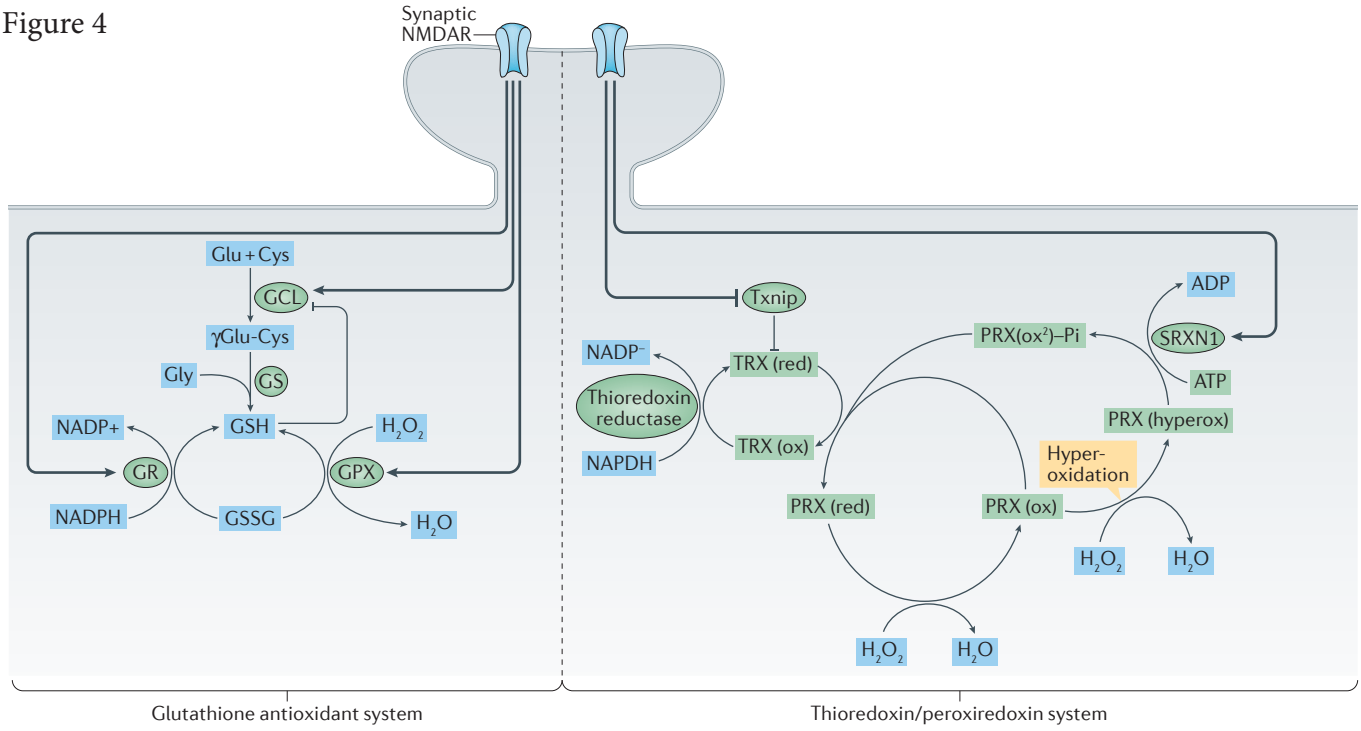

function of their associated networks, as well as high frequency neuronal synchrony [2, 5]. We also discuss the clinical studies that will be required to prove or disprove this hypothesis and to determine which therapeutic strategies may prove effective in targeting this pathological hub both before 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 signaling [6-8].

Human studies suggest NMDAR hypofunction

The hypothesis that NMDAR hypofunction might be a pathogenic trigger for schizophrenia [9, 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 antagonists [13]. Recently, autoimmune diseases associated with anti-NMDAR antibodies were reported to be associated with severe psychosis [14]. 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 schizophrenia [19], although this finding awaits confirmation with newer**, more specific** probes [20]. 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 hypofunction [20].

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 schizophrenia [21] as well as other genes functionally up- and down-stream 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

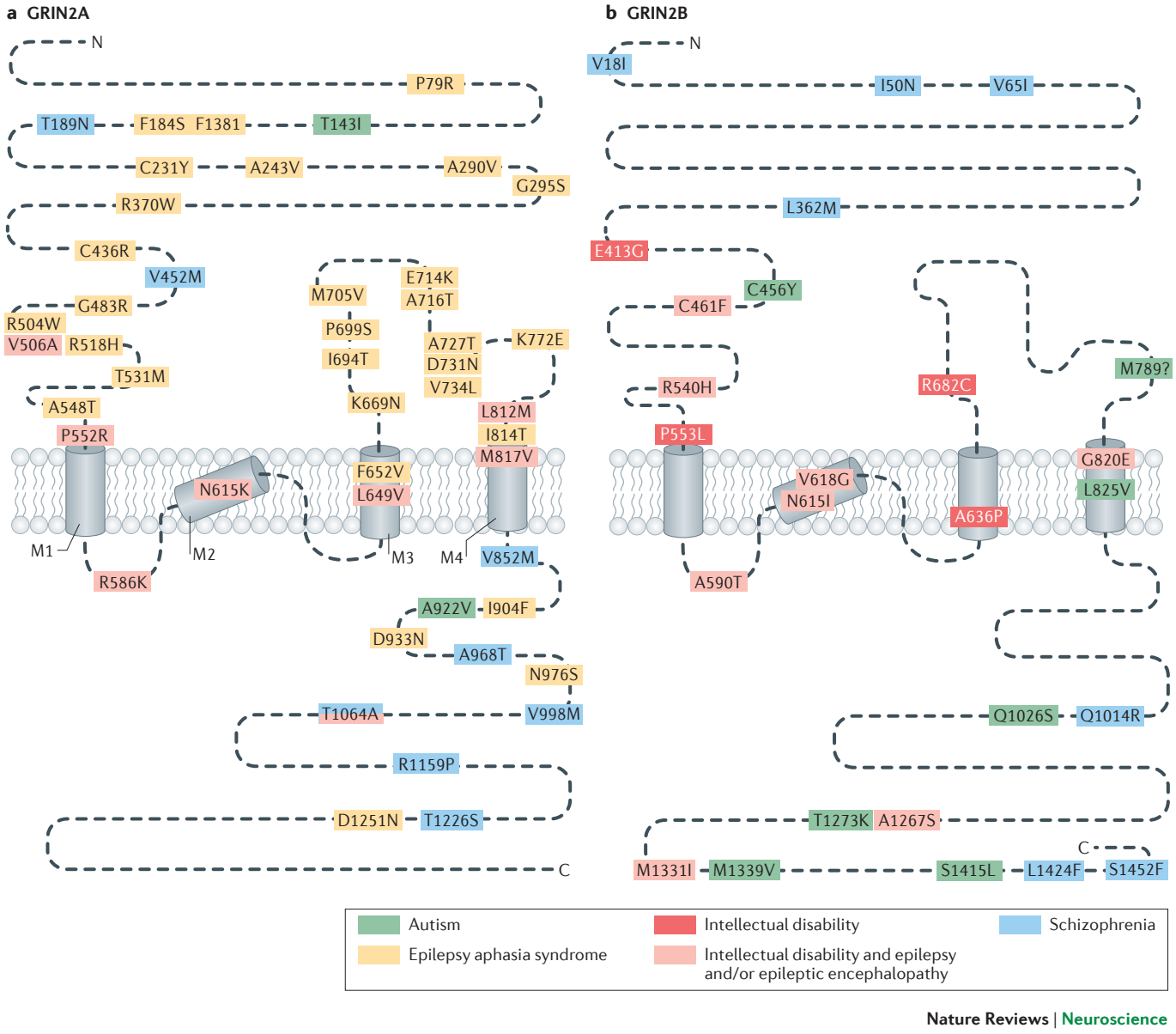

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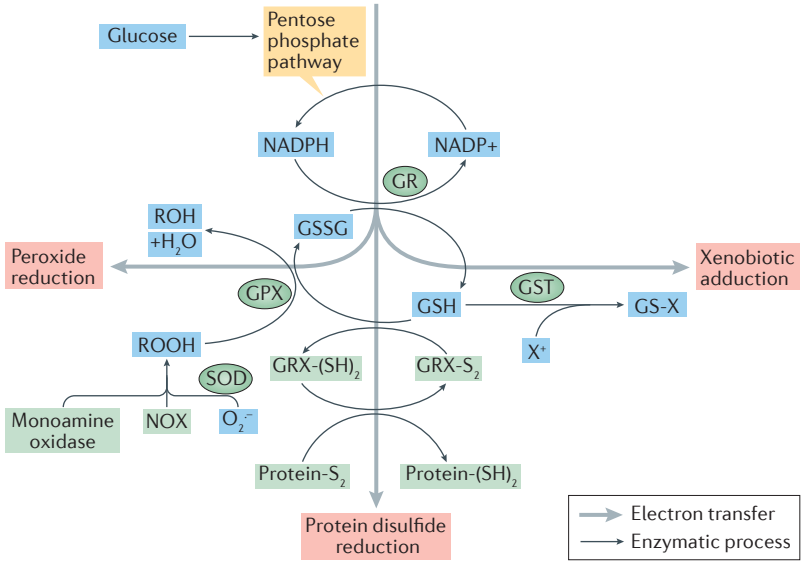
Figure 2



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 mouse [25] and the *Srr* knockout mouse [26] — 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 perturbations [1]. 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 week [28]. A milder antagonism regime caused long-lasting behavioural changes and a specific long-lasting decrease in cortical PVI numbers [29], mimicking a key pathological feature of schizophrenia [2].

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 behaviors [30], as well as spatial memory impairment [31]. The behavioural deficits induced by interneuronal *Grin1* deletion are exacerbated by social isolation stress[30], which models aspects of known environmental risks for schizophrenia, including childhood maltreatment and psychosocial adverse events [1].

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 neurons [32-34]. During development, even subtle peturbations to redox balance can affect the signaling pathways and processes involved in neurogenesis and neuronal differentiation [35].

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 levels [39]. Proteomic postmortem studies also point to the activation of oxidative stress responses in schizophrenia [40]. 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 dysregulated [36]. Importantly, low cortical GSH levels have been found to correlate with more severe negative symptoms in patients with schizophrenia [43].

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 fibroblasts [44]. Evidence that copy number variants and polymorphisms within glutathione-s-transferase (GST) genes may be susceptibility factors for schizophrenia has also been revealed [45].

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 hypofunction [70]. 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 GSH [72]. 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 defcits [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 brain [75]) 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 show 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 1α (*PPARGC1A*, also known as *PGC-1*α), a regulator of mitochondrial energy metabolism and antioxidant defences [78]. 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 H_2O_2 [29]. 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 astrocytes [34] 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 pathways [81]. 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 astrocytes [83].

Another consequence of relatively weak intrinsic antioxidant defences is that neurons rely on astrocytes to provide extrinsic support [82]. In response to oxidative stress, astrocytes increase GSH production (via NFE2L2-dependent mechanisms) and release. The release 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 schizophrenia [88]. Epidemiological evidence points to maternal infection being a risk factor in schizophrenia [1] and maternal infection in mice is sufficient to induce long-term PPI and social behavioural changes in the offspring, changes that are attributable to inflammation [89]. 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 Polyl:C causes a long-lasting sensitivity of the offspring to peri-pubertal stress and NMDAR antagonists [90]. Furthermore, PVI deficits in the DISC1-dominant negative transgenic mouse are exacerbated by a neonatal Polyl:C challenge [91].

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 inflammation [94]: disruption of NFE2L2 induces NF-κB activation, probably by modifying NF-κB inhibitor α (NFKB1A, also known as IkBα) 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 hypoacetylation [97]. 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 research [22]. 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 cohort [98]. 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 conditions [99] 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 hypofunction [100] 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 medication [100-103] 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 rodents [104]. 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 schizophrenia [56] 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 3s [105].

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 disease [82] and one, sulforaphane, has shown some promise in a preliminary open-label trial for SZ [106]. 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 activation [107]. 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 NF22L2 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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References

1. Brown, A.S. The environment and susceptibility to schizophrenia. *Prog Neurobiol* **93**, 23-58 (2011).
2. Lewis, D.A., Curley, A.A., Glausier, J.R. & Volk, D.W. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. *Trends Neurosci* **35**, 57-67 (2012).
3. Insel, T.R. Rethinking schizophrenia. *Nature* **468**, 187-93 (2010).
4. Mighdoll, M.I., Tao, R., Kleinman, J.E. & Hyde, T.M. Myelin, myelin-related disorders, and psychosis. *Schizophr Res* **161**, 85-93 (2015).
5. Uhlhaas, P.J. & Singer, W. Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* **11**, 100-13 (2010).
6. Paoletti, P., Bellone, C. & Zhou, Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat Rev Neurosci* **14**, 383-400 (2013).
7. Wyllie, D.J., Livesey, M.R. & Hardingham, G.E. Influence of GluN2 subunit identity on NMDA receptor function. *Neuropharmacology* **74**, 4-17 (2013).
8. Bell, K.F. & Hardingham, G.E. The influence of synaptic activity on neuronal health. *Curr Opin Neurobiol* **21**, 299-305 (2011).
9. Javitt, D.C. & Zukin, S.R. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* **148**, 1301-8 (1991).
10. Coyle, J.T., Basu, A., Benneyworth, M., Balu, D. & Konopaske, G. Glutamatergic synaptic dysregulation in schizophrenia: therapeutic implications. *Handb Exp Pharmacol*, 267-95 (2012).
11. Luby, E.D., Cohen, B.D., Rosenbaum, G., Gottlieb, J.S. & Kelley, R. Study of a new schizophrenomimetic drug; sernyl. *AMA Arch Neurol Psychiatry* **81**, 363-9 (1959).
12. Krystal, J.H. et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**, 199-214 (1994).
13. Anis, N.A., Berry, S.C., Burton, N.R. & Lodge, D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br J Pharmacol* **79**, 565-75 (1983).

14. Dalmau, J. et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* **7**, 1091-8 (2008).

15. Javitt, D.C. et al. Translating glutamate: from pathophysiology to treatment. *Sci Transl Med* **3**, 102mr2 (2011).

16. Schwarcz, R. et al. Increased cortical kynurenate content in schizophrenia. *Biol Psychiatry* **50**, 521-30 (2001).

17. Hashimoto, K. et al. Reduced D-serine to total serine ratio in the cerebrospinal fluid of drug naive schizophrenic patients. *Prog Neuropsychopharmacol Biol Psychiatry* **29**, 767-9 (2005).

18. Weickert, C.S. et al. Molecular evidence of N-methyl-D-aspartate receptor hypofunction in schizophrenia. *Mol Psychiatry* **18**, 1185-92 (2013).

19. Pilowsky, L.S. et al. First in vivo evidence of an NMDA receptor deficit in medication-free schizophrenic patients. *Mol Psychiatry* **11**, 118-9 (2006).

20. Howes, O., McCutcheon, R. & Stone, J. Glutamate and dopamine in schizophrenia: an update for the 21st century. *J Psychopharmacol* **29**, 97-115 (2015).

21. Group, S.W. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421-7 (2014).

22. Balu, D.T. & Coyle, J.T. The NMDA receptor 'glycine modulatory site' in schizophrenia: D-serine, glycine, and beyond. *Curr Opin Pharmacol* **20**, 109-15 (2015).

23. Carlsson, M. & Carlsson, A. The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. *J Neural Transm* **75**, 221-6 (1989).

24. Morris, R., Anderson, E., Lynch, G. & Baudry, M. Selective impairment of learning and blockade of long-term potentiation by an NMDA receptor antagonist, AP5. *Nature* **319**, 774-776 (1986).

25. Mohn, A.R., Gainetdinov, R.R., Caron, M.G. & Koller, B.H. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* **98**, 427-36 (1999).

26. Balu, D.T. et al. Multiple risk pathways for schizophrenia converge in serine racemase knockout mice, a mouse model of NMDA receptor hypofunction. *Proc Natl Acad Sci U S A* **110**, E2400-9 (2013).

27. Stefani, M.R. & Moghaddam, B. Transient N-methyl-D-aspartate receptor blockade in early development causes lasting cognitive deficits relevant to schizophrenia. *Biol Psychiatry* **57**, 433-6 (2005).

28. Ikonomidou, C. et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* **283**, 70-74 (1999).

29. Wang, X., Pinto-Duarte, A., Sejnowski, T.J. & Behrens, M.M. How Nox2-containing NADPH oxidase affects cortical circuits in the NMDA receptor antagonist model of schizophrenia. *Antioxid Redox Signal* **18**, 1444-62 (2013).

30. Belforte, J.E. et al. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat Neurosci* **13**, 76-83 (2010).

31. Korotkova, T., Fuchs, E.C., Ponomarenko, A., von Engelhardt, J. & Monyer, H. NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. *Neuron* **68**, 557-69 (2010).

32. Dringen, R., Pawlowski, P.G. & Hirrlinger, J. Peroxide detoxification by brain cells. *J Neurosci Res* **79**, 157-65 (2005).

33. Hardingham, G.E. & Lipton, S.A. Regulation of Neuronal Oxidative and Nitrosative Stress by Endogenous Protective Pathways and Disease Processes. *Antioxid Redox Signal* (2011).

34. Fernandez-Fernandez, S., Almeida, A. & Bolanos, J.P. Antioxidant and bioenergetic coupling between neurons and astrocytes. *Biochem J* **443**, 3-11 (2012).

35. Kennedy, K.A., Sandiford, S.D., Skerjanc, I.S. & Li, S.S. Reactive oxygen species and the neuronal fate. *Cell Mol Life Sci* **69**, 215-21 (2012).

36. Yao, J.K. & Keshavan, M.S. Antioxidants, redox signaling, and pathophysiology in schizophrenia: an integrative view. *Antioxid Redox Signal* **15**, 2011-35 (2011).

37. Do, K.Q., Cabungcal, J.H., Frank, A., Steullet, P. & Cuenod, M. Redox dysregulation, neurodevelopment, and schizophrenia. *Curr Opin Neurobiol* **19**, 220-30 (2009).

38. Flatow, J., Buckley, P. & Miller, B.J. Meta-analysis of oxidative stress in schizophrenia. *Biol Psychiatry* **74**, 400-9 (2013).

39. Coughlin, J.M. et al. Marked reduction of soluble superoxide dismutase-1 (SOD1) in cerebrospinal fluid of patients with recent-onset schizophrenia. *Mol Psychiatry* **18**, 10-1 (2013).

40. Martins-de-Souza, D., Harris, L.W., Guest, P.C. & Bahn, S. The role of energy metabolism dysfunction and oxidative stress in schizophrenia revealed by proteomics. *Antioxid Redox Signal* **15**, 2067-79 (2011).

41. Do, K. et al. Schizophrenia: glutathione deficit in cerebro spinal fluid and prefrontal cortex in vivo. *Eur J Neurosci* (2000).

42. Gawryluk, J.W., Wang, J.F., Andreazza, A.C., Shao, L. & Young, L.T. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *Int J Neuropsychopharmacol* **14**, 123-30 (2011).

43. Matsuzawa, D. & Hashimoto, K. Magnetic resonance spectroscopy study of the antioxidant defense system in schizophrenia. *Antioxid Redox Signal* **15**, 2057-65 (2011).

44. Gysin, R. et al. Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence. *Proc Natl Acad Sci U S A* **104**, 16621-6 (2007).

45. Rodriguez-Santiago, B. et al. Association of common copy number variants at the glutathione S-transferase genes and rare novel genomic changes with schizophrenia. *Mol Psychiatry* **15**, 1023-33 (2010).

46. Steullet, P. et al. Redox dysregulation affects the ventral but not dorsal hippocampus: impairment of parvalbumin neurons, gamma oscillations, and related behaviors. *J Neurosci* **30**, 2547-58 (2010).

47. Kulak, A., Cuenod, M. & Do, K.Q. Behavioral phenotyping of glutathione-deficient mice: relevance to schizophrenia and bipolar disorder. *Behav Brain Res* **226**, 563-70 (2012).

48. Jacobsen, J.P., Rodriguiz, R.M., Mork, A. & Wetsel, W.C. Monoaminergic dysregulation in glutathione-deficient mice: possible relevance to schizophrenia? *Neuroscience* **132**, 1055-72 (2005).

49. Cabungcal, J.H. et al. Transitory glutathione deficit during brain development induces cognitive impairment in juvenile and adult rats: relevance to schizophrenia. *Neurobiol Dis* **26**, 634-45 (2007).

50. Gokhale, A. et al. Quantitative proteomic and genetic analyses of the schizophrenia susceptibility factor dysbindin identify novel roles of the biogenesis of lysosome-related organelles complex 1. *J Neurosci* **32**, 3697-711 (2012).

51. Yap, M.Y., Lo, Y.L., Talbot, K. & Ong, W.Y. Oxidative stress reduces levels of dysbindin-1A via its PEST domain. *Neurochem Int* **79**, 65-9 (2014).

52. Natarajan, S.K. et al. Proline dehydrogenase is essential for proline protection against hydrogen peroxide-induced cell death. *Free Radic Biol Med* **53**, 1181-91 (2012).

53. Goldsmit, Y., Erlich, S. & Pinkas-Kramarski, R. Neuregulin induces sustained reactive oxygen species generation to mediate neuronal differentiation. *Cell Mol Neurobiol* **21**, 753-69 (2001).

54. Filiou, M.D., Teplytska, L., Otte, D.M., Zimmer, A. & Turck, C.W. Myelination and oxidative stress alterations in the cerebellum of the G72/G30 transgenic schizophrenia mouse model. *J Psychiatr Res* **46**, 1359-65 (2012).

55. Johnson, A.W. et al. Cognitive and motivational deficits together with prefrontal oxidative stress in a mouse model for neuropsychiatric illness. *Proc Natl Acad Sci U S A* **110**, 12462-7 (2013).

56. Cabungcal, J.H. et al. Juvenile antioxidant treatment prevents adult deficits in a developmental model of schizophrenia. *Neuron* **83**, 1073-84 (2014).

57. Otte, D.M. et al. N-acetyl cysteine treatment rescues cognitive deficits induced by mitochondrial dysfunction in G72/G30 transgenic mice. *Neuropsychopharmacology* **36**, 2233-43 (2011).

58. Wirth, E.K. et al. Neuronal selenoprotein expression is required for interneuron development and prevents seizures and neurodegeneration. *FASEB J* **24**, 844-52 (2010).

59. Cabungcal, J.H., Steullet, P., Kraftsik, R., Cuenod, M. & Do, K.Q. Early-life insults impair parvalbumin interneurons via oxidative stress: reversal by N-acetylcysteine. *Biol Psychiatry* **73**, 574-82 (2013).

60. Cabungcal, J.H. et al. Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proc Natl Acad Sci U S A* **110**, 9130-5 (2013).

61. Morishita, H., Cabungcal, J.H., Chen, Y., Do, K.Q. & Hensch, T.K. Prolonged Period of Cortical Plasticity upon Redox Dysregulation in Fast-Spiking Interneurons. *Biol Psychiatry* (2015).

62. Greenhill, S.D. et al. Adult cortical plasticity depends on an early postnatal critical period. *Science* **349**, 424-7 (2015).

63. Hikida, T. et al. Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. *Proc Natl Acad Sci U S A* **104**, 14501-6 (2007).

64. Schiavone, S. et al. Involvement of NOX2 in the development of behavioral and pathologic alterations in isolated rats. *Biol Psychiatry* **66**, 384-392 (2009).

65. Back, S.A. & Rosenberg, P.A. Pathophysiology of glia in perinatal white matter injury. *Glia* **62**, 1790-815 (2014).

66. Monin, A. et al. Glutathione deficit impairs myelin maturation: relevance for white matter integrity in schizophrenia patients. *Mol Psychiatry* (2014).

67. Lipton, S.A. et al. Cysteine regulation of protein function--as exemplified by NMDA-receptor modulation. *Trends Neurosci* **25**, 474-80 (2002).

68. Choi, Y.B. & Lipton, S.A. Identification and mechanism of action of two histidine residues underlying high-affinity Zn2+ inhibition of the NMDA receptor. *Neuron* **23**, 171-80 (1999).

69. Kohr, G., Eckardt, S., Luddens, H., Monyer, H. & Seeburg, P.H. NMDA receptor channels: subunit-specific potentiation by reducing agents. *Neuron* **12**, 1031-40 (1994).

70. Steullet, P., Neijt, H.C., Cuenod, M. & Do, K.Q. Synaptic plasticity impairment and hypofunction of NMDA receptors induced by glutathione deficit: relevance to schizophrenia. *Neuroscience* **137**, 807-19 (2006).

71. Guidi, M., Kumar, A. & Foster, T.C. Impaired attention and synaptic senescence of the prefrontal cortex involves redox regulation of NMDA receptors. *J Neurosci* **35**, 3966-77 (2015).

72. Bodhinathan, K., Kumar, A. & Foster, T.C. Intracellular redox state alters NMDA receptor response during aging through Ca2+/calmodulin-dependent protein kinase II. *J Neurosci* **30**, 1914-24 (2010).

73. Papadia, S. et al. Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat Neurosci* **11**, 476-87 (2008).

74. Radonjic, N.V. et al. Decreased glutathione levels and altered antioxidant defense in an animal model of schizophrenia: long-term effects of perinatal phencyclidine administration. *Neuropharmacology* **58**, 739-45 (2010).

75. Bell, K.F. & Hardingham, G.E. CNS Peroxiredoxins and Their Regulation in Health and Disease. *Antioxid Redox Signal* (2011).

76. Hardingham, G.E. & Bading, H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat Rev Neurosci* **11**, 682-96 (2010).

77. Baxter, P.S. et al. Synaptic NMDA receptor activity is coupled to the transcriptional control of the glutathione system. *Nat Commun* **6**, 6761 (2015).

78. Jiang, Z., Rompala, G.R., Zhang, S., Cowell, R.M. & Nakazawa, K. Social isolation exacerbates schizophrenia-like phenotypes via oxidative stress in cortical interneurons. *Biol Psychiatry* **73**, 1024-34 (2013).

79. Moghaddam, B. & Krystal, J.H. Capturing the angel in "angel dust": twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. *Schizophr Bull* **38**, 942-9 (2012).

80. Powell, S.B., Sejnowski, T.J. & Behrens, M.M. Behavioral and neurochemical consequences of cortical oxidative stress on parvalbumin-interneuron maturation in rodent models of schizophrenia. *Neuropharmacology* **62**, 1322-31 (2012).

81. Bell, K.F. et al. Neuronal development is promoted by weakened intrinsic antioxidant defences due to epigenetic repression of Nrf2. *Nat Commun* **6**, 7066 (2015).

82. Gan, L. & Johnson, J.A. Oxidative damage and the Nrf2-ARE pathway in neurodegenerative diseases. *Biochim Biophys Acta* **1842**, 1208-18 (2014).

83. Jimenez-Blasco, D., Santofimia-Castano, P., Gonzalez, A., Almeida, A. & Bolanos, J.P. Astrocyte NMDA receptors' activity sustains neuronal survival through a Cdk5-Nrf2 pathway. *Cell Death Differ* **22**, 1877-89 (2015).

84. Bell, K.F. et al. Mild oxidative stress activates Nrf2 in astrocytes, which contributes to neuroprotective ischemic preconditioning. *Proc Natl Acad Sci U S A* **108**, E1-2; author reply E3-4 (2011).

85. Bell, K.F., Fowler, J.H., Al-Mubarak, B., Horsburgh, K. & Hardingham, G.E. Activation of Nrf2-regulated glutathione pathway genes by ischemic preconditioning. *Oxid Med Cell Longev* **2011**, 689524 (2011).

86. Deighton, R.F. et al. Nrf2 target genes can be controlled by neuronal activity in the absence of Nrf2 and astrocytes. *Proc Natl Acad Sci U S A* **111**, E1818-20 (2014).

87. Lewerenz, J. et al. Phosphoinositide 3-kinases upregulate system xc(-) via eukaryotic initiation factor 2alpha and activating transcription factor 4 - A pathway active in glioblastomas and epilepsy. *Antioxid Redox Signal* **20**, 2907-22 (2014).

88. Reus, G.Z. et al. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience* **300**, 141-54 (2015).

89. Kneeland, R.E. & Fatemi, S.H. Viral infection, inflammation and schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* **42**, 35-48 (2013).

90. Giovanoli, S. et al. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. *Science* **339**, 1095-9 (2013).

91. Ibi, D. et al. Combined effect of neonatal immune activation and mutant DISC1 on phenotypic changes in adulthood. *Behav Brain Res* **206**, 32-7 (2010).

92. Beloosesky, R., Gayle, D.A. & Ross, M.G. Maternal N-acetylcysteine suppresses fetal inflammatory cytokine responses to maternal lipopolysaccharide. *Am J Obstet Gynecol* **195**, 1053-7 (2006).

93. Lante, F. et al. Late N-acetylcysteine treatment prevents the deficits induced in the offspring of dams exposed to an immune stress during gestation. *Hippocampus* **18**, 602-9 (2008).

94. Buelna-Chontal, M. & Zazueta, C. Redox activation of Nrf2 & NF-kappaB: a double end sword? *Cell Signal* **25**, 2548-57 (2013).

95. Jin, W. et al. Disruption of Nrf2 enhances upregulation of nuclear factor-kappaB activity, proinflammatory cytokines, and intercellular adhesion molecule-1 in the brain after traumatic brain injury. *Mediators Inflamm* **2008**, 725174 (2008).

96. Pan, H., Wang, H., Wang, X., Zhu, L. & Mao, L. The absence of Nrf2 enhances NF-kappaB-dependent inflammation following scratch injury in mouse primary cultured astrocytes. *Mediators Inflamm* **2012**, 217580 (2012).

97. Liu, G.H., Qu, J. & Shen, X. NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. *Biochim Biophys Acta* **1783**, 713-27 (2008).

98. Kantrowitz, J. et al. D-serine for the treatment of negative symptoms in individuals at clinical high risk of schizophrenia: a pilot, double-blind, placebo-controlled, randomised parallel group mechanistic proof-of-concept trial. *The Lancet Psychiatry* **2**, 10 (2015).

99. Li, Y. et al. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. *Nat Commun* **4**, 1760 (2013).

100. Lavoie, S. et al. Glutathione precursor, N-acetyl-cysteine, improves mismatch negativity in schizophrenia patients. *Neuropsychopharmacology* **33**, 2187-99 (2008).

101. Berk, M. et al. N-acetyl cysteine as a glutathione precursor for schizophrenia--a double-blind, randomized, placebo-controlled trial. *Biol Psychiatry* **64**, 361-8 (2008).

102. Carmeli, C., Knyazeva, M.G., Cuenod, M. & Do, K.Q. Glutathione precursor N-acetyl-cysteine modulates EEG synchronization in schizophrenia patients: a double-blind, randomized, placebo-controlled trial. *PLoS One* **7**, e29341 (2012).

103. Farokhnia, M. et al. N-acetylcysteine as an adjunct to risperidone for treatment of negative symptoms in patients with chronic schizophrenia: a randomized, double-blind, placebo-controlled study. *Clin Neuropharmacol* **36**, 185-92 (2013).

104. Farr, S.A. et al. The antioxidants alpha-lipoic acid and N-acetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice. *J Neurochem* **84**, 1173-83 (2003).

105. Amminger, G.P., Schafer, M.R., Schlogelhofer, M., Klier, C.M. & McGorry, P.D. Longer-term outcome in the prevention of psychotic disorders by the Vienna omega-3 study. *Nat Commun* **6**, 7934 (2015).

106. Shiina, A. et al. An Open Study of Sulforaphane-rich Broccoli Sprout Extract in Patients with Schizophrenia. *Clin Psychopharmacol Neurosci* **13**, 62-7 (2015).

107. Fox, R.J. et al. BG-12 (dimethyl fumarate): a review of mechanism of action, efficacy, and safety. *Curr Med Res Opin* **30**, 251-62 (2014).

108. Gupta, K. et al. Human embryonic stem cell derived astrocytes mediate non-cell-autonomous neuroprotection through endogenous and drug-induced mechanisms. *Cell Death Differ* **19**, 779-787 (2012).

109. Burnashev, N. & Szepetowski, P. NMDA receptor subunit mutations in neurodevelopmental disorders. *Curr Opin Pharmacol* **20**, 73-82 (2015).

110. O'Roak, B.J. et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* **338**, 1619-22 (2012).

111. Tarabeux, J. et al. Rare mutations in N-methyl-D-aspartate glutamate receptors in autism spectrum disorders and schizophrenia. *Transl Psychiatry* **1**, e55 (2011).

112. Myers, R.A. et al. A population genetic approach to mapping neurological disorder genes using deep resequencing. *PLoS Genet* **7**, e1001318 (2011).

113. Lemke, J.R. et al. GRIN2B mutations in West syndrome and intellectual disability with focal epilepsy. *Ann Neurol* **75**, 147-54 (2014).

114. Lemke, J.R. et al. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. *Nat Genet* **45**, 1067-72 (2013).

115. Freunscht, I. et al. Behavioral phenotype in five individuals with de novo mutations within the GRIN2B gene. *Behav Brain Funct* **9**, 20 (2013).

116. Endele, S. et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. *Nat Genet* **42**, 1021-6 (2010).

117. Lesca, G. et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. *Nat Genet* **45**, 1061-6 (2013).

118. Carvill, G.L. et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. *Nat Genet* **45**, 1073-6 (2013).

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 references[110-118]. **[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-S_2 (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 parvablumin-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; (ox^2)=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 the multidrug resistance protein (MRP1) and broken down to the Cys-Gly dipeptide by γ-glutamyl transpeptidase (γGT). 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 capacity [77] (see Figure. 3).