Schizophrenia: glutathione deficit as a new vulnerability factor for disconnectivity syndrome

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Summary


Schizophrenia, a major psychiatric disease, affects individuals in the centre of their personality. Its aetiology is not clearly established. In this review, we will present evidence that patients suffering of schizophrenia present a brain deficit in glutathione, a major endogenous redox regulator and antioxidant. We will also show that, in experimental models, a decrease in glutathione, particularly during development, induces morphological, electrophysiological and behavioural anomalies consistent with those observed in the disease.

In the cerebrospinal fluid of drug-naive schizophrenics, glutathione level was decreased by 27% and its direct metabolite of glutathione by 16%. Glutathione level in prefrontal cortex of patients, measured by magnetic resonance spectroscopy, was 52% lower than in controls. Patients' fibroblasts reveal a decrease in mRNA levels of the two glutathione synthesising enzymes, glutamate-cysteine ligase modulatory subunit (GCLM) and glutathione synthetase. GCLM expression level in fibroblasts correlates negatively with symptoms severity.

Glutathione is an important endogenous redox regulator and neuroactive substance. It is protecting cells from damage by reactive oxygen species generated, among others, by dopamine metabolism. A glutathione deficit-induced oxidative stress would lead to lipid peroxidation and micro-lesions at the level of dendritic spines, a synaptic damage responsible for abnormal nervous connections or structural disconnectivity. On the other hand, a glutathione deficit could also lead to a functional disconnectivity by depressing NMDA neurotransmission, in analogy to phencyclidine effects.

Present experimental data are consistent with the proposed hypothesis: decreasing pharmacologically glutathione level in experimental models, with or without blocking dopamine (DA) uptake (GBR12909), induces morphological, electrophysiological and behavioural changes similar to those observed in patients:

- **Dendritic spines and GABA interneurons:** (a) In neuronal cultures, low glutathione level and dopamine induce decreased density of neural processes; (b) in developing rats (p5–p16), glutathione-level deficit and GBR induce a decrease in normal spines in prefrontal pyramids and in GABA-parvalbumin but not in -calretinin immunoreactivity in anterior cingulated cortex.

- **NMDA-dependent synaptic plasticity:** Glutathione depletion impairs NMDA responses in neuronal cultures and long-term potentiation in hippocampal slices.

- **Cognition:** Developing rats with low glutathione level and GBR have deficits in olfactory integration and object recognition, deficit which appears earlier in males than females, in analogy to the delay of the psychosis onset between man and woman.

In summary, a deficit of glutathione and/or glutathione-related enzymes during early development would lead to both a functional and a structural disconnectivity, which could be at the basis of some perceptive, cognitive and behavioural troubles of the disease. It could constitute a major vulnerability factor for schizophrenia. Attempts to restore physiological glutathione functions could open new therapeutic avenues. This translational research, made possible by a close interaction between clinicians and neuroscientists, should also pave the way to the identification of biological factors.
markers for schizophrenia. In turn, they should allow early diagnostic and hopefully preventive intervention to this devastating disease.

*Keywords: schizophrenia; glutathione; oxidative stress; disconnectivity; dopamine; NMDA*

**Introduction**

Schizophrenia is an endogenous psychosis characterised by an array of symptoms classically dichotomised into positive symptoms (delusions, hallucinations, thought disorder, incoherence of speech and behaviour) and negative ones (deficits in cognitive and social abilities, poverty of speech, affective flattening, etc). However, among most schizophrenic patients, psychotic symptomatology is only transient (state feature as opposed to trait feature). The patients also present more discrete, but more permanent dysfunctions such as cognitive deficits (attention, specific forms of memory, executive functions) and perceptual instability (basic symptoms) [1, 2] that are now thought to be central to the behavioural disturbances and functional disability of the patients. While present antipsychotic treatments are relatively efficient against positive symptoms, there is no effective drug therapy for the negative and cognitive ones as well as for the basic symptoms. Indeed, even in patients stabilised with present antipsychotics, these cognitive and basic impairments prevent the social and professional integration of young individuals at and after the time of their education [3, 4]. While schizophrenia affects approximately 1% of the world population, the specific aetiological factors remain elusive.

Numerous studies have focused on identifying genetic and environmental vulnerability factors. Concerning the *genetic predisposition* to schizophrenia, family, twin and adoption studies have demonstrated that the morbidity risk of schizophrenia in relatives correlates with the degree of shared genes (incidence in general population 1%; second-degree relatives ~2–6%; first-degree relatives ~6–17%; dizygotic twins ~17%; monozygotic twins ~50%) [5]. However, the mode of inheritance of the disease is not simple [6]. Results from several genome-wide scans [7–11] have identified chromosomal regions of interest, and cumulative evidence from replication efforts suggest that schizophrenia-susceptibility genes may be found on chromosomes 1, 6, 8, 10, 13 and 22 (review, see [12–14]).

*Environmental factors* such as exposure to viral infectious [15], autoimmune, toxic or traumatic insults and stress during gestation, birth or childhood [16, 17] may also play a role in the pathogenesis of schizophrenia [18].

Despite formulation of several hypotheses, the pathophysiology of schizophrenia remains also in large part unknown. In the field of neurochemistry, although hypotheses that are based on the dysfunction of either the dopamine or the glutamate transmission systems have stimulated intense work, there has been no unequivocal support or clear rejection of either theory. Evidence for a dopamine-system dysfunction includes the psychosis-inducing effects of dopaminergic agonists and the antipsychotic potency of antagonists [19–21]. The glutamate-hypofunction hypothesis relies on the fact that phencyclidine, a psychotomimetic drug, blocks the NMDA glutamate receptor [22–25]. Moreover, indirect pharmacologic evidence also points to the implication of serotonergic, noradrenergic, cholinergic and GABA-ergic systems.

Multiple lines of evidence suggest that schizophrenia is associated with abnormalities in neural circuitry and impaired connectivity. Magnetic resonance spectroscopy studies reveal alterations of membrane phospholipid metabolism and reduction in N-acetyl aspartate, a marker for neuronal integrity, in frontal and temporal lobes (for review, [26, 27]). Post-mortem histological studies [28] have shown: (a) in prefrontal cortex (PFC) a reduction in cortical thickness (5–10%); (b) an increase in cell density without change in total neuron number [29, 30]; (c) a decrease (~25%) in the number of dendritic spines (layer III–IV) indicating a loss of synaptic connectivity in prefrontal cortex and superior temporal cortex [31, 32]; (d) alteration of synaptic protein expression [33]; (e) in dorsal prefrontal cortex a subset of inhibitory GABA neurons is affected [26]; the density of GABA transporter-immunoreactive axon cartridges of GABA-ergic chandelier neurons is selectively decreased; (f) a 30% decrease in the total number of neurons in the thalamic nuclei mediodorsal (projecting to prefrontal cortex) and anterior (projecting to prefrontal cortex and anterior cingulate cortex) indicating a degeneration of the thalamo-prefrontal connections in schizophrenia [34, 35]. Moreover, recent advances in diffusion tensor imaging allowed in vivo explorations of anatomical connectivity in human brain. It has pointed to connectivity abnormalities in fronto-parietal and fronto-temporal circuitry in schizophrenia [36], for review see [37]. It has been proposed that oligodendroglia, myelin and white matter abnormalities may be the underlying mechanisms [38].

Besides this anatomical evidence for a structural connectivity, anomalies in information
integration across brain networks are accumulating. Functional connectivity is based on study of dynamic, context-dependent processes, which require the preferential recruitment of some networks over others. Methods for analysis of these processes are based on the premise that functionally interacting regions will show correlated patterns of activity [39–41]. In schizophrenia there is emerging evidence for a failure of the gamma band synchronization (40 Hz range) of anterior-posterior neural circuits in response to gestalt stimuli [42] and for binding capacity [43]. Moreover, functional perturbation of the excitatory glutamate NMDA type of receptors have also been reported [44–46].

It is thus hypothesised that the perceptive, cognitive and behavioural troubles of schizophrenia are likely to reflect the above summarised structural and functional disconnectivity [3, 47]. Moreover, attempts to produce a unifying concept of the aetiology of schizophrenia have posited that these biological mechanisms have their origins in developmental processes that emerge prior to the onset of clinical symptoms. Indeed, evidence for pre- and perinatal epidemiological risk factors of schizophrenia and for premorbid dysfunction during infancy and childhood has led to the formulation of the so-called neurodevelopmental hypothesis: schizophrenia is viewed as resulting from aetiological events acting between conception and birth, and interfering with normal maturational processes of the central nervous system [48, 49]. Moreover, it is also hypothesised that the interaction of a genetic diathesis and early neurodevelopmental insults result in defective connectivity between a number of brain regions, including the midbrain, nucleus accumbens, thalamus, temporal-limbic and prefrontal cortices [50]. This defective neural circuitry is then vulnerable to dysfunction when unmasked by the developmental processes and events of adolescence (myelination, synaptic pruning and hormonal effects of puberty on the central nervous system) and exposure to stressors as the individual moves through the age of risk ([4, 51], for review see [52]).

In summary, the existing neuroanatomical, neurochemical, neurophysiological and psychopathological arguments converge to suggest that schizophrenia may be considered as a “disconnectivity syndrome”.

In this review, we will present evidence that patients suffering from schizophrenia present a brain deficit in glutathione, an important endogenous redox regulator and antioxidant. We will also show that, in experimental models, a decrease in glutathione, particularly during development, induces morphological, electrophysiological and behavioural anomalies analogous to those observed in the disease. Thus, brain deficit in glutathione system would lead to both a functional and a structural disconnectivity, which could be at the basis of some manifestations of the disease.

Clinical research

The observations reported below, made in three different collectives with various methodologies, converge to suggest that anomalies of glutathione metabolism represent a vulnerability factor in at least a subgroup of patients suffering from schizophrenia. They concern cerebrospinal fluid (CSF) and brain glutathione levels and mRNA expression in fibroblasts.

a) The cerebrospinal fluid of drug-naïve schizophrenic patients (n = 26; recruited in the Max Planck Institute of Psychiatry of Munich) was investigated. Using state-of-the-art methods, 27 compounds were analysed. A significant decrease in the levels of glutathione (~27%) [53] and of its direct metabolite γ-glutamyl-glutamine (γ-Glu-Gln, ~16%) [54] were observed in patients with schizophrenia compared to controls. In contrast, dopamine (DA) and serotonin metabolites were not different from controls. Moreover, the use of only 6 out of 27 substances analysed (glutathione, γ-Glu-Gln, glutamate, aspartate, taurine and isoleucine) as discriminating variables allowed us to classify 87.5% of the subjects correctly, with 96.2% of schizophrenic patients correctly diagnosed.

b) A new, non-invasive proton magnetic resonance spectroscopy (MRS) method that allows detection of glutathione with a high selectivity was developed [55]. In an MRS study with 14 patients, glutathione levels in the medial prefrontal cortex were found 52% (p = 0.0012) lower than controls [53] (patients: n = 14, recruited in the Psychiatric Department of Zurich University Hospital; control subjects: n = 14). With more subjects (n patients = 18; n controls = 20), a deficit of 51% (p = 0.05) of glutathione levels was confirmed (unpublished results). The drug-naïve status of most of the patients in the prefrontal cortex study and of 5 (out of 18) in the MRS study suggests that the deficit in glutathione may underlie the pathophysiology of the schizophrenic disease process and is not a consequence of treatment.

c) Gene expression of glutathione-related enzymes were investigated in fibroblast culture...
obtained from skin biopsy of patients (DSM IV criteria). The patients (n = 32) were recruited in the Department of Adult Psychiatry of Lausanne University, and the controls (n = 53), free of any psychiatric diseases, recruited in the Orthopaedic Hospital of Lausanne University. Both groups are submitted to a diagnostic interview for genetic studies. We analysed by quantitative real-time PCR the gene expression of 9 glutathione-related enzymes. Among these 9 genes, a decrease in the mRNA level of the following 3 genes was observed in patients compared to control subjects: γ-glutamyl-cysteine ligase (GCL) modulatory unit (GCLM), glutathione synthetase (GSS) and glutathione peroxidase 1 (GPX1) are significantly lower in patients compared to controls [56]. These three enzymes are directly involved in the metabolism of glutathione, particularly GCL and GSS, which are the two key enzymes for the synthesis of glutathione. GCL is made of two subunits, a catalytic one (GCLC) and a modulatory one (GCLM). Interestingly, while knock-out mice for the gene coding GCLC cannot survive, those for GCLM are viable, but their feedback inhibition by glutathione is drastically enhanced, leading to a low glutathione tissue level. The GCLM knock-out mice are also more sensitive to oxidative stress, leading to enhanced cellular damage [57]. Alterations of GCLM expression could thus be responsible for a lowered glutathione tissue level, as observed in the brain of schizophrenic patients. Although these results have been obtained from peripheral and not brain tissue, they are highly suggestive that genetic expression of enzymes of the glutathione metabolism is perturbed in the patients. The abnormal expression can result either from an abnormal gene or from an abnormal expression of the gene, or both. Interestingly, GCLM and GSS genes are localised in chromosome 1p21 and 20q11.2, two chromosomal regions previously shown by linkage studies to be critical for schizophrenia [58]. Genetic analysis is presently under way.

d) In a subgroup of patients, the mRNA expression of GCLM correlates negatively with the clinical scores of the Positive and Negative Symptoms Score (PANSS) [56]. For the positive, general psychopathology and two of the negative scores, the patients with the lowest GCLM mRNA levels have the most severe symptoms (high score), while those with more normal GCLM expression are less affected. This suggests that a low GCLM gene expression, likely to lead to an abnormality in the glutathione synthesising enzyme GCL and lower glutathione levels, is strongly related to the disease and that the fibroblasts represent an adequate surrogate for the nervous tissue. Taken together, these observations demonstrate that the glutathione brain deficit in schizophrenic patients could be due to a deficit in the gene expression of GCLM and GSS, the two key enzymes responsible for glutathione synthesis.

**Glutathione-schizophrenia hypothesis**

Based on a central role of glutathione in the pathophysiology of schizophrenia, we propose an hypothesis which could integrate many established biological aspects of this disorder. Glutathione, the major intracellular non-protein thiol, is known as a nucleophilic scavenger and an enzyme-catalysed antioxidant, and plays an important role in protecting the brain against oxidative stress and harmful xenobiotics [59–61]. On the other hand, glutathione is known to potentiate the NMDA receptors response to glutamate [62]. We have shown that glutathione is released into the extracellular space, predominantly in cortex [63] and glutathione has been proposed to play a neuromodulator/neurotransmitter role [64].

Although many of these functions might be involved in the disease, particularly during the development of the brain, two specific aspects will be emphasised here: (a) the glutathione effect on the NMDA receptor and (b) the protective action toward oxidative stress. Both may be related to the disconnectivity syndrome suspected to underlie the symptoms, the first implying a functional, the second a structural defect (fig. 1).

Functional disconnectivity due to glutathione deficit

A hypofunction of the NMDA receptor has been implied in schizophrenia for the following reasons: administration of phencyclidine or ketamine, both non-competitive antagonists of the NMDA receptor, induces a psychotic syndrome in normal subjects [65] and worsens the symptoms of schizophrenic patients. Furthermore, the mismatch negativity, a deficit in the evoked potential, observed 300 ms after an irregular sensory signal (oddball paradigm), is frequently found in schizophrenic patients [44–46, 66, 67]; this phenomenon can be induced by ketamine administration in normal subjects [46], suggesting that it is due to an underactivation of the NMDA receptor. Could a deficit in
glutathione possibly affect the NMDA-receptor function either by an extracellular or an intracellular mechanism, or by both?

a) **Extracellular NMDA-receptor redox site:** The NMDA receptor possesses an extracellular redox site which modulates the NMDA response [68]: in the absence of glutathione, the glutamate-induced depolarisation is minimal, while in its presence (100 µM–mM range) this response is maximally increased [62]. As glutathione is released by cell depolarisation [63] and assuming that an intracellular glutathione deficit reflects itself by an extracellular one (the exact synaptic concentration of it being difficult to estimate), it can be hypothesised that in case of a pathological glutathione deficit this potentiation would be perturbed, leading to an underactivation of NMDA receptors.

b) **Intracellular interaction between dopamine receptor and NMDA receptor:** It has been reported that the activation of dopamine receptors induces an increase in the NMDA response by a complex intracellular signalling [69–71]. Moreover, changes in the intracellular redox balance due to low glutathione levels alter the function of redox-sensitive proteins in the dopamine-mediated signalling pathways [72, 73]. A glutathione deficit could affect these signalling mechanisms, thus indirectly decreasing the efficacy of NMDA-receptor activation when dopamine receptors are stimulated. This idea is supported by the fact that glutathione is known to contribute to enzymatic phosphorylation/dephosphorylation which are potentially important for functional modifications of receptors. Such a mechanism would be of particular interest in view of the fact that most antipsychotic drugs are antagonists of the dopamine receptors.

In conclusion, at least two classes of mechanisms could relate a pathological glutathione deficit to an underfunction of the NMDA receptor and thus to abnormal brain function leading to schizophrenia symptoms.

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**Figure 1** Flow diagram describing the glutathione hypothesis.

- **Functional misconnectivity**
  - NMDA-R ↓
  - GABA-R ↓
  - Hallucinations, state symptoms?
- **Transcription factors**
  - Gene expression
  - DOPAMINE fronto-temporo-limbic
  - oxidants (OH\(^-\), O\(_2\)\(^-\), ONOO\(^-\))
- **Structural misconnectivity**
  - lipids, proteins, DNA peroxidation
  - Synaptic microlesions (terminals, membranes, mitochondria, dendritic spines)
  - fronto-temporal cortex neuropile connectivity ↓
  - Cortex ant. ↓, Hypofrontality, Ventricles ↑

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Structural disconnectivity due to glutathione deficit

As reviewed above multiple lines of evidence converge to demonstrate a disruption in anatomical connectivity in schizophrenia. Glutathione plays a major role in protecting cells against toxic effects of free radicals, produced among others by the metabolism of catecholamines, particularly dopamine [74], whose innervation is most abundant in the anterior parts of the cortex. In those cortical regions a glutathione deficit could be responsible for lipid membrane peroxidation [75] in the surroundings of dopaminergic terminals, leading to microlesions of synaptic terminals with the corresponding dendritic spines. These microlesions would generate a deficit in cortical connectivity which, in turn, could be at the origin of the cognitive, perceptual and behavioural troubles. Indeed, it has been proposed that oxidative stress-induced impairment of neuronal processes is involved in the pathophysiology of schizophrenia [76–78]. Furthermore, very little is known about the role of glutathione during brain development; there is a possibility that a glutathione deficit in specific critical periods would be responsible for the abnormal formation of some neurons, such as the parvalbumin immunoreactive inhibitory interneurons, as will be shown below. In turn, this neuronal anomaly might contribute to a loss of input on pyramidal dendrites, both from inhibitory and excitatory neurons, as they tend to maintain a constant balance [79].

Experimental research

Multiple experiments performed in our laboratory are consistent with the pathophysiological mechanism proposed: decreasing pharmacologically the level of glutathione in neuronal cultures, brain slices or developing animals, induces morphological, physiological and behavioural changes similar to those observed in schizophrenic patients [80–84]. The animal model is based on the inhibition, during the development, of the glutathione synthesising enzyme γ-glutamylcysteine ligase by buthionine sulfoximine (BSO) and an increase in extracellular dopamine level by a blocker of dopamine uptake (GBR 12909). This model has been performed in two rat strains: the non-mutant (Sprague-Dawley) rats which, unlike humans, are able to synthesize ascorbic acid (allowing them to partially compensate for a glutathione deficit) and the mutant rats “osteogenic disorder shionogi” (ODS) which, like humans, are unable to synthesize ascorbic acid.

Glutathione deficit and lipid peroxidation: In neuronal cultures under normal glutathione level, dopamine induced a 50% decrease in glutathione level through direct conjugation of glutathione with dopamine semiquinone/quinone; under conditions of decreased glutathione level, induced by ethacrynic acid treatment, dopamine further diminishes glutathione level via activation of dopamine receptors and generation of reactive oxygen species and reduction of the mitochondrial membrane potential (ΔΨ) [82]. This latter observation is consistent with the finding that depletion of brain glutathione is accompanied by impaired mitochondrial function and decreased N-acetyl aspartate [85]. The decreased glutathione level observed by us in schizophrenia might thus be related to the established N-acetyl aspartate reduction observed with MRS [53, 86]. In developing rats treated with buthionine sulfoximine with daily subcutaneous injections from postnatal day p5 until p16 there is a reduction by 50–62% of glutathione levels in various brain structures, including the frontal cortex. This effect, observed at day 16, is followed within a few days by a return to a normal glutathione level. Furthermore, an increase by 20–30% in lipid peroxidation products levels was observed in ODS rats in the diencephalon and pons/medulla [87].

Synaptic transmission, plasticity and NMDA hypofunction: In neuronal cultures depleted in glutathione by treatment during 24 h with low dose of ethacrynic and dopamine, the NMDA-induced intracellular [Ca2+] increase was selectively diminished or even abolished, an effect which can be reversed by addition of glutathione [Grima et al., unpublished results]. The fact that the addition of glutathione re-establishes the NMDA response suggests that restoring a normal brain glutathione level might have a therapeutic effect. Moreover, in rat hippocampal slices glutathione depletion impairs long-term potentiation, a mechanism of synaptic plasticity which is NMDA dependent in CA1 region [83]. This result suggests that a chronic glutathione level decrease leads to a hypofunction of the glutamate (NMDA) neurotransmission, in analogy to the effect of phencyclidine (PCP).

Dendritic spines: In neuronal cultures low glutathione level and dopamine application induce a decrease of the density of filopodia, neural processes possibly analogous to spines in mature neurons, without cell death [82]. In the animal model Golgi staining was performed with control non-mutant rats and rats treated with buthionine sulfoximine and dopamine uptake inhibitor (GBR 12909) from p5 until p24. The dendritic spines density in pyramidal neurons of layer III of pre-
frontal cortex is different from controls: a significant decrease in normal spines (−20%, p = 0.007 for class B, spine with enlargement) was observed concomitantly with an increase of abnormal spines (+30%, p = 0.003 for class A, elongated spine without enlargement and +100%, p = 0.03 for class C, spine with numerous enlargements) [58]. This is reminiscent of the decrease of the number of dendritic spines in prefrontal cortex reported in post-mortem brain of patients. These results are consistent with the concept of structural disconnectivity leading to cognitive deficits in schizophrenia.

Abnormal GABA neurons: Under the same condition, namely rats treated with buthionine sulfoximine and dopamine uptake inhibitor (GBR 12909) from p5 until p24, immunoreactivity of GABA neurons to parvalbumin is reduced in anterior cingulate cortex, but not that to calretinin [88]. This is of special interest as similar observations have been reported in post-mortem brain of schizophrenic patients, namely a decrease in parvalbumin immunoreactivity in anterior cingulate cortex while the calretinin one is normal [89, 90].

Memory deficit: In rats, treated with buthionine sulfoximine from p5 until p16, the motor development and adult-like walking is normal, suggesting that glutathione deficit is not associated with unspecified toxic consequences affecting the motor system. Their performances in an object recognition memory test were measured in adulthood. In contrast to buthionine sulfoximine-treated non-mutant rats, buthionine sulfoximine-treated mutant ODS rats did not discriminate familiar from novel objects, pointing to a deficit in episodic memory. This effect appeared earlier and was more important in male (day 65) than female (day 94) rats [80, 81]. Such an observation is of interest in view of the fact that the first psychotic episode appears about 5 years later in women than in men.

Sensory integration deficit: Rats were treated with buthionine sulfoximine from p5 to p16. Cognitive function was evaluated in the homing board task at age 260–400 days. Cognitive behaviour was assessed by the animal’s capacity to perform visual and olfactory place learning. We found that in buthionine sulfoximine-treated animals, place learning was impaired when only distant visual cues were available. Place learning was not impaired in the condition where only one olfactory cue was present (trained arena). However, the performance was impaired in buthionine sulfoximine-treated animals on the same homing table when five new differently controlled olfactory cues (one olfactory for each arena) were used [84]. These data suggest that the deficits are not attributable to sensory impairments but rather to problems arising at the level of information integration. The olfactory deficit observed in the proposed animal model is consistent with the reported olfactory recognition impairment in schizophrenia.

Thus a glutathione deficit has consequences consistent with the concept of functional disconnectivity, as a depression of NMDA-receptor function has been observed in neuronal cultures and hippocampal slices. When imposed to animals during development, it also induces a structural disconnectivity, as revealed by the decrease in dendritic spines and parvalbumine-immunoreactivity of inhibitory interneurons and suggested by the deficit in visual recognition and olfactory integration.

Discussion

The role of glutathione deficit proposed allows in a causal way to integrate many phenomenological aspects of schizophrenia. It is compatible with both the dopamine and the glutamate/NMDA hypotheses, with the neuropathological and neuroimaging indicating a decrease in prefrontal cortex thickness and dendritic spine loss. The gender differences could be explained by a differential protection against oxidative stress: the delayed appearance of initial episodes and the milder character of the disturbances in women compared to men could be related to the protective antioxidant effect of estrogens and progesterone [91, 92]. In addition, women tend to have a burst of symptoms around age 45–50, when, at the menopause, this hormonal protection tends to decrease. A potential role for viral infections (retrovirus) in the pathogenesis of schizophrenia has been proposed (see review, [93]). Glutathione is also involved in various viral infections. A decrease in glutathione was found in the corneal tissue of rabbits with Herpes Simplex 1-induced keratitis [94]. Human immunodeficiency virus (HIV) infection is associated with a systemic decrease in the glutathione content in humans [95, 96]). Of special interest is the observation that in HIV-1 tat transgenic mice the decrease in glutathione biosynthesis is accompanied by a decrease in the synthesising enzyme γ-glutamylcysteine ligase modulatory subunit (GCLM) mRNA and protein content, which resulted in an increased sensitivity of GCL to feedback inhibition by glutathione [97]. This is precisely the enzymatic subunit whose expression has been shown to be depressed in schizophrenic patients. Obstetrical and perinatal complications have been implied as risk factor for schizophrenia. In the context of our
hypothesis it is worth noting that preeclampsia pregnancies showed low glutathione levels as compared to normal pregnancy [98, 99]. Cataract is known to be related to a deficit in antioxidants, particularly in glutathione [100]. It is more frequent in patients affected by schizophrenia [101] and has been observed in animal models with reduced glutathione, including ours [80, 81]. Finally, seasonal birth influences schizophrenia incidence [102]: it is established that, in the northern hemisphere, individuals born in late winter months have a slightly higher risk to develop schizophrenia and those born in the late summer months a slightly lower risk. This correlation is reversed in the southern hemisphere. This phenomenon could be related to the fact that, during a winter pregnancy, the risk of viral infection is higher and the alimentation tends to provide less antioxidant than during a summer pregnancy.

The hypothesis also proposed that the glutathione deficit is due to a genetic anomaly affecting one or more glutathione-related enzymes from the early phases of brain development on. It is interesting to note that a polymorphism in the gene coding for the glutathione S-transferase has very recently been reported to be associated with schizophrenia [103]. This genetic component is compatible with the neurodevelopmental theory of schizophrenia according to which both genetic and environmental risk factors play a role in the disease. Indeed, the potential genetic origin of the glutathione deficit is consistent with the idea that the synaptic micro-lesions are taking place during brain maturation. These preclinical troubles exacerbate at late adolescence when stress enhances dopamine release. It is also established that the brain dopamine innervation increases toward the end of puberty and that socio-psychological stress induces a dopamine discharge. As the onset of the first disease outbreak is frequently triggered by a social stress situation, it is tempting to speculate that in a crisis situation the massive dopamine discharge outruns an already weakened glutathione antioxidant system, thus precipitating the psychotic symptoms.

In conclusion, available information obtained in our laboratory demonstrates that, in a group of patients suffering from schizophrenia, a deficit in mRNA expression of the glutathione-synthesising enzymes GCLM and GSS is likely to be responsible for the glutathione deficit in the brain. Furthermore, the results obtained in various models show that a glutathione deficit induces morphological, electrophysiological and behavioural changes analogous to those observed in the disease. Attempts to restore physiological glutathione functions could open new therapeutic avenues. This translational research, made possible by a close interaction between clinicians and neuroscientists, should also pave the way to the identification of biological markers for schizophrenia. In turn, they should allow early diagnostic and hopefully preventive intervention to this devastating disease.


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