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ORIGINAL ARTICLE

Social isolation stress and chronic glutathione deficiency have a common effect on the glutamine-to-glutamate ratio and *myo*-inositol concentration in the mouse frontal cortex

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Abstract

Environmental stress can interact with genetic predisposition to increase the risk of developing psychopathology. In this work, we tested the hypothesis that social isolation stress interacts with impaired glutathione synthesis and have cumulative effects on the neurochemical profile of the frontal cortex. A mouse model with chronic glutathione deficit induced by knockout (−/−) of the glutamate-cysteine ligase modulatory subunit (*Gclm*) was exposed to social isolation stress from weaning to post-natal day 65. Using magnetic resonance methods at high-field (14.1 T), we analysed the neurochemical profile in the frontal cortex, brain size and ventricular volume of adult animals. Glutathione deficit was accompanied by elevated concentrations of *N*-acetylaspartate, alanine, and glutamine, as well as the ratio of glutamine-to-glutamate (Gln/Glu), and by a reduction in levels of *myo*-inositol and choline-containing compounds in the frontal cortex of −/− animals with respect to wild-type littermates. Although there was no significant interaction between social isolation stress and glutathione deficiency, mice reared in isolation displayed lower *myo*-inositol concentration (−8.4%, $p < 0.05$) and larger Gln/Glu (+7.6%, $p < 0.05$), relative to those in group housing. Furthermore, glutathione deficiency caused a reduction in whole brain volume and enlargement of ventricles, but social isolation had no effect on these parameters. We conclude that social isolation caused neurochemical alterations that may add to those associated to impaired glutathione synthesis.

Keywords: magnetic resonance spectroscopy, metabolism, neurochemical profile, neurodevelopmental, social isolation.

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Chronic stress is a major risk factor for several neuropsychiatric disorders, including anxiety, depression and schizophrenia (de Kloet *et al.* 2005; Leuner and Shors 2013; Schiavone *et al.* 2013). Psychosocial stress disrupts oxidation-reduction (redox) homoeostasis by both impairing antioxidant defences and promoting free radical formation in the hippocampus and prefrontal cortex (Filipović *et al.* 2017). On the other hand, genetic predisposition for impaired glutathione synthesis increases susceptibility to oxidative stress, and has been proposed as a risk factor for

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Abbreviations used: Ala, alanine; Asc, ascorbate; Asp, aspartate; Cre, creatine; CRLB, Cramér-Rao lower bound; GABA, γ-aminobutyrate; Glc, glucose; Gln, glutamine; Glu, glutamate; Gly, glycine; GPC, glycerophosphorylcholine; GSH, glutathione; Ins, *myo*-inositol; Lac, lactate; Mac, macromolecule; MRS, magnetic resonance spectroscopy; MSUS, unpredictable maternal separation combined with unpredictable maternal stress; NAAG, *N*-acetylaspartylglutamate; NAA, *N*-acetylaspartate; NMDA, *N*-methyl-D-aspartate; PCho, phosphorylcholine; PCre, phosphocreatine; PE, phosphorylethanolamine; scyllo, *scyllo*-inositol; Tau, taurine; tCho, total choline-containing compounds; tCre, total creatine.

schizophrenia (Tosic *et al.* 2006; Gysin *et al.* 2007, 2011). Therefore, redox imbalance appears to be a converging hub for genetic and environmental risk factors in schizophrenia (Do *et al.* 2009). In particular, impairments in redox homoeostasis are linked to glutamatergic dysfunction because of hypoactive *N*-methyl-D-aspartate (NMDA) receptors, to degeneration of fast-spiking parvalbumin-positive GABAergic interneurons that are essential for fast local neuronal synchronization, to dysfunctional oligodendrocytes resulting in poor myelination and thus impairing axonal integrity and signal conduction across brain areas, and to neuroinflammation (Steullet *et al.* 2016).

Like in other tissues, cells in the brain are protected from oxidative stress by an antioxidant system that comprises a set of redox reactions, including the equilibrium between glutathione and glutathione disulphide (Dringen and Hirrlinger 2003). Glutathione levels are decreased in cerebrospinal fluid and medial prefrontal cortex of schizophrenia patients (Do *et al.* 2000; Matsuzawa and Hashimoto 2011). Moreover, subjects carrying polymorphisms in the gene coding for the catalytic subunit of the glutamate-cysteine ligase (*Gclc*) that are associated with high risk of developing schizophrenia (Gysin *et al.* 2007) display lower are lower glutathione concentration in the medial prefrontal cortex than low-risk genotype subjects (Xin *et al.* 2016). Transgenic mice have been generated to mimic this condition. While *Gclc* knockout mice are not viable (Dalton *et al.* 2000), mice with a functional deletion in the modulatory subunit of the glutamate-cysteine ligase (*Gclm*) display impaired glutathione synthesis leading to reduced glutathione levels, as well as reduced ratio of reduced-to-oxidized glutathione in the brain (Chen *et al.* 2012). Compared to wild-type mice, *Gclm*−/− mice were reported to have delayed oligodendrocyte maturation and myelination in the anterior cingulate cortex, and impaired white mater integrity (Monin *et al.* 2015; Corcoba *et al.* 2016). Furthermore, early-life insults inducing oxidative stress in *Gclm*−/− mice are detrimental to immature parvalbumin-immunoreactive interneurons and have consequences for anterior cingulate cortex functioning in adulthood (Cabungcal *et al.* 2013).

An environmental condition proposed very early as one of the possible causes of schizophrenia was social isolation (Faris 1934). In rodents, the exposure to social isolation is recognized to induce anxiety and depressive-like behaviours (*e.g.* Haj-Mirzaian *et al.* 2016; Ieraci *et al.* 2016). Interestingly, mice exposed to social isolation stress after weaning display mitochondrial dysfunction and increased oxidative stress in cortical areas (Jiang *et al.* 2013; Haj-Mirzaian *et al.* 2016), which further impacts oligodendrocytes in the prefrontal cortex, and results in impaired myelination (Liu *et al.* 2012; Makinodan *et al.* 2012). Indeed, oligodendrocytes are particularly susceptible to oxidative stress (Back *et al.* 1998). Their proliferation and differentiation is influenced by the intracellular redox state (Smith *et al.* 2000; French *et al.* 2009). Neurodegeneration of parvalbumine-positive neurons and neuroinflammation have also been shown in the prefrontal cortex of isolation-reared rats (Schiavone *et al.* 2009).

In this work, we tested the hypothesis that social isolation stress interacts with impaired glutathione synthesis and impacts the frontal cortex, resulting in local metabolic alterations. Regional concentrations of brain metabolites measured non-invasively by ^{1}H magnetic resonance spectroscopy (MRS), the so-called neurochemical profiles, have been widely used to identify specific biomarkers of neuropathology (Duarte *et al.* 2014b). Decrements of *N*-acetylaspartate levels across different brain regions have been observed in schizophrenia patients relative to healthy controls (Steen *et al.* 2005; Schwerk *et al.* 2014). However, others have demonstrated increased *N*-acetylaspartate levels in hippocampus of chronic patients (Lutkenhoff *et al.* 2010) and prefrontal cortex of high-risk adolescents (Keshavan *et al.* 2009). Bustillo *et al.* (2017) reported recently that, with age, *N*-acetylaspartate increases in cortical grey matter and decreases in white matter of schizophrenia patients (Bustillo *et al.* 2016). Alterations have also been abundantly reported for glutamine and glutamate concentrations: increased glutamine, glutamate and/or and the ratio of glutamine-to-glutamate (Gln/Glu) have been found in early stages of the disease (Tibbo *et al.* 2004; Hashimoto *et al.* 2005; Bustillo *et al.* 2009, 2017; de la Fuente-Sandoval *et al.* 2011; Brandt *et al.* 2016; Merritt *et al.* 2016), whereas decreased levels of these amino acids have often been observed in chronic patients (Tayoshi *et al.* 2009; Ohrmann *et al.*, 2005; Chiappelli *et al.* 2015; Brandt *et al.* 2016; Wijtenburg *et al.* 2017). Moreover, meta-analyses suggested a decline with age and disease duration in the levels of glutamate and glutamine (Schwerk *et al.* 2014), as well as *N*-acetylaspartate (Brugger *et al.* 2011). Schizophrenia has also been associated with alterations in *myo*-inositol levels (Chang *et al.* 2007; Chiappelli *et al.* 2015).

MRS can be applied in both clinical and pre-clinical settings, and thus represents a valuable method for translational research in the realm of schizophrenia. Indeed, we previously reported elevated Gln/Glu ratio in the frontal cortex of the *Gclm*−/− mouse, relative to controls (Duarte *et al.* 2012a; Corcoba *et al.* 2016). Napolitano *et al.* (2014) reported that social isolation results in an altered response to a ketamine (NMDA receptor antagonist) challenge in mice, namely an exacerbated ketamine-induced glutamine increase and a reduction of GABA concentration in the prefrontal cortex. Vernon *et al.* (2015), reported altered metabolite concentrations in the prefrontal cortex of adult mice born from females exposed to immune activation during gestation, namely decreased levels of glutathione, taurine and *N*-acetylaspartate.

In this study, we employed state-of-the-art, high-resolution MRS to measure brain neurochemical alterations in the

mouse frontal cortex caused by social isolation stress, redox dysregulation and their combination.

Methods

This study was conducted on male *Gclm*−/− (n = 21), +/− (n = 22) and +/+ (n = 19) mice from an in-house breeding colony (C57BL/6 background; details in Duarte *et al.* 2012a) according to the Swiss animal welfare legislation, and under approval of the local ethics committee (EXPANIM-SCAV). Mice were housed with controlled temperature (20–22°C) and humidity (50–56%) and free access to food and water, under a 12-h light-dark cycle (light off at 19 h 00). Upon weaning at post-natal day 21 ± 1, animals were housed alone or in groups of 3–5 individuals until they were scanned at post-natal day 65. To minimize litter effects in the data, animals from the same litter were randomly (based on coin tossing) assigned to both experimental groups whenever the litter size allowed it. This study focused on male mice avoid gender effects on the neurochemical profile (see Duarte *et al.* 2014a). Mice were taken from a total of 24 litters. From seven litters, only one male mouse was available. Mice allocated to be reared in isolation and group originated from 20 and 17 litters respectively. Sample size estimation was based on previous experiments (Duarte *et al*. 2012a; Corcoba *et al.* 2016; Gapp *et al.* 2017).

All experiments were carried out in a 14.1 T magnet with a horizontal bore of 26 cm (Magnex Scientific, Abingdon, UK), equipped with a 12-cm internal diameter gradient coil insert (400 mT/m, 200 μs), and interfaced to a DirectDrive console (Agilent Technologies, Palo Alto, CA, USA). Radio frequency transmission and reception were achieved with a home-built quadrature surface coil resonating at 600 MHz. Spontaneously breathing mice were anaesthetised with 1–1.5% isoflurane (Animalcare Ltd., York, UK) in a 1 : 1 O_2: air mixture, and fixed in a home-built mouse holder with a bite bar and two ear inserts. Body temperature was maintained at 37°C by a warm water circulation system receiving feedback from a rectal temperature probe. Respiration and temperature were continuously monitored using a MR-compatible system (Small Animal Instruments, Inc., Stony Brook, NY, USA). It should be noted that although blind scanning was not possible because mice were housed either alone (social isolation) or in group, spectra were analysed in an automated manner, without interference of the researchers.

T_2-weighted anatomical scans were acquired using a fast-spin-echo sequence with 3.3 s repetition time, 43.36 ms echo time, echo train length of 8, inter-echo spacing of 10.81 ms, field of view of 20 × 20 mm, matrix size of 128 × 128, 0.4 mm slice thickness, 37 slices, and 5 averages. Brain and ventricular volumes were assessed by manual segmentation of these anatomical images using FSLview (FMRIB'S Software Tools, Oxford, UK), as described previously (Corcoba *et al.* 2016).

The volume of interest (VOI) for MRS was precisely placed in the frontal cortex (4 × 0.9 × 1.6 mm^3) according to anatomical landmarks in fast-spin-echo images, reproducing the location in previous studies on the *gclm*−/− mouse (Duarte *et al.* 2012a; Corcoba *et al.* 2016). Field homogeneity in the VOI was achieved with FAST (EST)MAP (Gruetter and Tkác 2000). Spectra were acquired using SPECIAL with echo time of 2.8 ms and repetition time of 4 s (Mlynárik *et al.* 2006). The transmitter frequency was set on the water resonance for acquisition of a reference spectrum (8 scans) without VAPOR water suppression. The water-suppressed spectrum to analyse metabolites (240 scans) was acquired with transmission at 2.7 ppm. Chemical shift displacement errors were below 8%, 6% and 7% in the x, y and z directions respectively.

Metabolite concentrations were determined with LCModel (Stephen Provencher Inc., Oakville, Ontario, Canada), including a macromolecule (Mac) spectrum in the database and using the unsuppressed water signal measured from the same VOI as internal reference (Duarte *et al.* 2014a). The following metabolites were included in the analysis: alanine (Ala), ascorbate (Asc), aspartate (Asp), creatine (Cr), γ-aminobutyrate (GABA), glutamine (Gln), glutamate (Glu), glutathione (GSH), glycine (Gly), glycerophosphorylcholine, glucose (Glc), lactate (Lac), *myo*-inositol (Ins), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphorylethanolamine (PE), phosphorylcholine (PCho), phosphocreatine (PCr), *scyllo*-inositol (Scyllo), taurine (Tau). The Cramér-Rao lower bound (CRLB) was provided by LCModel as a measure of the reliability of the quantification for each metabolite. Glycerophosphorylcholine and phosphorylcholine displayed high spectral correlation and were reported as their sum, hereafter termed choline-containing compounds. In most measured spectra, *scyllo*-inositol was below the detection limit, and was excluded from subsequent analysis. With the exception of glutathione, metabolites with CRLB below 30% were also excluded from statistical analysis. Two wild-type mice were excluded from the MRS analysis because of abnormally high glutamine levels (outliers in a Tukey box plot), likely because of congenital portosystemic shunting (Cudalbu *et al.* 2013).

Statistical analyses were performed in R (R Foundation for Statistical Computing 2012). All variables were analysed by two-way ANOVAS using genotype, housing and their interaction as fixed factors. Significant effects of genotype were further investigated by Tukey honest significant difference *post hoc* pairwise comparisons. Visual inspection of residual plots revealed no deviation from normality and F-ratio tests were performed to assess homoscedasticity. Data are presented as mean±SEM unless otherwise stated. When exact p-values are not provided, significant differences were considered for $p < 0.05$.

Results

To determine major brain anatomical differences between groups, we assessed the total brain and ventricular volume from the T_2-weighted images that were acquired for VOI positioning in MRS scans at post-natal day 65, as well as the body weight before scanning (Fig. 1). We found a significant effect of genotype on body weight ($F_{2,56}$ = 7.6, p = 0.001), and *post hoc* analyses revealed lower weight in −/− animals when compared to +/− (−6.8 ± 3.0%, p = 0.02) or to +/+ mice (−8.3 ± 3.0, p = 0.004), but no differences between +/+ and +/− animals (−1.6 ± 3.0%, p = 0.8). Differences in total brain volume between genotypes ($F_{2,56}$ = 4.4, p = 0.02) were also analysed *post hoc*: −/− mice had smaller volumes relative to +/− mice (−3.5 ± 1.4%, p = 0.01), but not +/+ mice (−2.1 ± 1.5%, p = 0.2); whole brain volume was similar in +/− and +/+ mice (+1.5 ± 1.5%, p = 0.5). *post hoc* investigation of genotype

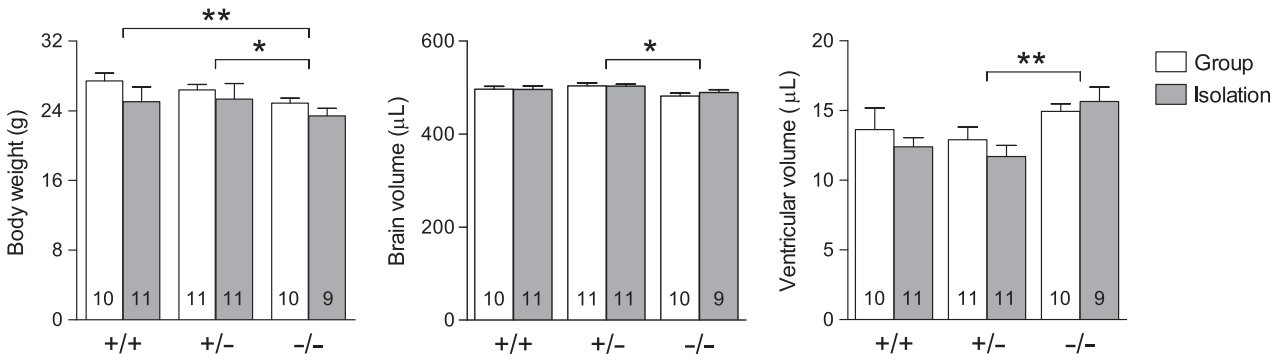

Fig. 1 Body weight, total brain volume and ventricular volume in *Gclm* +/+, +/– and –/– mice housed in either group (open bars) or isolation (filled bars). Data are shown as mean ± SEM; the number of mice per group is indicated at the bottom of each bar; *$p < 0.05$, **$p < 0.01$ from Tukey *post hoc* analyses of the genotype effects. Housing conditions had a significant effect only on the body weight ($F_{1,56} = 11.1$, $p = 0.002$).

effects on ventricular volume ($F_{2,56} = 5.3$, $p = 0.01$) revealed considerably bigger ventricles in –/– than in +/– (+24.4 ± 9.5%, $p = 0.007$) and also +/+ mice (+17.3 ± 9.3%, $p = 0.06$), but no differences between +/– and +/+ genotypes (–5.7 ± 9.2%, $p = 0.7$). The housing conditions had also an effect on the body weight ($F_{1,56} = 11.1$, $p = 0.002$), isolated animals showing a mean reduction of 6.4 ± 2.1% with respect to group-housed mice. No significant effects of housing on either brain ($F_{1,56} = 0.2$, $p = 0.7$) or ventricular ($F_{1,56} = 0.5$, $p = 0.5$) volumes were found, and interactions between genotype and housing did not reach statistical significance.

To determine metabolic alterations induced by glutathione deficiency and social isolation stress, we measured the neurochemical profile in the frontal cortex of mice of all three genotypes housed in either group or isolation (Fig. 2). Notably, glutathione was reduced to undetectable levels in the frontal cortex of *Gclm*–/– mice (CRLB> 30%), and ANOVA to *Gclm* +/+ and +/– mice revealed a significant genotype effect on its concentration ($F_{1,35} = 5.7$, $p < 0.05$). We further observed significant effects of genotype on the concentration of glutamine ($F_{2,54} = 9.3$, $p < 0.001$), the ratio Gln/Glu ($F_{2,54} = 8$, $p < 0.001$), *N*-acetylaspartate ($F_{2,54} = 5.2$, $p = 0.01$), *myo*-inositol ($F_{2,54} = 3.6$, $p = 0.03$), alanine ($F_{2,54} = 9.2$, $p < 0.001$) and choline-containing compounds ($F_{2,54} = 6.2$, $p = 0.004$), in general agreement with previous reports (Duarte *et al.* 2012a; Corcoba *et al.* 2016). The magnitude of each significant metabolic modification caused by the *Gclm* genotype in the frontal cortex is shown in Table 1. Housing conditions had an effect on the concentration of *myo*-inositol ($F_{1,54} = 6.8$, $p = 0.01$), with isolated animals displaying a 8.4 ± 3.3% reduction with respect to mice housed in groups. Gln/Glu was also affected by social isolation stress ($F_{1,54} = 4.6$, $p = 0.04$), with isolated animals showing a 7.6 ± 3.8% increase when compared to group-housed mice. This increase in Gln/Glu was mainly caused by the tendency for lower glutamate levels in isolated mice (–3.0 ± 1.7%, $F_{1,54} = 3.7$, $p = 0.06$). Notably, interactions between genotype and housing were not significant for any of the metabolites analysed.

Discussion

This study investigated for the first time the combined effect of genetically induced redox imbalance and psychosocial stress on the concentration of metabolites in the frontal cortex. Both insults resulted in increased ratio of glutamine-to-glutamate (Gln/Glu) and decreased *myo*-inositol levels, suggesting an overlapping action on cortical metabolism.

Social isolation caused an increase in Gln/Glu in the frontal cortex and a trend towards a decrease in glutamate concentration. In rats, social isolation has been reported to decrease glutamate as well as glutamine concentration in the hippocampus but not in the cortex, and to reduce antioxidant enzymatic capacity in both brain areas (Shao *et al.* 2015). Other studies have reported reduced levels of glutamate receptors in cortex and hippocampus upon chronic social isolation stress (Hermes *et al.* 2011; Sestito *et al.* 2011), suggesting alterations in glutamatergic neurotransmission. Glutamate, the most concentrated amino acid in the frontal cortex, is primarily located in neurons, and neuronal loss or reduced neuronal processes are likely to result in decreased tissue glutamate content (Duarte *et al.* 2012b). Indeed, chronic stress was reported to result in a marked reduction in the dendritic arborization in the medial prefrontal cortex (Radley *et al.* 2004; Brown *et al.* 2005; Liston *et al.* 2006).

Consistent with the results reported previously (Corcoba *et al.* 2016; Duarte *et al.* 2012a), we found an increase in glutamine (and Gln/Glu) in the frontal cortex of *Gclm*–/– with respect to +/+ and +/– mice. The common effect of social isolation and *Gclm* deletion thus highlights glutamatergic neurotransmission as one of the pathways on which genetic and environmental risk factors may converge to

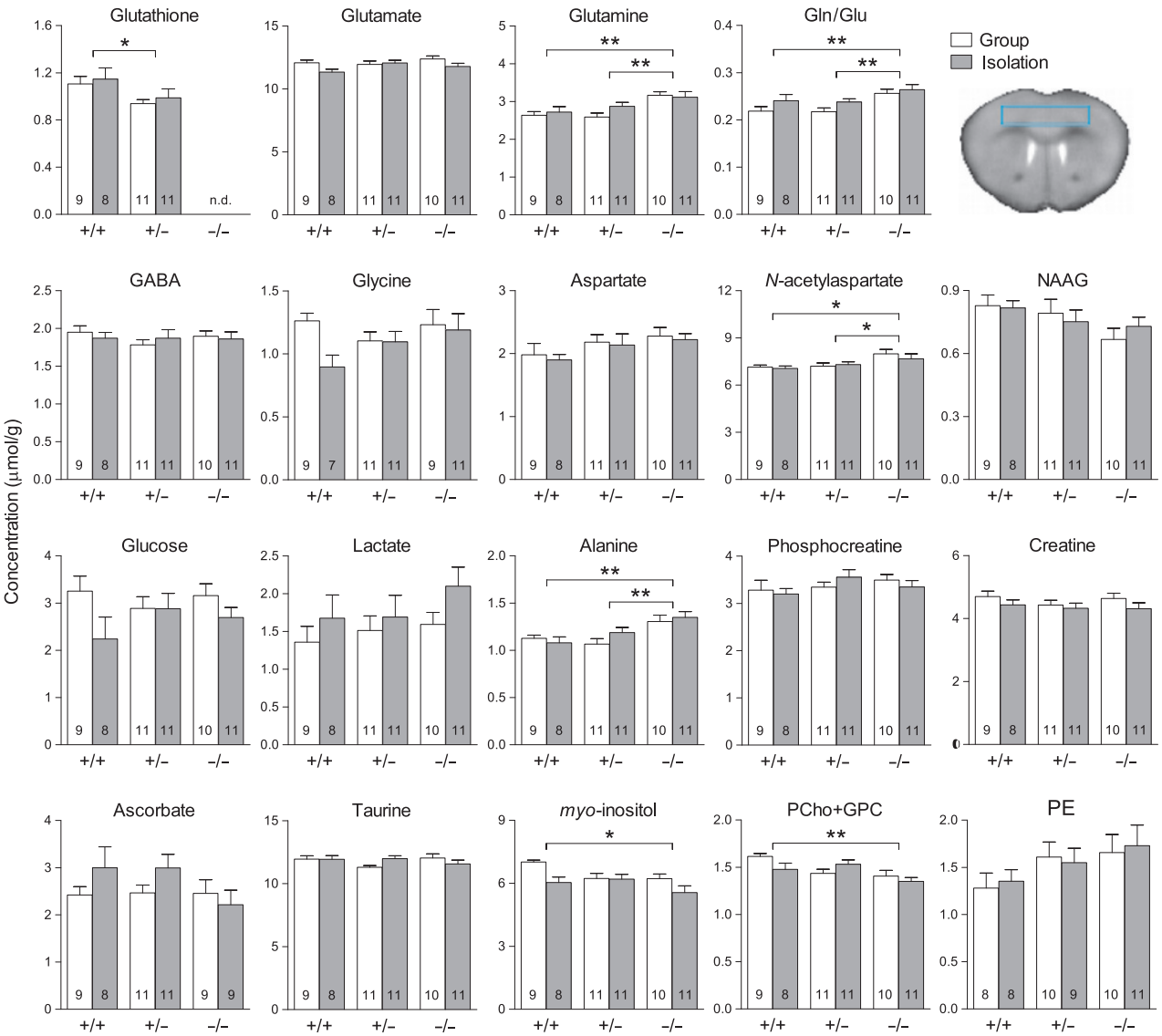

Fig. 2 Neurochemical profile in the anterior cortex of *Gclm* +/+, +/− and −/− mice housed either in group (open bars) or social isolation (filled bars). The brain image on the top right depicts the typical position of the voxel used for acquisition. Glutathione was not detectable (n.d.) in *Gclm*−/− mice. Data are shown as mean±SEM; the number of mice per group is indicated in each bar; *$p < 0.05$, **$p < 0.01$ from Tuckey *post hoc* analyses of the genotype effects. Significant housing effects were observed on the concentration of *myo*-inositol ($F_{1,54} = 6.8$, $p = 0.01$), and on Gln/Glu ($F_{1,54} = 4.6$, $p = 0.04$). Gln/Glu, glutamine-to-glutamate ratio; GABA, γ-aminobutyrate; NAAG *N*-acetylaspartylglutamate; PCho+ glycerophosphorylcholine (GPC), phosphorylcholine plus glycerophosphorylcholine; PE, phosphorylethanolamine.

trigger pathology in schizophrenia and other neuropsychiatric disorders. In line with our findings, increased Gln/Glu was found in the cerebrospinal fluid of schizophrenia patients, relative to healthy subjects (Hashimoto *et al.* 2005). An increase in glutamate plus glutamine levels was also found in the right medial frontal cortex of adolescents at high risk versus low risk for developing schizophrenia (Tibbo *et al.* 2004). Bustillo *et al.* (2009) reported higher anterior cingulate Gln/Glu in minimally treated patients than in control subjects. Interestingly, glutamate was reported to be higher in young schizophrenia patients, but to decrease with age, relative to healthy subjects (Schwerk *et al.* 2014; Brandt *et al.* 2016).

Other changes in the neurochemistry of *Gclm*−/− mice included an increase in *N*-acetylaspartate levels, also reported in previous experiments (Corcoba *et al.* 2016; Duarte *et al.* 2012a), which could be a consequence of impaired aspartoacylase activity in oligodendrocytes, with potential consequences for myelination (discussed in Corcoba *et al.* 2016; Monin *et al.* 2015). Indeed, the rate of glutathione synthesis is crucial to maintain normal balance between reduced and oxidized glutathione states (Dringen and Hirrlinger 2003),

Table 1 Differences in metabolite concentrations between *Gclm* genotypes calculated from *post hoc* pairwise comparisons (see Fig. 2)

Metabolite	Comparison	Difference ± SD (%)	*p*-value
Glutathione (GSH)[a]	−/− to −/+		
	−/− to +/+		
	−/+ to +/+	−14 ± 6	0.022
Glutamine (Gln)	−/− to −/+	15 ± 5	0.002
	−/− to +/+	17 ± 6	0.001
	−/+ to +/+	2 ± 6	0.889
Gln/Glu	−/− to −/+	14 ± 5	0.002
	−/− to +/+	14 ± 5	0.007
	−/+ to +/+	−1 ± 5	0.988
N-acetylaspartate (NAA)	−/− to −/+	8 ± 4	0.035
	−/− to +/+	10 ± 4	0.011
	−/+ to +/+	2 ± 4	0.804
myo-inositol (Ins)	−/− to −/+	−5 ± 5	0.384
	−/− to +/+	−10 ± 5	0.038
	−/+ to +/+	−5 ± 5	0.409
Alanine (Ala)	−/− to −/+	18 ± 6	0.002
	−/− to +/+	20 ± 7	0.001
	−/+ to +/+	2 ± 7	0.929
Choline-containing compounds	−/− to −/+	−7 ± 4	0.079
	−/− to +/+	−11 ± 4	0.004
	−/+ to +/+	−4 ± 4	0.395

p-values are from *post hoc* Tukey tests.
[a]Glutathione was undetectable in *Gclm*−/− mice.

which has a role on cell maturation, namely of oligodendrocytes (discussed in Monin *et al.* 2015; Corcoba *et al.* 2016). While this early *N*-acetylaspartate increase (our mice were young adults – P65) may be associated with myelination deficits, it is possible that it decreases at later ages, following what has been observed in patients. A meta-analysis of 64 clinical MRS studies (mostly including chronic patients) indicates decrease in brain *N*-acetylaspartate in schizophrenia (Steen *et al.* 2005). However, early drug naive or minimally treated patients were reported to display unaltered *N*-acetylaspartate in the anterior cingulate (Ohrmann *et al.* 2005). Interestingly, it was recently reported that while cortical *N*-acetylaspartate is unchanged in young patients relative to controls, it tends to increase with age in grey matter and to decrease in white matter (Bustillo *et al.* 2017).

Impaired myelination by oligodendrocytes (Monin *et al.* 2015) is also consistent with the observed reduction in levels of choline-containing compounds in −/− relative to +/+ mice. Phosphorylcholine and glycerophosphorylcholine are the major water-soluble choline-containing compounds observed in brain MRS, and their reduction has been associated with impaired turnover of cellular membranes (Duarte *et al.* 2012b). These choline-containing compounds are precursors of phosphatidylcholine and, in turn, of sphingomyelin, which is necessary for adequate myelination of axons (Oshida *et al.* 2003). In addition to integrating membranous myelin sheaths surrounding axons, sphingomyelin has been implicated in immune responses (Li *et al.* 2015).

Reduced levels of *myo*-inositol in *Gclm*−/− mice when compared to controls may also reflect impaired cell membrane turnover. *myo*-inositol is a precursor of the membrane lipid phosphatidylinositol, and the concentrations of both compounds increase steadily after birth in the mouse brain (Yao *et al.* 1999; Kulak *et al.* 2010). In addition, mice reared in social isolation displayed reduced *myo*-inositol concentration in the frontal cortex, compared to those in group housing. In line with a role of *myo*-inositol in cell membrane metabolism, mice exposed to social isolation stress after weaning display alterations in the morphology of oligodendrocytes and in the thickness of the myelin sheaths enwrapping axons of the prefrontal cortex, as well as reduced expression of myelin-related genes (Liu *et al.* 2012; Makinodan *et al.* 2012).

Impaired myelination during development is likely to affect connectivity across brain areas. Recently, neuroimaging methods were able to identify disrupted brain connectivity in mice housed in isolation for 1 month from post-natal day 35 onwards, prominently affecting the dorsolateral orbitofrontal cortex (Liu *et al.* 2016). In humans, controlled studies on the effect of severe social isolation are rare because of the ethical implications, but a recent follow-up study of children reared in orphanages showed that institutionalized children had smaller cortical white matter volumes (Sheridan *et al.* 2012) and reduced fractional anisotropy (a putative MRI marker of white matter integrity) in the uncinate fasciculus (Eluvathingal *et al.* 2006).

Anatomical images acquired for guidance upon VOI placement in MRS scans were further analysed for gross anatomical morphology. Volumetric measurements of the brain of *Gclm*−/− mice detected an increase in the ventricular volume with respect to +/− and a trend in the same direction when compared to +/+ animals. This increase stands out because −/− mice were smaller than the other two groups both in body weight and in brain volume. Moreover, this increased ventricular volume replicates previously published findings (Corcoba *et al.* 2016). Interestingly, ventricular enlargement has been suggested to constitute a neuroanatomical hallmark of schizophrenia (Wright *et al.* 2000; Shenton *et al.* 2001; van Erp *et al.* 2016) that appears already in first episode patients (Steen *et al.* 2006; Vita *et al.* 2006).

No effect of social isolation on ventricular or total brain volume was observed, even though isolated animals had consistently lower body weights than their group-housed littermates. A previous study reported increased size of the lateral but not third ventricles together with a decrease in brain size and body weight in male rats reared in isolation for 15 weeks after weaning (Fabricius *et al.* 2010). In our study, with mice reared in isolation after weaning during 6 weeks,

volumes of the whole brain and ventricles were similar to controls. The discrepancy between our study and that of Fabricius *et al.* (2010) is likely because of the shorter isolation period in our study, although it cannot be excluded that the effect of isolation may be different in the two animal species. Noteworthy, although mice exposed to social isolation stress were smaller than those reared in group, the lack of significant isolation-induced effects on the volumes of the brain and ventricles remained when body weight was used as covariate in the statistical analyses (not shown). Moreover, it has been reported that substantial variability in the brain anatomy exists in both inbred and outbred mouse strains (Scholz *et al.* 2016). When brain volume was used as a covariate in the analysis of ventricular volume, statistical outcomes remained unchanged, that is, there was an effect of genotype ($p = 0.010$) but not of isolation stress ($p = 0.499$; interaction $p = 0.536$).

In our study, MRS was performed on a VOI in the frontal cortex that included prefrontal as well as motor areas, matching previous work in this animal model (Duarte *et al.* 2012a; Corcoba *et al.* 2016). Despite not specific to a subcortical region, these results demonstrate that environmental stress impacts the neurochemical profile in the frontal cortex, and may accentuate metabolic alterations caused by redox imbalance in *Gclm*−/− mice. In particular, we further conclude that Gln/Glu and *myo*-inositol constitute good candidate biomarkers for schizophrenia research as they were prominently affected both by redox dysregulation and social isolation stress.

Acknowledgments and conflict of interest disclosure

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All experiments were conducted in compliance with the ARRIVE guidelines.

Author contributions

KQD conceptualized the study, AC and JMND designed the study. AC performed experiments. All authors interpreted results and contributed to write the manuscript.

References

Back S. A., Gan X., Li Y., Rosenberg P. A. and Volpe J. J. (1998) Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. *J. Neurosci.* **18**, 6241–6253.

Brandt A. S., Unschuld P. G., Pradhan S. *et al.* (2016) Age-related changes in anterior cingulate cortex glutamate in schizophrenia: a ^{1}H MRS Study at 7 Tesla. *Schizophr. Res.* **172**, 101–105.

Brown S. M., Henning S. and Wellman C. L. (2005) Short-term, mildstress alters dendritic morphology in rat medial prefrontal cortex. *Cereb. Cortex* **15**, 1714–1722.

Brugger S., Davis J. M., Leucht S. and Stone J. M. (2011) Proton magnetic resonance spectroscopy and illness stage in schizophrenia - a systematic review and meta-analysis. *Biol. Psychiatry* **69**, 495–503.

Bustillo J. R., Rowland L. M., Mullins P., Jung R., Chen H., Qualls C., Hammond R., Brooks W. M. and Lauriello J. (2009) ^{1}H-MRS at 4 Tesla in minimally treated early schizophrenia. *Mol. Psychiatry* **15**, 629–636.

Bustillo J. R., Jones T., Chen H., Lemke N., Abbott C., Qualls C., Stromberg S., Canive J. and Gasparovic C. (2017) Glutamatergic and neuronal dysfunction in gray and white matter: a spectroscopic Imaging study in a large schizophrenia sample. *Schizophr. Bull.* **43**, 611–619.

Cabungcal J. H., Steullet P., Kraftsik R., Cuenod M. and Do K. Q. (2013) Early-life insults impair parvalbumin interneurons via oxidative stress: reversal by *N*-acetylcysteine. *Biol. Psychiatry* **73**, 574–582.

Chang L., Friedman J., Ernst T., Zhong K., Tsopelas N. D. and Davis K. (2007) Brain metabolite abnormalities in the white matter of elderly schizophrenic subjects: implication for glial dysfunction. *Biol. Psychiatry* **62**, 1396–1404.

Chen Y., Curran C. P., Nebert D. W., Patel K. V., Williams M. T. and Vorhees C. V. (2012) Effect of chronic glutathione deficiency on the behavioral phenotype of Gclm-/- knockout mice. *Neurotoxicol. Teratol.* **34**, 450–457.

Chiappelli J., Rowland L. M., Wijtenburg S. A., Muellerklein F., Tagamets M., McMahon R. P., Gaston F., Kochunov P. and Hong L. E. (2015) Evaluation of *myo*-inositol as a potential biomarker for depression in schizophrenia. *Neuropsychopharmacology* **40**, 2157–2164.

Corcoba A., Steullet P., Duarte J. M. N., Van de Looij Y., Monin A., Cuenod M., Gruetter R. and Do K. Q. (2016) Glutathione deficit affects the integrity and function of the fimbria/fornix and anterior commissure in mice: relevance for schizophrenia. *Int. J. Neuropsychopharmacol.* **19**, 1–11.

Cudalbu C., McLin V. A., Lei H., Duarte J. M. N., Rougemont A. L., Oldani G., Terraz S., Toso C. and Gruetter R. (2013) The C57BL/6 mouse exhibits sporadic congenital portosystemic shunts. *PLoS ONE* **8**, e69782.

Dalton T. P., Dieter M. Z., Yang Y., Shertzer H. G. and Nebert D. W. (2000) Knockout of the mouse glutamate cysteine ligase catalytic subunit (Gclc) gene: embryonic lethal when homozygous, and proposed model for moderate glutathione deficiency when heterozygous. *Biochem. Biophys. Res. Commun.* **279**, 324–329.

Do K. Q., Trabesinger A. H., Kirsten-Krüger M., Lauer C. J., Dydak U., Hell D., Holsboer F., Boesiger P. and Cuénod M. (2000) Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *Eur. J. Neurosci.* **12**, 3721–3728.

Do K. Q., Cabungcal J. H., Frank A., Steullet P. and Cuenod M. (2009) Redox dysregulation, neurodevelopment, and schizophrenia. *Curr. Opin. Neurobiol.* **19**, 220–230.

Dringen R. and Hirrlinger J. (2003) Glutathione pathways in the brain. *Biol. Chem.* **384**, 505–516.

Duarte J. M. N., Kulak A., Gholam-Razaee M. M., Cuenod M. R., Gruetter R. and Do K. Q. (2012a) *N*-acetylcysteine normalizes neurochemical changes in the glutathione-deficient schizophrenia mouse model during development. *Biol. Psychiatry* **71**, 1006–1014.

Duarte J. M. N., Lei H., Mlynárik V. and Gruetter R. (2012b) The neurochemical profile quantified by *in vivo* ^{1}H NMR spectroscopy. *NeuroImage* **61**, 342–362.

Duarte J. M. N., Do K. Q. and Gruetter R. (2014a) Longitudinal neurochemical modifications in the aging mouse brain measured *in vivo* by ^{1}H MRS. *Neurobiol. Aging* **35**, 1660–1668.

Duarte J. M. N., Schuck P. F., Wenk G. L. and Ferreira G. C. (2014b) Metabolic disturbances in diseases with neurological involvement. *Aging. Dis.* **5**, 238–255.

Eluvathingal T. J., Chugani H. T., Behen M. E., Juhász C., Muzik O., Maqbool M., Chugani D. C. and Makki M. (2006) Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. *Pediatrics* **117**, 2093–2100.

van Erp T. G., Hibar D. P., Rasmussen J. M. *et al.* (2016) Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. *Mol. Psychiatry* **21**, 547–553.

Fabricius K., Helboe L., Steiniger-Brach B., Fink-Jensen A. and Pakkenberg B. (2010) Stereological brain volume changes in post-weaned socially isolated rats. *Brain Res.* **1345**, 233–239.

Faris R. E. L. (1934) Cultural isolation and the schizophrenic personality. *Am. J. Sociol.* **40**, 155–164.

Filipović D., Todorović N., Bernardi R. E. and Gass P. (2017) Oxidative and nitrosative stress pathways in the brain of socially isolated adult male rats demonstrating depressive- and anxiety-like symptoms. *Brain Struct. Funct.* **222**, 1–20.

French H. M., Reid M., Mamontov P., Simmons R. A. and Grinspan J. B. (2009) Oxidative stress disrupts oligodendrocyte maturation. *J. Neurosci. Res.* **87**, 3076–3087.

de la Fuente-Sandoval C., León-Ortiz P., Favila R., Stephano S., Mamo D., Ramírez-Bermúdez J. and Graff-Guerrero A. (2011) Higher levels of glutamate in the associative-striatum of subjects with prodromal symptoms of schizophrenia and patients with first-episode psychosis. *Neuropsychopharmacology* **36**, 1781–1791.

Gapp K., Corcoba A., vanSteenwyk G., Mansuy I. M. and Duarte J. M. N. (2017) Brain metabolic alterations in mice subjected to postnatal traumatic stress and in their offspring. *J. Cereb. Blood Flow Metab.* **37**, 2423–2432.

Gruetter R. and Tkác I. (2000) Field mapping without reference scan using asymmetric echo-planar techniques. *Magn. Reson. Med.* **43**, 319–323.

Gysin R., Kraftsik R., Sandell J. *et al.* (2007) Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence. *Proc. Natl Acad. Sci. USA* **104**, 16621–16626.

Gysin R., Kraftsik R., Boulat O. *et al.* (2011) Genetic dysregulation of glutathione synthesis predicts alteration of plasma thiol redox status in schizophrenia. *Antioxid. Redox Signal.* **15**, 2003–2010.

Haj-Mirzaian A., Amiri S., Amini-Khoei H. *et al.* (2016) Attenuation of oxidative and nitrosative stress in cortical area associates with antidepressant-like effects of tropisetron in male mice following social isolation stress. *Brain Res. Bull.* **124**, 150–163.

Hashimoto K., Engberg G., Shimizu E., Nordin C., Lindström L. H. and Iyo M. (2005) Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naïve schizophrenic patients. *BMC Psychiatry* **5**, 6.

Hermes G., Li N., Duman C. and Duman R. (2011) Post-weaning chronic social isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic-associated protein expression in female rats. *Physiol. Behav.* **104**, 354–359.

Ieraci A., Mallei A. and Popoli M. (2016) Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. *Neural. Plast.* **2016**, 6212983.

Jiang Z., Rompala G. R., Zhang S., Cowell R. M. and Nakazawa K. (2013) Social isolation exacerbates schizophrenia-like phenotypes via oxidative stress in cortical interneurons. *Biol. Psychiatry* **73**, 1024–1034.

Keshavan M. S., Dick R. M., Diwadkar V. A., Montrose D. M., Prasad K. M. and Stanley J. A. (2009) Striatal metabolic alterations in non-psychotic adolescent offspring at risk for schizophrenia: a ^{1}H spectroscopy study. *Schizophr. Res.* **115**, 88–93.

de Kloet E. R., Joëls M. and Holsboer F. (2005) Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* **6**, 463–475.

Kulak A., Duarte J. M. N., Do K. Q. and Gruetter R. (2010) Neurochemical profile of the developing mouse cortex determined by *in vivo* ^{1}H NMR spectroscopy at 14.1 T and the effect of recurrent anaesthesia. *J. Neurochem.* **115**, 1466–1477.

Leuner B. and Shors T. J. (2013) Stress, anxiety, and dendritic spines: what are the connections? *Neuroscience* **251**, 108–119.

Li M., Fan P. and Wang Y. (2015) Integrative approaches for lipid analysis. *Pharmacologia* **6**, 213–234.

Liston C., Miller M. M., Goldwater D. S., Radley J. J., Rocher A. B., Hof P. R., Morrison J. H. and McEwen B. S. (2006) Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J. Neurosci.* **26**, 7870–7874.

Liu J., Dietz K., DeLoyht J. M. *et al.* (2012) Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nat. Neurosci.* **15**, 1621–1623.

Liu C., Li Y., Edwards T. J., Kurniawan N. D., Richards L. J. and Jiang T. (2016) Altered structural connectome in adolescent socially isolated mice. *NeuroImage* **139**, 259–270.

Lutkenhoff E. S., van Erp T. G., Thomas M. A., Therman S., Manninen M., Huttunen M. O., Kaprio J., Lönnqvist J., O'Neill J. and Cannon T. D. (2010) Proton MRS in twin pairs discordant for schizophrenia. *Mol. Psychiatry* **15**, 308–318.

Makinodan M., Rosen K. M., Ito S. and Corfas G. (2012) A critical period for social experience-dependent oligodendrocyte maturation and myelination. *Science* **337**, 1357–1360.

Matsuzawa D. and Hashimoto K. (2011) Magnetic resonance spectroscopy study of the antioxidant defense system in schizophrenia. *Antioxid. Redox Signal.* **15**, 2057–2065.

Merritt K., Egerton A., Kempton M. J., Taylor M. J. and McGuire P. K. (2016) Nature of Glutamate Alterations in Schizophrenia: a Meta-analysis of Proton Magnetic Resonance Spectroscopy Studies. *JAMA Psychiatry* **73**, 665–674.

Mlynárik V., Gambarota G., Frenkel H. and Gruetter R. (2006) Localized short-echo-time proton MR spectroscopy with full signal-intensity acquisition. *Magn. Reson. Med.* **56**, 965–970.

Monin A., Baumann P. S., Griffa A. *et al.* (2015) Glutathione deficit impairs myelin maturation: relevance for white matter integrity in schizophrenia patients. *Mol. Psychiatry* **20**, 827–838.

Napolitano A., Shah K., Schubert M. I., Porkess V., Fone K. C. and Auer D. P. (2014) In vivo neurometabolic profiling to characterize the effects of social isolation and ketamine-induced NMDA antagonism: a rodent study at 7.0 T. *Schizophr. Bull.* **40**, 566–574.

Ohrmann P., Siegmund A., Suslow T., Spitzberg K., Kersting A., Arolt V., Heindel W. and Pfleiderer B. (2005) Evidence for glutamatergic neuronal dysfunction in the prefrontal cortex in chronic but not first-episode patients with schizophrenia: a proton magnetic resonance spectroscopy study. *Schizophr. Res.* **73**, 153–157.

Oshida K., Shimizu T., Takase M., Tamura Y., Shimizu T. and Yamashiro Y. (2003) Effects of dietary sphingomyelin on central nervous system myelination in developing rats. *Pediatr. Res.* **53**, 589–593.

R Foundation for Statistical Computing (2012) *R: a Language and Environment for Statistical Computing*. Vienna, Austria, ISBN: 3-900051-07-0, (http://www.r-project.org/).

Radley J. J., Sisti H. M., Hao J., Rocher A. B., McCall T., Hof P. R., McEwen B. S. and Morrison J. H. (2004) Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience* **125**, 1–6.

Schiavone S., Sorce S., Dubois-Dauphin M., Jaquet V., Colaianna M., Zotti M., Cuomo V., Trabace L. and Krause K. H. (2009) Involvement of NOX2 in the development of behavioral and pathologic alterations in isolated rats. *Biol. Psychiatry* **66**, 384–392.

Schiavone S., Jaquet V., Trabace L. and Krause K. H. (2013) Severe life stress and oxidative stress in the brain: from animal models to human pathology. *Antioxid. Redox Signal.* **18**, 1475–1490.

Scholz J., LaLiberté C., van Eede M., Lerch J. P. and Henkelman M. (2016) Variability of brain anatomy for three common mouse strains. *NeuroImage* **142**, 656–662.

Schwerk A., Alves F. D., Pouwels P. J. and van Amelsvoort T. (2014) Metabolic alterations associated with schizophrenia: a critical evaluation of proton magnetic resonance spectroscopy studies. *J. Neurochem.* **128**, 1–87.

Sestito R. S., Trindade L. B., de Souza R. G., Kerbauy L. N., Iyomasa M. M. and Rosa M. L. (2011) Effect of isolation rearing on the expression of AMPA glutamate receptors in the hippocampal formation. *J. Psychopharmacol.* **25**, 1720–1729.

Shao Y., Yan G., Xuan Y., Peng H., Huang Q. J., Wu R. and Xu H. (2015) Chronic social isolation decreases glutamate and glutamine levels and induces oxidative stress in the rat hippocampus. *Behav. Brain Res.* **282**, 201–208.

Shenton M. E., Dickey C. C., Frumin M. and McCarley R. W. (2001) A review of MRI findings in schizophrenia. *Schizophr. Res.* **49**, 1–52.

Sheridan M. A., Fox N. A., Zeanah C. H., McLaughlin K. A. and Nelson C. A. 3rd (2012) Variation in neural development as a result of exposure to institutionalization early in childhood. *Proc. Natl Acad. Sci. USA* **109**, 12927–12932.

Smith J., Ladi E., Mayer-Proschel M. and Noble M. (2000) Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. *Proc. Natl Acad. Sci. USA* **97**, 10032–10037.

Steen R. G., Hamer R. M. and Lieberman J. A. (2005) Measurements of brain metabolites by ^{1}H magnetic resonance spectroscopy in patients with schizophrenia: a systematic review and meta-analysis. *Neuropsychology* **30**, 1949–1962.

Steen R. G., Mull C., McClure R., Hamer R. M. and Lieberman J. A. (2006) Brain volume in first-episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. *Br. J. Psychiatry* **188**, 510–518.

Steullet P., Cabungcal J.-H., Monin A., Dwir D., O'Donnell P., Cuenod M. and Do K. Q. (2016) Redox dysregulation, neuroinflammation, and NMDA receptor hypofunction: a "central hub" in schizophrenia pathophysiology? *Schizophr. Res.* **176**, 41–51.

Tayoshi S. Y., Sumitani S., Taniguchi K., Shibuya-Tayoshi S., Numata S., Iga J., Nakataki M., Ueno S., Harada M. and Ohmori T. (2009) Metabolite changes and gender differences in schizophrenia using 3-Tesla proton magnetic resonance spectroscopy (^{1}H-MRS). *Schizophr. Res.* **108**, 69–77.

Tibbo P., Hanstock C., Valiakalayil A. and Allen P. (2004) 3-T proton MRS investigation of glutamate and glutamine in adolescents at high genetic risk for schizophrenia. *Am. J. Psychiatry* **161**, 1116–1118.

Tosic M., Ott J., Barral S. *et al.* (2006) Schizophrenia and oxidative stress: glutamate cysteine ligase modifier as a susceptibility gene. *Am. J. Hum. Genet.* **79**, 586–592.

Vernon A. C., So P. W., Lythgoe D. J., Chege W., Cooper J. D., Williams S. C. and Kapur S. (2015) Longitudinal in vivo maturational changes of metabolites in the prefrontal cortex of rats exposed to polyinosinic-polycytidylic acid in utero. *Eur. Neuropsychopharmacol.* **25**, 2210–2220.

Vita A., De Peri L., Silenzi C. and Dieci M. (2006) Brain morphology in first-episode schizophrenia: a meta-analysis of quantitative magnetic resonance imaging studies. *Schizophr. Res.* **82**, 75–88.

Wijtenburg S. A., Wright S. N., Korenic S. A. *et al.* (2017) Altered glutamate and regional cerebral blood flow levels in schizophrenia: a ^{1}H-MRS and pCASL study. *Neuropsychopharmacology* **42**, 562–571.

Wright I. C., Rabe-Hesketh S., Woodruff P. W., David a S., Murray R. M. and Bullmore E. T. (2000) Meta-analysis of regional brain volumes in schizophrenia. *Am. J. Psychiatry* **157**, 16–25.

Xin L., Mekle R., Fournier M. *et al.* (2016) Genetic polymorphism associated prefrontal glutathione and its coupling with brain glutamate and peripheral redox status in early psychosis. *Schizophr. Bull.* **42**, 1185–1196.

Yao F. S., Caserta M. T. and Wyrwicz A. M. (1999) *In vitro* proton and phosphorus NMR spectroscopic analysis of murine (C57Bl/6J) brain development. *NMR Biomed.* **12**, 463–470.