Developmental in vivo 1H NMR spectroscopy at 14.1 T in mice with genetic redox dysregulation: an animal model with relevance to schizophrenia

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Introduction: Glutathione (GSH), a major redox regulator and antioxidant, is decreased in cerebrospinal fluid and prefrontal cortex of schizophrenia patients [1]. The genes of the key GSH-synthesizing enzyme, glutamate-cysteine ligase catalytic (GCLC) and modifier (GCLM) subunits, are associated with schizophrenia, suggesting that the deficit in GSH synthesis is of genetic origin [2]. GCLM knock-out (KO) mice, which display a 80% decrease in brain GSH levels, have abnormal brain morphology and function [3].In schizophrenia, a developmental redox dysregulation may constitute one pathological "hub" on which converge genetic impairments of GSH synthesis and environmental risk factors generating oxidative stress [3]. Here, we used GCLM KO mice to investigate the impact of a genetically deregulated redox system on the neurochemical profile of the developing brain. The neurochemical profile of the frontal/anterior and occipital/posterior cortex of male and female GCLM KO and wild-type mice was determined by *in vivo* ^{1}H NMR spectroscopy on postnatal days 10, 20, 30, 60 and 90.

Methods: Localised ^{1}H NMR spectroscopy was performed on a 14.1 T, 26 cm VNMRS spectrometer (Varian, Magnex) using a home-built 8 mm diameter quadrature surface coil (used both for RF excitation and signal reception). Mice were anaesthetized under 1 to 2% isoflurane in oxygen gas. Field homogeneity was adjusted by FASTMAP [4], and ^{1}H NMR spectra were acquired from VOIs of 2.5 to 4 µL placed in anterior or posterior regions of the cortex, using SPECIAL [5,6] with TE of 2.8 ms and TR of 4 s. Typically, spectra were acquired with 480 scans. Metabolite concentrations were estimated with LCModel [7], using corrections for water content variation during development. Data was analyzed with a combined statistical linear model accounting for several variables including cortical area, genotype and age.

Results: The present study reports alterations of the neurochemical profile in the cortex of a mouse model of redox deregulation induced by genetic reduction of glutathione synthesis (figure 1). ^{1}H NMR spectroscopy at 14.1 T allowed the *in vivo* quantification of a neurochemical profile composed of 18 metabolites [6]. The evolution of this neurochemical profile was generally similar in both cortical regions of WT mice, with slight differences that were previously reported to the society [8]. GCLM KO mice displayed nearly undetectable GSH levels as compared to WT mice, demonstrating their drastic redox deregulation. Depletion of GSH triggered alteration of metabolites related to its synthesis, namely increase of glycine and glutamate levels during development. Concentrations of glutamine and aspartate that are produced from glutamate were also increased in GCLM KO animals relative to WT. In addition, GCLM KO mice also showed higher levels of *N*-acetylaspartate that originates from the acetylation of aspartate. These metabolites are particularly implicated in neurotransmission processes and in mitochondrial oxidative metabolism. Their increase may indicate impaired mitochondrial metabolism with concomitant accumulation of lactate in the adult mice (P60 and P90).

Conclusion: The observed metabolic alterations in the cortex of a mouse model of redox deregulation suggest impaired mitochondrial metabolism and eventually altered neurotransmission, both possibly triggering degeneration.

References: [1] Do *et al.* (2000) Eur J Neurosci 12:3721. [2] Gysin *et al.* (2007) PNAS 104:16621. [3] Do *et al.* (2009) Curr Opin Neurobiol 19 :220 . [4] Gruetter (1993) MRM 29:804. [5] Mlynárik *et al.* (2006) MRM 56:965. [6] Mlynárik *et al.* (2008) J Mag Reson 194:163. [7] Provencher (1993) MRM 30:672. [8] Duarte *et al.* (2009) Proc Intl Soc Mag Reson Med 17:3295.

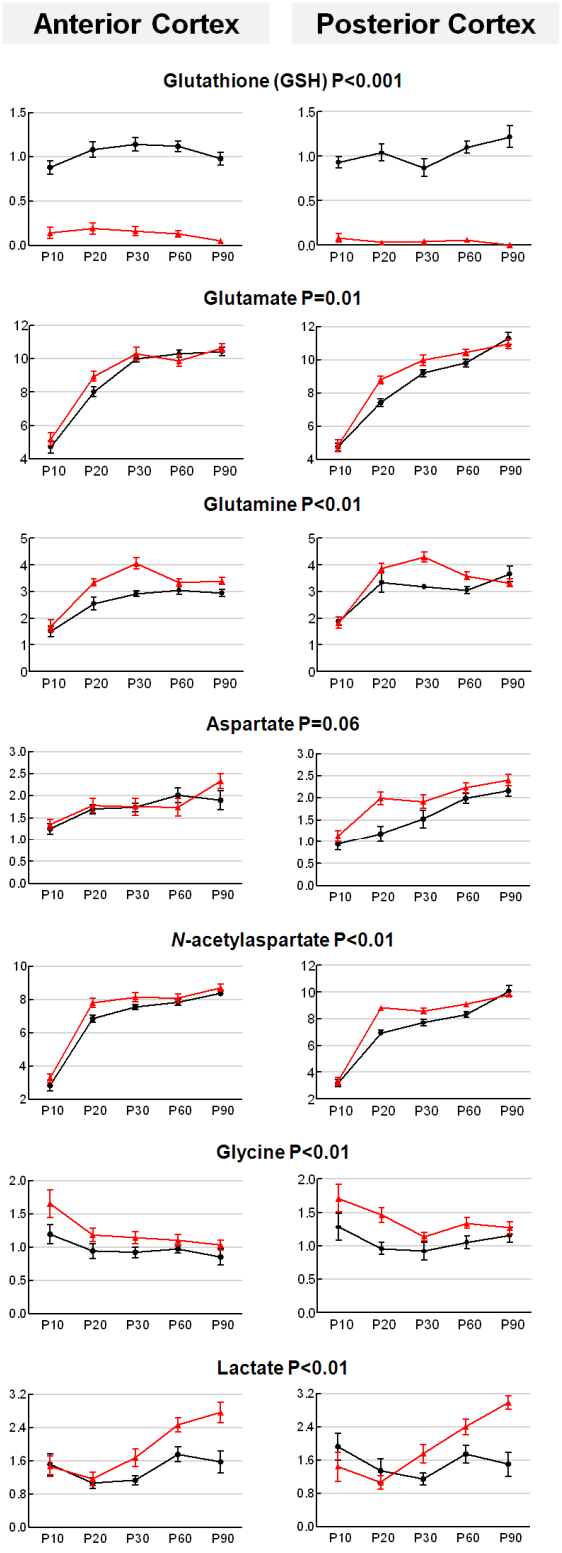

Figure 1. Alteration of the neurochemical profile by redox deregulation as determined by ^{1}H NMR spectroscopy at 14.1 T, in anterior and posterior regions of the developing cortex. Metabolite concentrations in µmol/g are shown as mean±SEM of 12 wild-type (in black) and 16 GCLM knock-out (in red) mice.