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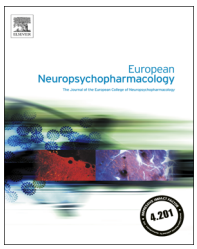

Glutamatergic synapse protein composition of wild-type mice is sensitive to in utero MTHFR genotype and the timing of neonatal vigabatrin exposure

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Abstract
The enzyme methylenetetrahydrofolate-reductase (MTHFR) is part of the homocysteine and folate metabolic pathways. In utero, *Mthfr*-deficient environment has been reported as a risk factor for neurodevelopmental disorders such as autism and neural tube defects. Neonatal disruption of the GABAergic system is also associated with behavioral outcomes. The interaction between *Mthfr* deficiency and neonatal exposure to the GABA-potentiating drug vigabatrin (GVG) in mice alters anxiety, memory, and social behavior in a gender-dependent manner. In addition, a gender-dependent enhancement of proteins implicated in excitatory synapse plasticity in the cerebral cortex was shown. Here we show that in utero MTHFR deficiency is sufficient to alter the levels of glutamate receptor subunits GluR1, GluR2, and NR2B in the cerebral cortex and hippocampus of adult offspring with a WT genotype. In addition, FMRP1, CAMKII α and γ, and NLG1 levels in WT offspring were vulnerable to the in utero genotype. These effects depend on brain region and the cellular compartment tested. The effect of in utero MTHFR deficiency varies with the age of neonatal GVG exposure to modify GluR1, NR2A, reelin, CAMKII α, and NLG1 levels. These changes in molecular composition of the glutamatergic synapse were associated with increased anxiety-like behavior.

Abbreviations: AED, antiepileptic drugs; AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CaMKII, Calcium/calmodulin-dependent kinase II; Dab1, Disabled-1; EPM, elevated plus maze; FMRP, Fragile X mental retardation 1 protein; GluR1, AMPA receptor subunit 1; GluR2, AMPA receptor subunit 2; GVG, Vigabatrin; KCC2, K^+-Cl^- transporter; MTHFR, Methylenetetrahydrofolate reductase; NLG1, Neuroligin 1; NKCC1, Na^+-K^+-2Cl, co-transporter; NMDA, N-methyl-di-aspartate; NR2A, NMDA receptor subunit 2A; NR2B, NMDA receptor subunit 2B; PKA, protein kinase A; PSD95, postsynaptic density protein 95; WT, wild type.

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Complex, multifactorial disorders of the nervous system show significant association with several genetic and environmental factors. Our data exemplify the contribution of an in utero MTHFR-deficient environment and early exposure to an antiepileptic drug to the basal composition of the glutamatergic synapses. The robust effect is expected to alter synapse function and plasticity and the cortico-hippocampal circuitry.

1. Introduction

Methylenetetrahydrofolate reductase (MTHFR) is a regulatory enzyme involved in the homocysteine and folate metabolic pathways. MTHFR is a critical enzyme in the one-carbon metabolism pathway as a source of the methyl donor S-adenosylmethionine (SAM), essential for DNA methylation. In humans, the 677C>T variant is a polymorphism in the *Mthfr* gene leading to reduced activity and impaired folate status of the enzyme, and it is a common genetic cause of mild hyperhomocysteinemia under impaired folate status (Frosst et al., 1995). The frequency of the polymorphism MTHFR677TT among Autism Spectrum Disorder children and their mothers is higher than in the general population (James et al., 2006; Mohammad et al., 2009; Goin-Kochel et al., 2009; Liu et al., 2011; Schmidt et al., 2012; Pu et al., 2013). An additive effect of additional gene variants in one carbon (C1) metabolism in the in utero environment was shown among newborns of mothers with MTHFR677TT genotype (Schmidt et al., 2011). In addition, the repeated finding that prenatal folic acid supplementation is associated with reduced risk for autism supports the link between the in utero C1 metabolism and normal brain development (Pu et al., 2013).

In mice, MTHFR activity is absent in *Mthfr*-deficient homozygous mice and reduced to 60–70% of that of wild type (WT) littermates in heterozygous mice. This level of MTHFR enzymatic activity is comparable to the levels of activity observed in humans with the 677C>T polymorphism.

Several antiepileptic drugs (AEDs) have been shown to interfere with homocysteine-folate metabolism, increasing homocysteine levels and decreasing those of folate, either dependent on or independent of the presence of the 677C>T *Mthfr* mutation (Sener et al., 2006; Vilaseca et al., 2000; Vurucu et al., 2008).

AEDs act via a variety of mechanisms, and the members of one AED category potentiate the efficacy of the GABAergic system. In the immature brain GABA is a developmental signal involved in various aspects of cell cycle, migration, morphogenesis, and synaptogenesis (Wang and Kriegstein, 2009). In addition, in the newborn brain GABA potentials are depolarizing compared to the adult brain where GABA potentials hyperpolarize the neuron membrane. This change in the nature of GABA is due to postnatal gradual increase in the expression of theK^+-Cl^- co-transporter KCC2 in neurons. In mice, KCC2 expression begins at postnatal days (P) 4–7 and by P12–P14 the expression of KCC2 in most neurons is sufficient to cause hyperpolarizing of GABA potentials (Ganguly et al., 2001); moreover, parallel decrease in NKCC1 expression at P10–P14 further supports the hyperpolarizing nature of GABA potentials (Dzhala et al., 2005).

Vigabatrin (GVG) is a new class of AEDs that blocks GABA degradation by inhibition of GABA-transaminase (Mumford and Cannon, 1994). Neonatal exposure to GABA-potentiating drugs was previously shown to induce long-lasting behavioral consequences, including learning and memory impairment in human and animal models (Dessens et al., 2000; Qume et al., 1995; Qiao et al., 2000; Levav et al., 2008) and modified synaptogenesis in mouse brain (Levav et al., 2008). Specific alteration of the GABA pathway by early neonatal GVG depends on the exact age of treatment (Levav-Rabkin et al., 2010).

Moreover, dopaminergic, noradrenergic, and glutamatergic transmission in the hippocampus and cerebral cortex of neonatal GVG-exposed mice undergo long-term alterations (Melamed et al., 2014).

A previous study from our group reported an interaction between *Mthfr* deficiency and neonatal exposure to the GABA-potentiating drug GVG in mice. Several domains of behavior were affected differentially in male vs. female mice; a. mice exploration in an open field test revealed gender-dependent effects of *Mthfr*+/− genotype and treatment; b. social behavior was impaired in female, but not male, mice; c. in contrast, recognition memory was impaired similarly by *Mthfr*+/− genotype and GVG treatment in both genders (Levav-Rabkin et al., 2011; Blumkin et al., 2011). The behavioral impairment was associated with a gender-dependent enhancement of proteins implicated in glutamatergic synapse plasticity in the female cortex. Reelin and fragile x mental retardation 1 protein (FMRP) levels and membrane GluR1/GluR2 ratios were elevated in mice treated neonatally with GVG and *Mthfr* +/− mice treated with saline, but not in *Mthfr*+/− mice treated with GVG, compared to control group. A minor influence on the levels of these proteins was observed in male mice cortices, possibly due to high basal protein levels.

Reports on the association between in utero MTHFR deficiency and the risk for autism in offspring (Schmidt et al., 2012, 2011) motivated us to explore the impact of maternal MTHFR genetic status and its outcome on proteins of the glutamatergic synapse. Moreover, since neonatal AED exposure was shown to modify proteins of the glutamatergic synapse and to interact with MTHFR to modify behavior both in humans (Dean et al., 2007) and mice (Levav-Rabkin et al., 2011; Blumkin et al., 2011), we examined here the interaction between the maternal MTHFR genotype and the age of neonatal GABA potentiation, and their impact on proteins critical for the glutamatergic synapse function. Specifically, this study examined if shortening the GABAergic intervention and limiting it to the time of GABA switch (postnatal days four to ten), compared to treatment during and after