



Short Communication

Methylenetetrahydrofolate reductase deficiency alters levels of glutamate and γ -aminobutyric acid in brain tissueN.M. Jadavji^{a,*}, F. Wieske^b, U. Dirnagl^{a,c}, C. Winter^b^a Department of Experimental Neurology, Center for Stroke Research Berlin, Charité University Medicine Berlin, Germany^b Department of Experimental Psychiatry, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany^c German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme key regulator in folate metabolism. Deficiencies in MTHFR result in increased levels of homocysteine, which leads to reduced levels of S-adenosylmethionine (SAM). In the brain, SAM donates methyl groups to catechol-O-methyltransferase (COMT), which is involved in neurotransmitter analysis. Using the MTHFR-deficient mouse model the purpose of this study was to investigate levels of monoamine neurotransmitters and amino acid levels in brain tissue. MTHFR deficiency affected levels of both glutamate and γ -aminobutyric acid in within the cerebellum and hippocampus. *Mthfr*^{-/-} mice had reduced levels of glutamate in the amygdala and γ -aminobutyric acid in the thalamus. The excitatory mechanisms of homocysteine through activation of the N-methyl-D-aspartate receptor in brain tissue might alter levels of glutamate and γ -aminobutyric acid.

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1. Introduction

Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism since it generates the main circulating form of folate, 5-methyltetrahydrofolate (5-methylTHF), which can serve as a methyl donor for the remethylation of homocysteine to methionine and subsequently the generation of S-adenosylmethionine (SAM) (Fig. 1). Through methylation SAM is involved in lipid metabolism and also donates a methyl group to catechol-O-methyltransferase (COMT), which catalyzes synthesis of neurotransmitters in the brain [1,2]. Increased levels of homocysteine, as a result of folate deficiency, have been linked to low levels of monoamine neurotransmitter levels in patients with depression [3].

Many polymorphisms of MTHFR have been described in the human population and the most common is a polymorphism at base pair 677, a conversion from C to T which results in a missense mutation causing an

alanine to valine conversion [4]. Homozygosity has been reported in 5–20% of North American and European populations [5]. These individuals have reduced enzyme activity (heterozygotes 65% and homozygotes 30% compared to controls [6]) and slightly elevated levels of plasma homocysteine, which is highly dependent on folate status [5,7]. Individuals with the TT genotype have increased risk of developing cognitive impairment [8], late onset Alzheimer's disease [9], as well as depression and schizophrenia [10]. Another condition involved in MTHFR deficiency is an inborn error of metabolism, which results in a severe deficiency in the enzyme and leads to homocystinuria. Patients with a severe MTHFR deficiency have significantly elevated levels of homocysteine and reduced levels of methionine and present with neurological and vascular complications [11–13].

In order to investigate the effects of MTHFR deficiency on neurological function in vivo, a knockout mouse model for MTHFR was developed, *Mthfr*^{+/-} and *Mthfr*^{-/-} mice have elevated levels of plasma homocysteine [14]. *Mthfr*^{+/-} mice model the polymorphism at base pair 677, as they have elevated levels of plasma homocysteine when compared to wildtype mice but are phenotypically normal. Whereas *Mthfr*^{-/-} mice model the severe form of MTHFR deficiency, these mice have significantly elevated levels of homocysteine and have motor and cognitive impairments [15]. In brain tissue, both *Mthfr*^{+/-} and *Mthfr*^{-/-} mice have significantly reduced levels of DNA methylation and S-adenosylhomocysteine [14] and *Mthfr*^{-/-} mice have reduced levels of 5-methylTHF [14,16] and SAM [14]. Additionally, behavioral studies have reported impairments in cognitive and motor function

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-methylTHF, 5-methyltetrahydrofolate; COMT, catechol-O-methyltransferase; HPLC, high performance liquid chromatography; MTHFR, methylenetetrahydrofolate reductase; SAM, S-adenosylmethionine; GABA, γ -aminobutyric acid; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin.

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[15,17]. The purpose of this study was to investigate whether levels of monoamine neurotransmitters and amino acids are altered in different brain regions of MTHFR-deficient mice.

2. Materials and methods

All experiments were approved by the Landesamt für Gesundheit und Soziales Berlin and performed in accordance with the German Animal Welfare Act. At 3-months of age blood and brain tissue from C57BL/6 male mice was collected and processed for post-mortem neurochemical analyses using high performance liquid chromatography (HPLC) as described previously [18]. The levels of dopamine, serotonin, and their metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) in the amygdala, caudate putamen, cerebellum, dorsal raphe, hippocampus, medial prefrontal cortex, nucleus accumbens, and thalamus, amygdalae were measured by HPLC with electrochemical detection. Glutamate and γ -aminobutyric acid (GABA) were determined by HPLC with fluorescence detection. We worked with male mice in this study to avoid sex differences that have been previously described in MTHFR mice [19] and neurotransmitter analysis [20].

Blood samples taken from the same mice were centrifuged at $7000 \times g$ for 7 min at 4°C to obtain the plasma. HPLC was used to measure plasma homocysteine concentrations by a diagnostic laboratory at Charite University Medicine (Labor 28, Berlin).

One-way analysis of variance (ANOVA) was used to compare genotype groups for each measurement followed by Tukey's post-hoc test if applicable. Significance level was set at $p \leq 0.05$.

3. Results

Mthfr^{+/-} and *Mthfr*^{-/-} had significantly higher plasma homocysteine levels when compared to *Mthfr*^{+/+} mice (Table 1, $p < 0.05$).

There were few changes in monoamine neurotransmitter levels and respective metabolites. Serotonin levels were increased in *Mthfr*^{-/-} in the amygdala when compared to *Mthfr*^{+/+} mice (Table 1, $p < 0.05$). Dopamine levels within the cerebellum were different between genotype groups (Table 1, one-way ANOVA, $F_{(2, 23)} = 8.45$, $p < 0.01$). 5-HIAA was increased in the cerebellum of *Mthfr*^{+/-} (Table 1, $p < 0.05$) and there was a genotype effect in the hippocampus (Table 1, one-way ANOVA, $F_{(2, 21)} = 3.35$, $p < 0.05$).

Glutamate levels were different between genotype groups within the amygdala (Table 1, one-way ANOVA, $F_{(2, 21)} = 3.45$, $p < 0.05$). *Mthfr*^{-/-} mice had reduced levels of glutamate in the cerebellum (Table 1, $p < 0.05$) compared to *Mthfr*^{+/-} mice, and hippocampus (Table 1, $p < 0.05$) compared to *Mthfr*^{+/+} mice. Cerebellar GABA levels were increased in *Mthfr*^{+/-} and *Mthfr*^{-/-} when compared to *Mthfr*^{+/+} mice (Table 1, $p < 0.05$). While, GABA levels were reduced in hippocampus (Table 1, $p < 0.05$) and thalamus (Table 1, $p < 0.05$) of *Mthfr*^{-/-} mice compared to *Mthfr*^{+/+}.

4. Discussion

Increased levels of plasma homocysteine have been linked to impaired monoamine synthesis as a result of reduced levels of SAM [1]. In the present study using a hyperhomocysteinemic MTHFR-deficient mouse model we investigated levels of the monoamine neurotransmitters, dopamine, 5-HT, and their respective metabolites, as well as the amino acids, glutamate and GABA in different brain areas. We confirmed that *Mthfr*^{+/-} (~14 μM) and *Mthfr*^{-/-} (~70 μM) have significantly elevated levels of plasma homocysteine as previously reported in earlier work [14,15,21]. We identified that *Mthfr*^{-/-} have increased levels of serotonin in the amygdala, whereas *Mthfr*^{+/-} have increased levels of cerebellar dopamine levels. Levels of 5-HIAA are increased in *Mthfr*^{+/-} mice in the cerebellum and reduced in the hippocampus of *Mthfr*^{+/-} and *Mthfr*^{-/-} mice. Additionally, hippocampal levels of HVA

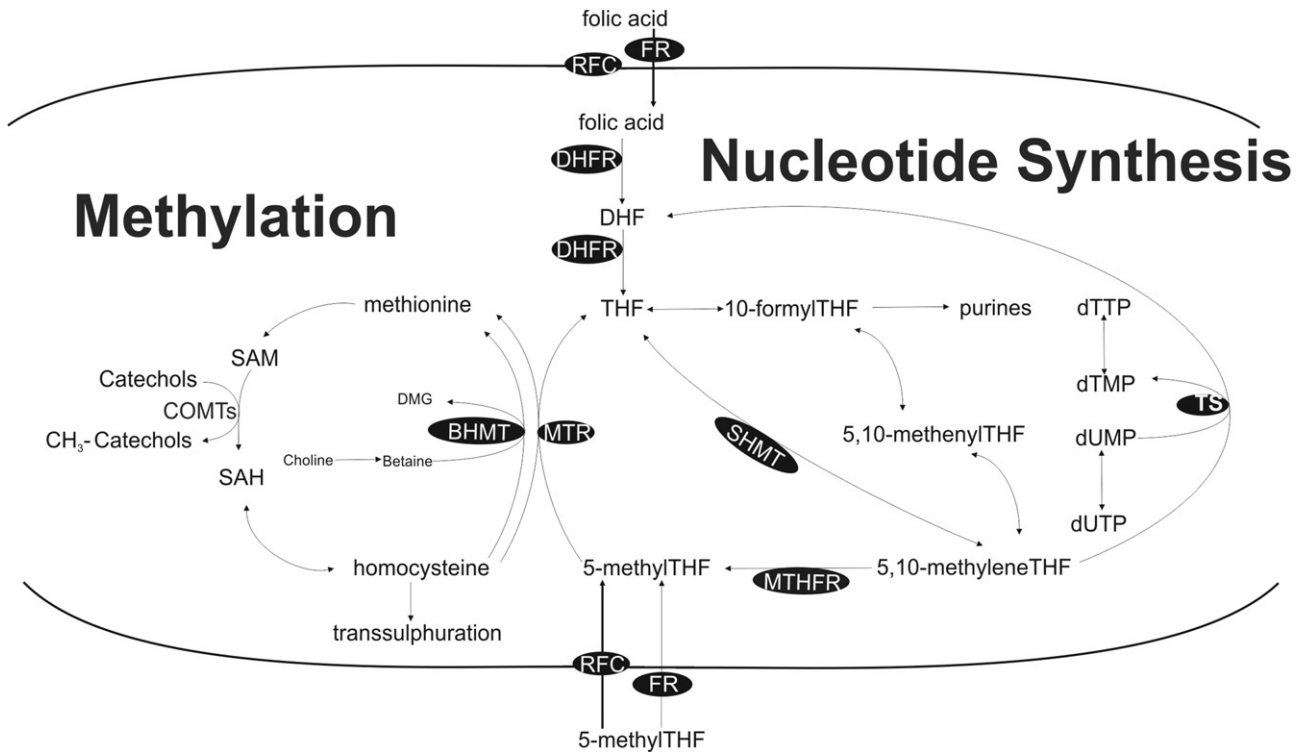


Fig. 1. One-carbon metabolism and related biological roles (methylation and nucleotide synthesis). Enzymes indicated in black circles. Abbreviations: BHMT – betaine homocysteine methyltransferase; CHDH – choline dehydrogenase; COMT – catechol-O-methyltransferase; DHFR – dihydrofolate reductase; dTMP – deoxythymidine monophosphate; dTTP – deoxythymidine triphosphate; dUMP – deoxyuridine monophosphate; dUTP – deoxyuridine triphosphate; FR – folate receptor; MTHFR – methylenetetrahydrofolate reductase; MTR – methionine synthase; MTRR – methionine synthase reductase; RFC – reduced folate carrier; SAM – S-adenosylmethionine; SAH – S-adenosylhomocysteine; and TS – thymidylate synthase.

Table 1

Plasma homocysteine concentrations and quantification of neurotransmitters and amino acids in different brain regions of *Mthfr*^{+/+}, *Mthfr*^{+/-} and *Mthfr*^{-/-} mice¹.

	<i>Mthfr</i> ^{+/+}	<i>Mthfr</i> ^{+/-}	<i>Mthfr</i> ^{-/-}	p value
Plasma homocysteine (μM)	8.2 ± 0.8	13.6 ± 1.4	71.1 ± 6.9	^{ab,c}
Brain area & neurotransmitter (μMol/g protein)				
Amygdala				
Dopamine	52.6 ± 12.3	74.8 ± 20.6	46.4 ± 10.5	NS
DOPAC	7.9 ± 1.7	10.1 ± 3.0	6.9 ± 1.1	NS
5-HT	57.0 ± 8.1	87.0 ± 10.9	94.3 ± 10.5	^{ac}
5-HIAA	17.2 ± 2.0	25.9 ± 4.0	23.6 ± 3.5	NS
HVA	14.9 ± 2.8	14.6 ± 2.3	10.8 ± 1.1	NS
Glutamate	101.4 ± 4.9	121.3 ± 5.8	113.9 ± 6.2	^{sa}
GABA	24.7 ± 1.7	27.6 ± 3.2	27.9 ± 4.0	NS
Caudate putamen				
Dopamine	739.8 ± 50.2	770.9 ± 30.4	728.4 ± 39.5	NS
DOPAC	46.6 ± 2.5	46.9 ± 2.5	49.1 ± 4.4	NS
5-HT	24.6 ± 2.6	16.1 ± 3.2	18.7 ± 4.0	NS
5-HIAA	10.1 ± 1.4	8.2 ± 1.3	7.7 ± 1.8	NS
HVA	55.4 ± 3.1	57.2 ± 3.2	51.1 ± 3.0	NS
Glutamate	92.1 ± 3.1	93.6 ± 4.8	81.2 ± 4.9	NS
GABA	17.5 ± 0.9	17.6 ± 0.4	15.6 ± 0.7	NS
Cerebellum				
Dopamine	1.3 ± 0.05	2.0 ± 0.2	1.4 ± 0.08	^{sa}
DOPAC	4.5 ± 0.5	3.7 ± 0.8	3.3 ± 0.5	NS
5-HT	3.8 ± 0.3	6.5 ± 1.4	5.1 ± 0.72	NS
5-HIAA	3.3 ± 0.3	5.8 ± 1.0	5.1 ± 0.4	^{sb}
HVA	0.6 ± 0.08	0.7 ± 0.1	0.6 ± 0.05	NS
Glutamate	69.7 ± 2.2	77 ± 2.2	68.3 ± 1.9	^{sd}
GABA	15.3 ± 0.5	18.0 ± 0.8	18.4 ± 0.8	^{ab,c}
Dorsal raphe				
Dopamine	11.5 ± 1.4	9.8 ± 1.2	9.6 ± 1.2	NS
DOPAC	5.0 ± 0.7	4.3 ± 0.6	4.3 ± 0.7	NS
5-HT	104.9 ± 20	85.3 ± 12.5	86.3 ± 15.0	NS
5-HIAA	54.2 ± 12.5	39.5 ± 6.7	35.2 ± 5.7	NS
HVA	13.9 ± 2.9	14.4 ± 2.9	8.3 ± 1.6	NS
Glutamate	67.1 ± 2.2	80.5 ± 4.4	77.5 ± 9.3	NS
GABA	33.6 ± 1.7	38.6 ± 2.6	28.7 ± 3.2	NS
Hippocampus				
Dopamine	2.9 ± 0.2	2.8 ± 0.3	3.6 ± 0.5	NS
DOPAC	5.4 ± 0.9	4.0 ± 1.0	3.6 ± 0.6	NS
5-HT	21.8 ± 1.3	21.7 ± 1.0	20.1 ± 1.9	NS
5-HIAA	19.8 ± 1.2	16.2 ± 1.0	15.3 ± 1.8	^{sa}
HVA	1.6 ± 0.1	1.2 ± 0.08	1.5 ± 0.2	^{sd}
Glutamate	90.2 ± 2.3	81.6 ± 3.5	76.0 ± 4.9	^{sc}
GABA	20.5 ± 1.0	18.4 ± 1.1	15.5 ± 1.0	^{sc}
Medial prefrontal cortex				
Dopamine	72.2 ± 31.0	71.0 ± 23.7	74.8 ± 29.2	NS
DOPAC	9.5 ± 1.8	8.4 ± 1.5	10.9 ± 2.3	NS
5-HT	31.6 ± 3.4	41.3 ± 6.7	41.8 ± 6.9	NS
5-HIAA	17.8 ± 3.3	20.1 ± 2.9	16.6 ± 3.1	NS
HVA	12.5 ± 1.8	10.5 ± 2.2	11.8 ± 1.5	NS
Glutamate	106.7 ± 7.7	121.5 ± 7.5	102.4 ± 10.0	NS
GABA	18.4 ± 1.7	21.1 ± 1.7	20.7 ± 2.8	NS
Nucleus accumbens				
Dopamine	633.0 ± 128.7	617.7 ± 171.2	1015 ± 115.4	NS
DOPAC	65.5 ± 12.1	56.4 ± 12.8	85.4 ± 12.8	NS
5-HT	80.2 ± 16.9	50.2 ± 9.4	111.0 ± 9.2	NS
5-HIAA	31.0 ± 6	18.5 ± 3.3	33.9 ± 5.1	NS
HVA	81.7 ± 10.2	52.3 ± 13.3	89.2 ± 6.1	NS
Glutamate	192.7 ± 24.6	147.0 ± 26.5	193.0 ± 7.9	NS
GABA	70.3 ± 6.1	56.0 ± 12.0	79.5 ± 9.5	NS
Thalamus				
Dopamine	11.4 ± 2.7	14.9 ± 2.7	14.1 ± 3.2	NS
DOPAC	2.9 ± 0.5	2.6 ± 0.6	2.7 ± 0.4	NS
5-HT	33.1 ± 5.4	39.5 ± 4.9	26.7 ± 3.9	NS
5-HIAA	18.1 ± 2.0	20.1 ± 3.4	14.5 ± 2.5	NS
HVA	7.3 ± 1.0	7.6 ± 0.3	6.4 ± 0.4	NS
Glutamate	97.6 ± 3.4	97.8 ± 3.9	91.9 ± 5.2	NS
GABA	21.3 ± 1.1	19.7 ± 1.4	15.4 ± 1.2	^{sc}

¹ All values are means ± SEM; n = 6–8 per group.

^a Significant one-way ANOVA.

^b p < 0.05, Tukey's post-hoc, *Mthfr*^{+/+} vs. *Mthfr*^{+/-}.

^c p < 0.05, Tukey's post-hoc, *Mthfr*^{+/+} vs. *Mthfr*^{-/-}.

^d p < 0.05, Tukey's post-hoc, *Mthfr*^{+/-} vs. *Mthfr*^{-/-}.

levels were reduced in *Mthfr*^{-/-} mice. More significantly, in *Mthfr*^{+/-} and *Mthfr*^{-/-} mice glutamate and GABA levels were changed in the amygdala, cerebellum, hippocampus and thalamus. Interestingly, when we examined whole brain levels of monoamine neurotransmitter and amino acid levels, no differences between genotype groups were observed (data not shown).

The cerebellum and hippocampus are susceptible to a deficiency in MTHFR as previously described [15,22–24]. More specifically, the cerebellum has the highest levels of homocysteine [25], which may make it more vulnerable to damage via homocysteine and change levels of metabolism of dopamine, serotonin and amino acids, specifically glutamate and GABA. Previous work has shown that homocysteine results in stimulation of the glutamate subunit on the N-methyl-D-aspartate receptor [26], it may be the increased levels of glutamate and GABA within the cerebellum are to counter the excitatory effects of homocysteine. On the other hand, deficiencies in MTHFR have been shown to impair hippocampal function [15,24]. There is ongoing neurogenesis within the dentate gyrus during adulthood [27] which may increase the demand for folate. In the present study we identified that glutamate is increased in amygdala and GABA is decreased in the thalamus as a result of severe MTHFR deficiency, this is novel data.

In the present study, a deficiency in MTHFR does not result in significant changes of monoamine neurotransmitter levels in majority of brain areas measured. This could be a result of compensation in *Mthfr*^{+/-} and *Mthfr*^{-/-} mice, since the deficiency is present from conception. In the peripheral nervous system, choline metabolism is significantly altered in MTHFR mice [28]. During folate deficiency choline can serve as an alternative methyl donor for remethylation of homocysteine to methionine via betaine S-homocysteine methyltransferase, however this enzyme is not highly expressed in brain tissue [29]. Interestingly, both serotonin and dopamine are also present in the peripheral nervous system. We propose that synthesis of serotonin and dopamine may be compensated in the peripheral nervous system, since previous data has shown that SAM levels are not changed in the liver of *Mthfr*^{+/-} and *Mthfr*^{-/-} mice possibly as a result of choline compensation [14].

This study has a number of limitations, the first being that tetrahydrobiopterin (BH4) which has been described as the rate limiting step required in the synthesis of monoamine neurotransmitters. Interestingly, BH4 structurally resembles folate and has been described to be reduced in endothelial cells when increased levels of homocysteine are present [30]. An additional study measuring levels of BH4 in brain tissue of MTHFR-deficient mice may be beneficial in understanding how this deficiency affects monoamine neurotransmission. Furthermore, studies measuring more monoamine neurotransmitters such as norepinephrine as well as catechol-A-methyltransferase (COMT) levels in different brain areas may help dissect the effect of MTHFR deficiency, elevated levels of plasma homocysteine and reduced SAM levels on neurotransmitter synthesis in this mouse model. C57Bl/6 mice have previously been shown to be less susceptible to MTHFR deficiency in comparison to BALB/c mice [21], therefore additional studies are may be warranted to investigate levels of monoamine neurotransmitter levels in BALB/c mice.

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