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Homocysteine, folate and vitamin B12 in neuropsychiatric diseases: review and treatment recommendations. — Source link

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Abstract: In Europe, neuropsychiatric diseases currently make up approximately a third of the total burden of disease. In 2004, 27% of the overall population was affected by at least one of the most frequent neuropsychiatric diseases such as Alzheimer's dementia, Parkinson's disease, stroke or depression. The annual costs of care exceed those of cancer, cardiovascular conditions and diabetes. In order to delay the onset or course of neurodegenerative diseases, the available potential should be utilized. As well as improving quality of life of patients and relatives, this may reduce the great financial burden caused by neurodegenerative disorders. However, the availability of established drugs or therapeutic agents is very limited. This paper reviews the state of current knowledge as to how homocysteine metabolism is relevant for neurodegenerative and other neuropsychiatric diseases, with particular emphasis on the evidence for prophylactic and therapeutic strategies. In the European countries, many people do not take the recommended daily minimum amount of folate and vitamin B12. Deficiency of these vitamins and secondary changes in the concentrations of associated metabolites, such as methylmalonic acid and homocysteine, may contribute to the onset and progression of neuropsychiatric diseases. This paper reviews the evidence regarding whether substitution of folate and vitamin B12 is beneficial, for example, in cerebrovascular disease, dementia and depression.

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Homocysteine, folate and vitamin B12 in neuopsychiatric diseases: review of the literature and recommendations for treatment

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Abstract

In Europe, neuropsychiatric diseases currently make up around one third of the total burden of disease. In 2004, 27% of the overall population was affected by at least one of the most frequent neuropsychiatric diseases such as Alzheimer dementia, Parkinson's disease, stroke or depression. The annual costs of care exceed those of cancer, cardiovascular conditions and diabetes. In order to delay onset or course of neurodegenerative diseases, the available potential should be utilized. As well as improving quality of life of patients and relatives, this may reduce the great financial burden caused by neurodegenerative disorders. However, the availability of established drugs or therapeutic agents is very limited. This paper reviews the state of current knowledge on to what extend homocysteine metabolism is relevant for neurodegenerative and other neuropsychiatric diseases with particular emphasis on the evidence for prophylactic and therapeutic strategies.

In the European countries, many people do not take the recommended daily minimum amount of folate and vitamin B12. Deficiency of these vitamins and secondary changes in the concentrations of associated metabolites, such as methylmalonic acid and homocysteine, may contribute to onset and progression of neuropsychiatric diseases. This paper reviews the evidence on whether substitution of folate and vitamin B12 is beneficial e.g. in several cases of cerebrovascular disease, dementia and depression.

Introduction

Worldwide, around 400 million people suffer from neurological and mental disorders [1]. The general consensus is that neuropsychiatric diseases make up around 35%

of the total burden of disease in Europe [2,3]. For example, in Europe, around 1.1 million new cases of cerebral ischaemia are reported every year [4]. The annual treatment costs for a total of 127 million Europeans suffering from at least one of the most frequent neuropsychiatric diseases amounted to €386 billion in 2004, which is more than the costs caused by cancer, cardiovascular disorders or diabetes [2]. Apart from those direct costs for hospitalisation, medical and nursing care, rehabilitation, drugs, psycho- and physiotherapy, laboratory costs and medical-technical services, there are additional indirect costs due to incapacity, disability and early death.

In European countries, many people do not take the recommended daily minimum amount of vitamin B12 and folate (naturally occurring biologically active form of the vitamin) or folic acid (precursor of folate, e.g. ingredient of synthetic vitamin drugs) [5-7]. Deficiency of these vitamins and consecutive changes in the concentration of associated metabolites such as methylmalonic acid (MMA) and homocysteine may contribute to the onset and progression of neuropsychiatric diseases [8-15].

1. Metabolism

1.1 Biochemistry

Folate and vitamin B12 play an important role in the development, differentiation and function of the central nervous system. Both vitamins are involved in methionine-homocysteine metabolism (figure 1). Methionine becomes activated to S-adenosylmethionine (SAM), which is indispensable for numerous reactions involving methylation, e.g. in the synthesis of nucleic acids [DNA, RNA], proteins, neurotransmitters, hormones, fatty acids, polysaccharides, phospholipids or DNA

methylation [16]. For example, several neuroendocrinologically important micro-molecules, such as noradrenaline and N-acetylserotonine, are converted by SAM dependent methylation reactions into biologically active neurotransmitters [17]. Others, such as L-DOPA, can be deactivated through SAM-dependent methylations. S-adenosylhomocysteine (SAH) results from SAM through the release of the methyl group. Due to antagonism, elevated SAH concentrations reduce the SAM-dependent methylation capacity and, by doing so, impair numerous metabolic processes in the brain [18]. Several cell dysfunctions, DNA damage and disturbed biosynthesis of myelin are potential consequences [19]. SAH is hydrolyzed into homocysteine. The reaction converting SAH into homocysteine is reversible and is favoured, when an increase in homocysteine levels occurs. Plasma homocysteine correlates closely with the SAH level in the cerebrospinal fluid and the brain in animals [20]. Homocysteine is a neuro- and vasculotoxic sulphur-containing intermediary product. Homocysteine can be transsulfurated to cystathionine and, subsequently, to cysteine, which is a component of glutathione. Transsulfuration of homocysteine depends on vitamin B6. Alternatively, homocysteine can be remethylated to methionine by addition of a methyl group from 5-methyltetrahydrofolate (5-MTHF), which is synthesized by 5,10-methylenetetrahydrofolate reductase (MTHFR). Remethylation can be catalyzed by methionine-synthase (MS), which requires vitamin B12 in the form of methylcobalamin as cofactor. By the remethylation reaction of homocysteine to methionine, 5-MTHF is regenerated to 5,10-MTHF, which is necessary for nucleic acid synthesis. Thus, vitamin B12 deficiency leading to a reduced methylation rate of homocysteine to methionine can lead to a functional deficiency of 5,10-MTHF and subsequent dysfunction e.g. of the haematopoietic system (“folate trap”). Reduced remethylation of homocysteine to methionine and

SAM due to a lack of vitamin B12 or folate can yield elevated levels of homocysteine. Reduced synthesis of SAM can lead to a state of “hypomethylation” e.g. resulting in disturbed synthesis of neurotransmitters and proteins important for the structural integrity of the brain (figure 1) [21,22].

The homocysteine metabolism in the brain is different from the systemic homocysteine metabolism. In the liver and the kidneys, there is an alternative pathway available for remethylation, apart from MS, the enzyme betaine-homocysteine-methyltransferase (BHMT), but this enzyme has not been detected in the brain [23]. Alternatively, in other organs such as liver, kidney and the gastrointestinal tract, homocysteine can be irreversibly broken down into cysteine and glutathione via cystathionine through condensation with serine (transsulfuration). The activities of the two involved enzymes cystathionine-β-synthase (CBS) and γ-cystathionase are each dependent on vitamin B6 as a cofactor. Similar to endothelial cells, neurons and other CNS cells do not seem to strongly express the complete homocysteine transsulfuration pathway, i.e., the activity of γ-cystathionase is low, although some brain transsulfuration activity has been reported, recently [24,25]. Hence, the capacity of homocysteine metabolism in the CNS is largely dependent on sufficient supplies of folate and vitamin B12 [26]. In particular, glia cells only have very small vitamin B12 stores that are quickly exhausted in cases of negative balance [27].

Due to the cofactor function of adenosylcobalamin for the mitochondrial methylmalonyl-CoA-mutase, vitamin B12 deficiency leads to the conversion of methylmalonyl-CoA to methylmalonic acid, which can be neurotoxic [8,28]. Another

CNS-specific feature is the dependence on the transport of folates through the blood-brain barrier. During this active transport process, 5-MTHF at the choroid plexus binds to folate receptor proteins and reaches the neurons through endocytosis, storage and release via the cerebrospinal fluid compartment [29,30]. If active folate transport or metabolism at the choroid plexus is disturbed, 5-MTHF levels can become low in the cerebrospinal fluid even in the presence of normal plasma folate concentrations [31]. By active transport, 5-MTHF occurs in higher concentrations in the cerebrospinal fluid [around 14-18 ng/mL] than in the blood [3-12 ng/mL] [32]. Similarly, there exist active transport mechanisms for vitamin B6 and B12 [33,34].

1.2 Genetics

In addition to the availability of vitamins, genetic variants contribute to the inter-individual differences in homocysteine metabolism [35,36]. Several of these variants have been reported to be associated with neuropsychiatric diseases. However, literature is conflicting, and numerous studies did not observe any association of genetic variants of homocysteine metabolism with disease. The frequent missense variant of methylenetetrahydrofolate reductase (MTHFR) c.677C>T is (A222V) is associated with reduced enzyme activity, and homozygous carriers of this variant have a mean increase of plasma homocysteine levels of approximately 25%, whereas the effect on homocysteine levels is generally stronger when folate plasma levels are low [37-42]. Despite the association between MTHFR c.677TT and elevated homocysteine plasma levels, the association of this variant with different neuropsychiatric diseases is inconsistently reported in numerous studies [43-45]. Because homocysteine levels are modulated by factors like age, gender, smoking

and renal function, the homocysteine increasing effect of MTHFR c.677C>T might be overridden or confounded by such factors [46-49]. Nevertheless, large meta-analyses have proven that the T-allele is associated with cardio- and cerebrovascular disease, and this polymorphism may also be associated with the incidence of dementia [50-53].

A lot of further studies investigated the association of other genetic variants of homocysteine metabolism with neuropsychiatric diseases. Although also negative results have been reported, there, e.g., may be associations between the variants CBS c.844_845ins68bp (p.-), MTHFR c.1298A>C (E429A), MS c.2756A>G (D919G), Tc2 c.776C>G (p.R259R), with plasma homocysteine levels, birth defects, cerebrovascular disease, neurodegeneration, (neuro)oncological and psychiatric disorders [54-64]. In the opinion of the authors, none of the genetic variants of homocysteine metabolism has yet been proven to be of sufficient relevance for an individual to justify analysis in the clinical routine beyond studies. Data on the clinical relevance in terms of consequences for therapy or (secondary) prevention of neuropsychiatric diseases may be achieved in the next years.

2. Vitamin deficiency in children, adults, and the elderly

2.1. Children

There is a need for a considerable amount of one-carbon groups for brain proliferation, cerebral maturation and myelination, particularly in newborns, children and adolescents during their growth period. Thus, disturbances of folate, vitamin B12 and homocysteine metabolism can lead to psychomotor retardation and to a variety

of unspecific neuropsychiatric symptoms [31,32,65-67]. Dysfunctional homocysteine metabolism caused by genetic deficiencies leads to greatly elevated homocysteine concentrations in the plasma (>100 µmol/L), often presenting with neuropsychiatric disorders [67-69]. Examples are CBS deficiency [68,69], disturbances of intracellular cobalamin metabolism (cblC, cblD, cblF, cblE-, cblG-defect) and MTHFR deficiency [67]. These rare congenital metabolism disorders need to be dealt with by a specialist department and are not the subject of the present review.

However, also nutritional conditions can lead to severe disturbances of folate, vitamin B12 and homocysteine metabolism in children. In particular, babies breastfed by mothers with vitamin B12 deficiency, e.g., due to vegan diet, can be affected by serious and irreversible CNS damage [70,71].

2.2. Adults

In adults, folate or vitamin B12 deficiency mostly develop for months and years, before e.g. disturbed DNA synthesis and disturbed methylation lead to symptoms of (megaloblastic) anemia and neurological impairment such as forgetfulness, sleeplessness, tiredness, irritability, lethargy and mood swings [72]. Further progression may involve cerebral demyelinisation, seizures, impairment of the peripheral nervous system like hypo- or paraesthesiae, pareses, depression and dementia [73-75]. Importantly, up to 30% of patients with vitamin B12 deficiency and normal folate levels exclusively show neurological symptoms [75]. In particular, diagnostic attention must be paid to risk groups of folate and vitamin B12 deficiency such as pregnant women, patients with inflammatory gastric conditions, people taking relevant drugs and alcoholics [76].

Vitamin B6 acts as a cofactor in more than 100 enzymatic reactions and is involved in the synthesis of various neurotransmitters [77] such as those occurring in the tryptophan-serotonin metabolism. Vitamin B6 deficiency was suggested to be associated with migraine, chronic pain, seizures and depression [78] as well as cardio-vascular diseases [79], but according to our knowledge there is currently no robust data supporting the idea that vitamin B6 deficiency is epidemiologically relevant for neuropsychiatric diseases in Western European Countries.

2.3 Elderly population

The prevalence of vitamin B12 and folate deficiency increases with age and is common in the elderly [47,80]. Whilst serum and CSF concentrations of folate and vitamin B12 fall, those of homocysteine rise with age [81]. The cause of vitamin deficiency in higher age has been variously ascribed to chronic illnesses, side effects of medications, malabsorption, increased demand and poor diet [82]. In elderly Europeans, the average intake of folate is clearly below the recommended daily dose of 400 µg/day [83].

Many neuropsychiatric diseases connected with the homocysteine metabolism, such as cognitive impairment, dementia, Parkinson's disease and polyneuropathy, have their highest prevalence in elderly persons. Therefore, disturbances of homocysteine metabolism that are associated with neuropsychiatric diseases may be of pronounced importance for the elderly.

3. Neuropsychiatric diseases

The pathophysiological mechanisms of neuropsychiatric diseases can be divided into disease-specific and disease-non-specific damage. Interactions between

mechanisms of neuropsychiatric diseases with folate, vitamin B12 and homocysteine, which have been suggested in the literature, are summarised in table 1.

3.1. Cerebral ischaemia

There is evidence that elevated plasma levels of homocysteine can affect the endothelium, promote growth of (vessel) smooth muscle cells and activate haemostasis [84] (see figure 2). Elevated plasma homocysteine is a confirmed risk factor for atherosclerotic diseases and thromboembolic events [76]. Meta-analyses have proven the association of elevated homocysteine levels with the risk of cerebral ischaemia [53,85,86]. The data collected in 30 retro- and prospective studies showed that a homocysteine difference of -3 µmol/L (~25%) is associated with an approximate 19-24% lower risk of cerebral ischaemia [53,87]. An exponential increase of risk for cerebral ischaemia was shown prospectively for lower dietary folate intake as well as for increased homocysteine levels [88,89]. Each µmol/L of homocysteine led to a risk increase of around 6-7% [90] with a 5 µmol/L rise in homocysteine levels raising the risk by 65% [87].

Further, the resulting potential to lower the risk of developing ischaemia by approximately 19% [53,87] was confirmed in randomised controlled therapy studies involving high-risk populations. Although results of the first intervention studies have been regarded as disappointing at first, the Vitamin Intervention for Stroke Prevention (VISP) study based on 3680 probands in whom cerebral ischaemia had occurred, the relative risk of having another ischaemia event was lowered by 21% after 2 years of therapy with 2.5mg folic acid and other B vitamins daily [91]. Substitution of 2.5mg folate/ day together with vitamin B6 and B12 lowered the

relative risk of stroke by 24% during 5 years in the Heart Outcomes Prevention Evaluation (HOPE)-2 study involving 5222 patients with vascular disease or diabetes [92,93]. However, within the NORVIT study, a daily dose of a lower dose of folic acid, i.e., 0.8mg per day, did not proof to be effective in preventing stroke in patients with myocardial infarction [94].

According to a meta-analysis including 16,841 participants from eight randomised studies, folic acid therapy lowered the relative risk of suffering cerebral ischaemia by 18% in all participants [86]. The effect correlated with the observed decrease of homocysteine levels. Importantly, analysis of subgroups showed that the effect of folate on the risk of cerebral ischemia was significant only for participants treated and followed-up for at least 36 months, whereas there was no significant effect in shorter treatment regimens indicating that intervention should be permanent and intervention studies should exceed duration of 36 months.
Food fortification with folic acid in the US and Canada was shown to be associated with a significant drop in the number of deaths by cerebral ischaemia [95].
In summary, folate supplementation has been proven to be effective in primary and secondary prevention of cerebral ischemia. Whether the effect of folate on homocysteine levels or other folate-dependent effects are the underlying mechanisms, remains unknown. The results of further, currently ongoing intervention studies may provide additional information in the near future [96,97].

→ *In conclusion, there is evidence that long-term supplementation of folate is effective in secondary stroke prevention (all patients) as well as for primary stroke prevention in patients at risk such as patients with diabetes mellitus or after*

myocardial infarction. The best dose or combination with other B-vitamins, if any, has not been evaluated yet, but a daily dose of 2.5mg folic acid was shown to be effective in the HOPE2 and the VISP study [evidence category IA].

3.2. Impairment of cognitive functions

Alzheimer's Dementia (AD) is the most frequent form of dementia followed by vascular and mixed (Alzheimer and vascular) dementia [98]. If AD onset could be delayed five years, the number of people affected would approximately halve [99]. Folate, vitamin B12 and homocysteine metabolism may have impact on both AD and vascular dementia. Similar to the association of elevated homocysteine levels with major stroke, elevated homocysteine levels are associated with cerebral microangiopathy and microvascular brain lesions as biological correlate of vascular dementia [100-102]. However, there are putative biological mechanisms for the association of homocysteine with dementia exceeding cerebrovascular disease. First of all, homocysteine has neurotoxic effects in cell culture and in animal experiments [9,103]. Possible mechanisms of neurotoxicity of homocysteine are multiple and include activation of NMDA receptors, DNA damage [104], and binding of copper and concomitant cytochrome C oxidase deficieny [105]. Therefore, the presence of elevated homocysteine brain levels may well promote neuronal cell death during neurodegenerative processes.

The AD brain is characterised by extracellular beta-amyloid (Aβ) deposition and intracellular neurofibrillary tangles. Homocysteine metabolism influences the progression of these two histological hallmarks of Alzheimer disease in experimental

models. The amount of Aβ production depends on expression of the amyloid precursor protein (APP) and different secretases splitting APP in an amyloidogenic or none amyloidogenic manner [106]. One of the most important mechanisms causing alteration of gene expression is disturbed DNA methylation. DNA methylation in general is accomplished through the specific enzymes, the DNA methyltransferases, which transfer a methyl group to the cytosine of CpG dinucleotides, and the degree of promoter gene CpG methylation is an important factor in gene silencing [107]. Due to the role of SAM as the ubiquitous methyl group donor and SAH as a strong inhibitor of SAM-dependent transmethylation reactions, lower levels of SAM and higher levels of SAH result in a reduced methylation capacity in general and in reduced DNA methylation in particular [108,109]. It has previously been shown that low SAM levels are associated with decreased DNA-demethylation followed by increased expression of presenilin 1 and β-secretase (amyloidogenic pathway), leading to an increase in Aβ production. Supplementation of SAM prevented these changes in cell culture experiments and mouse models [110-112] .

In AD, neurofibrillary tangles are thought to result from hyperphosphorylation of tau protein. High concentrations of hyperphosphorylated tau protein (P-tau) predict the development of dementia [113]. Tau is dephosphorylated by protein phosphatase 2A (PP2A), and methylation of PP2A is required for correct binding and dephosphorylation of tau [114,115]. Incubation of Neuro-2a cells with SAH is associated with a decrease in PP2A methylation and associated with enhanced tau phosphorylation, and PP2s methylation becomes down-regulated in the brains of hyperhomocysteinemic mice [116].

Thus, low plasma, CSF and brain levels of SAM and high levels of the methyltransferase-inhibitor SAH may promote both Aβ production and P-tau accumulation. In support of this hypothesis, SAM levels are decreased in brain tissue and cerebrospinal fluid of Alzheimer patients, [117,118] and increased SAH levels in brain tissues of Alzheimer patients correlate with disease progression and cognitive impairment [119]. Recent findings showed that oral substitution of SAM results in an increase in its plasma and CSF levels, and SAM substitution may lead to some clinical improvement in AD patients according to unconfirmed results [120].

Another link between homocysteine metabolism and neurodegenerative disorders in general and Alzheimer disease in particular is oxidative stress [121]. First of all, the brain is an organ with a limited baseline transsulfuration capacity, due to the limited activity of CBS and γ-cystathionase [122-124], which is crucial for the transsulfuration reaction of homocysteine to the glutathione component cysteine. Furthermore, the transsulfuration reaction is activated by SAM [125,126]. A lack of brain SAM may result in a reduced antioxidative capacity and increased oxidative stress (figure 1).

Patients with dementia and reduced memory show lower levels of folate and vitamin B12 and higher levels of homocysteine in plasma and cerebrospinal fluid [127-134]. Homocysteine levels are associated with the severity of cognitive, physical and social impairments in demented patients [134-138]. Low folate or high homocysteine levels were reported to be associated with atrophy of the brain, in particular with the cortex, the amygdale and the hippocampi [139-144]. Otherwise apparently healthy people with folate or vitamin B12 deficiency are at elevated risk to develop cognitive impairment and dementia [131,143,145-151]. Differences in homocysteine plasma

levels were suggested to explain 5 to 16% of the variance of cognitive function of healthy individuals [149,152]. Elevated homocysteine levels were shown prospectively to be an independent risk factor for mild cognitive impairment (MCI) and its conversion into Alzheimer's disease, and this association is dose-dependent [143,147-149,153-155]. The OR for the risk to develop dementia is increased by a factor of 2.8 to 4.6 in people with homocysteine levels of ≥14 µmol/L (compared with <10 µmol/L), and a 5 µmol rise in homocysteine levels is associated with a risk increase of approximately 40% [143,149,155]. Hence, a rise in homocysteine levels precedes the clinical onset of dementia, and persons with chronically elevated values have the highest risk of developing dementia. The available data cannot exclude that the rise of homocysteine levels in subjects developing dementia occurs after subclinical disease pathology onset. However, the association of low vitamin B12 and folate levels, which are associated with elevated homocysteine plasma levels, suggests that low folate, low vitamin B12 or homocysteine plasma levels are causally related with dementia.

In prospective studies using imaging procedures, it has been proven that both in subjects suffering from dementia and in healthy individuals, low folate and increased homocysteine values are prospectively associated with decreased cortical and hippocampal volume and, in subjects with dementia, also with faster disease progression [139,142,143].

An increased intake of folate or folic acid (diet or supplementation) was associated with a 50% lower risk of developing AD within 6 years [156]. Although literature is not univocal, some papers reported improved cognitive function in association with administration of folic acid or B vitamins. The largest study conducted so far involved

818 not demented, 50-70 years old participants with hyperhomocysteinemia, but normal vitamin B12 levels. The participants in the treatment group (0.8mg of folic acid over 3 years) performed significantly better in tests of sensomotoric speed, information processing speed and complex memory tasks) than those in the placebo group [157].

There are some limitations concerning the transferation of these results to the clinical practise. The studies differed concerning population criteria, vitamin dose, treatment duration and specification of cognitive measurements. In regard of several negative results, further and long term studies are necessary to reconfirm previous positive study results and establish optimal protocols of folate and vitamin B12 intake [158]. The possible protective effect of folate or vitamin B12 against dementia can be expected to be greater the earlier therapy is started, if homocysteine levels are elevated before treatment, and if the duration of therapy is sufficiently long [151,156,157,159,160].

-> We conclude that there is evidence that low levels of folate and vitamin B12 and high levels of homocysteine are risk factors for mild cognitive impairment and dementia. In addition, they dispose towards an unfavourable clinical course. Supplementation of folate and vitamin B12 has beneficial effects for patients with mild cognitive impairment or Alzheimer´'s disease with evidence of category IIa. One may speculate that B vitamin supplementation might also be benefical in the maintance of cognitive function of healthy elderly individuals with high homocysteine levels.

3.3 Depression

Depression is the most frequent psychiatric disease. It is underdiagnosed and undertreated, particularly in older patients [161]. Approximately one third of depressive patients show low levels of folate and elevated levels of homocysteine in serum or erythrocytes, and folate and SAM have been observed to be decreased in the cerebrospinal fluid [162,163]. Accordingly, neuropsychiatric disorders are frequent in patients with megaloblastic anaemia due to folate deficiency [164]. In subjects with severe folate deficiency in the cerebrospinal fluid, greatly altered levels of monoamine metabolites such as hydroxyindole acetic acid and homovanillic acid have been found [165]. Folate also affects the synthesis rate of tetrahydrobiopterin (BH4), a cofactor for the hydroxylation of phenylalanine and tryptophan and is, therefore, directly involved in the synthesis of monoamine neurotransmitters [166]. Vitamin B6 (pyridoxal-5`-phosphate; PLP) acts as cofactor in the metabolism of tryptophan and serotonine, and a deficiency could be associated with depressive symptoms [78]. However, alternatively, depression may lead to altered nutritional behaviour as the reason for changed folate levels in depressive patients. However, the efficacy of a drug-based treatment using antidepressants seems to be influenced by initial folate values, as treatmentt is less effective in presence of folate deficiency (delayed or weaker effect) [167-170]. Pre-treatment with folic acid [168] as well as the simultaneous administration of folic acid and fluoxetine leads to a significantly improved efficacy that correlates with changes in homocysteine levels [171], and low folate levels increase the risk of suffering a depressive relapse during fluoxetine therapy [172]. These associations were

stronger in women [173,174]. In a large sample of more than 5000 women 20-34 years old Kendrick et al found folate levels to be associated with anxiety and depression, but adjustment for socioecomic and lifestyle factors weakend the association considerably. The authors concluded that socioeconomic factors are much more important for depression in their population of young female participants [175]. However, even in young individuals, this does not rule out a relevant role of folate in case of low folate levels [175] or when moderately decreased folate levels are combined with additional risk factors like the TT genotype of MTFR c.677C>T [176]. In addition to fluoxetin, folate levels also seem to influence the response to treatment with other antidepressants such as imipramine [167,168], nortriptyline and sertraline [168,177]. Folic acid and SAM may improve the efficacy of an antidepressant-based therapy through better availability of neurotransmitters and via further methylation reactions in the nervous system [178]. Folate and vitamin B12 are necessary for SAM synthesis (figure 1). This relation may be a biological basis of the associations observed between folate and depression, as SAM is necessary for the synthesis of dopamine, noradrenaline, serotonine and 5-hydroxyindole-3-acetic acid in the brain [179,180]. Oral and intravenous SAM administration showed a mood-enhancing effect [181] and its antidepressant effect may be comparable with the classical tricyclical antidepressants [182]. However, currently, the clinical use of SAM itself for depression is not approved in most countries.

Although several categories of antidepressants are available, approximately 30–40% of patients suffer from depression refractory to therapy [183].Therapy with folic acid should be taken into account as adjunctive treatment and may be beneficial even in cases with normal blood folate levels [184,185].

→ *Concerning the majority of published results, folate deficiency favours depression and affects its duration and degree of clinical severity. Folic acid has antidepressant characteristics of its own and improves the therapeutic efficacy of antidepressant drugs. In cases of actual folate deficiency, folic acid administration can be particularly effective* [evidence category IA]. *In addition, SAM may have antidepressant effects (evidence category IIA).*

3.4. Parkinson's Disease and L-DOPA Therapy

Prospective studies in healthy subjects showed no connection between intake of folate and vitamin B12 and the risk of developing Parkinson's disease [186], although one study reported an association of low levels of vitamin B6 and Parkinson´s disease [187]. The elevated homocysteine values observed in patients with Parkinson's disease are due to L-DOPA therapy [188]. A large part of the administered L-DOPA receives methyl groups from SAM through the action of catechol-*O*-methyltransferase (COMT) being converted into 3-*O*-methyldopa. This reaction leads to a drop in SAM as well as an increase in SAH and homocysteine levels (the latter by 60–80%) [189-192]. Hence, administration of L-DOPA results in a rise in homocysteine values [193,194]. The group with the highest homocysteine concentrations also showed the highest concentrations of 3-OMT [194]. These changes in Parkinson patients can plausibly be reduced with COMT inhibitors [195]. Also in Parkinson patients, elevated homocysteine values in the plasma are

associated with a higher incidence of depression [189,196] and an increased risk of cardiovascular disease [188] and cerebral ischaemia [197,198]. The risk of Parkinson patients to develop dementia is four to six times higher [199] and is increased by the presence of hyperhomocysteinemia [196,200,201].

→ *There is no evidence that disturbances of folate, vitamin B12 or homocysteine metabolism are relevant for the etiology of Parkinson´s disease. However, elevated homocysteine levels secondary to L-DOPA treatment in patients with Parkinson´s disease may accelerate neurodegeneration and may dispose to vascular disease and, thus, should be treated with folate and vitamin B12. In addition, vitamin B6 may be included in the supplementation regimen as it is necessary for homocysteine transsulfuration supporting the synthesis of glutathione. Glutathione is used for the defence against oxidative stress that is supposedly involved in neurodegeneration such as that seen in Parkinson's disease [evidence category IIb].*

3.5. Schizophrenia

Compared with healthy individuals, patients with schizophrenia may have higher plasma homocysteine values [202], and elevated homocysteine or low folate levels seem to correlate with extrapyramidal motor symptoms induced by neuroleptic therapy and with negative symptoms of schizophrenia [203], although these results have not been sufficiently confirmed, yet. A meta-analysis of risk alleles for schizophrenia suggested that the T-allele of MTHFR c.677C>T, the most prevalent genetic disposition for elevated homocysteine plasma levels, may be associated with

schizophrenia [204]. However, the therapy studies published so far did not provide evidence that vitamin supplementation and homocysteine lowering had beneficial effects in the treatment of schizophrenia. A vitamin B6 supplementation was found to lower homocysteine levels, but there was no significant effect on schizophrenia symptoms [205]. A decrease in homocysteine levels in patients with initial values of > 15 µmol/L has allegedly led to a significant improvement of clinical symptoms after three months in a small study [206].

→ *The current data is insufficient to decide whether folate, vitamin B6, vitamin B12 and homocysteine metabolism may be relevant for schizophrenia incidence, clinical course or treatment.* [evidence category III].

3.6 Bipolar disorders

Recent studies observed disturbances of homocysteine metabolism also bipolar disorders (BD). Ozebek et al. showed that homocysteine levels are higher, and folate levels are lower in patients with a bipolar disorder in comparison to healthy controls [207]. Additionally, the variants MTHFR c.1298A>C and MTR c.2756 A>G were shown to be associated with bipolar disorders [207,208]. Hyperhomocysteinemia may also play a role in the pathophysiology of neurocognitive deficits in BD, with a higher impact in older patients, or in patients, who had a delayed onset of illness [209-211]. Prospective studies are required to further analyse the role of homocysteine metabolism in the pathophysiology of bipolar disorders.

→ *The current data is insufficient to decide whether folate, vitamin B6, vitamin B12 or homocysteine metabolism may be relevant for bipolar disorder incidence, clinical course or treatment.* [evidence category III].

3.7. Multiple Sclerosis

A connection between vitamin B12 deficiency and multiple sclerosis (MS) has been suspected due to the fact that the illness is frequently accompanied by macrocytosis [212]. Irrespective of the course or stage of the disease, elevated concentrations of homocysteine have been found in the plasma and cerebrospinal fluid of MS patients [213,214] as well as lowered concentrations of vitamin B12 [215], whilst folate levels are normal [213]. As alternative to vitamin deficiency or at different stages of disease and treatment, elevated homocysteine levels in multiple sclerosis may also be the consequence of chronic inflammation processes or of cellular immune activation [213,216,217]. Prospective studies on vitamin concentrations in blood and the cerebrospinal fluid before disease onset are lacking. Based on experimental tests, there is a possibility that homocysteine metabolism plays a part in the creation or maintenance of the chronic inflammation process in MS patients. In the animal model of MS, an inhibition of transmethylation suppresses the CD4 cell-mediated autoimmune reactions [218].

Weekly vitamin B12 injections (i.m.) in 138 patients led to an improved clinical picture in the therapy group after 24 weeks whilst a combination therapy mixing vitamin B12 with lofepramine and L-phenylalanine did not produce an additional

therapeutic effect [219]. In a very small therapy group that received multivitamin preparation, the neurological findings were judged to have improved in comparison with the control subjects [220].

→ *There is some evidence that folate, vitamin B12 and homocysteine metabolism is changed in patients with multiple sclerosis. Vitamin B12 and multivitamin substitution have been reported to be beneficial in single studies. However, the current data are insufficient to allow recommendations for diagnosis or treatment to be made* [evidence category III].

3.8. Epilepsy and antiepileptic therapy

Epileptic seizures are caused by the pathological stimulation or a lack of stimulation of nerve cells. NMDA receptors play an important part in the generation and maintenance of epileptic seizures. Homocysteine and other sulphur-containing metabolites (cysteine, homocysteine acid etc.) can trigger epileptic fits as agonists for NMDA receptors [221].

Several anticonvulsive drugs, even those of the newer generation, can interfere with folate metabolism [222]. Their intake lowers folate levels and is therefore associated with an increase in homocysteine concentrations [223]. Changes to plasma homocysteine levels have been observed above all during the application of phenobarbital, carbamazepine, primidone, phenytoin and valproate. Phenytoin and carbamazepine increase homocysteine by lowering folate and vitamin B6 levels [223,224]. Phenytoin, carbamazepine, phenobarbital and primidone induce the cytochrome (CYP) P450 enzyme system in the liver. Phenytoin is a substrate of CYP

2C9 as well as 2C19 and inhibits CYP 3A4, 5 and 7. Carbamazepine and phenobarbital induce CYP 2C19 or 2B6, whilst primidone is a substrate of CYP 2C19 and does not have an inhibitory effect. In animal trials, phenytoin inhibits MTHFR activity [225]. Disturbed folate metabolism observed during the administration of antiepileptic drugs particularly during the first trimester could be one mechanism for the teratogenity of some anticonvulsive drugs [226]. In particular, carbamazepine and valproic acid were found to be associated with lowered serum folate levels as a possible risk of neural tube defects, but sufficient data on several antiepileptic drugs are missing [227,228]. This is why folic acid supplementation for women of childbearing age, who are on antiepileptics, is particularly indicated and is urgently recommended by the specialist societies [229,230].

Further, elevated homocysteine and lowered folate concentrations can represent a risk factor for the occurrence of an interictal (occurring between seizures) psychosis [231]. Development of depressive symptoms has also been reported by patients on antiepileptic drugs [169,232]. In the animal model, adding folic acid supplements to anticonvulsive therapy has a mood-enhancing effect, improves cognitive functions and raises the seizure threshold [233].

In children [234] and adults [235] on anticonvulsive drugs, the daily intake of 0.4 to 1.0 mg of folic acid lowers elevated homocysteine values in most cases within 1-3 months. The treatment of folate-deficient epileptics with 5 mg folic acid over 1–3 years was reported to lead to improved initiative, attention, concentration, mood and social behaviour [236]. An adjuvant therapy with vitamin B6 lowered the severity of epileptic seizures even in those forms of paediatric epilepsy that do not belong to the congenital pyridoxine- and pyridoxal-5`-phosphate-dependent forms [77].

→ *Antiepileptic drugs can raise homocysteine concentrations supposedly due to their interaction with the folate metabolism. Since valid data are missing for several of the antiepileptic drugs, it is recommended that monitoring of folate and vitamin B12 levels should be performed in all patients treated with antiepileptic drugs in the long-term. In case of abnormal results and, concerning folate, in women of childbearing age, vitamins should be substituted [evidence category IIb and Ic-Ia for women of childbearing age].*

4. Safety of multivitamin therapy

Multivitamin therapy may be not safe for everyone. Folate therapy can have negative effects in persons with subclinical vitamin B12 deficiency [237]. This should be avoided by excluding vitamin B12 deficiency before folate therapy, or by supplementing folate together with vitamin B12 in a dose of least 400µg per day. Folate is essential for nucleotide synthesis and DNA methylation. Therefore, folate deficiency has been associated with increased risks of several cancer types. However, high amounts of folate intake may also increase liability to cancer consistent with the role of folate in cell proliferation [238]. Although a safe upper limit of folic acid intake of 1 mg/d for adults and 300–800 g/d for children, depending on age, has been proposed there is no consensus about what blood concentrations of folate might cause harm, if any [239].

Expert commentary

Elevated plasma homocysteine levels are a risk factor for stroke. Whereas, in the NORVIT study, daily substitution of 0.8mg folate did not prove to be effective in

secondary stroke prevention, 2.5mg folate significantly reduced the risk for secondary stroke in the HOPE2 and the VISP study. Accordingly, the meta-analysis of Wang and co-workers which included 8 intervention studies reported a significant preventive effect of folate supplementation against stroke. Most current guidelines, however, do not recommend vitamin substitution for homocysteine lowering in secondary stroke prevention, which supposedly means that some avoidable strokes are not prevented.

Elevated levels of plasma and CSF homocysteine may promote diseases like mild cognitive impairment, Alzheimer's disease and vascular dementia by several mechanisms. In addition, homocysteine as NMDA-receptor agonist may directly interfere with antidementive drugs like NMDA-receptor antagonists. In patients with cognitive deficits, folate, vitamin B12 and homocysteine should be determined, and abnormal levels should be treated. Substitution may be beneficial even in cases of values within the reference ranges.

Although a lack of folate and vitamin B12 and elevated levels of homocysteine promote neurodegeneration, there is no evidence at the clinical level that folate, vitamin B12 and homocysteine metabolism is involved in the specific aetiology or pathogenesis of Parkinson's disease. However, L-DOPA therapy leads to elevated homocysteine levels which may speculatively have adverse effects on Parkinson's disease in addition to the general risks associated with elevated homocysteine levels. Thus, measurement of plasma levels of homocysteine is recommended in L-DOPA treated patients, and elevated levels should be treated. Similarly, several antiepileptic drugs are associated with decreased levels of folate and increased levels of homocysteine, and they should also be treated. In addition, women of childbearing age treated with antiepileptic drugs should control folate and vitamin

B12 plasma levels and should, if levels are below the reference range, increase the folate prophylaxis for pregnancy to 2.5mg per day starting three months before possible conception in addition to supplementation of vitamin B12, e.g. 100µg per os per day, if necessary [240,241]. Women with instable epilepsy treated with phenytoin should consult a specialist prior to vitamin B12 substitution, as vitamin B12 supplementation may reduce phenytoin efficacy according to this paper´s authors´experience.

Depression is associated with low plasma levels of folate and high plasma levels of homocysteine. A reduced synthesis of SAM, which is related to such laboratory findings, may be an underlying mechanism of such associations, as SAM is necessary for neurotransmitter synthesis and may be relevant for the efficacy of several antidepressant drugs. We conclude that there is a case for determination of folate, vitamin B12 and homocysteine levels in patients suffering from depression, and abnormal levels should be treated. Even in patients with normal values, folate substitution may have antidepressant effects or may increase the effects of antidepressant drugs, especially in patients with otherwise drug-resistant depression. Thus, adjuvant treatment with folate is a considerable safe therapeutic option for several patients with depression.

Concerning other neuropsychiatric diseases such as multiple sclerosis or schizophrenia, there is speculation, but no evidence, that folate, vitamin B12 and homocysteine metabolism may have any impact.

Safety: Even after the prolonged use of high doses, the toxicity of folic acid remains low [242]. However, due to the risk of masking megaloblastic anaemia and irreversible neurological disorders, it is not recommended to carry out folic acid therapy without first excluding a causal vitamin B12 deficiency or co-supplementing

vitamin B12, in particular in elderly people [242]. In addition, folate supplementation is controversially discussed to promote tumour development or growth. Thus, therapeutic folate supplementation should be restricted to selected populations, until such issues have been solved. It is the opinion of the authors of this paper that folate supplementation is advisable for prevention of stroke in populations at risk, in patients with mild cognitive impairment or dementia and in selected patients suffering from depression in addition to persons with folate levels below the reference range and women who might become pregnant. Based on much therapeutic experience, vitamin B12 (cyanocobalamin and hydroxocobalamin) is considered to be well tolerated. Accordingly, the Food and Nutrition Board of the Institute of Medicine at the National Academy of Sciences has not issued an upper limit for vitamin B12 intake. However, high doses exceeding 900µg per os per day may lead to vitamin-B12-acne in rare cases in the experience of the authors.

Vitamin B6 is regarded as safe within the dosage range of 2-25 mg in the experience of the authors, which should be sufficient for treatment of levels below the reference ranges [76]. At doses over-exceeding 25 mg, or the more, exceeding 50 mg, side effects like paraesthesia were frequently reported by the respective patients.

Concerning laboratory analysis of the vitamins involved in homocysteine metabolism, the determination of the biologically available form of vitamin B12, holotranscobalamin, may be superior to the determination of total vitamin B12 [243]. However, this does not reflect the authors' experience. In questionable cases, determination of methylmalonic acid may be much more informative.

Five-year view

For decades, B-vitamins played an important role in the treatment of neurological diseases, and hyperhomocysteinemia has, for a long time, been seen as an important risk factor for cerebrovascular disease. Today, B-vitamins and homocysteine are often regarded as past topics that have lost their eligibility in the treatment and prevention of neuropsychiatric disease, which may in part be due to over-exaggerated expectations or lobbyism. There is evidence that folate, vitamin B12 and homocysteine metabolism can interact with the aetiology and pathology of neuropsychiatric diseases. Manipulation of this metabolism can be beneficial e.g. in relation to stroke prevention and improved clinical course of dementia or depression. Since simple therapies such as vitamin substitution may have clear positive effects on diseases as mentioned it is opportune to reconsider and change current guidelines basing on the evidence of large studies and meta-analyses published in high-ranked peer-reviewed. In five years, additional studies will have finished and, hopefully, we will be able to recommend more exact doses and protocols for treatment and prevention with folate and vitamin B12 for clearer defined populations.

Key issues (8-10 bullet points summarized in the review)

- Folate substitution is effective in selected primary and in secondary prevention of stroke. A dose of 2.5mg per day per os has been proven to be effective, although lower doses may be effective, too. The combination with vitamin B12 may have additional benefitial effects and may improve the safety of folate supplementation. Low doses of 15µg vitamin B12 per day per os may be sufficient, but also higher doses like 100µg can be expected to be safe. The preventive effects increase when supplementation is long-term or continuous. Patients of countries with folate fortification or with low baseline homocysteine levels have lower benefit.
- Folate, vitamin B12 and homocysteine levels should be determined in patients with dementia or depression and, repeatedly, e.g. once a year, in patients treated with antiepileptic drugs or with L-DOPA. Abnormal levels should be treated.
- Folate itself exerts antidepressant effects in animal models and promotes the effects of other antidepressant drugs, in particularly in patients with otherwise drug resistant depression. Folate supplementation maybe considered in patients with depression.
- Substitution of folate and vitamin B12 may improve cognitive functions even in the absence of folate or vitamin B12 deficiency.
- The costs of treatment with folate and vitamin B12 should be covered by health insurance schemes for prevention of vascular events in patients at risk as well as for cognitive impairment, dementia and depression. Treatment guidelines should be modified.

- The impact of folate, vitamin B12 and homocysteine metabolism on other common neuropsychiatric diseases including multiple sclerosis and schizophrenia has not been proven.

Evidence categories refer to therapeutic usefulness.

Classification:

Class I Conditions with regard to which there is evidence and/or general agreement that the procedure or treatment is beneficial, useful, and effective.

Class II Conditions with regard to which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment.

Class IIa Weight of evidence/opinion is in favour of usefulness/efficacy.

Class IIb Usefulness/efficacy is less well established by evidence/opinion.

Class III Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective and in some cases may be harmful.

Level of evidence A Data derived from multiple randomized clinical trials.

Level of evidence B Data derived from a single randomized trial or non-randomized studies.

Level of evidence C Only consensus opinion of experts, case studies, or standard-of-care.

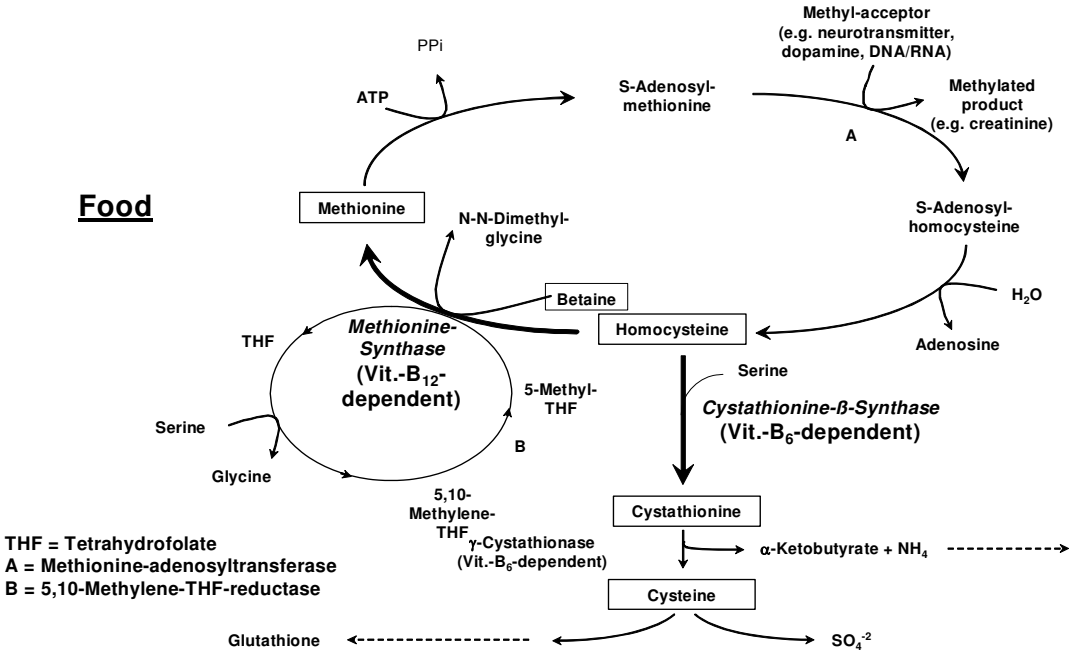

Figure 1. The Homocysteine Metabolism

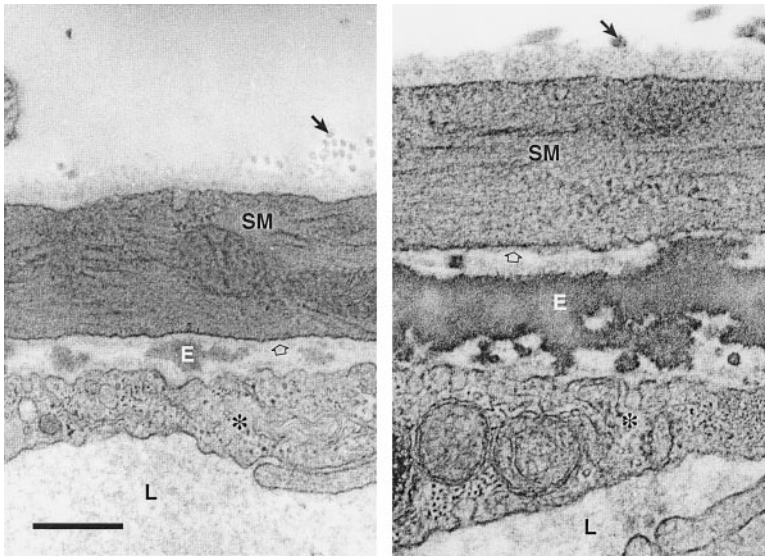

Figure 2. Electron micrographs of cerebral arterioles in a CBS +/+ mouse fed control diet (left) and a CBS +/- mouse fed high-methionine diet (right). Vascular lumina (L) are oriented toward the bottom. Components of the vessel wall include endothelium (asterisk), elastin (E), smooth muscle (SM), collagen (closed arrows), and basement membrane (open arrows). Bar = 1 μm. Reproduced from the article “Structure of Cerebral Arterioles in Cystathionine b-synthase deficient mice", Circ Res. 2002 Nov 15;91(10):931-7. With kind permission of Gary Baumbach.

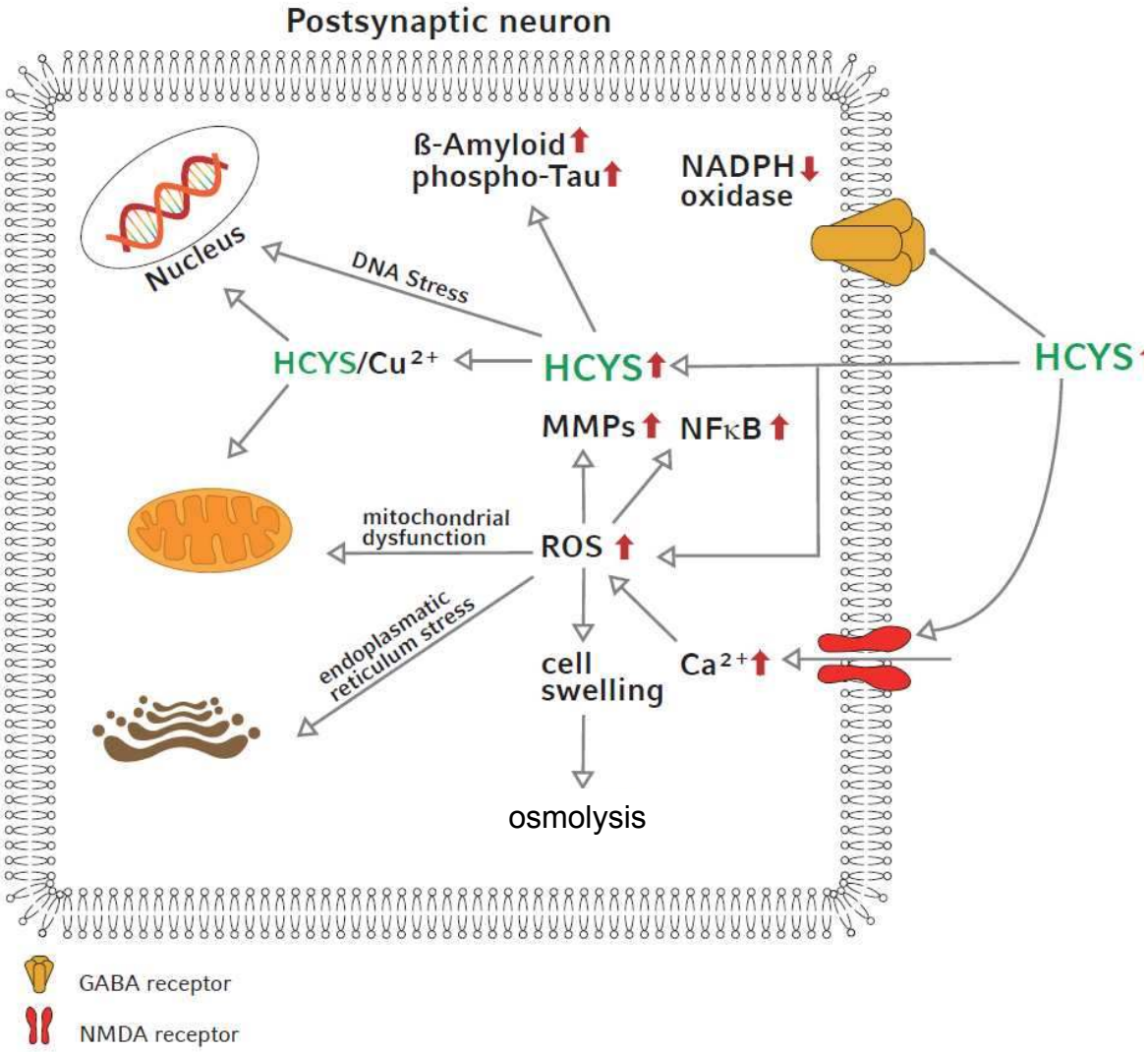

Figure 3. Selected mechanisms of neurotoxicity of homocysteine. Homocysteine (HCYS) can enter the cell from the extracellular space, but also activate the NMDA receptor. This leads to intracellular increase of Ca^{2+} and accumulation of reactive oxygen species (ROS). In addition, homocysteine itself can increase intraneuronal concentrations of ROS. ROS, e.g., increase the intracellular activation of matrix metalloproteinases (MMPs), increase NFκB and induce endoplasmatic reticulum stress and mitochondrial dysfunction, cell swelling and osmolysis of the cell. Homocysteine has inhibitory function at the GABA receptor

reducing NAPH oxidase activity and promoting oxidative stress. Furthermore, homocysteine forms toxic complexes with copper which can, e.g., induce DNA damage and can cause reduced activity of copper-dependent enzymes like the superoxide dismutase or the cytochrome C oxidase as part of the mitochondrial respiratory chain. Intracellular oxidative stress and interaction with expression, phosphorylation and activation of neuronal proteins are discussed as mechanisms of the association of elevated homocysteine levels with an increased formation of phospho-Tau and β-Amyloid in Alzheimer's disease.

	Hcy↑	FA↓	B12↓
B- and γ-secretases (then Aß ↑)	↑	↑	↑
Reactive (free) oxygen radicals	↑	↑	
Ca2+ concentration (cytosol)	↑	↑	
Glutamate excitotoxicity	↑	↑	
MPTP-induced neurotoxicity	↑	↑	
Iron-(copper-)induced neurotoxicity	↑	↑	
DEW-protein phosphorylation	↑	↑	
ATP concentration	↓	↓	
NMDA activation	↑		
Amyloid ß-induced neurotoxicity, oxygen radicals, apoptosis	↑	↑	
ADMA	↑		
NO bioavailability	↓	↓	
MPTP toxicity	↑	↑	
DNA strand breaks	↑	↑	
DNA repair	↓	↓	
Transmethylation	↓		
S-Adenosylmethionine (SAM)	↓	(↓)	(↓)
S-Adenosylhomocysteine (SAH)	↑	(↑)	(↑)
Methyltransferase activities	↓	(↓)	(↓)
Endoplasmic reticulum stress	↑	↑	
Cell cycle (Cyclin B and E)	↑		
Endothelial damage	↑		
Activity of Na^+/K^+-ATPase	↓		
Hypomethylation	↑	↑	
COMT activity	↓		
LDL oxidation, lipid peroxidation	↑		
Inflammation, protease activation (MMP, TNFα)	↑	↑	↑
Mitochondrial complex inhibition	↑		
Synapse dysfunction, degeneration	↑	(↑)	
Myelinisation	↓	↓	↓
Tetrahydrobiopterine synthesis		↓	
Neurotransmitter synthesis	↓	↓	
Microangiopathy, ischaemia, hypoxy	↑		
Presenilin ½ protein expression	↑	↑	↑
NTPDase* activity	↑		
5`-Nucleotidase activity	↑		
Respiratory chain complex I activity	↓		
Protein kinase-C activity	↑		
Methylation (e.g. DNA)	↓	↓	↓
Na+, K+-ATPase activity	↓		
Protein phosphatase 2A	↓	↓	

Tyrosinhydroxylase activity	↓	↓	
NF-κB activation	↑		
mGluR activity	↑		
Glutathione-oxidase activity	↓		
HERP protein expression	↑		
Leukoaraiosis	↑	↑	
GFAP mRNA expression			↑
EGF expression			↓
PARP activation	↑		
Caspase activation	↑		

Table 1. Metabolic conditions observed in association with increased homocysteine (Hcys), or decreased folic acid (FA) and vitamin B12 plasma levels

NMDA = *N*-methyl-*D*-aspartate-receptor; **NTPDase=** nucleoside triphosphate diphosphohydrolase; **COMT** = catechol-*O*-methyltransferase, **mGluR** = group 1 metabotropic glutamate receptors; **ADMA** = asymmetric dimethylarginine; **NO** = nitric oxide; **HERP** = homocysteine-inducable endoplasmic reticulum stress protein; **MPTP** = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; **MMP** = metalloproteinases, **TNFα** = tumor necoris factor α; **GFAP** = glial fibrillary acidic protein; **EGF** = epidermal growth factor; **PARP** = poly-ADP-ribose-polymerase. ↑ = reinforcement, stimulation, ↑ = reduction, inhibition.

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