



Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders

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Folate is a cofactor in one-carbon metabolism, during which it promotes the remethylation of homocysteine – a cytotoxic sulfur-containing amino acid that can induce DNA strand breakage, oxidative stress and apoptosis. Dietary folate is required for normal development of the nervous system, playing important roles regulating neurogenesis and programmed cell death. Recent epidemiological and experimental studies have linked folate deficiency and resultant increased homocysteine levels with several neurodegenerative conditions, including stroke, Alzheimer’s disease and Parkinson’s disease. Moreover, genetic and clinical data suggest roles for folate and homocysteine in the pathogenesis of psychiatric disorders. A better understanding of the roles of folate and homocysteine in neuronal homeostasis throughout life is revealing novel approaches for preventing and treating neurological disorders.

We are concerned with the metabolism of homocysteine, one aspect of a much broader array of biochemical reactions involved in one-carbon metabolism [1]. The reason for this is the increasing evidence that homocysteine plays a role in developmental and adult neurological disorders, and the fact that homocysteine metabolism is affected by dietary factors, most notably folate. The amino acid methionine plays a pivotal role in one-carbon metabolism, a series of biosynthetic pathways crucial for DNA synthesis and repair, and various methylation reactions (Fig. 1). The normal dietary supply of methionine does not meet the demands for the many different biochemical reactions that involve methyl groups; additional methyl groups are generated by *de novo* methyl synthesis from the one-carbon folate pool. Folate (5'-methyltetrahydrofolate) provides the methyl group for the conversion of methionine to S-adenosylmethionine (SAM), the major methyl donor for most methyltransferase reactions; when folate levels are low, SAM is depleted, resulting in a reduction in the methylation of cytosine in DNA. Thus, folate deficiency can decrease DNA methylation and thereby enhance gene transcription and DNA strand

breakage, and can impair DNA repair resulting in genetic mutations or triggering apoptosis [2,3]. SAM is also involved in the methylation of proteins, phospholipids and neurotransmitters [4]; consequences of deficiencies in SAM therefore radiate beyond effects on DNA alone. Folate deficiency might also have deleterious effects on cells by permitting the accumulation of homocysteine, a potentially toxic substance produced by demethylation of methionine (Fig. 1). Homocysteine–folate metabolism in the brain is generally similar to that in other tissues, although differences do exist [5]. Excess homocysteine is released from extrahepatic tissues that do not have the complete transsulfuration pathway and is taken up by the liver for remethylation to methionine; homocysteinuria occurs with pathological accumulation of homocysteine. The nervous system might be particularly sensitive to extracellular homocysteine, as it promotes excitotoxicity via stimulation of NMDA receptors and damages neuronal DNA, thereby triggering apoptosis [6,7] (Fig. 1). Homocysteine levels are normally kept low by remethylation to methionine in a reaction that requires folate and vitamin B12 (Fig. 1). Although we focus on folate, it should be recognized that vitamin B12 plays a role in homocysteine metabolism that is similar to that of folate. In addition, homocysteine can be converted to cystathionine by the activity of the enzyme cystathionine- β -synthase (CBS); action of CBS might increase levels of the antioxidant glutathione [8] (Fig. 1) in a possible compensatory mechanism that counteracts the potential oxidative damage resulting from increased homocysteine [9]. Thus, changes in the levels of expression or functional activity of methionine synthase and CBS can affect levels of homocysteine. The pathway for conversion of homocysteine to cystathionine is present in the brain, although the conversion of cystathionine to cysteine might be absent [10]. Impaired CBS activity or folate deficiency in brain cells would therefore be expected to increase local concentrations of homocysteine, independently of plasma levels of homocysteine. However, dietary folate deficiency in mice results in increased glutathione levels in brain tissue [11], suggesting the presence of one or more additional compensatory mechanisms to counteract the consequences of oxidative stress associated with folate deprivation.

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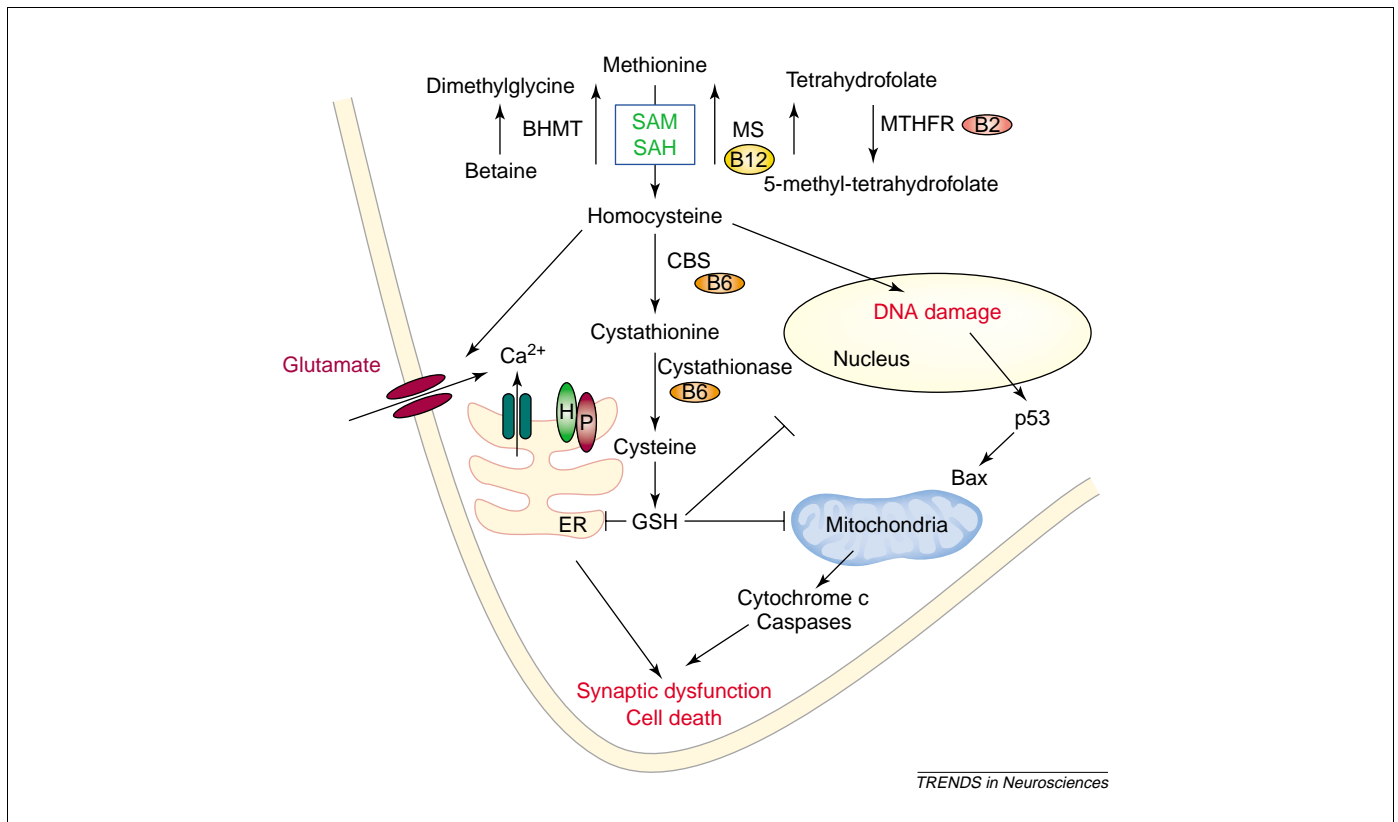


Fig. 1. The roles of homocysteine and folate in one-carbon metabolism and neuronal survival and death. Homocysteine is produced from the amino acid methionine by demethylation. Folate and vitamin B12 promote remethylation of homocysteine to regenerate methionine. Homocysteine can also be converted to cysteine (a precursor of glutathione) by the activities of the enzymes cystathionine- β -synthase (CBS) and cystathionase. Homocysteine can cause synaptic dysfunction and neuronal death by promoting DNA damage and activation of apoptotic signaling cascades involving p53, Bax, mitochondrial alterations, release of cytochrome c and caspase activation. Homocysteine may also have direct actions on glutamate receptors that result in enhanced Ca^{2+} influx. Finally, homocysteine induces endoplasmic reticulum stress, which might contribute to its pathogenic actions. Abbreviations: B2, vitamin B2; B6, vitamin B6; B12, vitamin B12; BHMT, betaine-homocysteine methyltransferase; ER, endoplasmic reticulum; GSH, glutathione; H, homocysteine-inducible endoplasmic reticulum stress protein; MS, methionine synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase; P, presenilin 1; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

Most mammals, including humans, cannot synthesize folate and therefore must obtain it from dietary sources such as green vegetables, some citrus fruits, liver and whole grains. The major dietary folates are 5-methyltetrahydrofolate and formyltetrahydrofolate; vitamin B12-dependent methionine synthase plays an important role in facilitating the conversion of extracellular 5-methyltetrahydrofolate to polyglutamate tetrahydrofolate, a form of folate that can be readily used in nucleotide biosynthesis. In addition, vitamin B6 is necessary for the formation of 5,10-methylenetetrahydrofolate from tetrahydrofolate. Cells throughout the body, including neurons and glial cells, express folate transporters, suggesting that folate and vitamin B12 play a crucial role in methionine and homocysteine metabolism in most cell types [12].

Folate and homocysteine in development and adult neuroplasticity

The importance of dietary folate in the nervous system was established in studies showing that pregnant women who are folate deficient have a greatly increased risk of neural tube defects in their babies [13]. The defects, which include spina bifida, meningocele, encephalocele and anencephaly, result from abnormalities in neural cell proliferation, differentiation and death (Fig. 2). Studies of embryonic brain cells in culture have shown that depriving

astrocytes and neural stem cells of folate inhibits their proliferation (I. Kruman and M.P. Mattson, unpublished). Methotrexate (an inhibitor of folate metabolism) inhibits the proliferation of neural progenitor cells (I. Kruman and M.P. Mattson, unpublished) and induces apoptosis in newly-generated neurons [14]. Although neurons in the adult are post-mitotic and might therefore be expected to suffer less from the DNA nucleotide misincorporation that accompanies folate deprivation in mitotic cells [15], it does appear that DNA repair is also very important in post-mitotic neurons [14]. Nucleotide excision DNA repair is likely to be an important source of uracil misincorporation in post-mitotic neurons. Mitotic neuroblasts also remain susceptible to nucleotide misincorporation during the S phase of the cell cycle as a result of folate deprivation, which could account for some of the inhibition of neural cell proliferation. Vitamins B12 and B6 are cofactors in the metabolic pathways that affect homocysteine levels. Vitamin B12 deficiency during pregnancy results in elevated homocysteine levels in the fetus and increases the incidence of developmental defects in its nervous system [16]. In infancy, vitamin B12 deficiency can result in psychomotor regression, sensory neuropathy, severe hypotonia, seizures and apathy, which could result from impaired myelination [17,18]. In addition, hereditary deficiency of transcobalamin II results in profound

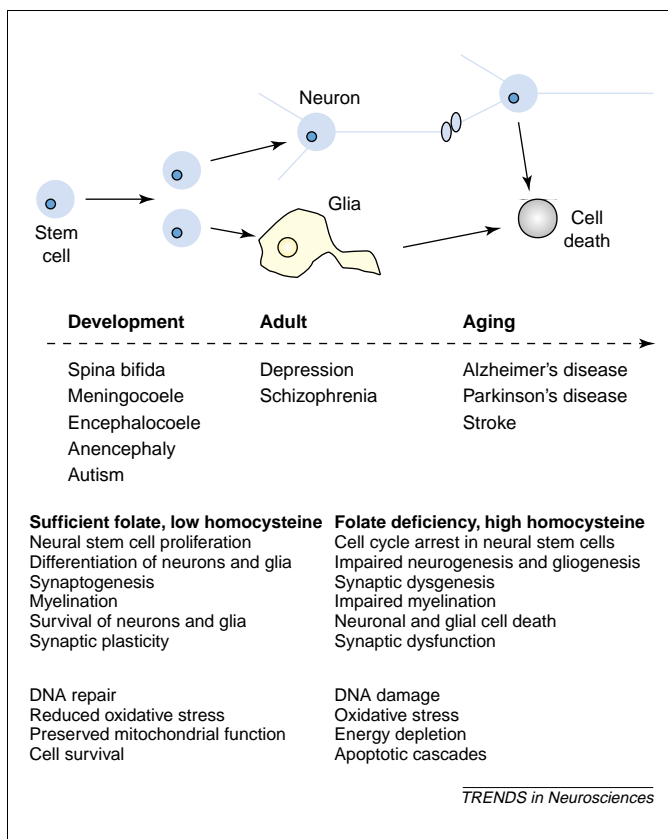


Fig. 2. Involvement of folate and homocysteine in development, adult plasticity and aging of the nervous system. Folate and homocysteine have been shown to affect fundamental processes of developmental and adult neuroplasticity, including stem cell proliferation and differentiation into neurons and glial cells, and cell survival. Synaptic plasticity might also be sensitive to homocysteine. By impairing neuronal plasticity and promoting neuronal degeneration, homocysteine could contribute to the pathogenesis of psychiatric and neurodegenerative disorders.

neurological abnormalities that are characterized by severely retarded intellectual development, ataxia and pyramidal deficit in the limbs [19]. Animal studies support the above findings in developing humans, in whom deficiencies of folate, vitamin B12 and vitamin B6 can result in severe developmental abnormalities in the nervous system [20]. Additional proteins that are likely to influence homocysteine levels under specific conditions include methionine synthase reductase [21], the folate transporter glutamate carboxypeptidase-II [22] and the vitamin B12 transporter transcobalamin [23].

Synaptic transmission and plasticity are very sensitive to environmental factors and, accordingly, synaptic dysfunction and degeneration occur early in the pathogenesis of several different neurological disorders [24]. Several findings suggest that elevated homocysteine levels might alter synaptic function. Overactivation of glutamate receptors is implicated in the pathogenesis of several neurodegenerative and psychiatric disorders, and homocysteine can activate synaptic glutamate receptors either directly [25] or indirectly after metabolism into L-homocysteic acid [26,27]. Homocysteine might also render neurons vulnerable to excitotoxicity by inducing DNA damage [7]. The possible effects of homocysteine on learning, memory and synaptic plasticity remain to be determined. Similarly, it is not known if and how folate modifies synaptic plasticity, although one study suggests

that folate can enhance excitability of hippocampal circuits by presynaptic disinhibition of GABAergic neurons [28].

Genetic variations in genes encoding enzymes involved in folate metabolism can influence development of the nervous system. Mice with a null mutation in the gene for the folate transporter exhibit profound abnormalities in development of the nervous system and die *in utero* [29]. A polymorphism in 5,10-methylenetetrahydrofolate reductase [MTHFR (an enzyme that converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; Fig. 1)], which results in decreased folate metabolism, increases homocysteine levels and the risk of neural tube defects [30]. One common such polymorphism is present in up to 40–45% of individuals in some populations [31]. Most common is a thermolabile variant ('C677T' or C/T) that exhibits reduced activity *in vitro*. Individuals homozygous for this variant exhibit mild hyperhomocysteinemia, which can be further augmented by diminished dietary folate [32,33]. Homozygous thermolabile MTHFR has been described in 10–12% of normal Caucasians, in 19% of individuals with arterial disease and in 11% of individuals with venous thrombosis [34]. Diminished activity of this enzyme also reduces production of tetrahydrofolate (required for DNA synthesis) and reduced adenosylmethionine (required for DNA methylation [35]). The importance of MTHFR in brain development is most clearly seen in individuals with a genetic deficiency of MTHFR, which leads to delayed psychomotor development in infancy, severe mental retardation and psychiatric symptoms [36].

Methionine synthase deficiency is associated with the rare *cb1E* and *cb1G* genotypes, which result in hyperhomocysteinemia, homocystinuria and diminished cellular methionine [37]. An additional methionine synthase polymorphism [A2756G (Asp → Gly)] with a homozygous frequency of $\geq 5\%$ might increase plasma homocysteine concentrations [38], although this has not been established in all studies. Another polymorphism involves CBS, which converts homocysteine to cystathionine, which is subsequently converted to cysteine [39] – the rate-limiting precursor of glutathione [40]. Deficiency in CBS leads to homocystinuria, a rare autosomal recessive disease of sulfur amino acid metabolism. Severe hyperhomocysteinemia is associated with CBS deficiency and results in multiple organ–system damage, including mental retardation [41]. Deficiencies in CBS activity might have the combined deleterious influence of forcing more homocysteine to be exported (Fig. 1), leading to increased excitotoxicity via increased stimulation of NMDA receptors, while possibly simultaneously decreasing compensatory oxidative buffering capacity by preventing glutathione synthesis [14,42]. CBS deficiency in children has been reported to increase total homocysteine in spinal fluid by over 10-fold and to induce abnormalities in brain function [43]. Thus, elevated homocysteine levels might mediate the adverse effects of folate deficiency on the developing nervous system as well as in the adult. Another link between folate, homocysteine metabolism and brain development comes from studies showing that alterations in enzymes involved in homocysteine and

folate metabolism occur in individuals with Down's syndrome, a disorder that is characterized by abnormal brain development and mental retardation [44–46].

Beyond dietary supplementation with folate, additional therapeutic approaches have been tested to prevent or correct neurological disorders associated with genetic aberrancies in homocysteine metabolism. Attempts to compensate for deficient CBS polymorphisms have included reducing the dietary intake of methionine (thereby reducing homocysteine production); dietary replacement of cysteine or NAC, or both, to maintain glutathione production; and stimulation of metabolic runoff of homocysteine by an alternative pathway via treatment with betaine [47]. Betaine treatment dramatically reduces spinal fluid homocysteine in children with CBS deficiency [36]. Importantly, betaine can reduce homocysteine even under conditions of folate deficiency; individuals deficient in MTHFR respond to betaine treatment [41]. Betaine (3 g twice daily for 24 weeks) was apparently tolerated in a pilot clinical study in individuals with Alzheimer's disease [48]. However, the required enzyme, betaine-homocysteine methyltransferase, is not detectable in the brain; the efficacy of betaine treatment might therefore be via reduction of systemic homocysteine burden [49].

Mechanisms of homocysteine-induced neuronal dysfunction and cell death

The adverse effects of folate/vitamin B12 deficiency and elevated homocysteine levels in the developing nervous system, taken together with the fact that hyperhomocysteinemia is a risk factor for cardiovascular disease and stroke, has prompted examination of potential roles for homocysteine and deficiencies in one-carbon metabolism in age-related neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). Early studies documented adverse effects of vitamin B12 deficiency on the adult nervous system. For example, experiments in non-human primates have shown that vitamin B12 deficiency can cause the degeneration of neurons in the spinal cord and brain of adults [50,51]. Folate deficiency and homocysteine can damage and kill neurons in cell culture, and can increase their vulnerability to being killed by various excitotoxic, oxidative and metabolic insults (Table 1). The available data suggest several possible mechanisms whereby homocysteine damages and kills neurons. Homocysteine induces DNA breakage in cultured neurons [7] by a mechanism that may

involve impaired transmethylation of DNA; because folate and vitamin B12 deficiencies retard methionine regeneration, SAM levels are also reduced as a consequence of folate or vitamin B12 deficiency [52]. Homocysteine induces apoptosis in part via DNA damage; cultured neurons treated with homocysteine eventually deplete their ATP reserves in attempting to repair homocysteine-induced DNA damage [7]. In this regard, depletion of cellular ATP is thought to be a pivotal factor in neurodegeneration in AD, PD and Huntington's disease (HD) [53–55]. As with considerations of homocysteine-induced oxidative stress, this maintains the possibility that moderate or even slight hyperhomocysteinemia might, especially over protracted periods, foster a crucial reduction in ATP in neurons in aging brains in which ATP is already depleted.

Oxidative damage resulting from folate deprivation and homocysteine might be caused by increased cytosolic Ca^{2+} levels and DNA damage [6,7]. Folate deprivation and homocysteine treatment each increase cytosolic Ca^{2+} levels in cultured neurons, and treatment with Ca^{2+} -channel blockers attenuates these increases, suggesting that at least the bulk of the increase results from influx across the plasma membrane [6]. Homocysteine potentiates glutamate neurotoxicity, and the toxicity of homocysteine itself is attenuated by antagonists of metabotropic glutamate receptors [7,41]. Folate deficiency and homocysteine also compromise glutathione peroxidase activity [56,57] and reduce tissue levels of vitamins A, C and E [58], suggesting that folate deficiency compromises antioxidant reserves at multiple levels. Indeed, *N*-acetyl-L-cysteine, vitamin C and vitamin E reduce homocysteine-mediated apoptosis in non-neuronal cells, apparently by scavenging hydrogen peroxide [59]. *N*-acetyl-L-cysteine and vitamin E can also prevent oxyradical generation in cultured neuroblastoma cells after folate deprivation [6]. Vitamin E diminishes the extent of neurodegeneration in the mouse brain after folate deprivation during oxidative challenge with dietary iron [60]. Folate supplementation itself is not as effective as *N*-acetyl-L-cysteine, vitamin C or vitamin E in protecting against homocysteine-induced apoptosis; this difference is thought to derive from the inability of folate itself to quench hydrogen peroxide effectively [61]. In contrast to its inability to quench hydrogen peroxide, folic acid is capable of reducing intracellular superoxide levels independently of any reduction in homocysteine levels [60].

One reason why folate supplementation might be less

Table 1. Examples of adverse effects of folate deficiency and homocysteine on neurons^a

Neurodegenerative agent or condition	Impact of folate deprivation	Impact of homocysteine	Neuroprotective agents	Refs
Reactive oxygen species	Increased	Increased	Vitamin E, NAC	[60]
Cytosolic Ca^{2+}	Increased	Increased	Channel blockers, chelators	[6]
Glutamate excitotoxicity	Increased	Increased	NMDA-receptor channel blocker	[6,25]
MPTP-induced neurotoxicity	Increased	Increased	Antioxidants, PARP inhibitors	[92]
Iron-induced neurotoxicity	Increased	Not tested	Vitamin E	[11]
Tau phosphorylation	Increased	Increased	NMDA-receptor channel blocker	[6]
ATP levels	Depleted	Depleted	PARP inhibitors	[6,7]
Apoptosis	Increased	Increased	PARP inhibitors, SAM, antioxidants	[6,7,14,41]
A β -induced neurotoxicity	Increased	Increased	Vitamin E, NAC	[14,41]
Impact of diminished ApoE function	Increased	Not tested	Vitamin E	[11]

^aAbbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAC, N-acetylcysteine; PARP, poly-(ADP-ribose) polymerase; SAM, S-adenosylmethionine.

effective than anticipated is that there is a 'feedback' mechanism within the methionine cycle. *S*-adenosylhomocysteine (SAH) hydrolase is a bi-directional enzyme that favors hydrolysis of SAH to homocysteine under normal conditions. However, in the presence of increased homocysteine (resulting from folate deficiency) the activity of this enzyme reverses, causing accumulation of SAH. Moreover, SAH, the demethylated product of SAM transmethylation, is itself a potent competitive inhibitor of SAM-mediated methylation reactions. Decreased intracellular methylation reactions can thus result from either a decrease in formation of SAM or an increase of SAH [62]. This line of reasoning is supported by the recent demonstration that homocysteine induces DNA breakage and resultant apoptosis [7,14], and that co-treatment with SAM prevents homocysteine-induced apoptosis [6].

Folate, homocysteine and neurodegenerative disorders

Increased oxidative stress, metabolic compromise, DNA damage, and the triggering of apoptotic and excitotoxic cell death pathways are involved in the pathogenesis of each of the major neurodegenerative disorders including AD, PD, HD and amyotrophic lateral sclerosis. The latter shared mechanisms occur despite the involvement of different disease-specific initiating factors – altered amyloid precursor protein (APP) processing in AD, accumulation of dopaminergic toxins in PD, polyglutamine expansions in the huntingtin protein in HD. Homocysteine levels increase during normal aging, and a high level of homocysteine is an independent risk factor for stroke – the most prevalent age-related neurodegenerative condition [63]. The epidemiological, genetic and experimental findings described below suggest that individuals with elevated homocysteine levels are at increased risk of two other major neurodegenerative disorders: AD and PD.

Alzheimer's disease

Studies of human populations and experimental models of AD suggest roles for folate deprivation and homocysteine in the disease process [14,64]. Individuals with AD exhibit elevated levels of homocysteine [65–68], which can occur before disease onset [69]. Plasma homocysteine levels are increased in most cases of AD and represent an early marker of cognitive impairment in the elderly [65,70,71]. Low serum folate levels are strongly associated with atrophy of the cerebral cortex [71]. In addition, although levels of folate in cerebral spinal fluid are normally three- to fourfold higher than in blood, spinal fluid levels of folate are significantly lower in individuals with AD [72]. SAM levels, as well as the activity of the enzyme responsible for its generation (methionine-*S*-adenosyltransferase), are

also decreased in the spinal fluid and brains of individuals with AD compared with age-matched controls [73]. Altered folate metabolism, including that arising from genetic aberrancies, might therefore represent one contributing factor to the association of Down's syndrome with AD [74].

Factors contributing to the onset and development of AD include genetic predisposition, amyloid β -peptide ($A\beta$) accumulation, oxidative stress, Ca^{2+} mismetabolism and excitotoxicity [75,76] (Table 2). A particularly insidious aspect of the neurotoxicity of folate deprivation and increased homocysteine is that they interact synergistically with, and potentiate, several of these factors (Table 1). For example, homocysteine or folate deprivation can potentiate $A\beta$ neurotoxicity in cultured neurons [14,77] and in transgenic mice overexpressing APP that develop amyloid deposits in their brains [14]. Simultaneous exposure of cultured neurons to $A\beta$ and homocysteine induces oxidative stress and apoptosis at concentrations which, when $A\beta$ and homocysteine are given individually, have marginal influence on either phenomenon [41]. Resultant levels of oxyradicals and apoptosis exceed that resulting from a twofold higher concentration of either $A\beta$ or homocysteine alone, indicating a synergistic effect of homocysteine and $A\beta$ on neurodegeneration. Because oxidative damage is at the core of neuronal degeneration in AD, increased oxidative stress represents one mechanism by which dietary or genetic folate deficiency contributes to neurodegeneration. Indeed, increased oxyradical production occurs in cultured neurons deprived of folate or exposed to homocysteine [7,41]. Moreover, tetrahydrofolate is very susceptible to oxidation [78], as is cob(I)alamin, an intermediate form of vitamin B12 [79], suggesting that depletion of folate and vitamin B12 might contribute to the oxidative damage to neurons. Although homocysteine can directly damage neurons, it is also possible that damage to cerebral blood vessels contributes to neurodegenerative effects of folate deficiency and hyperhomocysteinemia in individuals with AD [80].

An interesting link between homocysteine and AD comes from recent studies of a protein known as HERP (homocysteine-inducible endoplasmic reticulum stress protein). HERP was identified in a screen for cDNAs encoding proteins that increase γ -secretase cleavage of $A\beta$ [81]. The latter study provided evidence that HERP interacts with presenilins 1 and 2, and that overexpression of HERP in cultured cells increases the production of $A\beta$. More recently, it has been shown that HERP is present in hippocampal and cortical neurons where its expression is induced by endoplasmic reticulum stress [82]. Full-length 54 kDa HERP is cleaved by caspases in neurons undergoing apoptosis; this cleavage might compromise a

Table 2. Factors that cause, or increase the risk of, neurodegenerative disorders^a

Disease or condition	Genetic factors	Environmental factors	Refs
Alzheimer's disease	APP, PS1 mutations, apolipoprotein E4	Lack of education, high-calorie diet, folate deficiency	[14,54,66,75]
Parkinson's disease	α -Synuclein, mutations in the gene encoding parkin	Toxins, high-calorie diet, folate deficiency	[54,89,90,92]
Stroke	Apolipoprotein E4	High calorie–high-fat diet, sedentary lifestyle, folate deficiency	[63]
Psychiatric disorders	MTHFR mutations	Stressful events	[98,99,101–103]

^aAbbreviations: APP, amyloid precursor protein; MTHFR, methylenetetrahydrofolate reductase; PS1, presenilin 1.

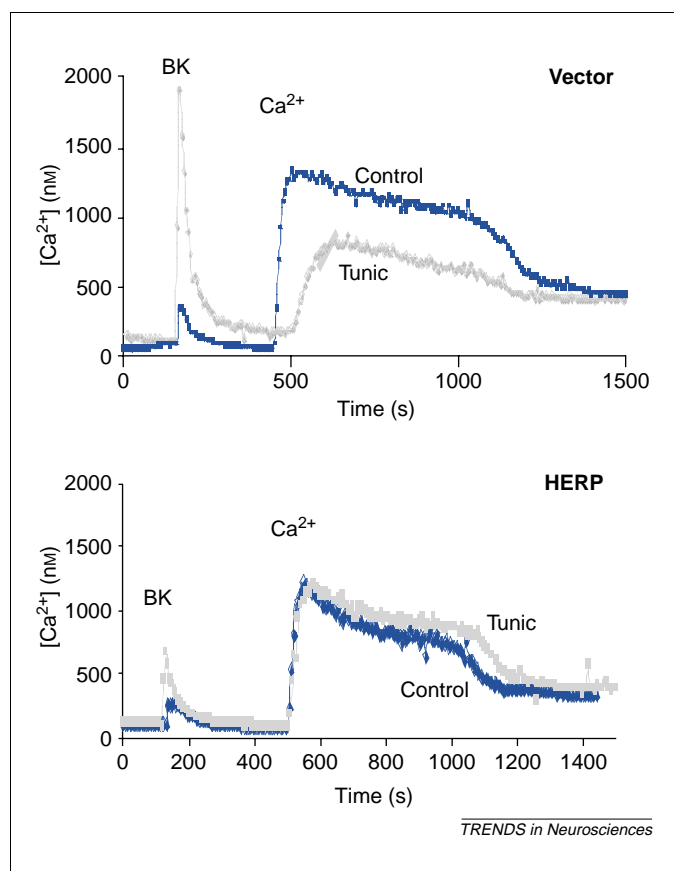


Fig. 3. Homocysteine-inducible endoplasmic reticulum stress protein (HERP) stabilizes cellular Ca^{2+} homeostasis. Vector-transfected control PC12 cells (top) and PC12 cells overexpressing HERP (bottom) were either exposed to tunicamycin (tunic; an inducer of endoplasmic reticulum stress) for 8 h or were untreated. The culture medium was then changed to medium lacking Ca^{2+} , and the intracellular free Ca^{2+} concentration was measured by imaging of the Ca^{2+} indicator dye fura-2 before and during sequential exposures to bradykinin (BK, 10 μ M) and Ca^{2+} (2 mM). Values are the mean of measurements made in cells in three separate cultures. Note that the Ca^{2+} response to bradykinin was greatly enhanced, and capacitative Ca^{2+} entry greatly reduced, in control cells treated with tunicamycin. By contrast, tunicamycin had very little effect on the Ca^{2+} responses in cells overexpressing HERP. Modified from Ref. [82].

neuroprotective function of HERP because its levels are increased in individuals with AD and because overexpression of HERP protects cultured neural cells against death induced by endoplasmic reticulum stress and $A\beta$ [82]. HERP stabilizes endoplasmic reticulum Ca^{2+} homeostasis, which could account for its neuroprotective actions (Fig. 3). Further work will be required to clarify the influence of homocysteine on HERP in neurons and the role of HERP in AD and other neurodegenerative disorders.

As described above, folate deprivation and increased homocysteine inhibit transmethylation reactions by reducing SAM. In this regard, impaired methylation pathways have been implicated in many neurological and psychological disorders, including dementia, depression and psychosis. SAM levels have been found to be low in the cerebral spinal fluid and brains of individuals with several neurological disorders, including AD [83,84]. The full range of factors that contribute to this reduction in SAM levels remain undisclosed. However, compromise in folate metabolism is likely to have a profound impact under conditions in which SAM levels are already lowered. Individuals with an E4 isoform of apolipoprotein (ApoE)

are at increased risk of AD, possibly because of a diminished antioxidant activity of this isoform compared with that of other isoforms [85,86]. An interaction between ApoE4 and MTHFR in AD risk has recently been reported [87]. Folate deprivation has a profound deleterious impact on the neurotoxic consequences associated with diminished ApoE function. Homozygous ApoE knockout (*ApoE*^{-/-}) mice deprived of folate for one month (while receiving dietary iron as a pro-oxidant) demonstrated a marked increase in oxidative damage in brain tissue when compared with *ApoE*^{-/-} mice maintained in the presence of folate; normal mice did not exhibit any increase in oxidative damage after folate deprivation [60]. Notably, although no detectable difference in oxidative damage was induced in *ApoE*^{-/-} mice after deprivation of vitamin E in the presence of folate, vitamin E deprivation augmented the increased oxidative damage accompanying folate deprivation. Consistent with findings in individuals with AD, *ApoE*^{-/-} mice displayed an increase in steady-state glutathione in brain tissue compared with normal mice [60]. Folate deprivation and iron challenge each increased glutathione levels in both normal and *ApoE*^{-/-} brains; however, this increase in glutathione was apparently incapable of compensating for the lack of ApoE after folate deprivation coupled with iron challenge, as indicated by increased oxidative damage [11]. These data are consistent with prior indications that oxidative stress can overwhelm the ability of glutathione to quench oxyradicals [88] and demonstrate how the combined influence of oxidative stress, genetic predisposition and folate deprivation can induce neurodegeneration under conditions in which no single factor is able to do so.

Parkinson's disease

Although not as extensively studied as AD, emerging data suggest that dietary folate and plasma homocysteine levels have an impact on PD (Table 1). Increased plasma homocysteine levels have been documented in individuals with PD [89,90]. Moreover, a further increase in homocysteine levels was detected in individuals with PD bearing the C677T mutation in MTHFR. Importantly, levodopa treatment might promote depletion of methyl groups and thereby cause an elevation of homocysteine levels [91], suggesting that this common treatment could actually accelerate the neurodegenerative process. Folate deprivation and administration of homocysteine enhanced MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced depletion of dopaminergic neurons in a mouse model of PD [92]. Folate deprivation and homocysteine both sensitized human dopaminergic neurons in culture to damage and death induced by rotenone and iron, two agents implicated in the pathogenesis of PD [92]. The latter findings suggest that folate deficiency and increased homocysteine levels could, by damaging dopaminergic neurons, hasten the onset and progression of PD.

Postscript: teetering on the edge

Recent experimental findings described above support the prior clinical association between folate deprivation, increased homocysteine levels and neurodegeneration. Potentiation of neurodegeneration induced by treatments

such as glutamate excitotoxicity and A β toxicity by homocysteine maintains the possibility that even moderate hyperhomocysteinemia can exert a deleterious impact on neuronal health far beyond what might be anticipated (Fig. 4). Whether or not polymorphisms in enzymes that regulate homocysteine levels contribute to age-related neurodegeneration remains to be clarified. Notably, polymorphisms that even mildly impair folate metabolism might place individuals at increased risk for developing neurodegenerative conditions, even in the presence of what is considered sufficient dietary folate. Thus, levels of folate that are considered adequate under normal circumstances might actually be deficient under conditions that promote neurodegeneration. The emerging impact of folate metabolism and homocysteine on nervous system homeostasis suggest that diagnostic and therapeutic approaches should include attention to these factors.

Folate and homocysteine in psychiatric disorders

Psychiatric disorders are common and often debilitating. For example, the overall prevalence of depression and anxiety disorders in the USA are ~15% and 17%, respectively, and at least 1% of the population suffers from schizophrenia during their lifetime. Genetic factors

contribute to the pathogenesis of major psychiatric disorders, but environmental factors also play major roles.

Depression

More than three decades ago it was reported that individuals suffering from depression have reduced folate levels compared with non-depressed controls [93]. Folate supplementation has further been shown to reverse depression in some cases [94,95]. Subsequent studies documented decreased levels of folate and vitamin B12 in individuals with clinical depression and have provided evidence for a mechanism involving hyperhomocysteinemia and altered methylation reactions [96]. Other studies have linked folate deficiency to the neurochemistry of depression; levels of the 5-HT metabolite 5-hydroxyindoleacetic acid were decreased in the cerebral spinal fluid of depressed individuals, and were restored to normal in individuals after folate supplementation [97]. In addition, vitamin B6 therapy was reported to be effective in reducing symptoms of depression and schizophrenia [98].

Schizophrenia

In one study there was an increased risk of schizophrenia when folate levels were low and homocysteine levels were elevated, whereas this risk was not increased in individuals with low folate levels, but normal homocysteine levels [99]. Homocysteine might mediate the effects of folate and vitamin B12 deficiencies in psychiatric disorders, because elevated levels of homocysteine in individuals with schizophrenia are not necessarily related to folate or vitamin B12 deficiencies [100]. Clinical improvement after folate supplementation has been documented in a double-blind placebo-controlled trial in schizophrenics and individuals with depression [101]. Genetic analyses revealed that individuals with the relatively common C677T polymorphism in the gene encoding MTHFR (which is present in 10–12% of the population and results in a greater than 70% reduction in enzyme activity) are at increased risk of schizophrenia [102]; homozygosity for the MTHFR mutation is associated with elevated homocysteine levels that are not normalized after folate supplementation. Folate and vitamin B12 have also been linked to autism and other childhood neuropsychiatric disorders [103].

Implications for the prevention and treatment of neurological disorders

The impact of dietary folate supplementation on the incidence of defects in the developing nervous system has been established in the USA, where the incidence of neural birth defects has dropped precipitously since the time that the government mandated folate supplementation of some commonly consumed processed foods. Regarding risk of cardiovascular disease and stroke, the relationship between plasma homocysteine levels and risk of cardiovascular disease and stroke is as follows: <7 μ M, low; 8–11 μ M, moderate; 12–16 μ M, high; >16 μ M, very high. Data from the Framingham study suggest a similar risk relationship for neurodegenerative disorders [64]. Dietary supplementation with folate at amounts up to 2 mg per day progressively decreases

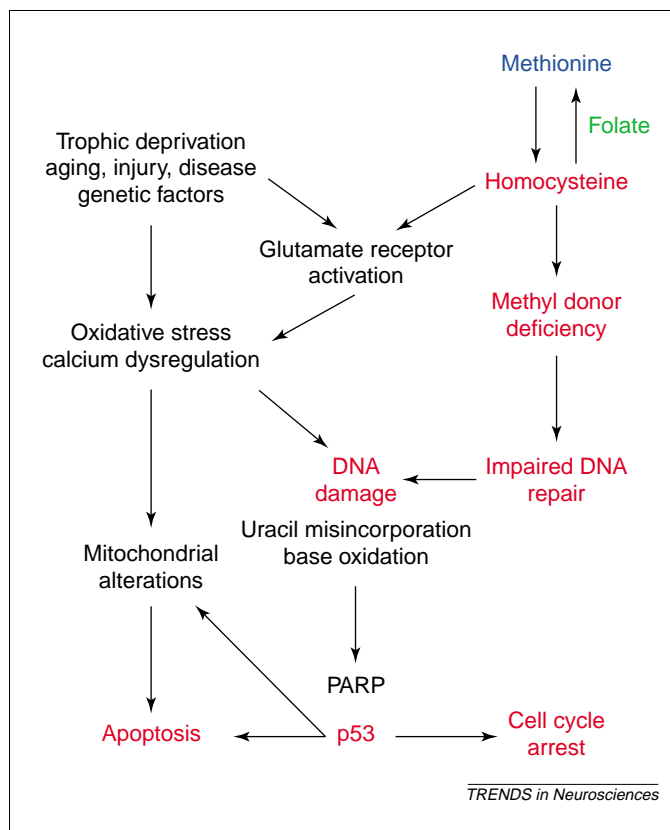


Fig. 4. Mechanisms by which homocysteine might induce the death of neurons, and cell cycle arrest in neural stem cells and glial cells. The pathway that has adverse effects on developing and mature neurons is shown in red. Key steps involve methyl donor deficiency resulting in increased DNA damage due to deficient DNA repair. DNA damage activates poly-(ADP-ribose) polymerase (PARP), resulting in ATP depletion and activation of the tumor suppressor protein p53, which can trigger cell-cycle arrest or apoptosis. These adverse effects of homocysteine can be enhanced by oxidative stress and perturbed cellular Ca²⁺ homeostasis that occurs during aging and in age-related neurodegenerative disorders.

plasma homocysteine levels, with significant decreases occurring with 200–400 µg per day [104]. Therefore, it seems likely that dietary supplementation with 0.4–1 mg of folate will reduce the risk of each of the neurodegenerative and psychiatric disorders described above. Vitamins B12 and B6 might provide additional protection. Individuals with some psychiatric disorders, most notably depression, have been shown to benefit from folate supplementation. Whether folate, vitamin B12 and/or vitamin B6 will prove effective in treating individuals with neurodegenerative disorders such as AD and PD remains to be determined in clinical trials that are ongoing or planned. The bottom line at this point is that each of us should know our homocysteine level and, if high, should modify our diet and take folate supplements so as to bring the level from a danger zone into a comfort zone.

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