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DIETARY AND GENETIC COMPROMISE IN FOLATE AVAILABILITY REDUCES ACETYLCHOLINE, COGNITIVE PERFORMANCE AND INCREASES AGGRESSION: CRITICAL ROLE OF S-ADENOSYL METHIONINE

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Abstract: Folate deficiency has been associated with age-related neurodegeneration. One direct consequence of folate deficiency is a decline in the major methyl donor, S-adenosyl methionine (SAM). We demonstrate herein that pro-oxidant stress and dietary folate deficiency decreased levels of acetylcholine and impaired cognitive performance to various degrees in normal adult mice (9-12months of age, adult mice heterozygously lacking 5',10'-methylene tetrahydrofolate reductase, homozygously lacking apolipoprotein E, or expressing human ApoE2, E3 or E4, and aged (2 – 2.5 year old) normal mice. Dietary supplementation with SAM in the absence of folate restored acetylcholine levels and cognitive performance to respective levels observed in the presence of folate. Increased aggressive behavior was observed among some but not all genotypes when maintained on the deficient diet, and was eliminated in all cases supplementation with SAM. Folate deficiency decreased levels of choline and N-methyl nicotinamine, while dietary supplementation with SAM increased methylation of nicotinamide to generate N-methyl nicotinamide and restored choline levels within brain tissue. Since N-methyl nicotinamide inhibits choline transport out of the central nervous system, and choline is utilized as an alternative methyl donor, these latter findings suggest that SAM may maintain acetylcholine levels in part by maintaining availability of choline. These findings suggest that dietary supplementation with SAM represents a useful therapeutic approach for age-related neurodegeneration which may augment pharmacological approaches to maintain acetylcholine levels, in particular during dietary or genetic compromise in folate usage.

Nutritional compromise often accompanies aging and can promote neurological deficiencies (1). Folate deprivation leads to age-related neurodegenerative disorders including Alzheimer's disease (AD; 2). Folate deficiency fosters a decline in S-adenosyl methionine (SAM; the major methyl donor), decreasing DNA methylation during aging and AD (3, 4) and increasing DNA breakage (5, 6). Folate deficiency and resultant SAM depletion potentiate several AD genetic risk factors, including increasing homocysteine (which potentiates Abeta neurotoxicity; 7-10), fostering presenilin-1 (PS-1) overexpression (11, 12), and compromising glutathione usage (which increases oxidative stress and potentiates the impact of ApoE deficiency; 13, 14). In this regard, the C677T polymorphism of 5',10' methylene tetrahydrofolate reductase (MTHFR; which utilizes folate to regenerate methionine from homocysteine) is an ApoE4-dependent AD risk factor (15-17). SAM declines, while its hydrolysis product S-adenosyl homocysteine (SAH) increases in AD; SAH inhibits methyl transferases that utilize SAM and therefore further inhibits methylation in AD (3, 18, 19). Finally, folate deprivation impairs cognitive performance in mice (20). Since SAM regulates neurotransmitter levels (21, 22), the decline in SAM that accompanies folate deprivation may play a role in cognitive impairment.

AD is accompanied by a decrease in several neurotransmitters including acetylcholine (ACH) (23). Strategies to maintain or increase acetylcholine production are therefore of interest, perhaps in concert with acetylcholinesterase inhibition (e.g., 24-27). Increasing availability of choline, the ACH precursor, represents one such strategy (28, 29). Increased consumption of choline or choline precursors has not been effective in AD but could be effective prior to its onset (30, 31). Polymorphisms in choline acetyltransferase, which generates ACH from choline, potentiate AD genetic risk factors including ApoE4 (32). Choline undergoes net efflux from brain, which is prevented by N-methyl nicotinamide (33). Since N-methyl nicotinamide is formed by SAM-dependent methylation (33, 34), SAM may regulate ACH generation. Since choline is a methyl donor (28, 29) it is likely scavenged in the absence of SAM. Furthermore, choline is converted to betaine in order to remethylate homocysteine (35); this pathway is absent from brain tissue, but is otherwise systemic, and therefore fosters choline efflux from brain. MTHFR deficiency depletes choline by increasing betaine-dependent homocysteine remethylation (32, 36, 37). Folate deprivation and SAM depletion may therefore compromise ACH generation by depleting available choline via multiple mechanisms.

We examine herein the impact of dietary and genetic folate deficiency and SAM supplementation on ACH, cognitive performance and aggression in several mouse models.

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Materials and Methods

Mouse strains and Diets

Mice used in this study were normal C57B/6 mice from our colony aged 9-12 months (defined as "adult") and 2-2.5 years (defined as "aged"; 38), adult mice lacking murine ApoE (ApoE -/- mice) or lacking murine ApoE and expressing human ApoE2, E3 or E4 on a C57Bl6 background, and adult BALB/cAnNCrlBR mice heterozygously lacking MTHFR (MTHFR+/-) along with normal littermates (MTHFR+/+; genotypes of which were determined by PCR as described previously; 39).

Mice received a diet ("AIN-76"; Purina/Mother Hubbard, Inc.) either lacking folate and vitamin E (defined as the "deficient diet") or supplemented with folic acid (4 mg/kg), and vitamin E (50IU/kg total diet wet weight) (defined as the "complete diet"). The deficient diet was also supplemented in all cases with iron (50g/500g total diet) as a pro-oxidant. Mice of the same genotype were maintained in groups of 3 or 4 on these diets for 1 month (20, 40, 41). Additional groups of mice maintained on the deficient diet received SAM for the entire month (100mg/kg diet; 14).

Cognitive performance and spontaneous aggression

Cognitive impairment was monitored after maintenance on the above diets for 1 month using a standard Y maze test as described (20). The pattern of exploration of the Y maze was recorded over 5 min intervals for each mouse. The frequency in which mice visited each of the 3 arms in succession during any 3-arm visitation sequence versus the total visitations defines the "% alternation."

During the course of these studies, spontaneous extreme aggressive behavior (self-mutilation and/or severe attacks among cage-mates) was noted among some strains of mice maintained on the deficient diet; milder forms of aggression were not quantified.

Biochemical Assays

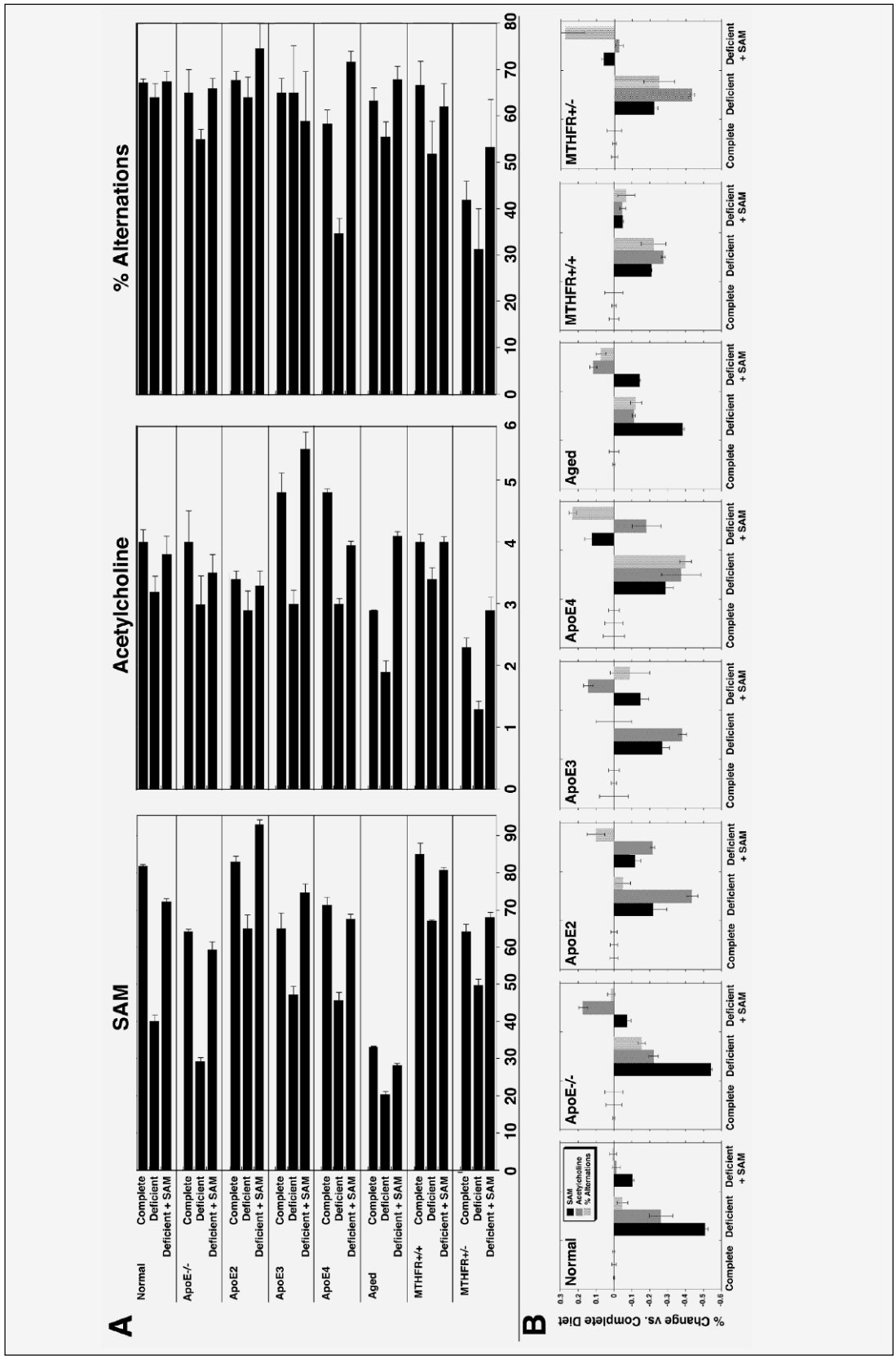
Following the above maze trials, mice were sacrificed by cervical dislocation, and the frontal portion of the brain (encompassing cortex and hippocampus) was immediately removed and frozen (-80^{o}C), and stored frozen until use. Choline and acetylcholine levels in homogenates of forebrain tissue were determined by the method of Erb et al. (34) using an Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (Molecular Probes Invitrogen Detection Technologies, Inc.) by comparison with standard curves in a Hitachi F2000 fluorescent spectrophotometer. According to the manufacturer's instructions, choline was quantified initially, then samples were treated with acetylcholinesterase (which converted all ACH to choline); subtraction of the initial choline readings from the post-acetylcholinesterase incubation yielded acetylcholine values. SAM levels in homogenates of forebrain were determined by comparison to SAM standards using a Bridge-It S-Adenosyl Methionine (SAM) Fluorescence Assay Kit (Mediomics, LLC). N-methyl nicotinamide (NMN) levels were assayed using the method of Clark et al. (42). Briefly, NMN was extracted from brain homogenates using 30% TCA. Extracts were cleared of TCA using ethyl acetate, and subsequently lyophilized. Prior to assaying, 10% acetophenone, 1N KOH, and 99% formic acid were added to the lyophilized samples. Following a 4-hour incubation at 25^{o} C, NMN levels were determined by comparison to NMN standards using a Hitachi F2000 fluorescent spectrophotometer. Fluorescence was measured at an excitation of 370 nm and emission of 430 nm. Spectrophotometric assays for SAM, acetylcholine and N-methyl nicotinamide were carried out on a Hitachi F2000 fluorescent spectrophotometer. All data were derived from at least 2 independent experiments with 3-4 mice from each strain on each diet per experiment, for a total of 7-8 mice per strain per diet. Statistical comparisons were carried out with Student's t test (for individual comparisons between two diets or genotypes), ANOVA (for comparisons of 3 or more diets and genotypes) and the Wilcoxon rank-sum test (for correlation of ACH and aggression among all mice used in this study). ANOVA and rank-sum are indicated where utilized; other values were derived from the t test.

Results

Folate deficiency for 1 month fostered a decline in SAM in brain tissue of all mouse lines tested (Fig. 1A). Consistent with prior studies (39, 41), adult MTHFR+/- and ApoE-/- mice each displayed statistically ($p<0.05$) reduced SAM even in the presence of dietary folate as compared to normal mice of the identical genetic backgrounds; dietary deficiency further reduced SAM levels in cortical tissue of ApoE-/- and MTHFR+/- mice. Mice expressing ApoE2 displayed identical levels of SAM on the complete diet as did normal mice. Mice expressing ApoE3 and E4 each displayed statistically ($p<0.05$) reduced levels of SAM when maintained on the deficient diet. Supplementation of the deficient diet with SAM restored SAM levels in ApoE2, E3 and E4 mice to levels equal or surpassing levels observed on the complete diet. Normal aged mice displayed reduced SAM as compared to normal adult mice ($p<0.05$) even when maintained in the presence of folate. ApoE-/- mice maintained on the complete diet also displayed reduced SAM as compared to normal adult mice ($p<0.05$), which declined further ($p<0.05$) following dietary folate deprivation. Supplementation of the folate-free diet with SAM restored the respective levels of SAM observed for these mice when maintained on the complete diet. These data confirm dietary supplementation with SAM can increase levels within brain tissue (43).

We next examined the influence of these diets on ACH levels. ACH levels were statistically identical in normal adult C57B/6, mice expressing ApoE2, or MTHFR+/+ mice regardless of diet (Fig. 1A). MTHFR+/- mice maintained on the

Figure 1
Folate deprivation and dietary supplementation with SAM regulate acetylchoine levels and cognitive performance



Normal adult and aged mice, adult ApoE-/- mice, mice expressing ApoE2, E3 and E4, adult MTHFR+/+ and MFTHR+/- mice maintained on the indicated diets for 1 month were subjected to Y maze analyses, after which brain tissue was harvested and spectrophotometrically analyzed for SAM and acetylcholine levels as described in Materials and Methods. Panel A presents the mean spectrophotometric values (± standard error) for SAM and acetylcholine, and the mean % alternations (± standard error) in the Y maze as indicated. Panel B presents the % change (± standard error) in values obtained for SAM levels, acetylcholine levels and % alternations for each mouse strain, calculated by dividing mean values obtained for the deficient diet and, separately, for the deficient diet + SAM, by the mean values obtained for the complete diet, and subtracting this value from 100%. Mean values obtained for mice on the complete diet are therefore defined as 0. See text for further description and statistical comparisons.

complete diet displayed statistically ($p<0.05$) reduced ACH versus MTHFR+/+ mice. Dietary deprivation of folate further reduced levels of ACH in MTHFR+/- mice versus those in MTHFR+/- mice maintained on the complete diet ($p<0.05$), while supplementation of the deficient diet with SAM prevented this further reduction. Mice expressing ApoE3 and E4 each displayed statistically higher ACH levels than normal mice when maintained on the complete diet; the reason for this is unclear. Nevertheless, like other mice in this study, both ApoE3 and ApoE4 mice displayed significant ($p<0.05$) reductions in ACH levels when maintained on the deficient diet; supplementation of the deficient diet with SAM prevented or attenuated ($p<0.05$) these decreases for mice expressing ApoE3 or ApoE4, respectively. ACH in aged normal mice maintained on the complete diet was statistically reduced ($p<0.05$) as compared to adult normal mice. ACH levels in aged mice were further reduced ($p<0.05$) following folate deprivation, which was prevented by supplementation of the deficient diet with SAM (Fig. 1A).

To probe the potential impact of the decline in this neurotransmitter on cognitive performance, we subjected mice to a standard Y maze test. Normal adult mice and MTHFR+/+ demonstrated an obvious and consistent trend towards impaired performance when deprived of folate, as shown previously (20), however, this trend, as in prior studies, did not differ significantly from mice maintained in the presence of folate (Fig. 1A). Mice expressing ApoE2 and E3 did not exhibit impaired performance on the deficient diet (Fig. 1A). ApoE-/- mice and mice expressingApoE4 performed as well as normal mice on the complete diet, but their % alternations declined significantly when maintained on the deficient diet. MTHFR+/- maintained on the complete diet demonstrated significantly ($p<0.05$) poorer performance than MTHFR+/+ mice. The performance of both MTHFR+/+ and MTHFR+/- mice declined significantly when maintained on the deficient diet ($p<0.05$), however, that of MTHFR+/- mice declined further ($p<0.05$) than that of MTHFR+/+ mice. Dietary supplementation with SAM restored the performance of all mouse strains to levels statistically identical to the respective levels observed for these mice maintained on the complete diet (Fig. 1A).

To facilitate comparison of the relationship among SAM levels, ACH levels and cognitive performance, we also calculated the % change in each of these parameters for each mouse strain maintained on the deficient diet and the deficient diet supplemented with SAM versus the respective values for each mouse strain maintained on the complete diet. While each mouse strain varied in the extent of change, in all cases ACH levels and cognitive performance varied in direct proportion to SAM levels (Fig. 1B).

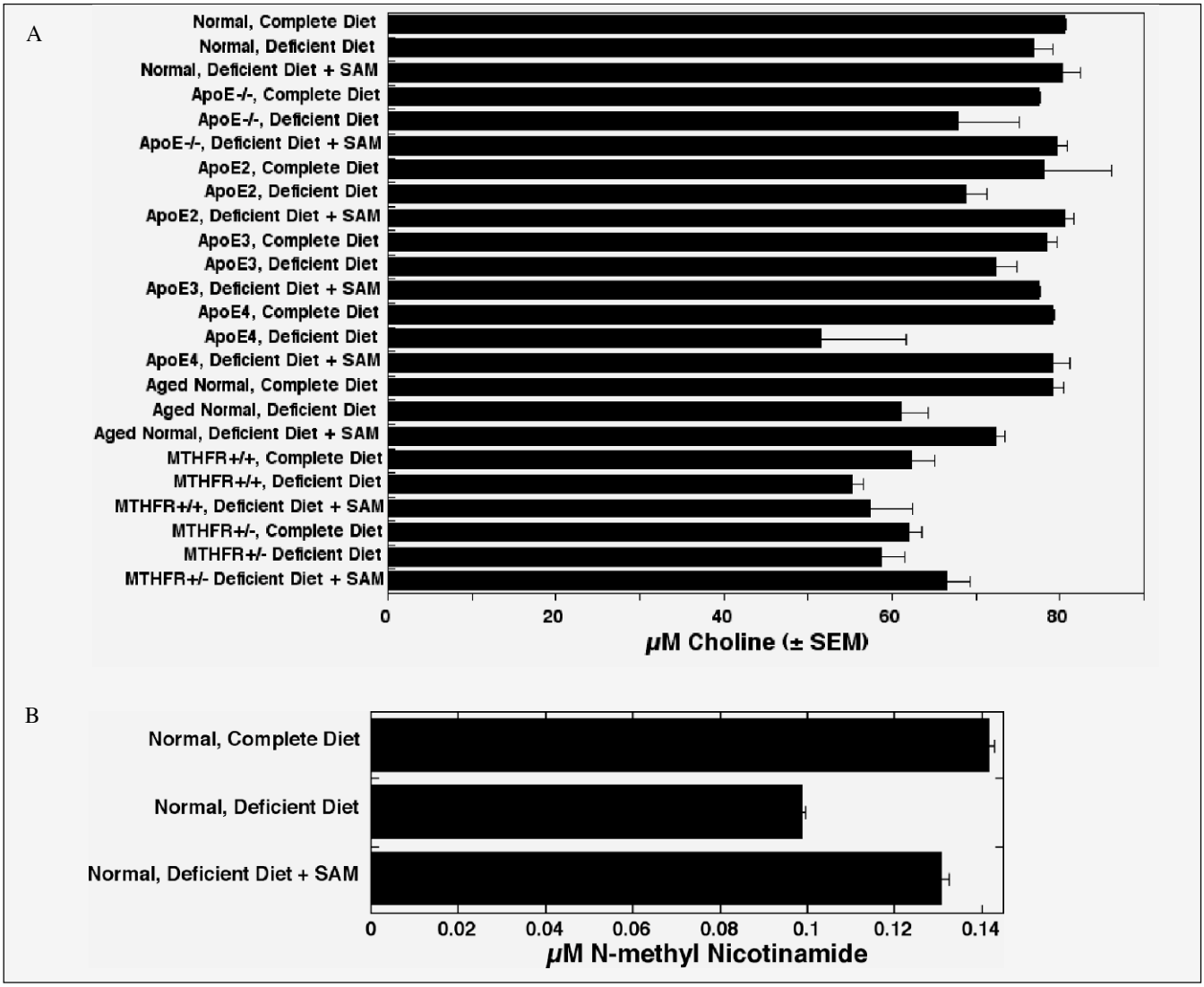
These data were consistent with the possibility that SAM played a role in ACH levels. One method by which SAM can regulate ACH levels in brain tissue is by maintaining levels within brain tissue of the ACH precursor choline (e.g., 28, 29), which it accomplishes by methylation of nicotinamide to generate N-methyl-nicotinamide, which inhibits transport of choline across the blood-brain barrier (34). We therefore quantified levels of choline and N-methyl-nicotinamide in mice maintained under these dietary regimens. Choline levels were reduced following maintenance of mice on the deficient diet. The extent of reduction was reduced or prevented following SAM supplementation of the deficient diet, in a pattern that paralleled that observed for ACH (Fig. 2A). N-methyl nicotinamide levels were also significantly ($p<0.05$) decreased following maintenance of mice on the deficient diet. However, supplementation of the deficient diet with SAM prevented this decrease (Fig. 2B).

It remained possible that the increase in reactive oxidative species in brain tissue resulting from maintenance on the deficient diet could foster the observed decrease in ACH levels via degeneration of cholinergic neurons. To address this possibility, ApoE-/- mice that had been subjected to the deficient diet for 1 month, which induced a decline in SAM and in cognitive performance (e.g., Fig. 1), were then returned to the complete diet for an additional month, and their performance was assessed in the Y maze. Prior to placing these mice on the deficient diet, they demonstrated a % alternation of 59.5 ± 7.4%; which declined significantly ($p<0.02$) to 37.7 ± 9.6% following maintenance for 1 month on the deficient diet. However, when these mice were returned to the complete diet for 1 month their performance increased to 62.1 ± 15.1%, which was statistically identical ($p>0.19$) to that prior to their maintenance on the deficient diet. Recovery of cognitive performance argues against depletion of cholinergic neurons or other permanent effects of this relatively short-term (1 month) induction of oxidative stress to brain tissue.

During the course of these studies, extreme aggressive behavior (self-mutilation and/or severe attacks among cage-mates) was noted among some mice maintained on the deficient diet. Out of the189 total mice utilized in this study, 41 exhibited extreme aggression, 39 of which had been maintained on the deficient diet. The remaining 2 mice exhibiting aggression had been maintained on the complete diet; aggression was not observed among mice maintained on the deficient diet supplemented with SAM (Fig. 3A). Since dietary deficiency under these conditions was apparently associated with aggression, we next compared acetylcholine levels with aggressive behavior. We first pooled all 189 mice independently of genotype; the average ACH levels for all mice regardless of genotype in this study exhibiting aggressive behavior (3.2±0.2μM; mean ± standard error of the mean; n=41) was 30% lower than acetylcholine levels of all mice not exhibiting aggression (4.6 ±0.2μM; n= 148; $p<0.001$, Rank-sum test; Fig. 3B).

To explore further the apparent correlation of diet, ACH and aggression, we compared these parameters for 4 randomly-selected individual mice from each genotype (Fig. 3C; Table 1) data for mice maintained on the deficient diet and the deficient diet supplemented with SAM are presented for simplicity only. This comparison was carried out since the only difference

Figure 2
Folate deprivation and dietary supplementation with SAM regulate levels of choline and N-methyl nicotinamide



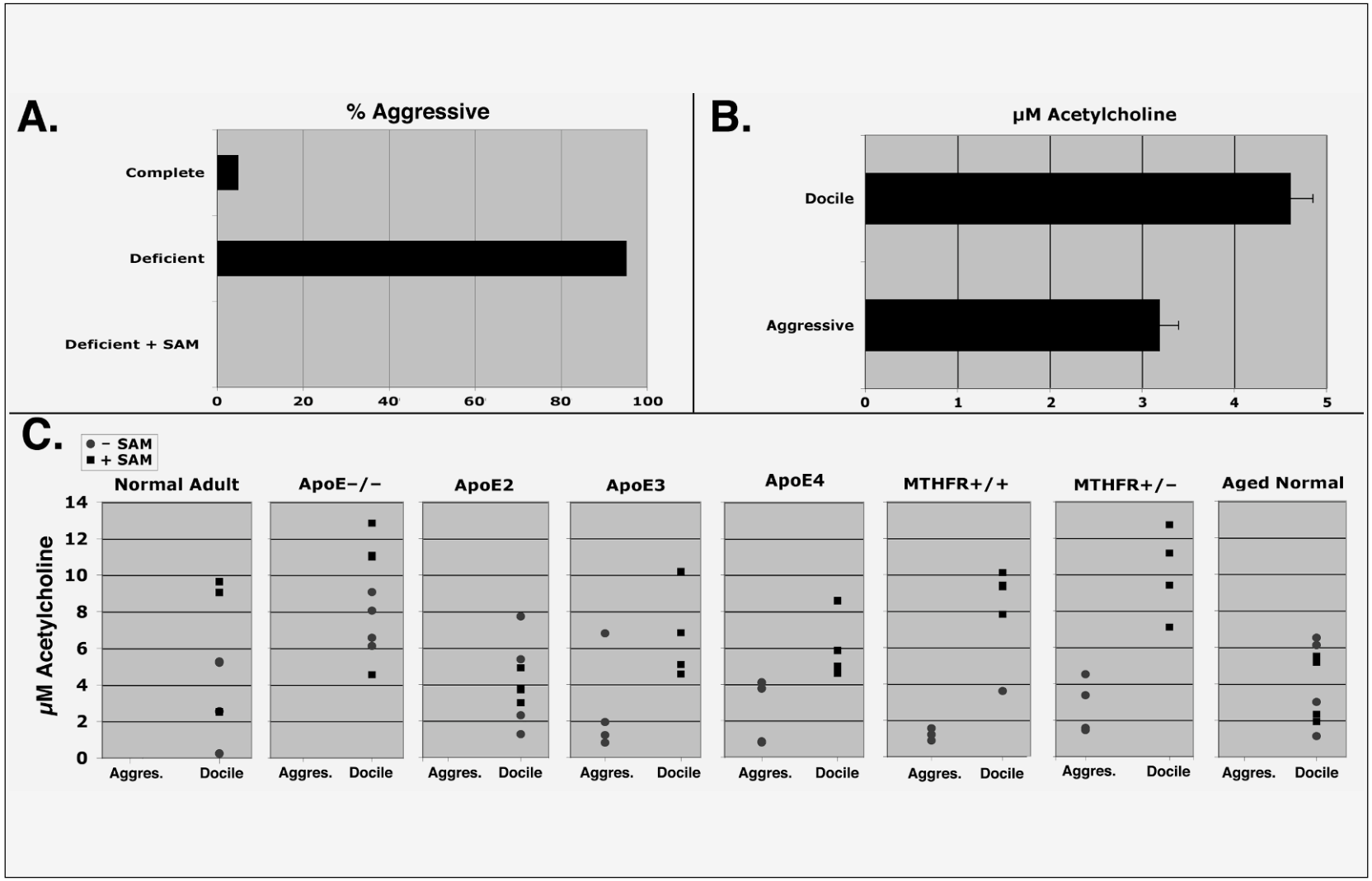
Panel A presents levels of choline in forebrain tissue from the indicated mouse strains maintained on indicated diets for 1 month determined as described in Materials and Methods. Panel B presents forebrain tissue from normal adult mice harvested and spectrophotometric analysis of forebrain tissue of normal adult mice for N-methyl nicotinamide as described in Materials and Methods. Note the decrease in levels in mice maintained on the deficient diet, and the partial prevention of this decrease by supplementation of the deficient diet with SAM. Values represent the mean spectrophotometric values (± standard error).

between the two diets is the presence and absence of SAM; similar correlation was observed in comparisons including the complete diet (not shown).

While all mouse genotypes exhibited some reduction in ACH following maintenance on the deficient diet, aggressive behavior was observed only among those genotypes in which a significant ($p<0.05$) reduction in ACH was observed (Fig. 3; Table 1). Comparison of acetylcholine levels versus aggressive behavior for mice maintained on the deficient diet with and without SAM supplementation demonstrated that reduced ACH levels were statistically correlated with aggressive behavior for each of these genotypes (Table 1). Notably, however, aggressive behavior was not observed among all genotypes. Moreover, aggression was correlated with the degree of difference in ACH levels in the presence and absence of SAM, rather than the absolute value of ACH; for example, normal mice on the deficient diet displayed ACH levels statistically identical to those observed in ApoE4 mice on the deficient diet, yet 100% of ApoE4 mice on this diet were aggressive, while none of the normal mice on the deficient diet exhibited aggression (Table 1; Fig. 3).

Figure 3

Folate and SAM regulate aggressive behavior; correlation with acetylcholine levels



Panel A presents the % of total mice (n= 189) of the total mice (i.e., summed from all genotypes used in this study) that displayed aggression for each diet. Note the significant ($p<0.001$; ANOVA) association of aggression with the deficient diet; 0% of mice displaying aggression had been maintained on the SAM-supplemented diet, and only 5% of mice displaying aggression had been maintained on the complete diet Panel B presents the mean acetylcholine levels (± standard error of the mean) for the total for the 41 total mice displaying aggression among all genotypes in the present study and the 148 mice not displaying aggression ("docile"); note the significant ($p<0.001$; Wilcoxon rank sum test) reduction in acetylcholine levels (approx. 30%) in mice displaying aggression. Panel C presents scatter plot analyses of acetylcholine levels among individual mice displaying aggressive behavior as described in Materials and Methods versus those that did not ("docile"); mice were maintained on the deficient diet or the deficient diet + SAM for one month as indicated. N=4 representative mice of each genotype for each diet (total of 8 mice) randomly selected from the total population on each diet. Note that no instances of aggressive behavior were observed following supplementation with SAM.

Table 1
Correlation of aggressive behavior with diet and acetylcholine levels

	μM Acetylcholine (± SEM)			% Aggressive	
	no SAM	SAM	P value	no SAM	SAM
Normal Adult	3.3 ± 1.2	5.9 ± 1.9	0.15	0	0
ApoE–/–	7.5 ± 0.7	9.9 ± 1.8	0.13	0	0
ApoE2	4.2 ± 1.5	3.8 ± 0.4	0.42	0	0
ApoE3	2.7 ± 1.4	6.7 ± 1.3	0.04	100	0
ApoE4	2.4 ± 0.9	6.0 ± 0.9	0.01	100	0
MTHFR+/+	1.8 ± 0.6	9.1 ± 0.5	0.01	75	0
MTHFR+/–	2.7 ± 0.7	10.1 ± 1.2	0.01	100	0
Aged Adult	4.2 ± 1.3	3.7 ± 0.9	0.39	0	0

Mean values of acetylcholine (± SEM) and the percentage of these mice displaying aggressive behavior are presented for each genotype and diet. These values are derived from the same mice presented individually in Fig. 3C. Note that, as shown in Fig. 1, acetylcholine levels declined in all mice when maintained on the deficient diet as compared to the deficient diet + SAM, but that significant ($P \leq 0.05$) reduction was observed only in ApoE3, ApoE4, MTHFR+/+ and +/- mice; note that aggression was confined to these mouse strains. Note further that no instances of aggressive behavior were observed following supplementation with SAM.

Discussion

Prior studies demonstrate that folate deprivation induces oxidative damage to brain tissue and cognitive impairment (20, 40, 41), and that supplementation with SAM prevented these deleterious effects (14). Herein, we demonstrate that dietary and genetic folate deprivation also reduces ACH levels, and that this effect is likely due to depletion of SAM, since dietary supplementation with SAM restored levels of ACH to those observed in mice receiving folate. Folate deprivation potentiates several risk factors for AD (see refs. in Introduction). A critical role for SAM in maintenance of ACH levels as demonstrated herein represents an additional mechanism by which dietary and genetic impairment in folate usage can contribute to the onset and progression of AD. The decline in ACH that accompanies folate deprivation is apparently due at least in part to depletion of N-methyl nicotinamide and resultant leakage of choline out of brain tissue, and that inhibition of choline transport out of the brain by increasing methylation of nicotinamide represents one method by which SAM maintains acetylcholine levels (44).

Since maintenance on the deficient diet induced both oxidative damage (40, 45) and a decline in ACH, it was initially unclear whether the accompanying decline in cognitive performance resulted from reduced ACH levels, increased oxidative damage, or both. Moreover, the observed reduction in ACH levels and decline in cognitive performance could have been derived from degeneration of cholinergic neurons due to oxidative damage. However, since mice that had been maintained on the deficient diet for 1 month exhibited recovery of cognitive performance when returned to the complete diet, it is unlikely that depletion of cholinergic neurons or other permanent neurodegenerative effects resulted from of this relatively short-term (1 month) induction of oxidative stress to brain tissue.

ACH levels in all cases declined following folate deprivation, and this decline was lessened or eliminated by supplementation with SAM, supporting the interpretation that SAM deprivation is a major factor responsible for the decline in ACH levels resulting from folate deprivation. Similarly, cognitive performance as ascertained by the Y maze declined significantly in all cases following folate deprivation except in normal adult mice (although they also exhibited a slight, but non-significant decline), and this decline was also lessened or eliminated in all cases by supplementation with SAM, supporting the interpretation that SAM regulates cognitive performance. Folate deprivation may induce nutritional compromises in addition to those resulting from SAM depletion, which may also compromise cognitive performance. The more severe impact of effect of folate deprivation and SAM supplementation on ApoE-/-, mice expressing ApoE3 and E4, MTHFR+/- and aged normal mice may relate to diminished endogenous oxidative buffering (e.g., 14, 38, 45-47). In addition, since SAM plays an indirect role in synthesis of the neurotransmitters dopamine, norepinephrine and serotonin (48, 49), levels of these neurotransmitters were likely reduced following folate deprivation. Differential depletion of these additional neurotransmitters, not assayed herein, may contribute to the differential impact of folate deprivation and SAM supplementation on cognitive performance observed among the various mouse genotypes utilized in this study.

Prior studies indicate that cholinergic depletion itself is sufficient to induce impairments in memory in rodents (50) while increased Abeta alone, which increases neuronal oxidative damage, is not by itself sufficient to induce cognitive impairment in experimental studies in rodents nor in AD patients (51, 52). In addition, cognitive deficiencies can be induced in mouse models of AD independently of oxidative stress and Abeta deposition (53). Taken together with our findings in the present study, we conclude that the decline in cognitive performance in maze performance is due to diminished production of ACH, as a consequence of choline efflux from brain, which in turn is regulated by availability of SAM. Notably, the impact of dietary supplementation with SAM on performance in maze trials (20) equals that obtained when mice receive donepezil to counteract a scopolamine-induced decline in cognitive performance (54). An effective therapeutic strategy to delay the progressive cognitive decline that accompanies AD may include dietary supplementation of SAM (well-tolerated in clinical studies; 22, 54-56) to boost acetylcholine production along with administration of cholinesterase inhibitor(s) to minimize ACH degradation.

Aggression was observed among some but not all genotypes when maintained on the deficient diet. Elimination of aggression in all cases following supplementation of the deficient diet with SAM indicates that SAM regulates aggression, which, as hypothesized above for cognitive performance, may be related to its regulation on acetylcholine and/or other neurotransmitters. This possibility is supported by

clinical studies indicating that aggressive behavior in AD may be related to serotonergic and cholinergic impairment (56, 57) and that donepezil lessened aggressive behavior in AD (58). A portion of this effect may derived from promotion of polyamine synthesis by SAM (44). Confinement of aggressive behavior to certain genotypes when maintained on the deficient diet, even when other strains displayed a statistically identical reduction in acetylcholine, indicates that aggression observed herein resulted from a combination of dietary deficiency and genetic background. This is supported by the observation of increased aggression among AD patients harboring the E4 allele of ApoE (59, 60).

Impaired cognitive performance in the Y maze also accompanied maintenance of mice on the deficient diet, but this did not correlate in all cases with aggressive behavior. For example, ApoE-/- and normal aged mice demonstrated impaired cognitive performance when maintained on the deficient diet, yet neither displayed aggressive behavior. Mice expressing ApoE3 did not exhibit impaired cognitive performance when maintained on the deficient diet, yet they exhibited aggressive behavior on this diet. Mice expressing ApoE4 displayed both impaired cognitive performance and aggressive behavior when maintained on the deficient diet, while mice expressing ApoE2 displayed neither. These results are consistent with E2 as being the most protective ApoE allele, since neither impaired cognition nor aggression was observed for ApoE2 mice, and with ApoE4 as the least protective, since both impaired cognitive performance and aggression was observed for ApoE4 mice. The observation of impaired cognitive performance ApoE-/- mice is consistent with a deleterious effect of diminished ApoE function (60, 61). What was perhaps unexpected was the observation of increased aggression in mice expressing ApoE3, since this allele is thought to be neuroprotective (63). It should be noted that, while the E4 allele is most strongly associated with AD, the presence of the E3 allele nevertheless confers significantly higher risk than does E2 (63-65). The generally-accepted notion that E3 is neuroprotective is largely derived from studies which compare only the E3 and E4 allele; while indeed E3 is significantly more neuroprotective than E4, it is far less neuroproctective than is E2 (63). Indeed, individuals bearing ApoE3/3 exhibited a higher incidence of AD than did individuals bearing ApoE 2/4 (65). Similarly, expression of human E2 was more effective at suppressing fibrillar Abeta deposition than was E3, which in turn was more protective than E4 (66, 67). These considerations are not presented herein to challenge the notion of ApoE4 as a risk factor for AD, but rather to provide insight into how expression of E3 in our transgenic mice can be associated with increased aggression as compared to mice expressing E2.

MTHFR+/+ exhibited aggressive behavior on the deficient diet, which was augmented by deletion of one MTHFR allele. These mice were included to investigate the consequences of direct genetic compromise in folate usage. Since they are on a different genetic background than the other mice in this study (all of which were C57B/6 derivatives), the degree of aggression exhibited by MTHFR+/+ mice should perhaps be considered as a different baseline, and be considered as controls for MTHFR+/- mice, rather than compared directly with the other mice of this study. Consistent with the other mouse strains utilized herein, cognitive performance and aggression of both MTHFR+/+ and +/- mice increased in response to dietary deficiency, and were alleviated by supplementation with SAM. The increased impact of MTHFR+/- versus MTHFR+/+ mice on dietary folate deprivation demonstrates the additive deleterious consequences of dietary and genetic compromise in availability of folate, and demonstrate that SAM supplementation is useful under such conditions. While our data demonstrate that folate supplementation is normally sufficient to prevent the onset of cognitive decline and aggression, our findings collectively suggest that SAM supplementation may be useful under conditions where folate is deficient, including potentially latent deficiencies in one or more enzymes of the methionine cycle. In this regard, individuals homozygous for the thermolabile C677T MTHFR polymorphism exhibit mild hyperhomocysteinemia, which is further augmented by diminished dietary folate (68-70). Homozygous thermolabile MTHFR has been described in 5% of normal Caucasians, in 19% of patients with arterial disease, and 11% with venous thrombosis (71). Notably, a 36% increase in C677CT and A1298C polymorphisms has recently been reported among young people; such polymorphisms were present in 4.63% of individuals >24yr of age, yet in 6.31% of those <24yr of age (72). The investigators considered that increased maternal dietary folate (confirmed in their samples) allowed an increase in fetal viability despite latent deficiencies in MTHFR; an increased latency within the population of critical genetic deficiencies in folate metabolism may therefore manifest only with age-related nutritional decline. Deficiencies in cysthathionine beta-synthase and methionine synthase each induce hyperhomocysteinemia (73, 74), and at least a portion of the impact of cystathionine beta-synthase deficiency is due to reduced levels of SAM (75).

Differential efficacy of SAM supplementation on cognitive impairment and aggression is consistent with prior studies in rodents demonstrating that cognitive performance can be improved independently of aggression (76, 77). Similarly, while both cognitive impairment and aggressive behavior accompany the progression of AD, yet are not necessarily temporally co-localized and may but do not necessarily respond to identical treatments (78-81). In all cases, however, as with elimination of aggressive behavior, dietary supplementation with SAM improved cognitive performance. This finding, along with the demonstration herein that SAM also indirectly contributes to ACH synthesis, and that prior demonstration that SAM facilitates glutathione usage in brain tissue (41) and regulates PS-1 expression and Abeta levels (11), collectively underscore the importance of this agent in maintaining overall brain health in aging and in AD.

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