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# Dietary Supplementation with S-Adenosyl Methionine was Associated with Protracted Reduction of Seizures in a Line of Transgenic Mice

**Sheryl Perry, James Levasseur, Amy Chan, and Thomas B Shea***

**Transgenic mice, although useful for analyses of gene function, can present unanticipated phenotypic manifestations, including behavioral problems, that may not be directly associated with the gene of interest but rather due to the complex interplay inherent in genomes. These unexpected events can present unique insight into gene function, leading to an advantage in some situations, yet in others can confound interpretation and compromise usefulness of the transgenic line. Here we document that short-term supplementation with S-adenosyl methionine (SAM)—a nutriceutical known to regulate neurotransmitter levels, improve working memory, and reduce aggression—reduced handling- and startling-induced seizures that otherwise precluded behavioral analyses in a transgenic line. This effect lasted for at least 1 mo after withdrawal of SAM and allowed mice to be used in standard maze analyses. These findings suggest that short-term administration of a neurotropic nutriceutical may provide a functional rescue for behavioral studies in an otherwise intractable transgenic mouse line as well as improve the welfare of similar lines.**

**Abbreviations:** SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine.

Site-directed mutagenesis, gene deletion, and the insertion of exogenous genes present powerful tools for genetic analyses. Incorporation of such genetic alterations into the murine germline, and the resultant generation of transgenic strains of mice, has provided novel insight into the roles of genes, and their interaction with other genes, at all stages of life. However, transgenic mice can present unanticipated behavioral problems that may not be associated directly with the gene of interest but rather are due to the complex interplay inherent in genomes.[20] This unexpected outcome can present unique insight into gene function, leading to an advantage in some situations, yet in others can confound interpretation and compromise usefulness of the transgenic line. Genetic variability of inbred strains can confound interpretation of behavior,[18] especially if behavioral analyses are part of the regimen to be studied.[26]

The presence of 1 or more ApoE4 alleles is associated with an increased risk of Alzheimer disease.[13] Transgenic mice in which the single murine ApoE allele has been ablated and replaced with human apolipoprotein E isoforms have been useful models for studying the impact of ApoE on age-related cognitive decline and Alzheimer disease. In addition to impaired cognition, mice lacking murine ApoE and expressing human ApoE4 (ApoE4 mice) display increased aggression as compared with normal mice, mice lacking murine ApoE, or mice lacking murine ApoE and expressing other human ApoE alleles.[5,6] These behavioral manifestations of ApoE4 mice are useful in that Alzheimer disease is often accompanied by behavioral trauma, including pyschosis and agitation,[7] and ApoE4 has been associated with an increase psychotic symptoms in humans.[27]

Recent shipments of ApoE4 mice displayed violent spontaneous and handling-induced convulsions (hereafter referred to as seizures for the sake of simplicity), which included jumping and eventual prostration due to overt exhaustion. These seizures occurred regardless of how quietly or slowly the handler or caregiver moved. These seizures, which persisted for more than 1 mo, had not previously been observed in ApoE4 mice or other mice in our facility and precluded the intended use of the ApoE4 mice in standard maze trials.[5]

In our ongoing studies, we had observed that dietary supplementation with the nutriceutical S-adenosyl methionine (SAM) reduced aggressive behavior in ApoE4 mice.[5] SAM also restores neurotransmitter balance, increases working memory, and modulates neuronal activity.[6,8,17,19] We therefore hypothesized that SAM supplementation would reduce or alleviate handling-induced seizures. Here we document that short-term administration of SAM reduced of seizures for extended periods, to the extent that these mice could be used in behavioral studies. We discuss the possibility that such an approach may be useful for habituation of other mouse lines displaying similar behavior difficulties.

## Materials and Methods

Transgenic mice lacking murine ApoE and expressing human ApoE4 on a C57Bl6 background (ApoE4 mice; B6.129P2-*Apoe*$^{tm3(APOE*4)Mae}$ N8) were obtained from Taconic Farms (Germantown, NY). Mice received a diet (AIN-76; Purina/Mother Hubbard), which was supplemented with SAM (100 mg/kg diet, Sigma-Aldrich, St. Lous, MO) under some conditions.[25] All treatments and procedures were approved by our Institutional Animal Care and Use Committee. Mice were housed under a

*Received: 27 Mar 2008. Revision requested: 30 Apr 2008. Accepted: 01 Aug 2008.*
*Center for Cellular Neurobiology and Neurodegeneration Research, University of Massachusetts Lowell, Lowell, Massachusetts.*
**Corresponding author. Email: Thomas_shea@uml.edu*

12:12-h light:dark cycle and were deemed healthy by our institution's veterinarian.

We monitored each mouse visually daily for 1 wk and recorded whether seizures occurred. Mice then received a diet supplemented with SAM for 2 wk, after which they received a diet without SAM for the next 2 wk. The presence or absence of 1 or more seizures daily for each mouse was monitored during this feeding regimen. Two independent trials of dietary supplementation with SAM were conducted with a total of 10 mice. Occurrence of seizures per week before, during, and after SAM treatment was compared by using paired *t* tests.

After completion of the described analyses, mice received SAM for 1 mo, and cognitive impairment was monitored after maintenance on the described diets for 1 mo by using a standard Y maze test as described.[16] The pattern of exploration of the Y maze was recorded over 5-min intervals for each mouse. In a 3-arm maze, mice normally will alternate the same sequence of exploration; for example, if we define the first arm explored as arm 1 and the second as arm 2, the next arm explored should be arm 3 rather than repeating arm 1, and mice tend to continue the sequence of 1, 2, 3. The frequency in which mice visited each of the 3 arms in succession during any 3-arm visitation sequence versus the total number of visitations defines the '% alternation.' Failure to continue the sequence is indicative of impairment in working memory.[11]

## Results

Over the course of 1 mo prior to SAM treatment, 100% of 2 groups of ApoE4 mice (n = 6 and n = 5, respectively) displayed violent seizures during every cage changing and handling procedure. The occurrence of handling-induced and spontaneous seizures was not quantified directly, but because cages were cleaned every other day, mice exhibited a minimum of 50% seizures per day for 4 wk. These observations were substantiated by quantifying seizures every day for 1 wk. During the first week of SAM supplementation, the occurrence of seizures was reduced by approximately 50% ($P < 0.01$ versus the week prior to SAM supplementation; paired *t* test, df = 9). Seizures continued to decline during SAM supplementation and did not recur over 6 wk after withdrawal of SAM (Figure 1).

A single mouse was withheld from SAM supplementation; this mouse underwent at least 1 seizure on 6 of the days during the first week of SAM supplementation for the test mice (that is, seizures on 86% of the days evaluated), on 2 of the 7 days (29%) during the second week of SAM supplementation for the test mice, and on 4 of the 7 days (57%) during the first week of SAM withdrawal for the test mice. Therefore, the values for the single untreated mouse do not show a progressive decline and differ substantially from those obtained from mice receiving SAM supplementation (Figure 1).

Prior to SAM supplementation, these mice could not perform in a standard Y maze analysis because simply by removing the lid from their cage provoked violent, protracted seizures leading to exhaustion. However, during 4 to 6 wk after withdrawal of SAM, seizures had not reappeared, and these mice performed as well as did mice of the same genetic background not expressing the transgene and ApoE4 mice from prior studies[6] (42.8% ± 5.9%, 48.9% ± 15.8%, and 39.0% ± 6.0% alternations, respectively) that had never exhibited seizures; why prior groups did not display seizures remains unclear

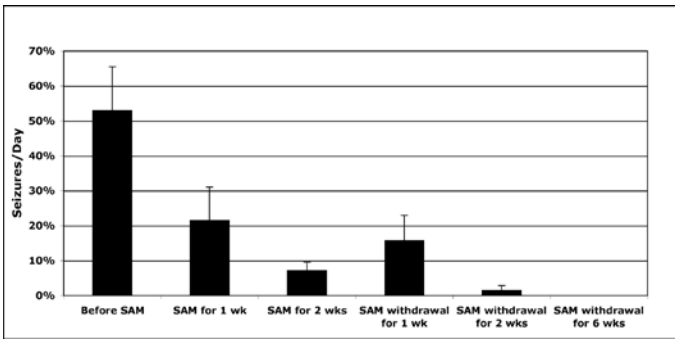


**Figure 1.** Quantification of the percentage (mean ± SEM) of seizures observed daily while mice were maintained in the presence or absence of SAM. Note the decline in seizures within 1 wk of SAM supplementation and the persistence of this decline for 6 wk after withdrawal of SAM.

## Discussion

Routine maintenance procedures, including cage changing and handling, can induce fear and distress, and animals do not readily habituate to these procedures.[12] Our findings demonstrated that dietary supplementation with SAM was associated with a reduction of handling-induced seizure. SAM and its downstream metabolites are metabolized rapidly and return to baseline within 24 h after oral administration of SAM.[18,24] SAM-dependent biochemical modifications within central nervous tissue, improved memory, and decline of aggression are transient and require continued consumption.[5,23,25] Accordingly, long-term reduction of seizures after SAM withdrawal are unlikely to be derived from any permanent metabolic alteration. Rather, we hypothesize that SAM-induced neurotransmitter balance, increase in memory, and restoration of neuronal activity[6,8,17,19] ameliorated the effect of handling on induction of seizures. This apparent accommodation is underscored by ability of SAM-treated mice to perform in maze trials in a manner equivalent to that of nontransgenic control mice.[16]

Because we did not maintain as controls a cohort of mice that did not receive SAM, our findings must be interpreted with caution. However, our intent was to alleviate seizures in our mouse colony rather than to conduct a study of the efficacy of SAM on seizures. Such a study could be conducted in other mouse strains. Reduced levels of SAM, and increased levels of its immediate metabolite, S-adenosyl homocysteine (SAH), are thought to contribute to the progressive development of seizures in another transgenic mouse line.[9,11,12] In this regard, increased levels of SAH inhibit SAM-dependent reactions, leading to a further functional SAM deficiency.[11,21,15] Correction of this ratio reduced seizures.[10] Mice deficient in ApoE display reduced SAM levels, and readily undergo an increase in SAH;[25] dietary supplementation with SAM was capable of correcting this ratio. The display of seizures by ApoE4 mice may have clinical relevance, as conflicting reports have appeared regarding the association of ApoE4 with seizures/epilepsy.[2-4,22] Notably, SAM improves mood in humans and has been useful for depression.[23]

Despite our positive results, the role of methylation in seizures is not straightforward. Administration of SAM in some cases promotes seizure activity.[14] We propose that in certain cases, inappropriate methylation, including that of bioamines, occurs after SAH accumulation,[2,3] perhaps due to SAH-induced inhibition of normal methylation pathways. In some but not all situations,

this inhibition may be alleviated by supplementation with SAM, which, by correcting the SAM:SAH ratio, may restore critical methylation reactions.[17] Mice lacking murine ApoE accumulate SAH more readily than do normal mice, possibly due to increased homocysteine levels,[23] which can prevent SAH metabolism.[11, 21] ApoE deficiency therefore is associated with an increased potential for SAH-induced inhibition of methylation.[25] Accordingly, although SAM administration was helpful in the case presented here, SAM may be ineffective or possibly exacerbate seizures in other transgenic lines.[5] The severe seizures we observed in our study have not been a universal finding for this transgenic line in our hands[5,6] Therefore, our findings may not be relevant for future generations of ApoE4 mice. Nevertheless, the findings presented herein indicate that deletion of 1 gene can induce unanticipated ancillary effects that include behavioral difficulties.[20] Routine maintenance procedures, including cage changing and handling, can precipitate seizures, and mice may not habituate to this effect.[1] Our findings indicate that short-term administration of SAM may provide a functional rescue for behavioral studies in an otherwise intractable transgenic mouse line and reduce adverse clinical events in such a line.[12]

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