

NOVIFIT® (NoviSAmE®) Tablets Improve Executive Function in Aged Dogs and Cats: Implications for Treatment of Cognitive Dysfunction Syndrome.

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ABSTRACT

A large number of aged dogs and cats demonstrate behavioral signs consistent with a clinical diagnosis of cognitive dysfunction syndrome (CDS), which likely is a consequence of pathological brain aging. Identification of treatments that prevent, halt, or reverse CDS, therefore, represents an unmet need in senior animal veterinary care. NOVIFIT Tablets are an S-adenosylmethionine (SAME) tosylate supplement currently marketed for improving cognitive health of aged dogs and cats. SAME is an endogenous metabolite involved in several biochemi-

cal pathways, and is deficient in humans with Alzheimer's disease. The current study examined the efficacy of NOVIFIT tablets on cognitive performance enhancement in cognitive domains impaired in canine and feline aging.

In the first study, aged dogs initially balanced for memory performance were divided into NOVIFIT tablet and placebo groups and tested on both the same memory task and an object discrimination learning and reversal learning task. No treatment effects on memory performance were found, but the NOVIFIT tablets-treated dogs did not show a significant increase in reversal learning errors compared to learning errors seen both under placebo and in previous aging studies, suggesting NOVIFIT tablets

selectively enhanced executive function. To confirm this interpretation, a study was then conducted in aged cats initially balanced for learning ability to more accurately assess the effects of NOVIFIT tablets on reversal learning. Compared to placebo, the least impaired cats at baseline showed significantly reduced reversal learning errors under NOVIFIT tablets administration compared to placebo, consistent with improved executive function. By contrast, the most impaired cats did not show a treatment effect. The current studies suggest that NOVIFIT tablets improve age-related deficits in executive function, but not necessarily in the most impaired subjects. Collectively, the results support the use of NOVIFIT tablets supplementation for cognitive health indications in aged dogs and cats especially in early stages of CDS.

INTRODUCTION

Like humans, pet dogs and cats are living longer, healthier, and more enriched lives.¹ In humans, the increase in lifespan has unfortunately resulted in what may be described as an epidemic increase in the frequency of dementias and particularly of Alzheimer's disease (AD).² In aged companion animals, cognitive dysfunction syndrome (CDS) is a neurodegenerative disease attributable to pathological brain aging that shares similar features with early AD, and presents clinically with age-related development of behavioral signs independent of medical or behavioral diagnoses.²⁻⁵ In dogs, the behavioral signs of CDS are often described using the acronym DISHA which includes: Disorientation; alterations in Interactions with owners, other pets and the environment; disturbances in Sleep-wake cycles; Housesoiling; and alterations in Activity. Additionally, the expression of novel behavioral signs in aged dogs consistent with diagnoses of anxiety, fear or phobias may also be attributable to CDS⁵. In cats, the existence of CDS was debatable, but recent evidence indicates that aged cats also show pathological brain aging, impaired cognitive function, and emergence of behavioral signs

consistent with CDS.^{4,5} Similar to dogs, common behavior signs in aged cats include housesoiling, altered sleep-wake cycles, disorientation, altered social interactions, alterations in activity and excessive vocalization.

In humans, deficits in specific cognitive domains, such as episodic memory, executive function and attention, are a consequence of aging and are significantly impaired in AD.⁶ For studying effects of (brain) aging and for evaluating potential cognitive enhancing therapeutics, we have developed several laboratory protocols for assessing domain specific cognitive function in dogs and cats.⁷⁻⁹ Compared to adult dogs, aged dogs are impaired on tests of working memory, complex learning, executive function (mainly assessed by reversal learning) and selective attention.¹⁰⁻¹² By contrast, age-related deficits on procedural learning and memory are not found. Moreover, some of these cognitive deficits are linked to neuropathology, including both amyloid-beta deposition and brain atrophy, similar in many respects to that seen in AD.^{14,15} More recently, we have adopted these protocols for cats and have described impairments in various cognitive domains including both executive function and working memory.⁹

The current study employed laboratory-based cognitive tests in aged dogs and cats to investigate the potential cognitive benefits of NOVIFIT® (NoviSAME®) Tablets, which is an S-adenosylmethionine (SAME) tosylate supplement currently marketed for support of cognitive function in aged companion animals. SAME is an endogenous methyl donor involved in numerous transmethylation reactions including nucleic acids, proteins, phospholipids and neurotransmitters.¹⁵ Treatment with SAME reportedly stimulates brain glutathione thereby decreasing oxidative stress, which is increased and strongly implicated in age-related cognitive dysfunction.¹⁶⁻¹⁹ Moreover, AD patients show SAME deficiency in both CSF and brain, and SAME supplementation is suggested as a potential therapeutic for AD.^{16,20,21} Our hypothesis

was that NOVIFIT tablets would improve cognitive function compared to placebo. In one previous canine clinical study, dogs diagnosed with cognitive impairment and treated with NOVIFIT tablets had significant improvements in activity and awareness over 8 weeks compared to placebo.²² Initially, we examined the effects of the NOVIFIT tablet supplementation compared to placebo control on tests of working memory, as well as on object discrimination and reversal learning, in aged dogs balanced for baseline working memory performance. Based on the results, we then examined the effects of NOVIFIT tablets on reversal learning performance of aged cats balanced for baseline learning ability.

MATERIALS AND METHODS

Two experiments were conducted; the first in aged dogs and the second in aged cats. In both, the experimental protocol was approved without reservation by the Local Institutional Animal Care and Use Committee, which operated under the guidelines of both the Ontario Ministry of Agriculture, Food and Rural Affairs and the Canadian Council on Animal Care. All procedures were conducted in accordance with the Animal for Research Act, the Animal Welfare Act, and the NRC Guide for Care and Use of Laboratory Animals. For both studies, technicians performing cognitive tasks were blinded to treatment conditions.

Experiment 1: Effects of NOVIFIT Tablets in aged dogs

Subjects: Fourteen aged Beagle dogs (9.2 – 12.8 years old) of adequate health and body condition were selected for inclusion in the study. Dogs were housed from 2-4 per pen based on compatibility with individual space greater than 1.9 m² per dog and environmental conditions controlled by an automated ventilation system. A combination of fluorescent and natural lighting was provided such that fluorescent lighting was available 12 hours daily. Water was provided ad libitum and standard commercial dog food was provided once daily in quantities sufficient to maintain body weight. Dogs were

observed twice daily for abnormal signs and no abnormal signs related to treatment or that compromised the study were found.

General Design: A blinded, parallel group, design was used with 7 dogs in each treatment group. All dogs initially were tested on 5 baseline variable-delayed non-matching to position task (DNMP) sessions, and dogs were allocated to two treatment groups (NOVIFIT tablets or placebo) balanced for DNMP performance. After a 15-day treatment wash-in, both groups were tested on an object discrimination and reversal learning task (maximum 30 days), and re-tested on the DNMP (10 days). Technicians administering the cognitive tests and conducting twice daily observations were blinded to treatment condition. NOVIFIT tablets were administered according to label such that dogs weighing less than 10 kg were administered 100 mg of SAME once daily and the remainder of dogs weighing between 10 and 20 kg were administered 200 mg of SAME once daily. Placebo animals received an empty capsule.

Experiment 2: Effects of NOVIFIT tablets in aged cats

Subjects: Sixteen aged cats (between 8.4 and 13.9 years old) of adequate health and body condition were selected for inclusion in the study. The cats were housed in rooms with multi-level resting areas and enrichment toys. A combination of fluorescent and natural lighting was provided such that the fluorescent lighting was available 12 hours daily. Water was provided ad libitum and standard commercial food was provided once daily in quantities sufficient to maintain body weight. Cats were observed twice daily for abnormal signs and no abnormal signs related to treatment or that compromised the study were evident over the course of the study.

General Design: A blinded, parallel group, design was used in the current study. All cats were tested at baseline on a discrimination learning task until the learning criterion was reached. Errors to learn the task was then used to allocate cats to two groups

(NOVIFIT tablets and placebo) balanced for learning performance. The NOVIFIT tablet group received 100 mg/day of SAME, and the placebo group received methylcellulose (5 mg/day) in an identical fashion. Cats then received a 15-day treatment wash-in period on their respective treatments. Following wash-in, subjects were re-tested on the same discrimination learning task used at baseline for 3 days and were then tested on a discrimination reversal task for a maximum 36 days or until the learning criterion was reached. Technicians administering the cognitive tests and conducting twice daily observations were blinded to treatment condition. The procedures for the learning and discrimination tasks were identical to those conducted in dogs and are described in more detail below.

Cognitive Test Methods

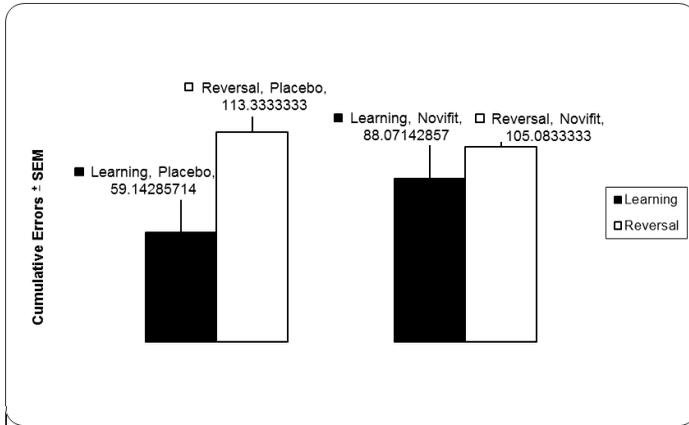
Cognitive Test Apparatus and General Test Procedures: All cognitive testing was conducted in a species-specific adaptation of the Wisconsin General Test Apparatus, which has been described previously for dogs.²³ The apparatus for cats was generally identical to that used for dogs, except it was smaller in size. Generally, the test apparatus was constructed from high density plastic and consisted of a test subject holding area and a presentation area, which were separated by stainless steel bars that could be adjusted to limit only the head of the test subject to enter the presentation area from the holding area. The tester could introduce the presentation tray to the test subject in the presentation area by way of a door controlled by the tester. Before each test trial presentation, the tester prepared the presentation tray with task appropriate objects over specified food wells and placed a food reward in the well covered by the correct object. Therefore the test subject was not able to observe preparation of the tray and had to displace the correct object to access the food reward beneath during the presentation. All objects were presented on coasters baited with unobtainable food, which prevented subjects from solving any task

using olfactory cues. Control of test specific parameters, such as randomization of correct food well locations, object placement, inter-trial intervals and delays were controlled using a dedicated computer program (DogCog and CatCog, CanCog Technologies, Inc, Toronto, ON); the responses of the test subject were recorded by the tester using a key press. Subjects were permitted to correct after their first error in a test session, but the presentation tray was immediately removed by the tester on subsequent incorrect responses.

Variable-Delayed Non-Matching to Position (DNMP): The variable-delayed DNMP has been described previously.¹¹ The DNMP consisted of two phases. During the first phase, a block was presented to the dog over one of three possible locations. After the dog displaced the block and retrieved the food reward in the food well beneath the block, the test tray was withdrawn and the delay was initiated. After the delay, the dog was then presented with two blocks identical to the first block; one over the food well used in the initial presentation and one over one of the remaining two wells. The correct response was to displace the block not covering the food well used in the initial trial. Each test session utilized delays of 5, 55, and 105 s, which occurred equally over the 18 test trials per daily test session, resulting in 6 trials at each delay. The delays occurred randomly within the test session.

Object Discrimination and Reversal Learning Task: This task consisted of a preference test, a learning phase and a reversal phase.¹⁰ In the preference test, two different objects were rewarded and the object chosen most frequently over 10 trials was considered the preferred object. If both objects were chosen equally, a coin toss was used to determine preferred object. The preferred object was always rewarded during the discrimination learning phase. For the reversal learning phase, the non-preferred object was rewarded. For the latter two phases, the correct object was presented both equally and randomly in the left and right positions. One

Figure 1: Mean errors on an object discrimination learning and reversal task in aged dogs. Dogs committed significantly more errors to learn the reversal task compared to the discrimination learning task. A trend for an interaction was also found which reflected the larger increase in errors from the learning to reversal tasks in the placebo group compared to the Novifit group. Error bars represent SEM.



test session was administered daily which consisted of 20 trials.

The learning criterion for both phases was identical and consisted of 2 stages. The first stage required subjects to perform with an accuracy of 18/20 or above on one day, or 16/20 or above over two consecutive test days. The second stage required subjects to obtain a combined score of 28/40 or above over the next two consecutive test days. Dogs were tested on both the learning and reversal phases until reaching the learning criterion on both phases, or for a maximum of 30 sessions, whichever occurred earlier. By study design, all cats reached the learning criterion on the learning phase, and were tested on the reversal learning phase until reaching the learning criterion, or for a maximum of 36 sessions, whichever occurred earlier.

Statistical Analyses

All statistical analyses were conducted using Statistica 6.0 (StatSoft, Tulsa, OK) and significance was set to $p < 0.05$. The data initially were analyzed with a repeated-measures analysis of variance (ANOVA) or

t-tests, as appropriate. For the DNMP, performance accuracy at each delay served as the independent variable. For discrimination and reversal learning, errors to reach the learning criterion, or total errors in cases where the learning criterion was not reached, served as independent variables. Post-hoc Fisher's test was used as appropriate to interpret the results of the ANOVAs.

RESULTS

Experiment 1: Effects of NOVIFIT

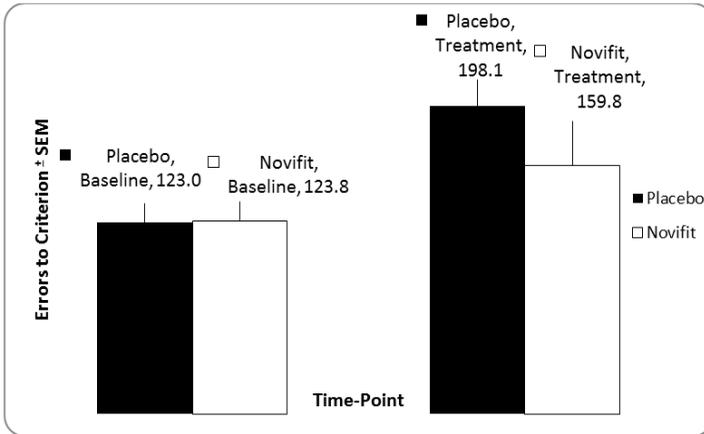
Tablets in Aged Dogs

DNMP: Groups were initially balanced for

baseline DNMP accuracy and a repeated-measures ANOVA was conducted with treatment group (NOVIFIT tablets and placebo) serving as a between-subject variable and with time-point (baseline and treatment), and delay (5, 55, and 105 s) serving as within-subject variables. A main effect of delay [$F(2,24)=354.9$; $p=0.002486$] was found, which was due to significantly lower performance at the 55 and 105 s delays compared to the 5 s delay [$p < 0.05$ in both cases]. However, no effects of treatment or time-point were found confirming groups did not differ for memory performance at baseline, or under treatment.

Object Discrimination Learning and Reversal: Cumulative errors to achieve the learning criterion on the learning discrimination phase and cumulative errors to achieve the learning criterion or over a maximum of 10 test sessions on the reversal discrimination task was analyzed using a repeated-measures ANOVA with treatment group as a between-subject measure and with task (learning and reversal) as a within-subject variable. Significant task effects [$F(1,$

Figure 2: Mean errors on an object discrimination learning and reversal task in aged cats. Cats committed significantly more errors to learn the reversal task compared to the discrimination learning task. A trend for an interaction was also found which reflected the fewer reversal errors under Novifit compared to placebo. No group differences were evident at baseline. Error bars represent SEM.



10)=36.8417; $p=0.000120$] and an interaction between task and treatment [$F(1, 10)=5.3846$; $p=0.042738$] were found. The task effect was due to increased errors on the reversal task compared to the initial learning phase (Figure 1). The task by treatment interaction was due to fewer errors in the placebo group compared to the treatment group on the learning phase [$p=0.091100$], but no difference in errors between the placebo and treatment groups on the reversal (Figure 1).

Experiment 2: Effects of NOVIFIT Tablets in Aged Cats

No treatment group differences at baseline were detected on discrimination learning errors, which confirmed the treatment groups were adequately matched for learning performance. Similarly, no group differences were found on the practice sessions, indicating no effect of treatment on long-term memory. An ANOVA was conducted examining errors with treatment group (treatment and control) serving as a between-subject variable and with time-point (baseline and treatment) serving as a within-subject variable. The analysis revealed only

a significant effect of time-point, which was due to an increased number of errors on the reversal learning phase compared to the baseline discrimination phase ($F(1, 14)=16.93$; $p=0.0011$; Figure 2). No treatment group differences were found, although there was a non-significant trend for a treatment by time-point interaction, which likely reflected the slight reduction in mean errors in the treatment group compared to the control group on the reversal phase (Figure 2).

A repeated-measures ANOVA was then conducted with the top half performers at baseline in each treatment group. The analysis revealed a significant effect of treatment group [$F(1, 6)=11.46$; $p=0.015$], which reflected reduced errors under the treatment condition compared to control (Figure 3). Post-hoc Fisher's revealed a significant increase in errors on the reversal phase in the control subjects compared to the treatment subjects [$p=0.003$]. By contrast, the groups did not differ at baseline nor did the treatment group show any differences in errors between the two time-points (Figure 3).

A repeated-measures ANOVA was also conducted with the bottom half performers at baseline in each group, but no treatment related effects were found (Fig 4).

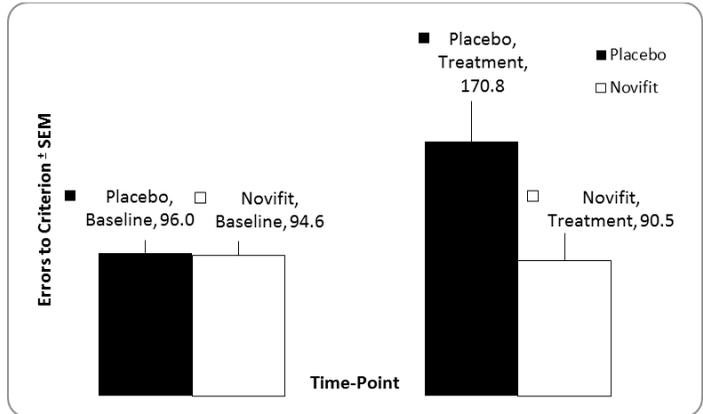
DISCUSSION

The current study sought to investigate if treatment with NOVIFIT tablets could improve laboratory-based measures of cognitive function in aged dogs and cats. The results of these studies suggest that NOVIFIT tablets improve executive function in both species, but has no effect on either short- or

long-term memory. Moreover, no abnormal observations attributable to NOVIFIT tablets were evident in either species. These results support the use of NOVIFIT tablets for improving cognitive health in aged dogs and cats. Moreover, this is the first published report we are aware of demonstrating cognitive benefits of a therapeutic in aged cats.

In the initial canine study, we examined the effects of NOVIFIT tablets in aged dogs on laboratory protocols assessing short-term working memory, discrimination learning and discrimination reversal learning ability. We elected to balance the NOVIFIT tablets and placebo groups according to baseline memory performance on the DNMP based on the rationale that DNMP learning and memory performance is impaired early in canine aging. The absence of treatment effects on DNMP performance indicates that the dose and treatment duration of NOVIFIT tablets used in the current study is ineffective on short-term working memory performance. By contrast, the significant task by treatment interaction on the object discrimination and reversal learning task suggests a beneficial effect of NOVIFIT tablets on executive function. Specifically, NOVIFIT tablets were associated with increased errors on the discrimination learning phase compared to placebo, but the increase in reversal learning errors over discrimination learning errors was reduced under NOVIFIT tablet treatment compared to the placebo control. Previous canine studies reveal an age-related increase in reversal learning errors compared to discrimination errors consistent with executive dysfunction and which are correlated with both frontal lobe atrophy and amyloid-

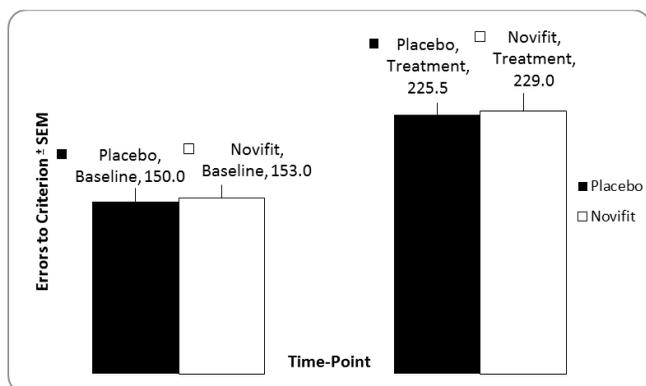
Figure 3: Mean errors on an object discrimination learning and reversal task in top performing aged cats. The aged cats performing in the top half of each group on the baseline learning task were compared across treatment. The cats receiving Novifit committed significantly fewer errors compared to those receiving placebo. No group differences were evident at baseline. Error bars represent SEM.



beta deposition.^{10,13} This interaction can, therefore, be explained by either a NOVIFIT tablet treatment-induced improvement in executive function (i.e. reduced errors on reversal learning compared to discrimination learning) or by a NOVIFIT tablet treatment-induced learning impairment (increased errors on discrimination learning under NOVIFIT tablet treatment compared to placebo). Because cumulative errors over a limited number of reversal learning sessions was used in the analysis, the former interpretation is more likely. However, the latter interpretation cannot be completely ruled out because treatment groups were not balanced initially for learning ability.

The rationale for the design of the feline study was to determine if NOVIFIT tablets improved age-related executive dysfunction.⁹ We therefore balanced the treatment groups according to errors on a baseline discrimination learning task to control for group differences in learning ability and no differences on baseline learning errors were found between groups. Prior to reversal learning, we also examined if treatment affected performance on the previously

Figure 4: Mean errors on an object discrimination learning and reversal task in low performing aged cats. The aged cats performing in the bottom half of each group on the baseline learning task were compared across treatment. No group differences were evident at baseline or on the reversal task. Error bars represent SEM.



learned discrimination task to assess treatment effects on long-term memory. Once again, no treatment differences were found. There was a trend for fewer errors on reversal learning in cats treated with NOVIFIT tablets compared to placebo. When cats were divided into top or bottom half performers according to baseline learning performance, NOVIFIT tablets significantly reduced reversal learning errors, consistent with improved executive function, in the subpopulation of cats with better learning ability, but no effect was evident in the poorer performers. This suggests that NOVIFIT tablets improve aged-related deficits in executive function, but that beneficial effects are least evident or absent in cats most affected.

The current studies were subject to individual limitations, as suggested above. Future studies could employ a young subject control group to better establish what subpopulation(s) of aged subjects would best respond to NOVIFIT tablet supplementation. It may also be prudent to investigate the mechanism of action of NOVIFIT tablets or SAME in future studies. We have previously reported that antioxidant therapies in dogs reduce oxidative stress and AD-like pathology in addition to improving cogni-

tive function.²⁴⁻²⁶ Although not investigated in the current study, the putative antioxidant effects of SAME, therefore, may mediate the beneficial functional effects reported here.¹⁶⁻¹⁹ Regardless, the consistency of results in both the aged dog and cat studies suggest that NOVIFIT tablets enhance age-related executive dysfunction.

CONCLUSION

The current study investigated the effects of NOVIFIT tablets, a supplement containing NoviSAME[®], on laboratory measures of cognitive function in both aged dogs and cats. Overall, NOVI-

FIT tablets were ineffective at improving measures of short- or long-term memory. However, studies in both aged dogs and cats consistently revealed potential benefits of NOVIFIT tablets on executive function. Collectively, this supports the use of NOVIFIT tablets for cognitive health indications in aged companion animals. Future studies could focus on elucidation of the mechanism of action of NOVIFIT tablets or SAME responsible for both cognitive and behavioral improvements previously reported in companion animal aging, as well as identifying subpopulations of cognitively impaired pets most likely to benefit from NOVIFIT tablet treatment.

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