Nutritional Modulation of Cognitive Structure in the Dog

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Abstract

Cognition in the dog is a loosely used term that refers to mental capabilities, which include several diverse processes that affect a dog's ability to adapt to its environment, to respond to training and to interact with humans. Cognitive abilities can change with age and develop into a form of dementia. This presentation examines the assessment and structure of cognition in the dog and evidence indicating that cognitive processes are sensitive to and can be modified by nutritional interventions. A specific focus will be on studies that attempt to manipulate levels of normal nutrients, with the goal of developing nutritional strategies that counteract the effect of age on cognition.

Glossary of Abbreviations
ALCAR: Acetyl-L-Carnitine
AOX: Antioxidants
BPB: Brain Protection Blend
DHA: Docosahexanoic Acid
DNMP: Delayed Non-Matching-To-Position-Task
LA: Lipoic Acid
MCT: Medium-Chain Triglycerides
OA: Osteoarthritis
TGTA: Toronto General Test Apparatus

Cognitive Structure in the Dog

Cognition in dogs, as in humans, refers to inferred processes that determine how the dog views the world and how it adapts to its environment and changes in its environment. Cognition is a dynamic rather than a static process, with the level of cognitive ability varying markedly among individuals. Cognitive level also varies with age.

Our approach toward understanding cognition in the dog borrows significantly from the approach taken by neuropsychologists to the study of cognition in the human. Neuropsychologists largely agree that cognition is not a unitary function but encompasses a set of components, referred to as domains, which are at least partially independent and can be linked to identifiable brain circuitry. For humans, the National Institute of Health has identified six distinct components: "executive function," "attention," "working memory," "episodic memory," "processing speed," and "language" (see *www.nihtoolbox.org*). To the extent possible, we have tried to develop a standardized collection of tests that can be used to assess each of these in the dog.

As a starting point, we first developed a standardized test apparatus, referred to as the TGTA (Toronto General Test Apparatus), based on an apparatus that had previously been used in assessing cognition in nonhuman primates.[1] The apparatus (See Figure 1) contains a holding area for the dog and a movable tray, which an experimenter can use to present various test problems to the dog.

Figure 2 illustrates how we use this test system to set up two related tasks and how we can use performance on the tasks to help understand cognitive structure. In both examples (concurrent spatial discrimination tasks), the subjects are

Figure 1. Current Version of Toronto General Test Apparatus

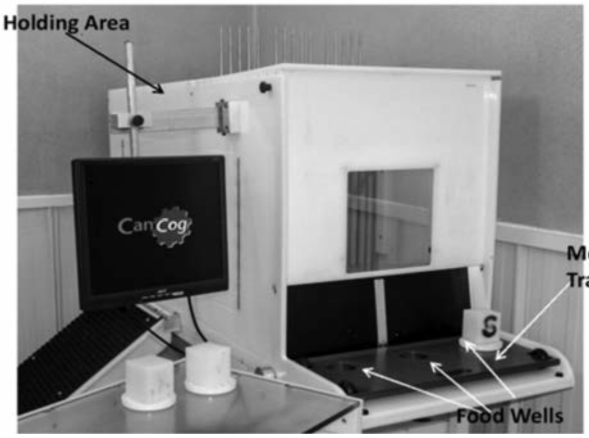
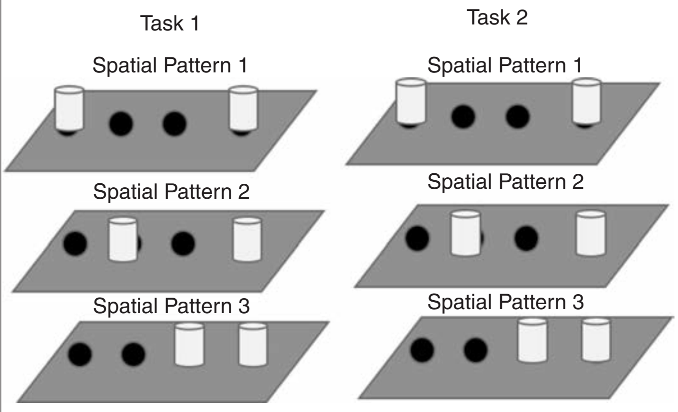


Figure 2. Two Different Versions of a Concurrent Discrimination Learning Task



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provided with three different problems. For each, the dog is presented with two identical object pairs at different locations, and for each pair, one position is designated to be correct. For the first variant, the correct location is the one closest to the dog's right. For the second variant, the correct location is to the dog's right on the first and third pattern but to the left for the second pattern. The subjects are given up to 15 training trials on each of the problems daily until they learn to correctly respond to all three problems.

To develop this task, we first tested 16 dogs on the first set of problems (Task 1). The dogs were subdivided into old (>9 years of age) and adult (<less than 6 years of age) groups. After completing the first part of Task 1, the subjects were tested on a reversal learning task, in which the correct position for each pattern was switched. In Task 2, we tested 7 old and 7 adult dogs on the initial task and on a reversal learning task.

All the dogs learned Task 1 within three test days (between 30 and 90 training trials), and there were no significant differences between the old and young dogs in the initial learning or reversal learning (Figure 3). The second task was much more difficult, and it also revealed large age effects, with the old dogs learning more slowly and making more errors than the young dogs.

Evidence for the Existence of Separate Cognitive Domains in the Dog

Thus far, we have shown we can set up different types of tasks that can differ markedly in difficulty as well as age sensitivity. In the above example, two tasks that appear to be virtually identical were strikingly different in difficulty. We believe that this is because the problems engage different cognitive processes. The first task is easy because the subjects have to learn to respond to the object closest to their right in order to perform accurately. This strategy won't work in the second task. If we look at each of the three problems separately, in the first task, the three problems were about equally difficult. In the second task, however, both groups of dogs made significantly more errors on the second problem than they did on either the first or third pattern (see Figure 4).

Figure 3. Performance of old and young dogs on two different concurrent spatial discrimination learning tasks. Learning is rated based on the number of errors made before reaching a learning criterion. Note differences in Y-axis scales.

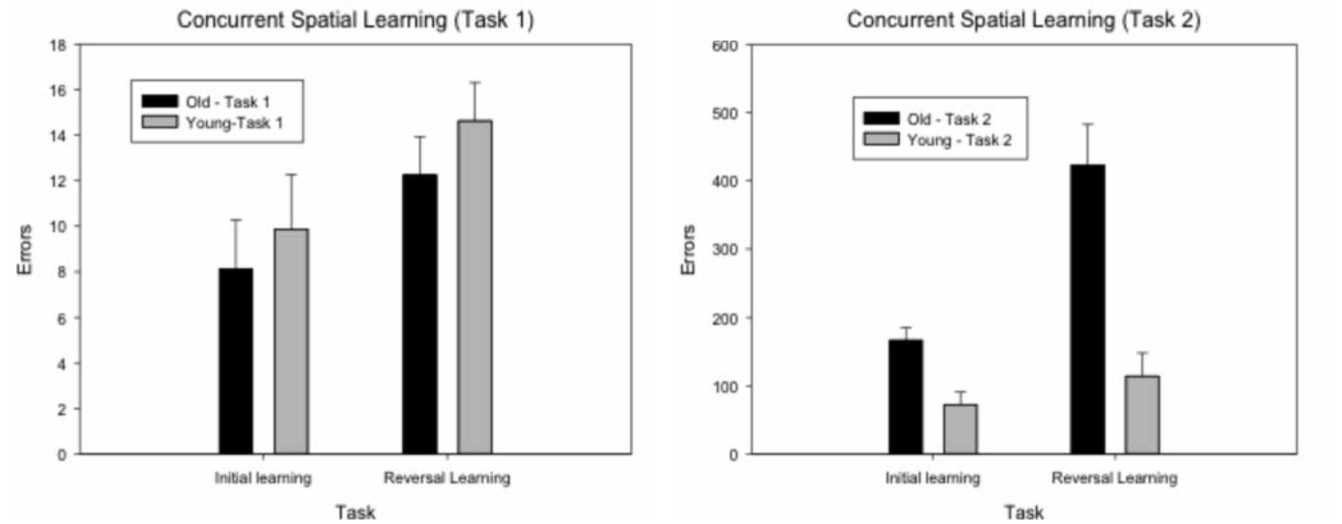
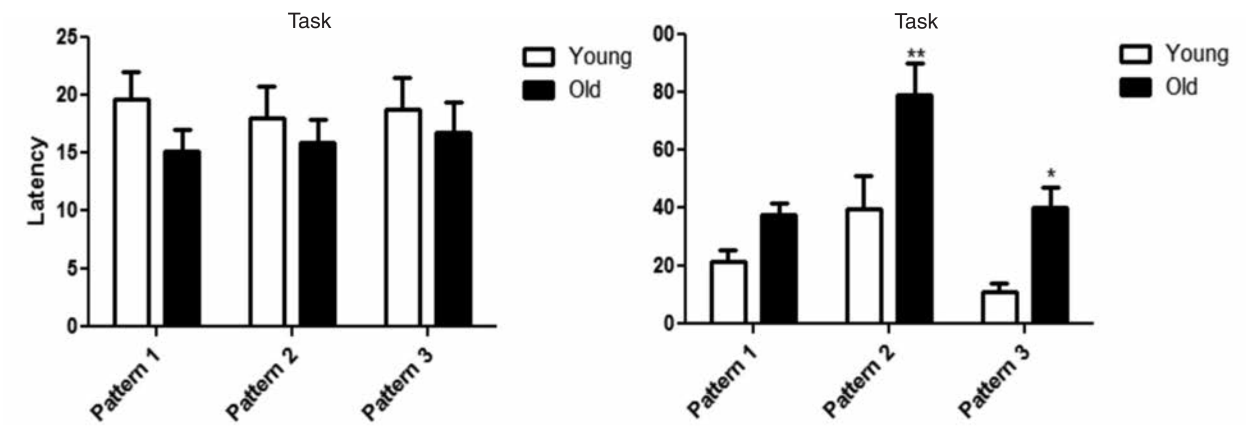


Figure 4. Errors as a problem of function and age on two different variants of a concurrent spatial discrimination task.



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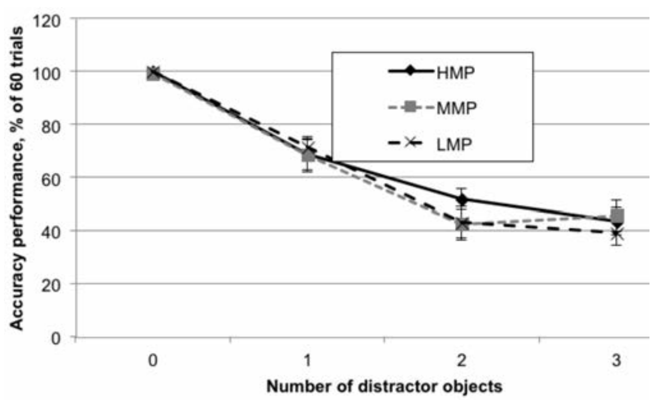
The above results provide two lines of evidence of the existence of distinct cognitive domains. First, the differences in difficulty and in pattern of errors suggest that different processes are required to learn Task 1 than Task 2. Second, Task 2, but not Task 1 shows sensitivity to age with old dogs performing more poorly than the young.

Zanghi, et al.[2] provided more direct evidence that cognitive domains are at least partially independent. They first ranked a group of aged dogs' performances on a delayed-nonmatching-to-position test (DNMP), which assess working memory. Based on accuracy of performance, the dogs were first characterized as high, medium or poor performers. The subjects were then trained on an attention task and a motor-learning task.

Figure 5. Performance on a test of selective attention as a function of performance on a spatial memory task and number of distractors. (HMP: high memory performers; MMP: moderate memory performers; LMP: low memory performers).



The attention task assessed the dogs' ability to detect and respond to a previously rewarded object, when given one, two or three alternative choices. Performance on this task depends on the total number of choices, and the larger the number, the greater the number of errors. Despite the significant differences among the groups in performance on the memory task, there were no significant differences on the attention task, indicating that dogs that differ in memory capabilities do not necessarily differ in their ability to selectively attend to a specific object (See Figure 5).

The motor-learning task involved training dogs to reach and pull in an object in order to obtain a food reward underneath the object. Performance on the task was based on the maximum distance between the object and the dog, at which the dog could respond successfully. On this task as well, there were no differences in performance among any of the three groups.

Collectively, these results show that performance on a memory task does not accurately predict performance on an attention or motor-learning task, which supports the supposition that the tasks reflect functionally separate cognitive domains.

Strategies for Developing Nutritional Interventions that Target Cognition

The potential value of nutritional modification in dogs, like in humans, is apparent in many areas. The composition and quantity of food consumed can be a key factor in the development of potentially pathological conditions, such as obesity and osteoarthritis, which affect quality of life.[3] Purina, for example, carried out a lifelong dietary restriction study examining the long-term effects of caloric restriction.[4] Labrador Retriever dogs, at 6 weeks of age, were assigned to either a control diet or a caloric-restricted diet and maintained on the diets throughout their lives. Later in life, there were numerous changes, including dramatic differences in the development of osteoarthritis (OA). Thus, by 8 years of age 77 percent of the dogs fed the control diet showed radiographic evidence of OA in two or more joints; by contrast, only 10 percent of the dogs on the caloric-restricted diet showed evidence of OA in two or more joints.

Our focus here is on a more restricted target, namely that of cognition. The previous discussion provides a necessary preamble by showing that cognition is not a unitary process, and this has important implications in the assessment of nutritional benefits. With companion animals, this is a new field of research, but it could have important implications for dogs as well as for humans. As we've previously seen, there is a marked age-dependent cognitive decline in acquisition of a spatial concurrent discrimination and reversal task. We have also carried out numerous studies using neuropsychological measures that demonstrate a selective pattern of cognitive decline, which is particularly notable in tests of complex learning, executive function and attention.[5,6]

Two different strategies have been followed in the attempt to provide nutritional supplements that have cognitive benefits. The first, the "cocktail approach," incorporates a combination of ingredients and the assumption that the ingredients will work cooperatively. The second focuses on individual supplements. We'll look at both, starting with two different types of cocktails and subsequently examine the potential benefits of the two different nutritional supplements, docosahexanoic acid (DHA) and medium-chain triglycerides (MCT).

Development of Nutritional Cocktails for Modification of Cognitive Function

Effects on Cognition of Dietary Supplementation with Antioxidants and Mitochondria Cofactors

Our first attempt to evaluate the effectiveness of a nutritional intervention on cognition in dogs was in 1999 when we set up a study in collaboration with the University of

California at Irvine, Hills Pet Food, and the Lovelace Respiratory Research Institute in New Mexico. The project was a longitudinal study designed to examine the effect of a diet supplemented with a cocktail of antioxidants (AOX) and mitochondirial cofactors on age-associated cognitive decline. The subjects were 48 aged, cognitively naïve Beagle dogs. We also ran a group of puppies in parallel on the same tasks and diets.

The initial results showed a positive effect of the AOX-supplemented diet on acquisition of a landmark discrimination learning test, which assesses visuospatial function,[7] on an oddity discrimination learning paradigm,[8] on size and intensity discrimination learning, and on discrimination reversal learning.[9,10] By contrast, over the first two years of the study, we found no group differences on performance of a working memory task, but we did obtain positive results on this task in the third year of the study.[11]

In accounting for all the positive data, we discovered that antioxidant supplementation provided only part of the story. The initial design also included another intervention, namely behavioral enrichment, which had extra cognitive training, increased exercise and social interaction. A 2x2 design was used, in which half the dogs on each diet were also provided with behavioral enrichment and half were given the antioxidant supplement. The positive results on the landmark and oddity tests were based on a comparison that was restricted to the dogs that also received behavioral enrichment. On the discrimination and reversal tasks, when the analysis took into consideration behavioral enrichment as well as AOX supplementation, it became clear that the positive AOX effect was largely driven by the group that received both AOX and behavioral enrichment; AOX alone did not produce significant improvement over the control diet. Similarly, the positive data on the DNMP after two years of treatment was also driven by the group that received the AOX-supplemented diet and behavioral enrichment.

Isolating Key Nutritional Supplements

A related follow-up study[12] sought to establish the importance of mitochondrial supplementation by restricting the supplementation to a combination of l-carnitine and alpha lipoic acid, which is a mitochondrial cofactor. The study used 12 dogs that were matched based on performance on a DNMP task and then placed into a control and treatment group.

The groups were then assessed for performance on a landmark discrimination protocol and retested on the DNMP task. The dogs receiving the supplement performed significantly better than the group administered the control compound on the landmark task. On the DNMP, by contrast, the treatment and control groups did not differ.

In an attempt to confirm and extend these results, Christie, et al.[13] obtained baseline data from a group of dogs showing equivalent levels of cognition based on performance on the DNMP. They then formed three equivalent groups: a control group, a group administered only acetyl-l-carnitine (ALCAR), and a third group administered alpha-lipoic acid (LA). They tested the dogs on a landmark discrimination learning protocol. After completing this initial single-supplement phase, the treatment groups were given an additional supplement so that the dogs initially provided ALCAR were now also given LA, and the dogs initially given LA were now also given ALCAR. To assess the effects of the combined supplements, subjects were tested on a black/white discrimination and reversal learning protocol. The results did not reveal any statistically significant group differences during either the single supplement or combined supplement stages, indicating that ALCAR, LA and the combination of the two had no significant cognitive benefits. However, these dogs had been undergoing cognitive assessment over several months when the study was performed, which provided them with considerable cognitive enrichment and could have obscured the appearance of any significant treatment effects. Another factor that could not be evaluated was the levels of carnitine in the normal diet, compared to the levels in the earlier study. There was some indication that basal levels were increased over those in the control diet of the previous study, raising the possibility of there being a threshold amount of carnitine in the diet necessary to support normal cognition.

Cognitive Benefits from a Specially Formulated Brain-Protection Blend of Nutrients

The second example of a cocktail-based approach was a report by Pan, et al.,[14] who developed a new diet referred to as the brain protection blend (BPB) based on a biomarker analyses. The specific strategy targeted risk factors known to be associated with aging and dementia in humans. The four main components were antioxidants, arginine, B vitamins, and fish oil. They first attempted to assess effectiveness in aged cats that had been trained on a collection of neuropsychological tests analogous to those we had developed for use in dogs. Subjects were tested after a 30-day washin. The BPB group showed significantly more accurate performance on three of the four test protocols (an egocentric learning and reversal task, a size discrimination and reversal learning task, and a spatial memory task). There were no significant differences on the landmark test.

Cognitive Modification by Addition of Single Supplements

Effect on Cognition in Aged Dogs of Dietary Supplementation with Medium-Chain Triglycerides

Medium-chain triglycerides were first proposed as a nutritional intervention for cognition by Reger, et al.,[15] who hypothesized that MCTs can be converted to ketones, which could provide the brain with an alternative to energy obtained from glucose metabolism. The idea is appealing because brain metabolism is known to decline with age and could be a factor in causing age-associated cognitive decline.

To assess the effectiveness of MCTs in dogs, we first administered a collection of cognitive tests to a group of aged Beagle dogs and used the results to establish cognitively equivalent groups on control and treatment diets. The dogs in the treatment group were maintained on a diet supplemented with 5.5% MCT and were maintained on the diet for eight months. During this time, they were tested sequentially on landmark discrimination learning, an egocentric visuospatial function test and an attention test. The groups did not differ on the initial component of the landmark task, which involved learning to discriminate between a coaster and a coaster with a plastic rod on top of it. They did differ significantly, however, on the next component, a true landmark task in which the dog was rewarded for approaching the coaster closest to the landmark. On the egocentric task, the groups performed at equivalent levels on the initial learning, but the MCT-supplemented group made significantly fewer errors on the reversal component. Finally, on the attention task, the groups did not differ on the first two phases, but they differed on the third phase, in which the dogs tested with a stimulus previously associated with reward and with a novel distractor. In each of the protocols, the group differences observed always reflected more accurate performance by the group on the MCT supplement.

We also found, as expected, that the group on the MCT supplement showed significantly elevated levels of beta-hydroxybutyrate, a ketone body. The cognitive data showed improved performance of the MCT-supplemented group on selective tasks from each of the three protocols. In each case, the significant improvement was seen in the more difficult tasks, though the groups did not differ in easier tasks. This suggests that supplementation with MCTs can produce a generalized enhancement of brain function.

DHA Supports Cognition in Aged Dogs

The final study (Hadley, et al., 2016, in submission) focused on possible benefits accruing from supplementation with DHA, an omega-3 fatty acid. The study used aged Beagle dogs, which prior to the start of the study were first switched to a new diet that lacked n-3 polyunsaturated fatty acids (Joy® dog food). The dogs were screened at baseline on the DNMP, and then assigned to two cognitively equivalent diet groups. One group was continued on the baseline diet, and the second group was given the control diet plus a supplement, *Schizochytrium* sp., which is a primary source of omega-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA). The study ran for almost six months after the start of the treatment phase, and during this time the dogs were tested on a collection of different neuropsychological tests that included DNMP, a concurrent discrimination learning task and a contrast sensitivity protocol.

The results showed that DHA supplementation produced task-specific benefits. Specifically, the results revealed statistically significant improvement in learning a contrast discrimination task and in performance on a variable contrast phase (See Figure 6). The dogs receiving DHA supplementation did not differ from the dogs on the control diet on the DNMP task (see Figure 7) or in learning a concurrent discrimination task.

Figure 6. Performance of control animals (Group 1) and animals on a diete supplemented with *Schizochytrium* sp. on a contrast discrimination protocol. During the initial learning phase, subjects were trained to distinguish between a black triangle and black circle on a white background. During the variable contrast phase, the contrast between background and object was varied, with performance decreasing in parallel with reduced contrast

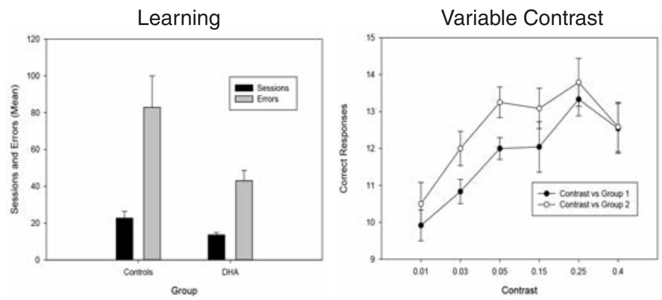
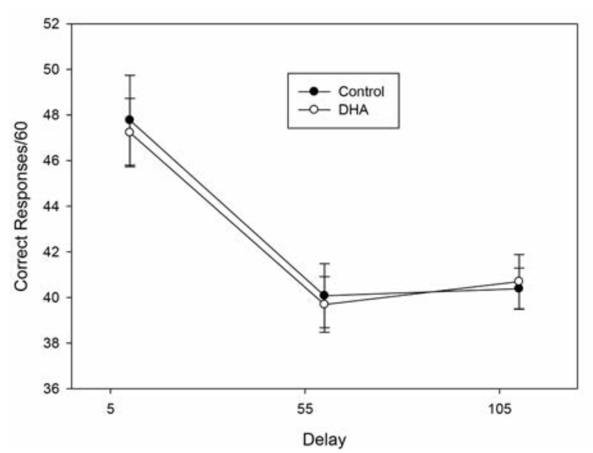


Figure 7. Performance on the DNMP task as a function delay for the control and treatment groups at baseline and at the end of the treatment phase



The contrast discrimination task was designed to assess the dogs' ability to both learn a difficult task and to detect objects on cards, in which the objects became difficult to distinguish from the background because of low contrasts. The superior performance of the supplemented group suggests that though DHA supports cognition in general, it also is selectively important for tasks involving complex visual processing.

Discussion and Conclusions

This article had two main goals. The first was to describe what we mean by cognition in dogs, and the second was to discuss evidence that canine cognition can be modified by nutritional interventions. Our approach toward understanding canine cognition is parallel to that taken by neuropsychologists who have attempted to characterize cognition in humans and assume that cognition consists of functionally distinct domains that can be selectively assessed with neuropsychological tests.

Evidence in support of this view comes from comparisons of performance on a collection of neuropsychological tests, which reveals that the correlations between some pairs of tests are small. Thus, a given dog may show a high level of accuracy on a test of working memory but perform poorly on a test of selective attention or motor learning. From a neuropsychological perspective, these findings are consistent with the suggestion that differences in test performance arise because the tests utilize different brain regions or systems.

The cognitive domain hypothesis also accounts for differences between tasks in age sensitivity. For example, we've consistently found that tasks requiring complex learning, such as the concurrent spatial discrimination task, show much greater age sensitivity than simple discrimination learning or working memory tasks.

Nutritional Supplementation and Cognitive Benefits

As previously mentioned, there has been little systematic research on the effectiveness of nutritional interventions in modifying cognition in dogs or cats. The data presented here, however, strongly suggests that nutrition can have beneficial effects and that further research may prove fruitful. We have discussed two different types of strategies, the cocktail approach and the single supplement approach. The cocktail approach uses a combination of supplements that are hoped to work synergistically. The first example that we discussed was a diet supplemented with a broad spectrum of antioxidants and mitochondrial cofactors, and the study went on for over two years. The results revealed that positive evidence was obtained only from aged dogs and that the effectiveness appeared to be linked to environmental enrichment. The absence of an effect on young dogs was not unexpected since oxidative damage is only thought to become functionally important later in life.

The second example was the brain protection blend diet, which was supplemented with antioxidants, arginine, fish oil, and B vitamins. In addition, the study was performed in cats. Unlike the antioxidant spiked diet, the rationale underlying the formulation of the BPB diet was to elevate levels of biomarkers that normally decline with age.

We also discussed two different single-supplement interventions: a medium-chain triglyceride and an algae containing high levels of omega-3 fatty acids. Both studies yielded positive results. The rationale for the addition of a medium-chain triglyceride was to increase availability of energy to the brain by providing an alternative source of energy produced by metabolism of ketones. The study that used algae as a supplement also demonstrated positive results and was informative in two respects. Before starting the treatment phase, the subjects were placed on a control diet that lacked significant levels of DHA. This provides a useful strategy for demonstrating the benefits of omega-3 fatty acids in the diet. It would be useful to know, however, what the maximum potential benefits could be and how much omega-3 must be present to produce optimal performance.

Prevention Versus Cognitive Enhancement

There are generally two reasons why a supplement could produce cognitive benefits. The first pertains to the possibility of supplementation being neuroprotective by slowing or possibly reversing degenerative processes, which could potentially explain why in the AOX study there was evidence of benefits that only began to emerge after three years on the diet. The second reason why a supplement could have beneficial effects is because it can enhance normal brain function. Thus, we suspect that MCT supplementation serves more as a cognitive enhancer, which would have cognitive benefits that only persist if the dog is maintained on the diet.

CDS and Age-Dependent Cognitive Dysfunction

A final question that warrants discussion is the clinical relevance of this laboratory-based research for companion animals outside the laboratory. We believe that the cognitive structure assessed in the laboratory utilizes the same processes available outside the laboratory, and the types of changes that we observe correspond to parallel changes that can occur in normal life. Companion animals may also show parallels in cognitive and behavioral changes associated with aging to those observed during the development of human dementia. Ruehl, et al.[17] were the first to identify a syndrome in dogs referred to as cognitive dysfunction syndrome (CDS) that is associated with disorientation, inactivity, sleep problems, disrupted social interactions, loss of housetraining, and anxiety. CDS can emerge in many different forms and is assumed if dysfunction is seen in more than one category of signs. Thus, CDS may encompass behavioral deficits, such as activity or sleep problems, that would not necessarily be described as cognitive, but similar to cognitive decline are also linked to changes in brain health.

Future Directions

Finally, it's important to ask where this research will eventually lead us. This is a particularly important question in the context of pharmaceutical research into human cognitive loss and dementia. Despite spending billions of dollars on research into drug development, we still are lacking any drug that can treat or prevent dementia. Nutritional interventions that enhance normal function provide a promising approach for targeting age-related cognitive decline in both humans and companion animals. In this context, it would be useful to have more multiyear studies that could definitively establish long-term benefits. For such studies, it also would be important that the assessment tests be carefully selected, keeping in mind the complex structure of cognition. A better understanding of the beneficial effects of nutritional supplementation could also have economic benefits by prolonging the useful life span of working dogs.

References

1. Milgram NW, Head E, Weiner E et al. Cognitive Functions and Aging in the Dog: Acquisition of Nonspatial Visual Tasks. *Behav Neurosci*. 1994;108:57-68.

2. Zanghi BM, Araujo J, Milgram NW. Cognitive Domains in the Dog: Independence of Working Memory from Object Learning, Selective Attention, and Motor Learning. *Anim Cogn*. 2015;18:789-800.

3. Laflamme DP. Nutrition for Aging Cats and Dogs and the Importance of Body Condition. *Vet Clin N Am-Small*. 2005; 35:713-742.

4. Lawler DF, Larson BT, Ballam JM, et al. Diet Restriction and Aging in the Dog: Major Observations over Two Decades. *Brit J Nutr*. 2008;99:793-805.

5. Milgram NW, Head E, Weiner E, et al. Cognitive Functions and Aging in the Dog: Acquisition of Nonspatial Visual Tasks. *Behav Neurosci*. 1994;108:57-68.

6. Snigdha S, Christie LA, De Rivera C, et al. Age and Distraction Are Determinants of Performance on a Novel Visual Search Task in Aged Beagle Dogs. *Age*. 2012;34:67-73.

7. Milgram NW, Head E, Muggenburg B, et. al. Landmark Discrimination Learning in the Dog: Effects of Age, an Antioxidant Fortified Diet, and Cognitive Strategy. *Neurosci Biobehav R*. 2002;26:679-95.

8. Milgram NW, Zicker SC, Head E, et.al. Dietary Enrichment Counteracts Age-Associated Cognitive Dysfunction in Canines. *Neurobiol Aging*. 2002;23:737-745.

9. Milgram NW, Head E, Zicker S, et al. Effect of an Antioxidant Diet, Cognitive Enrichment and Age on Size Discrimination Learning and Reversal Learning in Beagle Dogs. *Exp Gerontol*. 2004;39:753-755.

10. Milgram NW, Head E, Zicker SC, et al. Learning Ability in Aged Beagle Dogs Is Preserved by Behavioral Enrichment and Dietary Fortification: A Two-Year Longitudinal Study. *Neurobiol Aging*. 2005;26:77-90.

11. Nippak PM, Mendelson J, Muggenberg B, et al. Enhanced Spatial Ability in Aged Dogs following Dietary and Behavioural Enrichment. *Neurobiol Learn Mem*. 2007;87:610-623.

12. Milgram NW, Araujo JA, Hagen TM, et al. Acetyl-L-Carnitine and {Alpha}-Lipoic Acid Supplementation of Aged Beagle Dogs Improves Learning in Two Landmark Discrimination Tests. *FASEB J*. 2007;13:3756-3762.

13. Christie LA, Opii WO, Head E, et al. Short-Term Supplementation with Acetyl-L-Carnitine and Lipoic Acid Alters Plasma Protein Carbonyl Levels but Does Not Improve Cognition in Aged Beagles. *Exp Gerontol*. 2009;12:752-759.

14. Pan Y, Araujo JA, Burrows J, et al. Cognitive Enhancement in Middle-Aged and Old Cats with Dietary Supplementation with a Nutrient Blend Containing Fish Oil, B Vitamins, Antioxidants and Arginine. *Brit J Nutr*. 2013;110:40-49.

15. Reger MA, Henderson ST, Hale C, et al. Effects of Beta-Hydroxybutyrate on Cognition in Memory-Impaired Adults. *Neurobiol Aging*. 2004;25:311-314.

16. Pan Y, Larson B, Araujo JA, et al. Dietary Supplementation with Medium-Chain TAG Has Long-Lasting Cognition-Enhancing Effects in Aged Dogs. *Brit J Nutr*. 2010;12:1746-1754.

17. Ruehl WW, Bruyette DS, De Paoli A, et al. Canine Cognitive Dysfunction as a Model for Human Age-Related Cognitive Decline, Dementia and Alzheimer's Disease: Clinical Presentation, Cognitive Testing, Pathology and Response to L-Deprenyl Therapy. *Prog Brain Res*. 1995;106:217-225.