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Timely Topics in Nutrition

Nutritional management of brain aging in dogs

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Improved nutrition and medical care have contributed to prolonged life spans in people and their pets. Current estimates suggest that there are approximately 20 to 30 million senior and geriatric dogs > 7 years old in the United States.[1] Longer life spans may increase the prevalence of age-related medical conditions, including behavioral and cognitive problems. By use of systematic and controlled laboratory testing procedures, deficits in learning and memory have been documented in older dogs and appear similar to changes detected in older humans.[2-7] In client-owned older animals, behavioral signs such as disorientation, decreased social interaction, inability to remember housetraining, sleep disturbances, and altered amounts of activity have been reported.[8] Therapeutic intervention was often not considered in the past because the progression of behavioral problems in older dogs had no plausible explanation.

Cognitive dysfunction syndrome (CDS) has been used to describe behavioral changes evident in client-owned older dogs.[8,9] Numerous studies have been performed by use of owner-based observational questionnaires to assess the prevalence of CDS in dogs. In a study[8] involving responses of 26 pet owners, the most common complaints associated with older dogs included destructive behaviors, inappropriate urination and defecation, and excessive vocalization. In another study,[10] 22 of 80 (28%) dogs 11 to 12 years of age and 23 of 34 (68%) dogs 15 to 16 years of age had evidence of CDS. Furthermore, CDS may be progressive because older dogs with impairments in 1 behavioral category subsequently had impairments in 2 or more categories within 12 to 18 months.[10,11]

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Owner-evaluated, survey-based studies measure global brain dysfunction and may be insensitive to early and subtle changes in learning and memory associated with brain aging in dogs. An alternative method involves development of neuropsychologic tests that directly provide quantitative and objective measures of cognitive function without reliance on questionnaires. Neuropsychologic tests have been used to accomplish 3 objectives. They can be used to identify nonsubjective cognitive changes as a function of age in dogs, characterize the biological basis of age-dependent cognitive decline, and screen potential therapeutic interventions, including nutritional management. Although results of most neuropathologic and neuropsychologic studies in older dogs have been published in human neuroscience journals, many of the findings are of interest to the veterinary community. Thus, our intent was to review the proposed biological basis for brain aging and cognitive dysfunction in dogs. In addition, we intended to describe results of nutritional intervention with antioxidant-enriched foods that have been used in neuropsychologic laboratory tests and clinical trials in older dogs.

Pathologic Features of Brain Aging in Dogs

The brains of older dogs have several key features that have also been observed in the brains of older humans.[7] Many of these features are evident in conjunction with early pathologic changes in the brains of clinically normal older people, people with mild cognitive impairment, and patients with Alzheimer's disease.[7,12] Brains of older dogs have a number of morphologic changes similar to those observed in brains of older people, including cortical atrophy, widening of the ventricles, degeneration of myelin in the white matter, accumulation of degraded proteins, damage to DNA, and reduction of endogenous antioxidants (**Figure 1**).[13-17]

The brain of an older dog also accumulates proteins within and around neurons; these proteins may be toxic to the neurons.[18] Accumulation of diffuse proteinaceous plaques has received the most attention in older dogs because it is believed to play a causative role in development of Alzheimer's disease in humans.[19] Plaques contain a number of proteins, but the primary constituent is the **β-amyloid peptide (Aβ)**, which has identical amino acid sequences in people and dogs and

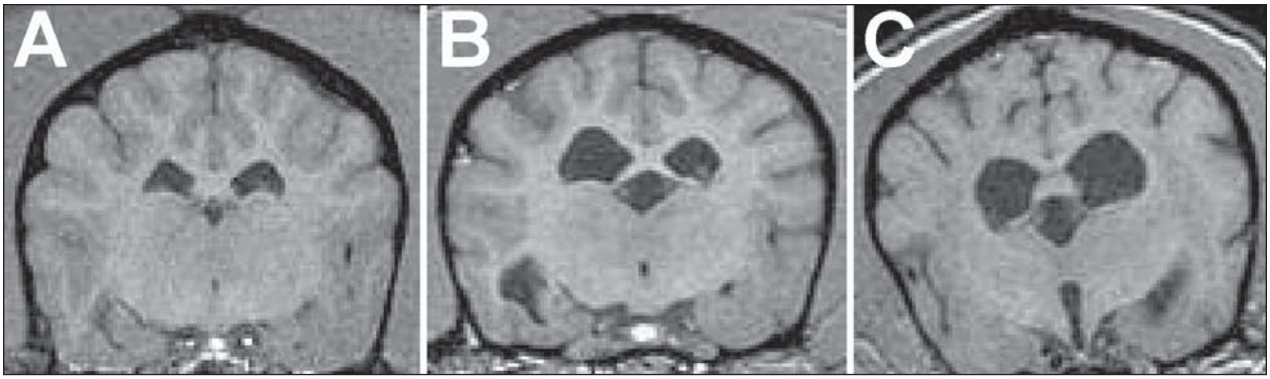

Figure 1—Magnetic resonance imaging scans of the brain of a 4-year-old dog (A), 9-year-old dog (B), and 15-year-old dog (C). Notice that there is marked enlargement of the ventricles and cortical atrophy (deeper gyri and wider sulci) in the older dogs. (Adapted from Head E, Milgram NW, Cotman CW. Neurobiological models of aging in the dog and other vertebrate species. In: Hof PR, Mobbs CV, eds. *Functional neurobiology of aging*. San Diego: Academic Press Inc, 2001;457–465. Reprinted with permission.)

may contribute to neuronal toxicosis.[20,21] The extent of Aβ deposition in the brain of dogs is linked to the severity of cognitive deficits and behavioral changes.[22,23] Amyloid precursor protein is the source of the Aβ, and the intact precursor protein also appears to contribute to neuronal dysfunction by targeting mitochondria of cortical neuronal cells.[24] Accumulation of amyloid precursor protein causes mitochondrial dysfunction and impaired energy metabolism in cortical neurons.

Not all brain regions are equally vulnerable to Aβ-associated pathologic changes. Deposits of Aβ are evident in the prefrontal cortex at an earlier age and in a more consistent manner than in other cortical regions (**Figure 2**).[25] Older dogs that are severely impaired when performing a reversal learning task, which measures the ability to inhibit a previously learned behavior (ie, sensitive to function of the frontal lobe), are also the same dogs that have the most extensive Aβ-associated pathologic changes in the prefrontal cortex.[26] This pattern of Aβ accumulation in older dogs parallels that seen in people.[7,27] However, Aβ deposition does not account for all the cognitive variability in older animals, which suggests that other mechanisms or events play a role in cognitive dysfunction.

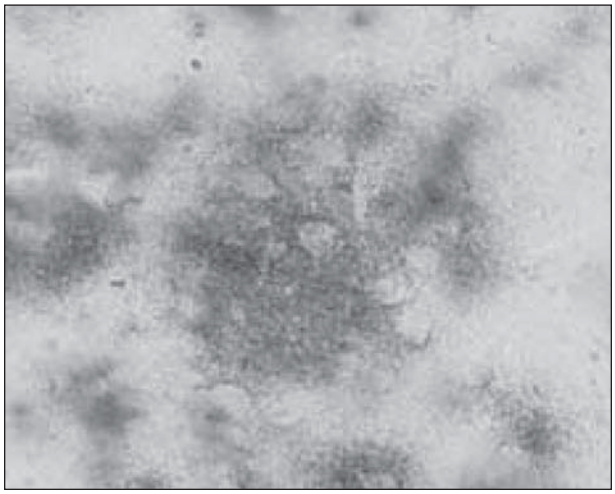

Figure 2—Photomicrograph of the prefrontal cortex of a 12-year-old cognitively impaired Beagle revealing diffuse plaques within the brain. Thin sections were immunostained with an antibody directed against β-amyloid 1-42. Bar = 50 μm. (Adapted from Head E, Milgram NW, Cotman CW. Neurobiological models of aging in the dog and other vertebrate species. In: Hof PR, Mobbs CV, eds. *Functional neurobiology of aging*. San Diego: Academic Press Inc, 2001;457–465. Reprinted with permission.)

Oxidative Damage and Brain Aging

A candidate mechanism that may contribute to neuronal dysfunction and progressive accumulation of neuropathologic lesions in brains of older animals is oxidative damage. The free-radical theory of aging was first proposed in 1956.[28] According to the theory, reactive-oxygen species that are formed as by-products of cellular metabolism cause cellular damage.[29] Aerobic metabolism in mitochondria has been implicated in the production of most reactive-oxygen species. As mitochondria age or become dysfunctional as a result of disease, additional reactive-oxygen species form that may then result in further uncontrolled reactions within that cell.[29-31] Brain aging is believed to be a cumulative response to these alterations, and the associated neuropathologic changes account for age-associated cognitive and behavioral changes.

The brain may be especially vulnerable to cumulative oxidative damage and effects of aging because of its high metabolic rate (ie, high demand for oxygen), limited capability for defense against antioxidants, and limited repair ability.[32] The aging process also appears to be accompanied by a decrease in the ability to counter the effects of oxidative stress and a decrease in mitochondrial function, which results in greater oxidative damage.[30,33,34] Production of free radicals leads to oxidative damage to proteins, lipids, and nucleotides, which may contribute substantially to neuronal dysfunction and, ultimately, neuronal death. Oxidative damage to lipids and proteins increases with age in the brain of dogs and may serve as a common mechanism initiating and linking several pathologic features of brain aging.[35] There is increased oxidative damage to brain lipids prior to overt Aβ deposition, which provides evidence that oxidative damage is an early event.[36] The Aβ is also able to directly generate oxidative damage to lipids and proteins.[29] Analysis suggests that oxidative changes actually are evident within canine neuronal mitochondria and affect mitochondrial function. Young (mean, 3.4 years old) dogs have improved mitochondrial bioenergetics in

brain tissue, compared with values for older (mean, 10.7 years old) dogs. Mitochondrial concentrations of reactive-oxygen species are substantially increased in brains of older dogs, and oxidation of mitochondrial proteins is increased in brains of older dogs.[a]

Typically, several mechanisms balance the production of free radicals. However, it is possible that these mechanisms begin to fail as animals become older. For example, the superoxide dismutase enzyme system that helps to reduce free radicals appears to diminish with age in the canine brain.[17]

Cognitive Impairment in Older Dogs

Advanced learning ability of dogs is evidenced by their use as assistance companions (eg, guide dogs) and working animals (eg, military and search-and-rescue dogs). Learning and memory can be tested systematically in dogs by the use of tasks developed for nonhuman primates. In parallel with human and primate studies, tasks are selected that are sensitive to the function of specific cortical circuits or regions of the brain. All testing is conducted by use of food rewards, which sufficiently motivates dogs to learn each task.

Use of these testing procedures has revealed that older dogs are typically able to learn simple skills to the same extent as younger dogs.[2] However, some older dogs can have pronounced impairment. Simple associative learning, such as visual discrimination (ie, learning that 1 of 2 dissimilar objects covers a food reward), typically remains intact with age.[2,26,37] However, substantial impairment is evident for more complex discrimination learning problems, such as discrimination on the basis of size or oddity.[26,38] Prefrontal-dependent tasks, such as reversal learning (reward contingencies are reversed and animals must shift from responding from 1 object to a second object), are also consistently impaired in older dogs.[2]

In addition to learning ability, memory is also compromised in older dogs. Forms of memory that appear to involve age-sensitive mechanisms include spatial memory (ability to remember the location of a food reward) and object-recognition memory (ability to recognize an object seen 10 to 120 seconds previously).[3,4,39] However, the variability in performance of these tasks is extensive. Older dogs appear to fit into 1 of 3 categories as determined on the basis of learning ability and memory testing. Dogs may be unimpaired or successfully aging, age-impaired, or severely impaired (**Figure 3**).[40] These clusters of older dogs may be similar to declines along a cognitive continuum in people with mild age-associated memory impairment followed by cognitive decline and dementia.[41] The decline in learning and memory documented in laboratory studies is also consistent with behavioral changes observed in dogs with CDS. Careful questioning of an owner is the best way for veterinarians to detect early signs of cognitive impairment in an older dog. A questionnaire is a useful diagnostic tool and an excellent means of tracking changes and response to treatment (**Appendix 1**).

Effects of Antioxidant-Enriched Foods on Cognitive Decline in Older Dogs

A series of studies[38,40,42,43] were used to test the hypothesis that a food enriched with complex mixtures of antioxidants and mitochondrial cofactors could result in improvements in learning and memory and reduce the extent of pathologic changes that accumulate in the brain of older dogs. In addition, a longitudinal investigation of the effects of dietary management on cognitive function of Beagles has been completed.[44]

In the longitudinal study,[44] the experimental subjects were groups of old (10 to 13 years old) and young (3 to 5 years old) Beagles. The study was conducted as a randomized, controlled clinical trial with each dog assigned to receive an extruded senior food (control diet) or an enriched food (test diet). The enriched food was supplemented with vitamins E and C, mitochondrial cofactors (L-carnitine and DL-α-lipoic acid), and a mixture of fruits and vegetables (**Appendix 2**). Vitamin E is a lipid-soluble vitamin that protects cell membranes from oxidative damage, whereas vitamin C is a water-soluble vitamin that helps replenish cellular concentrations of vitamin E.[30,45] L-Carnitine is a precursor to acetyl-L-carnitine, which is involved in mitochondrial lipid metabolism and maintenance of efficient mitochondrial function.[46,47] Lipoic acid is an antioxidant nutrient capable of reduction-oxidation recycling of other antioxidants and increasing intracellular concentrations of glutathione.[48-50] Glutathione is the primary intracellular water-soluble antioxidant. Fruits and vegetables are sources of carotenoids and flavonoids that appear to have additional antioxidant activity.[51,52]

Cognitive ability was similar between groups prior to dietary intervention. In 1 measure from the study, dogs were fed the control or enriched diet for 6 months and tested on 4 oddity-discrimination learning tasks

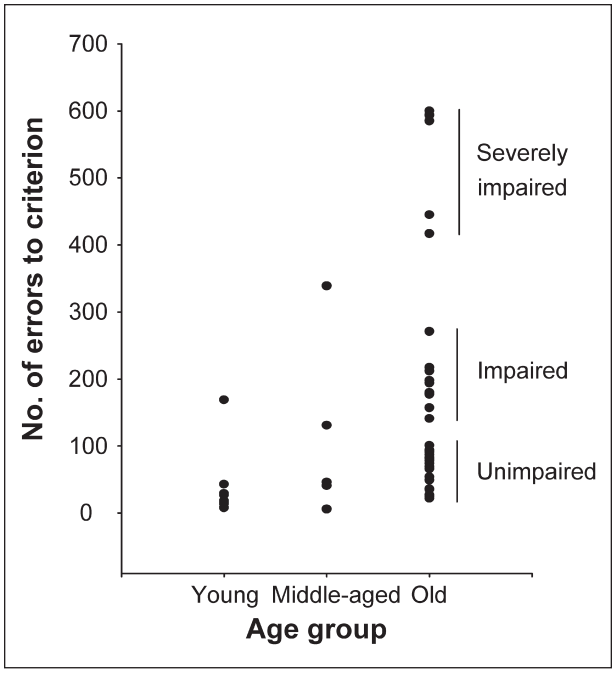

Figure 3—Number of errors for spatial-memory testing in 3 groups of dogs. Notice that young and middle-aged dogs were primarily unimpaired, whereas there were more old dogs that were impaired or severely impaired. (Adapted from Head E, Milgram NW, Cotman CW. Neurobiological models of aging in the dog and other vertebrate species. In: Hof PR, Mobbs CV, eds. *Functional neurobiology of aging*. San Diego: Academic Press Inc, 2001;457–465. Reprinted with permission.)

that had increasing difficulty.[42] Each task involved repeatedly providing the dogs with 3 objects, 2 of which were identical and 1 that was dissimilar (ie, odd). Use of progressively more similar objects for each new problem increased the difficulty of the task. Old dogs made significantly more errors than young dogs in all tasks (**Figure 4**). This supports the adage that you cannot teach an old dog new tricks. However, old dogs receiving the enriched diet performed significantly better on the more difficult tasks than old dogs receiving the control diet (**Figure 5**). The enriched diet caused the most substantial improvement in the ability of old dogs to learn complex tasks. Oddity-discrimination testing provides a sensitive measure of age-dependent cognitive deterioration in dogs, and this age-dependent effect may thus be partially mitigated by feeding a food enriched with a complex mixture of antioxidants and mitochondrial cofactors.

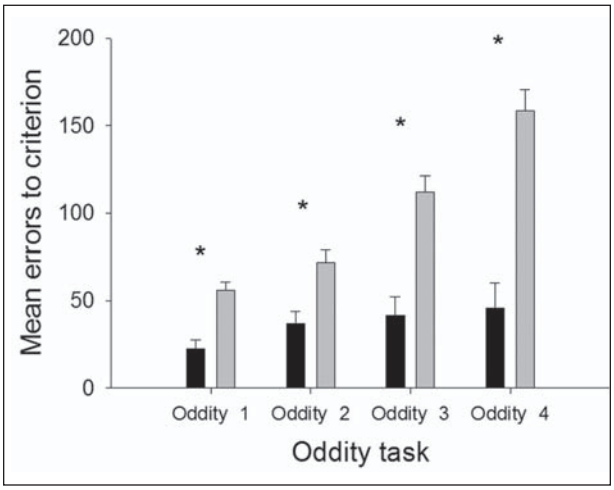

Figure 4—Mean number of errors to criterion made by young (black bars) and old (gray bars) dogs learning 4 progressively more difficult oddity-discrimination tasks. Error bars represent SEM. Asterisks indicate significant ($P < 0.05$) differences between young and old dogs. (Adapted from Milgram NW, Zicker SC, Head E, et al. Dietary enrichment counteracts age-associated cognitive dysfunction in canines. *Neurobiol Aging* 2002;23:737–745. Reprinted with permission.)

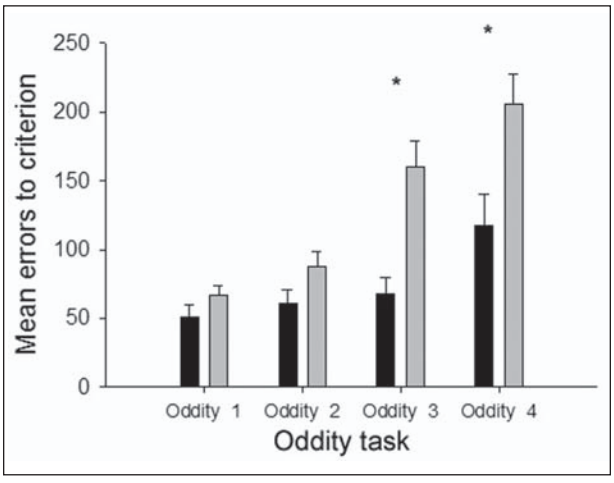

Figure 5—Mean number of errors to criterion made by old dogs learning 4 progressively more difficult oddity-discrimination tasks and receiving a control diet (gray bars) or a diet enriched in antioxidants and mitochondrial cofactors (black bars). Error bars indicate SEM. Asterisks indicate significant ($P < 0.05$) differences between control diet– and antioxidant diet–fed dogs. (Adapted from Milgram NW, Zicker SC, Head E, et al. Dietary enrichment counteracts age-associated cognitive dysfunction in canines. *Neurobiol Aging* 2002;23:737–745. Reprinted with permission.)

Other measures from longitudinal studies[38,40] evaluated dietary effects on landmark-discrimination learning in dogs. Tests of landmark-discrimination learning are used to assess the ability of animals to use spatial information to locate objects or targets. The available evidence suggests that spatial ability deteriorates with age and dementia. In humans with dementia and dogs with age-associated cognitive decline, spatial deficits are notable in the tendency of individuals to become lost in familiar surroundings. Specifically, these testing methods can be used to study factors affecting the ability of dogs to use external landmarks to indicate the location of food. In other studies,[3,4,37] older dogs have performed more poorly than younger dogs in tests of landmark-discrimination learning.

In 1 longitudinal study,[38] old and young dogs were fed a control or enriched diet for 1 month and trained on a series of landmark-discrimination tasks. In each task, dogs were provided with 2 objects and a landmark. The dogs were initially trained with the landmark on top of the correct object (ie, the object associated with a food reward) and then trained with the landmark placed at successive distances (1, 4, and 10 cm) from the correct object. To obtain a food reward, dogs were required to respond to the object associated with the landmark. Error and success rates for these tasks were determined. Age effects on the performance of all tasks were found with old dogs because they made significantly more errors and were successful less often, compared with results for young dogs. However, old dogs consuming the enriched diet performed significantly better on tasks with fewer errors and better success rates than did old dogs consuming the control diet. These results clearly document that age can affect learning in dogs but can be partially mitigated with appropriate nutritional management.

Environmental enrichment is an important factor in mitigating the effects of brain aging in dogs.[44,53] Learning ability is best preserved in older dogs by use of a combination of dietary fortification and behavioral enrichment.[44,53] This supports the adage of use it or lose it with respect to brain function and offers exciting new opportunities for exploring synergistic effects of nutritional and environmental therapeutic interventions.

Consistent with the hypothesis that oxidative damage contributes to brain aging in dogs, improvements in memory and learning of older dogs attributable to dietary enrichment parallel changes in mitochondrial function. It has been documented[a,b] that there are improvements in mitochondrial bioenergetics, reductions in mitochondrial concentrations of reactive-oxygen species, and reductions of Aβ accumulation in brain tissue of older dogs consuming food enriched with antioxidants and mitochondrial cofactors.

Effect of Antioxidant-Enriched Foods to Reduce Age-Related Behavioral Changes in Dogs

In addition to the extensive aforementioned laboratory tests, a randomized, double-blind, controlled

clinical trial was conducted to evaluate effects of dietary enrichment in dogs with age-related behavioral changes.[54] The study was conducted to evaluate pet dogs ≥ 7 years old that were consistently recognized by their owners as having at least 2 behavioral characteristics of age-related cognitive decline. Dogs were randomly assigned to receive an enriched diet[c] or control diet,[d] and neither the dog owners nor veterinarians were aware of the food assigned to each dog. Clinical features of age-related behavioral changes were measured by use of a standardized informant-based questionnaire completed by the owners. The questionnaire assessed numerous canine behaviors (Appendix 1). The decision to use a questionnaire completed by the pet owners as the outcome-measuring device was derived from the premise that aging and behavioral changes associated with cognitive decline are progressive and manifested primarily in the home environment. Therefore, owner assessments were deemed the most viable assessment of behavioral changes attributable to dietary intervention.

In that study,[54] investigators enrolled 142 dogs (71 for the enriched diet and 71 for the control diet). Of the 142 dogs, 125 (61 for the enriched diet and 64 for the control diet) completed a 60-day feeding period. At the time of enrollment, those 125 dogs ranged from 7 to 20 years of age (mean, 12 years; median, 11.5 years). During the 60-day feeding period, significant improvements were detected for 14 of 16 separate behavioral attributes for the group consuming the enriched diet but only 4 of 16 behavioral attributes for the control group, as assessed by use of the questionnaires completed by the owners. In addition, a significant advantage existed for dogs consuming the enriched diet, compared with dogs consuming the control diet, at day 60 for attributes of agility, recognition of family members, and recognition of other animals. Dogs consuming the enriched diet also had a significant improvement with regard to excessive licking and patterned pacing behaviors. These findings are consistent with the premise that dogs with age-related cognitive decline manifested as confusion or compulsive behaviors can benefit substantially from nutritional management offered by feeding a food enriched in antioxidants and mitochondrial cofactors. Results of that clinical trial support the hypothesis that dietary intervention with a food enriched with antioxidants and mitochondrial cofactors modulates behavioral manifestations of age-related cognitive decline. Veterinarians should include behavioral checklists for use in assessing senior pets and consider incorporating nutritional management in treatment programs for patients with evidence of age-related behavioral or cognitive changes.

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b. Pop V, Head E, Nistor M, et al. Reduced Aβ deposition with long-term antioxidant diet treatment in aged canines (abstr), in *Proceedings*. Soc Neurosci Conf 2003;525.4.
c. Prescription Diet Canine b/d, Hill's Pet Nutrition Inc, Topeka, Kan.
d. Purina Dog Chow, Nestle Purina Petcare Co, St Louis, Mo.

Appendix 1

Behaviors evaluated in dogs to assess age-related cognitive decline.

Confusion, awareness, and spatial orientation
- Gets lost in familiar locations
- Goes to wrong side of door (eg, hinge side)
- Gets stuck and cannot navigate around or over obstacles
- Is less responsive to stimuli

Relationships and social behavior
- Decreased interest in petting or contact
- Decreased greeting behavior
- Alterations or problems with social hierarchy
- In need of constant contact (ie, overdependent or clinging)

Activity—Increased or repetitive
- Staring, fixation, or snapping at objects
- Pacing or aimless wandering
- Licking owners or household objects
- Inappropriate vocalization
- Increase in appetite (volume of food consumed or speed of consumption)

Activity—Decreased
- Decreased exploration (apathy)
- Decreased responsiveness to stimuli
- Decreased self-care
- Decrease in appetite or disinterest in food

Anxiety or irritability
- Restlessness or agitation
- Separation anxiety
- Increased irritability

Sleep-wake cycle
- Restless sleep or waking during the night
- Increased sleep during the daytime

Learning and memory—housesoiling
- Indoor elimination at random sites or in view of owners
- Decreased or no signaling of need to eliminate
- Goes outdoors but eliminates indoors upon return
- Elimination in crate or sleeping area
- Incontinence

Learning and memory—work, tasks, or commands
- Impaired working ability
- Decreased recognition of familiar people or pets
- Decreased responsiveness to known commands or tricks
- Decreased ability to perform tasks
- Unable or slow to learn new tasks (must retrain)

Adapted from Landsberg GM, Hunthausen W, Ackerman L. The effects of aging on behavior in senior pets. In: Landsberg GM, Hunthausen W, Ackerman L, eds. *Handbook of behavior problems of the dog and cat*. 2nd ed. Philadelphia: Elsevier Science Ltd, 2003;273. Reprinted with permission.

Appendix 2

Amounts of nutrients or enhancements used in control and enriched (test) diets formulated for dogs used in neuropsychologic studies of brain aging.

Ingredient	Control food	Enriched food
Vitamin E	Approx 100 U/kg	Approx 1,000 U/kg
L-Carnitine	None added	Approx 260 mg/kg
DL-α-Lipoic acid	None added	Approx 120 mg/kg
Vitamin C	None added	Approx 80 mg/kg
Tomato pomace*	None added	1%
Dried spinach*	None added	1%
Dried carrot*	None added	1%
Dried citrus pulp*	None added	1%
Dried grape pomace*	None added	1%
Docosahexaenoic acid†	None added	0.01%

*Vegetables and fruit ingredients were added at 1% of the diet in place of corn. †Docosahexaenoic acid was added only to the diet used in the behavioral clinical study.

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