

Canine Cognitive Dysfunction (CCD), Alzheimer's Disease (AD), and  $\beta$ -Amyloid

Accumulation:

Using CCD as a Reference for the Development of AD Treatments and Therapies

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## CCD AS A REFERENCE FOR AD

**Abstract**

Alzheimer's Disease (AD) is a growing epidemic, with a new development of AD occurring every 65 seconds (Alzheimer's Association, n.d.). Previously in high-school, I completed surface-level research on the general similarities and differences between Canine Cognitive Dysfunction (CCD)—a disease with symptoms and progression pattern that parallels AD—and decided to continue my research more in-depth in college. I studied how  $\beta$ -amyloid accumulates in various brain regions—such as the prefrontal, occipital, entorhinal and parietal cortices—in canines to determine how  $\beta$ -amyloid accumulation is associated with the development of behaviors signifying cognitive decline observed in CCD and AD—such as changes in the sleep-wake cycle, social interaction, housetraining, and general orientation—in order to understand how neuroscientists may be able to derive new understandings from a canine model with CCD, and apply them to humans with AD to develop therapies and treatments for AD. In order to capture a variety of perspectives when researching CCD and AD, I developed a list of questions to help guide and focus my research. How are canines and humans similar? How are CCD and AD similar? What developmental and progression parallels can be drawn? What treatments are effective for CCD? What treatments are effective for AD? How can shared pathologies in canines and humans allow for the application of CCD treatment on AD? Through my research I discovered that domestic canines and humans share the same environmental stressors, develop oxidative stress in a similar manner, and  $\beta$ -amyloid accumulation in the canine brain parallels  $\beta$ -amyloid accumulation in the human brain. Additionally, canine response to pharmaceuticals mimics human response to pharmaceuticals. Because of this, conducting studies on domestic canines with CCD may be the best animal model for AD. I surmise that future research should be conducted through testing how known CCD treatments (with varying active ingredients and

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constituents) work to suppress  $\beta$ -amyloid accumulation in humans. Treatments previously found to be successful on canines with CCD should be restudied for the purpose of observing how the treatment interacts with canine pathology that is shared with humans in order to better determine how CCD treatments can be manipulated to be efficient in treating AD. The possibility of synthesizing treatments through the use of laboratory canines, testing these treatments on domestic canines to determine effectivity, testing these treatments on laboratory rats to determine safety for human use, and finally manufacturing these treatments for human use could be a treatment development path to make implementation possible.

*Keywords:* Alzheimer's Disease (AD), Canine Cognitive Dysfunction (CCD),  $\beta$ -amyloid, animal reference model, treatment manipulation, behavior, cortices

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Canine Cognitive Dysfunction (CCD), Alzheimer's Disease (AD), and  $\beta$ -Amyloid Accumulation:

Using CCD as a Reference for the Development of AD Treatments and Therapies

### **Introduction**

Alzheimer's Disease (AD) is a progressive form of dementia with no current cure (Alzheimer's Association, n.d.). 5.8 million Americans suffer from AD, with 1 in 3 seniors dying due to complications caused by AD (Alzheimer's Association, n.d.). The amyloid hypothesis is a popular yet controversial theory that identifies  $\beta$ -amyloid as a key cause of AD.  $\beta$ -amyloid is a small fragment of amyloid precursor protein (APP) that forms oligomers, fibrils and plaques that disrupt cell-to-cell communication and activate immune cells, triggering inflammation (Alzheimer's Association, 2017). The amyloid hypothesis states that AD can be guaranteed to occur in individuals with genetic mutation in genes associated with  $\beta$ -amyloid production, causing the formation of  $\beta$ -amyloid plaques (Alzheimer's Association, 2017). Very few treatments and antibodies have been proven to sufficiently slow  $\beta$ -amyloid accumulation in clinical trials, most notably, an aducanumab trial from 2016 was classified as the first successful clinical trial that evidenced slowed AD progression (Alzheimer's Association, 2017).

Canine Cognitive Dysfunction (CCD) is a progressive, neurobehavioral syndrome often referred to as 'doggy dementia' (Cain & Cain, n.d.). While awareness surrounding CCD's existence is minimal, studies have been conducted in which CCD is used to better understand AD, as the two diseases are developmentally similar, and canine pathology mirrors human pathology. CCD is considered a good animal model for AD because canine and human pathology is similar, CCD and AD progress complementary to each other, and canines respond to pharmaceutical treatment in a fashion akin to humans, providing strong predictive validity.

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An example of the shared pathology between canines and humans is the development of  $\beta$ -amyloid oligomers. In the  $\beta$ -amyloid pathology of canines and humans, both canines and humans develop  $\beta$ -amyloid oligomers. Breydo, Kurouski, Rasool, Milton, Wu, Uversky . . . and Glabe (2016) conducted tests—including peptide synthesis, labeling of A $\beta$ 40 with Acrylodan, general A $\beta$  preparation, Western blots, electron microscopy, FTIR, CD spectra, Raman spectroscopy, the determination of site-specific conformational stability, and the determination of global stability (p. 701)—in order to determine and further clarify the structure of A $\beta$  oligomers. Breydo et al. (2016) claimed that an in-depth understanding of the structure of A $\beta$  oligomers is necessary, as A $\beta$  peptides have the ability to penetrate cell membranes and can be classified as a neurotoxic entity that contributes to the progression of AD development (p.700). Breydo et al. claimed that because fibular oligomers (FOs) and fibrils are richer in  $\beta$ -sheet stacking, they are more stable and resistant to GdnSCN denaturation, with fibrils being more stable than FOs) (p. 702). Breydo et al. continued that PFOs are unstructured and disordered with weak intermolecular interactions due to the absence of  $\beta$ -sheets, causing PFOs to denature at lower concentrations of GdnSCN than both FOs and fibrils (p. 702). Breydo et al. stated that the N-terminals of PFOs were completely disordered, while C-terminals indicated the presence of antiparallel  $\beta$ -barrels (p. 705). Zahs and Ashe (2013) supported Breydo et al. (2016)'s claims, stating that identifying the oligomer that causes amyloid cascading is integral to developing treatments and therapies to prevent AD. Research regarding  $\beta$ -amyloid oligomer structure is critical in determining possible therapies, as it is necessary to establish the cause of  $\beta$ -amyloid oligomer deposition in order to determine options to block this spreading. Researchers must identify the structure of  $\beta$ -amyloid oligomers, as it would be extremely difficult to develop treatments and therapies to combat its neurotoxicity. Identifying the oligomer responsible for

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initiating amyloid growth in canines with CCD could be useful when developing treatments and therapies for AD, as oligomer functions may be monitored more easily, and treatments and therapies may be tested and observed before implementation on a test subject with similar pathology and pharmacological responses to humans. In “A Canine Model of Human Aging and Alzheimer's Disease,” Head (2013) conducted a study observing laboratory canines completing a set of complex tasks to determine how behavior dysfunction is linked to the brain's cortices. Head claimed that  $\beta$ -amyloid oligomers are inversely related to the total amount of  $A\beta$  accumulation in the brain in the study.

Because canines share a common environment and environmental stressors, both species develop oxidative stress in a similar manner, contributing to the similar manner in which both species accumulate  $\beta$ -amyloid.  $\beta$ -amyloid accumulation in the prefrontal, entorhinal, and occipital cortices in canines with Canine Cognitive Dysfunction (CCD) parallels that of humans with Alzheimer's Disease (AD), and canines' response to pharmaceuticals closely resembles that of humans, making CCD the ideal reference model to study the early stages of AD, and a solid foundation to begin developing and testing new treatments and therapies. A canine model may prove to be helpful when synthesizing new treatments, however new treatments could be tested first on laboratory rats due to ethical concerns. This is especially apparent in the modern 'cruelty-free' climate, in which drug manufacturers along with cosmetic companies have been criticized by organizations (such as PETA) for performing preliminary testing of products on animals such as rabbits. Texas A&M University faced harsh backlash from the general public after it was discovered that live canines were being used to study Duchenne Muscular Dystrophy (DMD) (People for the Ethical Treatment of Animals, n.d.). In order to comply with the ethical demands of the public, preliminary testing could be conducted on laboratory rats to determine if new

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treatments are safe for use on canines before secondary testing is conducted on laboratory canines and domestic canines.

By determining how  $\beta$ -Amyloid accumulates in various brain regions—such as the prefrontal, occipital, entorhinal and parietal cortices—in canines to determine how  $\beta$ -amyloid accumulation is associated with the development of behaviors signifying cognitive decline observed in CCD, and AD—such as changes in the sleep-wake cycle, social interaction, houstraining, and general orientation, neuroscientists may be able to derive new understandings from a canine model and apply them to AD to develop therapies and treatments for AD. Newly developed treatments could be developed using canine pathology, tested on laboratory rats to determine if the treatment can be safely administered to canines and humans, tested on laboratory canines with CCD to observe how the treatment interacts with canine pathology, tested on domestic canines to observe any differences in effectiveness and function, and human trials can be conducted to determine if any further manipulation of the treatment is needed.

### **Differentiating Between Being Aged vs. Impaired**

Because a definitive line between having the status of aged or impaired in canines and humans has not been exclusively defined, treatments and therapies aimed to slow CCD and AD progression are not prescribed during early stages of each respective disease, lowering the ability of the treatments and therapies to work efficiently. Establishing a specific boundary to mark when a canine or human is merely aging or suffering from cognitive impairment or CCD/AD is necessary when developing treatments and therapies to separate gentler, precautionary measures from harsher, more aggressive treatment intended to be used on those determined to be impaired.

Madari, Farbakova, Katina, Smolek, Novak, Weissova, . . . and Zilka (2015) conducted a study with 300 canines (85 ineligible for observation) in which canines were scored from zero to

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five (zero meaning abnormal behavior was never observed, and five meaning abnormal behavior was observed several times within a week) for 17 items separated into 4 categories; special orientation, social interactions, sleep-wake cycles and house soiling (p.139). Madari et al. (2015) identified the stages of CCD as mild cognitive impairment, moderate cognitive impairment, and severe cognitive dysfunction, and developed an assessment to measure the severity and progression of CCD named CADES (canine dementia scale) (p.138). Madari et al. claimed that the development of the CADES scale for canines was necessary due to the lack of information known about CCD stages and phenotypic variability (p.138). Colliot, Chételat, Chupin, Desgranges, Magnin, Benali, ... and Lehericy (2008) recognized a similar problem in distinguishing between humans with AD and humans with mild cognitive impairment (MCI). In their study, Colliot et al. (2008) segmented the hippocampi of 25 patients with AD, 24 patients with MCI, and 25 healthy elderly and measured the average hippocampal volume for each group to distinguish between patients with AD, patients MCI, and elderly controls (p. 195). Colliot et al. assessed the accuracy of automated hippocampal volumetry in response to the lack of definition between AD individuals and MCI, and determined that hippocampal volume loss was an accurate way to distinguish between those with AD and MCI, as 84% of AD patients were correctly classified by analyzing hippocampal volume loss (p. 200).

Brustrom and Ober (1998) conducted a study in which 40 elderly normal adults and 28 individuals with AD were measured for global cognitive functioning, memory, use of memory strategies, memory strategy efficacy, and depressive symptomatology in order to determine predictors of perceived memory impairment (p.404). Brustrom and Ober aimed to distinguish between aged brains and AD through a methodologically qualitative approach, as memory strategies and efficacy were measured in the elderly (average age of 75) in order to define the

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difference between aging/MCI individuals versus AD individuals (p.404). Brustrom and Ober concluded that while previous studies connecting objective and perceived memory have not been concrete in their findings, the study found that those with less severe AD are more likely to acknowledge deficits in memory when compared to those with more severe AD, and that those who are aging report memory consistent with their memory test scores (p. 408). Brustrom and Ober contest that further research should be done concerning the connections between objective and perceived memory in order to determine whether it may be used as a solid defining factor to separate those who are aging from those with AD, especially as qualitative measures to define aging and AD (p. 409).

Defining set standards to separate whether a canine or human is cognitively impaired rather than aging may significantly impact the efficiency of a treatment or therapy that is prescribed. Because there is not a definite line separating the loss of cognitive function due to natural aging versus having the status of being cognitively impaired, there are many missed opportunities to treat early stages of CCD and AD. Behavioral markers for CCD and AD—such as changes in the sleep-wake cycle, social interaction, housetraining, and general orientation—may be overlooked and attributed to natural aging, despite these symptoms being associated with early stage progression of CCD and AD. The missed diagnosis of early stages of CCD and AD, and the misdiagnosis of CCD and AD as natural aging eliminates the possibility of introducing less aggressive therapies—such as increasing antioxidant content in the diet through supplements such as Aktivait (for canines)—and treatments—such as Donepezil, Galantamine, and Rivastigmine (for humans)—to those affected in order to slow progression of CCD and AD and suppress behavioral symptoms.

### **Similarities Between Human and Canine Pathology**

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Because canines and humans share similar general pathologies, similar  $\beta$ -amyloid pathologies, and display complementary signs of oxidative damage due to sharing the same environmental stressors, canines and humans may share the same factors that contribute to CCD and AD and progress each disease.

$\beta$ -amyloid peptides share nearly identical pathology in canines and humans. In “Plasma B-Amyloid Peptides in Canine Aging and Cognitive Dysfunction as a Model of Alzheimer's Disease,” González-Martínez, Rosado, Pesini, Suárez, Santamarina, García-Belenguer, . . . and Sarasa (2011) conducted a study with 88 canines separated into four groups of (young, middle-aged, cognitively unimpaired aged, and cognitively impaired aged) (p.591). González-Martínez et al. (2011) defined young as one to four-year-old canines, middle-aged as five to eight-year-old canines, and cognitively impaired or unimpaired as less than nine-year-old canines (pp. 591). González-Martínez et al. stated that there were nine young canines, ten middle-aged canines, 31 cognitively unimpaired canines, and 38 cognitively impaired canines observed (88 canines total) (p.591). González-Martínez et al. identified sleep-wake cycle, social interaction, housetraining, and orientation as key areas of cognitive deficit in canines with cognitive impairment, measured levels of plasma  $A\beta$ 1-42 and  $A\beta$ 1-40 in canines with and without CCD, and hypothesized that the respective  $A\beta$  levels would correlate with the canines' level of cognitive impairment (p. 590). González-Martínez et al. claimed that  $\beta$ -amyloid peptide deposition present in CCD parallel plasma  $\beta$ -amyloid, a biomarker for AD, as both evidence similar neurodegeneration and oxidative damage patterns (p. 590). González-Martínez et al. identified plasma  $A\beta$ 1-42 as a relatively controversial biomarker that specifically distinguishes between non-cognitively impaired and mildly impaired humans (p. 591), and created a study to observe the  $A\beta$ 1-42 proposed biomarker in companion canines. González-Martínez et al. asserted that the cognitively

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impaired group of canines had a higher level of A $\beta$ 1-42 accumulation than the cognitively unimpaired group of canines, and claimed that while A $\beta$ 1-42 levels were high in cognitively impaired canines, the mildly impaired canines actually had the highest level of A $\beta$ 1-42 accumulation (pp. 594). González-Martínez et al. hypothesized that the decrease in A $\beta$ 1-42 levels in cognitively impaired canines aged nine years or more may be caused by increasing brain amyloid deposition, however, despite the decrease of A $\beta$ 1-42 in relation to age, mildly impaired canines had higher A $\beta$ 1-42 levels than both unimpaired and severely impaired canines (p. 594). González-Martínez et al. claimed that the amyloid hypothesis, often used to explain the development of AD, is reflected in both mild CCD and mild AD when observing A $\beta$ 1-42 accumulation, making mild CCD a good reference model for mild AD (p.594). Likewise to González-Martínez et al., Head (2013) identified the A $\beta$ 1-42/A $\beta$ 1-40 ratio in cerebrospinal fluid to be a good indicator of cognitive decline (p. 1386) in canines and humans.

The identification of A $\beta$ 1-42 as a potential biomarker in both CCD and AD could prove to be useful in the development of treatments for CCD that can be manipulated to treat for AD. In order to create effective treatments for any disease or condition, researchers (in the case of CCD and AD—neuroscientists) must first establish the cause(s) and biomarkers for the disease or condition. The establishment of biomarkers provides a foundation for neuroscientists to develop a treatment from, as the primary function of the treatment will most likely target the biomarkers of the disease or condition. The identification of a common biomarker between CCD and AD, A $\beta$ 1-42, should make it easier to develop a treatment that is applicable to canines with CCD and humans with AD, as the treatment would only have to target one shared biomarker (A $\beta$ 1-42) rather than multiple different biomarkers. Because A $\beta$ 1-42 exists as a potential biomarker in CCD and AD, a treatment could be developed that specifically targets A $\beta$ 1-42 in

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order to diminish the accumulation of A $\beta$ 1-42, subsequently slowing CCD or AD progression during early stages.

Head (2013) conducted studies with laboratory beagles with a median lifespan of 13.9 years (p.1384). Head identified beagles under five years old to be similar to humans under 40 years old, beagles five to nine-years-old as humans between 40-60 years old, and beagles over nine years as humans 66 years or older (pp. 1384). Head claimed that molecular cascades—such as accumulation of  $\beta$ -amyloid in both the cerebral vasculature and diffuse plaques—advance cognitive degeneration, as seen in mild CCD and AD (p. 1384). Head argued that canine models have more predictive validity when applying CCD test results on humans due to the extreme similarities in pathology, including the identical amino acid sequences coding for A $\beta$  in canines and humans, and the shared presence of A $\beta$  precursor protein APP in both canines with CCD and humans with AD (p. 1384). Head claimed that the spontaneous development of white matter is consistent in both AD and CCD, which is linked to laminin immunoreactivity deficits and increased iron deposits (p. 1385). Head asserted that a loss of myelin observed in aging dogs may leave the frontal cortex vulnerable to white matter, progressing CCD significantly (p. 1385).

In “Canine Cognitive Dysfunction and Alzheimer’s Disease – Two Facets of the Same Disease,” Prpar Michevc and Majdič (2019) claimed that  $\beta$ -amyloid accumulation occurs frequently in cerebral amyloid angiopathy (CAA), which is the accumulation of  $\beta$ -amyloid in blood vessels of the cerebral cortex (p. 6). Similarly, Head (2013) argued that CAA is observed in similar frequency in both canines and humans (p. 1386). Head stated that CAA may create vulnerability in the occipital cortex, leading to impaired vascular function and microhemorrhages, in both canines and humans (p. 1386).

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While canine and CCD pathology mirrors human and AD pathology nearly perfectly, there are minimal differences that should be taken into account when using canines and CCD as a reference for humans and AD. Schütt, Helboe, Pederson, Waldemar, Berendt, and Pederson (2016) conducted a study in which they observed 15 canine brains ranging from normal cognitive function, mild cognitive dysfunction, or CCD, observed amyloid- $\beta$  plaque deposition,  $\tau$  pathology, and inflammatory markers present in the prefrontal cortex, and compared observations from cognitively impaired canine brains to the brain of a young dog, and brain sections from AD patients (control). Schütt et al. (2016) stated that distinct  $\tau$  protein accumulation and histology in canines with CCD was inconclusive, and that no neuritic plaques were found in the canine brains studied, meaning cortical A $\beta$  deposition was denoted as a diffuse subtype. Despite this, Schütt et al. concluded the study by stating that CCD is a model that could provide more insight into AD, especially when concerning A $\beta$  pathology and progression during early AD stages.

Because canines and humans share similar pathology, treatments could be tested on canines with CCD to observe how the treatment could interact with pre-existing pathology. The observations of how treatments interact with the pathology of a canine with CCD could be used to predict how the treatment may interact with the pathology of a human with AD. The testing of new treatments on canines with CCD could prove useful in observing pathological interactions—such as how a treatment may suppress  $\beta$ -amyloid accumulation—to determine if a treatment is effective in slowing CCD and AD progression. If a treatment is not effective in slowing CCD progression in canines, there is a high chance that the treatment will not be effective in slowing AD progression in humans. Because canines and humans share similar pathology, canines with

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CCD provide predictive validity on how effective a treatment may be when introduced to humans with AD.

### **Canines and Humans Share the Same Environment and Environmental Stressors**

Because canines and humans share the same environment and environmental stressors they may subsequently display similar patterns in oxidative damage development. Canines and humans are socially dependent animals that must adapt to adhere to the changing social environment, this creates stress that can cause oxidative damage and contribute to the progression of CCD and AD. Because canines and humans develop oxidative damage complementary to each other, domestic canines may be the best canines to conduct studies to use CCD as a reference for AD.

Oxidative damage and neuroinflammation contribute significantly to the development of  $\beta$ -amyloid deposition, fueling the progression of CCD and AD. While it is most common for researchers investigating CCD and AD to use laboratory canines, it may be more beneficial to use domestic or companion canines because domestic canines share a common environment with humans. Therefore, companion canines and humans may share the same environmental stressors that contribute to oxidative damage and neuroinflammation. In "Identification and Management of Cognitive Decline in Companion Animals and the Comparisons with Alzheimer Disease: A Review," Cory (2013) stated that few studies have been conducted with companion animals rather than laboratory animals, and proposed that future studies should consider varying genetic, cognitive, and environmental factors in order to more similarly reflect AD environments (p. 292). Cory stated that CCD shares similar development patterns of  $\beta$ -amyloid to AD, and hypothesized that the development of  $\beta$ -amyloid is caused by neuroinflammation and oxidative stress caused by the environment (p. 292). Cory claimed that canine sociability mirrors human

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sociability, and contended that studies conducted on laboratory dogs fail to capture these social-environmental factors (cognitive challenges) due to the controlled environment laboratory canines are studied in (p. 292).

The use of laboratory canines excludes environmental stressors that cause oxidative damage, while canines that share the same environment as humans mirror the oxidative damage seen in humans (due to the shared environmental stressors). Cory (2013), and González-Martínez et al. (2011) claimed how the use of laboratory canines may limit the possible connections that could be made between CCD and AD. The development of oxidative stress is often attributed to environmental stressors, so using domestic canines to observe progression would serve as a more accurate model due to its increased similarity (González-Martínez et al., 2011). Since laboratory canines do not share the same environmental stressors as humans due to the extremely controlled conditions in labs, the use of domestic canines instead of laboratory canines may prove to be more useful. Canines with oxidative damage may respond differently to treatments and therapies when compared to canines without oxidative stress, so it is important to conduct studies concerning CCD on domestic canines as it would make results easier to translate to AD application, especially when considering the fact that domestic canines are exposed to the same environmental stressors as humans.

### **Similar Behavioral Changes in Humans and Canines**

Because canines and humans share similar pathology and environmental stressors, both species exhibit similar behavioral deficits—such as reversal learning failure, visuospatial difficulties, and acquisition and recognition decline—as CCD or AD progresses respectively.

Cory (2013) stated that in both canines and humans,  $\beta$ -amyloid accumulation in the cerebral cortex may be linked to behavioral changes (p. 294). Cory stated that specifying

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behavioral markers to identify as cognitive decline may increase the ability to diagnose AD and CCD earlier in progression, and named specific behaviors—such as decreases in adaptability, and increases in anxiety or repetitive behavior—as possible markers (p. 292). Cory identified memory, problem-solving ability, and adaptability to be main areas of decline in AD and CCD, along with social withdrawal (p. 293). Cory claimed the behavioral changes in canines are repetitive and incomplete behaviors, patterns which may repeat anywhere from within minutes to hours throughout a given day (p. 293). Cory claimed that one key behavioral change in the diagnosis of CCD and AD is changes in sleep-wake cycles (p. 294), and identified overattachment and isolation as two main factors relating to social changes that may be used to diagnose AD and CCD. Cory claimed that in both CCD and AD, canines and humans will fail to complete previously learned tasks—such as eating, potty-training, and moving around a known environment (p. 294). Cory asserted that as each respective disease develops, all factors build up to cause a change in behavior and character (p. 294).

González-Martínez et al. (2011)—Like Cory (2013)—identified four key areas of cognitive deficit in CCD canines; sleep-wake cycle, social interaction, housetraining, and orientation (pp. 590). González-Martínez et al. stated that the 2 key areas with the most reported dysfunction in their cognitive status questionnaire—which was completed by the owners of the companion canines observed in the study—were sleep-wake cycle and socio-environmental interactions (pp. 594).

Because canines and humans share four common, key areas of cognitive decline—changes in the sleep-wake cycle, social interaction, housetraining, and spatial orientation—treatments could be developed to target the key areas of decline and be implemented in canines with CCD and humans with AD.

### **$\beta$ -Amyloid Accumulation in Different Brain Regions**

Because  $\beta$ -amyloid accumulates in multiple brain regions—such as the prefrontal, frontal, occipital, parietal, and entorhinal cortices—each region  $\beta$ -amyloid accumulates in causes a specific aspect of cognitive dysfunction to occur—such as visual discrimination deficits, sleep-wake cycle disruptions, and retention errors—depending on which brain region evidenced  $\beta$ -amyloid accumulation.

Cummings, Head, Afagh, Milgram, and Cotman (1996) conducted a study in which eleven purebred beagles from New York and nine mixed breed mongrels from Canada were observed for reward approach and learning approach learning, followed up with discrimination, reversal, object recognition and spatial learning testing in order to determine a correlation with  $A\beta$  accumulation (p. 12). Cummings et al. (1996) claimed that declines in overall cognitive function as well as in behavioral tasks, such as orientation and recognition, were both correlated with  $A\beta$  accumulation (p. 18). Head (2013) came to a similar conclusion, and specified that tasks associated with the prefrontal cortex, such as reversal learning and visuospatial working memory are shown to decline in canines with CCD (p. 1384). Head claimed that memory decline as a function of age may be observed through acquisition, such as object recognition and spatial learning, and occurs early in the aging process in, when canines are approximately six or seven years old (p. 1385). Head asserted that each loss of function in canines may be attributed to cognitive decline in a specific domain of the brain (i.e. prefrontal decay is associated with reversal learning and visuospatial working memory decline) (p. 1385). Head stated that cortical atrophy and ventricular widening are consistent products of cognitive decline, while decline in other domains in the brain may vary, such as in prefrontal cortex and the hippocampus (p. 1385).

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When determining treatment options, connecting the cognitive deficiencies associated with CCD and AD (reversal learning, acquisition, recognition, visuospatial memory, etc.) with  $\beta$ -amyloid accumulation in the prefrontal, entorhinal and occipital cortices is critical when determining the appropriate prescription for canines and humans alike. Treatments and therapies target specific brain regions (such as the prefrontal, entorhinal, and occipital cortices) and work to suppress symptoms associated with each region's decline (such as reversal memory failure, recognition decline, etc.). Identifying the role  $\beta$ -amyloid plays in these cortices to cause these cognitive deficiencies in canines is necessary to begin making hypotheses concerning how CCD treatments may be manipulated to be implemented in AD patients.

A previous study conducted by Head, Callahan, Muggenburg, Cotman, and Milgram (1998)—in which 24 canines (ages ranging from 2.4 years to 13.3 years old) being kept individually in wooden boxes were observed in order to determine the effect of side and object preference on the accumulation of  $\beta$ -amyloid and behavioral dysfunction by the use of two objects and three food wells (p. 416)—elaborated on the prefrontal, entorhinal, and occipital cortices' and lobes' roles in instances of cognitive decline in canines. Head et al. (1998) classified canines five or less years old as young, canines six to nine years old as middle-aged, and canines ten or more years old as old (p. 416). Head et al. claimed that  $\beta$ -amyloid accumulation in the prefrontal cortex was significantly associated with the impairment of reversal learning,  $\beta$ -amyloid accumulation in the entorhinal cortex was significantly associated with the impairment of size-discrimination learning, and  $\beta$ -amyloid accumulation in the parietal and entorhinal cortices was significantly associated with reward-approach learning (p. 420). Head et al. determined that  $A\beta$  accumulation in the occipital lobe is associated with impairment in object-discrimination (p. 420). A study conducted by Head, McCleary, Hahn, Milgram, and

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Cotman (2000)—in which vibratome sections from 40 canines (one group of 20 canines aged 4.5 to 15.3-years-old, and one group of 20 canines aged 6.7 to 17.8-years-old) were collected from the dorsolateral prefrontal cortex, entorhinal cortex, occipital cortex and parietal cortex in order to measure A $\beta$  accumulation and determine a correlation between A $\beta$  deposition in brain cortices and behavioral dysfunction (p. 90)—provided a timeline for  $\beta$ -amyloid accumulation in these cortices. Head et al. (2000) stated that A $\beta$  deposition in the prefrontal cortex frequently began around the age of nine, while A $\beta$  deposition in the parietal, occipital, and entorhinal cortices began around the age of 14, with the lowest A $\beta$  deposition occurring in the parietal cortex (p. 92).

Head et al. claimed that A $\beta$  deposition relationship in the entorhinal and parietal cortices was virtually the same, while A $\beta$  deposition was significantly the highest in the prefrontal cortex and significantly the lowest in the occipital cortex (p.92). Head et al. claimed that the pattern of A $\beta$  deposition found in canines—beginning in the prefrontal cortex, spreading to the entorhinal and parietal cortices, and finally the occipital cortex—is consistent with the A $\beta$  deposition pattern found in humans (p.94). Head et al. stated that canines under 11 years resembled A $\beta$  deposition patterns of those in Stage A of Braak and Braak's (1991) staging scheme—a study in which the stages of AD were clarified—with older canines displaying A $\beta$  deposition in the cortical regions resembled those in Stage B, and older canines displaying A $\beta$  deposition in subcortical regions resembled those in Stage C (canines in Stage B and C were diagnosed with clinical dementia). Head et al. elaborated that only a subset of canines 14 years or younger will experience behavioral deficits associated with the hippocampal or entorhinal cortex functions, and asserted that intervention targeted towards the prefrontal cortex would be best implemented before the age of eight in canines (p. 95).

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Schütt et al. (2016) contested that A $\beta$  pathology in the prefrontal cortex is positively correlated with age, rather than the severity of cognitive decline, bringing into question if CCD and AD are guaranteed with age (p. 433). Contrary to Schütt et al., Head (2013) claimed that cognitive decline is not guaranteed as a result of aging in humans or canines. Both Schütt et al. (2016) and Head (2013) stated that more research should be conducted with the prefrontal cortex, along with other brain regions (such as the entorhinal, parietal, and occipital cortices) that contribute to behavioral dysfunction associated with CCD and AD (such as visuospatial disfunction and memory decline).

Making connections between  $\beta$ -amyloid accumulation in different brain regions and corresponding cognitive decline behaviors in canines and humans may be substantially beneficial when trying to determine if CCD treatments will be applicable to the same brain regions and behaviors or if they will have a different or no effect. AD treatments are often prescribed to decrease the intensity or frequency of a specific behavior associated with AD—such as size-discrimination, reward-approach and object-approach learning decline. Gaining familiarity with  $\beta$ -amyloid accumulation in the canine brain cortices to determine if new treatments and therapies would target the same brain cortex—and subsequently the same behavior—could be immensely useful as it provides a better understanding on how new treatments may differ in function in canines and humans. Determining if a treatment targets the same brain cortex in canines and humans may influence the amount of manipulation needed to make the treatment applicable in CCD and AD while performing the same function and offering the same benefits. Future studies should consider developing connections between how canine brain cortices react to constituents of CCD treatments in comparison to how canine brain cortices react to constituents of AD treatments. Establishing constituents that target the same brain cortex and provide the same

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benefits in canines with CCD and humans with AD could make the synthetization of new treatments that are effective in treating behavioral deficits in CCD more easily applicable to the treatment of the same behavioral deficits in AD.

### **Application of CCD Treatments for Canines on Humans with AD**

Because canines share general and  $\beta$ -amyloid pathology and respond to medicinal ingredients akin to humans (due to paralleling genomes), medication that is used to treat CCD may be manipulated and applied to AD.

In “Nutritional Supplementation in Cases of Canine Cognitive Dysfunction—A Clinical Trial,” Heath, Barabas, and Craze (2007) implemented the nutritional supplement Aktivait into the diets of 20 canines to observe its effects on traditional CCD behaviors in order to compare the therapeutic effects of Aktivait against 24 canines being administered a placebo (p. 287). Heath, Barabas, and Craze hypothesized that the similar pathology between CCD and AD suggests that treatments that work for one disorder could possibly be applied to the other with shared success (p. 284). Heath, Barabas, and Craze identified single ingredient supplements, such as fruit derived polyphenolics which have high antioxidant activity, as an example of treatment that has observed success in relieving AD symptoms that could be implemented in CCD canines, and asserted that nutritional manipulation in the form of adding antioxidants to the diet may improve behavioral defects caused by CCD and AD (p. 285). Heath, Barabas, and Craze identified the antioxidants in Aktivait, such as N-acetyl cysteine,  $\alpha$ -lipoic acid, Vitamins C and E, L-carnitine and Co-enzyme Q10, DHA and EPA, and phosphatidylserine (p. 285). Heath, Barabas, and Craze claimed that vitamins C and E, and NAC protect against oxidative stress, while Q10, selenium and  $\alpha$ -lipoic acid are efficient antioxidants (p. 293). Heath, Barabas, and Craze claimed that the introduction of L-carnitine, acetyl-L-carnitine, and  $\alpha$ -lipoic acid resulted

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in improved overall cognitive function, and should be used as a combination (p. 293). Heath, Barabas, and Craze claimed that behavioral errors previously observed in tasks such as sleep-wake cycle, recognition, and house-training were decreased after the introduction of Aktivait (p. 293). This study by Heath, Barabas, and Craze provides valuable information regarding antioxidant effects on AD and CCD, and may be implemented as a less harsh, more assessable and easily introduced treatment. The connection of each of the constituents of Aktivait (N-acetyl cysteine,  $\alpha$ -lipoic acid, Vitamins C and E, L-carnitine and Co-enzyme Q10, DHA and EPA, and phosphatidylserine) to a specific brain region—such as the parietal, prefrontal, or occipital cortices—is needed in order to fully grasp how nutritional therapy may improve AD and CCD symptoms—such as visuospatial, recognition, and acquisition behavioral deficits. Future studies could be conducted with the introduction of Aktivait into a human diet to determine whether the same benefits would be observed, and how the concentration of constituents can be manipulated to observe a more effective treatment option in humans.

Similar to Heath, Barabas, and Craze (2007)—Cory (2013) claimed in “Identification and Management of Cognitive Decline in Companion Animals and the Comparisons with Alzheimer Disease: A Review,” that canines show a positive response to cerebral perfusion, anxiolytic agents, antioxidants, lifetime cognitive enrichment, and behavioral training (pp. 292). Cory claimed that by participating in neuroprotection and neurogenic practices through cognitive stimulation, canines and humans may have a lessened chance of developing CCD of AD respectively (p. 296). Cory stated that cognitively and socially rich environments positively correlate with maintaining cognitive ability before and after preclinical symptoms have begun to occur (p. 296). Cory continued that as long as cognitively rich environments are introduced in an easing manner without exacerbating clinical symptoms, they would be beneficial in addition to

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medication or lifestyle modifications (p. 296). Cory stated that while biomarkers to identify CCD and AD early on exist, pharmacological treatment can only be prescribed when symptoms hinder a patient's ability to function (p. 297). Cory identified some pharmacological treatments such as memantine, which reduces neuronal death while maintaining physiological function (moderate to severe AD), donepezil which provides short-term solutions concerning cognitive function by increasing acetylcholine by inhibiting acetylcholinesterase, propentofylline, which increases blood flow through the brain and improves social behavior, diazepam and fluoxetine which improve overall function, and selegiline which inhibits monoamine oxidase-B (p. 299). Cory claimed that while nutritional and pharmacological treatments may improve a patient's physiological wellbeing post-diagnosis, nutraceuticals may serve as preventative strategies in the mild stages of AD and CCD (p. 299). Cory stated that because the relationship between physiology and behavior is so convoluted, a balance between neuropathophysiological and epidemiological treatment and therapy must be achieved in order to slow progression and treat symptoms most effectively (p. 298). Cory asserted that additionally, recognizing that pharmacological treatment of each disease in its earlier stages is the key to success, and studying the differences in pharmacological effectiveness on both diseases and species is also necessary (p. 299). Conducting studies in which canines are treated with pharmaceuticals—such as donepezil and fluoxetine—and have neuroprotective practices—such as an antioxidant supplement and cognitive stimulation—implemented simultaneously may assist in determining how the effectiveness of pre-existing treatments can be enhanced. Finding a balance between pharmaceutical treatment and neuroprotective practices in canines with CCD could provide a better understanding for the balance of pharmaceutical treatment and neuroprotective practices in humans with AD.

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In “Canine Cognitive Dysfunction Syndrome: Prevalence, Clinical Signs and Treatment with a Neuroprotective Nutraceutical,” Osella, Re, Odore, Girardi, Badino, Barbero, & Bergamasco (2007) observed 124 canines for the prevalence of clinical signs of CCD in aged canines, and conducted an open-label clinical pilot trial to determine whether clinical signs of CCD decreased with the implementation of neuroprotective nutraceutical Senilife (p. 299). Osella et al. (2007) claimed that canines must demonstrate one or more signs associated with CCD—such as spatial disorientation and confusion, learning and memory disorders, inability to recall previously learned commands, repetitive activity or hypoactivity, difficulties in social interaction, modified sleeping-wake patterns, states of anxiety or restlessness, change in appetite, decline in personal cleanliness, and reduced perception of response to stimuli—to be officially diagnosed with CCD (p.300). Osella et al. identified 39 items to be tested, which were grouped as disorientation (nine items), socio-environmental interaction (13 items), sleep-wake cycles (four items), house soiling (six items) and general activity (seven items) (p.301). Osella et al. stated canines introduced to Senilife showed a marked improvement CCD clinical signs (p.306). Osella et al. identified the constituents of Senilife as phosphatidylserine (PS), standardized Ginkgo bilobaextract (EGb), pyridoxine and d-alpha-tocopherol (p. 306). Osella et al. claimed that the implementation of PS in human patients and laboratory animals improved learning and memory, and it has been proven to be safe through canine testing and human testing (p. 306). Osella et al. stated that EGb stimulates the cholinergic, serotonergic, noradrenergic and glutaminergic system, inhibits MAO A and B, increases dopamine levels, protects the neurons against apoptosis induced by  $\beta$ -amyloid protein, increases brain metabolism, and promotes short-term retention of spatial memory in laboratory canines (p.306). Osella et al. claimed that these benefits may be reproduced when implemented in AD patients. Future studies should attempt to

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confirm Osella et al.'s claim that Senilife benefits may be reproduced in AD patients by implementing Senilife into the diets of humans with AD. Concentrations of the constituents of Senilife should be manipulated in canines with CCD order to determine predictions for the highest efficiency rates in humans with AD.

Laboratory canines and domestic canines may be used as a reference to test newly synthesized treatments for AD. Newly developed treatments could first be developed considering canines pathology, and tested on laboratory rats to determine if the treatment can be safely administered to canines and humans. The treatment could then be tested on laboratory canines with CCD in order to observe how the treatment interacts with canine pathology (which is strikingly similar to human pathology), and  $\beta$ -amyloid pathology. The treatment could move to testing on domestic canines in order to observe any differences in effectiveness and function because of oxidative damage present in domestic canines (caused by environmental stressors shared by humans). Finally, human trials can be conducted to determine if any further manipulation of the treatment needs to be conducted in order to maintain consistent function and efficiency across species. The efficiency of the treatment should be heavily tracked from the laboratory rat test phase to the human trial test phase.

### **Conclusion**

Canines with CCD share similar general and  $\beta$ -amyloid pathology, accumulate  $\beta$ -amyloid in the parietal, prefrontal, entorhinal, and occipital cortices in a similar manner, display common behavioral deficits, and respond to pharmaceuticals in a complementary fashion to humans with AD. Because of this, canines with CCD are the best animal reference to study the progression of AD, and provide a foundation for the synthesization of new treatments with predictive validity. Treatments for canines with CCD—such as Aktivait—should be researched further to determine

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how constituents from CCD treatments can be manipulated for implementation in humans with AD. New treatments for AD could be developed using laboratory canines (due to canines and humans sharing similar pathology), have preliminary testing conducted using laboratory rats (to determine if the treatment can be safely implemented in canines and humans), secondary testing on domestic canines (to observe how the treatment interacts with oxidative damage caused by environmental stressors that are shared with humans), and finally, complete human trials to establish the level of efficiency of the new treatment. From this process, the new treatment may be manipulated in any phase to enhance the efficiency of the treatment until the maximum effectiveness could be reached.

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