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Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging

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Title: Frontal Lobe Volume, Function, and Aβ Pathology in a Canine Model of Aging

Abbreviated Title: Frontal Lobe Volume and Aging in the Beagle

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Abstract

Application of MRI techniques reveals that human brain aging varies across cortical regions. One area particularly sensitive to normal aging is the frontal lobes. *In vitro* neuropathological studies and behavioral measures in a canine model of aging previously suggested that the frontal lobes of the dog might be sensitive to aging. In the present study, MRI scans were acquired to compare age-related changes in frontal lobe volume with changes in executive function and β-amyloid pathology in the frontal cortex of beagle dogs aged 3 months to 15 years. Decreases in total brain volume appeared only in senior dogs (age 12 and older) while frontal lobe atrophy developed earlier, appearing in the old dogs (aged 8-11 years). Hippocampal volume also declined with age but not occipital lobe volume past maturity. Reduced frontal lobe volume correlated with impaired performance on measures of executive function including inhibitory control and complex working memory, and with increased β-amyloid accumulation in the frontal cortex. Age-related hippocampal atrophy also correlated with complex working memory but not inhibitory control, whereas occipital lobe volume did not correlate with any cognitive measure. These findings are consistent with the frontal lobe theory of aging in humans, which suggests that the frontal lobes and functions subserved by this region are compromised early in aging.

Aging is associated with variable changes in brain volume, morphology, and cognitive function. One brain region that may be particularly vulnerable to aging is the frontal cortex. In humans, rates of cortical decline in the frontal lobes are typically greater than rates for the entire brain, hippocampal complex, amygdala, parietal, occipital, and insular cortices (Coffey et al., 1992; Murphy et al., 1996; Raz, 1996; Salat, et al., 1999, 2001; Resnick et al., 2000; Jernigan et al., 2001). Decline is also larger in the dorsolateral and orbitofrontal cortex compared to the frontal pole, anterior cingulate, or precentral gyrus (Jernigan et al., 1991; Raz et al., 1993, 1997; Tisserand et al., 2002).

Patterns of beta-amyloid (Aβ) deposition in healthy aged humans (Braak and Braak, 1997; Bussière et al., 2002) and non-human primates (Struble et al., 1985; Heilbroner and Kemper, 1990) also suggest frontal lobe integrity is compromised early in aging. In aging human brains, Aβ deposition occurs early in the prefrontal cortex (Braak and Braak, 1997; Bussière et al., 2002). *In vitro* studies in beagle dogs report similar variability in Aβ accumulation with the earliest and most consistent deposition occurring in the prefrontal cortex around 8 years of age (Yoshino et al., 1996; Hou et al., 1997; Satou et al., 1997; Head et al., 2000). By contrast, Aβ deposition in the entorhinal, parietal, and occipital brain regions does not appear consistently until after 12 years of age in the dog (Head et al., 2000).

Consistent with the frontal lobe theory of aging, complex cognitive processes such as categorical abstraction, monitoring, manipulation, shifting of set, and inhibitory control, decrease following damage to the frontal lobes or in parallel with age-related decreases in frontal lobe volume in humans, (Milner, 1982; Raz et al., 1998b; Stuss et al., 2001; Gunning-Dixon and Raz, 2003), monkeys (Petrides, 1991, 1995; Dias et al., 1996),

and rats (Iverson and Mishkin, 1970; Birrel and Brown, 2000; Delatour and Gisquet-Verrier, 2001). We previously reported impaired executive functions in aged beagle dogs on measures of inhibitory control (Tapp et al., 2003a), maintenance and manipulation (Tapp et al., 2003b), and concept abstraction (Tapp et al., 2004).

The present study examined the effect of age on frontal lobe volume in young and old dogs using *in vivo* MRI. In an earlier MRI study, decreased total brain volume and increased ventricular volume were observed in aging beagle dogs (Su et al., 1998). Although these findings are similar to ventricular and cortical changes in human MRI studies (Condon et al., 1988; Gur et al., 1991; Sullivan et al., 2002), regional differences in brain volume as a function of age in the dog were not examined. The present study is the first to apply MRI and image processing techniques to selectively examine *in vivo* frontal lobe aging in the beagle dog. Changes in frontal lobe volume were subsequently compared to Aβ deposition and executive functions to develop a more comprehensive analysis of frontal lobe aging in the canine model.

Materials and Methods

Subjects

The study was performed with beagle dogs housed at the University of Toronto (U of T; Ontario, Canada) and the Lovelace Respiratory Research Institute (LRRI; Albuquerque, New Mexico). Animals were housed singly or in pairs in vivarium cages at U of T or in indoor/outdoor kennel runs at the LRRI. Fresh water was provided *ad libitum* and each subject received 300 grams of food (Hill's Pet Nutrition®, Canine Maintenance®, Topeka, KS) daily. Animals were inspected daily by trained veterinary

and behavioral technicians and clinical neurological examinations were performed annually. All dogs were in good health prior to the study and procedures were conducted in accordance with local and federal Animal Care policies.

Imaging Procedures

Magnetic imaging procedures were conducted using a GE-LX 1.5T mobile MRI scanner. MRI scans were collected for 66 beagles (32 males, 34 females) aged 3 months to 15 years of age. During imaging procedures each dog was sedated by a subcutaneous injection of acepromazine (2 mg) and anesthesia was then induced by inhalation of 5% isoflurane and maintained using 2% isoflurane/oxygen gas mixture. Respiration was maintained with a Bellows respirator and heart rate was monitored through an esophageal balloon. Subjects were placed into the magnet bore headfirst in a prone position, and a quadrature-knee RF coil was secured over the head of the animal. Imaging parameters included two excitations (NEX = 2.0), a 256 x 256 matrix, and a 12 cm field of view. T_1-weighted brain images were acquired in the coronal plane using a spoiled gradient (SPGR) pulse sequence (repetition time [TR] = 40 msec; echo time [TE] = 9.0 msec; flip angle = 40^0; slice thickness = 1.2 – 1.4 mm; pixel size = 0.47). Sixty images were acquired for each dog during a 25-minute scan period. After completing the scan, animals were recovered in isolation cages under observation of the facility veterinarian.

Reformatting and Alignment of MR Images

Variations in head tilt, pitch, and rotation were corrected offline using the proprietary software, Analyze® (version 4.0; Mayo Clinic, Rochester, New York, USA)

with standard neuroanatomical landmarks (Talairach and Tournoux, 1988), to bring images into a unified co-ordinate system in all three orthogonal planes. Deviations in head rotation were corrected in the axial plane by adjusting the ocular orbits perpendicular to the interhemispheric fissure. Variations in pitch were corrected by aligning images in the axial and saggital planes along the anterior-posterior commissures. Head tilt was corrected in the axial plane by aligning images with the orbital and auditory canals. All region of interest analyses were performed on reformatted images (Figure 1).

Computer Analysis of MRI Scans

Manual planimetry techniques were used to trace the following regions of interest (ROIs); the frontal cortex (total, left and right), left and right ventricles within the frontal cortex (i.e. frontoventricular volume), total brain volume (including separate ROIs for left and right cerebral hemispheres), total intracranial volume, hippocampal volume and occipital lobe volume.

The frontal cortex was defined in the coronal plane beginning with the olfactory bulbs and continued rostro-caudally through contiguous slices to the anterior cruciate sulcus. The cruciate sulcus was demarcated from a 3-D surface rendering of the entire brain achieved by automatic erosion of nonparenchymal areas in Analyze®. Total frontal lobe volume was acquired by tracing the left and right frontal lobes separately on contiguous slices throughout the entire canine frontal cortex. When visible, left and right lateral ventricles in the frontal lobes were also traced as separate objects.

Total brain volume (TBV) and total intracranial volume (TIV) were defined in the transaxial plane beginning with the first slice at the level of the ventral pons. TBV and

TIV measures were acquired from every third slice ventrodorsally to the superior-most slice in the dorsal plane of the cerebral cortex.

The hippocampus was traced from the most anterior to the most posterior section where the structure was visible. Occipital lobes were demarcated using the superior colliculi as the anterior landmark to the most posterior section displaying the cortex.

TBV was calculated as a proportion of the TIV. Frontal lobe (FLV), frontoventricular (FVV), hippocampal (HCV), and occipital lobe (OCV) volumes were calculated as a ratio of TBV. This resulted in the following brain volume variables for statistical analysis: percent total brain volume [(TBV/TIV) X 100], percent total frontal volume [(FLV/TBV) X 100], percent left frontal lobe volume [(lFLV/left hemisphere) X 100], percent right frontal lobe [(rFLV/right hemisphere) X 100], percent frontoventricular volume [(FVV/FLV) X 100], percent hippocampal volume [(HCV/TBV) X 100] and percent occipital volume [(OCV/TBV) X 100].

Reliability of Regional Volumetric Measurements

All ROIs were performed by the same person (PDT). These measures were repeated by a second rater (CTS) on 30 randomly selected images to obtain intra-class correlation (ICC) measures for all ROIs. ICC reliability estimates for all ROIs exceeded 0.958.

Cognitive Tasks

Cognitive measures were performed within 3-12 months of the MRI scans and were conducted in a 0.609-m x 1.15-m x 1.08-m wooden canine-adaptation of the

Wisconsin General Test Apparatus (Milgram et al., 1994). The battery of cognitive tests included an initial size and reversal discrimination task (Head et al., 1998; Tapp et al., 2003a), a complex working memory task (Tapp et al., 2003b), and a set learning and size concept task (Tapp et al., 2004).

Size Discrimination and Reversal. Inhibitory control was measured using the size discrimination and reversal task. On the size discrimination (SD) task, subjects were presented with two identical red wooden blocks differing only in height. Responses to only one object (i.e. large or small block) resulted in a food reward. Each subject received a total of 10 trials per day and the location of the large and small object was randomized between trials. Half the animals on the SD task were rewarded for choosing the large object, the remaining half were rewarded for choosing the small object. After completing the SD task, all animals were tested on size discrimination reversal (SDR) procedures. Test procedures for this task were identical to the SD task except that the reward contingencies were reversed. Thus, animals previously rewarded for choosing the large block were now rewarded for selecting the small block and animals rewarded for choosing the small block were rewarded for choosing the large block. A total of 46 animals (22 males, 24 females) aged 3-14 years were tested on the SD and SDR tasks.

Spatial List Learning (SLL). The SLL task measured complex working memory processes including maintenance and manipulation of information. Two subtests, SLL and modified SLL (mSLL), were used. Each subtest consisted of three phases per trial. On the SLL task, subjects were presented with a single red coffee jar lid in one of three spatial

locations on phase one of each trial. After a fixed delay (5, 10, 20, or 50 seconds) a second identical coffee jar lid was presented on phase two of the same trial. This second stimulus was presented simultaneously with the first object, in one of the two remaining spatial locations. Only responses to the object in the new spatial location were rewarded. After a second delay period (5, 10, or 20 seconds) a third identical coffee jar lid was presented simultaneously with the first two objects, located in the last remaining spatial location. Only responses to this object were rewarded. Subjects were tested on the SLL subtest for a maximum of 50 days. Testing on the mSLL subtest began the first day following the SLL task. Testing procedures on the mSLL were identical to those used on the SLL task, with the following exception. On phase two of each trial, the second coffee jar lid was presented in the absence of the first coffee jar lid (i.e. only one coffee jar lid was present during phases one and two of each trial). Twelve animals (9 males, 3 females) aged 4-15 years completed the SLL task and 10 of those animals (8 males, 2 females) completed the mSLL task.

Size Learning Set Discrimination and Concept Learning. The size discrimination protocol described above was modified to assess rule induction and concept learning in the dogs. Measurement of these executive functions involved three related tasks: a 2-choice size discrimination learning-set task (2CSD), a 3-choice size discrimination task (3CSD) and a 3-choice size concept task (3CSC). The 2CSD task consisted of six different subtests each involving a size discrimination between two red blocks. Subtest one used the same large and small blocks from the initial SD task. After reaching criterion on subtest one, subjects were tested on the remaining five subtests using a fixed

order of testing. On subtest two, animals were presented with a discrimination problem between a 2- and 3-block stimulus; on subtest three a 3- and 4-block stimulus; subtest four, a 1- and 4-block stimulus; subtest five, a 2- and 4-block stimulus; and subtest six a 1- and 3-block stimulus. Twenty-two animals (9 males, 13 females) aged 4-15 years were tested on the 2CSD task.

Testing on the 3CSD began the first day following the last 2CSD subtest and included 19 of the 22 animals (7 males, 12 females) from the 2CSD task. Stimuli 1, 2, and 4 from the 2CSD subtests were used for this task. Subjects were rewarded for selecting the size-object that was rewarded during the 2CSD task. Spatial locations of all three objects were randomized across each daily session of 12 trials.

The final cognitive measure was the 3CSC task, which consisted of two subtests; the 3CSC-balls and 3CSC-bottles task. On the 3CSC-balls task dogs were presented with three toy soccer balls varying in size (small, medium, and large). Only responses to objects conceptually similar in size to those used on the 3CSD task were rewarded. Thus, animals rewarded for selecting the smallest block on the 3CSD task were rewarded for choosing the smallest toy soccer ball on the 3CSC task. Object locations were randomized between three spatial locations across 12 trials per session. After completing the 3CSC-balls task, subjects were tested on the 3CSC-bottles task in which stimuli consisted of three identical shampoo bottles each varying in height.

Beta-amyloid (Aβ) Quantification

Several months after the MRI scans, 23 dogs (12 males, 11 females; aged 2-15 years) were euthanized and the brains were removed according to a standard protocol.

The tissue was transferred into PBS pH 7.4 with 0.02% sodium azide and stored at 4°C. Aβ was detected using a rabbit polyclonal antibody $A\beta_{42}$, raised against a synthetic β-amyloid peptide (Biosource International, Camarillo, California) that detectsamino acids 1-42 of the Aβ peptide. Forty-μm thick vibratome sections were taken from the prefrontal cortex according to a canine brain atlas (Kreiner, 1966). Sections were pretreated for 4 min with 90% formic acid prior to overnight incubation at room temperature in $A\beta_{42}$ (1:5000) in Tris-saline with 2.0% bovine serum albumin (BSA) and 0.1% Triton X-100. Bound antibody was detected using a biotinylated anti-rabbit ABC peroxidase kit from Vector Labs (Burlingame, CA). $A\beta_{42}$ was visualized using a DAB substrate kit from Vector Labs.

The area occupied by β-amyloid was quantified using gray-scale thresholding procedures (Cummings et al., 1995, 1996). Ten semi-random fields, 5 in the superficial and 5 in the deep layers, of the frontal cortex were centered and digitized at 20X. Staining was captured using a 2.5X photo eyepiece, a Sony high-resolution CCD video camera (XC-77) and the built-in video capture capabilities of a Macintosh 8100/80AV. Using NIH Image 1.59, gray-scale thresholding at a cutoff level of 110 was selected to separate positive staining from background and to calculate the percentage of area occupied by β-amyloid immunoreactivity. Units of Aβ represent the average percentage of 10 individual 525 x 410 μm fields per region occupied by positive Aβ staining and are arbitrary because the magnification chosen directly affects the final value.

Statistics

Regression techniques were used to examine the relationship between frontal lobe volume with age, cognitive function, and Aβ-loads in the frontal cortex. Effects of age and sex on corrected TBV, FLV, FVV, HCV, and OCV were also examined using a general linear multivariate analysis of variance. Post hoc analyses were performed using the Tukey's HSD procedure. All analyses were performed using SPSS version 11.5. Differences were considered significant at $p < .05$ (two tailed) for all tests.

Results

Brain Volumes

Changes in TBV, FLV, FVV and HCV as a function of chronological age are shown in Figure 2. Increasing age was associated with smaller TBV [$r(30) = -.846$, $p = .0001$] and FLV [$r(66) = -.785$, $p = .0001$] and larger FVV [$r(66) = .511$, $p = .0001$]. Increasing age was also associated with decreased HCV [$r(55) = -.401$, $p = .001$]. OCV by contrast, did not vary with chronological age [$r(55) = -.130 = .362$]. To obtain a qualitative description of age on brain volume, male and female subjects were separated into five age groups based on previous criteria (Tapp et al., 2003a): puppies (3 months of age); young dogs (6 months-3.9 years of age); middle-aged dogs (4 7.9 years of age): old dogs (8-11.4 years of age); and senior dogs (11.5 years and older). A significant main effect of age was observed for TBV [$F(4,21) = 36.25$, $p = .0001$], FLV [$F(4,56) = 14.80$, $p = .0001$], FVV [$F(4,56) = 6.07$, $p = .0001$], HCV [$F(4,40) = 14.51$, $p = .0001$], and OCV [$F(4,40) = 2.97$, $p = .031$].

TBV was much smaller in senior dogs (Figure 3) compared to all other groups ($p < .001$). FLV (Figure 4) in senior dogs was also smaller compared to middle-aged ($p = .003$) and young dogs ($p = .0001$) and in old dogs compared to young dogs ($p = .004$). FVV (Figure 5) was also much larger in senior dogs compared to old ($p = .031$) and young ($p = .002$) dogs, and puppies ($p = .005$) but did not differ from middle-aged ($p = .811$). This was likely due to one animal in the middle-aged group whose percent FVV was 1.89 standard deviations greater than average frontoventricular volume for dogs in that group. Removing this animal from the analysis resulted in a significant difference in FVV between senior and middle-aged dogs ($p = .039$).

Corrected HCV (Figure 6) was smaller in puppies compared to young ($p < .001$), middle-aged ($p = .002$), and old dogs ($p = .033$), and in senior dogs HCV was smaller compared to middle-aged ($p = .020$) and young dogs ($p < .001$). HCV in old and senior dogs however, did not significantly differ ($p > .05$). Corrected OCV was larger only in the puppies compared to young ($p = .013$), old ($p = .022$), and senior dogs ($p = .032$).

In contrast to the main effect of age, there was no significant main effect of sex, or interaction between sex and age on any of the corrected brain measures ($p > .05$).

Brain-Behavior Relationships

A relationship between FLV and complex cognitive processes was observed on several indices of executive function. On the initial size and reversal discrimination tasks (Figure 7), errors increased as FLV decreased [$r(46) = -.285$, $p = .037$ and [$r(46) = -.325$, $p = .020$, respectively]. On the mSLL task (Figure 8) larger FLVs were associated with fewer total errors [$r(10) = -.516$, $p = .050$] and higher maximal memory scores [$r(10) =$

.677, $p = .011$]. Percent correct scores on the 2CSD set-learning task generally increased with FLV, but the relationship between FLV and percent correct scores was only significant on the last subtest [$r(22) = .592$, $p = .002$]. A similar trend was also observed on the 3CSC-balls and 3CSC-bottles tasks with percent correct scores on both tasks increasing with FLV (Figure 9) but the relationship was not statistically significant ($p > .05$).

Smaller HCV were associated with increased errors on subtest three of the 2CSD task [$r(35) = -.572$, $p = .008$] and the mSLL task [$r(11) = -.771$, $p = .003$], whereas larger HCV were associated with increased percent correct scores on subtest one of the 3CSC task [$r(18) = .464$, $p = .036$] and higher maximal memory scores on the mSLL task [$r(10) = .767$, $p = .003$]. By contrast, OCV was not related to performance on any cognitive measure ($p > .05$).

Frontal Lobe Volumes and Beta-Amyloid

Regression analyses also indicated a strong relationship between volume of the frontal cortex and Aβ loads in the superficial and deep layers of the frontal cortex (table 1). Smaller FLVs were associated with larger average amyloid loads and amyloid loads in the superficial layer of the frontal cortex ($p < .01$). A similar trend was observed between FLV and levels of amyloid in deeper layers of the frontal cortex, but the correlation did not reach statistical significance ($p > .05$).

Discussion

The present study used *in vivo* MRI and manual planimetry procedures to examine age-related changes in the FLV of beagle dogs. Consistent with an earlier study (Su et al., 1998), TBV decreased with age. From 3 months to 11 years of age, TBV remained stable in the dog. Decreases in TBV however, were observed in dogs aged 12 years and older (i.e. senior dogs). Increased ventricular volume is a hallmark of aging in human MRI studies (Condon et al., 1988; Sullivan et al., 2002). Two previous*in vivo* studies in aging dogs reported increased total ventricular volume with age (Vite et al., 1997; Su et al., 1998); a finding later confirmed *in vitro* (Gonzalez-Soriano et al., 2001). In the present study, the anterior horn of the lateral ventricle was selected as a region of interest because enlargement of this area was unique to older dogs. Quantification of this portion of the ventricles indicated that frontoventriculomegaly was almost two times greater in senior dogs compared to young, old, and middle aged dogs and 13 times larger compared to puppies. Dilation of the anterior portion of the lateral ventricle may represent a hallmark of advanced age in the dog, but it is unclear from the present study if the degree of dilation is any greater than the total dilation of ventricles in old dogs.

Decreased FLV with age was also observed in the dog, consistent with human MRI studies of frontal lobe aging (Coffey et al., 1992; Jenrigan et al., 2001; Murphy et al., 1996; Raz et al., 1993, 1997; Salat et al., 1999, 2001; Tisserand et al., 2002). Age-related changes in FLV in the present study were nonlinear. In puppies, the ratio of FLV to TBV was smaller compared to all other dogs, suggesting that at 3 months of age, the frontal lobes in these animals had not reached full maturity. These findings are comparable to the *in vitro* results reported by Fox (1971), indicating that the beagle brain

does not reach full maturation until 4-6 months of age when myelination of this region is complete. Beyond 6 months of age, frontal lobe volume remained stable until about 7 years of age. Around 8 years of age, a significant decrease in FLV was observed. This age-related decrease in FLV preceded decreases in TBV, which were greatest in the senior dogs aged 12 and older, and temporally corresponds to Aβ deposition in the frontal lobes of the dog (Head et al., 2000). Performance on tests designed to measure executive functions (Tapp et al., 2003a,b, 2004), comparable to those used in human studies, declined with decreasing FLV in aging dogs.

The occipital region undergoes the most rapid development postnatally and is fully matured before the frontal cortex (Fox, 1971). The larger occipital volume observed in the puppies likely reflects this development since no age-associated decrease in volume was observed in the adult dogs. There was no correlation between occipital volumes and cognitive task used in the current study. This suggests that the frontal lobe may be a cortical area particularly sensitive to aging in adult dogs.

Hippocampal volume also showed a decrease with age and similar correlations with cognitive performance as the frontal lobe. This suggests that the hippocampus is also sensitive to aging and may be involved in the cognitive processes engaged by the tasks used. This is not surprising given the importance of the hippocampus in learning and executive function tasks (Winocur and Moscovitch, 1990; Lombardi et al., 1999).

Taken together, the present study revealed several important and novel findings concerning the frontal lobes in aging dogs. First, changes in FLV follow a nonlinear pattern; increasing until middle age and declining thereafter. Second, age-related frontal lobe atrophy occurs early in aging. In the present study, declining FLV was observed at

10 years of age, but may occur even earlier in the dog since there were no animals between 7 and 10 years of age in this study. Third, shrinkage of the frontal lobes in the dog is concurrent with neuropathological changes in the same brain region. Fourth, cortical atrophy of the frontal lobes in aging dogs is associated with decreased performance on measures sensitive to frontal lobe functioning, which also involve other brain regions such as hippocampus.

Mechanisms of Age-related Frontal Lobe Atrophy in the Dog

The results from the MRI volumetric analysis provide the first *in vivo* evidence of decreasing FLV in the beagle dog beginning around 8-10 years of age. Additionally, these changes correlate with increased Aβ deposition in the frontal lobes and impaired executive function.

Our previous work shows that Aβ deposition is age-dependent and correlates with cognition (Head et al., 1998). Further, Aβ is toxic to neurons (Pike et al., 1993) and may be one mechanism mediating cell loss and thus atrophy in the prefrontal cortex. Our observation of a link between frontal atrophy and more extensive Aβ deposition provides further support for this hypothesis. Other structural changes in neurons are also plausible. For example, human MRI studies suggest age-related decreases in FLV represent gray matter loss (Murphy et al., 1996; Raz et al., 1997; Ge et al., 2002) resulting from decreased glycosphingolipids (Kracun et al., 1991, 1992), dendritic arbor (Coleman and Flood, 1987; Terry et al., 1987), or neuronal shrinkage (Coleman and Flood, 1987; Terry et al., 1987). Other studies indicate that white matter loss as early as 60 years of age could be responsible for reduced brain volume (Guttman et al., 1998; Salat et al., 1999,

2001; Jernigan et al., 2001). From the present study, it is unclear if changes in gray or white matter or both account for decreased frontal lobe volume in older dogs. Additional studies are underway to examine white and gray matter aging in dogs as well as markers representing neuron, glial or synapse loss in several brain regions.

Further work also needs to be done to determine whether subregions of the frontal cortex atrophy at equal rates in the aging dog. Studies of age-related changes in subdivisions of the human prefrontal cortex in young, old, and Alzheimer's subjects, indicate that the dorsolateral, inferior prefrontal, and orbitofrontal cortices are more vulnerable to age compared to other frontal subregions such as the precentral gyrus, frontal pole, and anterior cingulate gyrus (Raz et al., 1993, 1997; Xu et al., 2000; Salat et al., 2001; Tisserand et al., 2002).

Additional measures of non-frontal brain regions are also needed to compare rates of regional cortical atrophy across the dog brain. The aging human brain is characterized by shrinkage of the hippocampal complex (Jack et al., 1992; Golomb et al., 1996; Petersen et al., 2000; Wolf et al., 2001), temporal lobes (Coffey et al., 1992; Jack et al., 1998; Black et al., 2000), corpus callosum (Weis et al., 1993; Yamauchi et al., 2000; Hampel et al., 1998; Teipel et al., 2002; Black et al., 2000), and cerebellar vermis (Raz et al., 1992, 1998a). In the present study the occipital lobe showed no change in volume with age past maturity indicating that age-related cortical atrophy occurs only in vulnerable brain regions including the frontal lobes and the hippocampus.

Additional Brain-Behavior Measures in Aging Dogs

MRI studies of human brain aging indicate correlations between region-specific changes in brain volume and cognitive aging (Golomb et al., 1996; Fox et al., 1999; Petersen 2000; Wolf et al., 2001). For example, impaired executive function is concomitant with decreased prefrontal volume in elderly subjects (Raz et al., 1998b; Schretlen et al., 2000; Head et al., 2002; Gunning-Dixon and Raz, 2003), but executive functions also vary with volumetric changes in non-frontal brain regions (Berman et al., 1995; Lombardi et al., 1999; Stuss and Alexander, 2000). Recent tests developed to asses executive function in aging dogs suggested that inhibitory control (Tapp et al., 2003a) maintenance and manipulation of complex information in working memory (Tapp et al., 2003b), and rule induction and concept learning (Tapp et al., 2004), decrease with age. In the present study, decreased FLV was associated with impaired inhibitory control, maintenance and manipulation of complex working memory information, and, to a lesser degree, concept learning and rule induction. These functions are most likely not exclusive to the frontal lobes but involve cortical circuits that include other brain regions, such as the hippocampus. A similar relationship was observed between the cognitive measures and hippocampal volume suggesting that the age-related impairment in executive functions in the beagle could be due to decreased FLV, HCV, or both. Frontal lobe lesions studies are currently in progress to delineate the role of frontal lobes in executive functions in dogs.

The frontal lobes of the dog appear to be particularly sensitive to the aging process based on the neurological, neuroanatomical, and cognitive evidence presented. Further work is required to clarify brain-behavior relationships in the canine model of

aging but these findings strongly suggest that the frontal lobes of the dog play a similar role to that observed in humans and are sensitive to aging.

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Table 1. Relationship between brain volume and amyloid loads in the frontal cortex of the beagle dog

	r	p-value
Superficial Layer	-.504	.007**
Deep Layer	-.319	.069
Total Average	-.384	.035*

*$p < .05$

**$p < .01$

Figure Captions

Figure 1. Contiguous coronal slices from an MRI in a senior (A) and young (B) dog brain through the frontal lobes displayed in a caudal-rostral direction (left to right) from the cruciate sulcus to the anterior pole of the olfactory bulbs. Separate ROIs were calculated for left and right frontal hemispheres and ventricular volumes (arrow) for each subject. Identical planimetry procedures were used to calculate left, right, and total brain volumes brain volume in the transaxial plane for each dog.

Figure 2. TBV (A), frontal lobe volume (B), frontoventricular volume (C), and hippocampal volume (D) as a function of chronological age in the beagle dog.

Figure 3. Mean total brain volume as a proportion of total intracranial volume in puppies, young, middle-aged, old, and senior dogs. Individual values for animals within each group are presented and significantly different groups are indicated by an asterisk (**$p <$ .01).

Figure 4. Mean frontal lobe volume as a proportion of total brain volume in puppies, young, middle-aged, old, and senior dogs. Individual values for animals within each group are presented and significantly different groups are indicated by an asterisk (**$p <$ .01).

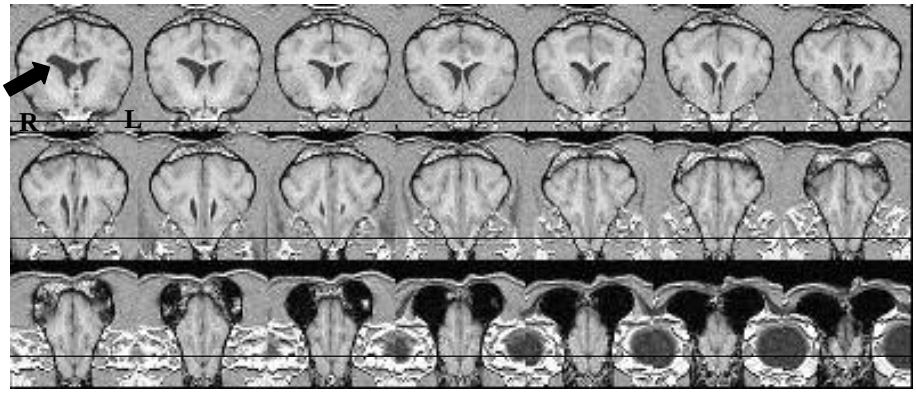
Figure 5. Mean frontoventricular volume expressed as a proportion of total frontal lobe volume in puppies, young, middle-aged, old, and senior dogs. Individual values for animals within each group are presented and significantly different groups are indicated by an asterisk (**$p < .01$).

Figure 6. Mean hippocampal volume expressed as a proportion of total brain volume in puppies, young, middle-aged, old, and senior dogs. Individual values for animals within each group are presented and significantly different groups are indicated by an asterisk (**$p < .01$).

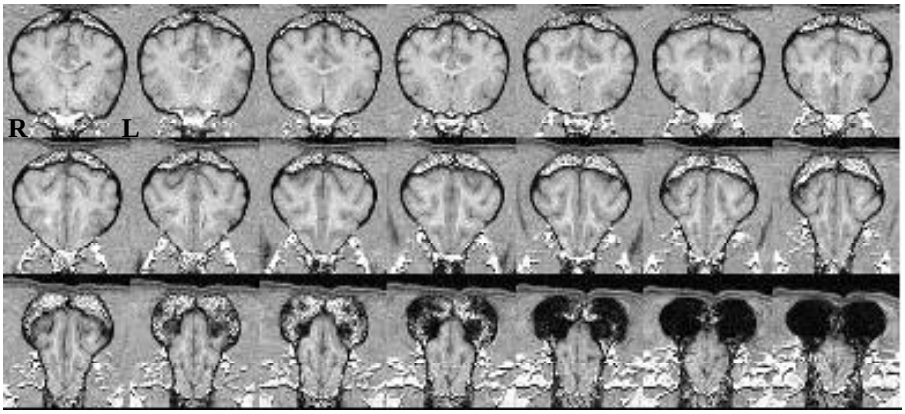
Figure 7. Total errors to criterion on an initial size (A) and size reversal discrimination (B) learning task in 46 beagle dogs as a function of percent frontal lobe volume.

Figure 8. Relationship between total errors to criterion (A) and maximal memory score obtained (B) on a modified spatial list-learning (mSLL) task with percent frontal lobe volume in 10 beagle dogs.

Figure 9. Relationship between percent correct scores on the 3CSC-balls (A) and –bottles (B) task as a function of percent frontal brain volume in 19 beagle dogs.

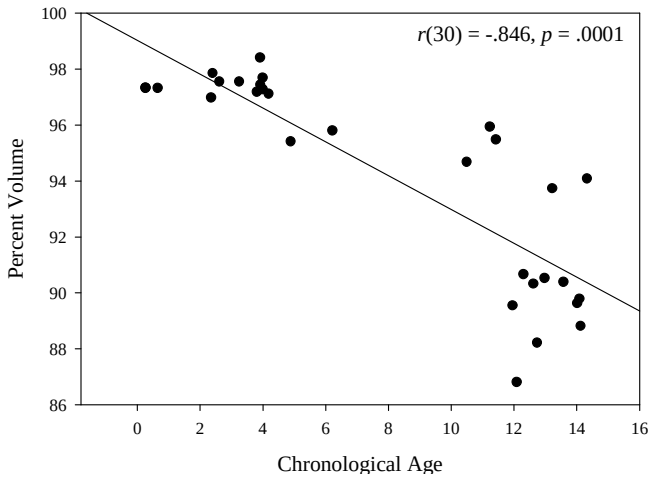


A

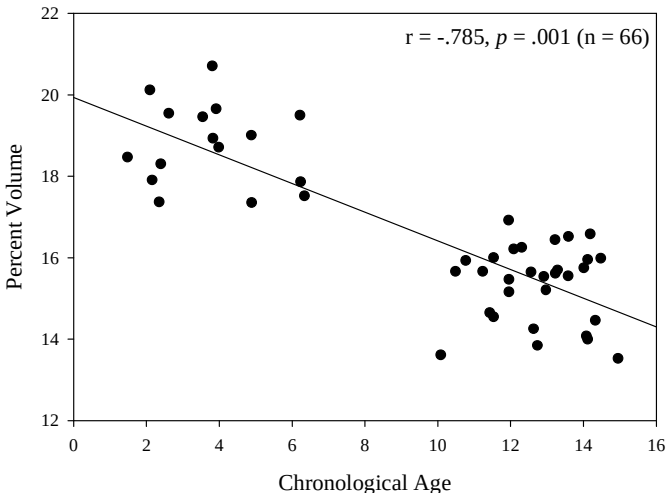


B

Figure 1



A



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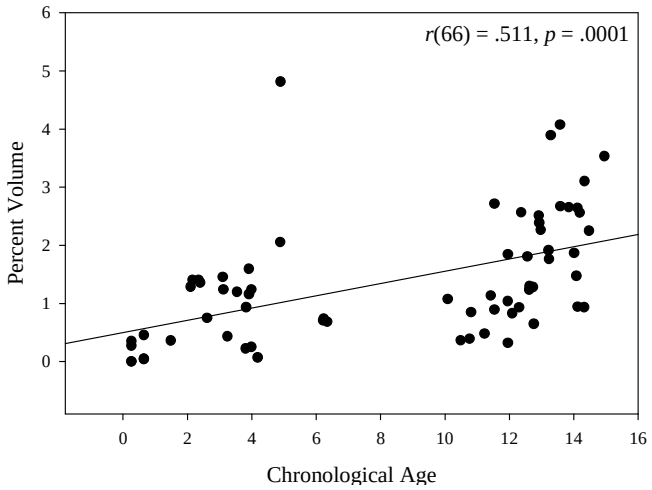
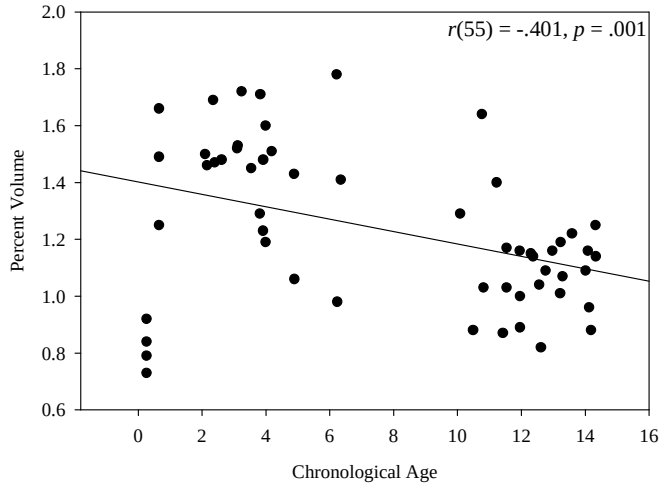


C



D

Figure 2

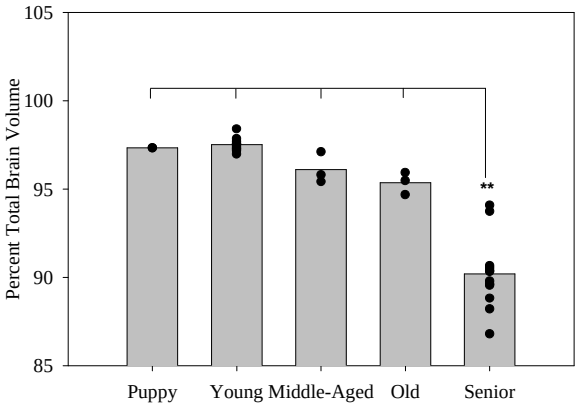
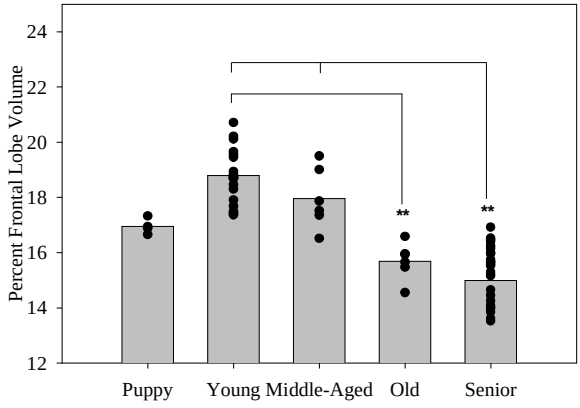


Figure 3



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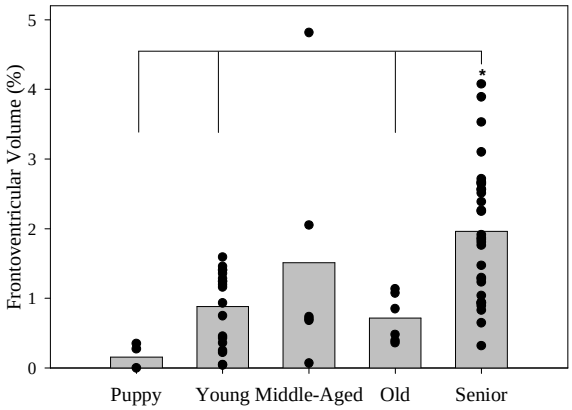

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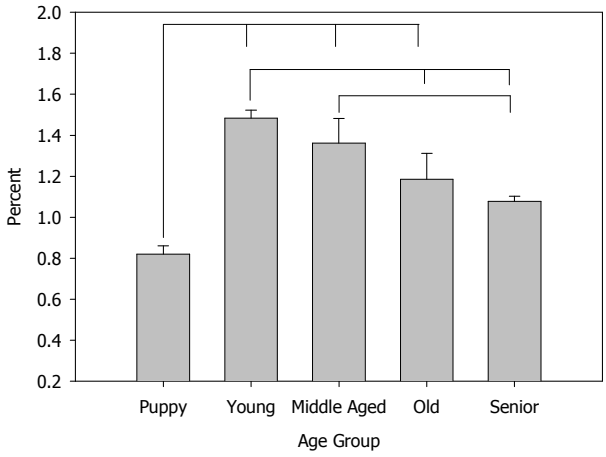

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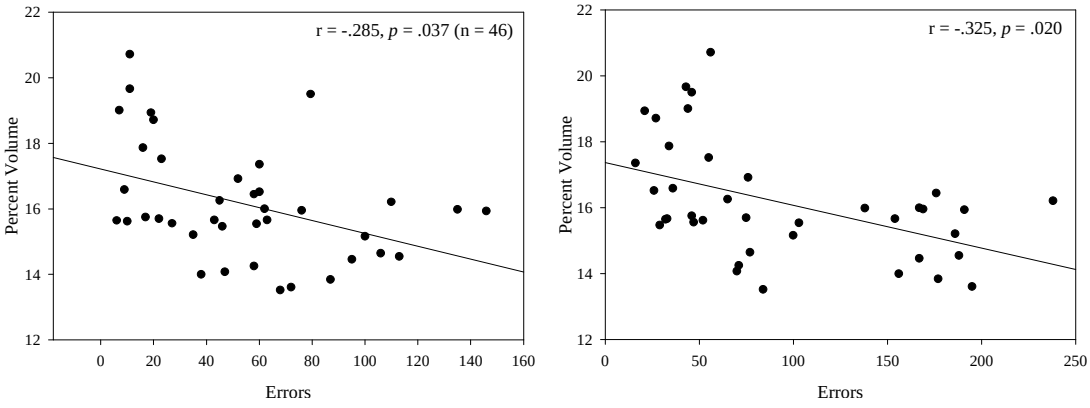
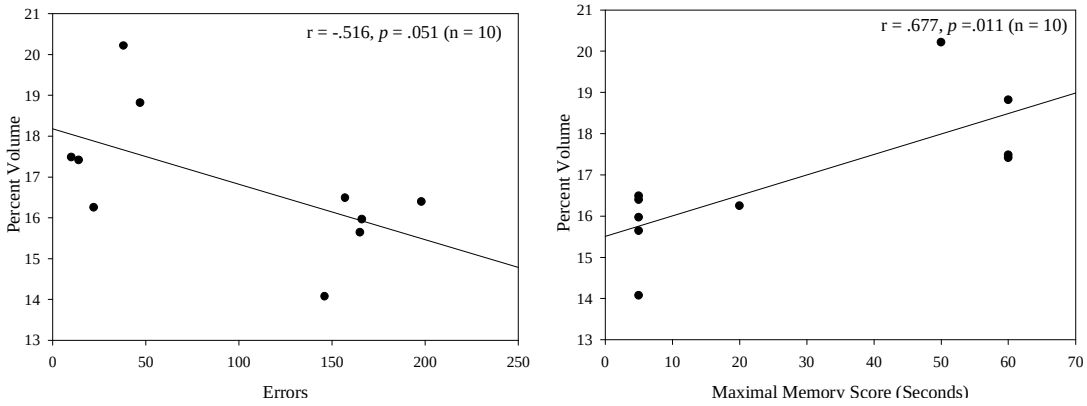


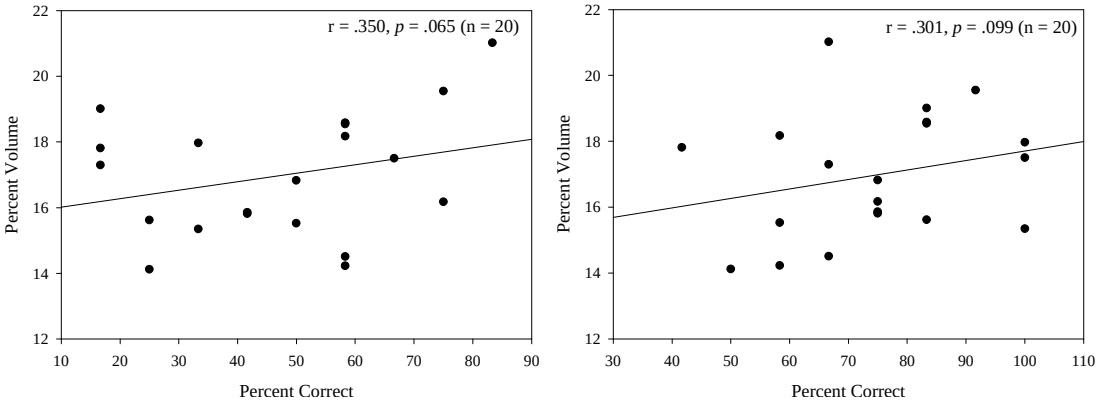
A B

Figure 7



A B

Figure 8


r = .350, p = .065 (n = 20)
Percent Volume
Percent Correct
22
20
18
16
14
12
10
20
30
40
50
60
70
80
90
r = .301, p = .099 (n = 20)
Percent Volume
Percent Correct
22
20
18
16
14
12
30
40
50
60
70
80
90
100
110

A

B

Figure 9