Scaling of the Corpus Callosum in Wild and Domestic Canids: Insights into the
Domesticated Brain

Running head: SCALING OF THE CORPUS CALLOSUM

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ABSTRACT

All domesticated mammals exhibit marked reductions in overall brain size, however, it is unknown whether the corpus callosum, an integral white matter fiber pathway for interhemispheric cortical communication, is affected by domestication differentially or strictly in coordination with changes in brain size. To answer this question, we used quantitative magnetic resonance imaging to compare the mid-sagittal cross-sectional areas of the corpus callosum in 35 carnivore species, including eight wild canids and 13 domestic dogs. We segmented rostro-caudal regions of interest for the corpus callosum and evaluated correlations with brain mass. The results of this study indicate that under the influence of domestication in canids, the corpus callosum scales to brain size in an allometric relationship that is similar to that of wild canids and other carnivores, with relatively high correlation coefficients observed for all regions, except the rostrum. These results indicate that architectural and energetic considerations are likely to tightly constrain variation in caudal components of the corpus callosum relative to overall brain size, however fibers passing through the rostrum, putatively connecting prefrontal cortex, are less constrained and therefore may contribute more towards species-specific

differences in connectivity. Given the species diversity of the Canidae and the resurgence of interest in the brain of the domestic dog, further studies aimed at characterizing the neural architecture in domesticated species is likely to provide new insights into the effects of domestication, or artificial selection, on the brain.

Key words: canids; domestication; scaling; corpus callosum; dogs, white matter; evolution, RRID:SCR-005988; RRID:SCR-003070, RRID:SCR-001905.

1.INTRODUCTION

Through his seminal publication, *The Variation of Plants and Animals under Domestication* (Darwin, 1868), Charles Darwin emphasized that domesticated species are united by a suite of shared behavioral and morphological characteristics that differ quite markedly from that of their ancestral progenitors. This broad, cross-species grouping of characteristics has been referred to as the “Domestication Syndrome” and includes amongst other features: depigmentation in the coat, changes in craniofacial morphology, increased docility, neotenus behavior and changes in the reproductive cycle (Wilkins, Wrangham & Fitch, 2014).

Not surprisingly the brains of domesticated species also share certain similarities, most notably all domestic avian and mammalian species show greater variability in brain size and have smaller absolute brain sizes than that of their wild ancestors and contemporary close relatives (see Kruska, 2005, for a review). While the effect of domestication on the brain and its subdivisions has been studied in many different species (Kruska, 1975; Kruska & Schott, 1977; Leybold, 2000; Schuchaer, 1963; Kruska, 1970; 1972; 1973; Kruska & Stephan, 1973; Schleifenbaum, 1973; Kruska, 1980; Ebinger,

1974; Plogman & Kruska, 1990), our understanding of the characteristic phenotype of the domesticated brain is not complete. For instance, we know very little about the effect of domestication on cortical and subcortical neural structures or if changes in the arrangement of fiber pathways or cellular architecture might explain the distinctive behavioral phenotype of domesticated species (i.e., decreased aggression, increased gregariousness and increased play). We are still far from developing a framework that characterizes the phenotypic response of the brain at different levels of organization under the influence of domestication. Nevertheless, significant advances are being made in comparative behavioral (e.g., Lampe et al., 2017; Naworth & McElligott, 2017; Werhahn et al., 2016; Nawroth, von Borell & Langbein, 2016; Nawroth, Brett, McElligott, 2016; Naworth, Ebersbach & von Borell, 2013; Beausoleil, Stafford & Mellor, 2006) and genomic studies of domesticated animals (e.g., Henricksen et al., 2016; Albert et al., 2012; Lindblad-Toh et al., 2005; Leonard et al., 2002), outpacing our understanding of how the underlying anatomy of the brain has changed.

The study of domestic species serves as a fertile, natural testing ground to evaluate the neurobiological effect of strong artificial selection for the production of behavioral variants in species to be easily handled and socialized by humans. One group of animals that has received a growing amount of attention in recent years has been the domestic dog (*Canis lupus familiaris*). Dogs belong to the phylogenetic group Canidae, a family of extant and extinct dog-like mammals comprising some 16 genera and 36 species (Nowak, 2005). Domestic dogs are thought to have been domesticated as early as 32,000 years ago (Garcia, 2005; Sablin & Khlopachev, 2002; Gemonpre et al., 2009; Lindblad-Toh et al., 2005) and have undergone a relative reduction in absolute brain size

of approximately 29% in comparison to their wolf progenitors (Rohrs & Ebinger, 1978; Schleifenbaum, 1973). This evolutionary transition has occurred relatively quickly on a geological timeframe and poses interesting questions concerning the flexibility and degree of modularity of the canid nervous system. Previous comparative quantitative investigations have revealed that brain structures in the dog have not undergone a uniform change in size as a result of domestication, but, rather are characterized by a mosaic pattern of reduction with certain regions appearing to be more greatly impacted than others (e.g., hippocampus 42% reduction; limbic lobe, olfactory and cerebellum approximately 30% reductions; mesencephalon a 10% reduction) (Kruska & Stephan, 1973; Kruska, 2005, 2007). While this similar pattern of mosaic reduction has been observed in at least six other domesticated species (i.e., rat, gerbil, mink, sheep, llama and pig), it is unknown if this holds true for all domesticated species or if there are particular brain components which are consistently invariant across species.

One brain structure that might prove a useful test case for addressing these questions is the corpus callosum (CC), a large white matter pathway which provides inter-hemispheric connectivity between cortical regions (LaMantia & Rakic, 1990; Wakana et al., 2004) and is known to play an important role in mediating complex behavior (Hofer & Frahm, 2006; Hinkley et al., 2012). The CC is unique to the eutherian mammal group (Johnson et al., 1982 a, b, 1994). Most of the fibers passing through the CC originate from the neurons of the isocortex (mainly dorsal isocortex) allowing for rapid transmission of information by reducing the wiring needed to connect these regions (Butler & Hodos, 2005, Ashwell, 2016). The CC consists of around 200-800 million fibers connecting the two hemispheres, depending on the species (Innocenti, 1986;

Sabine & Frahm, 2006) and even though the number of fibers remains fixed at birth, myelination of these fiber pathways continues throughout puberty (Luders et al., 2010). Although the CC has no clear internal anatomical landmarks or boundaries, the use of geometric partitioning schemes (Witelson, 1989) has helped to segment the CC into several functionally and morphologically distinct subregions (Witelson, 1989; Hofer & Frahm, 2006; Hofer et al., 2008; Chao et al., 2009).

In previous studies, the cross-sectional area of the CC has proved a useful proxy measure for overall CC size (Olivares, Michalland & Aboitiz, 2000; Manger et al., 2010; Ashwell, 2016) as it occupies a large section of the total commissural area (e.g., in the adult human this represents twice the magnitude of the sum of all other commissural structures, Goncalves-Ferreira et al., 2001). Using traditional regression analysis, interspecific comparisons of the CC's cross-sectional area have revealed a strong positive allometric scaling relationship with brain size (Manger et al., 2010), and have helped identify species which are clear outliers (Tarpley & Ridgeway, 1994; Gilissen, 2006). However, none of these earlier studies aimed at comparing CC dimensions across a wide range of mammalian Orders have used phylogenetic comparative methods (such as PGLS) to take into account evolutionary relatedness among species in this scaling relationship (see Phillips et al., 2015 for an application to Primates). In addition, the widely used segmentation system from Witelson (1989) has only been applied infrequently to the study of the CC in a cross-species comparison (e.g., Olivares et al., 2005) and to date no study has explicitly compared scaling relationships in the corpus callosums of wild and domestic species to evaluate if domestication resulted in any concomitant restructuring of this fiber pathway. Thus, the present study is aimed at

examining the scaling of the midsagittal cross-sectional dimensions of the corpus callosum relative to brain size in the Carnivora, including wild and domestic Canidae.

2. MATERIALS AND METHODS

2.1. Specimens

This dataset consists of 131 subject data points, representing 100 eutherian mammalian species (of which there are 20 Carnivora and 7 Canids). Data were derived from three major sources: 1) Primary data obtained through Magnetic Resonance Imaging (MRI) Image acquisition from whole brains scans; 2) Image analysis of the midsagittal surface of fixed mammalian brains housed in collections at Kent State University (M.A.R); the George Washington University (C.C.S) and Des Moines University (M.A.S); and 3) published data collated from the literature. Two of the wild canids included in this study (*Vulpes Vulpes* and *Canis latrans*) were wild-caught whereas the remaining four canid species (*Canis lupus lupus*, *Lycaon pictus*, *Chrysocyon brachyurus* and *Vulpes zerda*) were raised in captivity. A complete species list and relevant sources used in this study is included in Table 1, Figure 1 and Table 3. Below we provide detail on the image acquisition process.

2.2. MRI acquisition

Within 14 hours of each subject's death, the brain was removed and immersed in 10% formalin at necropsy. Magnetic resonance imaging of the postmortem brain specimens was performed on the whole brains of 28 Carnivora [including 13 domestic dogs; two maned wolves (*Chrysocyon brachyurus*), one fennec fox (*Vulpes zerda*), one red fox (*Vulpes vulpes*), one European wolf (*Canis lupus lupus*), one coyote (*Canis*

latrans), four African Painted dogs (*Lycaon pictus*), two Siberian tigers (*Panthera tigris*) and one snow leopard (*Panthera uncia*). MR images were obtained through ongoing collaborations with two imaging sources: 1) The Department of Radiology, Icahn School of Medicine at Mount Sinai using the following approach: All post mortem brain tissue was removed from the storage solution post fixation (10% buffered formalin), placed in Fomblin solution to mitigate the magnetic distortions and packed in guaze in a special container for MRI acquisition on a 7 T Bruker Biospec MR System. The anatomical scan used was a 3D FLASH (Fast Low Angle Shot) sequence with the following parameters: TR (Time to Repetition) = 36 ms, TE (Time to Echo) = 23 ms, flip angle=15°, FOV (Field of View) = 128×128×175, matrix size = 384×384×384, 20 averages, slice thickness = 0.5mm. 2) Images were also acquired through an ongoing collaboration with Fitzpatrick Referrals Ltd and the University of Surrey. All MR images were obtained for diagnostic purposes and with the owners consent on radiologically normal adult dogs (see Table 1) These remaining scans were obtained using a 1.5 T Siemens machine (Symphony, Enlargen, Germany). The sequence used was T2 weighted with the following parameters: TR = 3450 ms, TE = 95 ms, flip angle=150°, NEX (Number of Excitations) 2, FOV = 384×384×15, matrix size = 320x323, slice thickness = 1.5 mm,. Resulting DICOMS were converted to Analyze format and loaded into Analyze Version 10.0 (www.analyzedirect.com, RRID:SCR-005988) for post processing and image analysis. Using the Region of Interest (ROI) and editing tools in Analyze 10.0 the intensity was optimized and the image was rotated for both visualization and analysis.

To expand our sample size, we derived CC dimensions from digital images of the midsagittal hemi-sections of 10 fixed mammalian brains, comprising the following

species: African leopard (*Panthera pardus pardus*), bactrian camel (*Camelus bactrianus*), bongo (*Tragelaphus eurycerus*), clouded leopard (*Neofelis nebulosa*), coyote (*Canis latrans*), giraffe (*Giraffa camelopardalis*), Malay chevrotain (*Tragulus napu*), domestic sheep (*Ovis aries*), reindeer (*Rangifer tarandus*) and bontebok (*Damaliscus pygargus*). All images were taken with a standard digital camera with a scale bar included in the field of view. Images were imported into the image processing freeware ImageJ (<https://imagej.nih.gov/ij/>, RRID:SCR-003070) where images were calibrated before using the polygon tool to outline and measure the CC dimensions. Data on the cross-sectional area of the CC for other eutherian mammals were collated from the literature and included in our analysis (see Table 1).

2.3. Image preprocessing and tracing regions of interest

MR images were loaded into Analyze 10.0 and preprocessed by resectioning using the orthogonal sectioning module, to ensure that a horizontal line segment could be drawn directly through the anterior and posterior commissures in the sagittal view. Using the ‘fly tool’ the midbody of the CC was adjusted to lay straight horizontally in the sagittal view. Image intensities were adjusted for visualization. The output function was then used to reformat the entire volume and the reformatted scan was saved to the workstation. Using the ROI module an investigator (A.U.) scanned through the slices until a clearly visible midsagittal section of the CC, with accompanying fornix and cerebellum was found. The CC was then traced using the spline tool and the total area was measured and recorded for further comparison. Rostro-caudal ROIs were measured using the approach outlined in the Analyze guide for vertical division of the CC following protocols used in previous studies (e.g., Witelson, 1989; Olivares, Michalland

& Aboitiz, 2008). In accordance with this approach we used the grid tool to divide the selected total corpus callosum area into 30 equal sections for calculating the fractions of interest for segmentation: namely 1/2, 1/3 and 1/5th (see Fig 2a-e). Some examples of the comparative midsagittal cross-sectional areas for select species are shown in Figure 3.

2.4. Limitations, validation and inter-observer variability

This study is reliant on data generated from a number of different resources (i.e., published reports, 2D digital photographs and MRI for the Carnivores). The varied nature of these data raises the potential for some confounding effects due to variation in imaging protocol or measurement. In our analysis, caution was taken to ensure that all reported values from multiple sources were internally consistent (for each species) prior to inclusion in the final dataset (No data points were excluded in this analysis). Several recent studies have demonstrated the strong alignment between histological and MRI imaging data in the human and rat brain (e.g., Leergaard et al., 2010; Seehaus et al., 2015), thus validating our use of multiple imaging sources in the current investigation. Furthermore, primary data generated in the current study was compared between observers to make sure that the levels of inter and intraspecific repeatability were within an acceptable range. To validate our method of image alignment, resectioning and area measurement, five specimens were randomly selected for comparison and the borders of each area independently delineated by a second observer (M.A.S.) blind to the results of the first (A.U). Inter- observer variability was assessed using the concordance correlation coefficient of reproducibility (Lin, 1989). High levels of congruency were observed between each observer (i.e., all measurements fell within the accepted range of 0.90 and 0.99 for concordance correlation coefficient of reproducibility), suggesting that the

approach of image alignment and resectioning as well as the boundary designations between observers were reliable and covaried in a systematic fashion. Because of the wide range of mammalian brain sizes included in this dataset (i.e., orders of magnitude of approximately 10,000 fold differences), small errors or variation due to methodological differences are expected to have a minimal impact on the observed scaling relationships.

2.5. Data analysis

Linear regression analysis (Ordinary Least Squares and Phylogenetic Generalized Least Squares) was performed on the data, to evaluate the scaling of CC area against brain mass (in Mammals, Carnivores and within Canids). In accordance with the approach used in all previous comparative scaling studies of the CC (Rilling & Insel, 1999; Janke et al., 1997; Olivares et al., 2000; Manger et al., 2010), all areas and weights were normalized by logarithmic transformation to correct for dimension of units (Smith, 2005). To ensure that our results were directly comparable with that in the literature we did not undertake further geometric correction of the data (i.e., cube-root the areas and square-root the masses as performed by Manger et al., 2000). Thus our expected allometric relationship when comparing an area (e.g., CC area) against a volume (e.g., brain mass) is $2/3 = 0.67$ and all scaling exponent results (shown below) are compared relative to this value.

To account for the effects of species relatedness in the sample, phylogenetic generalized least squares (PGLS) analysis was performed using the ‘caper’ package (Orme et al. 2013) in R Version 3.4.1 (www.r-project.org/, RRID:SCR-001905) from data based on the mammalian super-tree (Bininda-Emonds et al., 2007, 2008). The phylogeny used in the current study is shown in Figure 1. We performed PGLS

regression analysis on three sets of data: mammals, carnivores, and canids. Domesticated dogs were excluded from all PGLS regressions. Preliminary tests were conducted on a combined set of wild and domestic canids with domestic canids in an unresolved polytomy as the sister taxon to wolves to incorporate phylogenetic uncertainty into the canid PGLS. The results of these preliminary tests differed minimally from those using wild canids only, and thus we report results from the analysis without the domestic canids. An analysis of covariance (ANCOVA) was used to test for differences in the slope and intercept of OLS regressions between wild and domestic canids and recovered no significant difference between the two groups. The raw data used to derive these relationships are shown in Table 1 and Table 3.

3. Results

3.1. Scaling of whole CC area with brain size across mammals and carnivores.

The expected allometric relationship between an area and a volume is $2/3 = 0.67$. Least squares regression analysis (OLS) revealed a positive allometric relationship between brain mass and whole CC area in mammals (slope = 0.88, 95% CI = 0.85–0.92, p-value < 0.001), whereas in carnivores, the slope was lower (slope = 0.66, p-value < 0.001). However, the 95% confidence interval for the slope in carnivores encompasses isometry (0.526–0.796). Brain mass explained 96% of the variance in CC area across mammals and 85% of the variance in carnivores (see Table 2 and Fig. 4). Similar patterns of correlation and scaling were observed when accounting for phylogeny using PGLS (see Table 2). Lambda values for both the Mammalian and Carnivore regression line indicate the presence of a phylogenetic signal in the data (i.e., $\lambda > 0$). After phylogenetic

correction, 89% of the variance in Mammalian CC area is explained by variation in brain mass whereas 85% of the variance in Carnivore CC area is explained by brain mass. The only mammalian species that appeared to be a clear outlier in our comparisons was the Pyrenean desman (*Galemys pyrenaicus*) which had a markedly larger CC area than would be predicted for its brain size (Fig 4). Speculation as to why this might be the case is beyond the scope of the current study but this observation points to further analysis in this unusual group of mammals.

3.2. Scaling of whole CC area with brain size in Canids (domestic vs wild).

To test whether the scaling of CC area in domestic dogs aligns with that predicted from wild Canids (e.g., wolves, foxes and coyotes), we derived regression analyses based solely on the subset of wild Canids and overlaid the domestic dog data points for visual comparison (See Fig. 4). Subsequent, regression analysis of whole CC area against brain mass for the Canidae revealed a positive allometric relationship (OLS slope = 0.82) with 92% of the variance in CC area attributed to variation in brain mass (both OLS and PGLS). Although the data points for the domestic canids were somewhat scattered around the canid regression line, all the data points lay well within the 95% prediction intervals (see Table 2 and Fig. 4). All PGLS slopes calculated in the above analyses had confidence intervals that encompass that for the OLS slopes and all the slopes and intercepts were significant. In the carnivore OLS and PGLS models, the slopes are ~ 0.67 in line with predictions for isometry. The confidence intervals for PGLS and OLS slopes in the canid and carnivore models also encompass 0.67, indicating that the two variables are defined by an isometric relationship. A visual inspection of the scatter plot (Fig. 4c) suggests slope differences in the scaling of CC area and brain mass for domestic canids

versus wild canids, but more data are required to specifically test this conclusion. An intraspecific comparison of whole CC area scaling within domestic dogs revealed a positive allometric relationship (OLS slope = 1.13) with 62% of the variation in the total mid sagittal area of the CC explained as a result of brain mass (Table 4 and Fig. 6h).

3.3. Scaling of CC subcomponents with brain size in canids (domestic vs wild).

For the Canidae, regression analysis once more revealed a positive allometric relationship between brain mass and CC sub-components (Fig. 5 a-g). The rostral mid-body (C3) had the highest coefficient of determination of 0.80 while all other divisions caudal to C3 had coefficients of determination ranging between 0.68 and 0.73, indicating that 68-73% of the variance in these CC regions could be explained by variation in brain mass. Components rostral to C3, the rostrum (C1) and the genu (C2) had coefficients of determination of 0.21 and 0.73 respectively, indicating that 21% and 73 % of the variance in size of these CC regions could be explained by brain mass. Intraspecific scaling in a sample of domestic dogs (Fig. 6, a-h, Table 4) indicated a similar pattern of low correlation coefficients for the more rostral Witelson regions with only 6% (C1) and 38% (C2) of the variation in CC area explained by variation in brain mass. More caudal Witelson regions (i.e., C3 to C7) had moderate coefficients of determination ranging between 0.47-0.62. Also observed were some notable breed differences in the size of the CC subcomponents (Fig. 6) as well as shape differences (Fig. 7).

4. Discussion

4.1. Scaling of whole CC area with brain size across mammals and carnivores.

To facilitate the direct comparison of our results with that in the literature we did

not perform geometric adjustment on the CC area and brain mass data. As a result isometry in our comparisons is expected to be $2/3$ (i.e., 0.67) in accordance with that of previous studies (e.g., Rilling & Insel, 1999; Janke et al., 1997; Olivares et al., 2000) but differs from that of Manger et al (2010). Here we discuss our findings relative to these earlier observations. Our findings show that the CC scales with brain size across mammals as well as within the carnivores, becoming proportionately larger in large brained animals due to positive allometric scaling (i.e., slope of > 0.67). While the OLS regression for the carnivores is slightly below isometry, phylogenetic correction using PGLS adjusts the slope to 0.71, which coincides with the pattern of positive allometry observed for the mammalian line. This pattern of scaling is in agreement with that reported in previous studies examining both inter and intraspecific variation in CC area in a wide range of species (e.g., Rilling & Insel, 1999; Janke et al., 1997; Olivares et al., 2000). Olivares et al (2000) obtained an allometric exponent of 0.76 (i.e., positive allometry on non geometrically corrected data) in their interspecific comparison of seven mammalian species, indicating as we have here, that CC area becomes proportionately larger in large brained animals. Similarly, Rilling and Insel (1999) obtained a slope of 0.70 for CC scaling against brain mass (using non-geometrically corrected data) using a sample of 11 species of primates. This observation of a 0.70 scaling exponent for the relationship between CC area and brain mass in primates, aligns well with our result of positive allometry (i.e., slope > 0.67) for all mammals. Although Manger and colleagues (2010) applied a geometric adjustment to their data, these authors also observed a positive allometry between the area of the CC and brain mass in eutherian mammals.

4.2. Scaling of whole CC area with brain size in Canids (domestic vs wild).

Our findings show that regression analysis of whole CC area against brain mass for the Canidae is also characterized by a positive allometric relationship with brain size becoming proportionately larger in large brained canids. Although the data points for the domestic dog were somewhat scattered around the canid regression line, they still lie well within the prediction intervals, suggesting that a similar positive allometric relationship may be extended to domestic canids. A visual inspection of the scatter plot (Fig. 4c) suggests slope differences in the scaling of CC area and brain mass for domestic canids versus wild canids. An inspection of the residuals confirmed that the scatter pattern for these two variables is random. One likely explanation for such a difference in scaling could be due to the taxon level effect (Pagel & Harvey, 1989) with the regression line for the Canidae representing an interspecific comparison which would differ from the intraspecific comparison for domestic dogs. Our analysis of whole CC scaling within domestic dogs, revealed a positive allometric relationship (OLS slope = 1.13) with 62% of the variation in the total mid sagittal area explained as a result of brain mass (Table 4 and Fig. 6h). This pattern of positive allometry aligns with that observed for the wild canidae, albeit at a much steeper slope for the domestic canids. Olivares et al (1999) also obtained similar correlation coefficients in their intraspecific comparison of CC scaling attributes for domestic dogs ($r = 0.67$; slope = 0.60).

4.3.1 Scaling of CC subcomponents with brain size in canids (domestic vs wild).

The use of a partitioning scheme subdivides the CC into functionally and morphologically distinct subregions, arranged from rostral to caudal (Hofer et al., 2008) and corresponding with the crossing points for fibers originating from particular brain areas (Witelson, 1989). In accordance with this scheme, comparative studies have

revealed that fibers originating from prefrontal, premotor and supplementary motor cortical areas cross the CC at the level of the rostrum, genu and rostral body respectively, whereas fibers originating in the motor cortex cross at the level of the rostral midbody (Olavarria et al., 1988; Hofer & Frahm 2006; Phillips & Hopkins, 2012). The caudal midbody serves as the crossing point for fiber bundles heading towards the somatosensory and caudal parietal cortex, whereas fibers passing through the isthmus and splenium connect the temporoparietal and occipital cortices, respectively (Fabri et al., 2014). The composition and fiber density of the CC is also known to vary in accordance with the topographical organization of the cortex. The most rostral part of the CC (i.e., rostrum and genu) is known to contain the highest density of thin myelinated axons connecting the prefrontal cortex and higher order sensory areas (Hofner & Frahm, 2006). Fiber density decreases along the rostro-caudal plane, moving from the genu towards the midbody of the CC before increasing again in the splenium, (i.e., the caudal part of the CC) connecting areas within the visual cortex/occipital lobe (Hofner & Frahm, 2006).

In our analysis of carnivores, we observed relatively low coefficients of determination for C1 (rostrum) but otherwise relatively high correlation coefficients for C2 (genu), C3 (rostral body), C4 (midbody), C5 (caudal body), C6 (isthmus) and C7 (splenium). Our findings indicate that regions C2- C7 are highly invariant from a scaling perspective and that species differences in the size of these subcomponents are largely a result of the robust scaling with brain size. In contrast, the lower correlation with brain mass observed for the C1 (rostrum) indicates that fibers emerging from rostral cortical areas (e.g., prefrontal cortex) are less constrained by their scaling relationship with brain size and may be more variable in density and composition. While preliminary, this

observation suggests that the rostral components of the CC may be a fruitful search space to uncover potential species-specific differences in connectivity or microstructure (i.e., axon composition) which might underlie some of the unique behavioral observations attributed to domestic dogs (e.g., Rossano et al., 2014; Persson et al., 2015; Bradshaw et al., 2015).

Within humans, several studies have shown that variation in behavioral as well as cognitive domains are directly related to variation in the underlying white matter. For example, differences in the underlying white matter have been associated with variation in reaction time (Walhovd & Fjell, 2007), attentional states (Niogi, 2010), reading performance (Schmithorst, 2005) as well as early learning (Als et al., 2004, Mabbott et al., 2006) and senescence (Salthouse, 2009, Zhao et al., 2015). In support of this, observations of white matter scaling across mammals have shown that the proportion of neural tissue dedicated to white matter also varies according to brain size, a point highlighted by the fact that white matter makes up only 12% of cortical volume in a mouse but more than 50% of cortical volume in the human (Zhang & Sejnowski, 2006). The fact that white matter makes up a disproportionately larger part of large brains has led to the suggestion that the enlargement of white matter volume in humans (especially in the prefrontal cortex) likely accounts for our unique cognitive attributes as a species (Schonemann et al., 2005; Smaers et al 2010).

These results illustrate the potential interplay of constraints and adaptation in patterning CC morphology, with the majority of variation in CC size being attributed to brain size, whereas the rostrum appears to display increased non-allometric variation under the influence of natural or artificial selection. The rigid scaling attributes of the

caudal components of the CC emphasize the strong constraints (developmental, energetic or architectural) governing the expansion of white matter, an observation in favor of the contingencies placed on organismal structure (e.g. Gould & Lewontin, 1979; Finlay & Darlington, 1995; Cheung & Darlington, 2005; Finlay, Darlington & Nicastro, 2008; Manger, Spocter & Patzke, 2013, Charvet et al., 2013). White matter volume and brain metabolic rate have been shown to scale predictably with brain and body size (Hofman, 1988; West et al., 1997; Harrison et al 2002), suggesting that variation in these components is governed by in relation to each other due to the energetic costs of maintaining the brain's long-range wiring. Among the several constraints to consider when looking at changes in the amount of underlying white matter are limitations on space, time and metabolic requirements, all of which uniquely challenge the evolution of mammalian brains, especially large brained mammals (Neves, 2017).

In large brains, action potentials must travel longer distances to their targets, thus imposing a limitation on the speed of interhemispheric transfer (Ringo et al., 1994; 1995). Several studies have looked at associated changes on axonal functioning (e.g., Wang et al., 2008; Hirsch, 1939; Hoffmester et al., 1991) and have collectively demonstrated that local adaptations (through the relaxing of scaling restrictions for certain white matter components) have played an important role in adjusting processing speed and function. Examples of this uncoupling of scaling relationships with brain size and associated behavioral specializations have been argued to be the result of adaptations (e.g., Barton & Harvey, 2000; Iwanuik et al 2004). It is likely that a similar process resulting in the uncoupling of scaling attributes for particular white matter components allowed for the increased variation observed in the rostrum of the canid CC. Here we postulate that the

observation of increased variation in the canid rostrum is an example of such local adaptations in the fiber pathway, likely related to changes in projection fibers heading towards the caudate nucleus and prefrontal cortex. In both dogs and humans, the caudate nucleus has been shown to play an integral role in behaviors involving social reward and reward-based behavioral learning (e.g., Haruno et al., 2004; Cook et al., 2015; 2016; Wake & Izuma, 2017) Although follow-up analysis using histological tracing and diffusion tractography (across a variety of canid species) is necessary to conclusively show a species level difference in the fiber pathways passing through the rostral CC our results taken in the context of prevailing literature strongly support this hypothesis.

4.3. Interpretational considerations and limitations of subcomponent comparisons

The C1 segment is considerably smaller than that of the other subcomponents, occupying on average only about 1.5% percent of the total midsagittal CC area (Table 5). This raises the possibility that the diminutive size of the C1 subcomponent might make it prone to measurement error, resulting in lower coefficients of determination. To test this possibility, we reran a series of OLS regressions on the combined C1 + C2 segment (i.e., rostrum & genu), to see if the low correlation coefficients persisted. C2 contributes around 20% of the total midsagittal CC area while C1 only contributes 6% of the total C1 + C2 area, thus occupying a relatively small area of the overall C1+C2 segment. We anticipated that if size was a contributing factor, the correlation coefficients would increase dramatically for this C1 + C2 segment, driven by the larger sized segment. However, correlation coefficients for the C1 + C2 segment in domestic dogs remained low ($r^2 = 0.064$) and in line with that observed for the C1 segment alone (Table 6). Similarly, in the wild canid sample, the correlation coefficients for the C1 + C2 segment

remained at around 0.20, indicating that the strength of the correlation coefficients were not driven by the size difference in the C1 segment. Furthermore, the delineation of the combined C1 + C2 segment in sub-structure definition II (See Figure 2) is more straightforward and reduces the effect of any measurement area on the much larger segment. A further limitation of the current study is the sample size for the domestic and wild canids, raising the question of whether we have adequately captured the species-specific variation for these regions. While this remains a general challenge for the field of comparative neuroanatomy, we believe our results align well with that of previous studies and serve as a good baseline comparison of the CC in wild and domestic canids.

In conclusion, the present study provides an important first step towards comparing the morphology of the CC in carnivores and evaluating the potential impact of domestication on brain structure and connectivity. Further comparative studies that examine white matter fiber pathways using other imaging techniques such as diffusion tensor imaging and electron microscopy are required to narrow down species differences in connectivity and how this might relate to differences in function.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: MAS, PRM. Collection of and qualitative analysis of data: AU, MAS, PRM, CCS, CT, JN, VW,

PRH, BW, KB, JH, MAR, CR, JJ. Statistical analysis and interpretation: RHD, MAS, CCS, PRM. Procurement, preparation, and fixation of tissue: AU, MAS, PRM, CCS, CT, JN, VW, PRH, BW, KB, JH, MAR, CR, JJ. Obtained funding: MAS, PRM. Drafting of the manuscript: MAS, PRM, CCS, CR, CT, PRH, MAR. Photomicrography and preparation of figures/tables: AU, MAS. Critical revision of the manuscript for important intellectual content: MAS, PRM, CCS, PRH, MAR, CR. Study supervision: MAS.

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FIGURE LEGENDS

FIGURE 1. Phylogeny used in the implementation of phylogenetic generalized least squares (PGLS). PGLS was performed using the caper package (Orme et al., 2013). The phylogeny was constructed using data based on the mammalian super-tree (Bininda-Emonds et al., 2007, 2008).

FIGURE 2. An outline of the process used for vertical division of the corpus callosum in Analyze 10.0. The approach is applied using the ROI tools Auto trace, Grid Divider and Placer modules which help to define the cross sectional border of the CC followed by division into 7 substructures. The approach of sub-dividing the CC into 7 sub-components is a standard approach in this area of research and is based on that documented by Witelson (1989). a) A representative image showing the use of the Auto Trace tool to define the borders of the CC in the mid-sagittal sections. Note prior to defining the borders of the CC, all MR images were first preprocessed by resectioning (ensuring that a horizontal line segment could be drawn directly through the anterior and posterior commissures in the sagittal view) so that the midbody of the CC was adjusted to lay straight horizontally in the sagittal view, b) A representative image and schematic showing the vertical division of the CC into 30 equally spaced regions as applied using the Grid Divider Tool in the ROI module, c) A representative image showing the initial sub-structure definition used to assign each of the 30 regions to their appropriate components (i.e., splenium, isthmus, caudal midbody, rostral midbody, rostral body, rostrum, genu). The reassignment of each region in the substructure definition I was

performed using the Objects Tool in the ROI module of Analyze 10.0. The six most caudal sections were assigned to the splenium, four sections rostral to the splenium were assigned to the isthmus, five sections rostral to the isthmus were assigned to the caudal midbody while 5 section rostral to this landmark were included in the rostral midbody. The remaining sections were assigned to the rostral body, d) A representative image showing the substructure definition II used to parcellate the object map of the rostral body into the genu and rostrum. Using the Placer Tool in the ROI module, a Placer window was placed over the genu and a new object was defined thus subdividing the area into three regions (i.e., genu, rostrum and rostral body). Each subcomponent was then renamed using the Object Define tool, e) A representative image showing the final completed midsagittal cross-sectional area of the corpus callosum as well as CC region subdivisions in the maned wolf (*Chrysocyon brachyurus*). Scale bar = 10 mm.

FIGURE 3. Images through the mid-sagittal area of the CC for select species. Note the shape differences. a) African leopard (*Panthera pardus pardus*); b) Siberian tiger (*Panthera tigris tigris*); c) African Wild dog (*Lycaon pictus*); d) Domestic pig (*Sus scrofa domestica*); e) domestic sheep (*Ovis aries*); f) Giraffe (*Giraffa camelopardalis*). Scale bar = 5 mm.

FIGURE 4. Regression analysis of the cross-sectional area of the corpus callosum plotted against brain mass in a range of mammals. Data used to derive these relationships are shown in Table 1. OLS = ordinary least squares regression; PGLS = phylogenetic generalized least squares. OLS lines are plotted in gray, PGLS lines are in black. Dashed

black lines represent 95% confidence intervals of the PGLS lines. The cross-sectional area is strongly correlated with brain mass both across all mammals and within carnivores. a) Cross-sectional area of the corpus callosum plotted against brain mass in all mammals with carnivores highlighted, lines represent the relationship across all mammals; b) Cross-sectional area of corpus callosum plotted against brain mass in carnivores, with wild and domestic canids highlighted, lines represent the relationship for all carnivores with domestic canids excluded; c) Cross-sectional area of corpus callosum in wild and domestic canids. Note that only OLS regression lines are plotted. OLS = ordinary least squares regression; PGLS = phylogenetic generalized least squares.

FIGURE 5. Regression analysis of the cross-sectional area of CC sub-components (i.e., Witelson regions) plotted against brain mass in canids. a) Cross-sectional area of CC sub-component (C1 = rostrum) plotted against brain mass in the Canidae. Note, the weak regression statistics with only 21% of the variation in area C1 being explained by brain mass. In contrast areas C2 to C7 are shown to be highly invariant and have high coefficients of determination with brain mass; b) Cross-sectional area of CC subcomponent (C2 = genu) plotted against brain mass in the Canidae; c) Cross-sectional area of CC subcomponent (C3 = rostral body) plotted against brain mass in the Canidae; d) Cross-sectional area of CC subcomponent (C4 = rostral midbody) plotted against brain mass in the Canidae; e) Cross-sectional area of CC subcomponent (C5 = caudal midbody) plotted against brain mass in the Canidae; f) Cross-sectional area of CC subcomponent (C6 = isthmus) plotted against brain mass in the Canidae; g) Cross-sectional area of CC subcomponent (C7 = splenium) plotted against brain mass in the Canidae. OLS =

ordinary least squares regression. Filled circles = wild canids; unfilled circles = domestic dogs.

FIGURE 6. Regression analysis of the cross-sectional area of CC subcomponents (i.e., Witelson regions) plotted against brain mass in domestic dogs. a) Cross-sectional area of CC sub-component C1 (rostrum) plotted against brain mass in the dogs. Note, the weak regression statistics with only 0.6% of the variation in area C1 being explained by brain mass. In contrast areas C2 to C7 have fairly moderate coefficients of determination ranging between 0.38 to 0.62; b) Cross-sectional area of CC subcomponent C2 (genu) plotted against brain mass in dogs; c) Cross-sectional area of CC subcomponent C3 (rostral body) plotted against brain mass in dogs; d) Cross-sectional area of CC subcomponent C4 (rostral midbody) plotted against brain mass in dogs; e) Cross-sectional area of CC subcomponent C5 (caudal midbody) plotted against brain mass in dogs; f) Cross-sectional area of CC subcomponent C6 (isthmus) plotted against brain mass in dogs; g) Cross-sectional area of CC subcomponent C7 (splenium) plotted against brain mass in dogs; h) Cross-sectional area of CC (C1 to C7) plotted against brain mass in dogs. OLS = ordinary least squares regression.

FIGURE 7. Representative images of the CC cross-sectional area in a sample of domestic dogs. Note all images were increased to the same size so that shape differences could be looked at visually. Although some shape differences were observed a larger sample size is required to determine if any of these are indicative of breed specific morphology. Note the English bull dog shown in “l” was not included in the study sample but is shown here

to highlight marked hydrocephaly and expanded lateral ventricle which distorts the CC morphology. Special care was taken to omit any animals from the study that had obvious neurological signs disturbing the underlying anatomy of the brain and ventricles. a) Maltese; b) Beagle ; c) Golden retriever; d) Cocker spaniel; e) Maltese cross; f) Poodle (Mini/Toy); g) Chihuahua; h) English springer spaniel; i) Curly coated retriever; j) English springer spaniel; k) Rhodesian ridgeback; l) English bull dog. Scale bar = 10 mm.

Corpus callosum definition

a

Corpus callosum division

b

Sub-structure definition I

c

Sub-structure definition II

d

e

a

b

c

d

f

g

h

i

j

k

l

V

Table 1: Species list, associated brain mass data and sources included in the current study. 1 = current study, 2 = Manger et al., 2010, 3 = Hakeem et al., 2005; 4 = Anthony, 1938; 5 = Shoshani et al., 2006; 6 = Rilling and Insel, 1999; 7 = Fears et al., 2009; 8 = Bauchot and

Order	Species	Brain mass (g)	CC area (cm2)	Source
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Stephan, 1961; 9 = Saban et al., 1990; 10 = Tarpley and Ridgway, 1994; 11 = Stephan et al., 1991.

Artiodactyla	<i>Hippopotamus amphibius</i>	530	1.79	4
Artiodactyla	<i>Camelus dromedarius</i>	526	1.74	4
Artiodactyla	<i>Giraffa camelopardalis</i>	509	1.80	2
Artiodactyla	<i>Rangifer tarandus</i>	287	1.35	4
Artiodactyla	<i>Oryx dammah</i>	244	1.17	1
Artiodactyla	<i>Ovis aries</i>	114	1.54	1
Artiodactyla	<i>Bos taurus</i>	183	0.95	1
Artiodactyla	<i>Sus scrofa domestic</i>	69	0.76	1
Artiodactyla	<i>Equus caballus</i>	306	1.91	1
Artiodactyla	<i>Bactrian camel</i>	659	2.46	1
Artiodactyla	<i>Tragelaphus eurycerus</i>	389	1.41	1
Artiodactyla	<i>Giraffa camelopardalis</i>	675	2.16	1
Artiodactyla	<i>Tragulus napu</i>	8.4	0.11	1
Artiodactyla	<i>Rangifer tarandus</i>	235	1.36	1
Afrotheria	<i>Loxodonta africana</i>	5145	8.52	2
Afrotheria	<i>Loxodonta africana</i>	5250	10.19	2
Afrotheria	<i>Loxodonta africana</i>	4835	9.19	2
Afrotheria	<i>Loxodonta africana</i>	4027	12.80	3
Afrotheria	<i>Elephas maximus</i>	4460	8.09	4
Afrotheria	<i>Elephas maximus</i>	5220	12.57	5
Carnivora	<i>Mustela putorius</i>	8.3	0.07	2
Carnivora	<i>Neofelis nebulosa</i>	73	0.52	1
Carnivora	<i>Panthera pardus pardus</i>	156	1.69	1
Carnivora	<i>Lutra lutra</i>	34	0.37	4
Carnivora	<i>felis caracal</i>	54	0.43	4
Carnivora	<i>Neofelis nebulosa</i>	68	0.56	1
Carnivora	<i>Hyaena hyaena (striata)</i>	198	0.82	4
Carnivora	<i>Helarctos malayanus</i>	217	1.16	4
Carnivora	<i>Panthera leo</i>	231	1.16	4
Carnivora	<i>Ursus arctos</i>	400	2.37	4
Carnivora	<i>Ursus maritimus</i>	470	2.33	4
Carnivora	<i>Vulpes zerda</i>	17	0.19	1
Carnivora	<i>Vulpes vulpes</i>	44	0.49	1
Carnivora	<i>Chrysocyon brachyurus</i>	84	0.93	1
Carnivora	<i>Chrysocyon brachyurus</i>	92	0.98	1
Carnivora	<i>Canis latrans</i>	67	0.48	1
Carnivora	<i>Lycaon pictus</i>	126	1.07	1
Carnivora	<i>Canis lupus</i>	130	0.98	1
Carnivora	<i>Canis lupus familiaris</i> (Toy Poodle)	45	0.42	1

Carnivora	<i>Canis lupus familiaris</i> (Chihuahua)	48	0.36	1
Carnivora	<i>Canis lupus familiaris</i> (Maltese cross)	56	0.42	1
Carnivora	<i>Canis lupus familiaris</i> (Mini Poodle)	59	0.40	1
Carnivora	<i>Canis lupus familiaris</i> (Cocker Spaniel)	60	0.47	1
Carnivora	<i>Canis lupus familiaris</i> (Beagle)	62	0.39	1
Carnivora	<i>Canis lupus familiaris</i> (Beagle)	70	0.87	1
Carnivora	<i>Canis lupus familiaris</i> (Beagle)	71	0.63	1
Carnivora	<i>Canis lupus familiaris</i> (Maltese)	41.76	0.42	1
Carnivora	<i>Canis lupus familiaris</i> (English spaniel)	75.16	0.72	1
Carnivora	<i>Canis lupus familiaris</i> (Golden retriever)	80.58	0.63	1
Carnivora	<i>Canis lupus familiaris</i> (Golden retriver)	80.78	0.62	1
Carnivora	<i>Canis lupus familiaris</i> (English spaniel)	82.39	0.68	1
Carnivora	<i>Canis lupus familiaris</i> (Curly coated retriever)	83.79	0.94	1
Carnivora	<i>Canis lupus familiaris</i> (Rhodesian Ridgeback)	98.33	0.76	1
Carnivora	<i>Canis lupus familiaris</i> (Pitbull mix)	55.7	0.26	1
Carnivora	<i>Canis lupus familiaris</i> (Pitbull)	77.8	0.44	1
Chiroptera	<i>Rousettus aegyptiacus</i>	2.6	0.02	2
Chiroptera	<i>Miniopterus schreibersii</i>	0.27	0.004	2
Insectivora	<i>Tenrec ecaudatus</i>	2.59	0.009	11
Insectivora	<i>Setifer setosus</i>	1.52	0.007	11
Insectivora	<i>Hemicentestes semispinosus</i>	0.84	0.005	11
Insectivora	<i>Echinops telfairi</i>	0.62	0.002	11
Insectivora	<i>Geogale aurita</i>	0.13	0.001	11
Insectivora	<i>Orzorictes talpoides</i>	0.58	0.01	11
Insectivora	<i>Microgale dobsoni</i>	0.56	0.01	11
Insectivora	<i>Microgale talazaci</i>	0.77	0.01	11
Insectivora	<i>Limnogale mergulus</i>	1.15	0.002	11
Insectivora	<i>Potamogale velox</i>	4.15	0.06	11
Insectivora	<i>Micropotamogale ruwenzorii</i>	1.13	0.01	11
Insectivora	<i>Chrysochloris stuhlmanni</i>	0.74	0.01	11
Insectivora	<i>Solenodon paradoxus</i>	4.72	0.03	11
Insectivora	<i>Echinosorex gymnurus</i>	6.08	0.07	11

Insectivora	<i>Hylmys suillus</i>	1.2	0.014	11
Insectivora	<i>Atelerix algirus</i>	3.26	0.03	11
Insectivora	<i>Erinaceus europaeus</i>	3.37	0.02	11
Insectivora	<i>Hemiechinus auritus</i>	1.88	0.02	11
Insectivora	<i>Desmana moschata</i>	4	0.05	11
Insectivora	<i>Galemys pyrenaicus</i>	1.33	0.21	11
Insectivora	<i>Talpa europaea</i>	1.02	0.01	11
Insectivora	<i>Parascalops breweri</i>	0.88	0.01	11
Insectivora	<i>Scalopus aquaticus</i>	1.31	0.02	11
Insectivora	<i>Sorex alpinus</i>	0.26	0.004	11
Insectivora	<i>Sorex araneus</i>	0.22	0.003	11
Insectivora	<i>Sorex cinereus</i>	0.17	0.003	11
Insectivora	<i>Sorex fumeus</i>	0.24	0.005	11
Insectivora	<i>Sorex minutus</i>	0.12	0.002	11
Insectivora	<i>Microsorex hoyi</i>	0.1	0.002	11
Insectivora	<i>Neomys anomalus</i>	0.28	0.003	11
Insectivora	<i>Neomys fodiens</i>	0.33	0.004	11
Insectivora	<i>Blarina brevicauda</i>	0.39	0.005	11
Insectivora	<i>Cryptotis parva</i>	0.25	0.003	11
Insectivora	<i>Anourosorex squamipes</i>	0.39	0.003	11
Insectivora	<i>Crociodura flavescens</i>	0.41	0.004	11
Insectivora	<i>crociodura russula</i>	0.2	0.002	11
Insectivora	<i>Suncus etruscus</i>	0.06	0.001	11
Insectivora	<i>suncus murinus</i>	0.38	0.004	11
Insectivora	<i>Scutisorex somereni</i>	0.64	0.006	11
Insectivora	<i>Sylvisorex granti</i>	0.17	0.002	11
Insectivora	<i>Sylvisorex megalura</i>	0.19	0.002	11
Insectivora	<i>Ruwenzorisorex suncoides</i>	0.37	0.005	11
Insectivora	<i>Myosorex babaulti</i>	0.36	0.006	11
Macroscelididae	<i>Elephantulus myurus</i>	1.27	0.02	2
Myrmecophaga	<i>Myrmecophaga jubata</i>	87	0.61	4
Perissodactyla	<i>Diceros bicornis</i>	531	2.52	2
Perissodactyla	<i>Diceros bicornis</i>	550	2.39	4
Perissodactyla	<i>Equus caballus</i>	585	2.43	4
Pinnipedia	<i>Odobenus rosmarus</i>	1250	1.89	10
Pinnipedia	<i>Phocartos hookeri</i>	488	1.75	4
Pinnipedia	<i>Monarchus albiventer</i>	345	1.01	4
Primates	<i>Homo sapiens</i>	1345.66	6.90	6
Primates	<i>Pan paniscus</i>	322.4	2.73	6

Primates	<i>Pan troglodytes</i>	349.44	2.77	6
Primates	<i>Gorila gorilla</i>	397.31	2.96	6
Primates	<i>Pan pygmaues</i>	421.55	3.19	6
Primates	<i>Hylobates lar</i>	85.99	1.07	6
Primates	<i>Papio cynocephalus</i>	148.46	1.24	6
Primates	<i>Macaca mulatta</i>	81.95	1.03	6
Primates	<i>Cercocebus atys</i>	102.77	1.02	6
Primates	<i>Saimiri scireus</i>	23.93	0.44	6
Primates	<i>Chlorocebus aethiops</i>	70.29	0.89	7
Primates	<i>Galago demidovii</i>	3.38	0.03	8
Primates	<i>Perodictus potto</i>	14	0.16	8
Primates	<i>Lemur macaco</i>	23.6	0.21	9
Primates	<i>Daubentonia madagascarensis</i>	45.15	0.37	9
Primates	<i>Lagothrix lagothrica</i>	101	0.39	9
Primates	<i>Papio hamadryas</i>	201	1.16	9
Rodentia	<i>Tatera brantsii</i>	1.61	0.03	2
Rodentia	<i>Thryonomys swinderianus</i>	13.75	0.13	2
Rodentia	<i>Hystrix africaeaustralis</i>	38.87	0.38	1
Rodentia	<i>Hydrochaeris hydrochaeris</i>	60	0.45	4
Sirenia	<i>Trichechus inguinus</i>	188	0.89	4
Tupaiaidae	<i>Tupaia glis</i>	3.2	0.03	8

Table 2: Summary of the regression statistics for CC area versus brain mass obtained using phylogenetic generalized least squares regression (PGLS) and ordinary least squares regression (OLS).

Group	Model	λ	Adjusted R^2	Slope	SE	CI Slope	t-value	P	Intercept	SE	t-value	P
Mammals	OLS		0.96	0.881	0.018	0.845– 0.916	49.020	0.000	–1.959	0.031	–64.22	0.000
Mammals	PGLS	0.607	0.89	0.828	0.030	0.769– 0.888	27.517	0.000	–1.911	0.065	– 29.599	0.000
Carnivores	OLS		0.85	0.661	0.064	0.526– 0.796	10.300	0.000	–1.487	0.138	–10.76	0.000
Carnivores	PGLS	0.583	0.85	0.711	0.070	0.564– 0.858	10.200	0.000	–1.618	0.161	– 10.075	0.000
Canids	OLS		0.92	0.823	0.105	0.531– 1.116	7.813	0.001	–1.713	0.193	–8.865	0.000
Canids	PGLS	0.000	0.92	0.823	0.105	0.531– 1.116	7.813	0.001	–1.713	0.193	–8.865	0.000

Table 3: Cross-sectional areas (mm²) for CC regions C1- C7 and associated brain mass (g) for the sample of wild and domestic canids shown in Figure 4 a-e. The total area (mm²) was mathematically determined by adding the sub-components (C1 -C7)

Species	C1	C2	C3	C4	C5	C6	C7	Total area	Brain mass (g)
<i>Canis lupus familiaris</i> (Beagle)	0.53	10.63	10.27	7.14	7.34	4.98	13.19	54.08	70.93
<i>Canis lupus familiaris</i> (Beagle/hound mix)	0.10	8.61	9.50	4.65	4.62	4.95	11.46	43.89	62.32
<i>Canis lupus familiaris</i> (Beagle/hound mix)	0.93	16.28	17.98	10.14	10.14	7.58	21.23	84.28	69.92
<i>Canis lupus familiaris</i> (Chihuahua)	0.68	9.56	10.92	6.83	5.01	4.55	14.00	51.55	47.85
<i>Canis lupus familiaris</i> (Coker Spaniel)	1.19	10.33	8.87	4.99	5.82	5.52	8.23	44.95	60.07
<i>Canis lupus familiaris</i> (Curly Coated Retriever)	0.30	12.88	27.67	11.62	9.79	10.33	28.85	101.44	83.79
<i>Canis lupus familiaris</i> (English Springer Spaniel)	0.96	17.23	17.02	8.53	7.76	6.90	17.75	76.15	82.39
<i>Canis lupus familiaris</i> (English Springer Spaniel)	1.60	7.60	16.22	7.13	5.88	4.17	17.50	60.10	75.16
<i>Canis lupus familiaris</i> (Golden Retriever)	1.48	12.75	14.15	7.49	5.07	5.32	19.22	65.48	80.58
<i>Canis lupus familiaris</i> (Maltese)	1.28	7.03	7.41	3.94	4.14	3.82	8.87	36.49	41.76
<i>Canis lupus familiaris</i> (Maltese Cross)	0.46	16.33	12.51	9.61	7.63	7.02	15.11	68.67	55.95
<i>Canis lupus familiaris</i> (Mini Poodle)	0.26	9.40	10.88	4.14	3.99	4.29	12.16	45.12	58.90
<i>Canis lupus familiaris</i> (Rhodesian Ridgeback)	2.52	19.76	18.88	11.96	12.57	16.56	29.13	111.38	98.33
Average <i>Canis lupus familiaris</i>	0.70	11.58	13.14	7.09	6.49	6.04	15.52	61.43	66.55
<u>Average <i>Vulpes vulpes</i></u>	1.66	8.47	8.91	4.65	5.72	5.35	17.51	52.27	44.00
<u>Average <i>Vulpes zerda</i></u>	0.14	4.39	4.90	2.95	2.78	2.46	5.66	23.28	16.69
<u>Average <i>Chrysocyon brachyurus</i></u>	1.78	18.48	19.35	10.75	11.62	8.11	23.39	93.94	87.71
<u>Average <i>Lycaon pictus</i></u>	0.64	23.67	23.76	11.76	12.59	9.58	31.33	113.33	125.76
<u>Average <i>Canis lupus lupus</i></u>	1.30	18.32	24.26	14.63	15.87	18.99	26.73	124.21	132.57

Table 4: Summary of the regression statistics (OLS) evaluating the relationship between the mid-sagittal areas for each CC region and brain mass in domestic dogs only. For ease of interpretation, all correlations were performed on logarithmic data so that the slope represents an allometric exponent. (C1 = rostrum, C2 = genu, C3 = rostral body, C4 = rostral midbody, C5 = caudal midbody, C6 = isthmus, C7 = splenium, OLS = ordinary least squares regression, CI slope = confidence interval of the slope).

CC region	r^2	Slope	Intercept	CI slope	<i>P</i>
C1	0.06	0.88	-1.75	-2.12, 2.81	0.45
C2	0.38	0.84	-0.48	0.42, 1.54	<0.05
C3	0.61	1.19	-1.05	0.52, 1.63	<0.01
C4	0.47	1.07	-1.12	0.27, 1.73	<0.05
C5	0.47	1.04	-1.08	0.44, 1.68	<0.05
C6	0.47	1.18	-1.37	0.32, 2.11	<0.05
C7	0.62	1.28	-1.15	0.47, 1.81	<0.01
Sum (C1 to C7)	0.63	1.13	-0.27	0.47, 1.61	<0.01

Table 5: Percentage of total cross-sectional area occupied by the relevant subcomponents of the CC. Note, that the C1 segment is considerably smaller than that of the other subcomponents, occupying on average only about 1.5% percent of the total midsagittal CC area, while other segments like C2 contribute around 20% of the total midsagittal CC area. C1 only contributes 6% of the total C1 + C2 area, so it occupies a relatively small area of the overall C1+C2 segment.

	C1 % of Total	C1 + C2 % of Total	C3 % of Total	C4 + C5% of Total	C6 + C7% of Total
<i>Canis lupus familiaris</i> (Beagle)	0.9%	20.6%	18.9%	26.8%	33.6%
<i>Canis lupus familiaris</i> (Beagle/hound mix)	0.2%	19.9%	21.7%	21.1%	37.4%
<i>Canis lupus familiaris</i> (Beagle/hound mix)	1.1%	20.4%	21.3%	24.1%	34.2%
<i>Canis lupus familiaris</i> (Chihuahua)	1.3%	19.9%	21.2%	22.9%	35.9%
<i>Canis lupus familiaris</i> (Cocker Spaniel)	2.7%	25.6%	19.7%	24.1%	30.6%
<i>Canis lupus familiaris</i> (Curly Coated Retriever)	0.3%	12.9%	27.9%	21.1%	38.6%
<i>Canis lupus familiaris</i> (English Springer Spaniel)	1.3%	23.9%	22.4%	21.4%	32.4%
<i>Canis lupus familiaris</i> (English Springer Spaniel)	2.7%	15.3%	26.9%	21.6%	36.1%
<i>Canis lupus familiaris</i> (Golden Retriever)	2.3%	21.7%	21.6%	19.2%	37.5%
<i>Canis lupus familiaris</i> (Maltese)	3.5%	22.8%	20.3%	22.1%	34.8%
<i>Canis lupus familiaris</i> (Maltese Cross)	0.7%	24.5%	18.2%	25.1%	32.2%
<i>Canis lupus familiaris</i> (Mini Poodle)	0.6%	21.4%	24.1%	18.0%	36.5%
<i>Canis lupus familiaris</i> (Rhodesian Ridgeback)	2.3%	20.0%	16.9%	22.0%	41.0%
Average dog	1.2%	20.0%	21.4%	22.1%	35.1%
Average <i>Vulpes vulpes</i>	3.2%	19.4%	17.1%	19.8%	43.7%
Average <i>Vulpes zerda</i>	0.6%	19.5%	21.1%	24.6%	34.9%
Average <i>Chrysocyon brachyurus</i>	2.1%	21.8%	20.6%	44.4%	33.8%
Average <i>Lycaon pictus</i>	0.6%	21.5%	42.4%	21.4%	36.1%

Average <i>Canis lupus lupus</i>	1.1%	15.8%	19.7%	44.5%	37.2%
Range	0.2 - 3.5%	15.3 - 24.4%	16.9 - 42.4%	18.0 - 44.5%	30.6 – 43.7%

Table 6: Regression statistics (OLS) of the combined rostrum and genu CC segments scaled against brain mass in domestic and wild canids. All correlations were performed on logarithmic data. (C1 + C2 = rostrum + genu, OLS = ordinary least squares regression, CI slope = confidence interval of the slope). Note the low correlation coefficients for the C1 + C2 area even though C1 only contributes ~ 6% of the total C1 + C2 area while C2 contributes around 20% of the total mid-sagittal CC area.

Taxa	CC region	r^2	Slope	Intercept	CI slope	<i>P</i>
Domestic Canids	C1+C2	0.06	0.11	1.02	-0.15, 0.36	0.4
Wild Canids	C1+C2	0.2	0.29	0.88	-2.78, 0.93	0.44

Graphical Abstract Image

The study of domestic animals provides an opportunity to evaluate the effect of artificial selection on the brain. Using image analysis techniques, we compared scaling of the corpus callosum in wild and domestic canids and show that the rostrum, putatively connecting the prefrontal cortex, is less constrained by brain size.