Significant neuroanatomical variation among domestic dog breeds

Abbreviated title: Neuroanatomical variation in dogs

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Abstract:

Humans have bred different lineages of domestic dogs for different tasks, like hunting, herding, guarding, or companionship. These behavioral differences must be the result of underlying neural differences, but surprisingly, this topic has gone largely unexplored. The current study examined whether and how selective breeding by humans has altered the gross organization of the brain in dogs. We assessed regional volumetric variation in MRI studies of 62 male and female dogs of 33 breeds. Notably, neuroanatomical variation is plainly visible across breeds. This variation is distributed non-randomly across the brain. A whole-brain, data-driven independent components analysis established that specific regional sub-networks covary significantly with each other. Variation in these networks is not simply the result of variation in total brain size, total body size, or skull shape. Furthermore, the anatomy of these networks correlates significantly with different behavioral specialization(s) such as sight hunting, scent hunting, guarding, and companionship. Importantly, a phylogenetic analysis revealed that most change has occurred in the terminal branches of the dog phylogenetic tree, indicating strong, recent selection in individual breeds. Together, these results establish that brain anatomy varies significantly in dogs, likely due to human-applied selection for behavior.

Significance statement:

Dog breeds are known to vary in cognition, temperament, and behavior, but the neural origins of this variation are unknown. In an MRI-based analysis, we found that brain anatomy covaries significantly with behavioral specializations like sight hunting, scent hunting, guarding, and companionship. Neuroanatomical variation is not simply driven by brain size, body size, or skull shape, and is focused in specific networks of regions. Nearly all of the identified variation occurs in the terminal branches of the dog phylogenetic tree, indicating strong, recent selection in individual breeds. These results indicate that through selective breeding, humans have significantly altered the brains of different lineages of domestic dogs in different ways.

Introduction

A major goal of modern neuroscience is to understand how variation in behavior, cognition, and emotion relates to underlying neural mechanisms. A massive "natural experiment" in this arena has been right under our noses: domestic dogs. Humans have selectively bred dogs for different, specialized abilities – herding or protecting livestock; hunting by sight or smell; guarding property or providing companionship. Significant breed differences in temperament, trainability, and social behavior are readily appreciable by the casual observer, and have also been documented quantitatively (e.g., (Serpell and Hsu 2005, Tonoike, Nagasawa et al. 2015)). Furthermore, recent genetic research indicates that this behavioral variation is highly heritable (MacLean, Snyder-Mackler et al. 2019).

This panoply of behavioral specializations *must* rely on underlying neural specializations. A small number of studies have investigated neural variation in dogs, including, for example, the effects of skull shape on brain morphology (e.g., (Carreira and Ferreira 2015, Pilegaard, Berendt et al. 2017) and anatomical correlates of aggression (e.g., (Jacobs, Van Den Broeck et al. 2007, Vage, Bonsdorff et al. 2010)). However, the neural underpinnings of behavioral differences between breeds remain largely unknown.

Most modern dog breeds were developed in an intentional, goal-driven manner relatively recently in evolutionary time; estimates for the origins of the various modern breeds vary between the past few thousand to the past few hundred years (Larson, Karlsson et al. 2012). This strong selection pressure suggests that brain differences between breeds may be closely tied to behavior. However, selection also occurred for outward physical appearance, including craniofacial morphology. This may have placed constraints on the internal dimensions of the skull, which in turn may have had secondary effects on brain morphology. There is substantial diversification of skull shape across dog breeds, and this has been linked to behavioral differences (Drake and Klingenberg 2010, McGreevy, Georgevsky et al. 2013). Alternatively, neuroanatomical variation may be explained primarily by body size rather than breed membership, with different breeds' brains representing minor, random, scaled-up or scaled-down variants of a basic species-wide pattern.

Any attempt to determine whether breeding for behavior has altered dog brains would have to be able to differentiate between these competing (and potentially interacting) hypotheses. A simple comparison of regional volumes would be insufficient for several reasons. First, a significant difference in the volume of, for example, the amygdala in pit bulls versus golden retrievers might seem intuitively meaningful, but in order to ascertain whether such a difference was truly the result of selection pressure on behavior, the phylogenetic structure of the dog family tree needs to be taken in to account in order to partition variance attributable to inheritance, and equal statistical priority needs to be given to the alternative hypotheses that observed variation in morphology. Second, and perhaps most importantly, a priori comparisons of regional gray matter volumes presuppose that experimenters can identify meaningful borders between regions. For highly conserved structures with clear anatomical boundaries, like the amygdala, this task is surmountable – but very little is known about the organization of higher-order cortical regions in dogs, and some complex behaviors that are the focus of selective breeding, like

herding or interspecies communication, almost certainly rely on some of these areas. For this reason, even creating the regional outlines for a simple ROI analysis would be problematic.

Therefore, the current study took a totally data-driven, whole-brain, agnostic approach to assessing morphological variation across dog brains. Our goal was to (a) determine whether significant non-random variation in brain anatomy exists across dogs, and if so, (b) differentiate between the competing and possibly interacting explanations for this variation.

Materials and Methods

Subjects

The dataset included T2-weighted MRI scans from 62 purebred dogs of 33 different breeds. These were grouped into 10 different breed groups as defined by American Kennel Club, which ostensibly represent groupings that were developed for similar behavioral specializations, such as herding or hunting. **Table 1** lists the breed, breed group, and other data for all dogs included in the study.

ID	Breed	Sex	Age (years)	Body mass (kg)	Cephalic index (from database)	Neuro-cephalic index	Brain volume (mm3)	Ostensible behavioral specialization / purpose
1	Basset Hound	Male	4.0	28.1	0.74	51.89	100070.10	scent hunting
2	Beagle	Male	14.3	17.0	0.74	61.82	82750.29	scent hunting
3	Beagle	Male	4.0	11.7	0.76	61.82	64887.65	scent hunting
4	Beagle	Male	ND	28.5	0.85	61.82	23259.63	scent hunting
5	Beagle	Male	4.0	8.3	0.82	61.82	66733.96	scent hunting
6	Beagle	Male	1.7	28.5	0.78	61.82	65738.93	scent hunting
7	Bichon Frise	Male	9.0	9.3	0.80	61.51	61849.71	explicit companionship
8	Border Collie	Male	6.1	28.2	0.65	54.38	83215.10	herding
9	Border Collie	Male	5.6	20.6	0.65	54.38	81668.60	herding
10	Boston Terrier	Male	11.9	12.5	0.90	92.62	66301.82	explicit companionship vermin control sport fighting
11	Boston Terrier	Male	5.8	8.9	0.90	92.62	76426.61	explicit companionship vermin control sport fighting
12	Boxer	Male	8.1	31.8	0.68	67.19	81555.33	guarding/protecting/sentinel work police/military work, war

								sport fighting
13	Boxer	Male	5.0	34.2	0.67	67.19	80814.97	guarding/protecting/sentinel work police/military work, war sport fighting
14	Boxer	Female	10.7	31.8	0.83	66.28	93337.26	guarding/protecting/sentinel work police/military work, war sport fighting
15	Boxer	Male	9.3	40.8	0.70	67.19	82323.66	guarding/protecting/sentinel work police/military work, war sport fighting
16	Bulldog	Male	1.0	16.8	0.74	90.18	63154.13	explicit companionship sport fighting
17	Bulldog	Male	4.4	30.0	0.77	90.18	80128.00	explicit companionship sport fighting
18	Cavalier King Charles Spaniel	Female	0.5	3.2	0.81	76.77	55777.97	explicit companionship
19	Cavalier King Charles Spaniel	Female	0.5	14.5	0.92	76.77	64695.16	explicit companionship
20	Cocker Spaniel	Female	6.4	18.1	0.75	61.01	66708.41	bird retrieval
21	Dachsund	Female	11.3	4.9	0.79	51.76	44076.29	vermin control scent hunting
22	Dachsund	Female	6.6	6.4	0.77	51.76	60492.56	vermin control scent hunting
23	Dachsund	Male	7.8	5.6	0.81	49.59	57168.79	vermin control scent hunting
24	Dachsund	Female	1.8	5.3	0.81	51.76	49716.87	vermin control scent hunting
25	Doberman Pinscher	Female	4.7	29.8	0.62	46.96	80287.44	guarding/protecting/sentinel work police/military work, war
26	English Pointer	Male	7.3	27.3	0.74	ND	91448.24	bird retrieval
27	German Short Haired Pointer	Female	6.2	27.0	0.73	48.30	75612.46	bird retrieval
28	Golden Retriever	Male	10.0	39.8	0.69	56.52	96010.49	bird retrieval
29	Golden Retriever	Male	6.0	42.2	0.70	56.52	96941.92	bird retrieval
30	Golden Retriever	Male	11.0	34.9	0.68	56.52	86438.69	bird retrieval
31	Greyhound	Female	7.5	36.7	0.65	45.83	97610.47	sight hunting
32	Greyhound	Male	3.8	37.1	0.65	46.84	97774.89	sight hunting
33	Greyhound	Female	2.2	36.0	0.66	45.83	101969.38	sight hunting

34	Jack Russell Terrier	Male	ND	14.0	0.80	59.28	70125.35	vermin control
35	Keeshound	Male	7.2	21.6	0.71	60.18	68766.94	explicit companionship guarding/protecting/sentinel work
36	Labrador Retriever	Male	9.7	32.6	0.65	55.82	94762.33	bird retrieval
37	Labrador Retriever	Female	5.0	30.5	0.66	56.11	84161.70	bird retrieval
38	Lhasa Apso	Female	10.7	13.2	0.93	ND	58177.18	guarding/protecting/sentinel work
39	Lhasa Apso	Female	4.0	7.6	0.86	ND	58152.92	guarding/protecting/sentinel work
40	Maltese	Male	6.6	6.0	0.81	65.29	46642.03	explicit companionship
41	Maltese	Male	10.0	3.0	0.84	65.29	35280.20	explicit companionship
42	Maltese	Male	5.5	6.6	0.77	65.29	46629.97	explicit companionship
43	Maltese	Male	6.0	8.9	0.88	65.29	47610.27	explicit companionship
44	Maltese	Female	6.0	2.0	0.92	68.83	28052.45	explicit companionship
45	Maltese	Female	4.9	3.4	0.85	68.83	46330.73	explicit companionship
46	Miniature Schnauzer	Male	9.4	12.8	0.77	51.79	62053.63	vermin control
47	Miniature Schnauzer	Female	6.3	5.0	0.80	54.99	53517.22	vermin control
48	Old English Sheepdog	Male	3.7	33.1	0.69	54.39	80709.26	herding
49	Pit Bull	Male	2.1	27.1	0.72	69.96	80571.31	sport fighting
50	Siberian Husky	Female	3.0	18.1	0.67	55.17	62094.04	running/racing
51	Silky Terrier	Male	3.0	4.4	0.84	58.23	46832.08	vermin control
52	Springer Spaniel	Female	1.1	18.4	0.75	49.34	72442.26	bird retrieval
53	Standard Poodle	Female	7.9	22.6	0.73	ND	80235.75	bird retrieval
54	Weimaraner	Male	3.3	48.4	0.66	49.05	110812.36	sight hunting
55	Welsh Corgi	Male	5.6	15.1	0.72	63.09	83234.19	herding
56	West Highland White Terrier	Male	5.9	11.0	0.78	60.84	72254.08	vermin control
57	Wheaton Terrier	Male	7.0	19.2	0.71	ND	70234.47	guarding/protecting/sentinel work herding vermin control bird retrieval
58	Whippet	Female	15.5	13.6	0.72	50.60	71357.64	sight hunting
59	Yorkshire Terrier	Female	3.8	3.9	0.82	ND	45103.02	explicit companionship vermin control
60	Yorkshire	Male	13.0	4.2	0.81	ND	45217.54	explicit companionship

	Terrier							vermin control
61	Yorkshire Terrier	Male	0.8	3.5	0.79	ND	38163.05	explicit companionship vermin control
62	Yorkshire Terrier	Male	11.5	3.2	0.82	ND	51760.84	explicit companionship vermin control

Table 1. Data for all dogs used in the study. Dogs from mixed/unknown breeds were excluded from analyses that used breed group as an independent variable. Cephalic indices are sex- and breed-specific averages from a large public database (Stone, McGreevy et al. 2016). Missing data denoted with "ND".

Image acquisition and preprocessing

T2-weighted MRI images were acquired on a 3.0T GE HDx MRI unit with a GE 5147137-2 3.0T HD T/R Quad Extremity Coil. Images were opportunistically collected at the Veterinary Teaching Hospital at the University of Georgia at Athens from dogs that were referred for neurological examination but were not found to have any neuroanatomical abnormalities. All scans were re-reviewed by a board-certified veterinary neurologist before inclusion.

The preprocessing pipeline was implemented using the NiPype workflow engine (Gorgolewski, Burns et al. 2011). Both transverse-acquired and sagittally-acquired images were available for each dog. Transverse-acquired images ranged from 0.234 mm^2 in-plane resolution and 2.699 mm slice distance to 0.352 mm^2 in-plane resolution and 3.499 mm slice distance. Sagittally-acquired images ranged from 0.273 mm^2 in-plane resolution and 3.200 mm slice distance to 0.430 mm^2 in-plane resolution and 3.200 mm slice distance. To maximize the use of all available anatomical information, the transverse and sagittal images were combined as follows. First, we manually performed skull-stripping on the transverse image. Next, we determined the smallest region of interest (ROI) that completely covered the brain from the brain mask image. The transverse image and transverse brain mask were then cropped using the computed ROI coordinates. Then, the transverse images were resampled to produce isotropic voxels in all three dimensions, the sagittal image was resliced so that it was in the same orientation as the transverse images, and a rigid registration was computed from the sagittally-acquired image to the original transverse image. The region containing the brain was then cropped in the sagittal image, and we then registered the smaller cropped sagittal image to the isotropically-resampled transverse brain image using a rigid registration. Finally, the cropped transverse and sagittal images were then rescaled so that the robust mean intensity of both images was 100, the images were averaged together, and then the brain mask applied to this combined image. A general diagram illustrating the overall processing pipeline is included in **Figure 1-1**, and a detailed NiPype registration workflow is included in **Figure 1-2**, both available in the extended data. Additionally, the accompanying registration code is available at https://gist.github.com/dgutman/a0e05028fab9c6509a997f703a1c7413.

Template creation

We produced a study-specific template representing the average brain morphology across the entire group, equally unbiased toward any particular image. This was accomplished using the *buildtemplateparallel.sh* script in the ANTS software package (Avants, Tustison et al. 2009), which nonlinearly registers each image into a common spatial framework.

Experimental design and statistical analyses

Morphological analyses

During nonlinear registration, a warpfield is produced that represents the mapping from the original image to the target image. The Jacobian of the warpfield represents the degree of warping that had to occur in each original image in order to bring it into alignment with the target image. To localize significant variation in gray matter morphology, we applied a one-sample t-test on the demeaned log Jacobian determinant images. This was accomplished using FSL's *randomise*, a tool for Monte Carlo permutation testing on general linear models (Winkler, Ridgway et al. 2014). This analysis permutes the sign of the log Jacobian and tests the null hypothesis that variation from the mean is random and therefore symmetrically distributed and centered around zero. The resultant t-statistic image was thresholded at $p<0.05$, after multiple comparisons correction was carried out using threshold-free cluster enhancement (Smith and Nichols 2009).

To calculate neurocephalic index, we identified maximally distant points on the left-right, rostral-caudal, and dorsal-ventral axes; neurocephalic index was computed the ratio of brain width to brain length x 100.

Cephalic index is defined as the ratio of skull width to skull length x 100. For many scans in our database, the exterior of the skull was not visible, but a large database of skull measurements is publicly available (Stone, McGreevy et al. 2016). We computed male and female average cephalic indices separately for each breed and used these sex-specific, breed-average measures in our analyses.

To identify regional co-variation in gray matter morphology, we used GIFT, a software package for Matlab (Calhoun, Adali et al. 2001). GIFT's toolbox for source-based morphometry (SBM) (Xu, Groth et al. 2009) is a multivariate alternative to voxel-based morphometry (VBM). It uses independent components analysis to identify spatially distinct, distributed networks of regions that covary across individuals, and computes their statistical relationship to other categorical or continuous variables. T2-weighted images underwent bias field correction using ANTS's Atropos N4 tool (Avants, Tustison et al. 2011) and segmentation into gray matter, white matter, and cerebrospinal fluid using FSL's FAST tool (Zhang, Brady et al. 2001). Gray matter segmentations were warped to the study-specific template and modulated by their log Jacobian determinants to produce per-subject maps of the degree of morphological divergence from the study-specific group-average template. In other words, the input to SBM consisted of gray matter maps for each subject, where intensity at each voxel corresponded to the degree of deformation required in order to come into alignment with the template (i.e., the demeaned log Jacobians). The number of sources was estimated using Akaike's information criterion (AIC, (Akaike

1974)); the application of AIC in SBM is described in (Xu, Groth et al. 2009). This procedure identified six components, each of which were thresholded at Z scores above 1.96 or below -1.96. Multiple regression and ANOVA analyses were then used to compute the relationship of each component to American Kennel Club-defined breed groups, with the statistical threshold set at $p<0.05$ after multiple comparisons correction.

Phylogenetic statistics

Because comparative data may be non-independent due to shared phylogenetic history, the assumptions of standard statistical methods may be violated (Harvey and Pagel 1991). We therefore used phylogenetic comparative methods that account for phylogenetic non-independence by including expected phylogenetic variance-covariance among species into the error term of generalized least-squares ('pGLS') linear models (Rohlf 2001). When quantifying linear models we additionally included a lambda parameter to account for phylogenetic signal (Pagel 1997). To test for differences in statistical fit among linear models that include different parameters (for example, the inclusion of grouping variables to test for differences among breed groups), we used least-squares phylogenetic analysis of covariance (pANCOVA) (Smaers and Rohlf 2016, Smaers and Mongle 2018). It should be noted that 'phylogenetic' approaches such as pGLS and pANCOVA are interpreted in the same way as standard least-squares approaches. The only difference between standard and phylogenetic least-squares approaches is that the phylogenetic approaches weight data points according to phylogenetic relatedness (Rohlf, 2001).

We further investigated the relationship between morphological components and the phylogenetic tree by estimating the amount of change that occurs on each lineage using a multiple variance Brownian motion approach (Smaers, Mongle et al. 2016, Smaers and Mongle 2018). This approach estimates phenotypic change along individual lineages of a tree and has been shown to provide more accurate estimates than traditional ancestral estimation methods (Smaers and Mongle 2017).

Lastly, we use multi-regime Ornstein-Uhlenbeck ('OU') approaches to estimate phylogenetic shifts in mean value directly from the data. This approach has become a standard approach in comparative biology to model trait change across a phylogeny. Specifically, this approach quantifies the evolution of a continuous trait 'X' as $dX(t)= \alpha[\theta - X(t)]dt + \sigma dB(t)$ where 'σ' captures the stochastic evolution of Brownian motion, 'α' determines the rate of adaptive evolution towards an optimum trait value 'θ' (90). This standard OU model has been modified into multiple-regime OU models allowing optima to vary across the phylogeny (Butler and King 2004). Such multi-regime OU models allow modelling trait evolution towards different 'regimes' that each display a different mean trait value. In other words, these approaches allow estimating directly from the data where in a phylogeny a shift in mean value of a trait has occurred. To overcome inherent difficulties with optimizing OU parameters (Ho and Ane 2014), several algorithmic improvements have been proposed. Here, we use the approach proposed by Khabbazian et al. (2016).

Results

Neuromorphological variation is plainly visible across breeds. Midline sagittal images from the raw, native-space scans of selected dogs are shown in **Figure 1A**. To provide a common spatial reference for measuring this variation, we created an unbiased, diffeomorphic template using the ANTS software package (Avants, Tustison et al. 2009). This template represents the average brain for the entire dataset, and is shown in **Figure 1B**.

To visualize morphological variation in a more standardized manner, we nonlinearly warped the template to each dog's native-space image. This allowed us to examine breed variation in brain morphology and size with invariant contrast and resolution. We also additionally re-scaled these images to have constant rostral-caudal lengths. This allowed us to more clearly visualize variation in morphology independent from variation in size. Both sets of scaled template images are shown in **Figure 1A**.

To carry out quantitative assessments of regional variation in gray matter morphology, we used the Jacobian determinants of the native-space-to-template spatial deformation fields to produce a variation intensity map. These fields represent a map of where and how much each dog's scan had to adjust in order to become aligned to the group-average template. The standard deviation of these maps thus indexes the extent to which brain anatomy varies across individuals, and is shown in **Figure 1C**.

To determine whether this variation was randomly distributed across the brain or focused in specific areas, we applied Monte Carlo permutation testing on the demeaned Jacobian determinant images. Importantly, this revealed that a large proportion of the brain shows significant gray matter morphological variation across subjects, illustrated in **Figure 1D**.

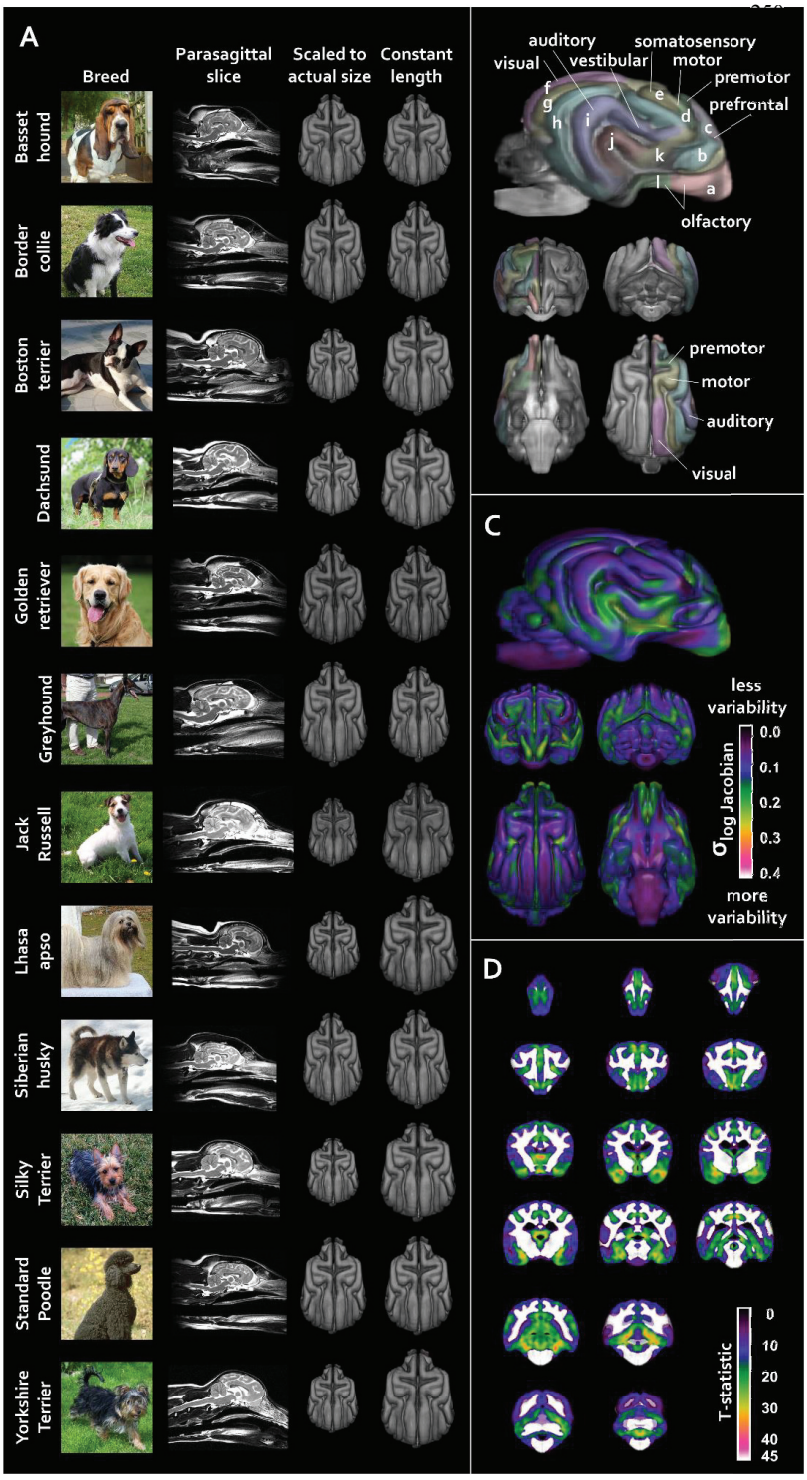

Fig. 1. Neuroanatomical variation in domestic dogs.
(A) MRI images and 3D reconstructions of warped template from 10 selected dogs of different breeds. Public-domain photos from Wikimedia Commons.
(B) Unbiased group-average template for this dataset. See Figures 1-1 and 1-2 for processing schematics. Neuroanatomical labels (based on (Palazzi 2011, Datta, Lee et al. 2012, Evans and de Lahunta 2013)): a) olfactory peduncle; b) orbital (presylvian) gyrus; c) proreal gyrus; d) pre cruciate gyrus; e) postcruciate gyrus; f) marginal (lateral) gyrus; g) ectomarginal gyrus; h) suprasylvian gyrus; i) ectosylvian gyrus; j) sylvian gyrus; k) insular cortex; l) piriform lobe.
(C) Brain-wide morphological variation, regardless of breed, as indexed by the standard deviation of all dogs' Jacobian determinant images.
(D) A Monte Carlo permutation test on demeaned gray matter Jacobian determinant images revealed that much of gray matter shows significant deviation from group-mean morphology. Colored regions are all p<.05 after multiple comparison correction; T statistic values are illustrated.

Given these results, we next sought to determine what accounts for this variation by probing the extent to which it is related to body size, head shape, and/or breed group membership.

Figure 2A shows the relationship between brain volume and body mass. The scaling coefficient of this relationship (pGLS; *b*=0.231, 95% CI=0.26-0.36) is significantly lower than that observed across most mammals (~0.67), indicating the occurrence of more variation in body size relative to variation in brain size than would be expected. Importantly, using the tree structure from a recent large-scale genomic analysis (Parker, Dreger et al. 2017), we were able to determine that the phylogenetic signal of the brain-body allometry is negative – i.e., that variation present at the tree's terminal branches is not predicted by the deeper structure of the tree. If grade shifts in the brain-body allometry exist, these would putatively show differences among different breeds. We tested this hypothesis by estimating putative grade shifts in the brain to body allometry directly from the data using an OU modelling approach (Khabbazian, Kriebel et al. 2016). This analysis revealed no grade shifts, thereby indicating that a one-grade allometry is the best explanation of the bivariate brain to body relationship.

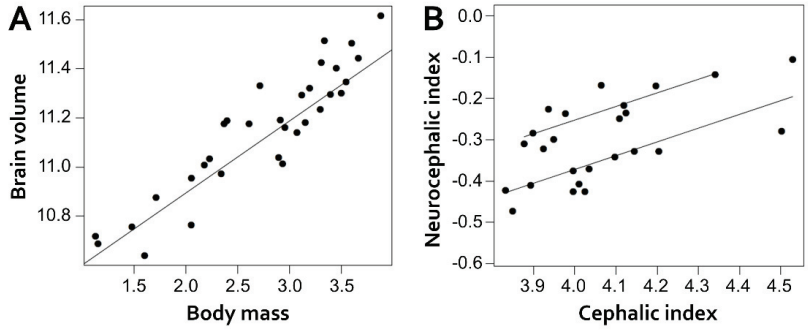

Fig. 2. Phylogenetic generalized least squares (pGLS) analyses on gross brain, body, and skull measurements. (A) Brain volume vs. body mass. (B) Neurocephalic index vs. cephalic index. Plotted points represent breed averages, not individuals.

In mammals, head shape is commonly measured using cephalic index (also known as skull index), calculated as maximum head width divided by maximum head length. We were interested in the possibility that human-driven selection on external craniofacial morphology may have had on the internal dimensions of the skull. To assess this, we computed an analogous *neurocephalic* index for each dog (maximum internal cranial cavity length divided by maximum internal cranial cavity width). **Figure 2B** shows the relationship between neurocephalic and cephalic index. Cephalic index is a significant predictor of neurocephalic index (pGLS: b=0.37, t=3.70, p<0.01). Also here we questioned whether grade shifts in this allometry exist, putatively showing differences among breeds. This analysis revealed that the neurocephalic-cephalic allometry was thus best explained by a two-grade model (F=31.19, p<0.001). The breeds on the higher grade, with a greater neurocephalic index for a given cephalic index, were as follows: Basset hound, beagle, German short-haired pointer, dachshund, cavalier King Charles spaniel, springer spaniel, west highland white terrier, silky terrier, bichon frise, and maltese. Importantly, this grade difference in the neurocephalic to cephalic index aligns with a significant difference in body size

(pANOVA: F=9.73, p<0.01; average body size 11kg, versus 23kg in other breeds). Smaller-bodied dogs hereby have a higher neurocephalic index (more spherical brains) for a given cephalic index (external head shape).

If variation in dog brain anatomy is unrelated to behavior, then variation should be randomly distributed across regions. Alternatively, if this variation represents heritable adaptations for behavior, then significant covariance should exist in separable, independent sub-networks of regions. To assess this, we performed source-based morphometry, a multivariate alternative to voxel-based morphometry which makes use of independent components analysis. This was accomplished using the GIFT software package (Xu, Groth et al. 2009). Results revealed 6 networks where regional volume covaried significantly across individuals. **Figure 3** shows these networks, along with factor loadings for each breed group. Major anatomical constituents of each network are labeled. Additional research is needed to definitively link the function of each network to its adaptive role in response to behavior selection. However, we note putative roles which may serve as initial hypotheses for future research.

Network 1 includes the nucleus accumbens, dorsal and ventral caudate, cingulate gyrus, olfactory peduncle, and gyrus rectus (medial prefrontal cortex). These regions are part of or connected to the mesolimbic reward system, a network implicated in reward signaling related to reinforcement learning, incentive salience, and motivation broadly across species (Alcaro, Huber et al. 2007, O'Connell and Hofmann 2011); in dogs, the caudate nucleus activates for both food reward and human social reward (Cook, Prichard et al. 2016). Tentatively, this network might be relevant for social bonding to humans, training, and skill learning.

Network 2 involves brain regions involved in olfaction and gustation, including the piriform lobe, which contains olfactory cortex, and the insula and pseudosylvian sylcus, where the cortical representation of taste is located (Evans and de Lahunta 2013). This component also involves regions of medial frontal cortex, which is involved in downstream or higher-order processing of chemosensation and shows activation in response to olfactory stimulation in awake but not sedated dogs (Jia, Pustovyy et al. 2014). We propose that this network might support volitional (as opposed to instinctive) responses to olfactory and gustatory stimuli.

Network 3 includes a distributed network of subcortical regions that are involved movement, eye movement, vision, and spatial navigation, including the lateral geniculate nucleus, pulvinar, hippocampus, cerebellum, oculomotor nucleus, interpeduncular nucleus, ventral tegmental area, and substantia nigra. It also involves cortical regions, including the medial part of the frontal gyrus (supplementary motor area) and the lateral gyrus (visual cortex). Tentatively, this network may reflect a circuit involved in moving through the physical environment.

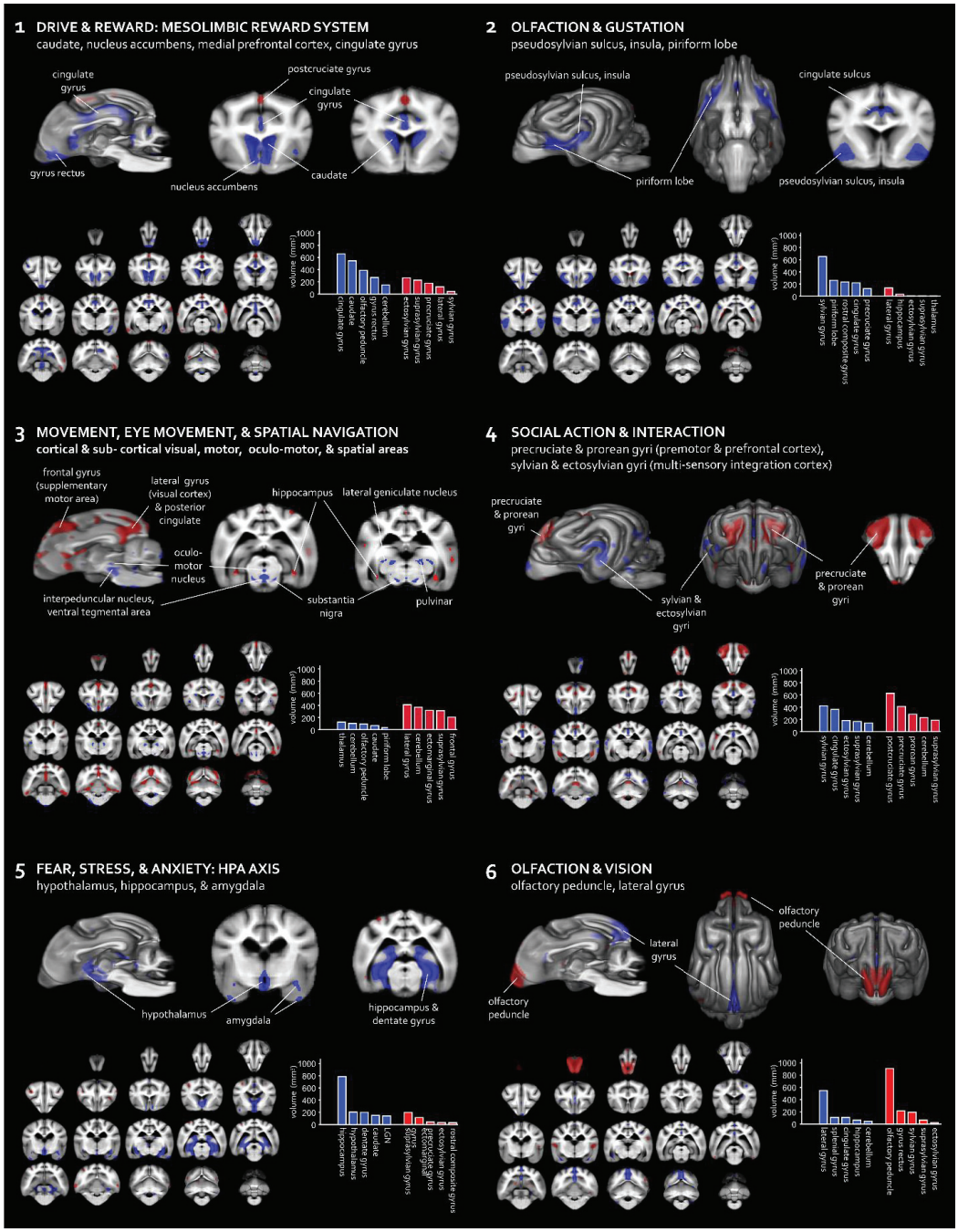

Fig. 3. Covarying regional networks in dog brain morphology. Independent components analysis revealed 6 regional networks where morphology covaried significantly across individuals. Red and blue regions are volumetrically anticorrelated: in

individuals where red is larger, blue tends to be smaller, and vice versa. Graphs represent volumetric quantification of the top 5 anatomical constituents of each of the 2 portions of each component.

Network 4 involves higher-order cortical regions that may be involved in social action and interaction. The precruciate and prorean gyri house premotor and prefrontal cortex, respectively, while the gyrus rectus is part of medial prefrontal cortex. The expansion of frontal cortex has been linked to increased sociality in extant hyena species (Holekamp, Sakai et al. 2007), and notably, the prorean gyrus has been linked to the emergence of pack structure in canid evolution (Radinsky 1969). The sylvian, ectosylvian, and suprasylvian gyri represent regions of lateral sensory cortex situated between gustatory, auditory, and somatosensory cortex (Evans and de Lahunta 2013), and likely contain higher-order association areas related to sensation and perception. In domestic dog fMRI studies, multisensory activation in these regions has been observed during the presentation of dog and human faces and vocalizations (Cuaya, Hernandez-Perez et al. 2016, Andics, Gacsi et al. 2017, Thompkins, Ramaiahgari et al. 2018).

Network 5 includes limbic regions that have a well-established role in fear, stress, and anxiety, including the hypothalamus, amygdala, and hippocampus and adjacent dentate gyrus (for a review, see (Tovote, Fadok et al. 2015)). These regions are involved in the HPA axis, which regulates behavioral and endocrine responses to environmental stressors and threats. Some of these regions are also involved in other affective and instinctual processes, including mating, memory, and aggression (O'Connell and Hofmann 2011).

Network 6 includes early sensory processing regions for olfaction and vision, including the olfactory peduncle and part of the lateral gyrus, which is the location of primary visual cortex (Evans and de Lahunta 2013).

Next, we investigated the relationship between these components, total brain size, and skull morphology. A significant relationship with total brain volume was present for all but Component 6, where it was marginal but did not meet significance (Component 1: $t = 3.663$, $p = 0.001$; Component 2: $t = -2.608$, $p = 0.014$; Component 3: $t = 6.219$, $p < .001$; Component 4: $t = -6.325$, $p < .001$; Component 5: $t = 3.938$, $p < .001$; Component 6: $t = 1.845$, $p = 0.076$). Components 3, 4, and 6 showed significant relationships with cephalic index, while Component 1 was marginal (Component 1: $t = -1.945$, $p = 0.064$; Component 3: $t = -2.165$, $p = 0.041$; Component 4: $t = 2.411$, $p = 0.024$; Component 6: $t = -2.171$, $p = 0.041$; pGLS). Components 1, 3, 4, and 6 showed significant relationships with neurocephalic index (Component 1: $t = -2.258$, $p = 0.032$; Component 3: $t = -3.823$, $p = 0.001$; Component 4: $t = 7.066$; $p < .001$; Component 6: $t = -2.890$, $p = 0.007$, pGLS).

We also investigated the relationship between these covarying morphological components and the phylogenetic tree. If variation in brain organization mainly reflects the deep ancestry of the tree, with little relationship to recent behavioral specializations, then brain morphometry should be highly statistically dependent on phylogenetic structure (i.e., high phylogenetic signal). Conversely, if brain organization is strongly tied to selective breeding for behavioral traits, then morphological traits should be divorced from the structure of the tree (i.e., low phylogenetic signal). We observed the latter (**Figure**

4). The majority of changes that occur in these components take place on the terminal branches of the phylogenetic tree.

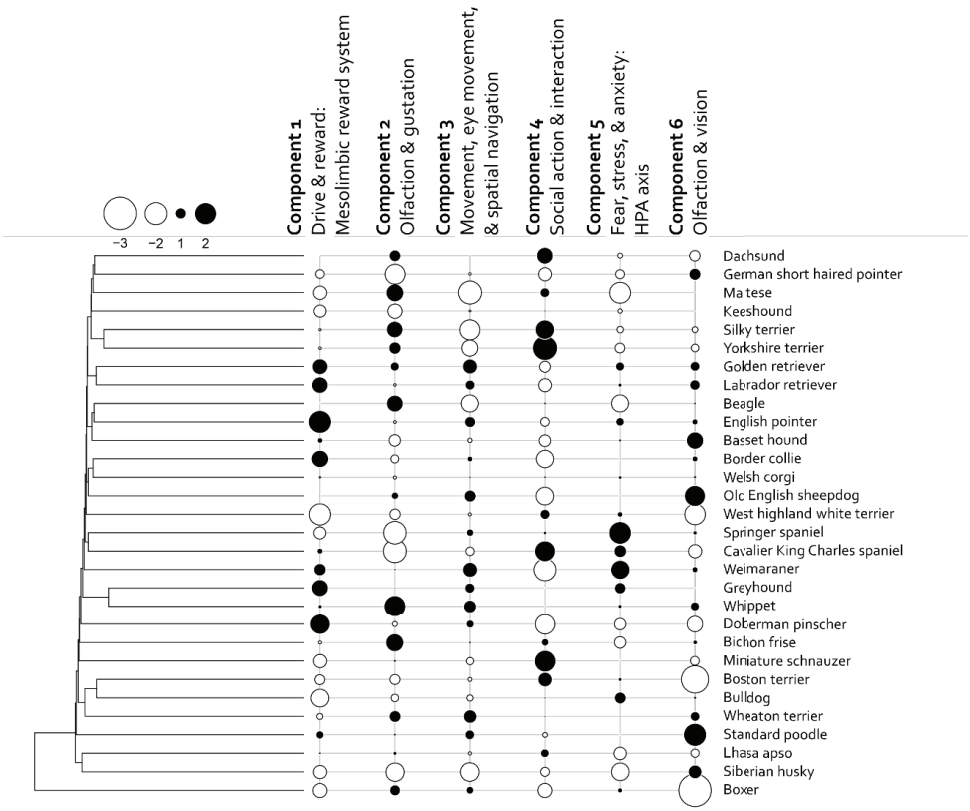

Fig. 4. Relationship between morphologically covarying regional brain networks and phylogenetic tree. Circles indicate factor loading. Phylogenetic tree from (Parker, Dreger et al. 2017).

Finally, we investigated whether these regionally covarying morphological networks were related to behavior. The American Kennel Club groups individual breeds into breed groups, but these breed groups change periodically and some groups contain breeds with disparate behavioral functions: for example, the non-sporting group includes both poodles and shar-peis. Therefore, rather using AKC breed groups, we identified each individual breed's ostensible behavioral specialization(s) as noted on the AKC website (www.akc.org). These were entered into in a multiple regression analysis using the GIFT Source Based Morphometry toolbox. Each of the 6 components showed significant correlation with at least one behavioral specialization (**Figure 5**). The behavioral specialization associated with the

most components (4 out of 6) was explicit companionship, and the component associated with the most behavioral specializations (6 out of 10) was Component 4, which involves regions involved in social action and interaction. Specific associations between associated brain networks and behavioral specializations are also apparent. For example, Component 3, which involves regions involved in movement, eye movement, and spatial navigation, showed a significant correlation with sight hunting, while Network 2, which involves regions involved in olfaction and gustation, showed a significant correlation with scent hunting.

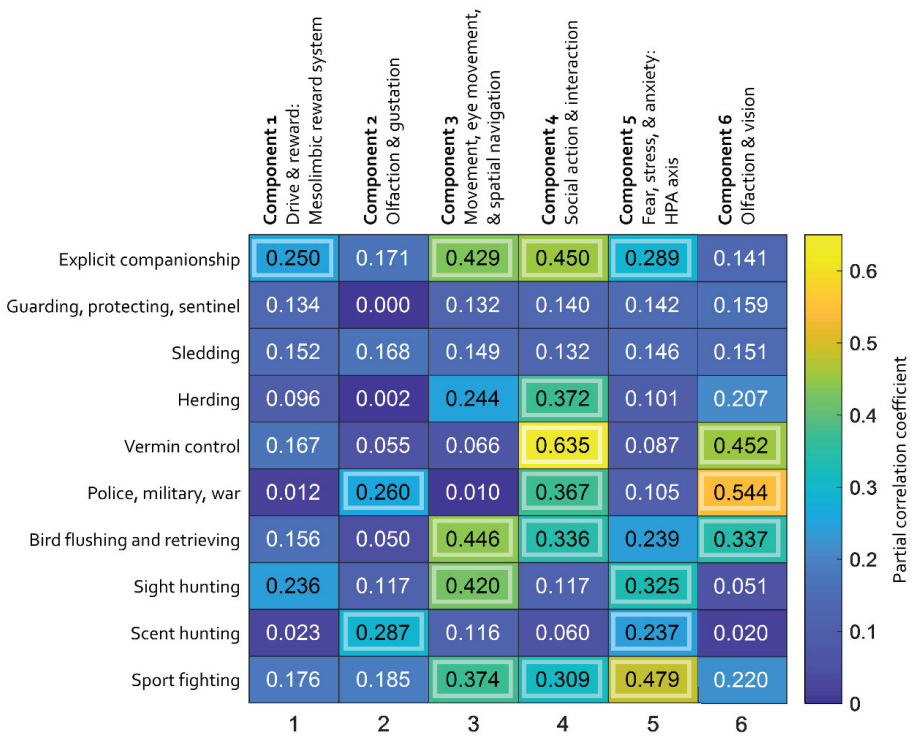

Fig. 5: Relationship between morphologically covarying regional brain networks and ostensible behavioral specializations. Colors indicate partial correlation coefficients resulting from multiple regression analysis on source-based morphometry results. Outlined boxes are significant at $p < .05$.

Discussion

The current study took a comprehensive, data-driven, agnostic approach to investigating neuroanatomical variation in domestic dogs. We first questioned whether significant variation in dog brain morphology even exists. The answer is a clear "yes": differences in gross brain anatomy are

readily appreciable (see **Figure 1A**). This observation was further confirmed by a whole-brain, multiple-comparison-corrected, voxelwise statistical analysis (**Figure 1C-1D**). Having established this basic finding, we then went on to probe the relationship between multiple, potentially interacting factors that might be linked to this variation: the total size of the body or brain, the external and internal morphology of the skull, the structure of the dog phylogenetic tree, and the organization of internal brain networks.

Dogs show intraspecific variation in morphology to a degree rarely seen in nature. There is a hundred-fold difference between the body mass of a Chihuahua (~1 kg) and the body mass of a Great Dane (~100 kg) (Sutter, Mosher et al. 2008). However, we found that dog brain sizes do not scale commensurately to dog body sizes, as indicated by a relatively low scaling coefficient for the relationship between brain size and body mass. To appreciate this effect, consider the adjacent dachshund and golden retriever images in **Figure 1A**: the dachsund's brain takes up most of the available endocranial space, while the golden retriever shows noticeably larger sinuses. A phylogenetic analysis revealed that changes in relative brain size are not predicated by relatedness and are more likely the result of selection on specific terminal branches of the phylogenetic tree (i.e., individual breeds).

In comparative animal cognition research, total brain size is often used as a gross index of cognitive capacity. Several previous studies have investigated the relationship between dog body size and cognition or behavior, with apparently contradictory results (see (Helton and Helton 2010, Stone, McGreevy et al. 2016) vs. (Broadway, Samuelson et al. 2017)). Additionally, a study that used a single scaling metric across breeds found that larger-brained (i.e., larger-bodied) dogs performed better on tests of executive function (Horschler, Hare et al. 2019). We found that larger dogs do tend to have larger brains, but that the brain:body allometry across breeds is low, indicating high variability in brain:body ratio across breeds (**Figure 2A**). Furthermore, we found that a substantial amount of variation in internal dog brain morphology is related to total brain size, suggesting that evolutionary increases or decreases in relative brain volume may be driven by changes in specific groups of regions. Moreover, we found that these networks differed across breed groups. Therefore, shifts in relative brain size may be related to expansion or contraction of specific networks, potentially leading to the presence or absence of correlations between body size and behavior depending on the specific breeds or behaviors being studied.

We also found that selection for smaller body size has significantly influenced the internal morphology of the cranial cavity. For a given cephalic index, or exterior skull shape, smaller-bodied dogs have more spherical brains (**Figure 2B**). This is consistent with a previous analysis linking foreshortening of the skull to ventral pitching of the brain and olfactory bulb, resulting in a more spherical brain (Roberts, McGreevy et al. 2010). We assessed the extent to which internal and exterior skull morphology were related to the covarying morphometric networks we identified. More networks showed a significant relationship with neurocephalic index than with cephalic index, suggesting that variation in brain morphology appears to be more tied to the internal morphology of the cranial cavity than to external craniofacial morphology – which is perhaps not surprising. Our results indicate that skull morphology is linked to the underlying anatomy of specific, different networks of brain regions; it is possible that this

could underlie the reported associations between behavior and head shape (e.g., (Gacsi, McGreevy et al. 2009, Helton 2009, McGreevy, Georgevsky et al. 2013)). Not all networks showed a significant relationship with either cephalic index or neurocephalic index, indicating that variation in dog brain morphology is partially but not totally dependent on variation in skull morphology. Importantly, we cannot say from the current analyses whether variation in skull morphology drives variation in brain morphology, the reverse, or both.

In addition to these analyses of the gross external shape and size of the brain and skull, we also investigated internal brain organization. This was accomplished using source-based morphometry to identify maximally independent networks that explain the variation present in the dataset. We identified six such networks (**Figure 3**). In the case of circuitry that is highly conserved across species – like circuitry for reward and motivation or fear and anxiety – it is a safe bet that research on other species is a good indicator of the functional role of these systems in dogs. This cannot be assumed to be the case for circuits that involve higher-order cortical association areas. Particularly in the case of our Network 4, it may be tempting to jump to conclusions about parallels with human cortical regions that are located in roughly the same location and are involved in similar tasks, e.g., the fusiform face area, Wernicke's area, or the mirror system. However, it is important to remember that primates and carnivores diverged further back in time than primates and rodents; humans are more closely related to mice than to dogs. Our last common ancestor with dogs likely had a fairly smooth, simple brain (Kaas 2011), and higher-order cortical association areas – along with whatever complex perceptual and cognitive abilities they support – have evolved independently in dogs and humans. Therefore, we stress that the functional roles of these networks, and their relationship to selection on behavior in specific breeds, should at this point still be considered an open question.

Having identified these six networks, we then investigated their relationship to the dog phylogenetic tree. We found that the majority of changes that occur in these components take place in the tree's terminal branches (i.e., individual breeds). This suggests that brain evolution in domestic dog breeds follows an "late burst model," with directional changes in brain organization being primarily lineage-specific. We also assessed whether these networks were related to selective breeding, as evidenced by the ostensible behavioral specialization(s) of each breed as noted by the AKC. In all six of the regionally covarying networks we found, significant correlations were found with at least one behavioral specialization. Associations between brain networks and related behavioral specializations are apparent. For example, Network 2, which involves regions that support higher-order olfactory processing, shows a significant correlation with scent hunting, while Network 3, which involves regions that support movement, eye movement, and spatial navigation, shows a significant correlation with sight hunting. These findings strongly suggest that humans have altered the brains of different breeds of dogs in different ways through selective breeding.

It is important to note that the current study was carried out on opportunistically-acquired data. The dataset included different numbers of dogs from different breeds, and some breeds are not represented at all. We used permutation testing for statistical hypothesis testing, which is a non-parametric approach appropriate for differing group sizes, but it is still possible that different patterns of variation

may have been obtained with a different sample makeup. Nonetheless, we expect the basic finding that this variation exists would remain.

Additionally, it should be noted that as dogs are increasingly bred to be house pets rather than working animals, selection on behavior is relaxing; significant behavioral differences have been found between working, show, and pet animals within a breed (e.g., (Lofgren, Wiener et al. 2014)). To our knowledge, the dogs in the current study were all house pets. Therefore, the findings reported here should be taken as representative of the innate breed-typical adaptations to brain organization that emerge without the input of specific experience – and may actually reflect relaxed or reduced versions of these adaptations. This might be akin to studying language circuitry in a lineage of language-deprived humans: humans almost certainly have some specialized "hard-wired" adaptations to this circuitry, but experience is required for the anatomical phenotype to fully emerge, and indeed it is difficult to consider language-related neural adaptations divorced from the context of language exposure and learning. Thus, future studies on purpose-bred dogs that are actively performing the tasks for which they are presumably adapted might expect to find additional or more pronounced neuroanatomical effects than we observed here.

Together, these findings have relevance to both basic and applied science. First and foremost, our findings introduce neural variation in domestic dog breeds as a novel opportunity for studying the evolution of brain-behavior relationships. Dogs represent a "natural experiment" in behavioral selection which has been ongoing for thousands of years; it seems remarkable that attempts to observe the neurological results of this experiment have so far been fairly minimal. Our findings also have implications for the current proliferation of fMRI studies in pet dogs, which nearly always group together dogs of varying breeds. The current study suggests that this approach might not be ideal, because there may be evolved breed differences in, e.g., functional responses to stimuli or anatomical distribution of receptors. In line with this possibility, one study has already found that border collies and Siberian huskies respond significantly differently to intranasal oxytocin (Kovacs, Kis et al. 2016). Additionally, on a practical level, our findings open the door to brain-based assessment of the utility of different dogs for different tasks. It might be possible, for example, to identify neural features that are linked to different breeds' specializations for specific behaviors, and to selectively breed or train dogs for enhanced expression of those neural features. Finally, on a philosophical level, these results tell us something fundamental about our own place in the larger animal kingdom: we have been systematically shaping the brains of another species.

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662 **Extended data: Figures 1-1 & 1-2**

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664 **Figure 1-1.** Conceptual schematic of neuroimaging analysis.

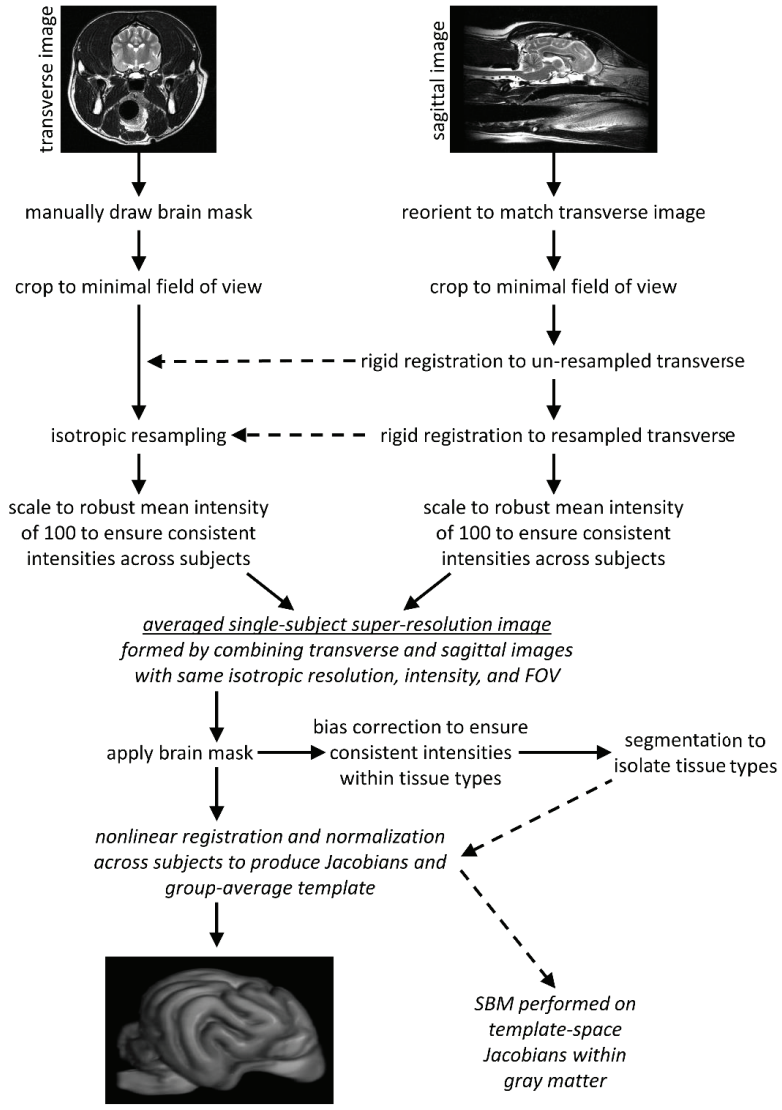

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Figure 1-2. NiPype pipeline for merging axial and sagittal images from each dog before registration to the template. Code is available online at https://gist.github.com/dgutman/a0e05028fab9c6509a997f703a1c7413.