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Neuropil Variation in the Prefrontal, Motor, and Visual Cortex of Six Felids

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Keywords
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Abstract

Introduction: Felids have evolved a specialized suite of morphological adaptations for obligate carnivory. Although the musculoskeletal anatomy of the Felidae has been studied extensively, the comparative neuroanatomy of felids is relatively unexplored. Little is known about how variation in the cerebral anatomy of felids relates to species-specific differences in sociality, hunting strategy, or activity patterns. ***Methods:*** We quantitatively analyzed neuropil variation in the prefrontal, primary motor, and primary visual cortices of six species of Felidae (*Panthera leo, Panthera uncia, Panthera tigris, Panthera leopardus, Acinonyx jubatus*, *Felis sylvestris domesticus*) to investigate relationships with brain size, neuronal cell parameters, and select behavioral and ecological factors. Neuropil is the dense, intricate network of axons, dendrites, and synapses in the brain, playing a critical role in information processing and communication between neurons. ***Results:*** There were significant species and regional differences in neuropil proportions, with African lion, cheetah, and tiger having more neuropil in all three cortical regions in comparison to the other species. Based on regression analyses, we find that the increased neuropil fraction in the prefrontal cortex supports social and behavioral flexibility, while in the primary motor cortex, this facilitates the neural activity needed for hunting movements. Greater neuropil fraction in the primary visual cortex may contribute to visual requirements associated with diel activity patterns. ***Conclusion:*** These results provide a cross-species comparison of neuropil fraction variation in the Felidae, particularly the understudied *Panthera*, and provide evidence for convergence of the neuroanatomy of *Panthera* and cheetahs.

Introduction

The Felidae are a diverse group of mammals consisting of 34 extant "cat-like" species that range in body size from large apex predators to smaller domesticated varieties [1].

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Despite considerable variation in body size and pelage [2], all felids share certain morphological adaptations, such as agile, muscular bodies, sharp, curved claws, well-developed facial vibrissae, visual, vestibular, auditory, and gustatory adaptations. These adaptations are employed during hunting when tracking or identifying prey species and aid in social interactions between conspecifics. Felids remain historically understudied [3], and we know very little about their comparative socio-cognitive behavior and neural architecture [4–7], which is surprising given their relative brain size and gyrencephalic cerebral cortices [8]. Arguably, this paucity in data is in part due to public perceptions concerning their predatory nature, as well as biases in the scientific community, which have tended to limit the scope of study in these species [9].

Although most members of the Felidae are solitary and only participate socially during mating or rearing [10], other members of this group are markedly social (e.g., African lions, *Panthera leo*). These observations point towards a unique theoretical vantage point that may help to disentangle the role of socio-cognitive and ecological factors on brain evolution. The *Panthera*, for example, provides a natural experiment where comparative studies could help elucidate predictions from either the domain-general or domain-specific social intelligence hypotheses [11]. At the root of these hypotheses is the question of whether social complexity is the major driver of cognitive differences between species or whether cognitive capacities rooted in other non-social domains have played a greater role in species behavioral and neuroanatomical differences. As argued by Borrego [12], *Panthera* is one of the few lineages where this question could be readily addressed because, despite the broad similarities in ecology between *Panthera* species, lions (*Panthera leo*) are widely regarded as the only truly social species within this clade [2, 13]. Such specific divergence in social behavior, against a backdrop of more homogenous ecological adaptations, is rare among such closely related species [14] (for another prime example in prairie voles).

Comparative studies of felids may also lend themselves to unique insight into the evolutionary process. For example, recent studies of comparative neural architecture in cheetahs (*Acinoyx jubatus*) have revealed a convergence in primary motor cortex pyramidal neuron morphology with that of the *Panthera*, highlighting the impact of shared ecological pressures required for hunting [15]. Furthermore, the study of domestic felids (*Felis sylvestris cattus*) compared to their wild counterparts may augment our understanding of artificial selection and its impact on the nervous system [16, 17]. Studies of felid nervous systems in a comparative context also provide ongoing support and refinement of our interpretations of the fossil record. For instance, there is still ongoing debate whether tarpit assemblages of Sabertooth cats (*Smilodon fatalis*) are indicative that these animals were social hunters like extant lions [18] or whether these accumulations are an artefact arising from solitary animals being collectively lured to their deaths through cues (olfactory, auditory, or visual) emitted from entrapped prey species [19].

We undertook the present study to assess potential regional and species differences in cortical microstructural organization across felids. The cortical column is considered the smallest functional unit of the cerebral cortex and consists of a vertically arranged cellular circuit which forms an essential building block of information processing in the neocortex [20]. The neuropil is an important subcomponent of the cortical column as it represents the tissue between the cell somata comprised of dendrites, axons, synapses, and microvasculature, where inter-neuronal connections are made. Thus, changes in neuropil structure may serve as a proxy for proportional connectivity, as it reflects overall changes in cortical column processing. Several earlier studies have compared cortical minicolumn morphology and neuropil in humans, nonhuman primates, and artiodactyls to analyze regional and species differences in cortical microstructure [21–28]; however, a quantitative comparison of cortical neuropil has yet to be conducted within the Felidae. It is unknown whether patterns of variation in neuropil observed in other species can be extrapolated to the Felidae or if associated morphological, behavioral, or ecological features have explanatory value in understanding cortical histology within this group.

In this study, we examined regional (prefrontal cortex, motor cortex, and primary visual cortex) and species differences in neuropil within the *Panthera* and two outgroup species (cheetah and domestic cat) and explored variation in association with brain size, behavior, and neuronal morphology. These results provide a comparative framework for understanding species differences in cortical column microcircuitry within the Carnivora.

Materials and Methods

Specimens and Histological Preparation

Histological sections from six felid species were used in this study. The phylogenetic relationship of these species is shown in Figure 1. Slide sets of four species (*Panthera leo* $N = 2$, adult male and female; *Panthera pardus* $N = 1$, male 22-year-old; *Acinonyx jubatus* $N = 1$, adult female; *Panthera tigris* $N = 1$, female 12-year-old) were obtained on loan from the University of Witwatersrand

(P.R.M.). The slides for the snow leopard (*Panthera uncia N* = 1, male 6-year-old) were obtained on loan from the George Washington University (C.C.S.), while the slides for the domestic cat (*Felis sylvestris cattus N* = 1, adult male) were obtained from the online data repository (brainmaps.org) as previously collated by Ted Jones. The tissue sections analyzed in the present study were used in earlier comparative investigations [29–31]. All animals included in these studies were treated and used in accordance with the guidelines of the NIH for care and responsible use of animals in scientific research. These studies were approved by the Colorado College Institutional Review Board (#011311-1) and the University of the Witwatersrand Animal Ethics Committee (2008/36/1; 2012/53/1) and the animal ethics committee of Des Moines University. For specific information on sample acquisition, we refer the reader to the materials sections of each of Jacobs et al. [15, 29] and Nguyen et al. [30]. In brief, specimens were obtained through collaboration with the Copenhagen Zoo (Denmark), the Smithsonian National Zoological Park (Washington, DC, USA), and the Boras Zoo (Sweden). Whole brains were removed and fixed in 4% paraformaldehyde using either immersion fixation or perfusion through the carotid arteries. Specimens were equilibrated in 30% sucrose before being transferred to an antifreeze solution prior to storage in a −20°C freezer. Homologous tissue blocks, 3–5 mm thick, were removed from the left hemisphere of each specimen: (1) a prefrontal block taken from the medial aspect of the frontopolar cortex; (2) the primary motor cortex block, located immediately posterior to the frontal pole and guided by the placement of the cruciate sulcus; and (3) the primary visual cortex block, located along the dorsal posterior aspect of the lateral gyrus (Fig. 2). Tissue blocks were frozen in crushed dry ice before being cut on a sliding microtome or cryostat at a section thickness of 50 μm. The entire tissue block was sectioned in the coronal plane, and sections were then mounted and stained for Nissl substance with cresyl violet.

Using the resultant Nissl sections, the neuropil fraction (NF) was sampled in cortical layers I–VI using an approach employed in previous comparative studies [25, 27, 31]. This image analysis approach is similar to methods used by other laboratories [28, 32–37] and measures the fraction of the projected profiles of stained cell bodies in each section versus the unstained profile (see Fig. 2). The stained component in each section represents the area occupied by the cell bodies of neurons, glia, and endothelial cells, whereas the unstained profiles in each section represent the neuropil (i.e., the area occupied by dendrites, axons, myelin, microvasculature, and synapses). Changes in the proportion of neuropil space have been shown to be a proxy for changes in connectivity and reflect overall changes in cortical column processing [26].

For the *Panthera* (i.e., *P. leo, P. pardus, P. tigris, P. uncia*) and cheetah (*Acinonyx jubatus*), high-resolution images were acquired in each region (prefrontal, primary motor, and primary visual cortex) using a Nikon Eclipse 80i microscope equipped with a motorized stage and a color video camera (Optronics, Golenta, CA, USA) coupled to a PC workstation running StereoInvestigator software (MBF Bioscience, Colchester, Vermont, Version 8.0). Contours were drawn at low magnification (×2 objective lens) around each region of interest, and digital images were obtained using a ×20 objective lens (Fig. 2). Image stacks consisting of ~20 images per section (6 sections per species) were collected from each neuroanatomical region before being converted to 8-bit gray-scale image frames for quantification. Sampling design parameters were kept in alignment with previous studies (i.e., grid spacing 1,000 μm × 1,000 μm; image size 1,600 × 1,200 pixels, resolution 0.37 pixels per μm, average target intensity 71%), and any counting frames that had artifacts or fell outside of the region of interest were omitted [25, 27]. For the domestic cat (*Felis sylvestris cattus*), images from each region were downloaded at ×20 magnification (Brainmaps.org) before being subjected to image analysis. As with the other specimens, each section consisted of approximately 20 images per stack, sampled from the region of interest.

To estimate the proportion of stained versus unstained tissue (i.e., the neuropil fraction NF), each image series was imported into ImageJ (v.1.32j) and processed through a pipeline that included background subtraction, binary conversion, and thresholding [38]. The percentage of the measuring frame occupied by the NF was calculated as the section-weighted mean. The mean coefficient of error (CE) of all NF measurements was 0.06.

Data Analysis

Linear regression analyses were conducted to evaluate potential scaling relationships between NF in each cortical region and brain mass (g) (Table 4) as well as neuronal cell parameters (Table 5) and average species behavioral and ecological characteristics (Table 6). Neuronal cell parameters were sourced from average species data for aspiny and pyramidal neurons as obtained from Neuromorpho.org. The cell parameters included in these analyses were: Asvol = Aspiny volume (μm^3); ASomaS = Aspiny Somal Size (μm^2); ASTDL = Aspiny Total Dendritic Length (μm); ASMSL = Aspiny Mean Segment Length (μm); ASDSC = Aspiny Number of Dendritic Segments; Pvol = Pyramidal volume (μm^3); PSomaS = Pyramidal Somal Size (μm^2); PTDL = Pyramidal Total Dendritic Length (μm); PMSL = Pyramidal Mean Segment Length (μm); PDSC = Pyramidal Number of Dendritic Segments; PDSN = Pyramidal Total Number of Spines on Dendritic Segments; PDSD = Pyramidal Average Number of Spines on Dendritic Length. Note that the snow leopard and domestic cat were omitted from these regression analyses as cell parameter data were not available for these species. In addition to exploring the bivariate relationships between NF and morphological characteristics, we also explored the relationship between NF and published average species behavioral and ecological characteristics. Species behavioral and ecological data (i.e., social complexity, behavioral complexity, dexterity score, species average group size, home range, geographic range) were sourced from species averages [4, 39]. The relationship between NF and continuous data (obtained from published sources) was compared using correlation coefficients (Pearson). The relationship between NF and categorical data (i.e., social complexity, activity patterns) were compared using analysis of variance. Species were split into two groups (social and solitary) as defined by Benson-Amram et al. [4] before testing for significant differences in group means. Similarly, species were split into diel groups (diurnal, nocturnal, and cathemeral-crepuscular) as coded by Michaud, Toussaint, and Gilissen [39] before significance testing. Based on the functions of the prefrontal cortex in planning, reasoning, and long-term memory in mammals [40], we predicted that prefrontal cortex NF would be correlated with social complexity, behavioral diversity, and species average group size. Similarly, we predicted that the primary motor cortex, given its role in initiating and executing movements [41], would likely be correlated with the home range, geographic range, and dexterity scores. Given the highly specialized function of the primary visual

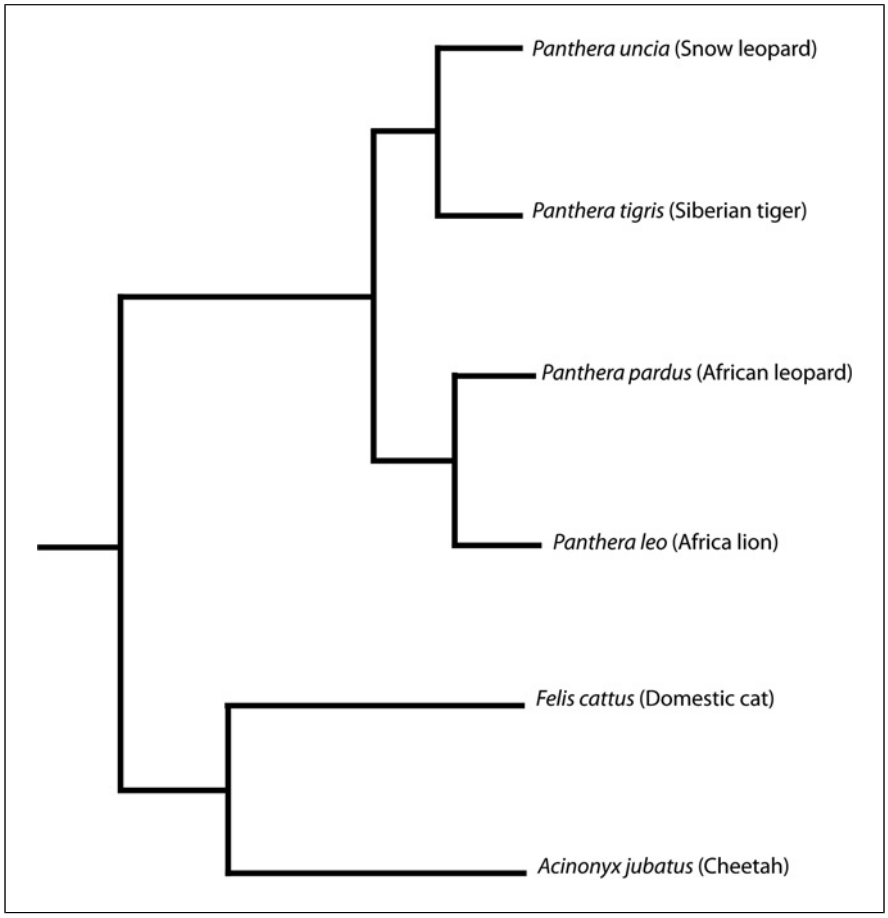

Fig. 1. Phylogeny used in the implementation of phylogenetic generalized least squares (PGLS). PGLS was performed used the caper package [43]. The phylogeny was constructed using data based on the mammalian super-tree [47].

cortex [42] in allowing sighted animals to make sense of visual input, we predicted that variations in species activity patterns would likely be correlated with primary visual cortex NF.

All data were log transformed (base 10) prior to model fitting and analyzed in R version 4.0.3 (www.r-project.org/, RRID:SCR-001905). To account for the effects of species relatedness in the sample, phylogenetic generalized least squares (PGLS) was performed using the "caper" [43] and Evomaps packages [44]. The phylogeny used in the current study (Fig. 1) was derived from [45–47]. Furthermore, we also evaluated differences in mean values using repeated measures analysis of variance (ANOVA) with species as the between-subjects factor and brain region as the within-subjects factor, followed by post hoc comparisons (Tukey). As an exploratory analysis, we also used principal component analysis to reduce the dimensionality of the data. The raw data used to derive these relationships are shown in Table 1 and Figure 3 and a summary of the regression statistics derived from the analyses is shown in Table 4 and 6.

Results

For the prefrontal cortex, the lion had the largest neuropil fraction (NF = 0.81), followed closely by the cheetah (NF = 0.81), tiger (NF = 0.79), snow leopard (NF = 0.71), African leopard (NF = 0.67), and domestic cat (NF = 0.67) (Table 1). We undertook significance testing using analysis of variance (ANOVA) to evaluate if there were any significant differences in mean NF between species and/or regions. These tests revealed that there were significant species ($p = 1.28 \times 10^{-30}$) and regional ($p = 1.77 \times 10^{-9}$) differences in neuropil fraction (Welch $F = 55.72$, df = 21.81, $p = 4.36 \times 10^{-14}$) (Table 2, 3, as well as Fig. 4a, b). The mean NF profiles for the lion, cheetah, and tiger were found to be significantly larger than those for the African leopard, snow leopard, and domestic cat. In addition, the mean NF profiles across species were significantly different from one another, indicating that NF in the prefrontal cortex was significantly greater than that for the primary motor and primary visual cortex, while the primary motor cortex NF was significantly larger than that of primary visual cortex. Post hoc testing of species differences in regional neuropil fraction (Table 3) revealed that for all three regions (i.e., prefrontal, primary motor, and primary visual cortex), the lion, cheetah,

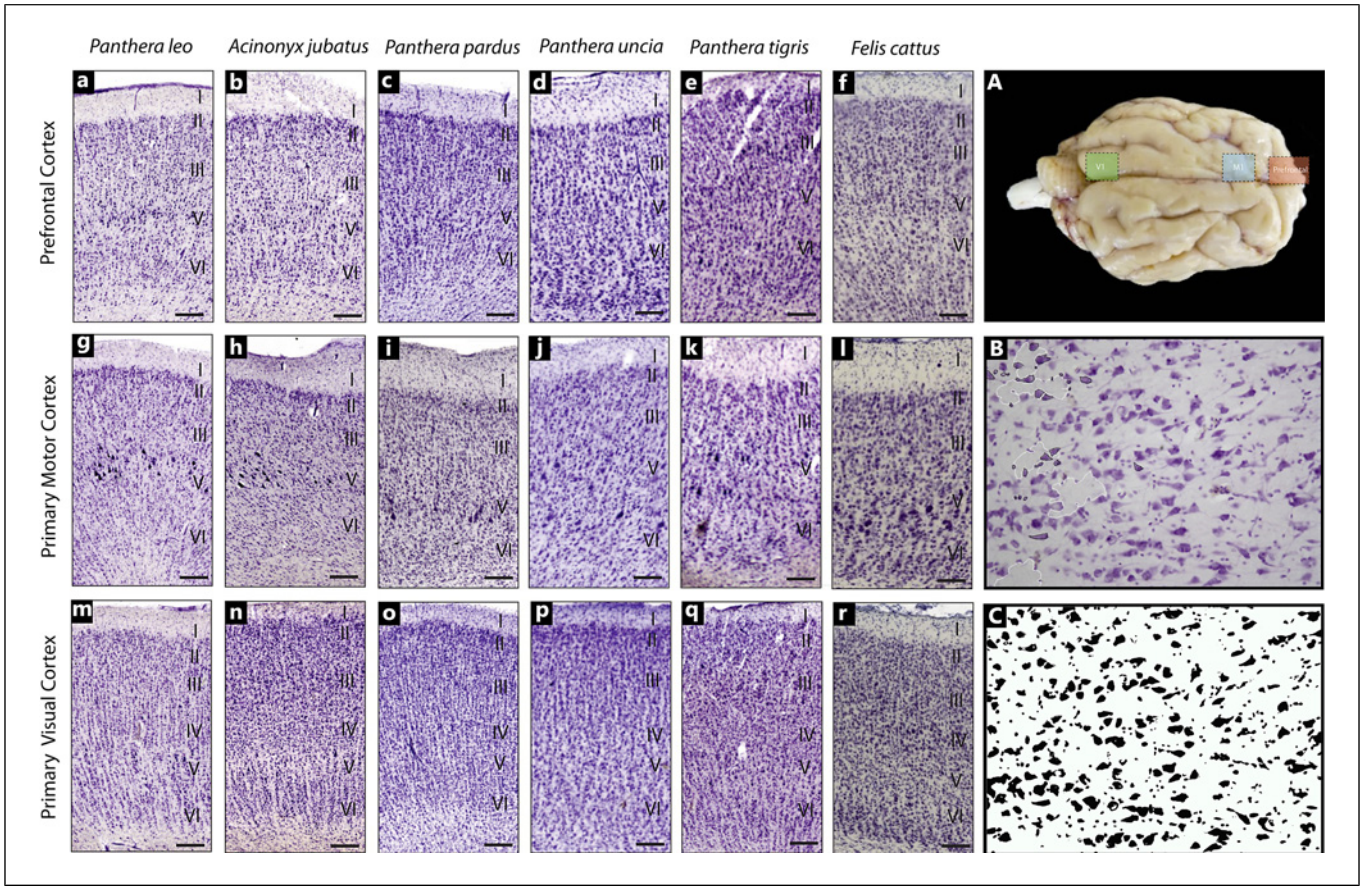

Fig. 2. An overview of the approach used for image acquisition and conversion used for measurement of the neuropil fraction (NF). In the panel (**a–r**), we show representative images through the prefrontal, primary motor, and primary visual cortex of each species sampled for the analysis. **A** A representative dorsal view of the felid cerebrum showing the approximate location of tissue sampling sites through the prefrontal, primary motor, and primary visual cortex. **B**, **C** Representative images of the neuropil space captured at ×20 magnification were followed by binary image conversion to measure the NF. Scale bar = 80 μm.

and tiger NF were significantly larger than that of the leopard, snow leopard, and domestic cat; however, the lion, cheetah, and tiger did not differ significantly from one another in terms of neuropil fraction. While the African leopard, snow leopard, and cat were largely similar in neuropil profile, post hoc testing revealed that the domestic cat had a significantly smaller neuropil fraction in the primary motor cortex as compared to the snow leopard and a significantly smaller neuropil fraction in the primary visual cortex as compared to the African leopard. We found that the neuropil fraction of the prefrontal cortex in the cheetah (NF = 0.81) was significantly larger than that found in its primary visual cortex (NF = 0.75). In the domestic cat, we found a similar statistically significant difference between the neuropil fraction of the prefrontal (NF = 0.67) and primary visual cortex (NF = 0.60), a pattern that was repeated for the neuropil fraction of the primary visual, primary motor, and prefrontal cortex of the snow leopard.

To explore and visualize these groupings further, we used principal component analysis (PCA) to reduce the parameters of NF prefrontal cortex, NF primary motor cortex, and NF primary visual cortex into a mean NF profile for each of the six felid species. These analyses revealed that the NF profiles for the lion, cheetah, and tiger clustered together while the domestic cat, snow leopard, and African leopard occupied a separate space in the PCA (Fig. 4c).

To evaluate scaling relationships between brain size and neuropil fraction, we used phylogenetic regression analysis (PGLS). The results of these scaling analyses are shown in Table 4 and Figure 5.

For the prefrontal cortex, 33% of the variation in neuropil fraction could be explained by variation in brain mass. Prefrontal cortex NF and brain mass, however,

Table 1. Summary of the mean neuropil fractions (NF) by cortical area for six felid species

Species	Region	Brain mass, g	Neuropil fraction	Std. error	95% CI lower	95% CI upper
Acinonyx jubatus	Prefrontal	139.50	0.81	0.01	0.79	0.83
Panthera leo	Prefrontal	166.40	0.81	0.01	0.79	0.83
Panthera pardus	Prefrontal	143.20	0.67	0.01	0.65	0.70
Panthera uncia	Prefrontal	67.46	0.71	0.01	0.69	0.73
Panthera tigris	Prefrontal	233.60	0.79	0.01	0.77	0.82
Felis cattus	Prefrontal	24.10	0.67	0.01	0.64	0.70
Mean prefrontal NF = 0.75; Coefficient of variation = 9.09						
Acinonyx jubatus	Primary motor	139.50	0.78	0.01	0.76	0.80
Panthera leo	Primary motor	166.40	0.80	0.01	0.78	0.82
Panthera pardus	Primary motor	143.20	0.66	0.01	0.64	0.68
Panthera uncia	Primary motor	67.46	0.71	0.01	0.69	0.73
Panthera tigris	Primary motor	233.60	0.80	0.01	0.77	0.82
Felis cattus	Primary motor	24.10	0.62	0.01	0.59	0.65
Mean primary motor NF = 0.73; Coefficient of variation = 10.37						
Acinonyx jubatus	Primary visual	139.50	0.75	0.01	0.73	0.77
Panthera leo	Primary visual	166.40	0.76	0.01	0.74	0.78
Panthera pardus	Primary visual	143.20	0.66	0.01	0.64	0.69
Panthera uncia	Primary visual	67.46	0.64	0.01	0.62	0.66
Panthera tigris	Primary visual	233.60	0.76	0.01	0.74	0.79
Felis cattus	Primary visual	24.10	0.60	0.01	0.56	0.62
Mean primary visual NF = 0.69; Coefficient of variation = 10.24						

were not significantly correlated ($p = 0.11$), and there was a small phylogenetic signal in the data ($\lambda = 0.49$). All the species datapoints lay well within the confidence and prediction intervals.

For the primary motor cortex, the lion had a slightly larger neuropil fraction (NF = 0.797), compared to the tiger (NF = 0.796), followed by the cheetah (NF = 0.78), snow leopard (NF = 0.71), African leopard (NF = 0.66), and domestic cat (NF = 0.62) (Table 1). Scaling analyses for the primary motor cortex revealed that 59% of the variation in neuropil fraction could be explained by variation in brain mass. There was a negligible phylogenetic signal in the data ($\lambda = 0.03$). Primary motor cortex NF and brain mass were significantly correlated ($p = 0.05$). As with prefrontal cortex NF, all the species datapoints for the primary motor cortex lay well within the confidence and prediction intervals.

For the primary visual cortex, the tiger had the largest neuropil fraction (NF = 0.765), followed closely by the lion (NF = 0.757), cheetah (NF = 0.747), African leopard (NF = 0.663), snow leopard (NF = 0.636), and domestic cat (NF = 0.598) (Table 1). Scaling analyses for the primary visual cortex revealed that 74% of the variation in neuropil fraction could be explained by variation in brain mass. There was a large phylogenetic signal in the data ($\lambda = 0.95$). Primary visual cortex NF and brain mass were found to be significantly correlated ($p = 0.004$), and all the species datapoints for the primary visual cortex lay well within the confidence and prediction intervals for the data, with an overall close fit to the model.

To explore the nature of the bivariate relationship between NF and neuronal cell parameters, we used least squares regression analysis to calculate the coefficients of determination and significance values for aspiny and pyramidal cell parameters (Table 5). Correlation analyses revealed that pyramidal somal size was significantly correlated with NF for the prefrontal ($p = 0.009$) and primary motor ($p = 0.005$) cortices, with 98 and 99% of the variation in pyramidal somal size explained by variation in prefrontal and primary motor NF, respectively; however, in the primary visual cortex, pyramidal somal size was not significantly correlated with NF ($R^2 = 0.59$, $p = 0.47$).

To investigate the relationship between NF and published species behavioral and ecological characteristics [4, 39], we used correlational analysis (Pearson) as well as analysis of variance (Table 6). Our analyses revealed that NF primary motor cortex was strongly and significantly correlated with species home range ($R^2 = 0.81$, $p = 0.01$). For the categorical data, analysis of variance revealed

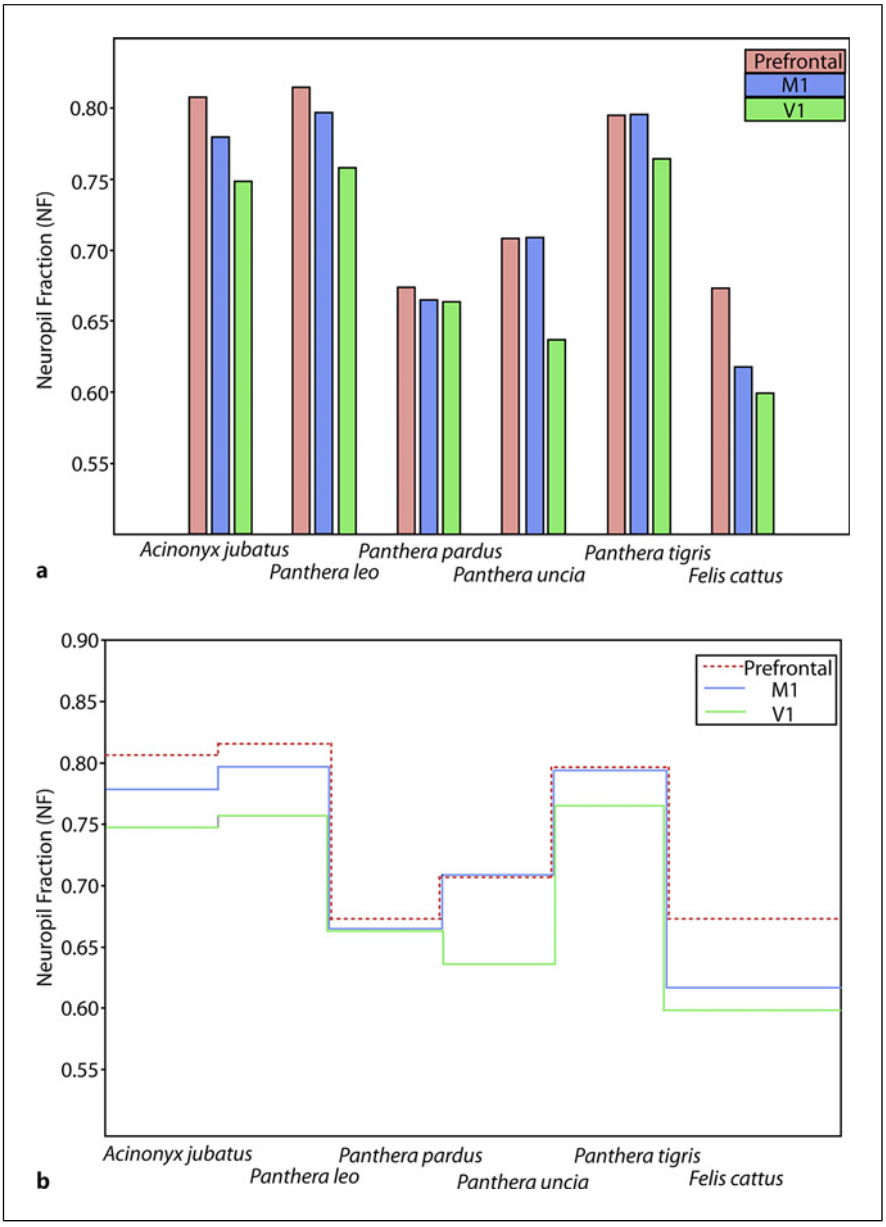

Fig. 3. Bar graph (**a**) of the mean neuropil fraction and steps chart (**b**) of the mean neuropil fraction in each species sampled in the prefrontal, primary motor and primary visual cortex.

Table 2. Results of repeated measures analysis of variance (ANOVA)

Source	Between groups df	*F*	*p* value
Dependent variable: neuropil fraction			
Species	5	101.13	1.28E-30
Region	2	27.25	1.77E-09
Species × region	10	1.79	0.07914

that there was a significant group difference in mean NF prefrontal cortex between the social and solitary felids (Welch $F = 59.55$, df = 18.22, $p = 3.7 \times 10^{-7}$) as well as significant differences in mean NF primary visual cortex between the diel groups (Welch $F = 8.14$, df = 14.26, $p = 0.0044$) (Fig. 6).

Discussion

Our analyses revealed significant species and regional differences in cortical neuropil among the Felidae. We observed that lions, cheetahs, and tigers had significantly greater neuropil than leopards, snow leopards, and domestic cats. On average, prefrontal cortex has the highest

Table 3. Summary of post hoc testing (Tukey) showing significant species and regional pairwise comparisons

Multiple comparisons					
Dependent variable: species regional neuropil fraction					
Prefrontal NF		**Sig.**	**Primary motor NF**		**Sig.**
Panthera leo	*Panthera pardus*	0.00E+00	*Panthera leo*	*Panthera pardus*	0.00E+00
Panthera leo	*Panthera uncia*	6.17E-08	*Panthera leo*	*Panthera uncia*	7.32E-06
Panthera leo	*Felis cattus*	4.25E-08	*Panthera leo*	*Felis cattus*	0
Acinonyx jubatus	*Panthera pardus*	5.68E-11	*Acinonyx jubatus*	*Panthera pardus*	1.43E-09
Acinonyx jubatus	*Panthera uncia*	3.13E-07	*Acinonyx jubatus*	*Panthera uncia*	1.45E-03
Acinonyx jubatus	*Felis cattus*	1.61E-07	*Acinonyx jubatus*	*Felis cattus*	0
Panthera tigris	*Panthera pardus*	1.82E-03	*Panthera tigris*	*Panthera pardus*	8.12E-09
Panthera tigris	*Panthera uncia*	1.13E-06	*Panthera tigris*	*Panthera uncia*	5.27E-04
Panthera tigris	*Felis cattus*	8.81E-05	*Panthera tigris*	*Felis cattus*	6.47E-11
			Felis cattus	*Panthera uncia*	0.0004059
Primary visual NF					
Panthera leo	*Panthera pardus*	5.44E-06	Frontal – *Felis cattus*	Visual – *Felis cattus*	0.001533
Panthera leo	*Panthera uncia*	5.20E-11	Frontal – *Acinonyx jubatus*	Visual – *Acinonyx jubatus*	0.008705
Panthera leo	*Felis cattus*	0	Frontal – *Panthera uncia*	Visual – *Panthera uncia*	1.02E-03
Acinonyx jubatus	*Panthera pardus*	1.71E-04	Frontal – *Panthera uncia*	Motor – *Panthera uncia*	6.11E-04
Acinonyx jubatus	*Panthera uncia*	8.20E-09			
Acinonyx jubatus	*Felis cattus*	0	**Dependent variable: mean neuropil fraction across all 6 species**		
Panthera tigris	*Panthera pardus*	8.95E-05			**Sig.**
Panthera tigris	*Panthera uncia*	4.54E-08	Prefrontal	Primary motor	0.00171,100
Panthera tigris	*Felis cattus*	5.06E-11	Primary motor	Primary misual	0.00004500
Felis cattus	*Panthera pardus*	0.02307	Primary Visual	Prefrontal	0.00000001

neuropil, followed by the primary motor and primary visual areas. Brain size was found to have a modest, positive correlation with regional neuropil, while at a cellular level, pyramidal neuron somal size was positively correlated with neuropil. Social and solitary felids differed significantly in the prefrontal cortex neuropil. We also observed significant group differences in neuropil for the primary visual area between diurnal felids and their nocturnal and cathemeral-crepuscular counterparts.

As is often the case for studies involving charismatic and difficult-to-acquire species, our results are limited by our sample size. In the current study, every effort was undertaken to include only adult specimens and exclude any animals with marked neurodegenerative changes. In humans, sexual dimorphism in neuropil volume has been reported at around 3% per cortical column [48], resulting in marginal differences in total neuropil volume between males and females [48]. While we emphasize caution in the interpretation of our results, in the absence of any sexual dimorphism data for neuropil in the Felidae and in the context of our current analyses, we are confident that these results could be generalized to the level of species. Below, we discuss these observations in more detail, with specific emphasis on the potential functional significance of our results.

Species-Specific Variation in Prefrontal Neuropil

In the prefrontal cortex, we observed that the 3 big cat species (i.e., lions, cheetahs, and tigers) had significantly greater neuropil space in comparison to the other cats, with lions forming the upper threshold of this group. In humans, regions within the prefrontal cortex are known to be involved in a wide range of behaviors including memory, decision making, and social functions [49–53]. The prefrontal cortex is strongly connected with the sensory-motor areas as well as sharing connections with limbic, hypothalamic, and striatal nuclei [40, 55–57]. Based on its neuroanatomical connections, the prefrontal cortex is partitioned into medial and lateral components [53] with the medial prefrontal cortex playing a role in processing social and affective information [58], while the lateral prefrontal cortex is mainly involved in supporting cognitive control processes [53]. The prefrontal cortex (PFC) has not been studied extensively in wild carnivores. However, studies on domestic cats and dogs have provided some insight into the connectional arrangement of this area. These studies have revealed that the carnivora prefrontal cortex is functionally and structurally similar to that of the macaque monkey albeit with some topographical changes [59–62]. Studies of afferent subcortical

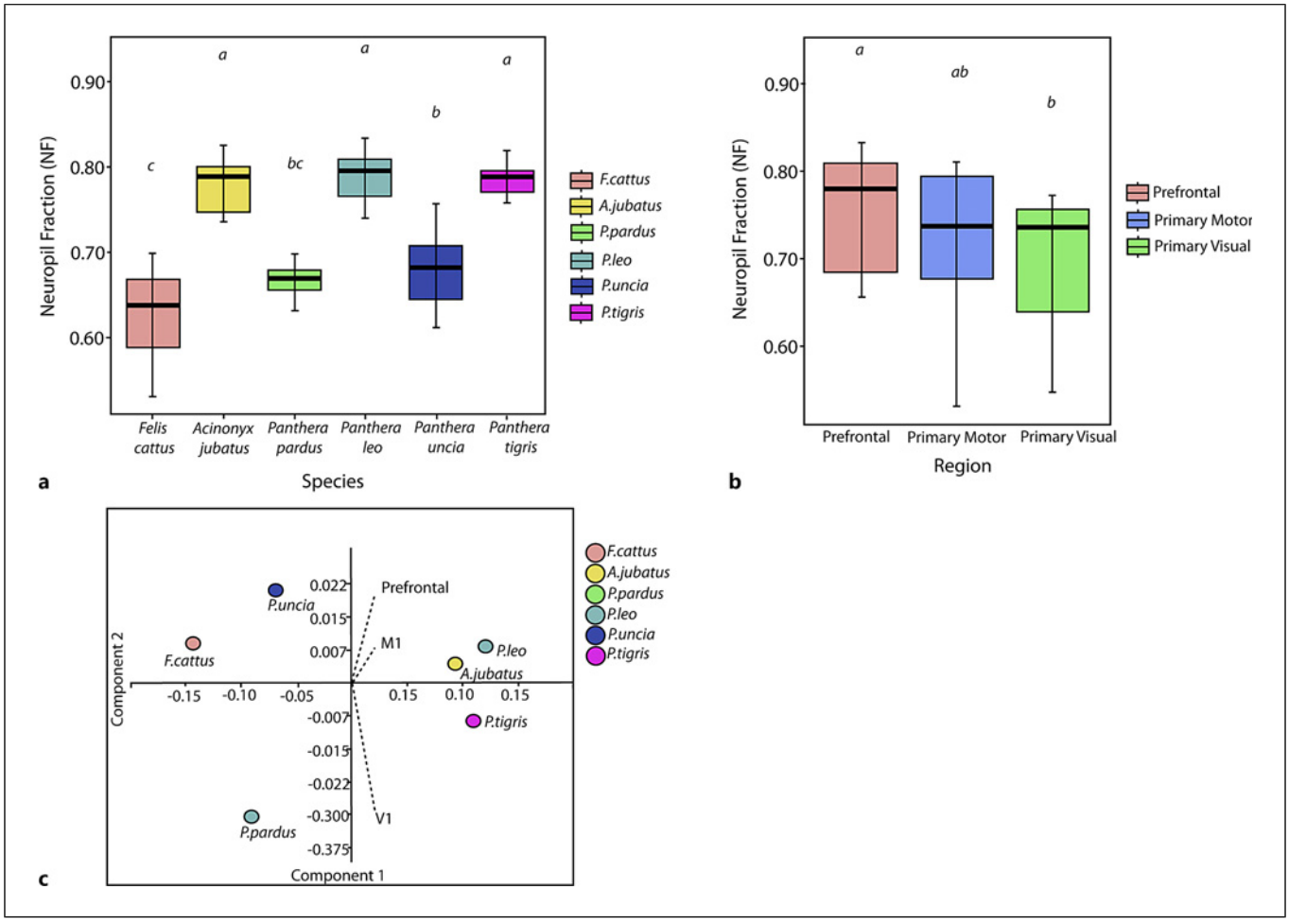

Fig. 4. Box and whisker plots by species (**a**) and region (**b**) with significant differences in neuropil fraction (obtained by ANOVA) indicated through compact letter display. Note as shown in Tables 3 and 4, significant species and regional differences were observed in mean neuropil fraction. The NF profiles for the lion, cheetah, and tiger were found to be significantly different from the leopard, snow leopard and domestic cat. In addition, the mean NF profiles from the three brain regions also differed significantly from one another. **c** Results of principal component analyses of brain mass, NF prefrontal, NF primary motor, and NF primary visual cortex in the six felid species. Note that based on these NF profiles the lion, cheetah, and tiger cluster into one group while the domestic cat, snow leopard, and African leopard occupy a separate morphospace.

Table 4. Summary of the regression statistics obtained using phylogenetic generalized least squares regression (PGLS)

Model (*x* variable; *y* variable)	λ	Multiple R^2	Adjusted R^2	Slope	SE	*t* value	*p* value	Intercept	SE	*t* value	*p* value	AIC
PGLS (brain mass, Prefrontal cortex NF)	0	0.46	0.33	0.06	0.03	2.03	0.11	−0.235	0.064	−3.669	0.021	−8.119
PGLS (brain mass, primary motor cortex NF)	0	0.67	0.59	0.09	0.03	2.68	0.06	−0.313	0.08	−4.12	0.0145	−6.761
PGLS. brain mass, primary visual cortex NF)	0	0.79	0.74	0.101	0.016	6.09	0.004	−0.358	0.038	−9.44	0.0007	−12.309

PGLS was performed using the Caper [43] and EvoMaps packages [44].

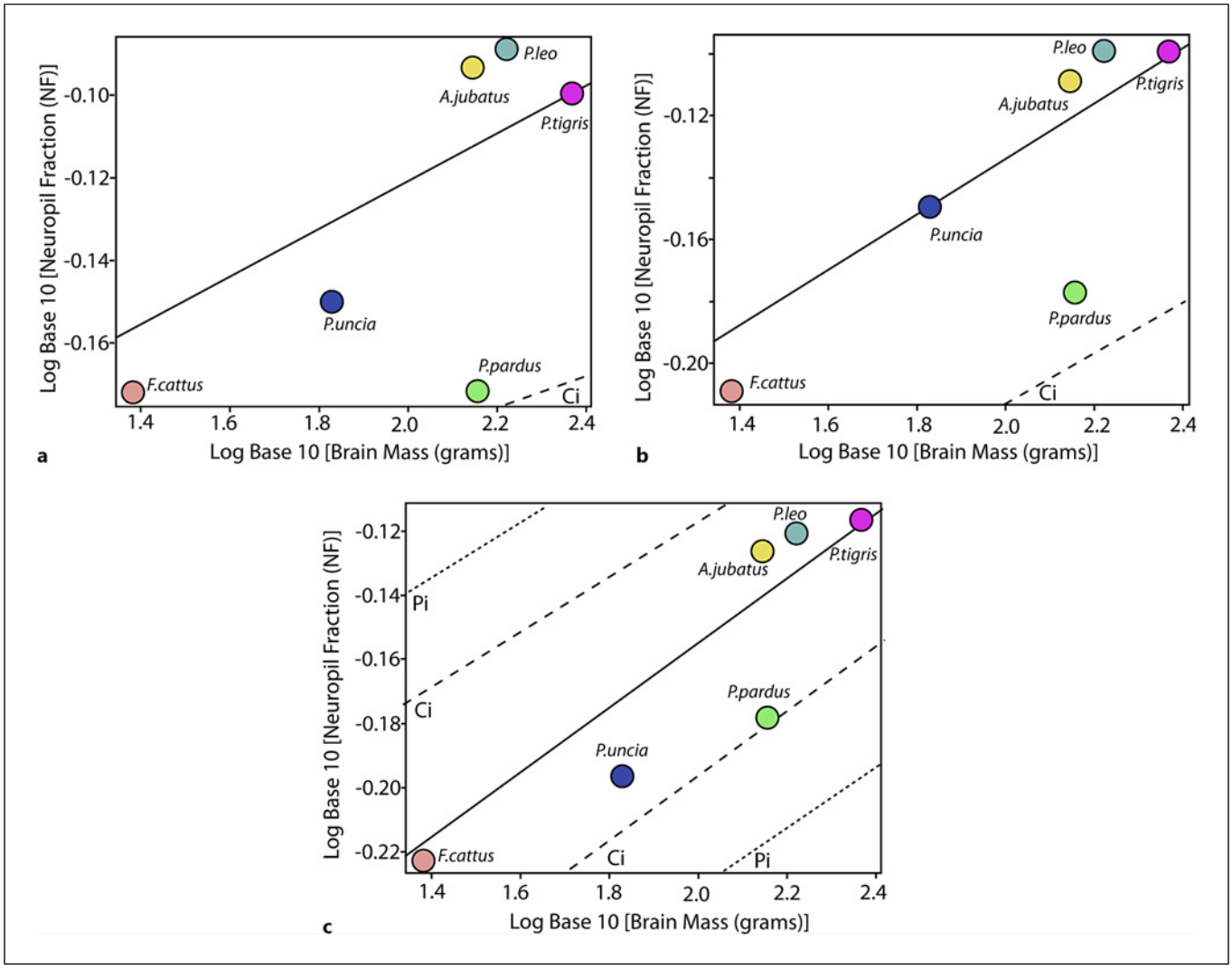

Fig. 5. Plots derived from phylogenetic generalized least squares regression (PGLS) analysis of Brain Mass plotted against mean Neuropil fraction (NF) for the prefrontal cortex (**a**), primary motor cortex (**b**), and primary visual cortex (**c**) across the six felid species. All data were logarithmically transformed (base 10) prior to inclusion in the regression analyses. Data used to derive these relationships are shown in Table 1. Dotted lines indicate the prediction and confidence intervals for each regression line derived using EvoMaps [44].

connections to the medial prefrontal cortex in dogs have revealed that the dorsal and ventral regions of the medial PFC receive projections from the mediodorsal and ventral thalamic nuclei, while the dorsomedial and ventromedial PFC receive projections from the mediodorsal thalamic nuclei [59]. The connectivity between the mediodorsal thalamus and the prefrontal cortex has long been considered an important, albeit debated, characteristic of the prefrontal cortex [63]. The axiom of this prefrontal-mediodorsal connectivity pattern has been challenged in recent years [64] but has remained popular as a distinguishing characteristic to separate prefrontal and motor regions. In carnivores, the prefrontal and motor cortex have been distinguished from one another based on their projections, either from the mediodorsal or ventrolateral thalamic nuclei, respectively [59]. This pragmatic definition has been supported by ablation experiments where ablations in the anteromedial PFC in dogs have resulted in impairments in Pavlovian differentiating tasks [65], while ablations to the dorsomedial PFC (i.e., medial precruciate surface) resulted in impairments in motor differentiating tasks, directional cues,

Table 5. Coefficients of determination and significance values for the neuropil fraction from the prefrontal, primary motor, and primary visual areas plotted against select neuronal cell parameters

OLS Model (*x* variable; *y* variable)	Prefrontal		Primary motor		Primary visual	
	r^2	*p* value	r^2	*p* value	r^2	*p* value
NF; Asvol	0.79	0.203	0.01	0.991	0.17	0.822
NF; ASomaS	0.57	0.429	0.32	0.674	0.29	0.706
NF; ASTDL	0.92	0.083	0.14	0.853	0.08	0.912
NF; ASMSL	0.84	0.163	0.35	0.649	0.44	0.554
NF; ASDSC	0.20	0.793	0.63	0.362	0.45	0.541
NF; Pvol	0.77	0.224	0.81	0.183	0.52	0.476
NF; PSomaS	**0.98**	**0.009**	**0.99**	**0.005**	0.59	0.407
NF; PTDL	0.40	0.605	0.78	0.218	0.54	0.454
NF; PMSL	0.65	0.346	0.64	0.359	0.57	0.429
NF; PDSC	0.11	0.882	0.78	0.213	0.55	0.446
NF; PDSN	0.34	0.657	0.37	0.656	0.79	0.212
NF; PDSD	0.43	0.561	0.03	0.966	0.00	0.977

Neuronal cell parameters were sourced from average data for aspiny and pyramidal neurons as obtained from Neuromorpho.org. NF, neuropil fraction; Asvol, aspiny volume (μm^3); ASomaS, AAspiny Somal Size (μm^2); ASTDL, aspiny total dendritic length (μm); ASMSL, aspiny mean segment length (μm); ASDSC, aspiny-number of dendritic segments; Pvol, pyramidal volume (μm^3); PSomaS, pyramidal somal size (μm^2); PTDL, pyramidal total dendritic length (μm); PMSL, pyramidal mean segment length (μm); PDSC, pyramidal number of dendritic segments; PDSN, pyramidal total number of spines on dendritic Segments; PDSD, pyramidal average mumber of spines on dendritic length; r^2, coefficient of determination.

Table 6. Correlation coefficients and *p* values for mean neuropil fraction (NF) in the frontalaand motor cortex compared against average species behavioral characteristics (i.e., behavioral diversity, dexterity score, species Average Group Size) and average species ecological characteristics (i.e., home range, geographic range)

Variable 1	Variable 2	r^2	*p* value
NF Prefrontal	Behavioral diversity	0.04	0.70
NF Prefrontal	Species avg. group size	0.28	0.28
NF Primary motor	Home range (km^2)	**0.81**	**0.01**
NF Primary motor	Geographic range (km^2)	0.64	0.06
NF Primary motor	Dexterity score	0.06	0.65

Behavioral and ecological data were sourced from [4, 39].

and directional responses [66–68]. In addition, the ventromedial prefrontal cortex is known to receive projections from anterior thalamic nuclei, while the caudal region of the ventromedial prefrontal cortex receives a richer stream of projections from "non-specific" thalamic nuclei as well as from the amygdaloid complex [59]. These connectional patterns of the medial prefrontal cortex in carnivores align with what we know for primates, where the medial prefrontal cortex is known to play a role in processing social and affective information [58]. Tissue sampling of the prefrontal cortex in the current study more closely aligns with the sampling of the medial prefrontal region. Our results suggest that the prefrontal cortex of lions, tigers, and cheetahs have relatively increased connectivity compared to that of the other felids. We hypothesize that this may be associated with the marked differences in sociality observed within the Felidae [69]. In support of this interpretation, below we review the social behavior of the Felidae included in our study.

Among the felids, lions are the only species that live in large social groups (prides), in extreme cases consisting of as many as 21 individuals [70]. The ability to support such a relatively high degree of group living may require improved cognitive faculties to monitor social hierarchy and group dynamics. Successful group living requires an ability to recognize the hierarchical organization within the group and to perceive and act on the changing status of self and others [71, 72]. In primates, our understanding of the neural mechanisms supporting these behaviors has highlighted the role of the prefrontal cortex acting alongside the amygdala and nucleus accumbens in responding to changes in group status (rewarding or stressful) and adjusting behavior appropriately [72, 73]. Furthermore, human patients with lesions to the medial prefrontal cortex have been shown to lack the ability to

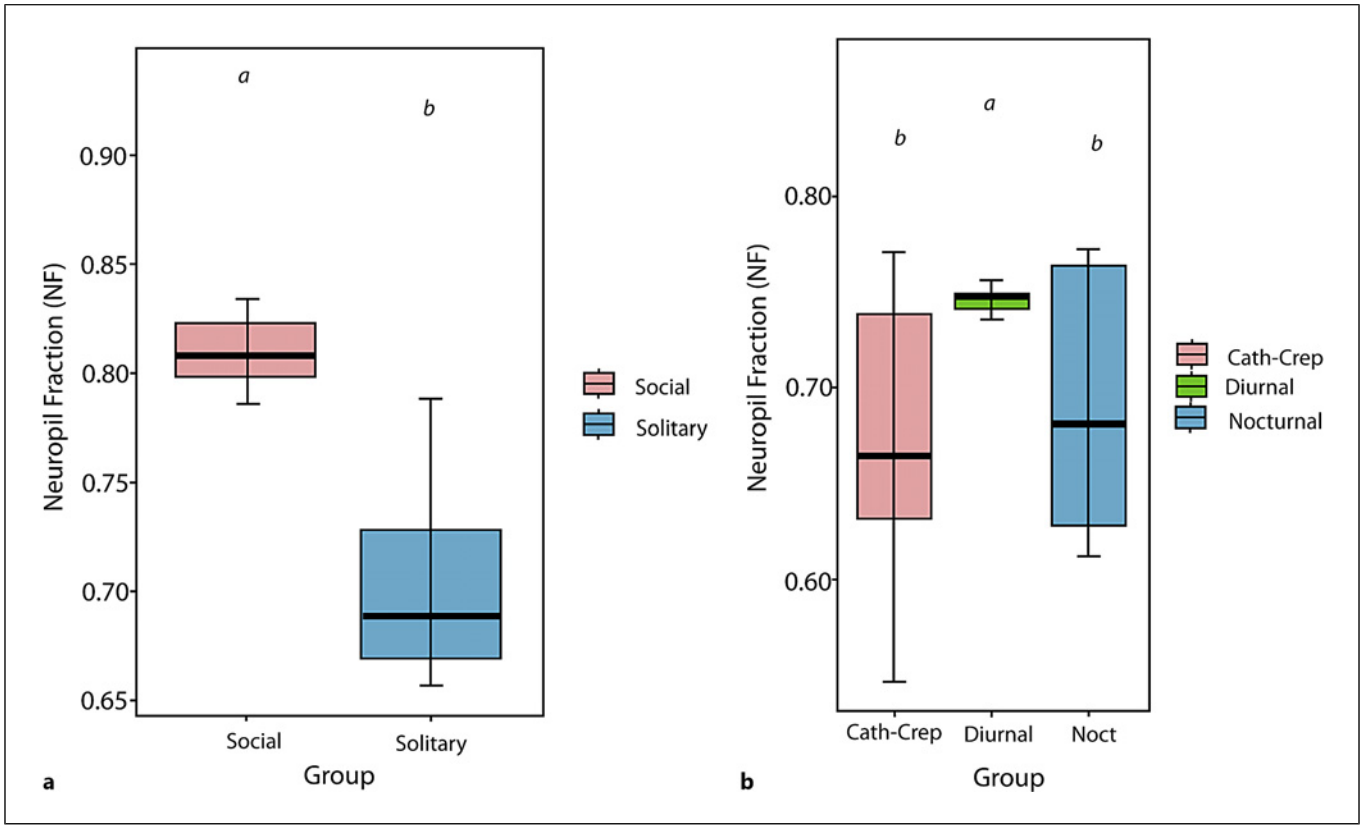

Fig. 6. Box plots of the neuropil fraction from each species datapoint grouped by Social Complexity (i.e., social vs. Solitary) and Activity patterns (diurnal, nocturnal, cathemeral-crepuscular). Behavioral and ecological data was sourced from [4, 39]. Significant differences in neuropil fraction are indicated through the use of the compact letter display above each boxplot.

make behavioral adjustments in response to shifting professional ranks [74] with data from both human and rodent studies converging on the observation that the medial prefrontal cortex helps to support monitoring of group dynamics [75]. The shared neural machinery of social monitoring in mammals is likely extended to the Felidae as well. For lions in particular, a relative increase in neuropil in the prefrontal cortex might provide a neuroanatomical basis for the elaboration of group living observed in this species; however, this would not exclude other felids (e.g., cheetahs and tigers) from having evolved similar changes in prefrontal cortex neuropil to support similar social behaviors.

For instance, cheetahs are known to form small social groups both in the wild and in captivity [76]. An adult female cheetah and her cubs will live socially up until about 18 months of age [77], whereafter cheetah cubs then break away from their mother to form sibling groups which persist for about 6–7 months [76]. After 7 months of age, female siblings separate from their littermates and lead a solitary lifestyle [77–79], while male siblings remain together in coalitions often throughout their lifetime [76, 80–82]. Like lions living within a pride, cheetah coalition groups are maintained by siblings living near one another and engaging in activities that affirm their affiliation through allogrooming and a marked reduction of within-group aggression [76]. These coalitions often consist of between 2 and 4 individuals and are not solely restricted to siblings as non-familiar members may also join coalitions [76, 83]. The formation and maintenance of coalition groups are thought to convey an adaptative advantage to cheetahs as they improve species reproductive success and survival through diversifying the genetic pool as well as helping to maintain territorial dominance [76, 77, 81, 82, 84]. We hypothesize that in cheetahs, the increased prefrontal cortex neuropil may have evolved in response to selection for increased sociality, likely driven by hunting (or reproductive) pressure as evidenced from the formation and maintenance of modern cheetah coalitions [76, 77, 80–84].

Given these observations of group living, our results on the prefrontal cortex neuropil in lions, tigers, and cheetahs are well supported by behavioral data and are aligned with our emerging understanding of the neural mechanisms of behavioral flexibility [85–89], a hallmark function of the prefrontal cortex. Our observations that leopards and snow leopards had significantly less prefrontal cortex neuropil (in comparison to the other three felids) also align well with behavioral observations of sociality for these species. Most notably, in contrast to the more social big cats, leopards are known to be solitary and rarely interact with other leopards except during mating and when rearing cubs [90–92]. They are solitary hunters, and once a kill is made, the individual will usually secure the kill in a tree to prevent kleptoparasitism [90]. When rearing cubs, females will also hunt alone and consume a small amount of the prey before leading the cubs to the kill site [92]. Both sexes are territorial and demarcate their territory using scent marking [91, 93], tree scratching [94], as well as vocalizations [91]. Male leopards tend to have larger territories than females, with territorial ranges varying depending on external factors such as prey density and resource availability [92, 95]. Similarly, snow leopards are also considered a solitary species, generally only coming together for mating during the breeding season [96], although monogamous pair bonding has also been reported [96, 97]. Pair bonding in these cases is considered a likely fitness strategy to increase reproductive and hunting success owing to the harsh, rugged environmental conditions within which snow leopards live [96]. These difficult environmental conditions are also thought to facilitate an appreciable amount of overlap between home ranges and prey sources, resulting in some increased tolerance for territorial sharing [98].

In contrast to the generally solitary lives of leopards, domestic cats show some degree of variability in sociality with factors such as rearing history and environmental factors affecting their tolerance for social living [99–101]. Studies on free-ranging domestic cats have emphasized this variability in social tolerance, with some groups showing little or no social interactions while others display strong social and affiliative bonds, indicating that free-ranging cats are likely social generalists [102].

Scaling analyses for the prefrontal cortex indicated a weak, non-significant positive trend with increasing brain size with only about a third of the variation in neuropil space explained by brain mass. This observation is consistent with that obtained in primates, where previous studies had found a lack of correlation between the neuropil space and brain size for prefrontal areas [103]. Our results from the prefrontal cortex neuropil are consistent with those from Golgi impregnation studies in Felidae which have highlighted species-specific differences including increased dendritic complexity in these larger brained felids [15, 30]. Results from significance testing of the prefrontal cortex neuropil in social and solitary felids (Fig. 6) also revealed a significant difference between the groups.

An earlier study of prefrontal scaling in carnivores demonstrated that neocortical scaling in this group is consistent with that observed in other mammalian orders [104]. Unfortunately, Bush and Allman [104] only included one felid species in their cross-species comparisons; however, they demonstrated significant differences in frontal scaling between carnivores and primates. Primates are characterized by hyperscaling of the PFC relative to the rest of the cortex [104], a pattern which has arguably been expanded within humans [105]. Despite these apparent differences, recent studies of connectivity profiles emerging from the frontal cortex have indicated strong homologies across species (i.e., between rodents and primates) in both the size and location of striatal emotional processing networks [106]. These observations highlight the conservation of form and function across mammals and add credence to our view that similar connectional homologies are expected within the Felidae (and Carnivora in general) and would support similar functions to those observed in primates.

Notably, our data on social complexity for the Felidae was obtained from published species values on group size [14]. While the use of group size as a proxy for social complexity has raised concerns [107], group size has been demonstrated to be an effective and robust measure of sociality in Carnivora and Primates [4, 108–110]. Our results highlight the link between the anatomical variance of the prefrontal cortex neuropil and species sociality. These observations are in line with what one would expect given our knowledge of the modularity of the brain [111, 112] and the mosaic nature of brain evolution [113].

Furthermore, scaling analyses also revealed a strong positive correlation between prefrontal cortex neuropil and pyramidal neuron soma size. This would suggest that lions, tigers, and cheetahs are predicted to not only have larger prefrontal cortex neuropil but also larger pyramidal neuron soma sizes within the prefrontal cortex due to the close relationship between these two variables. This result coincides with observations by [30], in which the authors found that lions had the largest (prefrontal) pyramidal neuron somal sizes and that this was also associated with increased dendritic complexity. The link between prefrontal pyramidal neuron size and dendritic complexity has been highlighted in studies on primates, where pyramidal

neurons in the prefrontal cortex are known to be more complex than those in other brain regions and are reflective of the central role of the prefrontal cortex in integrating cognitive and memory functions [114–116]. Similar to the conclusions of Nguyen et al. [30], who observed species differences in somal parameters and neuronal complexity between lions, cheetahs, and leopards, we also observed notable species differences. These cellular characteristics are likely to confer relevant species-specific cognitive and behavioral capabilities not seen in felids with smaller brains.

Species-Specific Variation in Primary Motor Cortex Neuropil

The primary motor cortex is an area located within the frontal lobe of the mammalian brain, and it is involved in generating, directing, and executing voluntary movements of the body [41]. Electrophysiological studies have revealed that a topographical map of the skeletal musculature is found across the primary motor cortical surface such that electrical stimulation of different parts will elicit different movements within the body [117, 118]. In addition, neural activity in the primary motor cortex has been shown to be correlated with several different patterns and components of movement including speed of movement [119], muscle force [120], direction of movement [121], and limb mechanics [122], both at the level of the single neuron or populations of neurons [120, 121, 123, 124]. Furthermore, long stimulation durations of a single area in the primary motor cortex have been shown to evoke complex, multi-muscle movements [125, 126]. Given the central role of the primary motor cortex in generating and directing movement of the mammalian body, we postulate that the pattern of neuropil variation observed in the felid primary motor cortex evolved in response to, or as a means of, supporting specialized movements, particularly as it relates to hunting behavior.

Unlike other felids which rely on ambush hunting or speed in pursuing prey, lions normally stalk their prey before charging with a short burst of speed [127, 128]. This strategy is reflective of the unique locomotory and energetic constraints imposed on lions as they lack the stamina to pursue prey over long distances, have a slow walking and running speed [127, 129], with a maximum running speed of only 28–37 mph [127, 128, 130], and are the largest cat species [131]. To compensate, lions have evolved a unique group hunting strategy that is particularly useful for bringing down large prey [128, 132]. Cooperative/group hunters are known to have greater hunting success rate than individual hunters [128, 132], emphasizing the adaptative value of cooperative hunting for lions. Group hunting is particularly useful when one considers the prey species which lions usually target. While lions are considered generalist hunters, they tend to rely on a small set of key prey species with select ungulates (i.e., zebra, wildebeest, impala, buffalo, and waterbuck) often targeted [133, 134]. Harward and Kerley [133] noted that lions often preferred prey weighing between 190 and 550 kg, while single lions are known to be able to take down animals almost twice their own body mass [135].

Given that neural activity in the primary motor cortex is known to be correlated with muscle force and speed of movement [119, 120], and that this neural activity is supported by the morphology of the underlying neuropil, it seems reasonable to assume that the observations of a relatively large neuropil fraction in the lion primary motor cortex are reflective of this enhanced predatory adaptation necessary for bringing down large prey (either in a group or single hunt) and assist in generating the speed needed for short charges. In addition to changes in the primary motor cortex, the evolution of cooperative, social living in lions (as reflected in changes to the frontal neuropil) may have also helped to support the emergence and/or maintenance of cooperative hunting within this group.

In the cheetah, we postulate that the larger neuropil fraction in the primary motor cortex evolved to support neural activity associated with an increase in the speed of movement, as exemplified in the highly developed running abilities of these animals. Cheetahs can reach a maximum running speed of 110 km/h [136], which is almost twice the maximum speed of a tiger or lion [137] and 1.7 times faster than that of the racing greyhound [138, 139]. From a locomotory perspective, the speed of movement in cheetahs is directly related to their unique musculoskeletal adaptations which include changes in the limb, tail, and cranial morphology [138–140]. Unlike the stalk-and-ambush tactics of the lion and tiger, cheetahs use a stalk-and-chase hunting strategy and would typically stalk their prey to within 50 m before giving chase, utilizing their comparatively enhanced speed and maneuverability [136]. In comparison to the racing greyhound, some of the enhanced functional adaptations for speed in cheetahs include changes in their hind and forelimb that enable longer strides, heavier bones to resist larger forces, as well as heavier musculature (particularly digital flexor muscles and extensor digitorum) that not only aids with traction during acceleration and movement but are also used in protraction of the dew claw during prey capture [138, 139]. In addition, their slight body size also aids their maneuverability. Cheetahs have a body mass of around 55 kg, which is 3–4 times less than

that of an adult tiger or lion [137]. As a result of their lighter body mass, as well as comparatively diminutive canine and masticatory morphology, cheetahs tend to prey on smaller (and younger animals) antelope such as springbok, impala, and Thomson's gazelle making up the majority of their diet [77, 141, 142]. These behavioral observations support the concept that the greater neuropil fraction in the cheetah's primary motor cortex is related to functional needs for increased speed that would be utilized during charging and pursuit of prey.

Hunting behavior in leopards (both African and snow leopard) is best characterized as stalk-and-ambush [92], with leopards usually stalking their prey over long distances [93] and then ambushing them through a fast charge over a short distance, usually 5 m [92, 143]. In terms of movement, anatomical adaptations in the forelimb and scapular regions of leopards allow them to complete vertical climbs [144, 145]. Climbing into trees is used to stash kills as well as rest between hunts [92]. Leopards are much smaller than tigers and lions, usually weighing about 60 kg [127] and only able to achieve a maximum running speed of 60 km/h, which is equivalent to that of the lion and tiger [137]. In comparison to cheetahs, leopards are relatively equivalent in terms of body mass (i.e., 55 vs. 60 kg), but the maximum running speed of cheetahs is 1.8 times faster than that of the leopard [137]. Unlike the elaboration of musculoskeletal adaptations for speed of movement as seen in the cheetah or the elaboration of muscle force for bringing down large prey as seen in the lion and tiger, we postulate that the significantly lower neuropil values in the primary motor cortex of leopards and domestic cats indicate that these species have remained rather ancestral in their neuropil fraction and have not undergone any marked deviations or elaborations of the underlying circuitry evident at this level of organization.

In contrast to the prefrontal cortex, scaling analyses for the primary motor cortex revealed a modest and significant positive correlation with overall brain size ($r^2 = 0.59$). We believe these observations are expected given the necessary expansion of primary motor control needed for a larger body. As pointed out by Seldon [146, 147], increased neuropil is also expected to allow for less overlapping in the dendritic trees, thus permitting a more segregated and modular function for the underlying region. It has been generally shown that species with larger brains tend to have a larger number of neurons with more elaborate dendritic trees [148–150]. With this in mind, we anticipate that scaling with brain size would explain a part of the variation in primary motor neuropil, with the remaining component of variation reflected in species variation in movement and muscle recruitment. In particular (as reviewed in the earlier section), we propose that the unique set of movements recruited during hunting would help to explain the remaining component of variation. In addition, given the close allometric relationship between brain and body mass [151], as well as body size and ecological parameters [152], we believe that the strong correlation between primary motor cortex neuropil fraction and home range is an expected outcome, as larger bodied species are forced to increase their activity budgets [153].

Scaling analyses also revealed a strong positive correlation between primary motor cortex neuropil fraction and pyramidal neuron soma size. This coincides with Golgi impregnation and stereological studies in which primary motor cortex gigantopyramidal neurons in the African lion and cheetah were found to be exceptionally large [15, 30]. Furthermore, Nguyen et al. [30] also point to the similarity in neuronal morphology between the cheetah and the *Panthera* as evidence for convergence in morphology to support the parallel evolution of enhanced hunting abilities in these two lineages. Jacobs et al. [15] suggested that the large size of primary motor cortex gigantopyramidal neurons in the *Panthera* and cheetah likely evolved to support the innervation of specialized fast-fatiguing type IIx muscle fibers which require large motor neurons [154, 155] and would aid in the fast movements and stalking behavior necessary for hunting. The current observations of convergence in the primary motor cortex neuropil in the cheetah are further evidence supporting this concept.

Species-Specific Variation in Visual Cortex Neuropil

In the current study, we observed a clear dichotomy in the neuropil fraction of the primary visual cortex, such that lions, tigers, and cheetahs had significantly more neuropil space in comparison to the other cats. Lions and tigers had equal but slightly greater primary visual cortex neuropil compared to cheetahs, although the difference was negligible between species. In contrast, the snow leopard, African leopard, and domestic cat (while differing only slightly from one another) had significantly less neuropil space in the primary visual cortex. As with the primary motor cortex, the primary visual cortex also contains a topographic organization, such that each primary visual area contains a retinotopic map of the contralateral visual field [156–158]. Neurons in the primary visual area are activated in response to specific characteristics of the visual stimuli including movement, spatial frequency, color, and orientation [42]. Given the role of the primary visual cortex, we hypothesize that the pattern of neuropil variation observed in felids may be indicative of differences in visual processing and acuity.

Depending on their relative activity, animal species can be classified as either diurnal, cathemeral, crepuscular, or nocturnal [159]. These activity patterns are strongly associated with behavioral ecology as well as several other life history parameters. In felids, activity patterns are known to be influenced by several external factors (including diet, light, and interaction with competitor species) [160–162]. Earlier studies have demonstrated a clear association between activity pattern and visual acuity in mammals, such that diurnal animals tend to have higher visual acuity than their nocturnal counterparts [163–167]. Similarly, studies of visual acuity in mammals that are cathemeral (i.e., active during hours of darkness and daylight) have found that these animals have visual capacities that occupy an intermediate space between nocturnal and diurnal species [165, 168, 169].

While 72% of extant Carnivora species are considered nocturnal [160], there is some notable inter- and intraspecific variation in activity patterns [91, 92, 127–129, 135, 161, 170–172]. Using published species activity patterns, we compared mean neuropil fraction in the primary visual cortex in each diel group (i.e., diurnal, nocturnal, cathemeral-crepuscular) based on the diel assignments of Michaud et al. [39] (i.e., cheetah = diurnal; lion and snow leopard = nocturnal; tiger, leopard, and cat = cathemeral-crepuscular). Mean neuropil in the primary visual cortex differed significantly between diel groups such that diurnal felids had greater neuropil space (Fig. 6). This observation aligns with our prediction that differences in the neuropil of the primary visual cortex are indicative of underlying changes in visual processing likely to support species-specific visual acuity.

Cheetahs have a visual acuity of 23.02 cycles/degree which, in comparison to that of domestic cat (8.85 cycles/degree), is 2.6 times greater [173] and adds support to our proposal that there are distinct differences in visual acuity between diel groups. Furthermore, cross-species studies of visual acuity and direct links with the morphology of the primary visual cortex have demonstrated a positive correlation between visual acuity and the number of neurons in the primary visual cortex, such that animals with a larger number of neurons or number of pinwheels (i.e., modular maps of the retinotopic space) in the primary visual cortex had greater visual acuity [174]. Earlier studies have also shown a close positive association between visual acuity and body size in mammals [175], which aligns with our observations related to larger brain size.

In contrast to our observations in the prefrontal and primary motor cortex, scaling analyses in the primary visual cortex revealed no correlation with pyramidal somal size. This suggests that primary visual cortex pyramidal neurons do not scale in size with the neuropil space. While species with larger brains tend to have larger numbers of neurons and more extensive dendritic trees [150], regional and cell-type deviations have been noted [148, 176]. Pyramidal neurons in the primary visual cortex are known to deviate from this "scaling-up effect" [177]. Here, we interpret our results as further evidence supporting this concept.

Conclusions

This study provides evidence in support of regional and species-level differences in felid cortical morphology, as well as testable hypotheses linking changes in neuropil fraction to cellular as well as ecological parameters. We hope that this will serve as a fruitful step towards fostering ongoing comparative studies on the neuroanatomy of complex behavior and the underlying neuroanatomical framework in this understudied group of mammals.

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Statement of Ethics

These studies were approved by the Colorado College Institutional Review Board (#011311-1) and the University of the Witwatersrand Animal Ethics Committee (2008/36/1; 2012/53/1) and the Animal Ethics Committee of Des Moines University.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Study concept and design: M.A.S., B.J., P.R.M., and C.C.S. Collection of and qualitative analysis of data: J.N., B.J., C.C.S., P.R.M., and M.A.S. Statistical analysis and interpretation: B.J., C.C.S., P.R.M., and M.A.S. Procurement, preparation, and fixation of tissue: J.N., E.M.W., K.O., K.B., B.J., C.C.S., P.R.M., and M.A.S. Obtained funding: M.A.S., P.R.M., and C.C.S. Drafting of the manuscript: M.A.S., P.R.M., C.C.S., B.J., B.K.B., and J.S. Photomicrography and preparation of figures/tables: J.N., P.R.M., and M.A.S. Critical revision of the manuscript for important intellectual content: M.A.S., P.R.M., B.J., and C.C.S. Study supervision: M.A.S.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Wozencraft WC. Order carnivora. In: Wilson DE, Reeder DM, editors. Mammal species of the world. A taxonomic and geographic reference. Baltimore: Johns Hopkins University Press; 2005. p. 532–628.
2 Nowak RM. Walker's Carnivores of the world. Baltimore, Maryland: Johns Hopkins University Press; 2005.
3 Manger PR, Cort J, Ebrahim N, Goodman A, Henning J, Karolia M, et al. Is 21st century neuroscience too focused on the rat/mouse model of the brain function and dysfunction? Front Neuroanat. 2005;2:5.
4 Benson-Amram S, Dantzer B, Stricker G, Swanson EM, Holekamp KE. Brain size predicts problem-solving ability in mammalian carnivores. Proc Natl Acad Sci U S A. 2016;113(9):2532–7.
5 Sakai ST, Arsznov BM, Hristova AE, Yoon EJ, Lundrigan BL. Big cat coalitions: a comparative analysis of regional brain volumes in Felidae. Front Neuroanat. 2016;10:99.
6 Sakai ST, Arsznov BM, Lundrigan BL, Holekamp KE. Brain size and social complexity: a computed tomography study in Hyaenidae. Brain Behav Evol. 2011;77(2):91–104.
7 Pérez-Barbería FJ, Shultz S, Dunbar RIM. Evidence for coevolution of sociality and relative brain size in three orders of mammals. Evolution. 2007;61(12):2811–21.
8 Radinsky L. Evolution of the felid brain. Brain Behav Evol. 1975;11(3–4):214–54.
9 Tensen L. Biases in wildlife and conservation research, using felids and canids as a case study. Global Ecol Conservation. 2018;15:e00423.
10 Macdonald D, Loveridge A. The biology and conservation of wild felids. Oxford, UK: Oxford University Press; 2010; p. 154–256.
11 Byrne RW, Whiten A. Machiavellian intelligence: social expertise and the evolution of intellect in monkeys, apes, and humans. Oxford: Clarendon Press; 1988.
12 Borrego N. Big cats as a model system for the study of the evolution of intelligence. Behav Process. 2017;141(Pt 3):261–6.
13 VanderWaal KL, Mosser A, Packer C. Optimal group size, dispersal decisions and postdispersal relationships in female African lions. Animal Behaviour. 2009;77(4):949–54.
14 Insel TR, Wang ZX, Ferris CF. Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. J Neurosci. 1994;14(9):5381–92.
15 Jacobs B, Garcia ME, Shea-Shumsky NB, Tennison ME, Schall M, Saviano MS, et al. Comparative morphology of gigantopyramidal neurons in primary motor cortex across mammals. J Comp Neurol. 2018;526(3):496–536.
16 Grewal JS, Gloe T, Hegedus J, Bitterman K, Billings BK, Chengetanai S, et al. Brain gyrification in wild and domestic canids: has domestication changed the gyrification index in domestic dogs? J Comp Neurol. 2020;528(18):3209–28.
17 Spocter MA, Uddin A, Ng JC, Wong E, Wang VX, Tang C, et al. Scaling of the corpus callosum in wild and domestic canids: insights into the domesticated brain. J Comp Neurol. 2018;526(15):2341–59.
18 Carbone C, Maddox T, Funston P, Mills MGL, Grether GF, Van Valkenburgh B. Parallels between playbacks and Pleistocene tar seeps suggest sociality in an extinct sabretooth cat, Smilodon. Biol Lett. 2009;5(1):81–5.
19 Kiffner C, Meyer B, Muhlenberg M, Waltert M. Plenty of prey, few predators: what is limiting lions (*Panthera leo*) in Katavi National Park, western Tanzania? Oryx. 2009;43(01):52–9.
20 Douglas RJ, Martin KA. Neuronal circuits of the neocortex. Annu Rev Neurosci. 2004;27:419–51.
21 Chance SA, Walker M, Crow TJ. Reduced density of calbindin-immunoreactive interneurons in the planum temporale in schizophrenia. Brain Res. 2005;1046(1–2):32–7.
22 Di Rosa E, Crow TJ, Walker MA, Black G, Chance SA. Reduced neuron density enlarged minicolumn spacing and altered ageing effects in fusiform cortex in schizophrenia. Psychiatry Res. 2009;166(2–3):102–15.
23 Buxhoeveden DP, Switala AE, Litaker M, Roy E, Casanova MF. Lateralization of minicolumns in human planum temporale is absent in nonhuman primate cortex. Brain Behav Evol. 2001;57(6):349–58.
24 Casanova MF, Trippe J, Tillquist C, Switala AE. Morphometric variability of minicolumns in the striate cortex of *Homo sapiens, Macaca mulatta*, and *Pan troglodytes*. J Anat. 2009;214(2):226–34.
25 Spocter MA, Fairbanks J, Locey L, Nguyen A, Bitterman K, Dunn R, et al. Neuropil distribution in the anterior cingulate and occipital cortex of artiodactyls. Anat Rec. 2018;301(11):1871–81.
26 Spocter MA, Raghanti MA, Butti C, Hof PR, Sherwood CC. The minicolumn in a comparative context. In: Casanova M, Opris I, editors. Recent advances on the modular organization of the cerebral cortex. Springer Publishing; 2015.
27 Spocter MA, Hopkins WD, Barks SK, Bianchi S, Hehmeyer AE, Anderson SM, et al. Neuropil distribution in the cerebral cortex differs between humans and chimpanzees. J Comp Neurol. 2012;520(13):2917–29.
28 Semendeferi K, Teffer K, Buxhoeveden DP, Park MS, Bludau S, Amunts K, et al. Spatial organization of neurons in the frontal pole sets humans apart from great apes. Cereb Cortex. 2011;21(7):1485–97.
29 Jacobs B, Johnson NL, Wahl D, Schall M, Maseko BC, Lewandowski A, et al. Comparative neuronal morphology of the cerebellar cortex in afrotherians, carnivores, cetartiodactyls, and primates. Front Neuroanat. 2014;8:24.
30 Nguyen VT, Uchida R, Warling A, Sloan LJ, Saviano MS, Wicinski B, et al. Comparative neocortical neuromorphology in felids: African lion, African leopard, and cheetah. J Comp Neurol. 2020;528(8):1392–422.
31 Sherwood CC, Holloway RL, Erwin JM, Schleicher A, Zilles K, Hof PR. Cortical orofacial motor representation in Old World monkeys, great apes, and humans. I. Quantitative analysis of cytoarchitecture. Brain Behav Evol. 2004;63(2):61–81.
32 Zilles K, Schleicher A, Kretschmann HJ. Automatic morphometric analysis of retrograde changes in the nucleus n. facialis at different ontogenetic stages in the rat. Cell Tissue Res. 1978;190(2):285–99.

33 Wree A, Schleicher A, Zilles K. Estimation of volume fractions in nervous tissue with an image analyzer. J Neurosci Methods. 1982; 6(1–2):29–43.
34 Buxhoeveden DP, Switala AE, Roy E, Litaker M, Casanova MF. Morphological differences between minicolumns in human and nonhuman primate cortex. Am J Phys Anthropol. 2001b;115(4):361–71.
35 Casanova MF, Buxhoeveden D, Gomez J. Disruption in the inhibitory architecture of the cell minicolumn: implications for autism. Neuroscientist. 2003;9(6):496–507.
36 Chance SA, Casanova MF, Switala AE, Crow TJ. Auditory cortex asymmetry altered minicolumn spacing and absence of ageing effects in schizophrenia. Brain. 2008;131(Pt 12):3178–92.
37 Schenker NM, Buxhoeveden DP, Blackmon WL, Amunts K, Zilles K, Semendeferi K. A comparative quantitative analysis of cytoarchitecture and minicolumnar organization in Broca's area in humans and great apes. J Comp Neurol. 2008;510(1):117–28.
38 Ridler TW, Calvard S. Picture thresholding using an iterative selection method. IEEE Trans Sys Man Cyber. 1978;8:630–2.
39 Michaud M, Toussaint SLD, Gilissen E. The impact of environmental factors on the evolution of brain size in carnivorans. Commun Biol. 2022;5(1):998.
40 Passingham RE. The frontal cortex: does size matter? Nat Neurosci. 2002;5(3):190–2.
41 Patestas MA, Gartner LP. Chapter 18: visual system. A textbook of neuroanatomy. Hoboken: Wiley-Blackwell; 2016. p. 350–75.
42 Haas J, Hass R, Spocter MA, de Sousa AA. Human visual neuroscience. In: Shackelford TK, Weekes-Shackelford VA, editors. Encyclopedia of evolutionary psychological science. New York, NY: Springer Cham; 2020.
43 Orme CDL, Freckleton RP, Thomas GH, Petzoldt T, Fritz SA, Isaac NJ. Caper: comparative analyses of phylogenetics and evolution in R. Methods Ecol Evol. 2013;3: 145–51.
44 Smaers JB, Mongle CS, Kandler A. A multiple variance Brownian motion framework for estimating variable rates and inferring ancestral states. Biol J Linn Soc Lond. 2016; 118(1):78–94.
45 Bininda-Emonds OR, Cardillo M, Jones KE, MacPhee RD, Beck RM, Grenyer R, et al. The delayed rise of present-day mammals. Nature. 2007;446(7135):507–12.
46 Bininda-Emonds OR, Cardillo M, Jones KE, MacPhee RD, Beck RM, Grenyer R, et al. Erratum: the delayed rise of present-day mammals. Nature. 2008;456(7219):274.
47 Nyakatura K, Bininda-Emonds ORP. Updating the evolutionary history of Carnivora (Mammalia): a new species-level supertree complete with divergence time estimates. BMC Biol. 2012;10:12.
48 Rabinowicz T, Petetot JM, Gartside PS, Sheyn D, Sheyn T. Structure of the cerebral cortex in men and women. J Neuropathol Exp Neurol. 2002;61(1):46–57.
49 Asplund CL, Todd JJ, Snyder AP, Marois R. A central role for the lateral prefrontal cortex in goal-direct and stimulus-driven attention. Nat Neurosci. 2010;13:507–14.
50 Adolphs R. The social brain: neural basis of social knowledge. Annu Rev Psychol. 2009; 60:693–716.
51 Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. Annu Rev Neurosci. 2001;24:167–202.
52 Duncan J, Owen AM. Common regions of the human frontal lobe recruited by diverse cognitive demands. Trends Neurosci. 2000; 23(10):475–83.
53 Wood JN, Grafman J. Human prefrontal cortex: processing and representational perspectives. Nat Rev Neurosci. 2003;4(2): 139–47.
55 Ongür D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb Cortex. 2000;10(3):206–19.
56 Croxson PL, Johansen-Berg H, Behrens TE, Robson MD, Pinsk MA, Gross CG, et al. Quantitative investigation of connections of the prefrontal cortex in the human and macaque using probabilistic diffusion tractography. J Neurosci. 2005;25(39):8854–66.
57 Wise SP. Forward frontal fields: phylogeny and fundamental function. Trends Neurosci. 2008;31(12):599–608.
58 Amodio DM, Frith CD. Meeting of minds: the medial frontal cortex and social cognition. Nat Rev Neurosci. 2006;7(4):268–77.
59 Kosmal A. Subcortical connections of the prefrontal cortex in dogs: afferents to the proreal gyrus. Acta Neurobiol Exp. 1981; 41(1):69–85.
60 Kosmal A. Subcortical connections of the prefrontal cortex in dogs: afferents to the medial cortex. Acta Neurobiol Exp. 1981; 41(4):339–56.
61 Kosmal A, Dabrowska J. Subcortical connections of the prefrontal cortex in dogs: afferents to the orbital gyrus. Acta Neurobiol Exp. 1980;40(3):593–608.
62 Stepniewska I, Kosmal A. Distribution of mediodorsal thalamic nucleus afferents originating in the prefrontal association cortex of the dog. Acta Neurobiol Exp. 1986; 46(5–6):311–22.
63 Preuss TM. Do rats have prefrontal cortex? The Rose–Woolsey–Akert program reconsidered. J Cogn Neurosci. 1995;7(1):1–24.
64 Carlén M. What constitutes the prefrontal cortex? Science. 2017;358(6362):478–82.
65 Brutkowski S. Prefrontal cortex and drive inhibition. In: Warren JM, Akert K, editors. The frontal granular cortex and behavior. New York: McGraw-Hill; 1964. p. 242–70.
66 Dabrowska J. On the mechanism of go-no go symmetrically reinforced task in dogs. Acta Neurobiol Exp. 1972;32(2):345–59.
67 Lawicka W. A proposed mechanism for delayed response impairment in prefrontal animals. Acta Biol Exp. 1969;29(3):401–14.
68 Stepien I, Stepien L. Effects of prefrontal lesions on left leg- right leg differentiation in dogs. Acta Neurobiol Exp. 1972;32(2): 331–43.
69 Bekoff M, Daniels TJ, Gittleman JL. Life history patterns and the comparative social ecology of carnivores. Annu Rev Ecol Syst. 1984;15(1):191–232.
70 Mosser A, Packer C. Group territoriality and the benefits of sociality in the African lion, Panthera leo. Animal Behaviour. 2009; 78(2):359–70.
71 Rowell TE. The concept of social dominance. Behav Biol. 1974;11(2):131–54.
72 Watanabe N, Yamamoto M. Neural mechanisms of social dominance. Front Neurosci. 2015;9:154.
73 Wang F, Kessels HW, Hu H. The mouse that roared: neural mechanisms of social hierarchy. Trends Neurosci. 2014;37(11): 674–82.
74 Karafin MS, Tranel D, Adolphs R. Dominance attributions following damage to the ventromedial prefrontal cortex. J Cogn Neurosci. 2004;16(10):1796–804.
75 Bricks LC, Koike H, Akbaraian S, Morishita H. Prefontal cortex and social cognition in mouse and man. Front Pyschol. 2015;6: 1805.
76 Chadwick CL, Rees PA, Stevens-Wood B. Captive-housed male cheetahs (*Acinonyx jubatus soemmeringii*) form naturalistic coalitions: measuring associations and calculating chance encounters. Zoo Biol. 2013; 32(5):518–27.
77 Caro TM. Cheetahs of the Serengeti plains: group living in an asocial species. Chicago: University of Chicago Press; 1994.
78 Wielebnowski NC, Ziegler K, Wildt DE, Lukas J, Brown JL. Impact of social management on reproductive, adrenal and behavioral activity in the cheetah (*Acinonyx jubatus*). Anim Conserv. 2002;5(4): 291–301.
79 Terio KA, Marker L, Overstrom EW, Brown JL. Analysis of ovarian and adrenal activity in Namibian cheetahs. South Afr J Wildl Res. 2003;33:71–8.
80 Caro TM, Collins DA. Male cheetahs of the serengeti. Natl Geogr Res. 1986;2:75–86.
81 Ruiz-Miranda CR, Wells SA, Golden R, Seidensticker J. Vocalizations and othe behavioral responses of male cheetahs (*Acinonyx jubatus*) during experimental separation and reunion trials. Zoo Biol. 1998; 17:1–16.
82 Durant SM, Kelly M, Caro T. Factors affecting life and death in Serengeti cheetahs: environment, age, and sociality. Behav Ecol. 2004;15(1):11–22.
83 Caro TM. Behavioral solutions to breeding cheetahs in captivity: insights from the wild. Zoo Biol. 1993;12(1):19–30.

84 Gottelli D, Wang J, Bashir S, Durant SM. Genetic analysis reveals promiscuity among female cheetahs. Proc Biol Sci. 2007; 274(1621):1993–2001.
85 Armbruster DJ, Ueltzhoffer K, Basten U, Fiebach CJ. Prefrontal cortical mechanisms underlying individual differences in cognitive flexibility and stability. J Cogn Neurosci. 2012;24(12):2385–99.
86 Jobson DD, Hase Y, Clarkson AN, Kalaria RN. The role of the medial prefrontal cortex in cognition, ageing and dementia. Brain Commun. 2021;3(3):fcab125.
87 Izquierdo A, Brigman JL, Radke AK, Rudebeck PH, Holmes A. The neural basis of reversal learning: an updated perspective. Neuroscience. 2017;345:12–26.
88 Bissonette GB, Powell EM. Reversal learning and attentional set- shifting in mice. Neuropharmacology. 2012;62(3):1168–74.
89 Floresco SB, Block AE, Tse MT. Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behav Brain Res. 2008;190(1):85–96.
90 Bailey TN. The African leopard: ecology and behavior of a solitary felid. New York: Columbia University Press; 1993.
91 Hunter L, Henschel P, Ray JC. Panthera pardus leopard. In: Kingdon J, Hoffmann M, editors. Mammals of Africa: carnivores, pangolins, equids and rhinoceroses. London (England): Bloomsbury; 2013. p. 159–68.
92 Stander PEP, Haden J, Kaqece I, Ghau I. The ecology of asociality in Namibian leopards. J Zool. 1997;242(2):343–64.
93 Bothma JP, Coertze RJ. Scent-marking frequency in southern Kalahari leopards. S Afr J Wildl Res. 2004;34(2):163–9.
94 Bothma JP, Le Riche EAN. Evidence of a flexible hunting technique in Kalahari leopards. S Afr J Wildl Res. 1989;19:57–60.
95 Bothma P, Knight MH, Le Riche EAN, van Hensbergen HJ. Range size of southern Kalahari leopards. S Afr J Wildl Res. 1997; 27(3/4):94.
96 Freeman H. Social behavior in the snow leopards and its implications for captive management. Int.Ped.Book of Snow Leopards. 1978;1:71–7.
97 Freeman H. Behavior in adult pairs of captive snow leopards (Panthera uncia). Zoo Biol. 1983;2(1):1–22.
98 Jackson R, Ahlborn G. Snow Leopards in Nepal-home range and movements. Natl Geogr Res. 1989;5:161–75.
99 Leyhausen P. The tame and the wild: another Just-So story? In: Turner DC, Bateson P, editors. The Domestic Cat. The biology of its behaviour. Cambridge: Cambridge University Press; 1988.
100 Mellen JD. A comparative analysis of scent marking, social and reproductive behavior in 20 species of small cats (Felis). Am Zool. 1993;33(2):151–66.
101 Turner DC. Social organization and behavioural ecology of free- ranging domestic cats. In: Turner DC, Bateson P, editors. The Domestic Cat. The biology of its behaviour. Cambridge: Cambridge University Press; 2014.
102 Vitale KR. The social lives of free-ranging cats. Animals. 2022;12(1):126.
103 Sherwood CC, Hof PR. The evolution of neuron types and cortical histology in apes and humans. In: Preuss TM, Kaas JH, editors. The evolution of primate nervous systems evolution of nervous systems. Oxford: Academic Press; 2007. p. 355–78.
104 Bush EC, Allman JM. The scaling of frontal cortex in primates and carnivores. Proc Natl Acad Sci U S A. 2004;101(11):3962–6.
105 Preuss TM, Wise SP. Evolution of prefrontal cortex. Neuropsychopharmacology. 2022; 47(1):3–19.
106 Heilbronner SR, Rodriguez-Romaguera J, Quirk GJ, Groenewegen HJ, Haber SN. Circuit-based corticostriatal homologies between rat and primate. Biol Psychiatry. 2016;80(7):509–21.
107 Bergman TJ, Beehner JC. Measuring social complexity. Anim Behav. 2015;103:203–9.
108 White DJ, Gersick AS, Snyder-Mackler N. Social networks and the development of social skills in cowbirds. Philos Trans R Soc Lond B Biol Sci. 2012;367(1597):1892–900.
109 Barton RA, Dunbar RIM. Evolution of the social brain. In: Whiten A, Byrne RW, editors. Machiavellian intelligence II: extensions and evaluations. Cambridge, United Kingdom: Cambridge Univ Press; 1997. p. 240–63.
110 Dunbar RIM. Neocortex size as a constraint on group size in primates. J Hum Evol. 1992; 22(6):469–93.
111 Healy SD, Rowe C. A critique of comparative studies of brain size. Proc Biol Sci. 2007;274(1609):453–64.
112 Healy SD, Rowe C. Costs and benefits of evolving a larger brain: doubts over the evidence that large brains lead to better cognition. Anim Behav. 2013;86(4):e1–3.
113 Barton RA, Harvey PH. Mosaic evolution of brain structure in mammals. Nature. 2000; 405(6790):1055–8.
114 Elston GN. Pyramidal cells of the frontal lobe: all the more spinous to think with. J Neurosci. 2000;20(18):RC95.
115 Jacobs B, Schall M, Prather M, Kapler E, Driscoll L, Baca S, et al. Regional dendritic and spine variation in human cerebral cortex: a quantitative Golgi study. Cereb Cortex. 2001;11(6):558–71.
116 Hauser MD. Perseveration, inhibition and the prefrontal cortex: a new look. Curr Opin Neurobiol. 1999;9(2):214–22.
117 Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain. 1937;60(4):389–443.
118 Neafsey E, Bold E, Haas G, Hurley-Gius K, Quirk G, Sievert C, et al. The organization of the rat motor cortex: a microstimulation mapping study. Brain Res. 1986;396(1):77–96.
119 Schwartz AB. Motor cortical activity during drawing movements: population representation during sinusoid tracing. J Neurophysiol. 1993;70(1):28–36.
120 Evarts EV. Relation of pyramidal tract activity to force exerted during voluntary movement. J Neurophysiol. 1968;31(1):14–27.
121 Georgopoulos A, Schwartz A, Kettner R. Neuronal population coding of movement direction. Science. 1986;233(4771): 1416–9.
122 Gribble PL, Scott SH, Graham KM, Cabel DW. Overlap of internal models in motor cortex for mechanical loads during reaching. Nature. 2002;417(6892):938–41.
123 Churchland MM, Shenoy KV. Temporal complexity and heterogeneity of single-neuron activity in premotor and motor cortex. J Neurophysiol. 2007;97(6): 4235–57.
124 Churchland MM, Cunningham JP, Kaufman MT, Foster JD, Nuyujukian P, Ryu SI, et al. Neural population dynamics during reaching. Nature. 2012;487(7405):51–6.
125 Graziano MSA, Taylor CSR, Moore T. Complex movements evoked by microstimulation of precentral cortex. Neuron. 2002;34(5):841–51.
126 Aflalo TN, Graziano MSA. Possible origins of the complex topographic organization of motor cortex: reduction of a multidimensional space onto a two-dimensional array. J Neurosci. 2006;26(23):6288–97.
127 Schaller GB. The serengeti lion. Chicago: University Chicago Press; 1972; p. 480.
128 Wilson DE, Mittermeier RA. Handbook of the mammals of the world. Barcelona (Spain): Lynx Edicions; Lion Panthera leo; 2009. p. 136–9.
129 Rudnai JA. The social life of the lion: a study of the behaviour of wild lions (*Pantera leo massaica* [Newmann]) in the Nairobi National Park, Kenya. Pennsylvania: Washington Square East; 1973.
130 Chassin PS, Taylor CR, Heglund NC, Seeherman HJ. Locomotion in lions: energetic cost and maximum aerobic capacity. Physiol Zool. 1976;49(1):1–10.
131 Seidensticker J, Christie S, Jackson P. Introducing the tiger: overview. In: Seidensticker J, Christie S, Jackson P, editors. Riding the tiger: tiger conservation in human-dominated landscapes. Cambridge (UK): Cambridge University Press; 1999. p. 1–3.
132 West P, Packer C. *Panthera leo* lion. In: Kingdon J, Hoffman M, editors, editors. Mammals of Africa: carnivores, pangolins, equids and rhinoceroses. London: Bloomsbury Publishing. 2013;149–58.
133 Hayward MW, Kerley GI. Prey preferences of the lion (*Panthera leo*). J Zool. 2005; 267(3):309–22.
134 Loveridge AJ, Hunt JE, Murindagomo F, Macdonald DW. Influence of drought on predation of elephant (*Loxodonta africana*) calves by lions (*Panthera leo*) in an African wooded savannah. J Zool. 2006;270(3): 523–30.

135 Estes RD. Lion *Panthera leo*. The behavior guide to African mammals: including hoofed mammals, carnivores, primates. Berkeley, CA: University of California Press; 2012; p. 369–77.
136 Walker EP. Mammals of the world. Baltimore: Johns Hopkins University Press; 1976.
137 Garland T. The relation between maximal running speed and body mass in terrestrial mammals. J Zool. 1983;199(2):157–70.
138 Hudson PE, Corr SA, Payne-Davis RC, Clancy SN, Lane E, Wilson AM. Functional anatomy of the cheetah (*Acinonyx jubatus*) hindlimb. J Anat. 2011;218(4):363–74.
139 Hudson PE, Corr SA, Payne-Davis RC, Clancy SN, Lane E, Wilson AM. Functional anatomy of the cheetah (*Acinonyx jubatus*) forelimb. J Anat. 2011;218(4):375–85.
140 Cuff AR, Sparkes EL, Randau M, Pierce SE, Kitchener AC, Goswami A, et al. The scaling of postcranial muscles in cats (Felidae) I: forelimb, cervical, and thoracic muscles. J Anat. 2016;229(1):128–41.
141 Dierenfeld ES. Nutrition of captive cheetahs: food composition and blood parameters. Zoo Biol. 1993;12(1):143–50.
142 Wildt DE, Brown JL, Bush M, Barone MA, Cooper KA, Grisham J, et al. Reproductive status of cheetahs *(Acinonyx jubatus*) in North American zoos: the benefits of physiological surveys for strategic planning. Zoo Biol. 1993;12(1):45–80.
143 Kruuk H, Turner M. Comparative notes on predation by lion, leopard, cheetah and wild dog in the Serengeti area, east Africa. Mammalia. 1967;31(1):1–27.
144 Kingdon J. East african mammals: an atlas of evolution in africa: carnivores. London: Academic Press; 1977; p. p476.
145 Turner A, Anton M. The big cats and their fossil relatives: an illustrated guide to their evolution and natural history. New York: Columbia University Press; 1997.
146 Seldon HL. Structure of human auditory cortex. I. Cytoarchitectonics and dendritic distributions. Brain Res. 1981;229(2):277–94.
147 Seldon HL. Structure of human auditory cortex. II. Axon distributions and morphological correlates of speech perception. Brain Res. 1981;229(2):295–310.
148 Bekkers JM, Stevens CF. Presynaptic mechanism for long-term potentiation in the hippocampus. Nature. 1990;346(6286):724–9.
149 Buckmaster PS, Amaral DG. Intracellular recording and labeling of mossy cells and proximal CA3 pyramidal cells in macaque monkeys. J Comp Neurol. 2001;430(2):264–81.
150 Mohan H, Verhoog MB, Doreswamy KK, Eyal G, Aardse R, Lodder BN, et al. Dendritic and axonal architecture of individual pyramidal neurons across layers of adult human neocortex. Cereb Cortex. 2015;25(12):4839–53.
151 Manger PR, Spocter MA, Patzke N. The evolutions of large brain size in mammals: the "over-700-gram club quartet". Brain Behav Evol. 2013;82(1):68–78.
152 Gruner OE, Herfindal I, Solberg EJ, Saether B. Home ranges, habitat and body mass: simple correlates of home range size in ungulates. Proc R Soc B. 2016;283:2016123420161234.
153 Van Moorter B, Rolandsen CM, Basille M, Gaillard J-M. Movement is the glue connecting home ranges and habitat selection. J Anim Ecol. 2016;85(1):21–31.
154 Goto M, Kawai M, Nakata M, Itamoto K, Miyata H, Ikebe Y, et al. Distribution of muscle fibers in skeletal muscles of the cheetah (*Acinonyx jubatus*). Mamm Biol. 2013;78(2):127–33.
155 Kohn TA, Burroughs R, Hartman MJ, Noakes TD. Fiber type and metabolic characteristics of lion (Panthera leo), caracal (Caracal caracal) and human skeletal muscle. Mol Integr Physiol. 2011;159(2):125–33.
156 Gazzaniga MS, Ivry RB, Mangun GR. Cognitive neuroscience, the biology of the mind. New York: W. W. Norton & Company; 2019.
157 Kremkow J, Jin J, Wang Y, Alonso JM. Principles underlying sensory map topography in primary visual cortex. Nature. 2016;533(7601):52–7.
158 Cang J, Rentería RC, Kaneko M, Liu X, Copenhagen DR, Stryker MP. Development of precise maps in visual cortex requires patterned spontaneous activity in the retina. Neuron. 2005;48(5):797–809.
159 Bennie JJ, Duffy JP, Inger R, Gaston KJ. Biogeography of time partitioning in mammals. Proc Natl Acad Sci U S A. 2014;111(38):13727–32.
160 Wu Y, Wang H, Wang H, Feng J. Arms race of temporal partitioning between carnivorous and herbivorous mammals. Sci Rep. 2018;8(1):1713.
161 Cozzi G, Broekhuis F, McNutt JW, Turnbull LA, MacDonald DW, Schmid B. Fear of the dark or dinner by moonlight? Reduced temporal partitioning among Africa's large carnivores. Ecology. 2012;93(12):2590–9.
162 Funston PJ, Mills MGL, Biggs HC. Factors affecting the hunting success of male and female lions in the Kruger National Park. J Zool. 2001;253(4):419–31.
163 Pettigrew JD, Dreher B, Hopkins CS, McCall MJ, Brown M. Peak density and distribution of ganglion cells in the retinae of micro- chiropteran bats: implications for visual acuity. Brain Behav Evol. 1988;32(1):39–56.
164 Kirk EC. Eye morphology in cathemeral lemurids and other mammals. Folia Primatol. 2006;77(1–2):27–49.
165 Kirk EC. Effects of activity pattern on eye size and orbital aperture size in primates. J Hum Evol. 2006b;51(2):159–70.
166 Heesy CP, Hall MI. The nocturnal bottleneck and the evolution of mammalian vision. Brain Behav Evol. 2010;75(3):195–203.
167 Hall MI, Kamilar J, Kirk EC. Eye shape and the nocturnal bottleneck of mammals. Proc Biol Sci. 2012;279(1749):4962–8.
168 Kay RF, Kirk EC. Osteological evidence for the evolution of activity pattern and visual acuity in primates. Am J Phys Anthropol. 2000;113(2):235–62.
169 Veilleux CC, Kirk EC. Visual acuity in the cathemeral strepsirrhine *Eulemur macaco flavifrons*. Am J Primatol. 2009;71(4):343–52.
170 Hayward MW, Slotow R. Temporal partitioning of activity in large African carnivores: tests of multiple hypotheses. S Afr J Wildl Res. 2009;39(2):109–25.
171 Seyrling I, Dierkes PW, Burger AL. Diurnal and nocturnal behaviour of cheetahs (*Acinonyx jubatus*) and lions (*Panthera leo*) in zoos. Animals. 2022;12(18):2367.
172 Sunquist M. The social organization of tigers (Panthera tigris) in royal chitawan national Park, Nepal. Smithsonian Contrib Zool. 1981;336:1–98.
173 Veilleux CC, Kirk EC. Visual acuity in mammals: effects of eye size and ecology. Brain Behav Evol. 2014;83(1):43–53.
174 Srinivasan S, Carlo CN, Stevens CF. Predicting visual acuity from the structure of visual cortex. Proc Natl Acad Sci U S A. 2015;112(25):7815–20.
175 Ross CF, Kirk EC. Evolution of eye size and shape in primates. J Hum Evol. 2007;52(3):294–313.
176 St John JL, Rosene DL, Luebke JI. Morphology and electrophysiology of dentate granule cells in the rhesus monkey: comparison with the rat. J Comp Neurol. 1997;387(1):136–47.
177 Gilman JP, Medalla M, Luebke JI. Area-specific features of pyramidal neurons-a comparative study in mouse and rhesus monkey. Cereb Cortex. 2017;27(3):2078–94.