

FORM, FIT, AND FUNCTION: BEHAVIORAL SPECIALIZATIONS AND BRAIN
VARIATIONS IN SELECTED CARNIVORE SPECIES

By

Bradley M. Arsznov

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

PSYCHOLOGY

2012

ABSTRACT

FORM, FIT, AND FUNCTION: BEHAVIORAL SPECIALIZATIONS AND BRAIN VARIATIONS IN SELECTED CARNIVORE SPECIES

By

Bradley M. Arsznov

Among mammals, brain size varies significantly even when body size is controlled. Although a number of hypotheses have been proposed to explain this variation, much of this research focuses on primates while relatively few studies have examined carnivore species. Carnivores provide an ideal group in which to examine factors that influence brain variation, as they experience a variety of social, environmental, and life history conditions. The present project examined some of these factors in combination with an analysis of brain volumes in selected species in the Felidae and Procyonidae families. Using computed tomography, the first study investigated the influence of social life history on intraspecific variation in brain size in African lions (*Panthera leo*), a gregarious species in which females are more social than males, in comparison to cougars (*Puma concolor*), a species where both males and females are solitary. The results indicated that, in African lions, females had a larger frontal cortical volume, a region involved in executive control of cognition and regulation of social behaviors, compared to males. In contrast, no sex difference in frontal cortical volume was found in cougars. These data demonstrate the importance of including sex as a variable in comparative analyses, and highlight the relationship between regional brain size (e.g. frontal cortex) and behavioral specializations (e.g. sociality). The second study examined the relative influence of social life history, forepaw dexterity, and arboreality on brain size and organization in selected species in the family Procyonidae. Procyonid species exhibit continuums of behaviors related to social and physical environmental complexities: the mostly solitary, semi-arboreal and highly dexterous raccoons

(*Procyon lotor*); the exclusively arboreal kinkajous (*Potos flavus*), which live either alone or in small polyandrous family groups; and the social, terrestrial coatimundi (*Nasua nasua*, *N. narica*). Interspecific analysis of virtual endocasts revealed that the social coatimundi had the largest relative frontal cortical volume compared to the other two species. The arboreal kinkajou had the largest relative cerebellum and brainstem volumes compared to the other two species. The dexterous raccoon had the largest relative posterior cerebrum volume which includes the somatosensory cortex compared to the other two species. Additionally, intraspecific analysis revealed female coatimundis possessed a larger relative frontal cortical volume than males, while no sex differences were present within the other two species. Social life histories differ in male and female coatimundis but not in either kinkajous or raccoons. This analysis in the three procyonid species supports the comparative neurology principle that behavioral specializations correspond to an expansion of neural tissue devoted to that function. The last study sought to determine if increased brain size and regional brain variations reflect an increase in neuron number. Nissl stained brain sections from a dog, *Canis familiaris*, raccoon, *P. lotor*, and spotted hyena, *Crocuta crocuta*, were analyzed using cytoarchitectonic criteria and stereology. The regions of interests included: prefrontal, orbital frontal, primary somatosensory, and primary visual cortices. Stereological analysis revealed that as brain size increases, neuron density decreases. Intraspecific comparisons revealed that regional neuron number and volume increased in brain regions that subserve behavioral specializations. Interspecific comparisons revealed that brain regions mediating specialized behaviors are not only larger in size but also contain more neurons. Overall, these findings in selected carnivore species support the idea that differences in sociality, environmental, and life history factors hypothesized to influence brain variations in primates, also correspond to regional and total brain variations in carnivores.

I dedicate this dissertation to my family. You have taught me that every journey begins with a single step and have provided me with love and support every step of the way.

ACKNOWLEDGEMENTS

First and foremost, I thank Dr. Sharleen Sakai for her role as my advisor and mentor. I will be forever grateful for the opportunity to be a part of such an incredibly supportive and encouraging environment. For their guidance and support over the years I thank my committee members Dr. Antonio Nuñez, Dr. Laura Smale and Dr. Kay Holekamp. I thank Dr. Kevin Berger and the Department of Radiology, Michigan State University, Dr. Anthony Pease, Veterinary Medical Center, Michigan State University and Comparative Mammalian Brain Collections (supported by the National Science Foundation). I also thank Dr. Barbara Lundrigan and the staff at the following museums for making their specimens available for this study: Michigan State University Museum, Field Museum of Natural History, and University of Michigan Museum of Zoology. Finally, I thank Dr. Cheryl Sisk for her generosity in sharing her laboratory space which allowed me to continue with my research.

TABLE OF CONTENTS

LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
INTRODUCTION.....	1
CHAPTER 1	
Pride Diaries: Sex, brain size and sociality in the African lion (<i>Panthera leo</i>) and cougar (<i>Puma concolor</i>)	
Introduction.....	8
Methods.....	10
Specimens.....	10
Digital measurements.....	11
Validation of virtual endocasts.....	13
Magnetic resonance imaging (MRI) and volume analysis.....	13
Magnetic resonance data acquisitions.....	13
Delineation of brain regions in virtual endocasts.....	14
Statistical analyses.....	19
Results.....	20
Whole endocasts.....	20
MRI data.....	21
Sex differences.....	24
Regional brain volume differences.....	24
Discussion.....	28
Endocranial measurements.....	28
Comparisons of endocranial volumes in males and females.....	30
Cougars.....	30
African lions.....	31
CHAPTER 2	
The Procyonid Social Club: Comparison of brain volumes in the coatimundi (<i>Nasua nasua</i> , <i>N. narica</i>), kinkajou (<i>Potos flavus</i>), and raccoon (<i>Procyon lotor</i>)	
Introduction.....	36
Methods.....	39
Specimens.....	39
Digital measurements.....	39
Validation of virtual endocasts.....	43
Delineation of brain regions in virtual endocasts.....	43
Statistical analyses.....	46

Results.....	47
Whole endocasts.....	47
Interspecific differences.....	48
Intraspecific comparisons.....	54
Discussion.....	58
Computed tomography (CT) technique.....	58
Endocranial measurements.....	59
Regional endocranial measurements.....	60
Intraspecific comparisons of endocranial and regional endocranial measurements.....	63
CHAPTER 3	
Is More Really More For Carnivores: Do larger brains have more neurons?	
Introduction.....	67
Methods.....	69
Specimens.....	69
Brain sectioning.....	69
Regions of interest and histological analysis.....	69
Endocranial volume.....	71
Statistical analyses.....	72
Results.....	72
Cytoarchitectonic descriptions of the ROIs.....	72
Correlations among selected carnivore species.....	80
Neuron number, regional volume, and neuron density comparisons between ROIs.....	82
Discussion.....	85
More is less: Brain size and neuron density.....	85
Differences between ROIs.....	86
Future directions and inquiries.....	88
GENERAL CONCLUSIONS.....	90
APPENDICES.....	94
Appendix 1.....	95
Appendix 2.....	97
REFERENCES.....	99

LIST OF TABLES

Table 1 - African lion (female and male) and cougar (female and male) averages $\pm$ standard deviations on measures for skull basal length (SBL), endocranial volume (Endo), anterior cerebrum volume (AC), posterior cerebrum volume (PC), cerebellum plus brain stem (CB+BS), anterior cerebrum neocortex surface area (AC neo.ctx), posterior cerebrum neocortex surface area (PC neo.ctx), anterior subcortical volume (AC sub.ctx), and posterior cerebrum subcortical volume (PC sub.ctx).23

Table 2 - Coatimundi (female and male), kinkajou (female and male), and raccoon (female and male) averages $\pm$ standard deviations on measures for skull basal length (SBL), basicranial axis length (BCAL), endocranial volume (Endo), anterior cerebrum volume (AC), posterior cerebrum volume (PC), cerebellum + brain stem (CB+BS), anterior cerebrum neocortex surface area (AC neo.ctx), posterior cerebrum neocortex surface area (PC neo.ctx), anterior subcortical volume (AC sub.ctx), and posterior cerebrum subcortical volume (PC sub.ctx).53

LIST OF FIGURES

Figure 1. A , C Lateral and ventral views of a CT-scanned skull of an adult male African lion (**A**) and cougar (**C**). Arrow indicates the linear measurement of skull basal length (SBL), defined as the distance from the anterior border of the median incisive alveolus to the mid-ventral border of the foramen magnum. SBL is used here as a proxy for body size. **B, D** Log cube root of endocranial volume regressed against log skull basal length for African lion (**B**) males (solid circles) and females (open circles) and cougar (**D**) males (solid triangles) females (open triangles). African lion: Pearson's $r = 0.59$, $p < 0.05$ and cougar: Pearson's $r = 0.67$, $p < 0.01$. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation12

Figure 2. A Photograph of a whole brain of an African lion (*P. leo*) from the Comparative Mammalian Brain Collection (No. 64352) and three-dimensional virtual endocast reconstructed from a African lion skull (Michigan State University specimen No. 11242). **B** Photograph of a whole brain of a cougar (*P. concolor*) from the Comparative Mammalian Brain Collection (No. 60206) and three-dimensional virtual endocast reconstructed from a cougar skull (Michigan State University specimen No. 12240). Major sulci and other anatomical features in both the whole brain and virtual endocast are shown. an = Ansate sulcus; cs = cruciate sulcus; la = lateral sulcus; pl = postlateral sulcus; pr = proreal sulcus; ss = suprasylvian sulcus.15

Figure 3. A Dorsolateral view of the virtual endocast reconstructed from an African lion. Outlines indicate boundaries of the anterior cerebrum (AC), posterior cerebrum (PC), and cerebellum brain stem (CB+BS). Arrows denote the location of proreal gyrus (pg), anterior sigmoid gyrus (asg), and posterior sigmoid gyrus (psg). **B** Dorsolateral view of the virtual endocast showing neocortical surface area (shaded) and subcortical volume (solid). Neocortical surface area was measured as a 1-pixel deep outer mask of the endocranium dorsal to the rhinal fissure. Regional neocortical surface area measures, AC and PC, were defined using the same landmarks used for regional endocranial AC and PC volumes. Subcortical volumes were measured by subtracting the regional neocortical volume from the corresponding regional brain volume.17

Figure 4. A Low-power photomicrograph of a Nissl-stained coronal section through the posterior sigmoid gyrus (No. 630) of the African lion from the Comparative Mammalian Brain Collection (No. 62-79). The arrows show the location of cruciate sulcus (cs), coronal sulcus (co), presylvian sulcus (ps) and rhinal sulcus (rh). **B** Higher magnification photomicrograph showing the cytoarchitectonic features of the dorsal bank of cruciate sulcus, including primarily large layer V pyramidal cells (No. 622). Boxed area in **A** shows the relative location within the pericruciate cortex. **A** Scale bar = 1 cm. **B** Scale bar = 25 μ m.18

Figure 5. A , B Line drawings of a dorsolateral view of the virtual endocast representing the African lion (**A**) and cougar (**B**), showing the location and relative position of prominent sulci. ae = Anterior ectosylvian sulcus; an = ansate sulcus; co = coronal sulcus; cs = cruciate sulcus; io = intraorbital sulcus; la = lateral sulcus; pc = postcruciate sulcus; pe = posterior ectosylvian; pl = postlateral sulcus; pr = proreal sulcus; ss = suprasylvian sulcus.22

Figure 6. Magnetic resonance image (MRI) of a sagittal section through an adult male African lion skull and brain. Comparisons of endocranial volume to total brain volume in three planes revealed that total endocranial volume was on average 3.65% greater than brain volume. Scale bar = 1 cm.22

Figure 7. A , B Proportional regional brain volumes: (AC, PC, and CB+BS, all relative to total endocranial volume, regional surface areas: AC and PC, all relative to total surface area dorsal to rhinal fissure, and regional subcortical volumes, AC and PC, all relative to regional total volume for African lion (**A**) males (solid bars) and females (open bars) and cougar (**B**) males (solid bars) and females (open bars). Error bars indicate 8 1 SEM. AC volume and AC surface area are significantly larger in female than male African lions ($p = 0.002$ and $p = 0.008$, respectively). PC volume and PC surface area are significantly larger in male than female African lions ($p = 0.012$ and $p = 0.007$, respectively). * $p < 0.05$, * * $p < 0.01$27

Figure 8. Photographs of the whole brain of **A** coatimundi (*N. nasua*, No. 58-360), **B** raccoon (*P. lotor*, No. 57-88), and **C** kinkajou (*P. flavus*, No. 58-365) from the Comparative Mammalian Brain Collection and three-dimensional virtual endocast reconstructed from a coatimundi skull (Michigan State University specimen No. 14371), raccoon (Michigan State University specimen No. 2246), and kinkajou (Michigan State University specimen No. 9355). Major sulci and other anatomical features in both the whole brain and virtual endocasts are shown. (1) cruciate sulcus, (2) postcruciate sulcus, (3) medial ansate sulcus, (4) lateral sulcus, (5) posterior suprasylvian sulcus; (6) proreal sulcus; (7) coronal sulcus; (8) anterior suprasylvian sulcus; (9) sylvian sulcus. Scale bar: 1 cm.41

Figure 9. A Lateral in situ views of virtual endocasts and skulls for coatimundi (Michigan State University specimen No. 14371), raccoon (Michigan State University specimen No.2246), and kinkajou (Michigan State University specimen No. 9355) (left to right). **B** Ventral view of a raccoon skull (shown in **A**) with arrow showing skull basal length, defined as the distance from the anterior border of the median incisive alveolus to the mid-ventral border of the foramen magnum and graph showing log cube root of endocranial volume regressed against log skull basal length for raccoons (solid circles), coatimundi (open squares), and kinkajou (x). Pearson's $r = 0.78$, $p < 0.01$. **C** Ventral view of a raccoon skull (shown in **A** and **B**) with arrow showing basicranial axis length, defined as the midventral border of the foramen magnum to the basisphenoid-presphenoid suture and graph showing log cube root of endocranial volume regressed against log basicranial axis length for procyonids (legend same as in **B**). Pearson's $r = 0.45$, $p = 0.002$. Scale bar: 1 cm.42

Figure 10. Isosurface rendering of a coatimundi (Michigan State University specimen No. 14371) endocast in **A** dorsal, **B** lateral (left), and **C** dorsolateral views. A.-B. Anterior cerebrum (AC - blue) was defined as the region rostral to the cruciate sulcus and caudal to the olfactory bulbs (OB - yellow). The posterior cerebrum (PC - green) was defined as the endocranial volume posterior to the cruciate sulcus and anterior to the tentorium osseum. The cerebellum and brain stem (CB + BS - orange) was defined as the endocranial volume within the cerebellar fossa including the area caudal to the tentorium osseum and rostral to the foramen magnum. C. Neocortex surface area (black lines) was defined as the endocranial surface area dorsal to the rhinal fissure (rh). Subcortical volume (purple) was defined as endocranial volume excluding the outermost 3mm of the virtual endocast dorsal to the rhinal fissure (green) and CB + BS. Scale bar = 1 cm.46

Figure 11. A Proportional regional brain volumes: AC, PC, and CB+BS, all relative to total endocranial volume. **B** Proportional regional surface areas: AC neocortical surface area and PC neocortical surface area, each relative to total surface area dorsal to rhinal fissure, and regional subcortical volumes, AC subcortical volume and PC subcortical volume, each relative to regional total volume for coatimundi (solid black bars), raccoon (open bars), and kinkajou (solid grey bars). Error bars indicate ± 1 SEM. AC volume and AC neocortical surface area are significantly larger in coatimundi than both raccoons and kinkajous ($p < 0.001$ and $p < 0.001$), while kinkajous have a significantly larger AC volume than raccoons ($p = 0.004$). Raccoons have a greater amount of PC volume than coatimundi and kinkajou ($p < 0.001$ and $p = 0.004$ respectively), while kinkajous have a greater amount of PC volume and PC neocortical surface area compared to coatimundi ($p < 0.001$) and a greater amount of PC neocortical surface than raccoons ($p = 0.050$). Additionally, kinkajous had the greatest relative CB+BS compared to both coatimundis and raccoons ($p = 0.001$ and $p < 0.001$). ** $p < 0.001$ and * $p \leq 0.05$52

Figure 12. Proportional regional brain volumes: (AC, PC, and CB+BS, all relative to total endocranial volume, regional surface areas (neoctx sa): AC and PC, all relative to total surface area dorsal to rhinal fissure, and regional subcortical volumes (subctx vol), AC and PC, all relative to regional total volume for **A** coatimundi females (solid bars) and males (open bars), **B** raccoon females (solid bars) and males (open bars), and **C** kinkajou females (solid bars) and males (open bars). Error bars indicate ± 1 SEM. AC volume is significantly larger in female than male coatimundi ($p = 0.005$) and CB+BS volume is significantly larger in male than female coatimundi ($p = 0.04$). No sex differences in regional brain volumes were found in either raccoons (B) or kinkajous (C).57

Figure 13. Dorsolateral view of whole brain photographs from the Comparative Mammalian Brain Collection (www.brainmuseum.org) of **A** raccoon #68-247, **B** dog #59-326, and **C** spotted hyena #64-352. Regional volume, neuron number, and neuron density were obtained in successive Nissl stained sections within regions of interest (ROIs) indicated by arrows and box outlines: orb – orbital frontal cortex, pr – preoreal frontal cortex, 3b – primary somatosensory cortex (area 3b), 17 – primary visual cortex (area 17). Scale bar = 1 cm.76

Figure 14. Photomicrographs showing cytoarchitectonic organization through **A** preoreal frontal cortex, **B** orbital frontal cortex, **C** primary somatosensory cortex (area 3b), and **D** primary visual cortex (area 17) in the dog. Scale bar = 25 μ m.77

Figure 15. Photomicrographs showing cytoarchitectonic organization through **A** prereal frontal cortex, **B** orbital frontal cortex, and **C** primary somatosensory cortex (area 3b), in the spotted hyena. Scale bar = 25 μ m.78

Figure 16. Photomicrographs showing cytoarchitectonic organization through **A** prereal frontal cortex, **B** orbital frontal cortex, **C** primary somatosensory cortex (area 3b), and **D** primary visual cortex (area 17) in the raccoon. Scale bar = 25 μ m.79

Figure 17. Graph showing the relationship between the estimated neuron density of each traced region of interest as a function of endocranial volume. Species labels correspond to endocranial volume columns. A strong relationship exists for average neuron density (Pearson's $r = -0.68$, $p = 0.02$), where larger endocranial volumes display a significant decrease in neuron density averaged over each ROI.81

Figure 18. Bar graphs showing **A** neuron number, **B** regional volume, and **C** neuron density for prereal frontal cortex (prereal), orbital frontal cortex (orbital), primary somatosensory cortex (primary sensory) and primary visual cortex (primary visual) in the dog (black bar), spotted hyena (grey bar) and raccoon (white bar).84

INTRODUCTION

It is well known that brain size varies greatly among mammals and that this variation is principally due to a positive allometric relationship between brain size and body size [Jerison, 1973]. The allometric relationship suggests that bigger animals require larger brains to maneuver larger muscle mass and that variations in brain size reflect an increase in somatomotor cortex and overall body size [Nopoulos *et al.*, 2000; Changizi, 2003]. However, relative brain size can vary significantly even after body size is controlled [Finlay *et al.*, 2001]. This increase in relative brain size beyond that expected for a given body size is known as encephalization [Jerison, 1973]. Primates are an exemplary encephalized group possessing larger brains relative to body size compared to most all other vertebrates [Jerison, 1973]. The development and maintenance of exceedingly large brains is not without energetic costs [Aiello and Wheeler, 1995]. The large primate brain is metabolically expensive, accounting for approximately 20% of resting oxygen consumption in humans, with approximately 10% of this energy driving the exchange of sodium and potassium [Laughlin *et al.*, 1998]. Given this cost, it seems plausible that increased brain size must confer advantages. However, the underlying selection pressures favoring the development of large and complex brains among particular mammalian species remain unclear. A variety of potential factors have been hypothesized to explain variation in relative brain size [Healy and Rowe, 2007]. Two of the most prominent hypotheses propose that either factors associated with complexity in the social environment [Jolly, 1966; Humphrey, 1976; Byrne and Whiten, 1988; Joffe and Dunbar, 1997; Dunbar, 2003] or complexity in the physical environment [Jerison, 1973; Parker and Gibson, 1977; Clutton-Brock and Harvey, 1980; Milton, 1981; Povinelli and Cant, 1995] may account for increased relative brain size.

The former, known as the 'social brain hypothesis' posits; that the relatively large brain size found in primates is due to factors associated with living in large complex social environments [Jolly, 1966; Humphrey, 1976; Byrne and Whiten, 1988; Dunbar, 2003]. Therefore, among primates, relative brain size is an indicator of an individual's ability to process information related to cognitively demanding aspects of the social environment including the social behavior of conspecifics [Dunbar, 2003]. Variables such as social group size [Sawaguchi and Kudo, 1990; Dunbar, 1992; Dunbar, 1998; Barton, 1996; Barton and Dunbar, 1997], grooming clique size [Kudo and Dunbar, 2001], the extent to which social skills are used in male mating strategies [Pawlowski *et al.*, 1998], the frequency of tactical deception [Byrne, 1995], and the frequency of social play [Lewis, 2001] have been used as measures of social complexity and are found to positively correlate with increases in relative brain size. However, in primates, as brain size increases not all brain regions increase proportionally, rather the greatest expansion is in the relative amount of neocortex [Finlay and Darlington, 1995]. While neocortex is present in all extant mammals, primates exhibit an especially large amount of neocortex, compared to the brain as a whole [Jerison, 2007]. Among primates, relative neocortex size has been shown to exhibit even stronger correlations with measures of social complexity, e.g. social group size compared to measure of relative brain size [Dunbar, 2011]. As neocortex increases in size [Kaas, 1993] or surface area [Krubitzer *et al.*, 1997], there is an associated increase in the number of neocortical areas [for review see Roth and Dicke, 2005]. In primates, when social complexity is correlated with regional neocortex measures, the association is strongest in the frontal cortex [Dunbar 2011], the brain region known to mediate complex social behaviors in humans and other mammals [Adolphs, 2001; Amodio and Frith, 2006]. Damage to the prefrontal cortex elicits profound behavioral deficits including inappropriate behavioral displays in a social setting, e.g.

unsolicited threatening or aggressive behaviors [Deets *et al.*, 1970; Synder, 1970]. These findings along with comparisons between primates and other mammalian species reveal that the relatively large brains in primates is due to an expansion in neocortex, specifically frontal cortex, and suggests that selection pressures related to the social environment may act on functionally distinct neural systems, e.g. prefrontal cortex, resulting in changes in the relative size of the brain regions [Barton and Harvey, 2000].

The second major hypothesis regarding factors influencing large brains suggests that increases in relative brain size are due to factors associated with physical environmental complexity. The relatively large brains of primates are hypothesized to be the result of increased cognitive demands associated with solving complex environmental problems, such as exploiting environmental resources and navigating a large home range size [Allman 1977; Clutton-Brock and Harvey 1980]. Additionally, complexity in the physical environment include navigating a three-dimensional arboreal environment [Povinelli and Preuss, 1995; Povinelli and Cant, 1995], degree of forepaw use in object manipulation and tool use [Parker and Gibson, 1977], foraging for food [Jerison, 1973; Clutton-Brock and Harvey, 1980; Milton, 1981], or behavioral flexibility in response to novel environments [Lefebvre *et al.*, 1997]. Thus, selective factors influencing increases in relative brain size may result from the need to navigate a three-dimensional arboreal environment [Povinelli and Preuss, 1995; Povinelli and Cant, 1995]. The ‘arboreal theory’ suggests that having to navigate an arboreal environment places a greater cognitive demand as a result of negotiating an unstable, unpredictable, and non-continuous arboreal environment [Smith, 1912]. Arboreal mammals including primates possess relatively larger brains compared to terrestrial mammals [McNab and Eisenberg, 1989]. In addition to factors related to aspects of an arboreal lifestyle, Parker and Gibson [1977] suggested increases in primate brain size are

associated increased cognitive demands related to sensorimotor functions involved in complex object manipulation and tool use related that tool use and extractive foraging. In primates, a positive correlation exists between relative brain size and the complexity of resource extraction strategies on seasonally available embedded foods [Boesch and Boesch, 1984]. An increase in the complexity of the foraging strategy involves behaviors that require extensive sensorimotor coordination, mediated by cortical control and an increase in cortex-mediated coordination. All could result in greater encephalization [Gibson, 1986]. Another source of physical environmental complexity relates to home range size and resource availability, where there is an increase in cognitive demands related to processing complex spatiotemporal mapping in order to successfully learn and recall when and where food resources become available [Clutton-Brock and Harvey, 1980]. Lefebvre *et al.* [1997] suggested that increased cognitive demands related to complexity in the physical environment are associated to the extent that an individual is able to adapt to novel environmental situations.

In addition to hypotheses examining the influence of complexity in either the social or physical environment, life history factors are also suggested to influence variations in relative brain size. In primates, life history variables such as prolonged development and increased longevity correlate with greater encephalization [Barrickman *et al.*, 2008]. Increased longevity allows for increases in relative brain size as species with larger brains are able to successfully adapt to changes in their environments during an extended lifespan [Allman *et al.*, 1993]. Large brain size has also been suggested to be related to an extended juvenile period of development where individuals require an extension in the learning period to acquire skills in order to navigate a dynamic social environment [Joffe, 1997]. Another prominent life history factor is gestation length, which suggests that the rate of brain growth during the prenatal period exceeds that of

postnatal period and could account for the exceedingly large brains of primates [Jerison, 1973; Gould, 1975].

While all these factors are hypothesized to influence brain size in the primate species studied, the role of the factors in affecting brain size in other mammals is less clear. The majority of studies examining mammalian brain size have focused on primate species [Gould, 1975; Clutton-Brock and Harvey, 1980; Dunbar and Shultz, 2007] while comparatively few studies have sought to investigate these factors in other mammals [Gittleman, 1986; Marino, 1996; Dunbar and Bever, 1998; Bush and Allman, 2004; Perez-Barberia *et al.*, 2007; Finarelli, 2006; Finarelli and Flynn, 2009; Swanson *et al.*, 2012]. Non-primate species that face the same selection pressures as shown in primate species should also exhibit corresponding increases in either relative brain size or regional brain volumes. Carnivores offer an alternative mammalian model in which to further investigate the influence of factors related to social and environmental complexity on variations in brain size.

The order Carnivora includes 13 extant families and approximately 270 species [Vaughan *et al.*, 2000] that display variations in brain and body size, as well as variation in social and non-social physical environments. Therefore, carnivores are an excellent model in which to investigate the influence of complexities in the social and physical environments on variations in total and regional brain size. While carnivores have been a focal point of earlier comparative studies of brain-body allometry [Jerison, 1973] and brain size evolution [Radinsky, 1969; Radinsky, 1978], additional comparative investigations in carnivores have examined the role of: life history on relative brain size [Bekoff *et al.*, 1984; Gittleman 1986], degree of forepaw dexterity on brain size [Iwaniuk, 1999], and social complexity on both relative brain and relative neocortex size [Dunbar and Bever, 1998; Perez-Barberia *et al.*, 2007]. These data support the

notion that similar factors proposed to influence brain size in primates may also influence brain size in carnivores. However, details of the association of some of these factors with total and regional relative brain size in carnivores remains to be determined. For example, Finarelli and Flynn [2009] suggest that among the 86 carnivore species examined by Perez-Barberia *et al.* [2007], the family Canidae are responsible for the significant relationship between larger than expected brain size and social complexity in the order Carnivora. It is important to highlight that these investigations were performed at broad taxonomic levels of analyses and a considerable degree of variation in encephalization patterns is observed both between species and between higher taxonomic groups [van Dongen 1998; Iwaniuk *et al.*, 1999]. One potential explanation for these variations in encephalization patterns among carnivore species is that the variations could be directly related to measures of body size [see Deaner *et al.* 2000 for review]. Swanson *et al.* [2012] noted differing interspecific effects relative to body size measures, skull size and body mass in an analysis of 36 carnivore species. This suggests that, while brain size does allometrically scale with body size, alternative relationships can arise depending on the measure used for body size when performing broad comparisons across taxonomic groups.

The possibility that the same factors influencing relative brain size or regional brain size, e.g. neocortex, at broad taxonomic levels might also influence variations in family level (interspecific) analysis or within species (intraspecific) analyses has been largely overlooked. Previously, an interspecific analysis of the influence of social complexity on relative and regional brain size within the carnivore family Hyaenidae revealed that among the four extant species, the highly gregarious spotted hyena (*Crocuta crocuta*) possess relatively larger brains and a relatively larger amount of frontal cortex compared to the less social species [Sakai *et al.*, 2011a]. Additionally, while intraspecific comparisons within spotted hyenas failed to reveal a

sex difference in relative brain size, regional comparisons revealed males possess a greater relative amount of frontal cortex than females, a finding attributed to the divergent social life histories between the sexes [Arsznov *et al.*, 2010]. These findings suggest that family level comparative analysis between closely related extant species exhibiting divergent behaviors can identify potential brain structures involved in the mediation of species-specific behaviors.

The present dissertation investigated the relationship between brain size and regional brain volumes in selected carnivore species known to exhibit specialized behaviors related either to the social or physical environment. The first study utilizes computed tomography (CT) to investigate the influence of social life history, an account of the series of events in an individual's life directly related to aspects of the social environment, on intraspecific variation in brain size in African lions (*Panthera leo*), a gregarious species where females are more social than males, in comparison to the cougar (*Puma concolor*), a species where both males and females are solitary. The second study examines interspecific and intraspecific variations in the relative influence of social life history, forepaw dexterity, and arboreality on brain size and organization in three species in the Family Procyonidae which exhibit continuums of behaviors related to social and physical environmental complexities. The last study examines three carnivore species: dog (*Canis familiaris*), raccoon (*Procyon lotor*), and spotted hyena (*Crocuta crocuta*) using cytoarchitectonic criteria and stereology to determine if an increase in total and regional brain size reflects an increase in the number of neurons. Together, these studies provide insight into the influence of sociality, environmental, and life history, factors hypothesized to influence brain variation in primates, on brain variations in selected carnivore species.

CHAPTER 1: Pride Diaries: Sex, Brain Size and Sociality in the African Lion (*Panthera leo*) and Cougar (*Puma concolor*)

Introduction

The social brain hypothesis advocates that sociality is the primary selection pressure responsible for the relative increase in brain size [Byrne and Whiten, 1988; Dunbar, 1992; Dunbar, 1998]. In primates, increased sociality is related to an increase in brain size; including expansion of frontal cortex. Frontal cortex is involved in executive control of cognition and regulation of social behaviors in humans and other mammals [Adolphs, 2001; Amodio and Frith, 2006], and its relative enlargement may reflect enhanced processing of social information. Analysis of relative brain size in other mammalian taxa including artiodactyls, ungulates, carnivores and bats [Shultz and Dunbar, 2007] has revealed a relationship between sociality and increased brain size.

Despite the support for the social brain hypothesis in comparative studies, few studies have examined if differences in social life history experienced by males and females is associated with differences in brain size. In humans, a portion of the ventral frontal cortex associated with social perception is larger in females than males [Wood *et al.*, 2008]. In contrast, we recently found that males possess proportionately more frontal cortex than females in spotted hyenas [Arsznov *et al.*, 2010]. This frontal cortical difference might be attributed to the differential social life history experienced by male and female spotted hyenas. Spotted hyenas are highly gregarious living in clans of up to 90 individuals [Kruuk, 1972; Holekamp *et al.*, 2007]. Female spotted hyenas are usually philopatric [Henschel and Skinner, 1987; Mills, 1990; Smale *et al.*, 1997; Boydston *et al.*, 2007], while most postpubertal male hyenas emigrate from their natal clans. Male spotted hyenas join neighboring clans at the bottom of the dominance hierarchy [East and Hofer, 2001] and behave submissively to all hyenas encountered in the new

clan [Holekamp *et al.*, 2007]. Thus, the larger frontal cortex possessed by male spotted hyenas may correspond either to the enhanced experience associated with frontal cortical processing or a sex-specific effect. The experience effect may be in response to the greater inhibitory control required of male spotted hyenas in the presence of larger, more aggressive females. Immigrant males may require enhanced inhibitory control over inappropriate behaviors, such as aggression, in order to negotiate the social system in the new clan. Alternatively, relative enlargement of frontal cortex may reflect enhanced processing of social information required of males as they immigrate from the natal clan to a new clan. Since spotted hyenas remember the identities and ranks of their clan mates throughout their lives [Holekamp *et al.*, 2007], immigrant males would typically have a greater social memory requirement than natal females. Finally, the sex difference in frontal cortex volumes may be the result of sex-specific genes including hormonal effects [Luders *et al.*, 2009]. The present study sought to assess each of these questions by examining regional brain volumes in two species: the African lion (*Panthera leo*) and the cougar (*Puma concolor*), members of two separate felid clades [Johnson *et al.*, 2006].

Among the extant felids, only the African lion is consistently gregarious [Packer and Pusey, 1987]. African lions live in complex fission-fusion social groups called prides [Packer and Pusey, 1987]. Social life history patterns in the pride vary between the sexes in both dispersal and dominance behavior. Male offspring emigrate from the natal pride upon sexual maturity where they enter a solitary nomadic phase or form a coalition with other male kin mates, whereas female offspring typically remain in the maternal pride [Packer and Pusey, 1987]. Female lions live in prides of up to 21 lions, and are subordinate to males. Male lions are highly aggressive towards female lions [Mosser and Packer, 2009]. In contrast to the social organization exhibited in the African lion, most other felid species including cougars [Sunquist

and Sunquist, 2002] are primarily solitary and only interact with conspecifics during the breeding season when they form pairs that last for a few weeks [Nowak and Paradiso, 1983]. Here, we compared total and regional brain volumes in male and female lions and cougars in order to determine if intraspecific variation in social life history correspond to brain volume differences. Three predictions regarding sex differences in frontal cortex were examined. (1) If the demands for inhibitory control in the presence of dominant aggressor underlie sex differences in frontal cortex, we predicted that female lions would be faced with greater demands for inhibitory control and would possess proportionately more frontal cortex than male lions. (2) If increased frontal cortical volume is related to increased social information processing, the cognitive demands resulting from emigrating to a new social group should correspond to an increase in frontal cortical volume in male lions compared to female lions, similar to our findings in male spotted hyenas. Like male spotted hyenas, male African lions also disperse from their natal pride and learn the identities of new pride members. (3) Finally, if sex-specific effects determine the difference in frontal cortex, intraspecific variation should reveal an absence of frontal cortical sex differences in the cougar, a species in which both males and females maintain a solitary life style. Here we used computed tomography (CT) to create virtual endocasts from lion and cougar skulls. The virtual endocasts are based on serial analysis of coronal images and yield quantitative regional and total brain volume data that were used to examine intraspecific variations.

Methods

Specimens

Skulls of 14 adult African lions (8 male, 6 female) and 14 cougars (7 male, 7 female) were obtained from the collections of the Michigan State University Museum, Field Museum of Natural History and University of Michigan Museum of Zoology (see Appendix 1).

Digital Measurements

Each skull was aligned along an anterior-posterior axis and scanned using either a General Electric Lightspeed 4 slice scanner or a General Electric Discovery ST 16 slice scanner at the Department of Radiology at Michigan State University (see Appendix 1). The following scanning parameters were used: a slice thickness of 0.625 mm, a table speed of 5.62 mm/rotation, a pitch of 0.562: 1 and a 30-cm field of view. The CT data were saved in the Digital Imaging and Communications in Medicine (DICOM) Centricity Version 2.2 format and the virtual endocasts were created using MIMICS 13.1 software (Materialise, Inc., Ann Arbor, Mich., USA). Virtual endocasts were created for each scanned skull using the procedures described in Sakai *et al.* [2011b]. Briefly, the skull was isolated from the surrounding air space, defined as a pixel value of –1024 Hounsfield units (HU). Next, the endocranial air space was filled for each slice in the coronal plane starting where the cribriform plate forms the floor of the endocranial cavity and extending caudally through the foramen magnum. The filled coronal sections were then stacked to create a three-dimensional reconstruction of the endocranial cavity (virtual endocast) using the MIMICS 3D object operation. Detailed external brain morphology, including gyral and sulcal patterns, can be seen in the virtual endocasts.

Skull basal length, defined as the distance from the anterior border of the median incisive alveolus to the mid-ventral border of the foramen magnum, was collected from each specimen as a measure of body size (fig. 1). Since skull basal length is highly correlated with body weight, the former is a reasonable proxy for body size when other measures (e.g., individual body weight, postcranial dimensions) are not available [Janis, 1990; Van Valkenburgh, 1990]. A single observer (B.M.A.) collected all linear skull measurements from the CT images.

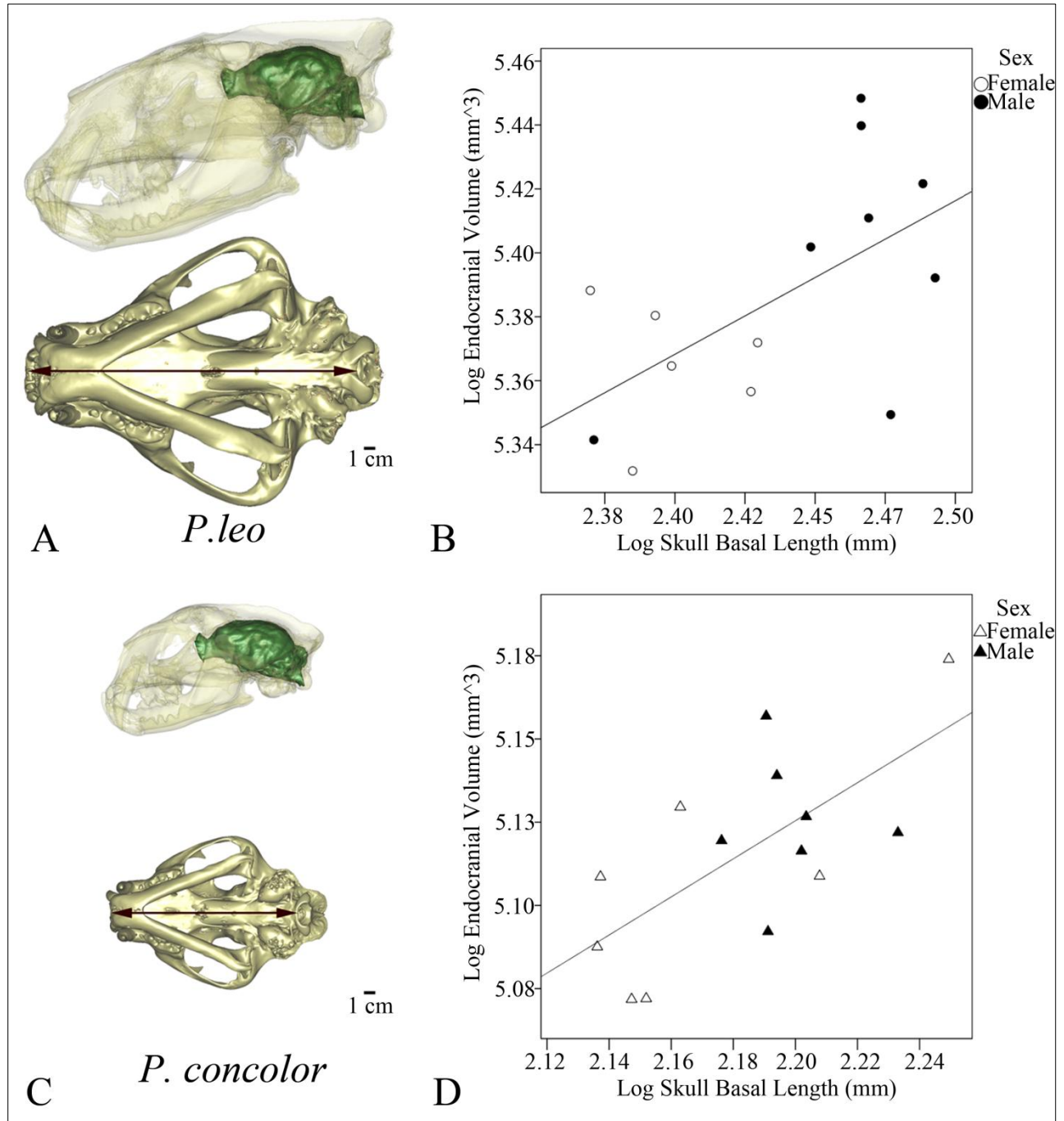


Figure 1. A, C Lateral and ventral views of a CT-scanned skull of an adult male African lion (A) and cougar (C). Arrow indicates the linear measurement of skull basal length (SBL), defined as the distance from the anterior border of the median incisive alveolus to the mid-ventral border of the foramen magnum. SBL is used here as a proxy for body size. B, D Log cube root of endocranial volume regressed against log skull basal length for African lion (B) males (solid circles) and females (open circles) and cougar (D) males (solid triangles) females (open triangles). African lion: Pearson's $r = 0.59$, $p < 0.05$ and cougar: Pearson's $r = 0.67$, $p < 0.01$. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

Validation of Virtual Endocasts

Whole Endocasts In order to confirm the general brain morphology obtained from the endocast, the gyral and sulcal patterns seen in the virtual endocasts were directly compared to the external morphological features on whole brain photographs of standard anatomical orientations, including dorsal, ventral, left lateral, and right lateral views, of a formalin-fixed African lion brain (specimen No. 62-79) and cougar (specimen No. 60-206) from the Comparative Mammalian Brain Collection (www.brainmuseum.org) (fig. 2). All volumetric data were made by a single observer (B.M.A.) and were obtained using the MIMICS 3D volume measurement operation. These measurements were assessed for reliability by comparing whole endocast volumes from a subset of 11 specimens obtained from 2 separate raters. An inter-rater reliability analysis revealed no significant difference in volumetric assessment from the endocasts created from each individual ($t(20) = 0.07, p = 0.95$). CT files were coded by animal number only, and the analysis and demarcation of brain regions was conducted blind with regard to the sex of the specimen.

Magnetic Resonance Imaging (MRI) and Volume Analysis

In order to assess the difference between endocranial volume and brain volume, we analyzed archived MR images of a 10-year old captive male African lion (John Ball Zoo, Grand Rapids, Mich., USA).

Magnetic Resonance Data Acquisitions

The lion was imaged at the Veterinary Medical Center, College of Veterinary Medicine, Michigan State University. The lion was darted to induce anesthesia and maintained using sevoflurane. The brain was imaged in a 1.5-T Siemens Espree (Siemens, Munich, Germany). T₁ - and T₂ -weighted images in the transverse and sagittal planes were acquired with pre- and

post-contrast medium T 1 sequences obtained in axial (transverse), sagittal and coronal (dorsal) planes. The image sequences were obtained using the following parameters: T 1 sequence: TR = 615 ms, TE = 15 ms, FOV = 24 cm, slice thickness = 5 mm, resolution: 256 x 256 x 16, flip angle of 150 degrees and interslice gap = 1.5 mm. T 2 sequence: TR = 6,920 ms, TE = 79 ms, FOV = 24 cm, slice thickness = 5 mm, resolution: 320 x 320 x 16, flip angle of 150 degrees and interslice gap = 1.5 mm. The animal used in this study was treated as a hospital patient according to guidelines set by the American Veterinary Medical Association.

Post-processing, reconstruction of the whole brain and endocranium and volume acquisition were performed using MIMICS 13.1 software (Materialise, Inc., Ann Arbor, Mich., USA). Volumetric measurements were made in three separate anatomical orientations: coronal, sagittal and axial. In each of these orientations, masks for total brain volume and endocranial volume were created by tracing the region of interest each serial section. This masking process was similar to the selection process of total endocranial volume for the CT data starting where the cribriform plate forms the floor of the endocranial cavity and extending caudally through the foramen magnum.

Delineation of Brain Regions in Virtual Endocasts

The gyral and sulcal pattern as well as bony landmarks were used as a guide to further subdivide the endocranium into 3 regions: anterior cerebrum, posterior cerebrum, and cerebellum/brain stem using the procedures described in Arsznov *et al.* [2010] (fig. 3). A brief description of the criteria employed follows.

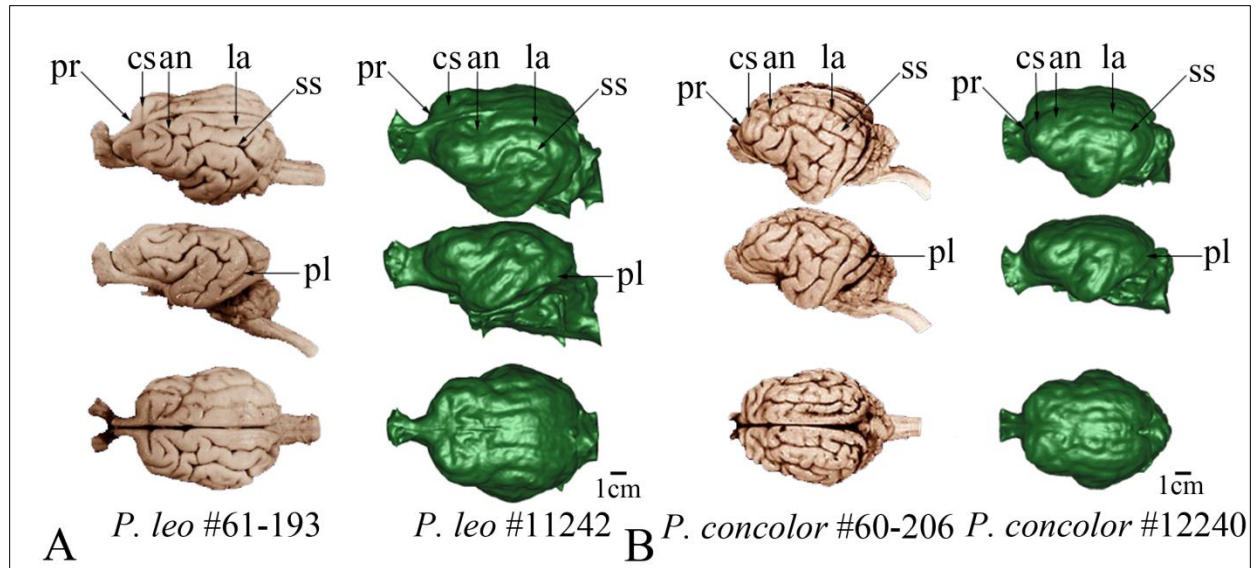


Figure 2. **A** Photograph of a whole brain of an African lion (*P. leo*) from the Comparative Mammalian Brain Collection (No. 64352) and three-dimensional virtual endocast reconstructed from a African lion skull (Michigan State University specimen No. 11242). **B** Photograph of a whole brain of a cougar (*P. concolor*) from the Comparative Mammalian Brain Collection (No. 60206) and three-dimensional virtual endocast reconstructed from a cougar skull (Michigan State University specimen No. 12240). Major sulci and other anatomical features in both the whole brain and virtual endocast are shown. an = Ansate sulcus; cs = cruciate sulcus; la = lateral sulcus; pl = postlateral sulcus; pr = proreal sulcus; ss = suprasylvian sulcus.

Anterior Cerebrum Volume (AC)

To our knowledge, no cortical map is available for the African lion or cougar. Therefore, we relied on the identification of anterior cortical areas reported in other carnivores and applied these criteria to the African lion and cougar. Anterior cortex, specifically, the frontal cortex is defined in primates as cortex rostral to the central sulcus. However, the central sulcus is absent in carnivores and its putative homologue, the postcruciate dimple or sulcus, which delimits the boundary between motor and somatosensory cortex in some carnivores [Hardin *et al.*, 1968; Gorska, 1974], is not present in all carnivore species and, where present, can be highly variable between hemispheres, even within the same individual [Hassler and Muhs-Clement, 1964; Kawamura, 1971]. Here, we use the cruciate sulcus as a landmark for demarcating anterior from posterior cerebrum, as it is a prominent feature that demonstrates less intra- and interspecific

variation than the postcruciate dimple [Radinsky, 1969; Myasnikov *et al.*, 1997]. The cruciate sulcus is coincident with the rostral most portion of the motor cortex (cytoarchitectonic areas 4 and 6) in the cat [Hassler and Muhs-Clement, 1964], dog [Gorska, 1974; Stanton *et al.*, 1986; Tanaka, 1987; Sakai *et al.*, 1993], raccoon [Sakai, 1982; Sakai, 1990], and spotted hyena [Arsznov *et al.*, 2010].

A histological series of sections through the pericruciate region of the African lion (62-79) was examined to determine if the cruciate sulcus displays the characteristic cytoarchitectonic features as those described in other carnivore species. A similar histological series in the cougar brain was not available. Analysis of the Nissl-stained lion brain sections revealed that the fundus of the postcruciate dimple delimits the boundary between cytoarchitectonic area 4 and area 3a. Area 4 is primarily defined by the absence of a granular cell layer IV and the presence of giant and large pyramidal cells in layer V. In the somatosensory cortex, area 3a is characterized by the presence of a granular cell layer IV and small pyramidal cells located in layer V [Hassler and Muhs-Clement, 1964]. The dorsal bank of the cruciate sulcus contains large and giant pyramidal cells in layer V consistent with cytoarchitectonic area 4 and the primary motor cortex as mapped in other carnivores [Hardin *et al.*, 1968; Gorska, 1974; Nieoullon and Rispal Padel, 1976] (fig. 4).

Anterior cerebral volume was calculated from the endocranial slices and was defined as the region rostral to the junction of the cruciate sulcus and midline, but caudal to the olfactory bulbs. The anterior cerebrum volume here is thus comprised of the frontal cortex and subcortical structures, including a small portion of the rostral most head of the caudate nucleus, ventral pallidum, olfactory tubercle and prepiriform cortex (fig. 3, 4).

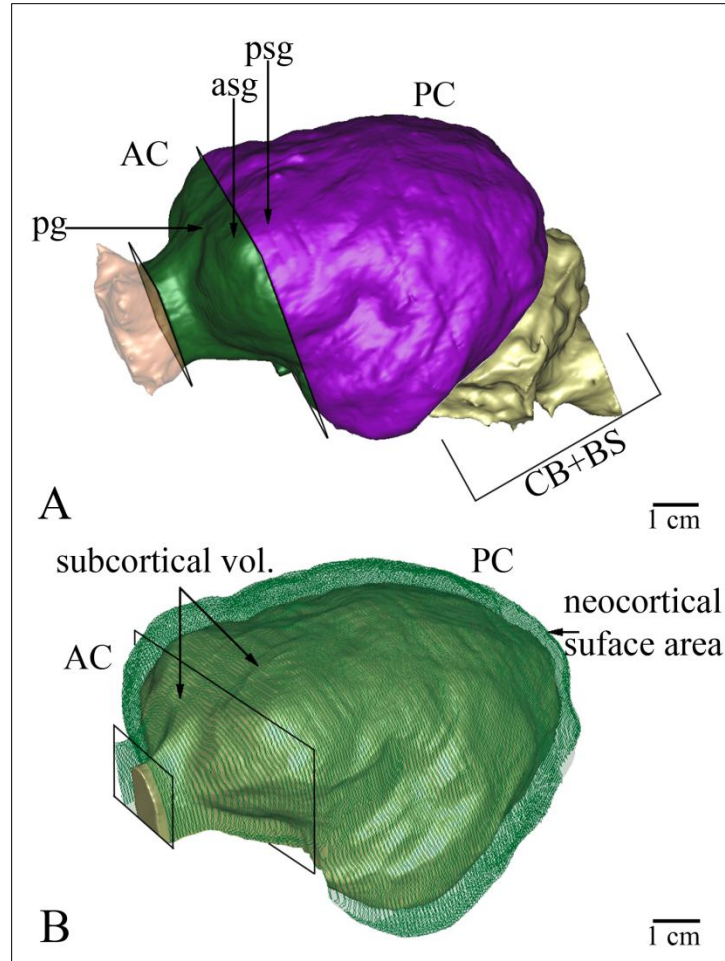


Figure 3. **A** Dorsolateral view of the virtual endocast reconstructed from an African lion. Outlines indicate boundaries of the anterior cerebrum (AC), posterior cerebrum (PC), and cerebellum brain stem (CB+BS). Arrows denote the location of proreal gyrus (pg), anterior sigmoid gyrus (asg), and posterior sigmoid gyrus (psg). **B** Dorsolateral view of the virtual endocast showing neocortical surface area (shaded) and subcortical volume (solid). Neocortical surface area was measured as a 1-pixel deep outer mask of the endocranium dorsal to the rhinal fissure. Regional neocortical surface area measures, AC and PC, were defined using the same landmarks used for regional endocranial AC and PC volumes. Subcortical volumes were measured by subtracting the regional neocortical volume from the corresponding regional brain volume.

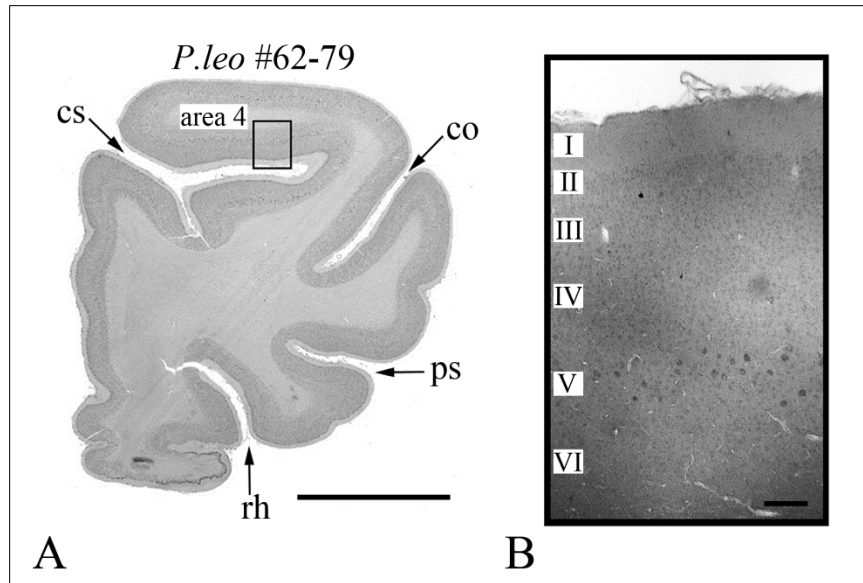


Figure 4. **A** Low-power photomicrograph of a Nissl-stained coronal section through the posterior sigmoid gyrus (No. 630) of the African lion from the Comparative Mammalian Brain Collection (No. 62-79). The arrows show the location of cruciate sulcus (cs), coronal sulcus (co), presylvian sulcus (ps) and rhinal sulcus (rh). **B** Higher magnification photomicrograph showing the cytoarchitectonic features of the dorsal bank of cruciate sulcus, including primarily large layer V pyramidal cells (No. 622). Boxed area in **A** shows the relative location within the pericruciate cortex. **A** Scale bar = 1 cm. **B** Scale bar = 25 μ m.

The endocranial volume posterior to the cruciate sulcus, but anterior to the tentorium cerebelli, is here referred to as the posterior cerebrum (fig. 3). This region included all cortex posterior to the cruciate sulcus as well as underlying diencephalic and rostral mesencephalic structures.

Cerebellum plus Brain Stem Volume (CB+BS)

The cerebellum and brain stem are housed within the posterior cranial fossa (fig. 4). This region was defined on the CT images of the skull as the area between the foramen magnum and the tentorium cerebelli, which covers the superior surface of the cerebellum. Thus, the volume measurement for cerebellum/brain stem included cerebellum, medulla, pons, and brain stem as far caudal as its junction with the spinal cord.

Whole and Regional Surface Areas

Since the endocast is based on the surface impression of the endocranium, it is limited in providing information regarding detailed brain structure. However, one measure, endocranial surface area dorsal to the rhinal fissure, has been used as an indicator of neocortical area [Jerison, 2007]. Here, neocortical surface area was measured from a 1-pixel deep outer mask of the endocast. Surface area located ventral to the rhinal fissure was excluded from this measure. Regional neocortical surface areas were obtained by segmenting the whole surface area mask at the same coordinates used for the regional endocranial volume masks for anterior cerebrum and posterior cerebrum.

Subcortical Regional Volumes

In order to determine if differences in either total or regional brain volume might be due to relative enlargement of the subcortical areas, we estimated subcortical volumes in the endocast by subtracting from the total volume the outermost 3 mm of the endocast. Neocortical depth was measured in cortex dorsal to the rhinal fissure in Nissl-stained coronal sections of the African lion (62-79) (fig. 4). The average depth of cortex was 2.5 mm. This measure was combined with an approximation of the dura mater thickness ranging from 0.2 mm in the cat to 0.25 mm in the dog [McComb *et al.*, 1981]. Then, a measure for cortical volume was calculated using the MIMICS morphology operation to dilate the 1-pixel neocortex surface area mask to a depth of 8 pixels or approximately 3 mm. Subcortical volumes were calculated by subtracting the regional neocortical volume from the corresponding regional brain volume.

Statistical Analyses

Statistical analyses were based on the endocasts of 8 male and 6 female African lions and 7 male and 7 female cougars. Prior to statistical analyses, skull basal length and endocranial volumes were log-transformed in order improve graphical representation of the data. The

allometric relationship between total endocranial volume and skull basal length was assessed using a bivariate correlation coefficient, Pearson's r for each species. Two sets of statistical analyses were employed to determine sex differences in endocranial or regional brain volumes. First, mean residual values obtained from linear regressions of log cube root of total endocranial volume plotted against log skull basal length, regional brain volume plotted against endocranial volume, regional surface area plotted against total neocortical surface area and regional subcortical volume plotted against regional volume were compared using independent t tests. Second, an analysis of variance (ANOVA) was employed comparing the mean residual values obtained from a linear regression of log cube root of total endocranial volume plotted against log skull basal length, and the following proportions: regional brain volume relative to total endocranial volume, regional neocortical surface area relative to total neocortical surface area and regional subcortical volume relative to regional volume. All statistical analyses were performed using the statistical software package PASW Statistics 18 (SPSS Inc., Chicago, Ill., USA).

Lastly, based on lion MRI analysis, the percentage difference between total endocranial and brain volume was calculated for each orientation plane (axial, sagittal, and coronal), and these measurements were averaged.

Results

Whole Endocasts

The virtual endocast and surface brain morphology is compared in the African lion and cougar in figure 2. Major morphological features are visible in both whole brain photographs and virtual endocasts. These include preoreal gyrus, cruciate sulcus, ansate sulcus, lateral sulcus and suprasylvian sulcus (fig. 2, 5). Virtual endocasts of both the African lions and cougars share

similar external morphology, with all of the major sulci present in both species (fig. 2). Despite the similarities in overall sulcal pattern, morphological differences are notable between the species. The shape of the rostral brain including the pericruciate region and proreal gyrus appears larger both mediolaterally and rostrocaudally in the African lion compared to the cougar. This is most apparent by comparing the dorsal views of the endocast and whole brain in the two species. The anterior sigmoid gyrus and proreal gyrus in the lion is clearly visible from this view but the same region is quite limited in the cougar (fig. 2, 3, 5). Additionally, the postcruciate sulcus is elongated in the African lion and extends parallel to the ansate sulcus, whereas in comparison the postcruciate sulcus is inconsistent in its length and extent varying both between individuals and between hemispheres in the same cougar. Finally, the extent to which the posterior cerebrum overlies the cerebellum differs in the two species. As seen from a dorsal view, the cerebellum is clearly visible in the cougar, but the posterior cerebrum largely overlies the cerebellum in the African lion (fig. 2).

MRI Data

Analysis of the MRI data of an African lion revealed the following percent differences between brain volume and total endocranial volume in each of 3 planes: axial – 5.60%; sagittal – 3.19%; coronal – 2.25%. On average, total endocranial volume was 3.65% greater than brain volume. An image of a sagittal section through the lion skull is shown in figure 6.

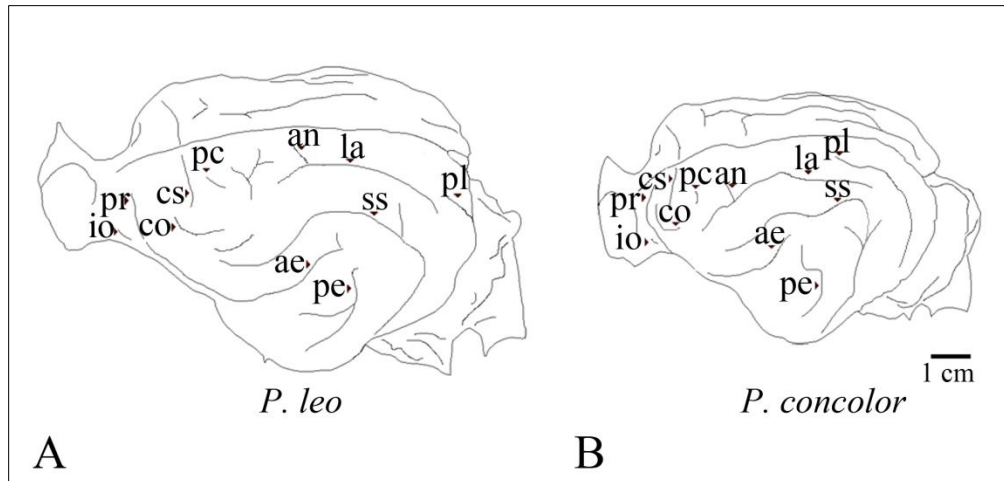


Figure 5. A , B Line drawings of a dorsolateral view of the virtual endocast representing the African lion (**A**) and cougar (**B**), showing the location and relative position of prominent sulci. ae = Anterior ectosylvian sulcus; an = ansate sulcus; co = coronal sulcus; cs = cruciate sulcus; io = intraorbital sulcus; la = lateral sulcus; pc = postcruciate sulcus; pe = posterior ectosylvian; pl = postlateral sulcus; pr = proreal sulcus; ss = suprasylvian sulcus.

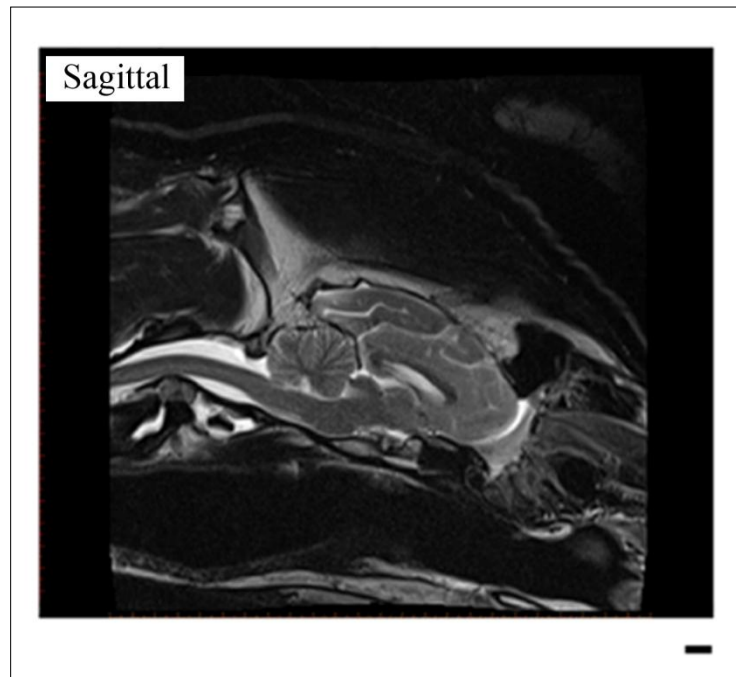


Figure 6. Magnetic resonance image (MRI) of a sagittal section through an adult male African lion skull and brain. Comparisons of endocranial volume to total brain volume in three planes revealed that total endocranial volume was on average 3.65% greater than brain volume. Scale bar = 1 cm.

Species	<i>P. leo</i>		<i>P. concolor</i>	
Sex	♀	♂	♀	♂
SBL, mm	251.81 ± 13.75	289.31 ± 23.90	148.67 ± 15.25	158.10 ± 6.53
Endo, mm ³	232,257.54 ± 10,538.41	252,464.38 ± 22,155.70	128,465.46 ± 110,15.45	133,359.40 ± 6,159.23
AC, mm ³	36,173.40 ± 5,924.53	26,511.25 ± 6,619.60	9,480.45 ± 2,077.50	10,727.17 ± 1,096.37
PC, mm ³	136,756.60 ± 15,101.13	165,082.30 ± 12,421.10	91,570.95 ± 7,405.97	92,372.98 ± 5,630.02
CB+BS, mm ³	46,767.36 ± 2,628.64	52,059.72 ± 5,298.10	23,692.99 ± 3,660.59	27,007.52 ± 1,586.12
AC neo.ctx, mm ²	5,068.30 ± 1,566.17	4,030.60 ± 1,328.99	998.98 ± 542.70	1,028.46 ± 309.25
PC neo.ctx, mm ²	17,060.52 ± 2,411.01	20,358.11 ± 1,531.65	10733.44 ± 2504.51	10,857.83 ± 822.05
AC sub.ctx, mm ³	24,347.02 ± 4,486.73	16,632.30 ± 5,832.82	6,230.71 ± 1,384.98	6,971.40 ± 706.95
PC sub.ctx, mm ³	98,152.31 ± 12,807.58	117,347.77 ± 13,443.04	69,384.45 ± 7,549.77	68,682.95 ± 4,174.90

Table 1. African lion (female and male) and cougar (female and male) averages ± 8 standard deviations on measures for skull basal length (SBL), endocranial volume (Endo), anterior cerebrum volume (AC), posterior cerebrum volume (PC), cerebellum plus brain stem (CB+BS), anterior cerebrum neocortex surface area (AC neo.ctx), posterior cerebrum neocortex surface area (PC neo.ctx), anterior subcortical volume (AC sub.ctx), and posterior cerebrum subcortical volume (PC sub.ctx).

Sex Differences

Endocranial volume and skull basal length are highly correlated in African lions and cougars (Pearson's $r = 0.59$, $p < 0.05$ and Pearson's $r = 0.67$, $p < 0.01$, respectively). In lions, total endocranial volume was on average $252,464 \text{ mm}^3$ (SD 22,155) in males and $232,257 \text{ mm}^3$ (SD 10,538) in females. In cougars, total endocranial volume was on average $133,359 \text{ mm}^3$ (SD 6,159) in males and $128,465 \text{ mm}^3$ (SD 11,015) in females. However, these sex differences in endocranial volume were not significant in either African lions ($t(12) = 2.05$, $p = 0.063$) or cougars ($t(12) = 1.03$, $p = 0.325$). Endocranial volume, skull basal length and regional brain volumes are presented in table 1. Residual analysis from separate linear regressions for each species of the influence of sex on endocranial volume as a function of skull basal length revealed no significant difference between the sexes in either the African lion or cougar ($t(12) = 0.40$, $p = 0.69$ and $t(12) = 0.080$, $p = 0.94$, respectively) (fig. 1). Additionally, an ANOVA comparing sex differences in endocranial volume relative to skull basal length revealed no significant differences in either African lions or cougars ($F(1,13) = 0.162$, $p = 0.69$ and $F(1,13) = 0.006$, $p = 0.94$, respectively).

Regional Brain Volume Differences

Sex differences in regional brain volumes were examined in two ways: proportional brain volume differences were analyzed using ANOVA and by using t tests on residual values obtained by regressing regional brain volumes as a function of total endocranial volume, regional neocortical surface area as a function of total neocortical surface area, and regional subcortical volumes as a function of regional volume. Analysis of the proportional regional brain volumes revealed significant differences between male and female African lions in AC ($F(1,13) = 16.39$, $p = 0.002$) and PC ($F(1,13) = 8.83$, $p = 0.012$), such that females have a greater amount of AC

and males have a greater amount of PC (fig. 7). No significant difference was present for the relative amount of CB+BS ($F(1,13) = 0.35, p = 0.56$). In contrast, no significant differences in either AC or PC were found between female and male cougars ($F(1,13) = 0.90, p = 0.36$ and $F(1,13) = 3.75, p = 0.08$, respectively). However, CB+BS volumes significantly differed such that male cougars had a greater relative amount of CB+BS than female cougars ($F(1,13) = 8.85, p = 0.012$) (fig. 7).

Analysis of residuals of regional brain volumes as a function of total endocranial volume revealed results similar to those reported above. Significant differences between male and female African lions in AC ($t(12) = 2.94, p = 0.012$) and PC ($t(12) = 2.31, p = 0.03$) volumes were found, but the relative amount of CB+BS volumes did not significantly differ between the sexes ($t(12) = 0.81, p = 0.44$). In cougars, there were no significant differences in AC or PC relative volumes between females and males ($t(12) = 1.14, p = 0.28$ and $t(12) = 1.63, p = 0.13$, respectively). However, male cougars have significantly more relative CB+BS volume than female cougars ($t(12) = 2.47, p = 0.03$).

Surface Area and Subcortical Volume Differences

Analysis of regional neocortical surface areas as a proportion of total neocortical surface area revealed that female African lions have a greater amount of AC neocortical surface area compared to males, while males have a greater amount of neocortical surface area devoted to PC ($F(1,13) = 10.02, p = 0.008$ and $F(1,13) = 10.32, p = 0.007$, respectively) (fig. 7). No significant differences were detected in regional neocortical surface areas in male compared to female cougars.

Interestingly, no sex differences were present in either the African lions or cougars when comparing the regional AC subcortical volumes as relative to total regional AC volume ($F(1,13)$

= 2.40, $p = 0.15$ and $F(1,13) = 0.096$, $p = 0.76$, respectively) (fig. 7). Similarly, no sex differences were present in either species when comparing the regional PC subcortical volume relative to total PC volume ($F(1,13) = 0.16$, $p = 0.70$ and $F(1,13) = 0.39$, $p = 0.56$, respectively). These findings suggest that the difference in regional volumes for AC and PC is not due to differences in subcortical volumes.

Additionally, independent t tests comparing residual differences between the sexes in regional neocortical surface area as a function of total neocortical surface area and regional subcortical volumes as a function of total regional volume produced similar results to those reported above using ANOVA. Among lions, females possess greater AC neocortical surface area than that found in males, while males have a greater PC neocortical surface area than that found in females ($t(12) = 4.06$, $p = 0.002$ and $t(12) = 3.78$, $p = 0.003$, respectively). No significant differences were detected in regional surface areas in the cougars. Finally, comparison of the residual differences of AC subcortical volumes as a function of total AC volume ($t(12) = 0.26$, $p = 0.80$ and $t(12) = 0.08$, $p = 0.94$, respectively) or of PC subcortical volumes as a function of total PC volumes ($t(12) = 1.05$, $p = 0.31$ and $t(12) = 0.66$, $p = 0.52$, respectively) did not result in a sex difference among either lions or cougars.

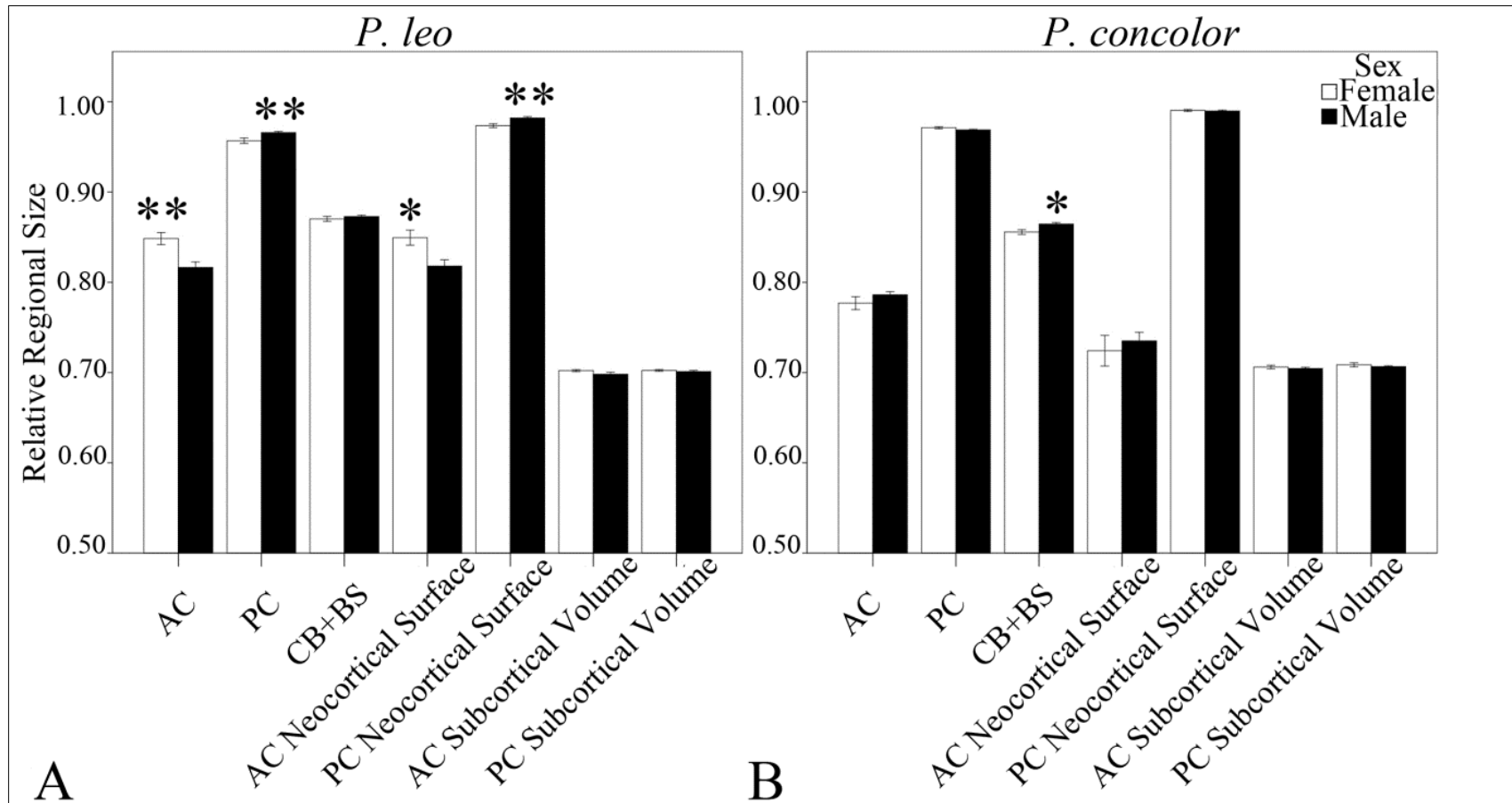


Figure 7. A, B Proportional regional brain volumes: (AC, PC, and CB+BS, all relative to total endocranial volume, regional surface areas: AC and PC, all relative to total surface area dorsal to rhinal fissure, and regional subcortical volumes, AC and PC, all relative to regional total volume for African lion (A) males (solid bars) and females (open bars) and cougar (B) males (solid bars) and females (open bars). Error bars indicate ± 1 SEM. AC volume and AC surface area are significantly larger in female than male African lions ($p = 0.002$ and $p = 0.008$, respectively). PC volume and PC surface area are significantly larger in male than female African lions ($p = 0.012$ and $p = 0.007$, respectively). * $p < 0.05$, ** $p < 0.01$.

Discussion

The present study utilized CT imaging techniques to create virtual 3-dimensional brain endocasts in two extant species from the family Felidae, the African lion and cougar. Previously, this nondestructive imaging technique has been shown to be useful in creating virtual endocasts in species where preservation of the brain is difficult or impossible, but skull specimens are readily available [Arsznov *et al.*, 2010; Sakai *et al.*, 2011a; Sakai *et al.*, 2011b]. The virtual endocasts show the external morphology of the African lion and cougar brain (fig. 2). We found no sex difference in brain size relative to skull basal length in either African lions or cougars. No sex differences in the relative amounts of anterior cerebrum volume, anterior cerebrum surface area, or anterior subcortical volume were found in cougars where both males and females are primarily solitary. However, we found female African lions possess significantly greater anterior cerebrum volume, including anterior cerebrum surface area, than that found in male African lions. Interestingly, no sex difference was found in the relative amount of anterior cerebrum subcortical volume in lions. Collectively, these findings suggest that the observed sex differences may be due to differences in frontal cortex and thus may be concomitant with differences in the social life histories of African lions. These findings lend support to previous findings that differences in social behaviors may correlate to dimorphisms in the amount of neural tissue devoted to the mediation of social behaviors [Arsznov *et al.*, 2010; Sakai *et al.*, 2011a].

Endocranial Measurements

Virtual endocasts have previously been used to examine sex differences in overall and regional brain volumes in carnivores [Arsznov *et al.*, 2010]. These analyses are greatly informative regarding both encephalization, the relative increase in brain size as a whole, and the ‘principle of proper mass’: the relative importance of a function to a species is related to the

amount of neural tissue devoted to that function [Jerison, 1973]. However, in many mammalian species, encephalization is accompanied by an increase in the relative amount of neocortex, known as neocorticalization [Jerison, 2007]. Furthermore, in mammals, as brain size increases, the neocortex increases in surface area and displays a greater degree of neocortical gyrification, a marked increase in gyral and sulcal convolutions [Welker, 1990]. Although our methodology using CT analysis of skulls prevents detailed analysis of brain structures, we address the question of whether differences may be attributed to neocorticalization by including two additional measures: relative neocortical surface area and relative subcortical volume. Here, neocortical surface area was an indicator of neocorticalization based on surface area measures dorsal to the rhinal fissure, a landmark readily identifiable on the virtual endocasts. These measurements allow us to assess whether observed sex differences in the regional brain endocasts are differentially related to neocortex or subcortical structures.

As with any derived measures, these additional measurements are not without their caveats. First, sulci as seen on the virtual endocast do not extend to the full cortical depth as observed in whole brain specimens. Thus, it does not reflect the total neocortical folding that is present in a whole brain specimen. Nevertheless, this measure provides an approximation of neocortical surface area from a virtual endocast when a whole brain specimen is difficult to obtain. Finally, our measure of subcortical volume does not provide information regarding the volume of particular subcortical structures. Instead, it is an approximation of the cerebral hemispheric volume excluding the outer 3 mm dorsal to the rhinal fissure. Despite these drawbacks, these indirect estimates of neocortical surface area and subcortical volume provide important information in making intra- and interspecies brain comparisons using the CT endocast methodology. Endocranial volumes obtained from our analyses of the CT data provide estimates

of brain volume. Since endocranial volume includes meninges, vasculature, cerebrospinal fluid and cranial nerves as well as the brain, this volume overestimates total brain volume.

Comparisons of endocranial and brain volumes in humans using CT have reported an average difference of 0.87% with increasing differences as a function of age [Ricard *et al.*, 2010]. In an analysis of 82 bird species, brain mass and endocranial volume did not significantly differ [Iwaniuk and Nelson, 2002]. However, similar studies in carnivores are lacking. Here, MRI analysis revealed endocranial volume exceeds brain volume by 3.65% in a live African lion. While this difference is relatively small in view of the estimated brain shrinkage of 32–58% based on histological tissue section analysis [Stephan *et al.*, 1981; Bush and Allman, 2004], this difference suggests caution in interpreting these volumes as brain measures. At the same time, we suggest that endocranial volume serves as useful and robust estimate of brain volume in comparative analyses.

Comparison of Endocranial Volumes in Males and

Females

Cougars

The cougar is primarily solitary, with the exception of mating and periods of juvenile dependence as typical of members of Felidae [Kleiman and Eisenberg, 1973; Ewer, 1976; Sunquist and Sunquist, 2002]. The absence of major differences in the social behavioral repertoires of male and female cougars may suggest that the cognitive demands also do not differ between the sexes. Indeed, the present study found no sex difference in overall endocranial volume relative to skull length in cougars. Furthermore, no sex differences were present in relative anterior cerebrum volume, relative posterior cerebrum volume, relative anterior cerebrum surface area, relative posterior cerebrum surface area, relative anterior subcortical

volume, or relative posterior subcortical volume. However, the relative cerebellum plus brain stem volume was significantly greater in male than female cougars. The cerebellum plays an important role in voluntary movement, gait, posture, and motor functions [Ghez and Fahn, 1985]. Cougars display incredible balance and agility, enabling them to effortlessly navigate a variety of difficult mountainous terrains [Busch, 1996]. Since the size of the cerebellum is typically conserved [Finlay and Darlington, 1995], our finding that male cougars possess a greater proportion of cerebellum plus brain stem even after controlling for skull basal length than female cougars is surprising. In primates, cerebellum size has been shown to vary independent of brain size, and species differences in relative cerebellum volume have been correlated with locomotion versatility [Rilling and Insel, 1998]. On average, an adult male cougar is 1.4 times larger in body mass than an adult female. The larger stature and more muscular build of males might require a greater degree of motor coordination than females, particularly in negotiating challenging physical environments. Additionally, male cougars occupy territories up to three times that of females [Logan and Sweanor, 2001]. Thus, it might be that male cougars require greater motor coordination and agility due to difficult physical environments such mountainous terrain while navigating a larger home range than their smaller female counterpart.

African Lions

Comparison of total endocranial volumes relative to skull basal length in female and male African lions revealed no significant differences. These findings are similar to our previous ones in the spotted hyena (*Crocuta crocuta*); total endocranial volume relative to body size did not differ between the sexes [Arsznov *et al.*, 2010]. In humans, males are reported to have a larger brain size compared with females [Rodrigues, 1991; Pakkenberg and Gundersen, 1997; Nopoulos *et al.*, 2000; Allen *et al.*, 2002; Leonard *et al.*, 2008]. However, this sex difference in

brain size is small when body size is controlled [Breedlove, 1994; Ellis *et al.*, 2008]. At the same time, sex differences in human social cognitive skills have been linked to observed sexual dimorphisms in brain regions known to mediate social behavior. Females possess proportionately greater orbital frontal cortex [Gur *et al.*, 2002] and ventral frontal cortex [Wood *et al.*, 2008] than males. These results are equivocal since other studies conclude that the ratio of frontal lobe volume to total intracranial volume in humans does not differ between the sexes [Allen *et al.*, 2002; DeCarli *et al.*, 2005; Ellis *et al.*, 2008].

Notably, the present study found that the relative volume and surface area of anterior cerebrum are significantly larger in female than male African lions. Moreover, there was no sex difference in the amount of relative anterior subcortical volume. Thus, our data suggest that frontal cortex may be significantly greater in female than male African lions. A potential explanation for this finding is that sexual differences exist in the neural processing associated with different underlying cognitive demands. This explanation supports the ‘principle of proper mass’: the amount of neural tissue devoted to a function is related to the relative importance of that function [Jerison, 1973]. Therefore, an expansion in a particular brain is indicative of greater behavioral capacity associated with that brain region. The frontal cortex is associated with the mediation of complex social behaviors in humans and other mammals [Adolphs, 2001; Amodio and Frith, 2006], and its relative enlargement may be related to an increase in the cognitive demands of social information processing that differ between the sexes.

Additionally, frontal cortex is related to the inhibition of inappropriate behavior in monkeys [Mishkin, 1964; Iversen and Mishkin, 1970; Fuster, 2002]. Moreover, in humans, impulsive aggression, as evidenced by acts of violence, is associated with reduced frontal lobe functioning [Bufkin and Luttrell, 2005]. Patients suffering damage to the ventral prefrontal

cortex show inappropriate social responses and disinhibition in addition to other deficits [Adolphs, 2001]. It is intriguing to speculate that the frontal cortex in the African lion may play a role in the mediation of appropriate social behavior. Indeed, male African lions are not only dominant to females, but are also much more aggressive than females. During the inception of a coalition's reign, subadult males and those females that have not yet reached sexual maturity will disperse or be killed by the immigrant males [Hanby and Bygott, 1987]. In addition to aggression towards subadult males and females, male lions are highly aggressive and have been observed using lethal aggression towards adult females [Mosser and Packer, 2009]. Conversely, females are philopatric and are typically recruited into the maternal pride [Pusey and Packer, 1987]. Female lions achieve many benefits from group living; they are an interesting example of social structure in carnivores, in that they are egalitarian and lack a formal dominance hierarchy [Packer *et al.*, 2001]. Female lions form symmetrical relationships and have a communal cub rearing system with multiple reproducing females [Packer *et al.*, 2001]. This relationship provides protection for their young against attacks from outside males [Packer *et al.*, 1990]. Thus, it seems plausible that the greater expanse of frontal cortex in female lions may be related to the mediation of appropriate social behaviors in the presence of a dominant male aggressor and not due to social information processing related to immigrating behaviors or navigation of a social hierarchy. While the precise roles of the frontal cortex in the mediation of African lion behavior are, of course, unknown, behavioral studies report previously learned inhibitory responses are disinhibited following lesions of the prefrontal cortex in dogs [Brutkowski and Davrowska, 1963; Brutkowski, 1965] and cats [Warren *et al.*, 1969]. Thus, it is tempting to hypothesize that the larger anterior cerebrum volume and surface area found in female lions

reflect their unique social conditions, and resulting need for greater inhibitory control, as they cope with selection pressures imposed by the socially dominant and more aggressive males.

We found that the relative volume of the posterior cerebrum is significantly greater in male than female African lions. The posterior cerebrum volume was delineated in such a manner to include the cortex posterior to the cruciate sulcus and the underlying subcortical regions. However, we also found that the relative amount of posterior neocortical surface area is larger in male than female African lions, while there is no sex difference in the relative amount of posterior subcortical volume. These findings suggest that the observed sex difference in posterior cerebrum in African lions is due to greater posterior neocortex in males than females. Posterior cortex including the posterior parietal area has been implicated in visuospatial processing [Lomber *et al.*, 1996]. It is intriguing to speculate that since male lions occupy and defend larger home range and territory than female lions [Pusey and Packer, 1987], visuospatial demands may be greater in males than female lions. At the same time, the larger posterior cortex may simply be a consequence of smaller frontal cortex found in males.

The African lion provides a unique model where the cognitive demands of life in the social pride appear to differ between the sexes. The sex difference in anterior cerebrum found in the African lion lends support to our previous finding in the spotted hyena [Arsznov *et al.*, 2010]. The data suggests that sex differences in social cognitive demands, marked by the presence of a dominant aggressor, seem to be related to differences in brain morphology, specifically frontal cortex. Additionally, the absence of a sex difference in anterior cerebrum volumes in the cougar, where both males and females are solitary, suggests that this volume difference is related to the degree of sociality and not the sex of the animal. These data provide support for the comparative neurological principle that behavioral specializations, e.g. inhibitory control in the presence of a

dominant aggressor, correspond to an expansion of the neural tissue mediating that function, e.g. frontal cortex. Whether other carnivore species in which sex differences in social behavior exist also possess similar sexual dimorphisms in brain morphology awaits further study.

CHAPTER 2: The Procyonid Social Club: Comparison of brain volumes in the coatimundi (*Nasua nasua*, *N. narica*), kinkajou (*Potos flavus*), and raccoon (*Procyon lotor*)

Introduction

One principle of comparative neurology is that behavioral specialization corresponds to an increase in neural processing and a concomitant expansion of the neural tissue devoted to that behavior [Jerison, 1973]. Species that exhibit specialized complex behaviors require a greater amount of neural tissue devoted to such processing (and perhaps larger brains) than that found in related species that do not exhibit complex behavioral specializations [Jerison, 1973; Eisenberg, 1981]. Relative increases in regional brain size have been associated with increases in sensory specializations [Welker and Campos, 1963; Radinsky, 1969; Joffe and Dunbar, 1997; Barton 1998], motor functions [Whishaw *et al.*, 1998; Changizi, 2003] and cognitive ability [Reader and Laland, 2002; Byrne and Corp, 2004; Dunbar and Shultz, 2007]. This positive correlation between behavioral specializations and regional brain size has provided a framework for examining a number of potential factors to explain relatively large brains, independent of body size, observed in a broad range of mammalian taxa including primates [Jolly 1969; Humphrey 1976; Byrne and Whiten 1988; Dunbar, 1992], bats [Baron *et al.*, 1996], carnivores [Finarelli, 2006; Finarelli and Flynn, 2009], cetaceans [Marino, 1996], ungulates [Perez-Barberia *et al.*, 2007] and some insectivores [Dunbar and Bever, 1998].

Two prominent factors hypothesized to be related to an increase in brain size are social complexity [Humphrey, 1976; Dunbar and Shultz, 2007] and environmental complexity [Parker and Gibson, 1977; Clutton-Brock and Harvey, 1980]. The former, commonly known as the ‘social brain’ hypothesis, posits that increased complexity in the intraspecific social environment acts as the primary selection pressure for a relative increase in brain size [Byrne and Whiten,

1988; Dunbar, 1992; Dunbar, 1998]. Therefore, living in a complex social environment requires a specialized behavioral repertoire that is associated with an increase in neural processing and an expansion of the brain areas that mediate those behaviors. Among primates, including humans, increased sociality is related to an increase in brain size marked by an expansion of frontal cortex, a brain region known to be involved in the executive control of cognition and regulation of social behaviors [Adolphs, 2001; Amodio and Firth, 2006; Powell *et al.*, 2010]. In addition to these selection pressures, selection pressures resulting from increased complexity in the physical environment have also been suggested to influence large brain size. Factors including arboreality [Lemen, 1980; Eisenberg and Wilson, 1981], habitat complexity [Budeau and Verts, 1986; Shultz & Dunbar, 2006], and complex foraging strategies [Parker and Gibson, 1977; Clutton-Brock and Harvey, 1980; Dechmann and Safi, 2009], all have been shown to positively correlate with brain size.

Studies examining the relationship between mammalian brain size and social or environmental complexity have largely focused on primate species [Byrne, 1994]. Carnivore species have been largely understudied in this context. This may be partially due to the lack of variation within a carnivore family. For example, members of the Felidae family are primarily solitary while most members of the Canid family are social [Gittleman, 1986]. However, one carnivore family, Procyonidae, offers a model family in which to examine variations in regional brain size as a function of species-specific behaviors. Procyonidae exhibit a continuum of social behavior: from the mostly solitary raccoons [Kaufmann, 1982]; kinkajous live either alone or in small polyandrous family groups [Kays and Gittleman, 2001]; and the social coatimundis live in gregarious bands of up to 40 individuals [Gompper, 1996; Romero and Aureli, 2007]. Additionally, these procyonids occupy varied habitats ranging from the exclusively arboreal

kinkajou [Kays and Gittleman, 1995]; the semi-arboreal raccoon [Hauver *et al.*, 2010]; and the mostly terrestrial coatimundi [Hirsch, 2011]. Finally, the raccoon is noted for its dexterous forepaw use and enhanced representation of the forepaw in the somatosensory cortex relative to other procyonids [Welker and Campos, 1963]. McClearn [1992] described that kinkajou possess greater grasping ability compared to other procyonid species and suggested that it might be an adaptation related to their arboreal exclusivity. However, Welker and Campos [1963] also described that the kinkajou displays enhanced grasping ability, but lacks the enhanced forepaw representation within somatosensory cortex. Thus, here it is important to distinguish that forepaw dexterity (i.e. manipulative ability) is distinct from grasping ability (i.e. convergence of digits toward the center of the palm). The present study examined these three closely related species in order to determine whether increased regional brain size is associated to species-specific behavioral specializations related to social (e.g. sociality) or environmental (e.g. habitat) complexity. Three predictions regarding species differences in regional brain volume were examined: 1) if the demands from living in a complex social environment underlie the expansion in frontal cortex, then the social coatimundi faced, with greater cognitive demands related to processing social information, would possess proportionately more anterior cerebrum (the region of the virtual endocast that contains frontal cortex) than that found in either raccoons or kinkajous, 2) if the demands from living in a complex three dimensional physical environment corresponds to regional brain volume changes, then the exclusively arboreal kinkajou would possess proportionately greater cerebellum + brain stem volume than that found in the raccoon or coatimundi 3) if the behavioral specialization of forelimb dexterity is related to an expanse of somatosensory cortex, then the highly dexterous raccoon would processes proportionately more posterior cerebrum (the region of the virtual endocast that contains the somatosensory cortex)

than that found in either the coatimundi and kinkajou. Lastly, in addition to intrafamily level comparisons of species-specific behaviors and regional brain size, differences in anterior cerebrum volume have been related to differing social life histories between the sexes [Arsznov *et al.*, 2010; Arsznov and Sakai, 2012]. This sex difference is hypothesized to be related to the differing social cognitive demands, experienced by the sexes [Arsznov *et al.*, 2010; Arsznov and Sakai, 2012]. Interestingly, a divergent social life history pattern exists between male and female coatimundi [Gompper, 1996]. Given the presence of sexually dimorphic social life histories experienced by coatimundi, we examined if the relative amount of frontal cortex in the coatimundi differed in males and females. We predicted that female coatimundi would possess proportionally more anterior cerebrum (a measure of frontal cortex) due to social life experiences than that found in male coatis. Here we used computed tomography (CT) to create virtual endocasts from raccoon, coatimundi, and kinkajou skulls. The virtual endocasts are based on serial analysis of coronal images and yield quantitative regional and total brain volume data that were used to examine both inter- and intraspecific variations.

Methods

Specimens

A total of 45 adult skulls including: 17 coatimundi (9 male, 8 female), 14 raccoon (7 male, 7 female), and 14 kinkajou (7 male, 7 female) were obtained from the collections of the Michigan State University Museum, University of Michigan Museum of Zoology and National Museum of Natural History, Smithsonian Institution (see Appendix 2).

Digital Measurements

Each skull was positioned along an anterior-posterior alignment and then scanned using either a General Electric Lightspeed 4 slice scanner or a General Electric Discovery ST 16 slice

scanner at the Department of Radiology at Michigan State University. Each scan was performed using the following parameters: a slice thickness of 0.625mm, a table speed of 5.62mm/rotation, a pitch of 0.562:1 and a 30cm field of view. CT data were saved in the Digital Imaging and Communications in Medicine (DICOM) Centricity Version 2.2 format and the virtual endocasts were created using MIMICS 13.1 software (Materialise, Inc., Ann Arbor, MI, USA). Three-dimensional virtual endocasts were created for each scanned skull using the procedures previously described in detail in Sakai *et al*, [2011]. Briefly, isolation of the cranial bone from surrounding air space was based on a defined pixel value of -1024 Hounsfield units (HU). The endocranial cavity was selected for and filled along the coronal plane beginning rostrally at the cribriform plate and extending caudally through the foramen magnum. Selected sections were then stacked to create a three-dimensional reconstruction of the endocranial cavity (virtual endocast) using the MIMICS 3D operation. These virtual endocasts provide clearly identifiable external brain morphology, including detailed gyral and sulcal patterns (fig. 8).

Skull basal length, defined as the distance from the anterior border of the median incisive alveolus to the mid-ventral border of the foramen magnum, and basicranial axis length, defined as the distance from the foramen magnum to the basisphenoid-presphenoid suture, were collected in each specimen as a proxy for body size (fig. 9). These two skull measures are strongly correlated with body weight [Radinsky, 1984] and are used here as reasonable proxies for measures of body size when alternative measures (e.g. individual body weight, postcranial dimensions) are not available. Linear skull measurements taken from the CT images were collected by a single observer (B.M.A.).

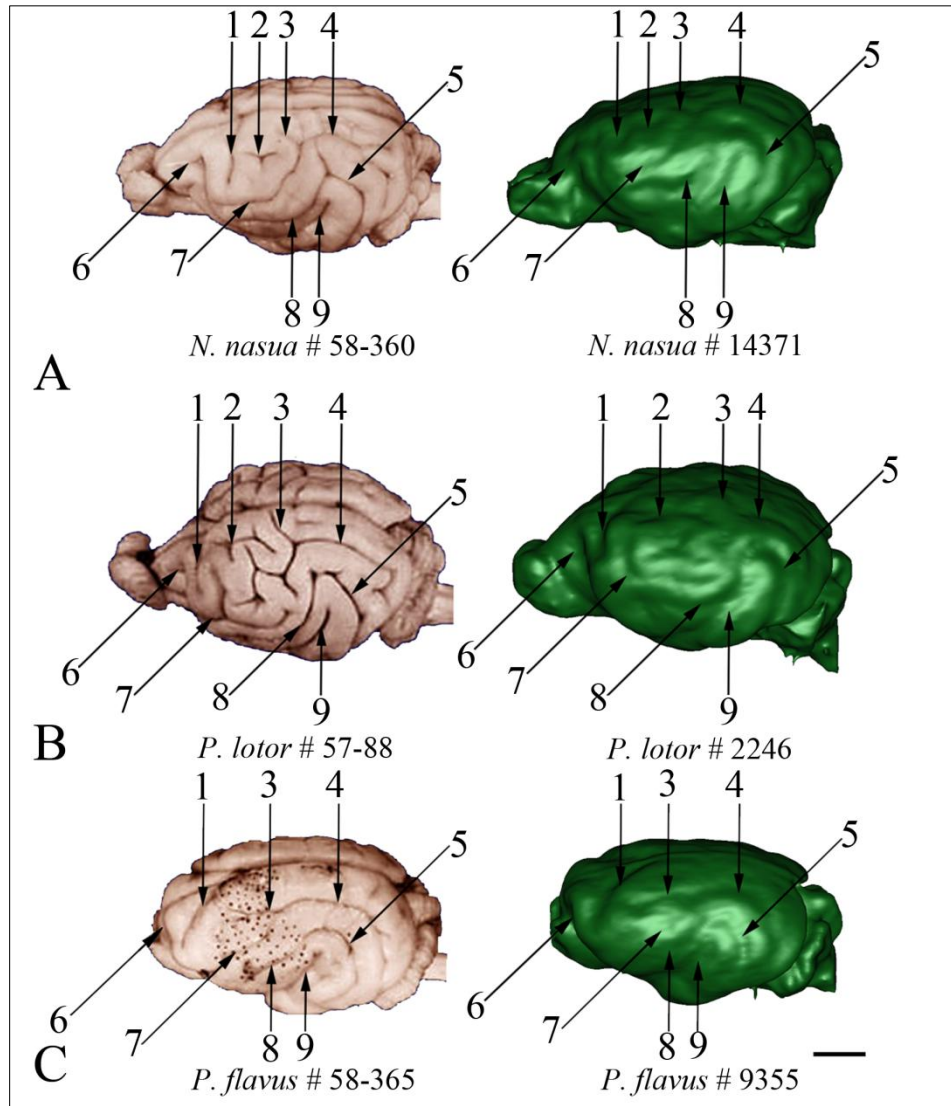


Figure 8. Photographs of the whole brain of **A** coatimundi (*N. nasua*, No. 58-360), **B** raccoon (*P. lotor*, No. 57-88), and **C** kinkajou (*P. flavus*, No. 58-365) from the Comparative Mammalian Brain Collection and three-dimensional virtual endocast reconstructed from a coatimundi skull (Michigan State University specimen No. 14371), raccoon (Michigan State University specimen No. 2246), and kinkajou (Michigan State University specimen No. 9355). Major sulci and other anatomical features in both the whole brain and virtual endocasts are shown. (1) cruciate sulcus, (2) postcruciate sulcus, (3) medial ansate sulcus, (4) lateral sulcus, (5) posterior suprasylvian sulcus; (6) proreal sulcus; (7) coronal sulcus; (8) anterior suprasylvian sulcus; (9) sylvian sulcus. Scale bar: 1 cm.

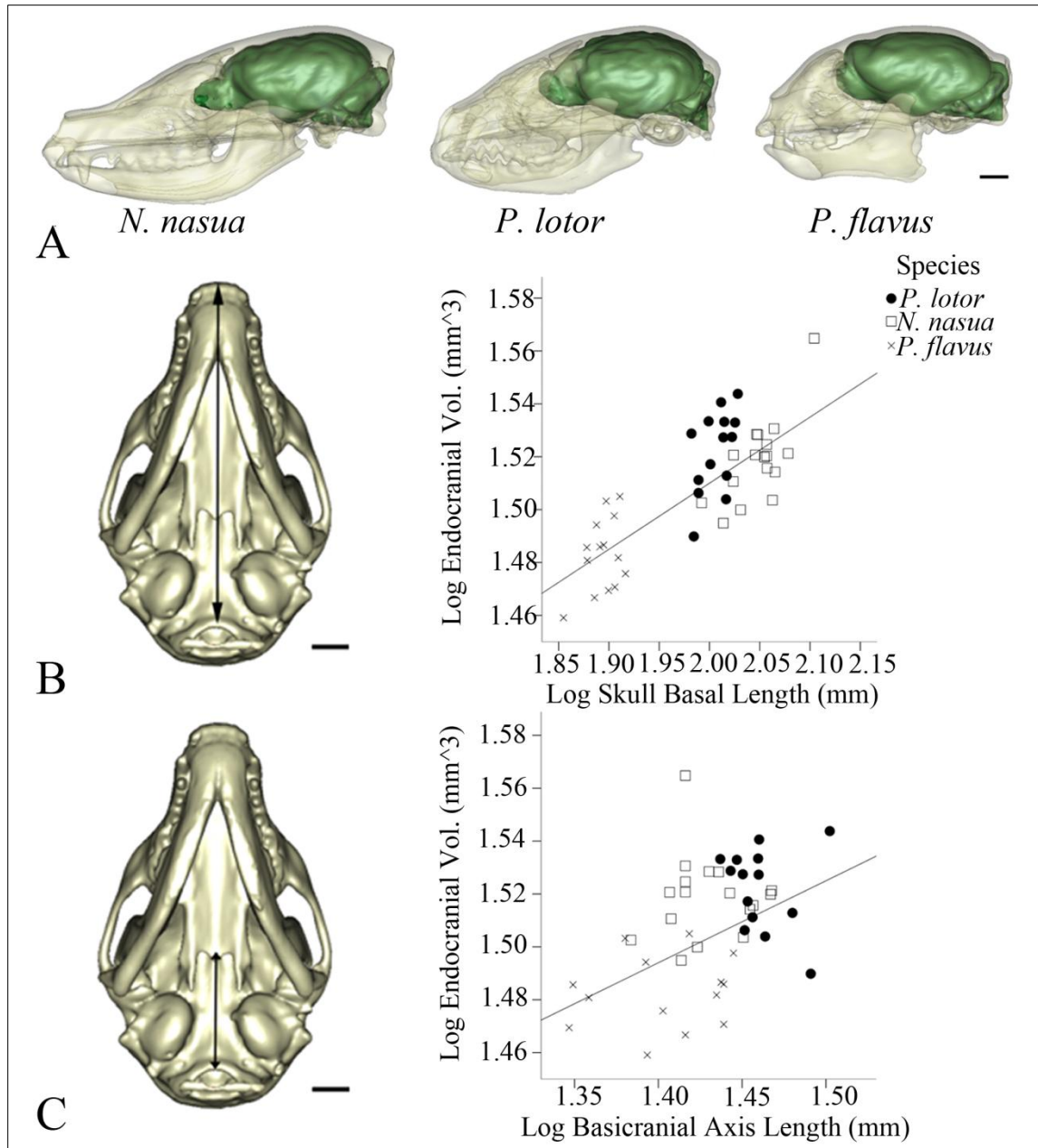


Figure 9. **A** Lateral in situ views of virtual endocrasts and skulls for coatiundi (Michigan State University specimen No. 14371), raccoon (Michigan State University specimen No.2246), and kinkajou (Michigan State University specimen No. 9355) (left to right). **B** Ventral view of a raccoon skull (shown in **A**) with arrow showing skull basal length, defined as the distance from the anterior border of the median incisive alveolus to the mid-ventral border of the foramen magnum and graph showing log cube root of endocranial volume regressed against log skull basal length for raccoons (solid circles), coatiundi (open squares), and kinkajou (x). Pearson's $r = 0.78$, $p < 0.01$. **C** Ventral view of a raccoon skull (shown in **A** and **B**) with arrow showing basicranial axis length, defined as the midventral border of the foramen magnum to the basisphenoid-presphenoid suture and graph showing log cube root of endocranial volume regressed against log basicranial axis length for procyonids (legend same as in **B**). Pearson's $r = 0.45$, $p = 0.002$. Scale bar: 1 cm.

Validation of Virtual Endocasts

Whole Endocasts

The external brain morphology, including the gyral and sulcal patterns, identifiable on the virtual endocasts were directly compared to whole brain photographs of standard anatomical orientations, including dorsal, ventral, left lateral and right lateral views, of a formalin-fixed raccoon brain (specimen No. 57-88), coatimundi (specimen No. 58-360), and kinkajou (specimen No. 58-365) from the Comparative Mammalian Brain Collection (www.brainmuseum.org) (fig. 8). All volumetric data were collected by a single observer (B.M.A.) and were obtained using the MIMICS 3D volume measurement operation. CT files were coded by animal number only, and the analysis and demarcation of brain regions was conducted blind with regard to the sex of the specimen.

Delineation of Brain Regions in Virtual Endocasts

Using procedures described in Arsznov *et al.* [2010], the endocranium was divided into 3 regions of interest: anterior cerebrum, posterior cerebrum, and cerebellum/brain stem, using gyral and sulcal patterns as well as bony landmarks as a guide with the exception of the delineation of the anterior cerebrum in the kinkajou (described below (fig. 10). A brief description of the criteria employed follows.

1. Anterior Cerebrum Volume (AC)

In primates, frontal cortex is defined as the region of cortex rostral to the central sulcus. The central sulcus is not present in carnivores; however, its analogue is identifiable as the postcruciate dimple or sulcus, which delimits the boundary between motor and somatosensory cortex in some carnivores [Hardin *et al.*, 1968; Gorska, 1974]. Cortical maps of somatosensory cortex in the raccoon [Welker and Seidenstein, 1959], coatimundi [Welker and Campos, 1963],

and kinkajou [Brodmann, 1909; Welker and Campos, 1963], showed that the motor and somatosensory transitional cortical boundary occurs along the postcruciate dimple or sulcus. The presence of a postcruciate dimple or sulcus is highly variable even between hemispheres in the same specimen [Hassler and Muhs-Clement, 1964; Kawamura, 1971]. Instead, the cruciate sulcus is a readily identifiable landmark that exhibits less intra- and interspecific variation compared to the postcruciate dimple or sulcus [Radinsky, 1969; Myasnikov *et al.*, 1997]. The cruciate sulcus is identified as being coincident with the rostral most portion of the motor cortex (cytoarchitectonic areas 4 and 6) in the cat [Hassler and Muhs-Clement, 1964], dog [Gorska, 1974; Stanton *et al.*, 1986; Tanaka, 1987; Sakai *et al.*, 1993], raccoon [Sakai, 1982; Sakai, 1990], spotted hyena [Arsznov *et al.*, 2010] and lion [Arsznov and Sakai, 2012]. The cruciate sulcus is used here as the boundary for separating anterior and posterior cerebrum.

Anterior cerebral volume was defined as the area rostral to the cruciate sulcus and caudal to the olfactory bulbs. Anterior cerebrum volume here is thus comprised of the frontal cortex and subcortical structures, including a small portion of the rostral most head of the caudate nucleus, ventral pallidum, olfactory tubercle and prepiriform cortex (fig. 10).

2. Posterior Cerebrum Volume (PC)

The endocranial volume posterior to the cruciate sulcus, but anterior to the tentorium cerebelli, is here referred to as the posterior cerebrum (fig. 10). This region included all cortex posterior to the cruciate sulcus as well as underlying diencephalic and rostral mesencephalic structures.

3. Cerebellum + Brain Stem Volume (CB+BS)

The cerebellum and brain stem are housed within the posterior cranial fossa. This region was defined on the CT images of the skull as the area between the foramen magnum and the

tentorium cerebelli, which covers the superior surface of the cerebellum (fig. 10). Thus, the volume measurement for cerebellum/brain stem included cerebellum, medulla, pons, and brain stem as far caudal as its junction with the spinal cord.

Whole and Regional Surface Areas

Since the endocast is based on the surface impression of the endocranium, it is limited in providing information regarding detailed brain structure. However, the endocranial surface area dorsal to the rhinal fissure has been used as an indicator of neocortical area [Jerison, 2007]. Here, whole and AC and PC regional surface areas were obtained using the procedures described in Arsznov and Sakai [2012]. Briefly, the rhinal fissure was located on each endocast and a 1-pixel deep mask was created that included only the endocast surface area dorsal to the rhinal fissure. Regional surface areas were obtained by delineating the whole surface area mask into AC neocortical surface area and PC neocortical surface area based on boundaries determined for regional AC and PC volumes (fig. 10).

Subcortical regional volumes.

In order to determine if total or AC and PC regional brain volume might be due to relative enlargement of the subcortical areas, we estimated neocortical depth in cortex dorsal to the rhinal fissure and calculated subcortical volumes regionally excluding the outermost 3 mm of the endocranium dorsal to the rhinal fissure (fig. 10). These procedures described in Arsznov and Sakai [2012]. First, the surface area mask was dilated to a 3mm deep mask using the MIMICS morphology operation. Then subcortical regional volumes were calculated by subtracting the 3mm deep mask from the corresponding AC and PC volume masks.

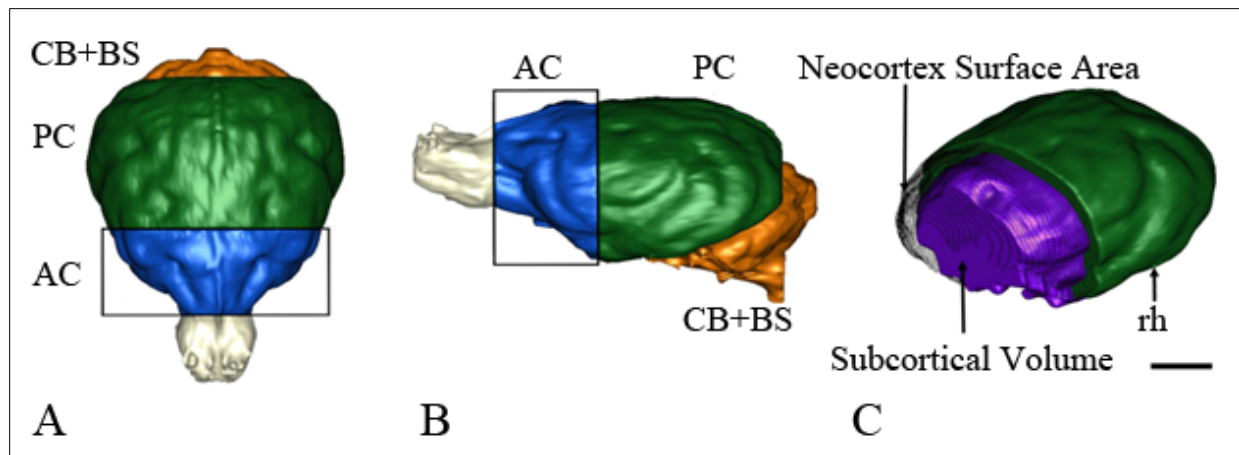


Figure 10. Isosurface rendering of a coatimundi (Michigan State University specimen No. 14371) endocranial volume in **A** dorsal, **B** lateral (left), and **C** dorsolateral views. A.-B. Anterior cerebrum (AC - blue) was defined as the region rostral to the cruciate sulcus and caudal to the olfactory bulbs (OB - yellow). The posterior cerebrum (PC - green) was defined as the endocranial volume posterior to the cruciate sulcus and anterior to the tentorium osseum. The cerebellum and brain stem (CB + BS - orange) was defined as the endocranial volume within the cerebellar fossa including the area caudal to the tentorium osseum and rostral to the foramen magnum. C. Neocortex surface area (black lines) was defined as the endocranial surface area dorsal to the rhinal fissure (rh). Subcortical volume (purple) was defined as endocranial volume excluding the outermost 3mm of the virtual endocranial volume dorsal to the rhinal fissure (green) and CB + BS. Scale bar = 1 cm.

Statistical Analyses

Statistical analyses were based on the endocranial volumes of 17 adult coatimundi (9 male, 8 female), 14 raccoon (7 male, 7 female), and 14 kinkajou (7 male, 7 female). Prior to statistical analyses: skull basal length, basicranial axis length, and endocranial volumes were log-transformed in order to improve graphical representation of the data. The allometric relationship between total endocranial volume and the two measures of body size were assessed using a bivariate correlation coefficient, Pearson's r . Two sets of statistical analyses were employed to determine both species and sex differences in endocranial or regional brain volumes. Comparisons among species were performed using an analysis of variance (ANOVA) on mean residual values from linear regressions and on regional proportions. First, mean residual values were obtained from linear regressions of log cube root of total endocranial volume plotted

against log skull basal length and log basicranial axis length. Residual values were also obtained from linear regressions of regional brain volume plotted against endocranial volume, regional surface area plotted against total neocortical surface area and regional subcortical volume plotted against regional volume. Second, relative values were obtained for the following proportions: regional brain volume relative to total endocranial volume, regional neocortical surface area relative to total neocortical surface area and regional subcortical volume relative to regional volume. Sex differences within each species were compared using independent *t* tests on mean residual values obtained from linear regressions of log cube root of total endocranial volume plotted against log skull basal length and log basicranial axis length, as well as regional brain volume plotted against endocranial volume, regional surface area plotted against total neocortical surface area and regional subcortical volume plotted against regional volume. Additionally, sex differences were compared using independent *t* tests on the proportional values of regional brain volume relative to total endocranial volume, regional neocortical surface area relative to total neocortical surface area and regional subcortical volume relative to regional volume. All statistical analyses were performed using the statistical software package IBM SPSS Statistics 19 (IBM Corporation, Armonk, New York, USA).

Results

Whole endocasts

The virtual endocast and external brain morphology is compared in the raccoon, coatimundi, and kinkajou in fig. 8. Major morphological landmarks including gyral and sulcal patterns are readily identifiable in virtual endocasts and whole brain photographs within each species. These features include preareal gyrus, cruciate sulcus, ansate sulcus, lateral sulcus and suprasylvian sulcus (fig. 8). Although a general pattern of major gyral and sulcal features is

found across species, variations in the sulcal pattern in within the pericruciate region and proreal gyrus, and the postcruciate sulcal or dimple region were observed. The pericruciate region and proreal gyrus is larger both mediolaterally and rostrocaudally in the coatimundi compared to the kinkajou and the raccoon (fig 8). Whereas the postcruciate sulcal or dimple region extending is larger in the mediolateral and rostrocaudal directions in the raccoon compared to the coatimundi and the kinkajou (fig 8).

Interspecific differences

1. Brain volume

Endocranial volume and the two measures of body size are strongly correlated in the three procyonid species (skull basal length, Pearson's $r = 0.78$, $p < 0.01$; basicranial axis length, Pearson's $r = 0.45$, $p = 0.002$). In the raccoon, coatimundi, and kinkajou, the average endocranial volume was 37019.05 mm^3 (s.d. 3864), 36242.00 mm^3 (s.d. 4242), and 28245.64 mm^3 (s.d. 2703) respectively. Endocranial volume, skull basal length, and basicranial axis length are presented in Table 2. An ANOVA of the mean residual values from separate linear regressions of endocranial volume plotted as a function of these body size measures revealed varying results (fig 9). Residual comparisons of the influence of skull basal length on endocranial volume revealed a significant difference between the species ($F(2, 42) = 4.85$, $p = 0.013$). Bonferroni post hoc analysis showed that raccoons had a relatively larger endocranial volume compared to the coatimundi ($p = .01$), but were not significantly different from the kinkajou. Furthermore, there was no significant difference between the coatimundi and kinkajou. Residual comparisons of the influence of basicranial axis length on endocranial volume resulted in significant differences between species ($F(2, 42) = 11.34$, $p < 0.001$), where the coatimundi and raccoon

had relatively larger endocranial volumes compared to the kinkajou ($p < 0.001$ and $p = 0.004$ respectively), but were not significantly different from each other ($p = 0.95$) (fig. 9)

2. Regional brain volume differences

Differences in regional brain volumes across species were examined as proportional regional brain volume and mean residual values obtained by regressing regional brain volumes as a function of total endocranial volume, regional neocortical surface area as a function of total neocortical surface area, and regional subcortical volumes as a function of regional volume. Analysis of the proportional regional brain volumes revealed significant differences between raccoons, coatimundi, and kinkajous in AC ($F(2, 42) = 92.82, p < 0.001$), PC ($F(2, 42) = 65.94, p < 0.001$), and CB+BS ($F(2, 42) = 13.34, p < 0.001$). Post hoc Bonferroni comparisons of these significant differences revealed that coatimundi have a greater amount of AC than raccoons or kinkajous ($p < 0.001$ and $p < 0.001$), while kinkajous have a greater amount of AC than raccoons ($p = 0.004$). Raccoons have a greater amount of PC than coatimundi or kinkajou ($p < 0.001$ and $p = 0.004$ respectively), and kinkajous have a greater amount of PC compared to coatimundi ($p < 0.001$). Analysis of CB+BS revealed that the kinkajou have a greater amount of CB+BS compared to raccoons and coatimundi ($p < 0.001$ and $p = 0.001$), while no significant difference was present in the relative amount of CB+BS between the raccoon and coatimundi ($p = 0.78$) (fig. 11).

Analysis of residuals of regional brain volumes as a function of total endocranial volume revealed results similar to those reported above, with exception of CB+BS. Significant differences between the coatimundi, raccoon, and kinkajou in AC ($F(2, 42) = 91.28, p < 0.001$) and PC ($F(2, 42) = 68.58, p < 0.001$) volumes were found, however the relative amount of CB+BS volumes did not significantly differ between the species ($F(2, 42) = 2.73, p = 0.077$).

3. Surface area and subcortical volume differences

Analysis of regional neocortical surface areas as a proportion of total neocortical surface area revealed a significant difference for AC ($F(2, 42) = 140.66, p < 0.001$), such that coatimundi have a greater amount of AC neocortical surface area compared to raccoons and kinkajous ($p < 0.001$ and $p < 0.001$). No significant difference was detected for relative amount of AC neocortical surface area between raccoons and kinkajous ($p = 0.07$). Significant differences for PC neocortical surface area were also found ($F(2, 42) = 149.44, p < 0.001$), where kinkajous have a greater amount of PC neocortical surface area compared to coatimundi and raccoons ($p < 0.001$ and $p = 0.050$), while raccoons have a greater amount compared to coatimundi ($p < 0.001$) (fig. 11).

Comparing residual differences in AC neocortical surface area as a function of total neocortical surface area produced similar results to those reported above using proportional data. Coatimundis have a greater amount of neocortical surface area devoted to AC than raccoons or kinkajous ($p < 0.001$ and $p < 0.001$), while no significant difference is present between raccoons and kinkajous ($p = 0.76$). Residual comparisons for PC neocortical surface area reveal that while coatimundi have relatively less PC neocortical surface area than raccoons or kinkajous ($p < 0.001$ and $p < 0.001$), there is no significant difference between raccoons and kinkajous ($p = 0.80$).

Analysis comparing the relative regional AC subcortical volumes to total regional AC volume produced a significant difference ($F(2, 42) = 94.83, p < 0.001$), such that kinkajous have a greater amount of subcortical volume than raccoons or coatimundi ($p < 0.001$ and $p < 0.001$) and there was no difference between raccoons and coatimundi ($p = 0.24$). Relative regional PC subcortical volumes to total regional PC volume a significant difference was produced ($F(2, 42)$

= 5.08, $p = 0.01$) where coatimundi have a greater amount of subcortical volume than both raccoons and kinkajous ($p = 0.047$ and $p = 0.02$). There was no significant difference in relative regional PC subcortical volume between the raccoon and kinkajou ($p = 1.00$) (fig. 11).

Mean residual analysis of values for relative AC and PC subcortical volumes as a function of regional AC and PC volumes revealed significant differences ($F(2, 42) = 42.10$, $p < 0.001$ and $F(2, 42) = 7.38$, $p = 0.002$). However, post hoc Bonferroni comparisons revealed slightly different results than above. Relative AC subcortical volume is significantly greater in the kinkajou compared to the coatimundi and raccoon ($p < 0.001$ and $p < 0.001$), and coatimundi have a greater amount of AC subcortical volume than raccoons ($p = 0.001$). Residual analysis of PC subcortical volume revealed coatimundi possess a greater amount compared raccoons ($p = 0.001$) but are not significantly different from kinkajous ($p = 0.13$). Furthermore, there is no significant difference between raccoons and kinkajous in comparisons of PC subcortical volume.

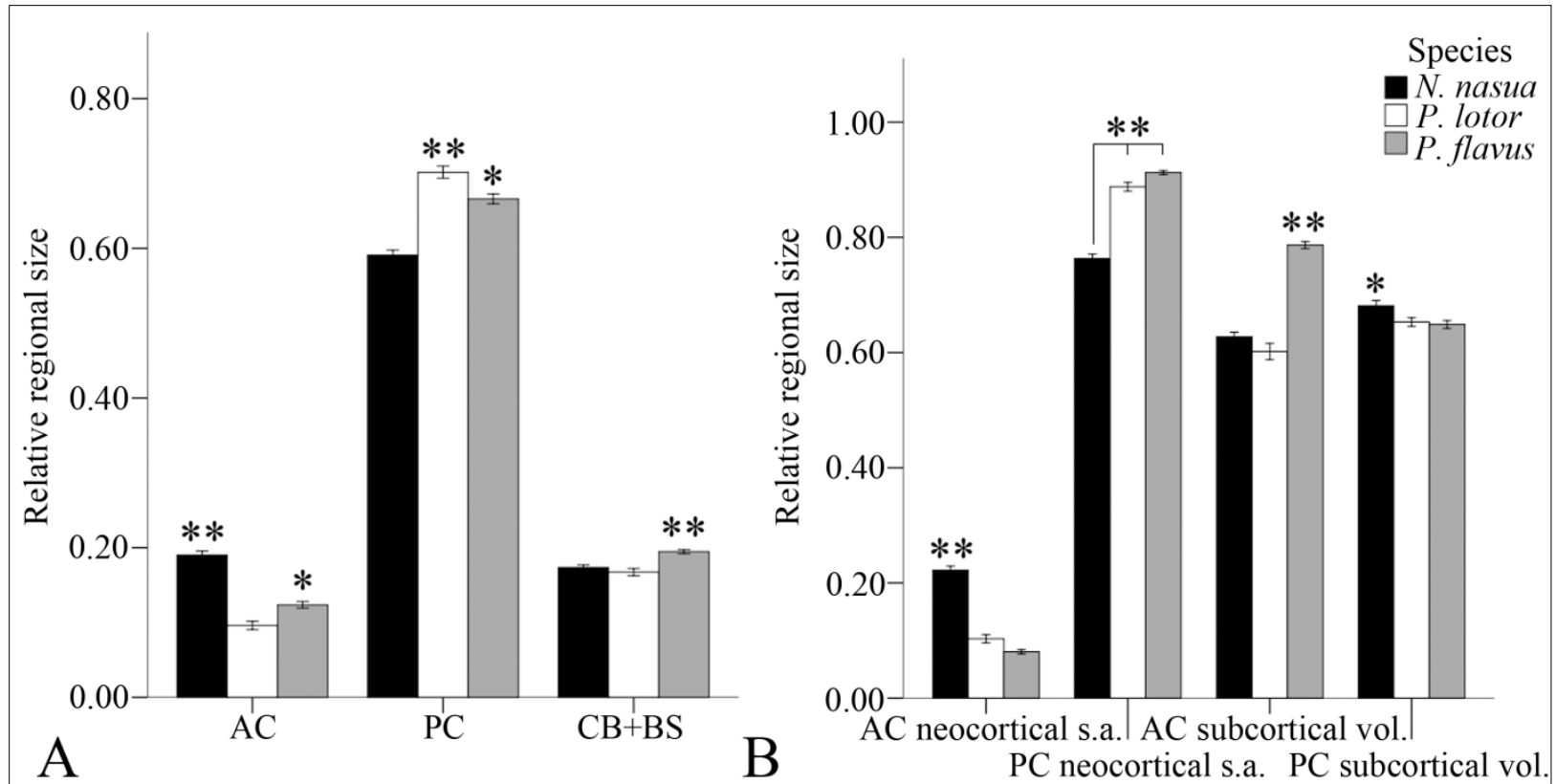


Figure 11. A Proportional regional brain volumes: AC, PC, and CB+BS, all relative to total endocranial volume. **B** Proportional regional surface areas: AC neocortical surface area and PC neocortical surface area, each relative to total surface area dorsal to rhinal fissure, and regional subcortical volumes, AC subcortical volume and PC subcortical volume, each relative to regional total volume for coatimundi (solid black bars), raccoon (open bars), and kinkajou (solid grey bars). Error bars indicate ± 1 SEM. AC volume and AC neocortical surface area are significantly larger in coatimundi than both raccoons and kinkajous ($p < 0.001$ and $p < 0.001$), while kinkajous have a significantly larger AC volume than raccoons ($p = 0.004$). Raccoons have a greater amount of PC volume than coatimundi and kinkajou ($p < 0.001$ and $p = 0.004$ respectively), while kinkajous have a greater amount of PC volume and PC neocortical surface area compared to coatimundi ($p < 0.001$) and a greater amount of PC neocortical surface than raccoons ($p = 0.050$). Additionally, kinkajous had the greatest relative CB+BS compared to both coatimundis and raccoons ($p = 0.001$ and $p < 0.001$). ** $p < 0.001$ and * $p \leq 0.05$.

Species	<i>N. nasua</i>		<i>P. flavus</i>		<i>P. lotor</i>	
Sex	♀	♂	♀	♂	♀	♂
SBL (mm)	108.89 ± 5.34	114.80 ± 6.79	77.68 ± 3.54	79.08 ± 2.17	100.36 ± 3.40	102.90 ± 3.70
BCAL (mm)	26.36 ± 1.52	27.44 ± 1.31	25.41 ± 1.74	25.41 ± 2.37	28.94 ± 0.94	28.94 ± 1.58
Endo (mm ³)	36236.33 ± 2170.91	36247.02 ± 5644.41	27179.57 ± 2783.85	29311.70 ± 2328.95	36396.87 ± 4326.88	37641 ± 3567.21
AC (mm ³)	7404.58 ± 713.00	6449.52 ± 1268.46	3247.93 ± 460.06	3734.57 ± 630.21	3800.62 ± 952.82	3305.47 ± 714.30
PC (mm ³)	21191.53 ± 1635.88	21567.76 ± 3089.72	18179.12 ± 1780.72	19448.75 ± 2086.98	25465.68 ± 3087.24	26479.74 ± 2716.39
CB + BS (mm ³)	6016.77 ± 660.65	6545.72 ± 1221.65	5343.65 ± 772.55	5664.63 ± 509.20	5821.86 ± 835.63	6578.79 ± 1065.02
AC neo.ctx (mm ²)	1268.40 ± 201.38	1227.09 ± 288.45	337.22 ± 30.48	420.44 ± 106.13	644.43 ± 209.02	560.94 ± 124.31
PC neo.ctx (mm ²)	4054.27 ± 379.84	4462.63 ± 601.01	4070.87 ± 526.42	4547.14 ± 708.82	5055.81 ± 431.64	5315.60 ± 458.73
AC sub.ctx (mm ³)	4580.52 ± 446.92	4095.43 ± 818.70	2538.43 ± 373.34	2951.58 ± 451.02	2339.50 ± 696.30	1983.51 ± 540.31
PC sub.ctx (mm ³)	14176.74 ± 1264.94	15035.58 ± 3130.37	11667.71 ± 1307.83	12817.08 ± 1995.02	16716.84 ± 2412.04	17256.86 ± 2134.06

Table 2: Coatimundi (female and male), kinkajou (female and male), and raccoon (female and male) averages ± standard deviations on measures for skull basal length (SBL), basicranial axis length (BCAL), endocranial volume (Endo), anterior cerebrum volume (AC), posterior cerebrum volume (PC), cerebellum + brain stem (CB+BS), anterior cerebrum neocortex surface area (AC neo.ctx), posterior cerebrum neocortex surface area (PC neo.ctx), anterior subcortical volume (AC sub.ctx), and posterior cerebrum subcortical volume (PC sub.ctx).

Intraspecific comparisons

1. Brain volume

The average endocranial volume in male coatimundis was $36,247\text{mm}^3$ (s.d. 5,644) and was $36,236\text{mm}^3$ (s.d. 2171) in females. In raccoons, total endocranial volume was on average $37,641\text{mm}^3$ (s.d. 3,567) in males and $36,396\text{mm}^3$ (s.d. 4,323) in females. Kinkajou males had an average total endocranial volume of $29,312\text{mm}^3$ (s.d. 2,329) and females had an average of $27,180\text{mm}^3$ (s.d. 2,784). Independent *t*-tests on the mean residual values from separate linear regressions of endocranial volume as a function of skull basal length revealed a significant difference within coatimundis ($t(15) = 2.38, p = 0.03$), where females had a relatively larger endocranial volume compared to males. No sex differences were present within raccoons or kinkajous ($t(12) = 0.31, p = 0.76$ and $t(12) = 1.37, p = 0.20$, respectively). However, independent *t*-tests on the residuals of endocranial volume as a function of basicranial axis length revealed no sex difference in coatimundis ($t(15) = 0.75, p = 0.46$), raccoons ($t(12) = 0.56, p = 0.60$), or kinkajous ($t(12) = 1.37, p = 0.20$).

2. Regional brain volume differences

Sex differences in regional brain volumes were examined in two ways: proportional regional brain volumes were compared as well as comparisons of residual values obtained by regressing regional brain volumes as a function of total endocranial volume, regional neocortical surface area as a function of total neocortical surface area, and regional subcortical volumes as a function of regional volume. In the coatimundi, an analysis of the proportional region volumes revealed significant differences between male and female coatimundi in AC ($t(15) = 3.13, p = 0.005$) and CB+BS ($t(15) = 2.29, p = 0.04$), where females have a greater amount of AC and males have a greater amount of CB+BS. No significant differences were present for the relative

amount of PC ($t(15) = 0.87, p = 0.40$). No significant sex differences in AC, PC, or CB+BS were found in raccoons (AC $t(12) = 1.52, p = 0.15$; PC $t(12) = 0.22, p = 0.83$; CB+BS $t(12) = 1.52, p = 0.16$) or kinkajous (AC $t(12) = 0.91, p = 0.38$; PC $t(12) = 0.51, p = 0.62$; CB+BS $t(12) = 0.45, p = 0.66$) (fig. 12).

Analysis of residuals of regional brain volumes as a function of total endocranial volume revealed similar results in that a significant sex difference in AC volume ($t(15) = 3.24, p = 0.005$) and CB+BS volume ($t(15) = 2.02, p = 0.06$) was found in coatimundis ($t(15) = 3.24, p = 0.005$). However, no significant sex difference was present in the relative amount of CB+BS ($t(15) = 2.02, p = 0.06$). There were no significant sex differences in AC, PC, or CB+BS in the raccoon (AC $t(12) = 1.74, p = 0.11$; PC $t(12) = 0.42, p = 0.68$; CB+BS $t(12) = 1.56, p = 0.15$) or kinkajou (AC $t(12) = 0.40, p = 0.70$; PC $t(12) = 0.02, p = 0.99$; CB+BS $t(12) = 0.13, p = 0.90$).

3. Surface area and subcortical volume differences

An analysis of regional neocortical surface areas as a proportion of total neocortical surface area revealed no sex differences in coatimundi (neocortical AC $t(15) = 1.65, p = 0.12$ and neocortical PC $t(15) = 1.70, p = 0.11$), raccoon (neocortical AC $t(12) = 1.15, p = 0.27$ and neocortical PC $t(12) = 1.18, p = 0.26$), and kinkajou (neocortical AC $t(12) = 1.10, p = 0.30$ and neocortical PC $t(12) = 1.34, p = 0.21$). Additionally, no sex differences were detected in proportional AC subcortical or PC subcortical volumes in the coatimundi (AC subcortical $t(15) = 0.93, p = 0.37$ and PC subcortical $t(15) = 1.32, p = 0.21$), raccoon (AC subcortical $t(12) = 0.48, p = 0.64$ and PC subcortical $t(12) = 0.31, p = 0.76$), or kinkajou (AC subcortical $t(12) = 0.89, p = 0.39$ and PC subcortical $t(12) = 1.15, p = 0.28$). Analysis of mean residuals from linear regressions yielded similar results where no sex differences were present in either the amount of

AC neocortical surface areas in coatimundi ($t(15) = 1.87, p = 0.08$), raccoon ($t(12) = 1.43, p = 0.18$), or kinkajou ($t(12) = 0.81, p = 0.44$) or the amount of PC neocortical surface areas in coatimundi ($t(15) = 1.92, p = 0.08$), raccoon ($t(12) = 1.45, p = 0.17$), or kinkajou ($t(12) = 0.80, p = 0.44$). Lastly, residual analysis of regional subcortical volumes also revealed no sex differences among coatimundi (AC subcortical $t(15) = 0.60, p = 0.56$; PC subcortical $t(15) = 1.50, p = 0.16$), raccoon (AC subcortical $t(12) = 0.75, p = 0.47$; PC subcortical $t(12) = 0.52, p = 0.62$), or kinkajou (AC subcortical $t(12) = 2.17, p = 0.05$; PC subcortical $t(12) = 0.90, p = 0.39$).

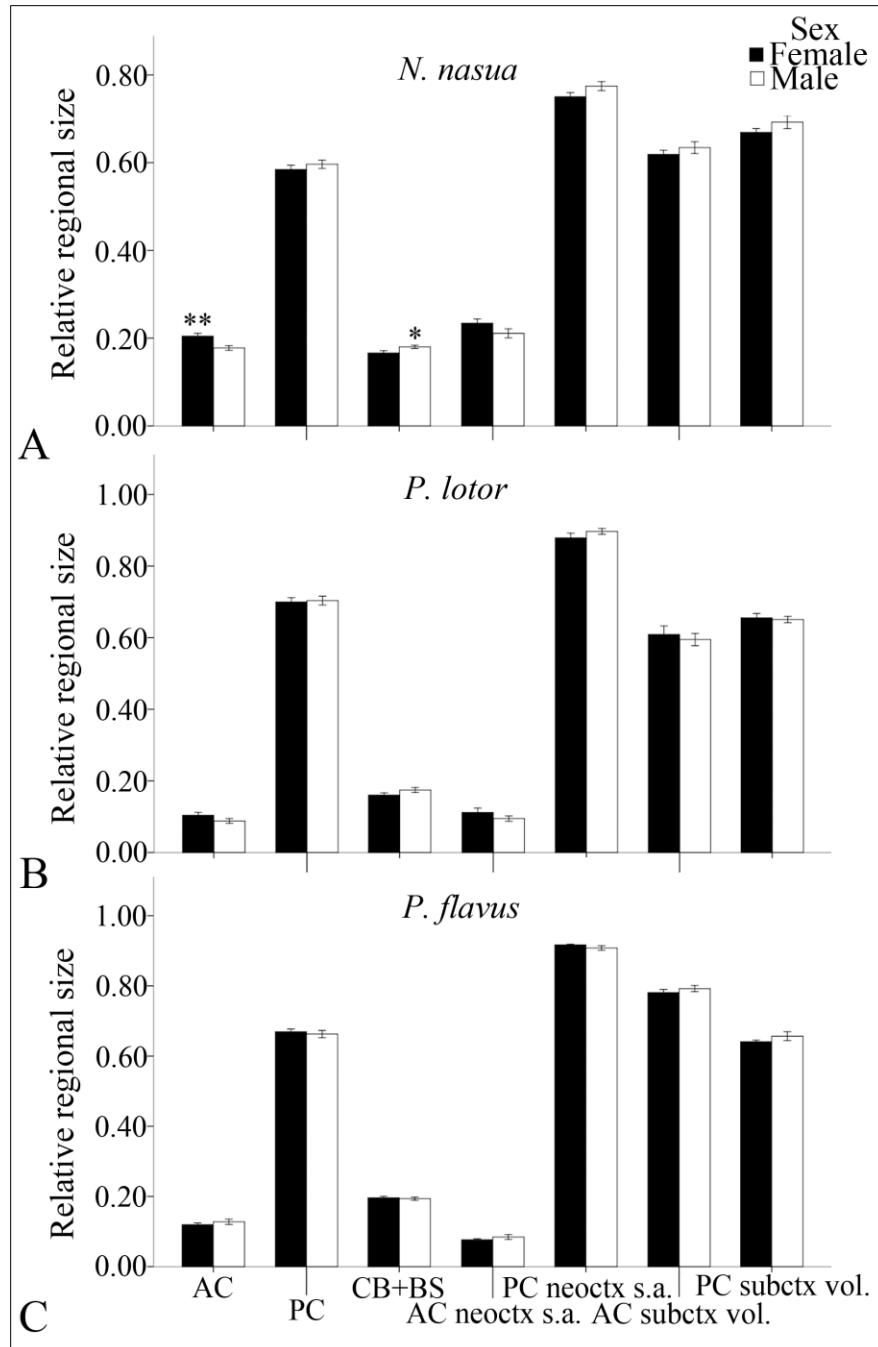


Figure 12. Proportional regional brain volumes: (AC, PC, and CB+BS, all relative to total endocranial volume, regional surface areas (neoctx sa): AC and PC, all relative to total surface area dorsal to rhinal fissure, and regional subcortical volumes (subctx vol), AC and PC, all relative to regional total volume for **A** coatimundi females (solid bars) and males (open bars), **B** raccoon females (solid bars) and males (open bars), and **C** kinkajou females (solid bars) and males (open bars). Error bars indicate ± 1 SEM. AC volume is significantly larger in female than male coatimundi ($p = 0.005$) and CB+BS volume is significantly larger in male than female coatimundi ($p = 0.04$). No sex differences in regional brain volumes were found in either raccoons (B) or kinkajous (C).

Discussion

Using CT imaging techniques, we found an association between regional brain size and behavioral specializations related to both the social and physical environments in three procyonid species. The social coatimundi possessed the largest relative anterior cerebrum volume and neocortical surface area in comparison to the less social kinkajou and the solitary raccoon. The raccoon, a species noted for its dexterous forepaw use, possessed the largest relative posterior cerebrum volume which also includes somatosensory cortex, compared to either the coatimundi or kinkajou. In addition, the exclusively arboreal kinkajou had the largest relative cerebellum and brain stem volume in comparison to the semi-arboreal raccoon and the terrestrial coatimundi. Lastly, a sex difference in the relative anterior cerebrum volume which includes the frontal cortex was found in the coatimundi whereas no sex differences were found in regional brain volumes in the raccoon or kinkajou. We hypothesize that social life history differences experienced by female and male coatimundis may account for the difference in relative anterior cerebrum volumes. Our findings provide support for the comparative neurology principle that behavioral specializations correspond to the expansion of neural tissue devoted to that function [Jerison 1973].

Computed tomography (CT) technique

The CT technique has been previously used to create virtual endocasts in a variety of species where preservation of the brain is difficult or impossible, but skull specimens are readily available [Arsznov *et al.*, 2010; Sakai *et al.*, 2011a; Sakai *et al.*, 2011b, Arsznov and Sakai, 2012]. The present study utilized this technique combined with MIMICS image processing software (Materialise, Inc., Ann Arbor, MI, USA) to create virtual 3-dimensional endocasts in three extant species from the family Procyonidae: the coatimundi, raccoon, and kinkajou. The virtual endocasts exhibit details of brain surface morphology, including gyrification, which were

directly comparable to the gyral and sulcal pattern of the brain in each species (fig 8). Since virtual endocasts created from CT scans are directly comparable with external brain morphology in these procyonid species as well as other carnivore species including the lion, mountain lion, and spotted hyena [Arsznov *et al.*, 2010, Arsznov and Sakai, 2012] we suggest that this method has great potential and provides a unique opportunity to expand comparative brain studies to include species not typically studied and in whom brain tissue preservation is not feasible.

Endocranial measurements

In order to examine relative brain size in Procyonidae, the present study examined the relationship between total endocranial volume and two estimates of body size: skull basal length and basicranial axis length (fig 9). These skull measures are reasonable proxies for individual body size given the absence of post-cranial bones or other individual measures of body size [Radinsky, 1984]. Although total endocranial volume was positively correlated with each skull measure (skull basal length - Pearson's $r = 0.78$, $p < 0.01$ and basicranial axis length - Pearson's $r = 0.45$, $p = 0.002$), relative brain size by species varied. When skull basal length was used as a proxy for body size, raccoons possess a significantly larger relative endocranial volume than the coatimundi, but not compared to the kinkajou. Additionally, there is no significant difference between the relative endocranial volumes of the coatimundi and the kinkajou. However, when relative endocranial volume was compared using basicranial axis length, a measure that extends from the basisphenoid-presphenoid suture line to the foramen magnum, the kinkajou possessed relatively smaller endocranial volume compared to both the coatimundi and raccoon. Relative brain volume using this measure did not significantly differ between the coatimundi and raccoon. Since the coatimundi possesses an elongated snout in comparison to either the raccoon or kinkajou [Welker and Campos, 1964], relative endocranial volume relative to either skull

measure would be expected to differ (fig. 9). Although Radinsky [1984] proposed that basicranial axis length can be used to help eliminate variation due to differences in the lengths of the splanchnocranium compared to the neurocranium, it remains unclear which scaling factor, skull basal length or basicranial axis length, is correct.

The differing patterns in relative endocranial volume within Procyonidae highlight one major problem in comparative studies that use body mass or other measures of body size in calculating relative brain size [see Striedter, 2005]. A recent analysis of endocranial volume in 36 carnivore species reported family level variation when relative endocranial volume was calculated as a function of mean species body mass or utilizing a principal component analysis of 3 skull measures [Swanson *et al.*, 2011]. This study [2011] demonstrates the difficulty in selecting a body size scaling factor when brain size covaries with body size. On the other hand, comparison of the regional brain areas relative to total brain size [Clark *et al.*, 2001] avoids the use body size measures and provides a more robust data set for comparative analyses [Iwaniuk, 2010].

Regional endocranial measurements

We found that regional brain size corresponds to behavioral specializations among the three procyonid species studied. The social coatimundi possessed the largest relative anterior cerebrum volume and neocortical surface area in comparison to the less social kinkajou and the mostly solitary raccoon. The relatively larger anterior cerebrum volume, largely composed of frontal cortex, found in the coatimundi is hypothesized to be related to enhanced cognitive processing required for social group living. In other mammals, including humans, frontal cortex is involved in the mediation of cognitive processes related to a complex social environment including: social decision making, task switching, updating working memory representations,

and inhibition of prepotent responses [Adolphs, 2001; Amodio and Frith, 2006]. The coatimundi social group, or band, consists of adult females along with juvenile males and females [Kaufmann, 1982]. This social structure can contain upwards of 40 individuals and is comprised of linear dominance hierarchies [Gompper 1995; Gompper and Decker 1998; Hirsch, 2011]. The female bonded bands have been observed to contain subgroups that will forage independently and may be similar to the fission-fusion clans of spotted hyenas [Gompper, 1995], another highly social carnivore species noted for both a relatively large brain and frontal cortex [Sakai *et al.*, 2011a]. In contrast to the gregarious coatimundi, kinkajous are typically solitary or found in small polyandrous family groups [Kays and Gittleman, 2001], while the raccoon is predominately solitary [Kaufmann, 1982].

The raccoon, a species noted for its dexterous forepaw use, possessed the largest relative posterior cerebrum volume in comparison to the coatimundi and kinkajou. The posterior cerebrum volume includes the somatosensory cortex as well as a number of other cortical, thalamic and subcortical structures. The raccoon is known for its prominent forepaw use during tactile exploration. Each neural pathway subserving forepaw use in the raccoon corresponds to an expansion and specialized representation of the forepaw in the somatosensory cortex [Welker and Campos, 1963; Welker and Seidenstein, 1959], thalamus [Welker and Johnson, 1965] and dorsal column [Pubols *et al.*, 1965]. Here, the relative enlargement of posterior cerebrum volume in the raccoon compared to the coatimundi and kinkajou includes the expanded somatosensory cortex and thalamus and is hypothesized to be related to forepaw dexterity exhibited by the raccoon. It is not clear whether these species differences in posterior cerebrum volumes is related to cortical expansion or overall increase subcortical volume since the additional analysis of

posterior cerebrum surface area and subcortical volume yielded inconsistent results based on both residual analysis and proportional comparisons.

Lastly, the exclusively arboreal kinkajou had the largest relative cerebellum and brain stem volume in comparison to the semi-arboreal raccoon and the mostly terrestrial coatimundi. While both coatimundis and raccoons are capable of climbing behavior, kinkajous are known for their exclusively arboreal existence marked by specialized behaviors including the ability to hang suspended by the tail and hind feet while handling food, locomotor flexibility, and locomotor stability in a complex arboreal environment [McClearn, 1992]. We hypothesize that the larger relative cerebellum + brain stem volume found in the kinkajou is related to such specialized behaviors related to locomotion within a complex three-dimensional arboreal environment. Taken together, we found that these three procyonid species exhibit specialized behavior related to complexities in both the social and physical environments with concomitant changes in regional brain volumes. These data provide support for the comparative neurology principle that behavioral specializations correspond to the expansion of neural tissue devoted to that function [Jerison, 1973].

The influence of multiple ecological, social and physical environmental factors on total and regional brain size has been investigated at multiple taxonomic levels and various patterns of association have been identified depending on the level of investigation. In a comparative study investigating cortical organization in the family Procyonidae, Welker and Campos [1963] demonstrated that the raccoon possesses the greatest enlargement of somatosensory cortex, compared to other species, including the coatimundi and kinkajou, and that this expansion is correlated to the highly dexterous forepaw specialization exhibited by the raccoon. Interestingly, Iwaniuk *et al.* [1999] did not find a significant correlation between relative brain size and

forelimb dexterity in his analysis of a broader range of fissiped carnivore species, including the Procyonidae family. Another intrafamily analysis examining the four extant species of the family Hyaenidae found a relationship between the relative amount of frontal cortex and sociality, where the most social species, the spotted hyena (*Crocuta crocuta*) had a greater relative amount of frontal cortex compared to less social hyaenids [Sakai *et al.*, 2011a]. However, a recent broad taxonomic analysis of 36 carnivore species failed to find a similar relationship between frontal cortex volume and sociality [Swanson *et al.*, 2012]. Although a positive relationship between relative cerebrum volume and sociality was found, Swanson *et al.* [2012] identified diet, not sociality, as the major factor influencing overall brain volume within the order Carnivora. These data suggest that intrafamily level comparisons might identify particular patterns in regional brain size that may not be evident when considering species in broader taxonomic context. While broad taxonomic comparisons of regional brain size provide vital insight into the potential factors influencing brain evolution patterns, they do not directly examine the brain structures involved in the mediation of species-specific behaviors. Instead, intrafamily level comparisons between closely related extant species exhibiting divergent behaviors can identify potential brain structures involved in the mediation of particular behaviors. We suggest that intrafamily level comparisons may reveal variations in morphological and ecological traits not detected by broader taxonomic comparative studies.

Intraspecific comparisons of endocranial and regional endocranial measurements

Interestingly, intraspecific comparisons revealed an association exists between relative and regional endocranial volume and sociality, specifically divergent social life history, in the coatimundi. The coati social group, or band, consists of philopatric adult females along with juvenile males and females [Kaufmann, 1982]. Adult females show a high level of tolerance for

juvenile aggressive behaviors with juvenile males displaying higher levels of aggression than females [Hirsch *et al.*, 2012]. Adult male coatis older than 2 years emigrate from their natal band and become solitary [Kaufmann, 1982]. Furthermore, adult males are known to behave aggressively toward bands and in some instances prey on juvenile coatis [Russell, 1981]. Although our analysis of relative endocranial volume found that female coatis have a greater relative endocranial volume when skull basal length is used as the body size scaling factor, no sex difference was found in endocranial volumes relative to basicranial length. However, our analysis of regional endocranial volumes revealed that female coatimundis possess a relatively larger proportion of frontal cortex volume in comparison to males and male coatimundis possess relatively larger cerebellum and brain stem volumes than females. Although females tended to have a relatively greater amount of anterior cerebrum surface area than males (0.23 ± 0.03 and 0.21 ± 0.03 , respectively), this difference was not statistically significant. Regional subcortical volumes did not differ by sex in the coatimundi. The sex difference in frontal cortex volume may be related to differing social cognitive demands experienced by male and female coatimundis. Specifically, since coati female social life history is marked by the presence of a dominant aggressor and tolerance to juvenile aggression, these experiences may require enhanced demand for inhibitory control, a frontal cortical function identified in a number of primate species [Mishkin, 1964]

We also found a sex difference in the relative size of cerebellum and brain stem in coatimundis based on proportional analysis. This finding was not supported based on residual analysis. While the male coatimundis tend to be larger than females [Allen, 1987] and it is possible that males require greater motor coordination than females, these discrepant results suggest that further investigation is warranted.

We did not find any additional sex differences in our analysis of regional brain volumes in the raccoon and kinkajou. In contrast to the sexual dimorphic life histories present in the coatimundi social structure, social life history does not significantly differ in raccoons or kinkajous. These findings are similar to those found in the mountain lion in that both female and male mountain lions are solitary, share similar life histories and no sex difference was found in relative frontal cortex volume [Arsznov and Sakai, 2012]. It is noteworthy that analysis of brain volume differences in other carnivore species where similar divergent social life history patterns exist between males and females have also found sex differences. Female lions live in prides of up to 21 lions while sexually mature male lions disperse from the pride and either live a solitary life or form a coalition with male kin [Packer and Pusey, 1987]. Frontal cortex volume is proportionately greater in female than male lions [Arsznov and Sakai, 2012]. Frontal cortex volume was also sexually dimorphic in the highly gregarious spotted hyena. However, in this species, males possess more frontal cortex than females [Arsznov *et al.*, 2010]. In spotted hyenas, the females live in a philopatric society [Smale *et al.*, 1997] and sexually mature males disperse from their natal clans to join another clan at the bottom of the dominance hierarchy [Smale *et al.*, 1997; East and Hofer, 2001; Boydston *et al.*, 2005]. In all three of these social carnivore species, the individuals that require enhanced inhibitory control in the presence of an aggressive conspecific possessed relatively greater frontal cortex volume. Although we do not currently know the functional role of the frontal cortex in these carnivore species, lesion studies in dogs report loss of inhibition to previously conditioned responses [Brutkowski, 1965].

As a whole, Carnivora represent a diverse mammalian order that exhibit tremendous variation in behavioral specializations related to the social and physical environments, as well as life history patterns [see Bekoff *et al.*, 1984]. Family-level comparisons highlight interspecific

variations in total and regional endocranial measures where unique cases of behavioral specialization are present. In addition to interspecific comparisons, intraspecific comparisons reveal patterns of variation related to divergent life history patterns between the sexes. Here, the carnivore family Procyonidae provides an exceptional model for investigating the relationship between endocranial measures and behavioral specializations related to social and physical environmental complexities at both interspecific and intraspecific levels of analyses. These data suggest that specialized behaviors and regional expansion relate to demands imposed by both the social environment (sociality) and physical environment (tactile exploration/manipulation and arboreality). These findings lend support to the comparative neurological principle whereby behavioral specializations correspond to an increase in the amount of neural tissue devoted to that behavior.

CHAPTER 3: Is more really more for carnivores: do larger brains have more neurons?

Introduction

A fundamental question that has gained considerable interest in the field of comparative mammalian neurology is: Do larger brains have more neurons? Neocortex, the uniformly thick gray matter covering the entire cortical mantle, is present in all extant mammals [Jerison, 2007]; however, mammals exhibit great variation in relative neocortex size [Kaas, 2000]. While relative brain size as a whole increases over evolutionary time [Jerison, 1973], the neocortex is the structural property of the brain that exhibits the most significant concomitant increase in relative size [Clark *et al.*, 2001]. Among primates, a mammalian order noted for exhibiting exceedingly large brains, the relatively large brain is due primarily to an expansion of neocortex [Jerison, 2007]. This increase in the size of neocortex is mostly attributed to an increase in neuron number and not an increase in neuronal size [see Kaas, 2000]. Larger brains also display a relative decrease in neuron density [Ringo, 1991]. The relative decrease in neuron density reflects an associated increase in the number of white matter axonal connections among neurons [Allman, 1999].

It is generally thought that an increase in neuron number is associated with an increase in cognitive abilities across a variety of species [Roth and Dicke, 2005]. The neocortex consists of several structurally distinguishable and presumably functionally distinct regions that subserve different aspects of behavior. Distinct cortical regions are identifiable based on the cytoarchitectonic organization of neurons that reflects the information processing characteristic of that region [Shepherd, 2011]. The principle of proper mass states that the relative amount of neural tissue subserving a particular behavior corresponds to the amount of information processing required for that function to occur [Jerison, 1973]. An expansion in relative regional

brain size is presumed to be associated with an increase in the ability to process or integrate cognitive information related to the behavioral function associated with that brain region. It is hypothesized that specialized behaviors arise as species respond to increased cognitive challenges imposed by the physical environment [Jerison, 1973; Clutton-Brock and Harvey, 1980; Povinelli and Preuss, 1995; Reader and Laland, 2002] or social environment [Jolly, 1966; Humphrey, 1976; Byrne and Whiten, 1988; Dunbar, 2003]. This implies that species that exhibit complex specialized behaviors should possess relatively greater expansion, and possibly a greater number of neurons, in brain regions that mediate specialized behaviors compared to species that do not exhibit behavioral specializations. Previously, family level interspecific analyses in Hyaenidae and Procyonidae examining regional brain size suggest that brain regions that mediate behavioral specializations related to both the social and physical environments are relatively larger in species that exhibit those behaviors [Sakai *et al.*, 2011a; Arsznov and Sakai, *in prep.*]. However, these results were based on computed tomographic (CT) three-dimensional virtual endocasts and the correlation between a relative increase in virtual endocast measures, e.g. volume and surface area, and neuron number or density remains unclear.

Stereological methods such as the optical fractionation [West *et al.*, 1991; West, 1999] can provide estimates for the numbers of neurons and other cell types within distinct brain regions [Korbo *et al.*, 1990; Andersen *et al.*, 1992]. The purpose of this study was two fold: 1) to examine the relationship between brain size and neuron number in selected carnivore species including the dog (*Canis familiaris*), spotted hyena (*Crocuta crocuta*), and raccoon (*Procyon lotor*), in order to determine if larger brains are associated with an increase in neuron number, and 2) to examine the relationship between regional brain size and behavioral specializations in order to determine if increases in regional brain size is a result of an increase in neuron number.

Methods

Specimens

Nissl stained brain sections of selected carnivore species including a dog (*Canis familiaris*), spotted hyena (*Crocuta crocuta*), raccoon (*Procyon lotor*) were analyzed using cytoarchitectonic criteria and stereology.

Brain sectioning

The dog brain was blocked in the stereotaxic coronal plane *in situ* then placed in a 30% sucrose-formalin solution and sectioned at 40µm as described in Stanton *et al.* [1986]. The raccoon brain was removed and placed in 30% sucrose in 0.1M phosphate buffer prior to being sectioned at 40µm along the coronal plane as described in Sakai [1990]. The brain of the spotted hyena was removed and immersion fixed in 4% para-formaldehyde. One hemisphere was blocked, and sunk in 4% paraformaldehyde in 0.1M phosphate buffer with 30% sucrose prior to frozen sectioning at 40µm thick in the coronal plane. All sections were stained with cresyl violet, dehydrated in a graded alcohol series and coverslipped.

Regions of interest and histological analysis

Regions of interest (ROI) included: prereal frontal cortex, orbital frontal cortex, primary somatosensory cortex, and primary visual cortex. From the Nissl-stained sections, each ROI were identified based on gyral and sulcal patterns and previous cytoarchitectonic descriptions. Prereal frontal cortex and orbital frontal cortex were identified based on cytoarchitectonic criteria previously described in the dog [Kosmal, 1981; Kosmal *et al.*, 1984; Tanaka, 1987]. Primary somatosensory cortex was identified based on cytoarchitectonic criteria previously described in the raccoon [Welker and Seidenstein, 1959; Welker and Campos, 1963; Welker *et al.*, 1964; Sakai, 1990]. Primary visual cortex was identified based on cytoarchitectonic criteria

as described in the cat [Hassler and Muhs-Clement, 1964; Kalia and Whitteridge, 1973] and ferret [Law *et al.*, 1988; Innocenti *et al.* 2002] (Note: visual cortex sections were not available for the spotted hyena). Each ROI was traced unilaterally in eight successive sections (except where otherwise noted) throughout the rostrocaudal axis using StereoInvestigator software (version 9; Microbrightfield, Williston, VT) at 4x objective and include cortical layers I-VI. Individual neurons were counted at 100x oil-immersion (1.4 N.A.) objective. The average section thickness was determined for each species by measuring section thickness for each counting frame interval. Overall, the average measured section thickness for each species was 9µm for the dog, 8µm for the raccoon, and 10µm for the spotted hyena. The following parameter settings were used to obtain the neuron counts: dissector height, 5µm; guard zone distance 1.5µm; counting frame width (x), 40µm; counting frame height (y), 40µm. The sampling grid parameters were set to ensure 15 counting frames per trace throughout each ROI. Parameters were set to obtain a coefficient error ≤ 0.1 for each of ROIs in the three species [Gundersen *et al.* 1999, m=1]. Volume measurements were obtained for each of the ROIs from the planimetry output function and neuron density was calculated for each ROI by dividing the estimated population (using mean section thickness) by the total measured volume (mm³). Criteria for neuron identification included the presence of a distinct Nissl-stained nucleolus, presence of distinct cytoplasm, and visible extension(s). Neuron number estimations were performed with StereoInvestigator software (version 9; Microbrightfield, Williston, VT) using the optical fractionator method [Morris *et al.*, 2008]. This method estimates the total number of selected objects (N_{obj}) according to the following equation: $N_{obj} = (\sum N)(1 / ssf)(1 / asf)(1 / tsf)$. Where section sampling fraction (ssf) is the number of sections sampled divided by the total number of sections within the reference space, area sampling fraction (asf) is the total area within the

sampled counting frames divided by the total area of all sampled sections, and the thickness sampling fraction (tsf) is the dissector height divided by the average total section thickness.

Endocranial volume

Average total endocranial volumes for were obtain from previously published data for the spotted hyena [Arsznov *et al.*, 2010] and raccoon [Arsznov and Sakai, *in prep.*, see Chapter 3]. Briefly, skulls were scanned using a General Electric Lightspeed 4 slice scanner or a General Electric Discovery ST 16 slice scanner at the Department of Radiology at Michigan State University. Parameters for each scan included: a slice thickness of 0.625mm, a table speed of 5.62mm/rotation, a pitch of 0.562:1 and a 30cm field of view. CT data were saved in the Digital Imaging and Communications in Medicine (DICOM) Centricity Version 2.2 format and the virtual endocasts were created using MIMICS 13.1 software (Materialise, Inc., Ann Arbor, MI, USA). From each scanned skull a three-dimensional virtual endocasts was created following procedures described in detail in Sakai *et al.*, [2011]. The endocranial cavity was identified and isolated from the surrounding cranial bone based on a defined pixel value of -1024 Hounsfield units (HU). The endocranial cavity, defined rostrally by the cribiform plate and caudally by the foramen magnum, was selected and filled along the coronal plane. Virtual endocasts were created using the MIMICS 3D operation. Additionally, endocranial volume for the dog was obtained from a three-dimensional volumetric reconstruction a from magnetic resonance image (MRI) scan brain from a dog following the procedures described in detail in Arsznov and Sakai [2012]. Briefly, the brain was imaged in a 1.5-T Siemens Espree (Siemens, Munich, Germany). The parameters for the obtained MRI sequences included: FOV = 16 cm, slice thickness = 5 mm, resolution: 192 x 192 x 16, flip angle of 0 degrees and interslice gap = 0.8 mm. This subject was part of another study and was treated according to guidelines set by the American Veterinary

Medical Association. Three-dimensional virtual reconstruction of the brain and volumetric measures were performed and obtained using MIMICS 13.1 software (Materialise, Inc., Ann Arbor, Mich., USA). The brain was selected for by tracing the exterior extent of the brain in the coronal plane using the MIMICS livewire function. This procedure was applied to serial sections from where the cribiform plate forms the floor of the endocranial cavity and extending through the foramen magnum.

Statistical analyses

A bivariate correlation between endocranial volume and neuron density was assessed using a Pearson correlation coefficient. Statistical analyses were performed using the statistical software package IBM SPSS Statistics 19 (IBM Corporation, Armonk, New York, USA).

Results

Cytoarchitectonic descriptions of the ROIs

- Proreal cortex

Proreal frontal cortex is located on along the proreal gyrus which occupies the dorsal, dorsolateral, and dorsomedial regions of the prefrontal cortex in the dog [Tanaka, 1987] (fig. 13). Here, proreal cortex was compared to descriptions previously made by Kosmal *et al.* [1984] and Tanaka [1987] in the dog. Briefly, cytoarchitectonic patterns in the dog were consistent with those previously described. Cytoarchitectonically, the cells in layer II of proreal cortex are densely packed and layer II is relatively thin (fig. 14A) compared to orbital frontal cortex (fig. 14B). Proreal layer III contains uniformly spaced medium sized neurons. Underlying layer III is a thin layer IV that is comprised of clusters of medium sized neurons amidst smaller granular cells that gradually merges with layer V that is primarily composed of medium sized pyramidal

cells. The layer V pyramidal cells are linearly arranged providing a clear transition between layers V and VI. Proreal layer VI contains palisades, vertically arranged columns of cells.

Layers II and III of the proreal cortex in the spotted hyena is similar cytoarchitectonically to the dog (fig. 15A). Interestingly, layer IV is more distinctive in the spotted hyena than in the dog due to the presence of a distinctive granular cell layer. Layer IV gradually merges inferiorly with layer V which contains medium sized pyramidal cells. Layer VI contains short columns of cells that create a well demarcated border with the underlying white matter in the spotted hyena (fig. 15A).

Raccoon proreal cortex also contains a densely packed layer II which transitions into a broad layer III (fig.16A) similar to dog and spotted hyena. The raccoon lacks a distinct layer IV, instead layer III containing small to medium sized pyramidal cells appears to transition into layer V as marked by the presence of larger sized pyramidal cells. Layer VI in the raccoon also is relatively thin and contains short palisades that border the underlying white matter (fig. 16A).

- Orbital frontal cortex

The orbital gyrus is located along the lateral surface of the prefrontal cortex. Here, orbital frontal cortex was delineated in the frontal plane and included cortex ventral to orbital sulcus and dorsal to rhinal fissure (fig 13). In each of the examined species, orbital frontal cortex was readily identifiable utilizing the criteria of Kosmal *et al.* 1984] and Tanaka [1987] in the dog. Briefly, the orbital frontal cortex in the dog contains relatively thin and moderately packed layer II that consisted of medium size neurons that smoothly transitioned with layer III (fig. 14B). Underlying layer III is a discernibly thin layer IV that is distinguishable by a combination of medium size neurons among clusters of smaller neurons, but does not contain the widely

dispersed medium sized pyramidal cells identifiable in layer V. Layer VI consists of palisades of cells that extend into the underlying white matter (fig 14B).

The orbital frontal cortex in the spotted hyena is similar to that of the dog. The spotted hyena orbital frontal cortex contains a moderately packed layer II that merges inferiorly with layer III containing uniformly distributed medium sized pyramidal cells (fig. 15B). However, layer II is relatively wider than in the dog. Layer IV is readily distinguishable and is composed of granular cells, interspersed with medium sized pyramidal cells. The inferior part of layer V contains medium sized pyramidal cells that smoothly transition into layer VI. Similar to the dog, layer VI of orbital frontal cortex in the spotted hyena consists of palisades that extend into white matter (fig. 15B).

In the raccoon, orbital frontal cortex layers II and III displayed a smooth transition consisting of medium sized neurons. Layer IV is not readily identifiable in the raccoon. Instead it appears that layer III merges with thin layer V consisting of a medium to large sized neurons occurring along the border with layer VI. Layer VI exhibits palisades with neuron columns extending into the white matter (fig. 16B).

Somatosensory cortex

The primary somatosensory cortex was identifiable in each species based on known gyral and sulcal patterns in the raccoon [Welker *et al.*, 1964] and ferret [Juliano *et al.*, 1996; McLaughlin *et al.*, 1998], including post cruciate dimple, coronal sulcus, and the medial and lateral branches of ansate sulcus. Here, detailed cytoarchitectonic analysis was based on a region delineated along the frontal plane as cortex lateral to the medial branch of ansate sulcus and medial to the coronal sulcus (fig. 13). In each of the 3 species, area 3b, the cytoarchitectonic area included in the primary somatosensory cortex [Kaas *et al.*, 1983] was readily identifiable

based on criteria previously described in the raccoon [Johnson *et al.*, 1983]. In the raccoon area 3b consists of a layer II that contains densely packed small pyramidal cells. Layer III contains small pyramidal cells near layer II and medium size pyramidal cells near layer IV (fig. 16C). These small pyramidal cells merge with a distinct densely packed granular layer IV. Underlying layer IV is an acellular band that delineates the junction between layers IV and V. This acellular band has previously been identified as a distinguishing characteristic of area 3b of somatosensory cortex in raccoons [Johnson *et al.*, 1982]. Inferior to the acellular band is layer V which contains medium sized pyramidal cells but lacks large pyramidal cells. The cells of layer VI in the raccoon have a columnar arrangement and extend into the underlying white matter. These cytoarchitectonic features of area 3b in the raccoon including a distinctive granular layer IV that lies superior to an acellular band that delineates the border between layers IV and V were also present in the spotted hyena (fig. 15C) and the dog (fig. 14C)

Primary Visual cortex

Primary visual cortex was located along the most caudal portion of neocortex and is situated around the crown of the lateral gyrus based on descriptions in the cat [Hassler and Muhs-Clement, 1964; Kalia and Whitteridge, 1973] and ferret [Law *et al.*, 1988; Innocenti *et al.* 2002; Manger *et al.* 2002]. Primary visual cortex (area 17) and the adjacent secondary visual cortex (area 18) [Kalia and Whitteridge, 1973] were identified in the dog and raccoon as cortex dorsal to the splenial sulcus and medial to lateral sulcus (fig. 13). In area 17 of the raccoon, cortical layer II is densely packed and contains small and medium sized pyramidal cells. Layer III consists of pyramidal cells that gradual increase in size from medium to distinct large pyramidal cells that are present at the junction between layers III and IV. Layer IV is relatively wide and contains densely packed granular cells. Located in layer IV is a relatively thin acellular

horizontal line known as the outer band of Baillarger. Inferior to the granular layer IV is layer V that contains medium to moderately large pyramidal cells (fig 14D). This inner pyramidal layer also contains the inner band of Baillarger. Layer VI is wide and consists of neurons arranged in clear columnar palisades that extend into the underlying white matter (fig. 14D). Similar cellular organization was present in area 17 of primary visual cortex in the dog (fig. 16D.)

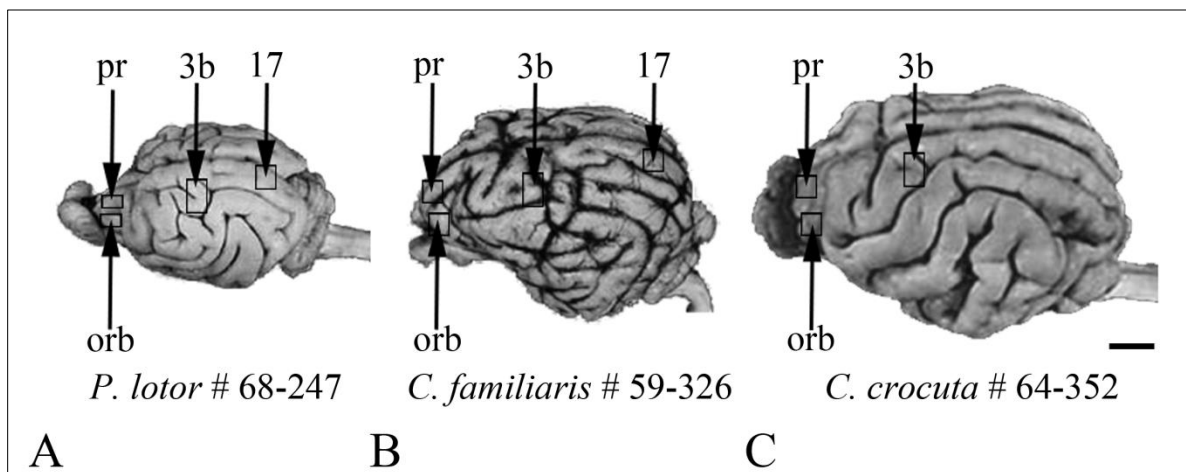


Figure 13. Dorsolateral view of whole brain photographs from the Comparative Mammalian Brain Collection (www.brainmuseum.org) of **A** raccoon No. 68-247, **B** dog No. 59-326, and **C** spotted hyena No. 64-352. Regional volume, neuron number, and neuron density were obtained in successive Nissl stained sections within regions of interest (ROIs) indicated by arrows and box outlines: orb – orbital frontal cortex, pr – preareal frontal cortex, 3b – primary somatosensory cortex (area 3b), 17 – primary visual cortex (area 17). Scale bar = 1 cm.

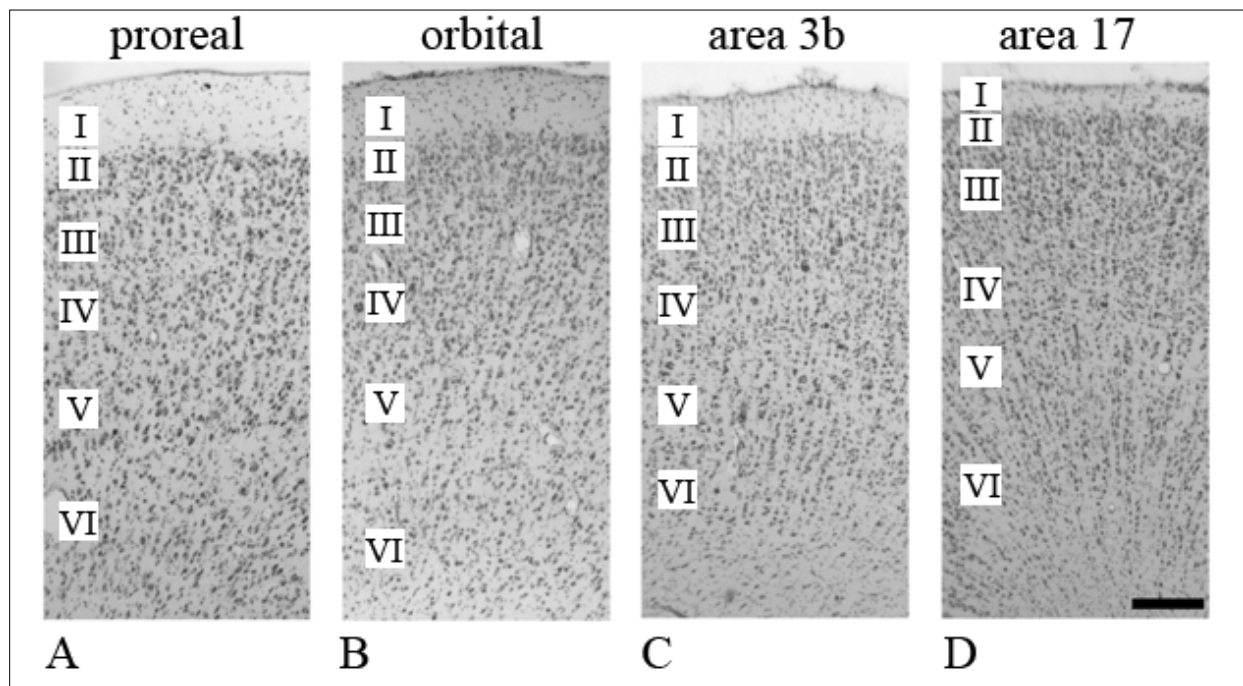


Figure 14. Photomicrographs showing cytoarchitectonic organization through **A** prefrontal frontal cortex, **B** orbital frontal cortex, **C** primary somatosensory cortex (area 3b), and **D** primary visual cortex (area 17) in the dog. Scale bar = 25 μ m.

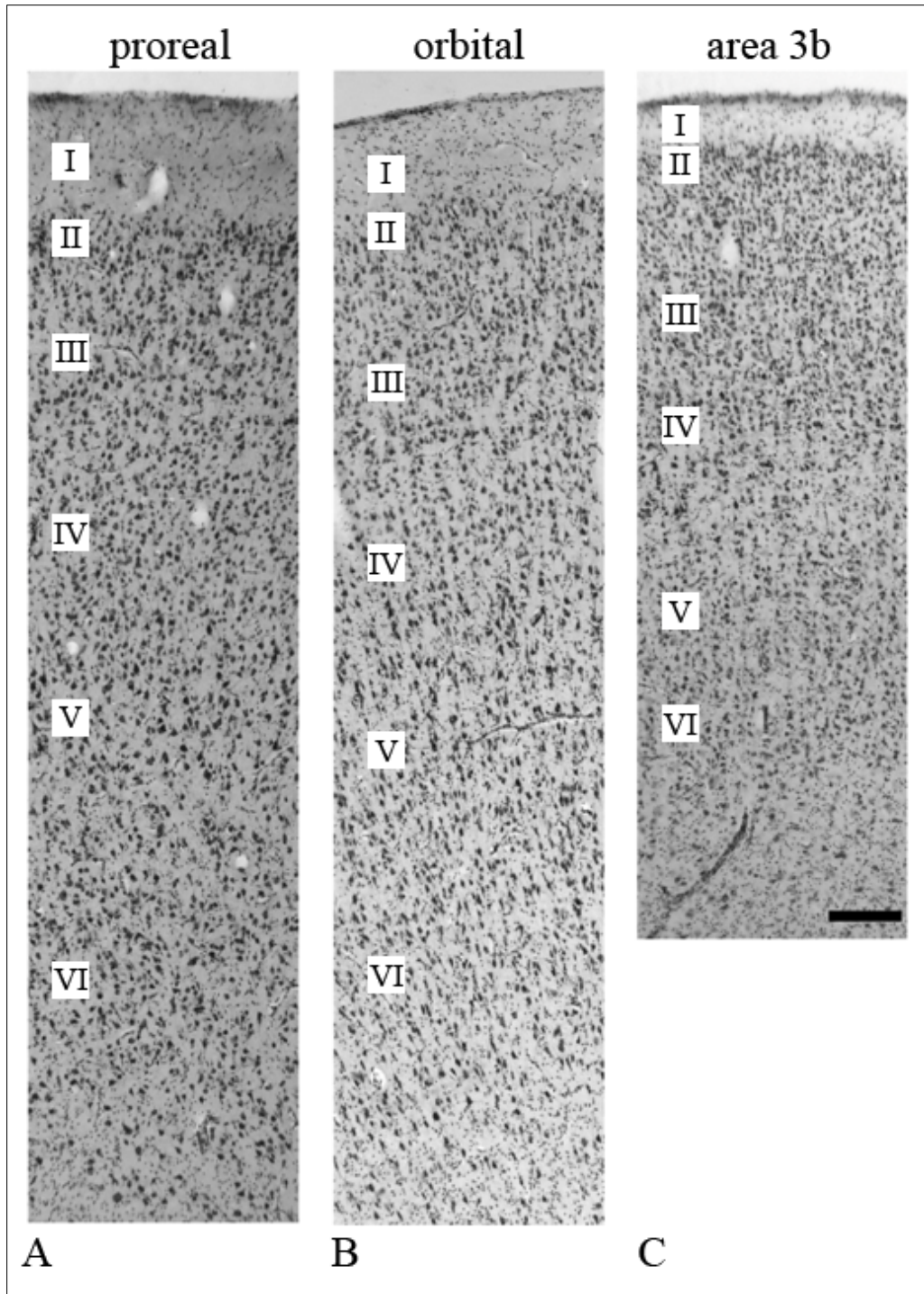


Figure 15. Photomicrographs showing cytoarchitectonic organization through **A** preoreal frontal cortex, **B** orbital frontal cortex, and **C** primary somatosensory cortex (area 3b), in the spotted hyena. Scale bar = 25 μ m.

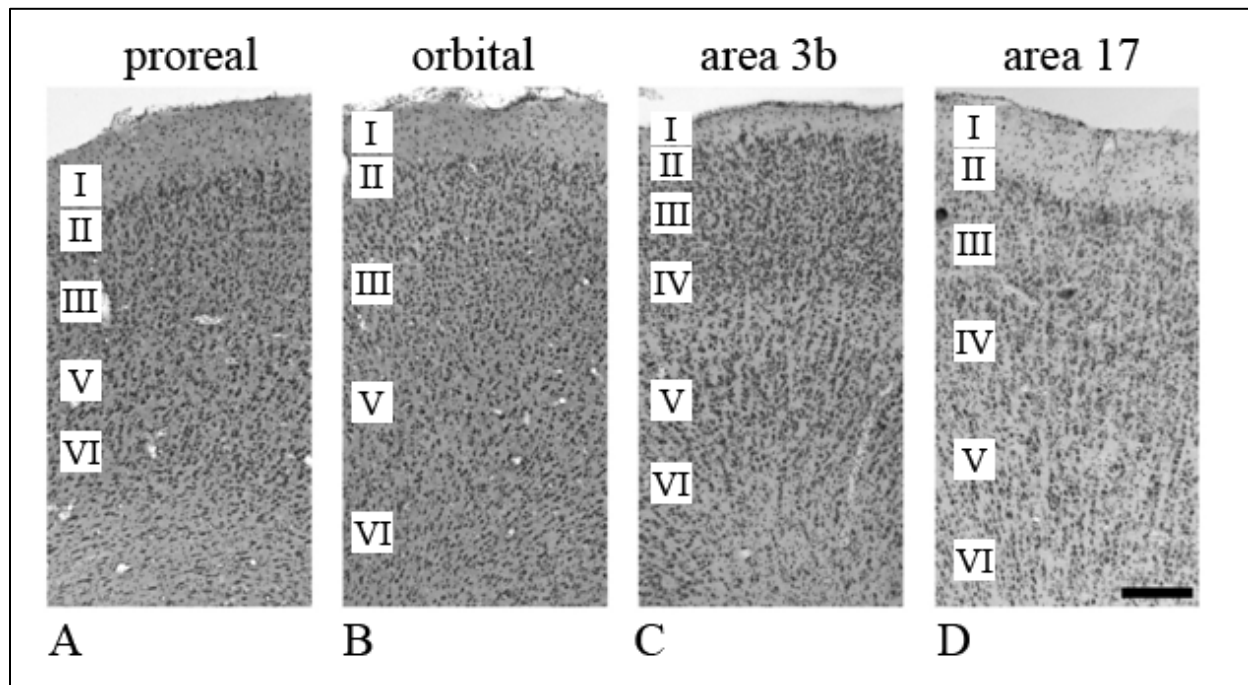


Figure 16. Photomicrographs showing cytoarchitectonic organization through **A** prefrontal frontal cortex, **B** orbital frontal cortex, **C** primary somatosensory cortex (area 3b), and **D** primary visual cortex (area 17) in the raccoon. Scale bar = 25 μ m.

Correlation among selected carnivore species

A bivariate, analysis of the endocranial volume and neuron density revealed a significant correlation (Pearson's $r = -0.68$, $p = 0.02$), where larger endocranial volumes negatively correlate with neuron density (fig.17).

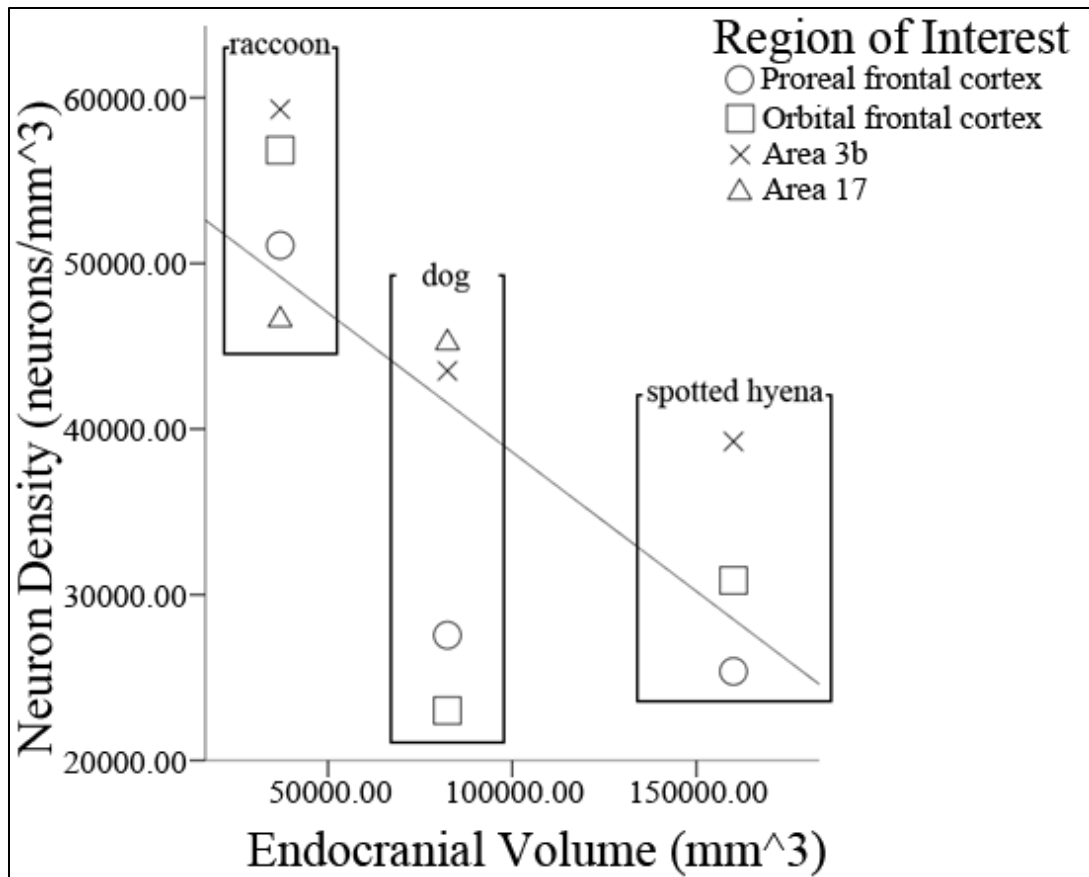


Figure 17. Graph showing the relationship between the estimated neuron densities of each traced region of interest as a function of endocranial volume. Species labels correspond to endocranial volume columns. A strong relationship exists for average neuron density (Pearson's $r = -0.68$, $p = 0.02$), where larger endocranial volumes display a significant decrease in neuron density averaged over each ROI.

Neuron number, regional volume, and neuron density comparisons between ROIs

In the dog, neuron number was relatively similar for each ROI (proreal frontal cortex, neuron no. = 2,063,790; orbital frontal cortex, neuron no. = 1,562,083; primary somatosensory cortex, neuron no. = 2,615,034; primary visual cortex, neuron no. = 1,943,751) (fig. 18A). Regional volumes were also similar in preoreal frontal cortex (74.9 mm^3), orbital frontal cortex (67.9 mm^3), and primary somatosensory cortex (60.1 mm^3), whereas the regional volume of visual cortex is comparatively smaller (43.0 mm^3) (fig. 18B). Interestingly, neuron density is similar in primary somatosensory cortex and primary visual cortex ($43,511 \text{ neurons/mm}^3$ and $45,203 \text{ neurons/mm}^3$ respectively) while similarities in neuron density also were present between preoreal frontal cortex ($27,553 \text{ neurons/mm}^3$) and orbital frontal cortex ($23,005 \text{ mm}^3$) (fig.18C).

In the spotted hyena, neuron number from highest to lowest was as follows: orbital frontal cortex (neuron no. = 4,138,262), primary somatosensory cortex (neuron no. = 3,453,787), and preoreal frontal cortex (neuron no. = 2,255,373) (fig. 18A). Regional volume of orbital frontal cortex was also greater (134.1 mm^3) than preoreal frontal cortex (88.9 mm^3) and primary somatosensory cortex (88.0 mm^3) (fig. 18B). Neuron density was greater in primary somatosensory cortex ($39,248 \text{ neurons/mm}^3$) compared to both preoreal frontal cortex ($25,370 \text{ neurons/mm}^3$) and orbital frontal cortex ($30,860 \text{ neurons/mm}^3$) (fig. 18C).

In the raccoon, neuron number was noticeably greater in primary somatosensory cortex (neuron no. = 5,888,491) compared to each of the other ROIs (primary visual cortex, neuron no. = 2,623,383; preoreal frontal cortex, neuron no. = 1,604,063, orbital frontal cortex, neuron no. = 1,688,087) (fig. 18A). A similar relationship between ROIs was also reflected in regional volume where primary somatosensory cortex had the largest regional volume (99.3 mm^3) followed by

primary visual cortex (56.3 mm^3) and prereal frontal cortex and orbital cortex (31.4 mm^3 and 29.7 mm^3 respectively (fig. 18B). Additionally, neuron density was greater in primary somatosensory cortex ($\text{neurons/mm}^3 = 59300$) compared to the other ROIs (primary visual cortex, $\text{neurons/mm}^3 = 46596$; prereal frontal cortex, $\text{neurons/mm}^3 = 51084$, orbital frontal cortex, $\text{neurons/mm}^3 = 56837$) (fig. 18C).

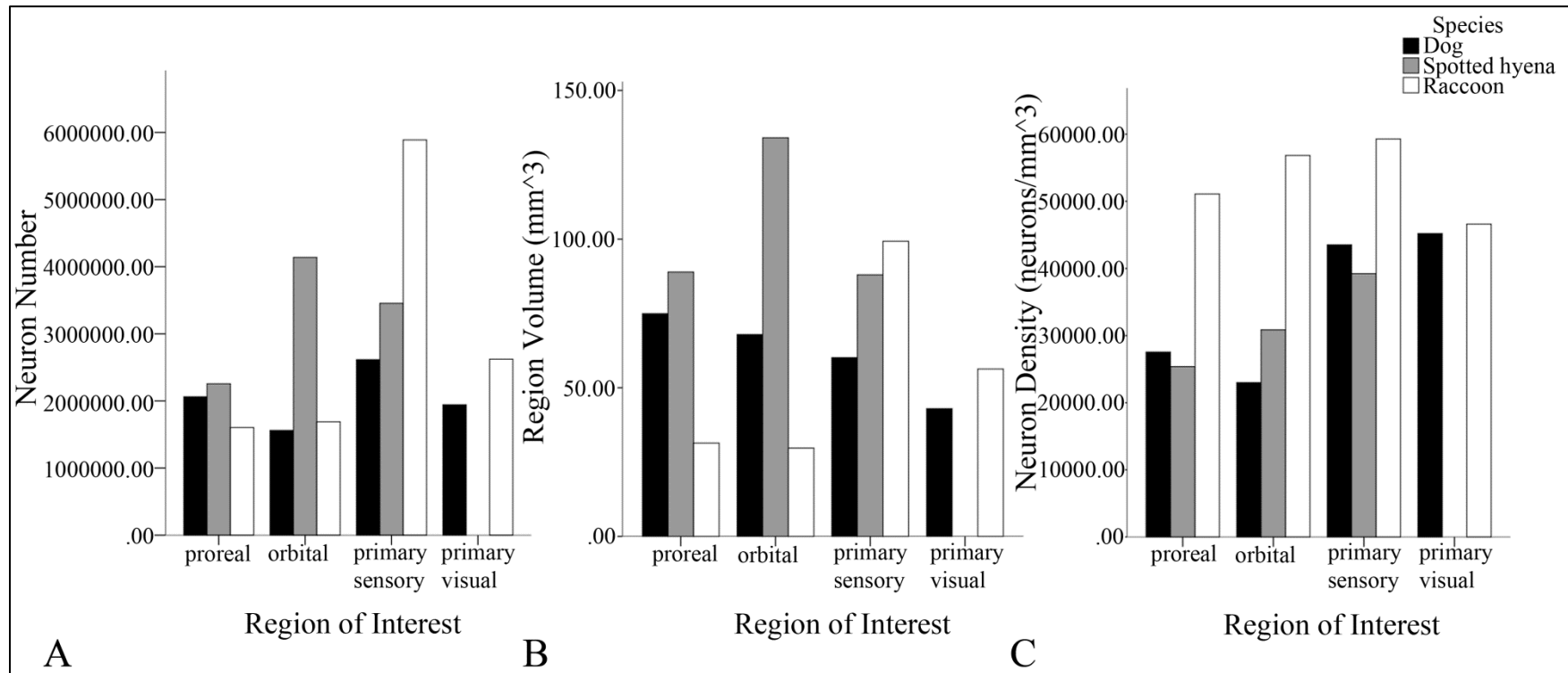


Figure 18. Bar graphs showing **A** neuron number, **B** regional volume, and **C** neuron density for prefrontal cortex (proreal), orbital frontal cortex (orbital), primary somatosensory cortex (primary sensory) and primary visual cortex (primary visual) in the dog (black bar), spotted hyena (grey bar) and raccoon (white bar).

Discussion

Stereological methodologies allow for detailed examination of neuron number and neuron density in discrete cortical regions identified based on cytoarchitectonic criteria. Here, the optical fractionator technique was used to examine the relationship between neuron number and brain size in selected carnivore species. This study provides estimations of neuron number and neuron density within 4 regions of cortex including prereal frontal cortex, orbital frontal cortex, primary somatosensory cortex (area 3b), and primary visual cortex (area 17) in three carnivore species. Despite our small sample size, these results lend support to three major assumptions underlying brain organization in mammals. Here, we found an increase in brain size is associated with a decrease in neuron density, as indicated by a significant negative correlation between endocranial volume and neuron density. Second, variations in neuron density patterns occur where primary sensory regions exhibit higher neuron densities compared to more anterior regions of cortex including prereal cortex and orbital frontal cortex. Third, these results lend further support to one principle of comparative neuroanatomy that states that behavioral specializations, e.g. forepaw dexterity in the raccoon, are reflected in both increased neuron number and an expansion of neural regions subserving that function, e.g. primary somatosensory cortex (area 3b).

More is less: Brain size and neuron density

Until recently, it was assumed that functional areas of cortex consist of relatively constant neuron densities despite variations in total size of the brain in various mammals [Rockel *et al.*, 1980]. In brief, the absolute number of neurons within a cortical strip was similar across cortical areas and species with the one exception that visual cortex, area 17, in primates contained a larger number of neurons [Rockel *et al.*, 1980]. However, further investigation has suggested that

cortical density exhibits a great degree of variation both within and across a variety of mammals including primates, proboscids, and cetaceans [Haug, 1987; Herculano-Houzel *et al.*, 2008]. In addition, an investigation of 28 mammalian species belonging to 3 clades revealed that that increases in brain size are associated with a decrease in neuron density [Herculano-Houzel *et al.*, 2011]. Cahalane *et al.* [2012] suggest that, in primates, regional cortical densities may be related to the earlier cessation of neurogenesis in anterior regions of cortex allowing for the development of elaborate neuronal processes [Goldman-Rakic, 1987] and dendritic arborizations [Elston *et al.*, 2009].

These findings suggest that in the three carnivore species studied, neuron density varies across the four cortical areas. Additionally, a significant relationship exists between endocranial volume and neuron density, where larger endocranial volumes exhibit a decrease in neuron density. In our sample, the spotted hyena possessed the greatest endocranial volume with the lowest neuron density whereas the raccoon with the smallest endocranial volume had the highest neuron density (Fig. 17). These findings are consistent with newer methodologies including isotropic fractionation in a broad range of mammals [Herculano-Houzel and Lent, 2005; Krubitzer *et al.*, 2011].

Differences between ROIs

The present findings indicate that regional cortex size, indicated by volume of ROIs, increases as brain size increases. Interestingly, where ROIs are related to known behavioral specializations, the regional volumes and regional neuron numbers displayed the greatest increases. For example, the raccoon exhibited the largest relative volume, neuron number, and neuron density in primary somatosensory cortex compared to all other examined ROIs. The raccoon is known for its behavioral specialization of forepaw dexterity and enlarged neocortical

forepaw representation [Welker and Seidenstein, 1959]. These results are consistent with the idea that specialized behavior such as the enhanced forepaw tactile processing in the raccoon is accompanied by not only an increased in the total cortical area devoted to the forepaw representation [Welker and Seidenstein, 1959] in comparison to other carnivores [Welker and Campos, 1963], but also that this cortical area contains a greater number and density of neurons.

Interestingly, the spotted hyena, a species noted for its social behavior, exhibited the relatively largest cortical volume and neuron number in orbital frontal cortex. The spotted hyena is a highly gregarious and aggressive species [Holekamp *et al.*, 2007] that have been shown to possess a relatively large frontal cortex compared to other extant hyaenids [Sakai *et al.*, 2011a]. Sakai *et al.* [2011a] hypothesized that the relatively large frontal cortex in the spotted hyena may be due to inhibition of socially inappropriate behaviors in the presence of a dominant aggressor. Orbital frontal cortex is involved in a variety of behaviors including value based judgment in decision making [Fellows, 2007] and inhibition of reactive aggression [Blair, 2004]. It is notable that the orbital frontal cortex in the spotted hyena was both greater in size and neuron number than in the raccoon and dog. In addition, the spotted hyena orbital cortex consisted of the most distinctive granular layer IV compared to the other two species (Fig. 15B).

It is tempting to hypothesize that the increased size and neuron number in orbital frontal cortex may be related to the complex social behavior, specifically inhibition of inappropriate behaviors in a social environment, in the spotted hyena. However, in the spotted hyena, increases in the regional size and neuron number in orbital frontal cortex were not associated with an increase in neuron density. Instead neuron density is greatest in somatosensory cortex (area 3b) compared to either regions of frontal cortex (Fig. 18 C). Previous investigations of neuron densities and neuron number in primates report a general gradient of high neuron density and

neuron number in primary sensory areas extending from posterior cortex (visual cortex) rostrally as far as the somatosensory cortex and a subsequent decline in both density and neuron number in anterior cortex [Finlay and Slattery, 1983; Collins *et al.* 2010; Cahalane *et al.* 2012].

Cahalane *et al.* [2012] suggest that the posterior-anterior neuron density gradient is the result of an earlier completion of neurogenesis in anterior cortical regions that allows for elaborate neuronal processes and increased cognitive processing in anterior regions of cortex. Thus, variations in neuron density patterns in the raccoon and spotted hyena may be associated with a more general pattern of cortical density where there is an increase in neuron density in primary sensory regions compared to more anterior regions of cortex. However, the increase in regional size and neuron number in cortical regions that mediate behavioral specializations are related to an increase in the information processing demands of that region.

Lastly, these findings support one principle of comparative neurology that species that exhibit behavioral specializations also display an increase in the neocortical representation in regions mediating those functions. Moreover, these preliminary results suggest that the principle of proper mass [Jerison, 1973] applies to both sensory (posterior) and association (anterior) cortical regions.

Future directions and inquiries

In order to further investigate these relationships, neuron counts of the primary visual cortex in the spotted hyena are necessary. Unfortunately, this block of cortex was not available in time for this analysis. However, it is vital that this cortical region is analyzed in order to determine if the relative patterns of neuron number, regional volume, and neuron density persist within these selected carnivore species. Interestingly, during the course of this study, it was noted that frontal cortex of the spotted hyena contains an additional gyral crown not present in

the dog or raccoon. This region of cortex is situated medial to orbital frontal cortex and lateral to prefrontal cortex. With no direct cortical comparison present in the other investigated species, this region is currently undergoing detailed cytoarchitectonic analysis. Finally, it is of great interest to extend this stereological and cytoarchitectonic analysis further by including additional carnivore species that exhibit both characteristic behaviors and behavioral specializations mediated by either sensory and association cortical areas in order to provide further understanding into how the underlying cytoarchitecture, relative size of a cortical area, the number of neurons, and neuron density relate to specific behaviors in different carnivore species.

GENERAL CONCLUSIONS

Understanding the factors that influence regional and total brain variations is an essential component of comparative neuroscience. Although a number of hypotheses have been proposed to explain variations in brain size, much of this research focuses on primates while relatively few studies have examined carnivore species [Healy and Rowe, 2007]. Where these hypotheses have been tested in carnivores, they have been conducted using broad taxonomic comparisons [Gittleman, 1994; Iwaniuk *et al.*, 1999; Pérez-Barbería *et al.*, 2007; Swanson *et al.* 2012], and the possibility that these factors might also influence variations within families or between sexes has been greatly overlooked. The research performed in this dissertation suggests that the factors hypothesized to influence brain variations in primates including sociality, environmental, and life history factors, also correspond to regional and total brain variations in selected carnivore species.

Based on previous evidence in the spotted hyena suggesting that sexually dimorphic variations in the relative amount of frontal cortex is associated with enhanced social cognition and divergent social life histories [Arsznov *et al.*, 2010], additional intraspecific comparisons sought to determine if divergent social life histories were associated with sex differences in regional and total brain variations in selected carnivore species in Felidae and Procyonidae. The results from the intraspecific comparisons revealed that sex differences in relative size of frontal cortex exist within species that exhibit divergent social life histories, e.g. African lions and coatimundi. Here, the results suggest that social life history factors including living in social groups marked by the presence of a dominant aggressor may be associated with an increase in relative size of frontal cortex. These results are further supported by the absence of sex differences in the relative amount of frontal cortex in species that do not exhibit differences in

social life history between the sexes e.g. cougars, raccoons, kinkajous. Still, until the influence of differing social life history on variations in regional brain size is investigated in additional carnivore species, our interpretation of these findings is tentative.

Although previous research suggested that both social and environmental complexity may be related to variations in brain size among primates, the generality of how these hypotheses, e.g. the ‘social brain hypothesis’ [Dunbar, 2003], relate to potential interspecific variations in carnivores remains unknown. Based on previous evidence that interspecific differences in total and regional brain variations are related to social complexity in the family Hyaenidae [Sakai *et al.*, 2011a], additional interspecific comparisons sought to determine if differences in social complexity, as well as physical environmental complexity, were associated in regional and total brain variations in selected carnivore species in Procyonidae. The results from these interspecific comparisons suggest that species that exhibit behavioral specializations display associated increases in the relative size of brain regions that mediate particular adaptive behaviors. Overall, these results suggest that behavioral specializations related to both the social, e.g. sociality, and physical environment, e.g. forepaw dexterity and arboreality, are associated with interspecific variations in regional brain size among selected species in the family Procyonidae. These results provide further support to one principle of comparative neurology that behavioral specializations correspond to the expansion of neural tissue devoted that function [Jerison, 1973]. Still, it remains to be seen if these factors influence similar interspecific patterns of regional brain variations in additional carnivore families where member species exhibit highly specialized behaviors related to the social and/or physical environment.

In order to examine if an increase in regional or total brain size is related to an increase in the number of neurons, cytoarchitectonic and stereological methodologies were applied to three

carnivore species including the dog, spotted hyena, and raccoon. The results confirm previous findings that increased brain size is correlated to an overall decrease in neuron density. Moreover, species that exhibit behavioral specializations display both relative increases in regional size and neuron number in brain regions subserving those functions. Differing patterns emerge depending on the behavioral function the region subserves. Carnivore species that express behavioral specializations (forepaw dexterity in the raccoon) known to be mediated by primary sensory regions (area 3b of primary somatosensory cortex), exhibited an increase in regional volume, neuron number, and neuron density. For the first time, stereological results suggest that species that express specialized behaviors associated with living in a complex social environment (inhibition of socially inappropriate behavior in the spotted hyena), exhibit increases in the regional volume and neuron number in brain regions known to mediate social behaviors (orbital frontal cortex), but are not associated with an increase in neuron density. Interestingly, these results suggest that variations in neuron densities between primary sensory regions and association regions of cortex may be related to developmental gradients in neurogenesis where anterior regions of cortex, e.g. frontal cortex, are less dense than posterior cortical regions, e.g. primary visual cortex [Cahalane *et al.*, 2012]; however, in regions that mediate behavioral specializations the increase in regional size and neuron number are related to an increase in the information processing demands of that region. It is possible to consider that in the selected carnivore species, behavioral specializations mediated by association cortical regions may reflect not only an increase in neuron number and neuron size, but also may relate to an increase in underlying white matter connections, dendritic arbors and spines, as well as an increase in the number of synapses [Kaas, 2000].

The interspecific and intraspecific comparative analyses performed throughout this dissertation project utilized measures obtained from three-dimensional virtual endocasts created from computed tomographic scans from skull specimens. Therefore, variations in regional or total brain size relate directly to measures of volume or surface area of the virtual endocast. While the field of comparative neuroanatomy has long recognized the utility of natural fossilized and latex endocranial casts [Radinsky 1969; Jerison, 1973; Jerison 2007], the application of imaging technologies such as computed tomography to create three-dimensional virtual models of the endocranial cavity is a relatively recent and exciting method to investigate comparative questions [Sakai *et al.* 2011b]. Coupling this newer imaging technique with established stereological methods proved a valuable combination in order to effectively investigate the questions posed for this dissertation project. Taken all together, these results suggest that behavioral specializations related to both the social and physical environment are associated with increases in the regional size and number of neurons in brain regions principally mediating those specialized functions.

APPENDICES

Appendix 1:

Field Museum (FMNH); Michigan State University Museum (MSUM); University of Michigan
Museum of Zoology (UMMZ)

Location	Catalog No.	Genus and species	Sex	Scanner
MSUM	14954	<i>Panthera leo</i>	Female	16
MSUM	8046	<i>Panthera leo</i>	Female	16
MSUM	36073	<i>Panthera leo</i>	Female	4
FMNH	20756	<i>Panthera leo</i>	Female	16
FMNH	20758	<i>Panthera leo</i>	Female	16
UMMZ	114803	<i>Panthera leo</i>	Female	16
MSUM	24411	<i>Panthera leo</i>	Male	16
MSUM	11241	<i>Panthera leo</i>	Male	16
MSUM	11242	<i>Panthera leo</i>	Male	16
MSUM	11675	<i>Panthera leo</i>	Male	16
MSUM	3949	<i>Panthera leo</i>	Male	16
MSUM	29988	<i>Panthera leo</i>	Male	16
FMNH	20762	<i>Panthera leo</i>	Male	16
MSUM	21884	<i>Panthera leo</i>	Male	16
MSUM	10658	<i>Puma concolor</i>	Female	4
MSUM	3810	<i>Puma concolor</i>	Female	16
MSUM	3811	<i>Puma concolor</i>	Female	16
MSUM	6321	<i>Puma concolor</i>	Female	16
MSUM	6322	<i>Puma concolor</i>	Female	16
MSUM	6325	<i>Puma concolor</i>	Female	16
MSUM	12387	<i>Puma concolor</i>	Female	16
MSUM	10659	<i>Puma concolor</i>	Male	16
MSUM	12240	<i>Puma concolor</i>	Male	16
MSUM	13071	<i>Puma concolor</i>	Male	16
MSUM	14361	<i>Puma concolor</i>	Male	16

MSUM	14363	<i>Puma concolor</i>	Male	16
MSUM	14365	<i>Puma concolor</i>	Male	16
MSUM	35996	<i>Puma concolor</i>	Male	16

Appendix 2:

Michigan State University Museum (MSUM); University of Michigan Museum of Zoology (UMMZ); National Museum of Natural History, Smithsonian Institution (NMNH)

Location	Catalog No.	Genus and species	Sex
MSUM	2861	<i>Nasua nasua</i>	Female
MSUM	3564	<i>Nasua nasua</i>	Female
MSUM	9350	<i>Nasua nasua</i>	Female
NMNH	36609	<i>Nasua narica</i>	Female
UMMZ	41773	<i>Nasua nasua</i>	Female
UMMZ	61301	<i>Nasua nasua</i>	Female
UMMZ	63163	<i>Nasua nasua</i>	Female
UMMZ	107922	<i>Nasua nasua</i>	Female
NMNH	305	<i>Nasua narica</i>	Male
MSUM	9346	<i>Nasua nasua</i>	Male
MSUM	9348	<i>Nasua nasua</i>	Male
MSUM	9349	<i>Nasua nasua</i>	Male
MSUM	11921	<i>Nasua nasua</i>	Male
MSUM	11937	<i>Nasua nasua</i>	Male
MSUM	14369	<i>Nasua nasua</i>	Male
MSUM	14370	<i>Nasua nasua</i>	Male
MSUM	14371	<i>Nasua nasua</i>	Male
MSUM	9358	<i>Potos flavus</i>	Female
MSUM	9359	<i>Potos flavus</i>	Female
MSUM	9374	<i>Potos flavus</i>	Female
MSUM	11923	<i>Potos flavus</i>	Female
MSUM	11932	<i>Potos flavus</i>	Female
MSUM	14729	<i>Potos flavus</i>	Female
MSUM	14730	<i>Potos flavus</i>	Female

MSUM	9352	<i>Potos flavus</i>	Male
MSUM	9355	<i>Potos flavus</i>	Male
MSUM	9357	<i>Potos flavus</i>	Male
MSUM	9365	<i>Potos flavus</i>	Male
MSUM	9376	<i>Potos flavus</i>	Male
MSUM	11933	<i>Potos flavus</i>	Male
MSUM	14719	<i>Potos flavus</i>	Male
MSUM	2279	<i>Procyon lotor</i>	Female
MSUM	2331	<i>Procyon lotor</i>	Female
MSUM	2332	<i>Procyon lotor</i>	Female
MSUM	12841	<i>Procyon lotor</i>	Female
MSUM	20148	<i>Procyon lotor</i>	Female
UMMZ	44934	<i>Procyon lotor</i>	Female
UMMZ	111168	<i>Procyon lotor</i>	Female
MSUM	2246	<i>Procyon lotor</i>	Male
MSUM	2280	<i>Procyon lotor</i>	Male
MSUM	2281	<i>Procyon lotor</i>	Male
MSUM	4653	<i>Procyon lotor</i>	Male
MSUM	5108	<i>Procyon lotor</i>	Male
UMMZ	62907	<i>Procyon lotor</i>	Male
UMMZ	170525	<i>Procyon lotor</i>	Male

REFERENCES

REFERENCES

- Adolphs R (2001): The neurobiology of social cognition. *Curr Opin Neurobiol* 11:231-239.
- Aiello LC and Wheeler P(1995): The expensive tissue hypothesis—the brain and the digestive-system in human and primate evolution *Curr. Anthropol* 36 199–221.
- Allen, Thomas B (1987): Wild Animals of North America. The National Geographic Society, 1987. 246-247.
- Allen JS, Damasio H, Grabowski TJ (2002): Normal neuroanatomical variation in the human brain: an MRI-volumetric study. *Am J Phys Anthropol* 118:341-358.
- Allman J, McLaughlin T, and Hakeem A (1993): Brain weight and life-span in primate species. *Proceedings of the National Academy of Sciences USA* 90: 118–122.
- Allman JM (1999): *Evolving Brains*. W.H. Freeman and Co., New York.
- Amodio DM, Frith CD (2006): Meeting of minds: the medial frontal cortex and social cognition. *Nat Rev Neurosci* 7:268-277.
- Andersen BB, Korbo L, Pakkenberg B (1992): A quantitative study of the human cerebellum with unbiased stereological techniques. *J Comp Neurol* 326:549 –560.
- Arsznov BM, Lundrigan BL, Holekamp KE, Sakai ST (2010): Sex and the frontal cortex: A developmental CT study in the spotted hyena. *Brain Behav Evol* 76:185-197.
- Arsznov BM and Sakai ST (2012): Pride diaries: sex, brain size and sociality in the African lion (*Panthera leo*) and cougar (*Puma concolor*). *Brain Behav Evol* 79:275-89.
- Baron GH, Stephan H, and Frahm HD (1996): Comparative neurobiology. In *Chiroptera*. 3 Volumes. Birkhäuser, Basel.
- Barrickman NL, Bastian M, Isler K, van Schaik, C (2008): Life history costs and benefits of increased brain size: a comparative test using primates. *Journal of Human Evolution* 54:568-590.
- Barton RA (1996): Neocortex size and behavioural ecology in primates. *Proceedings of the Royal Society of London Series B-Biological Sciences* 263:173-177.
- Barton RA and Dunbar RIM (1997): Evolution of the social brain. In *Machiavellian intelligence II: extensions and evaluations*. Evolution of the social brain. In *Machiavellian intelligence II: extensions and evaluations*.

- Barton RA (1998): Visual specialization and brain evolution in primates. *Proceedings of the Royal Society B-Biological Science* 265, 1409: 1933-1937.
- Barton RA and Harvey P (2000): Mosaic evolution of brain structure in mammals. *Nature* 405:1055 - 1058.
- Bekoff M, Daniels TJ, and Gittleman JL (1984): Life history patterns and the comparative social ecology of carnivores. *Annu. Rev. Ecol. Syst* 15: 191-232.
- Bertram R and Peters A. (2002): *The cat primary visual cortex*. San Diego, CA: Academic Press.
- Blair RJ (2004): The roles of orbital frontal cortex in the modulation of antisocial behavior. *Brain Cogn.* 55(1): 198-208.
- Boesch C and Boesch H (1984): Possible causes of sex-differences in the use of natural hammers by wild chimpanzees. *Journal of Human Evolution* 5:415 - 440.
- Boydston E, Kapheim K, Van Horn R, Smale L, Holekamp K (2007): Sexually dimorphic patterns of space use throughout ontogeny in the spotted hyaena (*Crocuta crocuta*). *Journal of Zoology* 267:271-281.
- Breedlove SM (1994): Sexual differentiation of the human nervous system. *Annu Rev Psychol* 45:389-418.
- Brodmann K (1909): *Localisation in the cerebral cortex*, trans. Garey LJ (1999). London: Imperial College Press.
- Brutkowski S (1965): Functions of prefrontal cortex in animals. *Physiol Rev* 45:721-746.
- Brutkowski S, Davrowska J (1963): Disinhibition after prefrontal lesions as a function of duration of intertrial intervals. *Science* 139:505-506.
- Budeau DA and Verts BJ (1986): Relative brain size and structural complexity of habitats of chipmunks. *Journal of Mammalogy*, 67:579-581.
- Bufkin JL and Luttrell VR (2005): Neuroimaging studies of aggressive and violent behavior: current findings and implications for criminology and criminal justice. *Trauma Violence Abuse* 6:176-191.
- Busch R (1996): *The cougar almanac*. New York, New York, USA: Lyons and Burford.
- Bush EC and Allman JM (2004): The scaling of frontal cortex in primates and carnivores. *P Natl Acad Sci USA* 101:3962-3966.
- Byrne R and Whiten A (1988): Machiavellian intelligence: Social expertise and the evolution of intellect in monkeys, apes, and humans.

- Byrne RW (1994): The evolution of intelligence. In: Slater PJB, Halliday TR, editors. Behaviour and Evolution. Cambridge: Cambridge University Press. pp. 223-265.
- Byrne RW (1995): *The Thinking Ape*. Oxford, UK: Oxford Univ. Press.
- Byrne R, Corp N (2004): Neocortex size predicts deception rate in primates. *Proc. R. Soc. B.* 271:1693 - 1699.
- Cahalane DJ, Charvet CJ, and Finlay BL (2012): Systematic, balancing gradients in neuron density and number across the primate isocortex. *Frontiers in Neuroanatomy.* 6(28): 1-12.
- Changizi MA (2003): The brain from 25,000 feet: High level explorations of brain complexity, Perception, Induction and Vagueness. Kluwer Academic, Dordrecht.
- Clark DA, Mitra PP, and Wang SS. (2001): Scalable architecture in mammalian brains. *Nature.* 10;411(6834):189–193.
- Clutton-Brock T and Harvey P (1980): Primates, brains and behavior. *Journal of Zoology* London 190:309 - 323.
- Collins CE, Airey DC, Young NA, Leitch DB, and Kaas JH (2010): Neuron densities vary across and within cortical areas in primates. *Proc.Natl.Acad.Sci. U.S.A.* 107,15927–15932.
- Deaner RO, Nunn CL, and van Schaik CP (2000): Comparative tests of primate cognition: different scaling methods produce different results. *Brain Behav Evol* 55: 44–52.
- DeCarli C, Massaro J, Harvey D, Hald J, Tullberg M, Au R, Beiser A, D'Agostino R, and Wolf PA (2005): Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. *Neurobiol Aging* 26:491-510.
- Dechmann DKN and Safi K (2009): Comparative studies of brain evolution: a critical insight from the Chiroptera. *Biological Reviews* 84: 161-172.
- Deets AC, Harlow HF, Singh S D, and Blomquist AJ (1970): Effects of bilateral lesions of the frontal granular cortex on the social behavior of Rhesus monkeys. *Journal of Comparative and Physiological Psychology*, 72, 452–61.
- Dunbar RIM (1992): Neocortex size as a constraint on group size in primates. *Journal of Human Evolution* 20:469-493.
- Dunbar RIM (1998): The social brain hypothesis. *Evol Anthro* 6:178-190.
- Dunbar RIM and Bever J (1998): Neocortex size predicts group size in carnivores and some insectivores. *Ethology* 104:695-708.

- Dunbar RIM (2003): The social brain: mind, language and society in evolutionary perspective. *Annu. Rev. Anthropol.* 325:163-181.
- Dunbar RIM and Shultz S (2007): Evolution in the social brain. *Science* 317: 1344-1347.
- Dunbar RIM (2011): Evolutionary basis of the social brain. In *Oxford handbook of social neuroscience* (eds J. Decety & J. Cacioppo) pp. 28–38. Oxford, UK: Oxford University Press.
- East M and Hofer H (2001): Male spotted hyenas (*Crocuta crocuta*) queue for status in social groups dominated by females. *Behav Ecol* 12:558-568.
- Eisenberg JF and Wilson DE (1978): Relative brain size and feeding strategies in the Chiroptera. *Evolution* 32: 740-751.
- Eisenberg JF (1981): *The Mammalian Radiations - An Analysis of Trends in Evolution, Adaptation, and Behavior*, The University of Chicago Press.
- Ellis L, Herschberger S, Field E, Wersinger S, Pellis S, Geary D, Palmer C, Hoyenga K, Hetsroni A, and Karadi K (2008): *Sex Differences: Summarizing More Than a Century of Scientific Research*. New York: Psychology Press.
- Elston GN, Oga T, and Fujita I (2009): Spinogenesis and pruning scales across functional hierarchies. *J. Neurosci.* 29, 3271–3275.
- Ewer R (1976): *The carnivores*. Ithaca, NY: Cornell University Press.
- Fellows LK (2007): The role of orbitofrontal cortex in decision making. *Ann N Y Acad Sci* 1121: 421-430.
- Finarelli JA and Flynn JJ (2009): Brain size evolution and sociality in Carnivora. *Proceedings of the National Academy of Sciences of the United States of America* 106: 9345-9349.
- Finarelli JA (2006): Estimation of endocranial volume through the use of external skull measures in the Carnivora (Mammalia). *Journal of Mammalogy* 87: 1027-1036.
- Finlay BL and Slattery M (1983): Local differences in the amount of early cell death in neocortex predict adult local specializations. *Science* 219:1349 –1351.
- Finlay BL and Darlington RB (1995): Linked regularities in the development and evolution of mammalian brains. *Science* 268:1578-1584.
- Finlay BL, Darlington RB, and Nicastro N (2001): Developmental structure in brain evolution. *Behavioral and Brain Sciences* 24:263-308.

- Frank L (1986): Social organization of the spotted hyaena (*Crocuta crocuta*): II. Dominance and reproduction. *Animal Behavior* 35:1510-1527.
- Fuster JM (2002): Frontal lobe and cognitive development. *J Neurocytol* 31:373-385.
- Ghez C and Fahn S (1985): The cerebellum. In: *Principles of Neural Science* (Kandel E, Schwartz J, eds), pp 502-522. New York: Elsevier.
- Gibson K (1986): Cognition, brain size, and the extraction of embedded resources. In: *Primate ontogeny, cognition and social behaviour* (Else J, Lee P, eds), pp 93 - 104. Cambridge, UK: Cambridge University Press.
- Gittleman JL (1986): Carnivore brain size, behavioral ecology, and phylogeny. *Journal of Mammalogy* 67: 23-36.
- Gittleman JL (1994): Female brain size and parental care in carnivores. *Proc. Natl Acad. Sci. USA*. 91, 5495–5497.
- Goldman-Rakic PS (1987): Development of cortical circuitry and cognitive function. *Child Dev*. 58, 601–622.
- Gompper ME (1995): *Nasua narica*. *Mammalian Species* 487:1-10.
- Gompper ME (1996): Foraging costs and benefits of coati (*Nasua narica*) sociality and asociality. *Behavioral Ecology* 7:254-263.
- Gompper ME and Decker D (1998): *Nasua nasua*. *Mammalian Species* 580:1-9.
- Gorska T (1974): Functional organization of cortical motor areas in adult dogs and puppies. *Acta Neurobiol Exp (Wars)* 34:171-203.
- Gould SJ (1975): Allometry in primates, with emphasis on scaling and the evolution of the brain. *Contributions to Primatology* 5: 244-292.
- Gundersen HJ, Jensen EB, Kieu K, and Nielsen J (1999): The efficiency of systematic sampling in stereology—reconsidered. *J. Microsc.* 193, 199–211.
- Gur RC, Gunning-Dixon F, Bilker WB, and Gur RE (2002): Sex differences in temporo-limbic and frontal brain volumes of healthy adults. *Cereb Cortex* 12:998-1003.
- Hanby J and Bygott J (1987): Emigration of subadult lions. *Anim Behav* 35:161-169.
- Hardin WB Jr, Arumugasamy N, and Jameson HD (1968): Pattern of localization in 'precentral' motor cortex of raccoon. *Brain Res* 11:611-627.

- Hart BL, Hart LA, and Pinter-Wollman N (2008): Large brains and cognition: Where do elephants fit in? *Neuro. & Biobehav. Rev.* 32(1): 86-98.
- Hassler R and Muhs-Clement K (1964): [Architectonic Construction Of The Sensomotor And Parietal Cortex In The Cat]. *J Hirnforsch* 20:377-420.
- Haug H (1987): Brain sizes, surfaces, and neuronal sizes of the cortex cerebri: A stereological investigation of man and his variability and a comparison with some mammals (primates, whales, marsupials, insectivores, and one elephant) *American Journal of Anatomy* 180 (2), 126-142.
- Hauver SA, Gehrt SD, Prange S, and Dubach J (2010): Behavioral and genetic aspects of the raccoon mating system. *Journal of Mammalogy* 91: 749-757.
- Healy SD and Rowe C (2007): A critique of comparative studies of brain size. *Proc. R. Soc. B.* 274 (1609): 453-464.
- Henschel J and Skinner J (1987): Social relationships and dispersal patterns in a clan of spotted hyaenas (*Crocuta crocuta*) in the Kruger National Park. *S Afr J Zool* 22:18-24.
- Herculano-Houzel S and Lent R (2005): Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. *J. Neurosci.* 25, 2518–2521.
- Herculano-Houzel S, Collins CE, Wong P, Kaas JH, and Lent R (2008): The basic non-uniformity of the cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12593–12598.
- Herculano-Houzel S (2011): Not all brains are made the same: new views on brain scaling in evolution. *Brain Behav Evol.* 78(1):22-36.
- Hirsch BT (2011): Within-group spatial position in ring-tailed coatis: balancing predation, feeding competition, and social competition. *Behavioral Ecology and Sociobiology* 65:391-399.
- Hirsch BT, Stanton MA, and Maldonado JE (2012): Kinship shapes affiliative social networks but not aggression in ring-tailed coatis. *PLoS One.* 7(5): e37301.
- Holekamp KE, Sakai ST, and Lundrigan BL (2007): Social intelligence in the spotted hyena (*Crocuta crocuta*). *Philos Trans R Soc Lond B Biol Sci* 362:523-538.
- Humphrey NK (1976): The social formation of intellect. In: *Growing Points in Ethology* (P. Bateson & R. Hinde E, ed), pp 303-317. Cambridge: Cambridge University Press.
- Innocenti GM, Manger P, Masiello I, Colin I, and Tettoni L (2002): Architecture and callosal connections of visual areas 17, 18, 19 and 21 in the ferret (*Mustella putorius*). *Cerebral Cortex* 12, 411–422.

- Iversen SD and Mishkin M (1970): Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity. *Exp Brain Res* 11:376-386.
- Iwaniuk AN, Pellis SM, and Whishaw IQ (1999): Brain size is not correlated with forelimb dexterity in fissiped carnivores (Carnivora): A comparative test of the principle of proper mass. *Brain Behavior and Evolution* 54: 167-180.
- Iwaniuk AN and Nelson JE (2002): Can endocranial volume be used as an estimate of brain size in birds? *Can J Zool* 80:16-23.
- Iwaniuk AN (2010): Comparative brain collections are an indispensable resource for evolutionary neurobiology. *Brain Behav Evol* 76:87-88.
- Janis C (1990): Correlation of cranial and dental variables with body size in ungulates and macropodoids. In: *Body Size in Mammalian Paleobiology, Estimations and Biological Implications* (Damuth J, BJ M, eds), pp 255-300. Cambridge: Cambridge University Press.
- Jerison H (1973): *Evolution of the Brain and Intelligence*. London: Academic Press.
- Jerison H (2007): What fossils tell us about the evolution of the neocortex. In: *Evolution of Nervous System* (Kaas J, Krubitzer L, eds). New York: Elsevier.
- Joffe TH (1997): Social pressures have selected for an extended juvenile period in primates. *Journal of Human Evolution*. 32(6): 593-605.
- Joffe TH and Dunbar RIM (1997): Visual and socio-cognitive information processing in primate brain evolution. *Proceedings of the Royal Society B-Biological Science* 264, 1386: 1303-1307.
- Johnson WE (2006): The Late Miocene Radiation of Modern Felidae: A Genetic Assessment. *Science* 311: 73-77.
- Jolly A (1966): Lemur social behavior and primate intelligence. *Science* 153:501-506.
- Juliano SL, Palmer SL, Sonty RV, Noctor S, and Hill GF, 2nd (1996): Development of local connections in ferret somatosensory cortex. *J Comp Neurol* 374:259-277.
- Kaas JH, Merzenich MM, and Killackey HP (1983): The reorganization of somatosensory cortex following peripheral nerve damage in adult and developing mammals. *Annu. Rev. Neurosci.* 6: 325-56.
- Kaas JH (1993): The functional organization of somatosensory cortex in primates. *Anat Embryol* 175:509-518.

- Kaas JH (2000): Why is brain size so important: design problems and solutions as neocortex gets bigger or smaller. *Brain and Mind* 1: 7-23.
- Kalia M and Whitteridge D (1973): The visual areas in the splenial sulcus of the cat. *J. Physiol.* 232, 275-283.
- Kaufmann JH (1962): Ecology and social behavior of the coati *Nasua narica*, on Barro Colorado Island, Panama. *University of California Publications of Zoology* 60: 95-222.
- Kaufmann JH (1982): Raccoon and allies. Pp. 567-585. *In: Wild Mammals of North America: Biology, Management, and Economics*. J. A. Chapman, and G.A. Feldhamer, (eds.). The Johns Hopkins Univ. Press, Baltimore.
- Kawamura J (1971): Variations of the cerebral sulci in the cat. *Acta Anat* 80:204-221.
- Kays RW and Gittleman JL (1995): Home-range size and social behavior of kinkajous (*Potos flavus*) in the Republic of Panama. *Biotropica* 27: 530-534.
- Kays R and Gittleman J (2001): The social organization of the kinkajou *Potos flavus* (Procyonidae). *Journal of Zoology London* 253:491-504.
- Kleiman DG and Eisenberg JF (1973): Comparisons of canid and felid social systems from an evolutionary perspective. *Anim Behav* 21:637-659.
- Korbo L, Pakkenberg B, Ladefoged O, Gundersen HJ, Arlien-Soborg P, and Pakkenberg H (1990): An efficient method for estimating the total number of neurons in rat brain cortex. *J Neurosci Methods* 31:93–100.
- Kosmal A (1981): Subcortical connections of the prefrontal cortex in dogs: Afferents to the preorel gyrus. *Acta Neurobiol. Exp.* 41: 69-85.
- Kosmal A, Markow G, and Stepniewska I (1984): The presylvian cortex as a transitional prefronto-motor zone in dog. *Acta Neurobiol. Exp.* 44: 273-287.
- Krubitzer L, Kunzle H, and Kaas J (1997): Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J. Comp. Neurol.* 379: 399-414.
- Krubitzer L, Campi K, and Cooke DF (2011): All rodents are not the same: A modern synthesis of cortical organization. *Brain Behav Evol* 78:51-93.
- Kruuk H (1972): *The Spotted Hyena: A Study of Predation and Social Behavior*. Chicago: University of Chicago Press.
- Kudo H and Dunbar RIM (2001): Neocortex size and social network size in primates. *Animal Behavior* 62:711 – 722.

- Laughlin SB, de Ruyter van Steveninck RR, Anderson JC (1998): The metabolic cost of neural information. *Nat Neurosci* 1:36–41.
- Law MI, Zahs KR, and Stryker MP (1988): Organization of primary visual cortex (area 17) in the ferret. *J Comp Neurol* 278: 157-180.
- Lefebvre L, Whittle P, Lascaris E, and Finkelstein A (1997): Feeding innovations and forebrain size in birds. *Animal Behaviour*, 53, 549–560.
- Lemen C (1980): Relationship between relative brain size and climbing ability in *Peromyscus*. *J. Mamm.*, 61:360-364.
- Leonard CM, Towler S, Welcome S, Halderman LK, Otto R, Eckert MA, and Chiarello C (2008): Size matters: cerebral volume influences sex differences in neuroanatomy. *Cereb Cortex* 18:2920-2931.
- Lewis K (2001): A comparative study of primate play behaviour: implications for the study of cognition. *Folia Primat.* 71:417–21.
- Logan K and Sweanor L (2001): Desert puma: evolutionary ecology and conservation of an enduring carnivore. Covelo, California: Island Press.
- Lomber SG, Payne BR, Cornwell P, and Long KD (1996): Perceptual and cognitive visual functions of parietal and temporal cortices in the cat. *Cereb Cortex* 6: 673-695.
- Luders E, Toga AW, Lepore N, and Gaser C (2009): The underlying anatomical correlates of long-term meditation: larger hippocampal and frontal volumes of gray matter. *Neuroimage* 45:672-678.
- Marino L (1996): What can dolphins tell us about primate evolution? *Evol. Anthropol.* 5:81-86.
- McClearn D (1992): Locomotion, posture, and feeding behavior of kinkajous, coatis, and raccoons. *Journal of Mammalogy*, 73(2): 245-261.
- McComb JG, Withers GJ, and Davis RL (1981): Cortical damage from Zenker's solution applied to the dura mater. *Neurosurgery* 8:68-71.
- McLaughlin DF, Sonty RV, and Juliano SL (1998): Organization of the forepaw representation in ferret somatosensory cortex. *Somatosens Mot Res* 15:253–268.
- McNab BK, and Eisenberg J F (1989): Brain size and its relation to the rate of metabolism in mammals. *American Naturalist*, 133, 157-167.
- Mills M (1990): Kalahari hyenas: the behavioural ecology of two species.: Unwin Hyman, London.

- Milton K (1981): Distribution patterns of tropical plant food as evolutionary stimulus to primate mental development. *American Anthropology* 83:534 - 548.
- Mishkin M (1964): Perseveration of central sets after frontal lesions in monkeys. In: *The Frontal Granular Cortex and Behavior* (Warren J, Akert K, eds), pp 219-241. New York: McGraw Hill.
- Morris J, Jordan C, and Breedlove M (2008): Sexual Dimorphism in Neuronal Number of the Posterodorsal Medial Amygdala Is Independent of Circulating Androgens and Regional Volume in Adult Rats. *Journal of Comparative Neurology* 506:851 - 859.
- Mosser A and Packer C (2009): Group territoriality and the benefits of sociality in the African lion, *Panthera leo*. *Animal Behavior* 78:359-370.
- Myasnikov AA, Dykes RW, and Leclerc SS (1997): Correlating cytoarchitecture and function in cat primary somatosensory cortex: the challenge of individual differences. *Brain Res* 750:95-108.
- Nieoullon A and Rispal-Padel L (1976): Somatotopic localization in cat motor cortex. *Brain Res* 105:405-422.
- Nopoulos P, Flaum M, O'Leary D, and Andreasen NC (2000): Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. *Psychiatry Res* 98:1-13.
- Nowak R and Paradiso J (1983): *Walker's Mammals of the World*. Baltimore: Johns Hopkins University Press.
- Packer C and Pusey A (1987): Intrasexual Cooperation and the Sex Ratio in African Lions. *The American Naturalist* 130:636-642.
- Packer C, Pusey AE, and Eberly LE (2001): Egalitarianism in female African lions. *Science* 293:690-693.
- Packer C, Scheel D, and Pusey AE (1990): Why lions form groups: Food is not enough. *American Naturalist* 136:1-19.
- Pakkenberg B and Gundersen HJ (1997): Neocortical neuron number in humans: effect of sex and age. *J Comp Neurol* 384:312-320.
- Parker ST and Gibson KR (1977): Object manipulation, tool use, and sensorimotor intelligence as feeding adaptations in cebus monkeys and great apes. *Journal of Human Evolution* 6: 623-641.
- Pawlowski BP, Lowen CB, and Dunbar RIM (1998): Neocortex size, social skills and mating success in primates. *Behaviour* 135:357-68.

- Perez-Barberia FJ, Shultz S, and Dunbar RIM (2007): Evidence for coevolution of sociality and relative brain size in three orders of mammals. *Evolution* 61: 2811-2821.
- Povinelli D and Cant J (1995): Arboreal clambering and the evolution of self-concept. *Quarterly Review Biology* 70:392 - 421.
- Povinelli D and Preuss T (1995): Theory of mind: evolutionary history of a cognitive specialization. *Trends in Neuroscience* 18:418 - 424.
- Powell J, Lewis PA, Dunbar RIM, García-Fiñana M, and Roberts N (2010): Orbital prefrontal cortex volume correlates with social cognitive competence. *Neuropsychologia* 48, 3554–3562.
- Pubols BH, Welker WI, and Johnson JI (1965): Somatic sensory representation of fore-limb in dorsal root fibers of raccoon, coatimundi, and cat. *J. Neurophysiol* 28:312-341.
- Pusey A and Packer C (1987): The evolution of sex-biased dispersal in lions. *Behaviour* 101:275-310.
- Radinsky L (1969): Outlines of canid and felid brain evolution. *Ann NY Acad Sci* 167:277-288.
- Radinsky L (1978): Evolution of brain size in carnivores and ungulates. *American Naturalist* 112:815–831.
- Radinsky L (1984): Basicranial axis length v. skull length in analysis of carnivore skull shape. *Biological Journal of the Linnean Society* 22:31-41.
- Reader S and Laland K (2002): Social intelligence, innovation, and enhanced brain size in primates. *Proc. Natl. Acad. Sci.* 99:4436 - 4441.
- Ricard AS, Desbarats P, Laurentjoye M, Montaudon M, Caiz P, Dousset V, Majoufre-Lefebvre C, and Maureille B (2010): On two equations about brain volume, cranial capacity and age. *Surg Radiol Anat* 32: 989-995.
- Rilling JK and Insel TR (1998): Evolution of the cerebellum in primates: differences in relative volume among monkeys, apes and humans. *Brain Behav Evol* 52:308-314.
- Ringo JL (1991): Neuronal interconnection as a function of brain size. *Brain Behav. Evol.* 38,1-6.
- Rockel AJ, Hiorns RW, and Powell TP (1980): The basic uniformity in structure of the neocortex. *Brain* 103:221–244.
- Rodrigues C (1991): Anatomical differences involving the archicortex and the neocortex of male and female brains: A quantitative study of 20 brains of each sex. *Hum Evo* 6:451-459.

- Romero T and Aureli F (2007): Spatial association and social behaviour in zoo-living female ring-tailed coatis (*Nasua nasua*). *Behaviour* 144: 179-193.
- Roth G and Dicke U (2005): Evolution of the brain and intelligence. *Trends Cogn Sci* 9(5):250-7.
- Russell JK (1981): Exclusion of adult male coatis from social groups: Protection from predation. *Journal of Mammalogy* 62:206-208.
- Sakai ST (1982): The thalamic connectivity of the primary motor cortex (MI) in the raccoon. *J Comp Neurol* 204:238-252.
- Sakai ST (1990): Corticospinal projections from areas 4 and 6 in the raccoon. *Exp Brain Res* 79:240-248.
- Sakai ST, Stanton GB, and Isaacson LG (1993): Thalamic afferents of area 4 and 6 in the dog: a multiple retrograde fluorescent dye study. *Anat Embryol (Berl)* 188:551-559.
- Sakai ST, Arsznov BM, Lundrigan BL, and Holekamp KE (2011a): Brain size and social complexity: a computed tomography study in hyaenidae. *Brain Behav Evol* 77:91-104.
- Sakai ST, Arsznov BM, Lundrigan BL, and Holekamp KE (2011b): Virtual endocasts: an application of computed tomography in the study of brain variation among hyenas. *Ann N Y Acad Sci* 1225 Suppl 1:E160-170.
- Sakai ST, Stanton GB, and Isaacson LG (1993): Thalamic afferents of area 4 and 6 in the dog: a multiple retrograde fluorescent dye study. *Anat Embryol (Berl)* 188:551-559.
- Sawaguchi T and Kudo H (1990): Neocortical development and social-structure in primates. *Primates* 31:283 - 289.
- Shepherd GM (2011): The microcircuit concept applied to cortical evolution: from three-layer to six layer cortex. *Front. Neuroanat.*, 5 (30): 1-15.
- Shultz S and Dunbar RIM (2007): The evolution of the social brain: anthropoid primates contrast with other vertebrates. *Proc Biol Sci* 274:2429-2436.
- Smale L, Nunes S, and Holekamp KE (1997): Sexually dimorphic dispersal in mammals: Patterns, causes, and consequences. In: *Advances in the Study of Behavior* (Slater P, Rosenblatt J, Snowden C, Milinski M, eds), vol 26, pp 181-250. San Diego: Academic Press.
- Smith GE (1912): The evolution of man. *Smithsonian Institution Annual Report*. Washington DC: Smithsonian Institution.

- Snyder D (1970): Fall from social dominance following orbital frontal ablation in monkeys. Proc. 78th Annu. Convention (Washington), Amer. Psychol. Ass., 235-236.
- Stanton GB, Tanaka D Jr, Sakai ST, and Weeks OI (1986): Thalamic afferents to cytoarchitectonic subdivisions of area 6 on the anterior sigmoid gyrus of the dog: a retrograde and anterograde tracing study. J Comp Neurol 252:446-467.
- Stephan H, Frahm H, and Baron G (1981): New and revised data on volumes of brain structures in insectivores and primates. Folia Primatol 35:1-29.
- Striedter GF (2005): Principles of brain evolution. Sunderland, MA: Sinauer Associates.
- Sunquist M and Sunquist F (2002): Wild cats of the world: University of Chicago Press.
- Swanson EM, Holekamp KE, Lundrigan BL, Arsznov BM, and Sakai ST (2012): Multiple determinants of whole and regional brain volume among terrestrial carnivorans. PLoS One. 7(6):e38447.
- Tanaka D Jr (1987): Neostriatal projections from cytoarchitectonically defined gyri in the prefrontal cortex of the dog. J Comp Neurol 261:48-73.
- Tilson R and Hamilton W (1984): Social dominance and feeding patterns of spotted hyaenas. Animal Behavior 32:715-724.
- Vaughan T, Ryan J, and Czaplewski N (2000): *Mammalogy, 4th Edition*. Toronto: Brooks Cole.
- Van Dongen PAM (1998): Brain size in vertebrates. In *The Central Nervous System of Vertebrates* (Vol. 3) (Nieuwenhuys, R. *et al.*, eds), pp. 2099–2134, Springer Berlin
- Van Valkenburgh B (1990): Skeletal and dental predictors of body mass in carnivores. In: *Body Size in Mammalian Paleobiology, Estimations and Biological Implications* (Damuth J, MacFadden B, eds), pp 181-206. Cambridge: Cambridge University Press.
- Warren JM, Coutant LW, and Cornwall PR (1969): Cortical lesions and response inhibition in cats. Neuropsychologia 7: 245-257.
- Welker WI and Seidenstein S (1959): Somatic sensory representation in the cerebral cortex of the raccoon (*Procyon lotor*) The Journal of Comparative Neurology 111:469-501.
- Welker WI and Campos GB (1963): Physiological significance of sulci in somatic sensory cerebral cortex in mammals of the family Procyonidae. The Journal of Comparative Neurology 120: 19-36.
- Welker WI, Johnson JJ, and Pubols BH (1964): Some Morphological and Physiological Characteristics of the Somatic Sensory System in Raccoons. American Zoologist 4:75-94.

- Welker WI and Johnson JI (1965): Correlation between nuclear morphology and somatotopic organization in ventrobasal complex of the raccoon's thalamus. *J. Anat.* 99: 761-790.
- Welker WI (1990): The significance of foliation and fissuration of cerebellar cortex. The cerebellar folium as a fundamental unit of sensorimotor integration. *Arch Ital Biol* 128:87-109.
- West MJ, Slomianka L, and Gundersen HJ (1991): Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec* 231:482–497.
- West MJ (1999): Stereological methods for estimating the total number of neurons and synapses: issues of precision and bias. *Trends Neurosci* 22:51–61.
- Whishaw IQ, Sarna JR, and Pellis SM (1998): Evidence for rodent-common and species-typical limb and digit use in eating, derived from a comparative analysis of ten rodent species. *Behav Brain Res.* 96 (1-2): 79-91.
- Wood JL, Heitmiller D, Andreasen NC, Nopoulos P (2008): Morphology of the ventral frontal cortex: relationship to femininity and social cognition. *Cereb Cortex* 18:534-540.