

Primate Brain Anatomy: New Volumetric MRI Measurements for Neuroanatomical Studies

Ana F. Navarrete^{a, b} Erwin L.A. Blezer^c Murillo Pagnotta^a
Elizabeth S.M. de Viet^b Orlin S. Todorov^b Patrik Lindenfors^{d, e}
Kevin N. Laland^a Simon M. Reader^{b, f}

^aCentre for Social Learning and Cognitive Evolution, School of Biology, University of St. Andrews, St. Andrews, UK; ^bDepartment of Biology and Helmholtz Institute, Utrecht University, Utrecht, The Netherlands; ^cBiomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, University Medical Center Utrecht, Utrecht, The Netherlands; ^dInstitute for Future Studies, Stockholm, Sweden; ^eCentre for Cultural Evolution & Department of Zoology, Stockholm University, Stockholm, Sweden; ^fDepartment of Biology, McGill University, Montreal, QC, Canada

Keywords

Primates · Brain architecture · Brain volume · Neocortex · Isocortex · Magnetic resonance imaging · Cognitive evolution · Comparative neuroanatomy

Abstract

Since the publication of the primate brain volumetric dataset of Stephan and colleagues in the early 1980s, no major new comparative datasets covering multiple brain regions and a large number of primate species have become available. However, technological and other advances in the last two decades, particularly magnetic resonance imaging (MRI) and the creation of institutions devoted to the collection and preservation of rare brain specimens, provide opportunities to rectify this situation. Here, we present a new dataset including brain region volumetric measurements of 39 species, including 20 species not previously available in the literature, with measurements of 16 brain areas. These volumes were extracted from MRI of 46 brains of 38 species

from the Netherlands Institute of Neuroscience Primate Brain Bank, scanned at high resolution with a 9.4-T scanner, plus a further 7 donated MRI of 4 primate species. Partial measurements were made on an additional 8 brains of 5 species. We make the dataset and MRI scans available online in the hope that they will be of value to researchers conducting comparative studies of primate evolution.

© 2018 The Author(s)
Published by S. Karger AG, Basel

Introduction

Comparative neuroanatomy has been used to address numerous evolutionary questions, from understanding the co-evolution of different brain areas to inferring the socio-ecological factors that have shaped brain evolution [Finlay and Darlington, 1995; Barton and Harvey, 2000; Finlay et al., 2001; Jerison, 2001; Dunbar and Shultz, 2007; Mars et al., 2014]. Much of this work has focussed on volumetric comparisons, although many other aspects of

neuroanatomy have been examined too [Herculano-Houzel et al., 2015; Mota and Herculano-Houzel, 2015]. While many comparative studies of brain enlargement focus on overall brain size, volumetric data on brain components is also vital, not least for establishing whether whole brain, network, or brain component measures provide better predictors for behavioural differences between species.

Brain anatomy in primates has been particularly extensively studied compared to most other vertebrate orders [although see, e.g., Baron et al., 1996], with numerous influential studies. However, volumetric data for specific brain areas is only available for a small percentage of the species belonging to the primate order [Reader and MacDonald, 2003; Mars et al., 2014]. The most complete dataset on primate brain anatomy, which included measures of a significant number of brain regions obtained from serial sections of primate brains, was published by Stephan and colleagues in the 1980s [Stephan et al., 1970, 1981, 1984, 1986, 1987; Frahm et al., 1984; Matano et al., 1985]. We henceforth refer to this as the “Stephan et al. dataset.” While it is an outstanding resource representing extensive and expert work, this dataset nonetheless has limitations [Powell et al., 2017]. In particular, only 44 species are covered, i.e., just 11% of known primate species [Wilson and Reeder, 2005]. Moreover, much of the data in this sample relies on just one or two individuals per species to provide measurements. For almost 25 years, the Stephan et al. dataset has been the largest source of brain component volumes for comparative analyses of primates, used in many studies of primate evolution, including very recent studies [e.g., Dunbar, 1998; Barton and Harvey, 2000; Finlay et al., 2001; Reader and Laland, 2002; Reader et al., 2011; Sandel et al., 2016].

This does not mean that no new primate data has become available over that period [Zilles et al., 2011; see Reader and MacDonald, 2003, for a compilation and discussion]. While many (perhaps most) publications on brain size have focused on macroscopic brain measures, such as overall brain mass [Clutton-Brock and Harvey, 1980; Harvey and Krebs, 1990; Pagel and Harvey, 1990] or endocranial volume [Isler et al., 2008; Smaers et al., 2012], in more specialized studies, especially those concerning research on brain functionality, measurements of specific brain structures of interest have been published [e.g., MacLeod et al., 2003; Bush and Allman, 2004; Sherwood et al., 2005; Smaers et al., 2010]. However, these studies rarely include species that were not already included in the Stephan et al. dataset, they tend to cover relatively few species, and differences in the methodology

used to obtain the measures of the Stephan et al. dataset and these studies render the combination of both problematic. Nevertheless, these studies have made substantial contributions to the Stephan et al. dataset, particularly in the addition of data on multiple individuals for certain primate species, in particular the great apes. Such additions have led to, e.g., reassessment of the assumption that the ratio of the different brain components is constant between conspecifics and has contributed to a greater understanding of intraspecific variation in brain anatomy in primates [Bogart et al., 2014].

One reason why the Stephan et al. dataset has not been greatly extended is the problem of specimen accessibility. Studies applying brain-scanning methods to live primate specimens are of considerable value in simultaneously testing cognitive performance and identifying the brain areas related to specific actions or forms of cognition. However, access to live animals is limited to a low number of primate species, such as marmosets, capuchins, macaques, and great apes, and the availability of species for such studies is expected to become more limited, rather than to increase, in the future [Rilling, 2014]. There are also ethical and practical problems in scanning live individuals for brain volumetric measurement. In contrast, post-mortem material for a wide range of primate species, in particular non-model species, derived from animals reared in zoological gardens and research institutes, is being eagerly collected by specialized institutions. Access to these primate brain collections has recently been made available for brain researchers [Kaas and van Eden, 2011; Zilles et al., 2011].

Traditional processing techniques for neuroanatomy provide precise and high-quality information but typically render specimens unavailable for further and/or complementary studies. For instance, Stephan and colleagues obtained their measures from serial sections of primate brains [Stephan et al., 1981]. This technique is time consuming and demands adequate infrastructure investments, and, once an area has been stained, it typically cannot be stained differently. More recently, there has been a rise in brain measurement studies using magnetic resonance imaging (MRI) as the primary tool, which resolves some of the problems associated with more traditional approaches [Mars et al., 2014]. In particular, MRI-based techniques have the advantage of not being destructive. MRI methods allow the use of living animals as specimens, which is of particular interest to researchers studying functional anatomy, as well as the repeated use of post-mortem samples using different and innovative techniques without damaging the sample [Rilling, 2014].

Another advantage of MRI is a reduction in the time needed to collect the image data, although the time required to analyse and summarise data from the images remains high. Thus, the use of post-mortem MRI in studies of primate brain evolution offers considerable promise [Mars et al., 2014]. However, there are still some weaknesses to MRI scans. Images obtained from living animals, where the specimen is sedated for a short period of time while its brain is being scanned, typically have poorer image quality and resolution than post-mortem MRI, and the accurate removal of non-brain tissue from images may be challenging [Wang et al., 2014]. Post-mortem MRI have the advantage of a higher resolution, and hence are better suited for extracting volumes, although unlike in vivo MRI there are concerns about damage caused by brain extraction, distortions generated by different extraction methods, delays between death and preservation, and the length of preservation of the brain [Mars et al., 2014]. Additionally, while Stephan's regions of interest could be delimited by changes in histological structure between regions, this finesse of detail cannot be applied to MRI, where the borders of an area can sometimes only be assessed by its position in relation to neighbouring structures. Nonetheless, these concerns are to some extent compensated by the greater reliability of analyses afforded by a larger sample of brains and could potentially be addressed in the future through statistical analyses.

Here, drawing on specimens from primate brain collections, we obtained high-resolution post-mortem MRI scans for a final sample of brain component volumes of 53 primate individuals from 39 primate species, 20 of which had never been detailed before in the literature. From these scans we extracted brain region volume data for 16 separate brain regions. This data is summarized in tabular form, both in this article and in the associated online databases, which also present the MRI scans themselves. We believe that the addition of these high resolution MRI to the pool of primate brain anatomical volumes will be a valuable contribution for the field of comparative neuroanatomy.

Materials and Methods

The Primate Brain Bank

Specimens were loaned with permission from the Netherlands Institute of Neuroscience Primate Brain Bank (PBB; <http://www.primatrainbank.org/>). At the time of this study the PBB contained 285 specimens of 48 species obtained from primates living in captivity donated by Dutch zoos and primate centres. No indi-

viduals are sacrificed for PBB brain issue. Instead, brains are collected from individuals that died from natural causes or that had to be humanely euthanized for reasons unrelated to the tissue collection. Thus the PBB allows brain tissue to be preserved that would otherwise go to waste. PBB preservation protocols consist of fixation, preferably by perfusion, with 4% buffered formaldehyde (followed by immersion in 4% buffered formaldehyde overnight if necessary), and storage at 4 °C in a buffered 15% sucrose solution. These conditions allow high-resolution post-mortem imaging. The main goal of the PBB is to facilitate research on primate behaviour and brain evolution [Kaas and van Eden, 2011].

Individual information made available by the PBB, depending on the specimen, included social and medical history, age, sex, post-mortem delay until brain fixation, and brain mass after fixation (online Suppl. Table S1; for online suppl. material, see www.karger.com/doi/10.1159/000488136). Our aims were to increase the number of primate species for which comparative brain component data were available and to increase the number of individuals per species measured. However, sample damage and availability meant that it was typically impossible to measure multiple individuals per species for this study. We prioritized the scanning of adult primate brains with minimal damage and minimal post-mortem delay. We chose species so as to have a sample that was taxonomically diverse, included species for which no published volumetric data was available [Stephan et al., 1981; Reader and MacDonald, 2003], and covered taxa of interest to researchers of cognitive evolution. If more than one specimen was available for a given species, we selected at least two individuals of different sex, if available. Brains were photographed and subjected to visual inspection prior to collection to account, if possible, for unnoticed damage or deterioration, and impaired specimens were not scanned.

Brain Scanning

Fifty-one PBB brains were imaged, i.e., 50 at the Neuroimaging Centre of Utrecht University and 1 (sample PB0111) at the F.C. Donders Centre for Cognitive Neuroimaging (Nijmegen, The Netherlands). Utrecht brain magnetic resonance imaging took place over two periods, i.e., February 2009 to July 2010 (approximately half of the samples imaged) and April 2012 to March 2013. For the scans conducted in Utrecht, brain specimens were transported from the PBB to the Center for Image Sciences at Utrecht University. Utrecht-imaged brains were prepared for imaging by a wash in sucrose solution and then placed inside an appropriately sized syringe and submerged in the zero MRI signal oil Fomblin (Solvay Specialty Polymers, Brussels, Belgium) to maintain the shape of the brain, to avoid the brains becoming compressed under their own weight, and to avoid air/tissue susceptibility MRI artefacts. Vacuum was used to extract air bubbles, which would have interfered with the imaging. Within the syringe, brains were supported in position with plastic rigid tubes. For Utrecht imaging, a Varian small-bore 9.4-T scanner (Varian NMR Instruments, Palo Alto, CA, USA) was used to obtain T1-weighted images. In total, each brain was scanned three times using different parameters for repetition time (TR) and echo time (TE). The first scan was exploratory, with a matrix of $512 \times 128 \times 128$ voxels, a TE of 5 ms, a TR of 30 ms, and an α of 10° , and it was used to assess image quality and internal brain damage. Severely damaged brains were not scanned further. After the first scan, the brains were scanned two additional times using a matrix of $512 \times 256 \times 256$ voxels, a TE of 5 ms, and an α of 10° . These scans only differed in TR (30 and 100

ms), with scan durations around 4.5 and 11 h, respectively. In the present study, the T1-weighted images taken with a TR of 100 ms, a TE of 5 ms, and an α of 10° were found to allow better discrimination of brain areas and they were used to obtain volumetric measures. Image resolution was proportional to the size of the brains, with voxel sizes ranging from 0.068 mm (*Microcebus*) to 0.37 mm (*Pan*). The PB0111 sample was imaged with a Siemens Magnetom trio 3-T scanner, used to obtain T1-weighted images, with a TR of 2,500 ms, a TE of 2.6 ms, an α of 10° , a matrix of $256 \times 256 \times 256$ voxels, and a voxel size of 0.7 mm.

MRI Scan Donations

C.C. Sherwood (George Washington University) provided MRI of 10 further primate brains (capuchins and great apes), which we measured to increase the number of species or the number of specimens per species in our sample (see online suppl. Table S1 for details). These images included post-mortem and in vivo MRI and varied in resolution. Because of this variability, we could not extract all volumes of interest from all samples and three samples were excluded from our main data compilation (Table 1; see online suppl. Table S1 for details).

Image Processing and Volume Extractions

Brain images were processed and measured by Ana Navarrete using the freeware MiPAV 7.0.1 (<http://mipav.cit.nih.gov/>) with a Wacom (Portland, OR, USA) PL-720 LCD tablet. Before delineating the regions of interest, all of the brain images were inspected for damage using the MiPAV tri-planar view. Most brains showed some kind of imperfection (see online suppl. Table S1 for details on each brain). Deformation of the inner structures, granulated surfaces, air bubbles, damaged and missing areas, and incomplete medullas were taken into consideration and reported to the PBB for future reference. After this assessment, 3 brains were discarded from measurement and are not included in the dataset. Five brains with lesser damage (PB0516, PB0203, PB0902, PB0720, and PB0609) were still measured to increase the sample and to obtain measures for regions of interest that were undamaged (see online suppl. Table S1 for details). These 5 brains were not included in our main data compilation (Table 1) or our analyses of measurement reliability.

Brains were realigned using the anterior and posterior commissures on the coronal axis (AC-PC axis) and the interhemispheric fissure and middle section of the medulla oblongata on the sagittal axis. Resolution for each axis was extracted for later calculations, and raw measurements of brain height, width, and length were obtained. File size was reduced by deleting blank slides in front of and behind the brain to facilitate processing. Longitudinal measures for height, width, and length of the brain were extracted using the tri-planar view in MiPAV. The next step was to recognize the structures of interest visually (see below for details), and record the first slide, the last slide, and the number of slides where the given structure appears. Using this information, at least 20 equidistantly spaced slides were selected to be worked on. This number of slides is sufficient to determine the volume of any object without exceeding an error of 5% [Zilles et al., 1982]. For smaller structures (e.g., lateral geniculate nuclei; LGN), or for larger structures in low-resolution MRI which were only visible in less than 20 slides, all slides were processed. The inter-slide space (i.e., the number of slides between sampled slides multiplied by the resolution of the sagittal axis) was recorded, and each region of interest was manu-

ally delineated. Afterwards, the sum of areas of the region of interest was extracted using the MiPAV statistics window. Volumes were obtained using the Cavalieri formula [Zilles et al., 1982]:

$$\text{Volume (mm}^3\text{)} = \text{sum of areas (mm}^2\text{)} \times \text{inter-slide space (mm)}.$$

We measured the following 13 brain regions: hemispheres (right and left), corpus callosum, striatum (right and left), claustrum (right and left), hippocampus (right and left), isocortex (i.e., neocortex, right and left), diencephalon, thalamus, LGN (right and left), cerebellum, mesencephalon, pons, and medulla oblongata. Three additional brain volumes were calculated by summing constituent regions. Telencephalon volumes were obtained by adding the volumes of the hemispheres and the corpus callosum. Brainstem volumes were obtained by adding the volumes of the mesencephalon, the pons, and the medulla oblongata. The total brain volume was obtained by adding the volumes of the telencephalon, the diencephalon, the cerebellum, and the brainstem.

To delimitate our regions of interest, we used primate stereological atlases and multiple other sources as references [Stephan et al., 1981; Semendeferi et al., 1997, 2001, 2002; Paxinos et al., 1999; Rilling and Insel, 1999; Semendeferi and Damasio, 2000; Semendeferi, 2001; Matochik et al., 2004; Sherwood et al., 2004; Crick and Koch, 2005; Schoenemann et al., 2005; Looi et al., 2008; Palazzi and Bordier, 2008; Tammer et al., 2009; Frey et al., 2011; Hikishima et al., 2011; Bauernfeind et al., 2013]. For the regions measured by Stephan et al. [1981], we followed their notes on which component parts to include, except where we measured these component parts individually. The definition of Stephan et al. [1981] of medulla oblongata included two regions, i.e., medulla oblongata and pons [Armstrong, 1985], that we measured individually. Stephan et al. [1981] noted that they measured only “pure” brain tissue, excluding ventricles, nerves, meninges, and other tissues. Working with MRI allowed us to exclude ventricles and meninges, but the image resolution and/or contrast was insufficient to distinguish nerves from brain tissue.

At the end of the data extraction from the MRI, the dataset included volumetric region measurements of 46 brains from the PBB collection (omitting the 5 brains that were excluded from our main dataset; see above) and 7 donated brain scans (omitting the 3 brains that were excluded from our main dataset). In total, we were able to collect data from 53 brains belonging to 39 species, including 20 species with no previously published brain component measurements. Not all volumetric measurements could be collected in all brains, either because the specimen MRI resolution was too low to identify the volume of interest satisfactorily or because the structure was damaged.

The inter-observer reliability of volumetric measures was assessed by comparison with three independent observers. We compared our measurements with those of a subset of 18 brains detailed in two unpublished Master's theses [de Viet, 2009; Todorov, 2010]. Additionally, we selected 5 brains (1 strepsirrhine, 2 platyrrhines and 2 catarrhines) to be measured again by an independent observer, i.e., Murillo Pagnotta, using the same references, software, and technological tools. Due to difficulties in reliably identifying landmarks across species, we do not report measurements for the pre-frontal cortex, frontal lobes, the amygdala, the insula, or the hypothalamus. We calculated inter-observer reliability as the percent difference between the separate measurements of different observers [Sherwood et al., 2004]. Inter-observer reliability was satisfactory (0–17% mean variance) for most structures. Large

Table 1. Mean brain component volumes for 39 primate species measured in the present study

Species	n	Source	Total brain	Telencephalon	Neocortex	Hippocampus	Striatum	Diencephalon	Cerebellum	Mesencephalon	Medulla	Pons
<i>Microcebus murinus</i>	1	PBB	1,696.29	1,036.36	763.02	70.31	63.74	174.61	257.44	94.87	125.08	7.93
<i>Galago senegalensis</i>	1	PBB	3,574.89	2,341.69	1,674.90	145.81	135.52	287.01	578.05	143.78	199.11	25.26
<i>Aotus lemurinus griseimembra</i>	1	PBB	14,646.57	11,045.66	7,632.45	403.77	564.73	971.92	1,701.99	304.00	524.07	98.93
<i>Aotus trivirgatus boliviensis</i>	1	PBB	16,920.08	13,091.83	9,675.47	443.08	631.77	1,122.09	1,623.46	320.79	630.78	131.13
<i>Ateles fusciceps robustus</i>	1	PBB	104,988.09	83,409.38	53,484.99	1,460.71	4,010.13	5,292.56	12,382.19	1,179.27	1,856.39	868.29
<i>Lagothrix lagotricha</i>	1	PBB	91,676.70	73,727.19	46,869.06	1,043.86	3,347.81	4,821.70	9,707.56	1,011.69	1,743.14	665.42
<i>Callimico goeldii</i>	1	PBB	12,112.34	9,129.15	6,191.43	220.57	474.49	877.72	1,270.39	328.47	446.25	60.37
<i>Callithrix geoffroyi</i>	1	PBB	7,098.63	5,444.31	4,057.73	166.26	282.56	432.39	713.35	210.90	259.50	38.17
<i>Callithrix jacchus</i>	1	PBB	8,124.76	6,314.91	4,541.98	203.19	334.26	521.46	686.23	219.00	347.28	35.88
<i>Callithrix penicillata</i>	2	PBB	6,335.88	4,694.76	3,264.95	160.39	252.38	451.90	727.00	203.04	222.24	36.94
<i>Callithrix pygmaea</i>	1	PBB	4,308.33	3,202.87	2,241.11	81.48	160.59	338.98	478.12	161.21	93.60	33.55
<i>Cebus apella</i>	1	PBB	74,229.03	61,387.98	37,879.40	548.09	2,802.43	3,784.61	5,931.26	854.00	1,715.21	555.96
<i>Leontopithecus rosalia</i>	2	PBB	11,355.29	8,746.57	6,427.27	225.98	402.20	747.79	1,089.90	281.74	416.43	72.86
<i>Saguinus fuscicollis weddelli</i>	1	PBB	8,200.26	6,176.23	4,246.02	210.48	324.77	592.40	827.38	222.27	344.62	37.36
<i>Saguinus imperator</i>	1	PBB	9,370.88	7,145.58	5,012.38	188.04	405.39	682.04	824.25	283.58	378.95	56.48
<i>Saguinus midas</i>	1	PBB	10,782.87	8,265.36	5,863.16	217.45	418.35	703.47	1,045.43	301.07	410.82	56.71
<i>Saguinus oedipus</i>	1	PBB	10,673.28	8,442.91	5,996.43	213.38	447.39	706.25	874.42	259.20	345.88	44.62
<i>Saimiri boliviensis</i>	2	PBB	21,657.87	18,065.35	12,418.81	205.94	903.13	1,213.45	1,378.18	381.27	512.42	107.20
<i>Saimiri sciureus</i>	1	PBB	21,642.56	17,757.59	12,190.01	196.01	783.93	1,191.16	1,671.98	356.31	559.06	106.46
<i>Cercopithecus hamlyni</i>	1	PBB	70,315.19	56,609.12	35,766.25	1,069.19	2,196.63	3,730.70	6,783.83	970.99	1,738.51	482.04
<i>Cercopithecus mitis albogularis</i>	1	PBB	66,443.85	54,545.09	34,895.38	782.98	2,277.88	3,513.38	5,433.24	912.63	1,539.89	499.62
<i>Cercopithecus nictitans</i>	1	PBB	73,183.47	59,159.74	37,132.31	806.42	2,124.61	3,367.65	7,283.92	996.65	1,944.00	431.51
<i>Colobus guereza</i>	1	PBB	77,247.81	60,744.88	33,524.27	1,127.59	2,160.50	4,331.59	7,926.48	998.71	2,320.87	925.27
<i>Erythrocebus patas</i>	1	PBB	97,005.79	81,518.03	49,024.07	994.69	2,429.86	4,474.39	7,298.02	1,165.77	1,958.27	591.31
<i>Lophocebus albigena</i>	1	PBB	89,810.41	73,890.25	44,892.22	1,070.65	2,949.11	4,349.64	8,125.75	931.39	1,808.51	704.87
<i>Macaca arctoides</i>	3	PBB	90,976.42	75,646.51	47,218.07	824.57	3,249.67	4,524.12	7,583.76	955.89	1,688.46	577.70
<i>Macaca fascicularis</i>	2	PBB	53,845.39	43,435.73	26,848.31	534.01	1,908.77	2,851.41	5,145.91	718.12	1,315.62	378.61
<i>Macaca fuscata</i>	2	PBB	89,294.87	72,835.99	45,235.73	850.76	3,370.82	4,396.61	6,413.89	999.07	1,756.12	493.19
<i>Macaca mulatta</i>	1	PBB	88,347.15	72,877.16	44,557.89	731.60	2,811.77	4,201.34	7,677.54	1,024.30	1,907.76	659.05
<i>Macaca nemestrina</i>	1	PBB	94,813.84	77,846.96	42,872.64	793.17	3,148.98	5,478.69	7,126.30	1,194.62	2,462.04	705.23
<i>Macaca nigra</i>	1	PBB	75,427.17	61,089.79	34,402.60	780.20	2,650.00	3,954.33	6,995.10	954.77	1,867.89	565.28
<i>Macaca silenus</i>	1	PBB	95,121.19	78,735.70	51,109.63	886.79	2,515.04	4,412.88	8,282.98	1,098.63	1,914.11	676.90
<i>Macaca sylvana</i>	1	PBB	83,284.63	68,216.51	45,352.78	923.45	3,096.24	4,470.40	7,259.82	1,124.14	1,763.79	449.96
<i>Mandrillus sphinx</i>	1	PBB	128,292.08	103,719.19	66,858.27	1,167.36	4,711.30	5,791.53	13,347.95	1,191.20	2,561.02	1,681.19
<i>Papio hamadryas</i>	1	PBB	168,266.30	139,593.78	85,309.20	1,513.50	4,566.84	7,643.95	14,639.32	1,580.78	3,505.81	1,302.66
<i>Gorilla gorilla gorilla</i>	1	PBB	368,395.55	295,932.13	138,853.21	1,474.35	7,049.35	16,726.38	43,960.74	2,879.56	5,890.55	3,006.19
<i>Gorilla gorilla gorilla</i>	2	GWU	432,243.52	341,380.47	200,844.97	1,892.57	8,681.21	16,636.19	60,476.48	2,689.30	7,726.00	3,335.08
<i>Pan paniscus</i>	1	PBB	286,618.26	224,578.36	128,612.97	2,179.85	6,703.00	12,158.48	38,571.67	1,971.74	6,142.02	3,195.98
<i>Pan paniscus</i>	1	GWU	325,918.11	257,896.80	158,461.10	2,387.77	8,186.92	13,986.56	45,665.06	2,090.34	4,770.64	1,508.71
<i>Pan troglodytes troglodytes</i>	2	PBB	320,578.92	253,708.88	153,386.69	2,692.90	7,106.19	12,877.36	44,830.51	2,082.10	4,591.31	2,488.75
<i>Pan troglodytes troglodytes</i>	2	GWU	378,562.79	298,489.30	179,591.89	2,392.97	10,647.51	16,868.70	50,909.77	2,456.84	5,832.13	4,006.05
<i>Pongo pygmaeus</i>	2	GWU	323,450.54	263,834.03	164,492.87	1,628.69	7,347.14	12,184.13	37,797.51	1,994.97	4,723.10	2,916.80

All values are volumes (in mm³) unless otherwise stated. Online supplementary Table S1 provides the individual-level morphological dataset including additional samples, additional brain areas (hemispheres, corpus callosum, claustrum, thalamus, lateral geniculate nuclei, and brainstem), lateralized measurements, specimen and individual details, and the list of specimens included in the means. Note that the medulla was frequently incomplete in PBB specimens (see online suppl. Table S1). PBB, Primate Brain Bank, Netherlands Institute of Neuroscience; GWU, donation of C.C. Sherwood, George Washington University, USA.

brain components of the brain (whole brain, telencephalon, cerebellum, and neocortex) had high inter-observer reliabilities (1–6% variance). Smaller structures showed a decreased but still satisfactory inter-observer variance (6–18% variance).

During selection of the areas of interest, we were able to identify several difficult “grey” border areas, i.e., borders or contact areas between structures for which observers showed consistent differences or landmarks were difficult to identify. These grey areas included: (1) the border between the diencephalon and the hemispheres, (2) the border between the mesencephalon and the diencephalon, and (3) the border of the pons, the medulla oblongata, and the mesencephalon.

Results

Table 1 summarizes the mean brain volume measures for 10 brain regions for 53 individuals belonging to 39 species. Means exclude specimens which were evaluated as significantly damaged or with specific measures that should be regarded with caution (see online electronic suppl. material Table S1 for details). A more detailed dataset, comprising individual level measurements of 16 brain regions (with bilateral measurements for 6 of these regions; the regions not in Table 1 are the hemispheres, the corpus callosum, the claustrum, the thalamus, the LGN, and the brainstem) from the same sample, as well as an additional 8 samples, is given in the online electronic supplementary material, together with associated source information and comments. These data, as well as the scans themselves, are available via www.karger.com/doi/10.1159/000488136. Additional resources will be posted to <http://primate.research.mcgill.ca>.

Preservation Effects

To test any effect of shrinkage in the sample due to storage in sucrose, we examined the brain mass prior to storage, where this measure was available in the PBB archives (33 specimens of the PBB sample). Brain mass measures were reported after extraction of the brain and fixation and prior to beginning the storage protocol. Note that brains originated from a variety of sources, had variable post-mortem delays, and may have been fixed by perfusion or immersion, potentially increasing variance between measures. Brain volume can be converted to brain mass by multiplying brain volume by a factor of 1.036 g/cm³ (the density of brain tissue) [Stephan et al., 1981; Rehkamper et al., 1991]. Here, we used this factor to transform brain mass to brain volume prior to storage and compared this with the total brain volume measured from MRI. The volume measured from MRI was significantly smaller than the brain volume prior to storage

(mean percent decrease = 9.1%, range = –1.0 to 44.9%; Wilcoxon signed-rank test: $N = 33$, $W = 269.5$, $p < 0.0001$). The *Cercopithecus mitis alboregularis* sample (PB0310) is unusual, with a significant post-mortem delay and a brain volume 44.9% smaller than expected based on its stated brain mass. However, its brain volume was similar to the average endocranial volumes presented by Isler et al. [2008] for *C. mitis* and *C. alboregularis*, while the brain mass is higher than the 75 g reported for the species by Stephan et al. [1981], suggesting that the sample brain mass was overestimated. The volumes measured from MRI were nonetheless still significantly smaller than the brain volumes before storage after excluding PB0310 (mean percent decrease = 8.0%, range = –1.0 to 18.5%; Wilcoxon signed-rank test: $N = 32$, $W = 253$, $p < 0.0001$).

Compatibility with Data in the Literature

We compared our Table 1 dataset with volumetric data based on measurements of serial sections, i.e., the Stephan et al. [1981] dataset, supplemented by Zilles and Rehkamper [1988], which provides data for the orangutan *Pongo pygmaeus*. Three species in the Stephan et al. [1981] dataset are only specified by genus [see Reader and Macdonald, 2003, for discussion and compiled data]. The datasets share 12 volumetric measures: overall brain volume, telencephalon, neocortex, striatum, hippocampus, diencephalon, LGN, thalamus, cerebellum, brainstem, mesencephalon, and medulla oblongata. Volumetric measures are complete for all regions, except the LGN and the thalamus, which were not compared. The medulla oblongata in the Stephan et al. [1981] dataset includes the pons [Armstrong, 1985]. So, for comparison, we added our measures of the medulla oblongata and the pons. Brainstem volume is the sum of the mesencephalon, the medulla, and the pons, and thus it was excluded from the analyses.

We selected those species which were present in the serial section and our Table 1 datasets ($n = 17$). Stephan et al. [1981] corrected brain component volumes in two steps, first by applying a conversion factor to account for brain shrinkage during sample preparation (based on the expected brain volume given the fresh brain mass of the specimen) and second by applying a conversion factor to account for within-species variation (based on the mean species brain mass). Zilles and Rehkamper [1988] applied a similar procedure. Combining these steps, corrected brain component volumes can be calculated as:

Corrected component volume = measured component volume \times (mean species brain volume/measured total brain volume).

To compare our datasets, we applied this correction to our brain component measurements (online suppl. Table S2) using the mean species brain masses provided by Stephan et al. [1981] and Zilles and Rehkamper [1988]. We calculated percent errors by comparing to the serial section dataset, and thus positive percent errors indicate larger volumes in the serial section dataset. We did not apply a correction to total brain volume given concerns about the estimates of fresh brain mass (see above), but nonetheless total brain volumes were generally similar to the serial section data (mean percent error = 3.5%, range = -14.1 to 23.7%; serial section data is corrected to species mean brain masses). For the component volumes, telencephalon volumes were similar (mean percent error = -4.5%, range = -10.0 to 7.0%). Cerebellum and diencephalon volumes were also similar, but with considerable variation between datasets for some species (cerebellum: mean percent error = 8.2%, range = -25.2 to 20.8%; diencephalon: mean percent error = 3.1%, range = -32.0 to 15.3%). In contrast, neocortex, striatum, hippocampal, and mesencephalon volumes were generally smaller in our dataset, and often considerably so (neocortex: mean percent error = 18.1%, range = -4.4 to 46.7%; striatum: mean percent error = 22.1%, range = 7.0–36.6%; hippocampus: mean percent error = 31.5%, range = 13.1–59.6%; mesencephalon: mean percent error = 23.5%, range = -12.8 to 50.4%). The combined medulla oblongata and pons was generally larger in our dataset than expected given the serial section datasets (mean percent error = -13.3%, range = -55.2 to 36.2%). Wilcoxon signed-rank tests indicated statistically significant differences between the datasets for all of these brain components apart from the diencephalon ($n = 17$; telencephalon: $W = 60.5$, $p = 0.003$; cerebellum: $W = 54.5$, $p = 0.008$; diencephalon: $W = 39.5$, $p = 0.06$; neocortex: $W = 73.5$; $p < 0.0001$; striatum: $W = 76.5$, $p < 0.0001$; hippocampus: $W = 76.5$, $p < 0.0001$; mesencephalon: $W = 75.5$, $p < 0.0001$; combined medulla and pons: $W = 54.5$, $p = 0.008$).

Discussion

Over the last three decades, relatively little new neurovolumetric data on primates has been gathered with broad species sampling, leaving comparative researchers heavily reliant on the primate brain dataset collected by Stephan and colleagues [1981]. Advances in MRI techniques potentially facilitate the study of primate brain anatomy and evolution, but thus far scans have mostly been used to study differences in anatomy and function-

ality in a small number of species. Moreover, until recently, restricted access to primate specimens or collections hindered the use of MRI techniques to obtain digital files adequate to take volumetric values of specific brain regions. Here, we were able to obtain measurements of 16 brain regions from 53 brains of 39 primate species. Our sample includes brain region measurements not recorded by Stephan et al. [1981], as well as specimens from 20 species without published brain component volumes [see compilation in Reader and MacDonald, 2003]. Measures of lateralized structures are also listed, potentially aiding future studies of primate brain asymmetry.

The work involved is labour intensive and, in spite of our efforts to increase the sample per species, 29 species are still represented by only one individual. This is a clear limitation of our dataset. For a few brains, volumes of some regions of interest could not be obtained, either because the resolution and/or contrast was insufficient to ascertain the border of the brain region, or because the structure had suffered substantial damage. Most brains have some degree of damage, attributed either to mechanical extraction prior to preservation or to preservation effects. The most commonly damaged structure was the medulla oblongata, which was incomplete or deformed in 23 specimens. Granulated surface, softness, air bubbles in the ventricles, and fragmentation of the hemispheres and cerebellum were also common problems. Small brains typically showed greater degrees of damage than larger brains. Collectively, this damage suggests that improvements are required in brain extraction and preservation methods. Specimen damage may be a recurrent issue in primate brain collections.

The relationship between brain mass and brain volume in those specimens where both measures are available suggests on the other hand that only a slight volumetric shrinkage has occurred during storage in the PBB sample. However, we, like Stephan et al. [1981], assume that shrinkage affects all brain areas equally, which may not be the case [Kretschmann et al., 1982]. When measures from our MRI sample were compared to the Stephan et al. [1981] and Zilles and Rehkamper [1988] datasets, which are based on serial section measurements, we observe comparable measurements for the total brain volume, the telencephalon, the cerebellum, and the diencephalon, whereas the neocortex, the striatum, the hippocampus, and the mesencephalon were typically smaller in our dataset, and the combined medulla oblongata and pons generally bigger. Moreover, we observed considerable variation across the dataset, with some species volumes being very different between the two datasets. This

is an obvious concern and emphasizes the need to investigate within-species variation in brain volume and, ideally, to compare different measurement techniques on the same brains directly. Moreover, note that our sample comes from captive specimens, while much of the Stephan et al. dataset comes from wild specimens [Reader and MacDonald, 2003]. Captive rearing and rearing conditions may affect both total and brain component volumes [Röhrs, 1985; Sallet et al., 2011; Kotschal et al., 2012]. Nonetheless, the potential to combine this data with that of Stephan et al. and other serial section data, as well as with growing MRI datasets [e.g., MacLeod et al., 2003; Reader and MacDonald, 2003; Bush and Allman, 2004; Sherwood et al., 2005; Smaers et al., 2010], while taking dataset differences into account, affords an opportunity to construct a far richer, representative, and more reliable primate brain database.

We hope that this new data will prove of value to future studies of primate neuroanatomy, and make it available

both as summary tables and as a complete electronic database amenable to further analysis. The MRI scans themselves are also made available so other researchers might use them to obtain additional variables of interest, for instance, in studies of gyrencephaly and the distribution of grey and white matter, which are attracting recent interest [Sherwood et al., 2006; Hopkins et al., 2008; Hopkins and Nir, 2010].

Acknowledgements

For invaluable assistance we thank Dr. Corbert van Eden, PBB coordinator, and Dr. Ido Toxopeus for his support of the early PBB. For donation of MR images and/or advice on measurement, we thank Dr. Chet Sherwood, Dr. Alexandra de Sousa, Dr. Atsushi Iriki, and Dr. Scott Fears. We thank the PBB for permission to scan the reported brains. For funding, we thank the John Templeton Foundation, the Canada Foundation for Innovation, and the Anna-Greta och Holger Crafoords Stiftelse.

References

- Armstrong E (1985): Allometric considerations of the adult mammalian brain, with special emphasis on primates; in Jungers WL (ed): *Size and Scaling in Primate Biology*. New York, Springer, pp 115–146.
- Baron G, Stephan H, Frahm HD (1996): *Comparative Neurobiology in Chiroptera*. Basel, Birkhäuser.
- Barton RA, Harvey PH (2000): Mosaic evolution of brain structure in mammals. *Nature* 405: 1055–1058.
- Bauernfeind AL, de Sousa AA, Avasthi T, Dobson SD, Raghanti MA, Lewandowski AH, Zilles K, Semendeferi K, Allman JM, Arthur D (2013): A volumetric comparison of the insular cortex and its subregions in primates. *J Hum Evol* 64:263–279.
- Bogart SL, Bennett AJ, Schapiro SJ, Reamer LA, Hopkins WD (2014): Different early rearing experiences have long-term effects on cortical organization in captive chimpanzees (*Pan troglodytes*). *Dev Sci* 17:161–174.
- Bush EC, Allman JM (2004): The scaling of frontal cortex in primates and carnivores. *Proc Natl Acad Sci USA* 101:3962–3966.
- Clutton-Brock TH, Harvey PH (1980): Primates, brains and ecology. *J Zool* 190:309–323.
- Crick FC, Koch C (2005): What is the function of the claustrum? *Phil Trans R Soc B Biol Sci* 360: 1271–1279.
- de Viet S (2009): The co-evolution of the neocortex and intelligence. Unpublished MSc thesis, Utrecht University, Utrecht, The Netherlands.
- Dunbar RIM (1998): The social brain hypothesis. *Evol Anthropol* 6:178–190.
- Dunbar RIM, Shultz S (2007): Understanding primate brain evolution. *Phil Trans R Soc B Biol Sci* 362:649–658.
- Finlay BL, Darlington RB (1995): Linked regularities in the development and evolution of mammalian brains. *Science* 268:1578–1584.
- Finlay BL, Darlington RB, Nicastro N (2001): Developmental structure in brain evolution. *Behav Brain Sci* 24:298–308.
- Frahm HD, Stephan H, Baron G (1984): Comparison of brain structure volumes in insectivora and primates. 5. Area striata (AS). *J Hirnforsch* 25:537–557.
- Frey S, Pandya DN, Chakravarty MM, Bailey L, Petrides M, Collins DL (2011): An MRI based average macaque monkey stereotaxic atlas and space (MNI monkey space). *Neuroimage* 55:1435–1442.
- Harvey PH, Krebs JR (1990): Comparing brains. *Science* 249:140–146.
- Herculano-Houzel S, Catania K, Manger PR, Kaas JH (2015): Mammalian brains are made of these: a dataset of the numbers and densities of neuronal and nonneuronal cells in the brain of glires, primates, scandentia, eulipotyphlans, afrotherians and artiodactyls, and their relationship with body mass. *Brain Behav Evol* 86:145–163.
- Hikishima K, Quallo MM, Komaki Y, Yamada M, Kawai K, Momoshima S, Okano HJ, Sasaki E, Tamaoki N, Lemon RN, Iriki A, Okano H (2011): Population-averaged standard template brain atlas for the common marmoset (*Callithrix jacchus*). *Neuroimage* 54:2741–2749.
- Hopkins WD, Nir TM (2010): Planum temporale surface area and grey matter asymmetries in chimpanzees (*Pan troglodytes*): the effect of handedness and comparison with findings in humans. *Behav Brain Res* 208:436–443.
- Hopkins WD, Taglialatela JP, Meguerditchian A, Nir TM, Schenker NM, Sherwood CC (2008): Gray matter asymmetries in chimpanzees as revealed by voxel-based morphometry. *Neuroimage* 42:491–497.
- Isler K, Kirk CE, Miller JMA, Albrecht GA, Gelvin BR, Martin RD (2008): Endocranial volumes of primate species: scaling analyses using a comprehensive and reliable data set. *J Hum Evol* 55:967–978.
- Jerison HJ (2001): Archaeological implications of paleoneurology; in Nowell A (ed): *The Mind's Eye: Multidisciplinary Approaches to the Evolution of Human Cognition*. Ann Arbor, International Monographs in Prehistory, pp 83–96.
- Kaas JH, van Eden CG (2011): Brain banks provide a valuable resource for comparative studies. *Brain Behav Evol* 77:65–66.
- Kotschal A, Sundstrom LF, Brelid D, Devlin RH, Kolm N (2012): Inside the heads of David and Goliath: environmental effects on brain morphology among wild and growth-enhanced coho salmon *Oncorhynchus kisutch*. *J Fish Biol* 81:987–1002.
- Kretschmann HJ, Tafesse U, Herrmann A (1982): Different volume changes of cerebral cortex and white matter during histological preparation. *Microscop Acta* 86:13–24.

- Looi JCL, Lindberg O, Liberg B, Tatham V, Kumar R, Maller J, Millard E, Sachdev P, Högberg G, Pagani M, Botes L, Engman EL, Zhang Y, Svensson L, Wahlund LO (2008): Volumetrics of the caudate nucleus: reliability and validity of a new manual tracing protocol. *Psychiatry Res* 163:279–288.
- MacLeod CE, Zilles K, Schleicher A, Rilling JK, Gibson KR (2003): Expansion of the neocerebellum in hominoidea. *J Hum Evol* 44:401–429.
- Mars RB, Neubert F-X, Verhagen L, Sallet J, Miller KL, Dunbar RIM, Barton RA (2014): Primate comparative neuroscience using magnetic resonance imaging: promises and challenges. *Front Neurosci* 8:298.
- Matano S, Stephan H, Baron G (1985): Volume comparisons in the cerebellar complex of primates. 1. Ventral pons. *Folia Primatol* 44: 171–181.
- Matochik JA, Chefer SI, Lane MA, Roth GS, Mattison JA, London ED, Ingram DK (2004): Age-related decline in striatal volume in rhesus monkeys: assessment of long-term calorie restriction. *Neurobiol Aging* 25:193–200.
- Mota B, Herculano-Houzel S (2015): Cortical folding scales universally with surface area and thickness, not number of neurons. *Science* 349:74–77.
- Pagel MD, Harvey PH (1990): Diversity in the brain sizes of newborn mammals. *Bioscience* 40:116–122.
- Palazzi X, Bordier N (2008): *The Marmoset Brain in Stereotaxic Coordinates*. New York, Springer.
- Paxinos G, Huang X-F, Toga AW (1999): *The Rhesus Monkey Brain in Stereotaxic Coordinates*. London, Academic Press.
- Powell LE, Isler K, Barton RA (2017): Re-evaluating the link between brain size and behavioural ecology in primates. *Proc Biol Sci* 2017: 284:20171765.
- Reader SM, Hager Y, Laland KN (2011): The evolution of primate general and cultural intelligence. *Phil Trans R Soc B Biol Sci* 366:1017–1027.
- Reader SM, Laland KN (2002): Social intelligence, innovation, and enhanced brain size in primates. *Proc Natl Acad Sci USA* 99:4436–4441.
- Reader SM, MacDonald K (2003): Environmental variability and primate behavioural flexibility; in Reader SM, Laland KN (eds): *Animal Innovation*. New York, Oxford University Press, pp 83–116.
- Rehkaemper G, Frahm HD, Zilles K (1991): Quantitative development of brain and brain structures in birds (galliformes and passeriformes) compared to that in mammals (insectivores and primates). *Brain Behav Evol* 37:125–143.
- Rilling JK (2014): Comparative primate neuroimaging: insights into human brain evolution. *Trends Cogn Sci* 18:46–55.
- Rilling JK, Insel TR (1999): The primate neocortex in comparative perspective using magnetic resonance imaging. *J Hum Evol* 37:191–223.
- Röhrs M (1985): Cephalization, neocorticalization and the effects of domestication on brains of mammals. *Fortschr Zool* 30:545–547.
- Sallet J, Mars RB, Noonan MP, Andersson JL, O'Reilly JX, Jbabdi S, Croxson PL, Jenkinson M, Miller KL, Rushworth MFS (2011): Social network size affects neural circuits in macaques. *Science* 334:697–700.
- Sandel AA, Miller JA, Mitani JC, Nunn CL, Patterson SK, Garamszegi LZ (2016): Assessing sources of error in comparative analyses of primate behavior: intraspecific variation in group size and the social brain hypothesis. *J Hum Evol* 94:126–133.
- Schoenemann PT, Sheehan MJ, Glotzer LD (2005): Prefrontal white matter volume is disproportionately larger in humans than in other primates. *Nat Neurosci* 8:242–252.
- Semendeferi K (2001): Advances in the study of hominoid brain evolution: magnetic resonance imaging (MRI) and 3-D reconstruction; in Falk D, Gibson KR (eds): *Evolutionary Anatomy of the Primate Cerebral Cortex*. New York, Cambridge University Press, pp 257–289.
- Semendeferi K, Armstrong E, Schleicher A, Zilles K, van Hoesen GW (2001): Prefrontal cortex in humans and apes: a comparative study of area 10. *Am J Phys Anthropol* 114:224–241.
- Semendeferi K, Damasio H (2000): The brain and its main anatomical subdivisions in living hominoids using magnetic resonance imaging. *J Hum Evol* 38:317–332.
- Semendeferi K, Damasio H, Frank R (1997): The evolution of the frontal lobes: a volumetric analysis based on three-dimensional reconstructions of magnetic resonance scans of human and ape brains. *J Hum Evol* 32:375–388.
- Semendeferi K, Lu A, Schenker N, Damasio H (2002): Humans and great apes share a large frontal cortex. *Nat Neurosci* 5:272–276.
- Sherwood CC, Cranfield MR, Mehman PT, Lilly AA, Garbe JAL, Whittier CA, Nutter FB, Rein TR, Bruner HJ, Holloway RL, Tang CY, Naidich TP, Delman BN, Steklis HD, Erwin JM, Hof PR (2004): Brain structure variation in great apes, with attention to the mountain gorilla (*Gorilla beringei beringei*). *Am J Primatol* 63:149–164.
- Sherwood CC, Hof PR, Holloway RL, Semendeferi K, Gannon PJ, Frahm HD, Zilles K (2005): Evolution of the brainstem orofacial motor system in primates: a comparative study of trigeminal, facial, and hypoglossal nuclei. *J Hum Evol* 48:45–84.
- Sherwood CC, Stimpson CD, Raghanti MA, Wildman DE, Uddin M, Grossman LI, Goodman M, Redmond JC, Bonar CJ, Erwin JM, Hof PR (2006): Evolution of increased glia-neuron ratios in the human frontal cortex. *Proc Natl Acad Sci USA* 103:13606–13611.
- Smaers JB, Dechmann DKN, Goswami A, Soligo C, Safi K (2012): Comparative analyses of evolutionary rates reveal different pathways to encephalization in bats, carnivores, and primates. *Proc Natl Acad Sci USA* 109:18006–18011.
- Smaers JB, Schleicher A, Zilles K, Vinicius L (2010): Frontal white matter volume is associated with brain enlargement and higher structural connectivity in anthropoid primates. *PLoS One* 5:e9123.
- Stephan H, Bauchot R, Andy OJ (1970): Data on size of the brain and various brain parts in insectivores and primates; in Nobak CR, Montagna W (eds): *The Primate Brain*. New York, Appleton, pp 289–297.
- Stephan H, Baron G, Frahm HD, Stephan M (1986): Comparison of size in brains and brain structures of mammals. *Z Mikrosk Anat Forsch* 100:189–212.
- Stephan H, Frahm HD, Baron G (1981): New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol* 35: 1–29.
- Stephan H, Frahm HD, Baron G (1984): Comparison of brain structure volumes in insectivora and primates. 4. Non-cortical visual structures. *J Hirnforsch* 25:385–403.
- Stephan H, Frahm HD, Baron G (1987): Comparison of brain structure volumes in insectivora and primates. 7. Amygdaloid components. *J Hirnforsch* 28:571–584.
- Tammer R, Hofer S, Merboldt JD, Frahm J (2009): *Magnetic Resonance Imaging of the Rhesus Monkey Brain*. Göttingen, Vandenhoeck & Ruprecht.
- Todorov OS (2010): The evolution of the prefrontal brain areas in primates: a volumetric study. Unpublished MSc thesis, Utrecht University, Utrecht, The Netherlands.
- Wang Y, Nie J, Yap P-T, Li G, Shi F, Geng X, Guo L, Shen D (2014): Knowledge-guided robust MRI brain extraction for diverse large-scale neuroimaging studies on humans and non-human primates. *PLoS One* 9:e77810.
- Wilson DE, Reeder DM (2005): *Mammal Species of the World: A Taxonomic and Geographic Reference*, ed 3. Baltimore, Johns Hopkins University Press.
- Zilles K, Amunts K, Smaers JB (2011): Three brain collections for comparative neuroanatomy and neuroimaging. *Ann NY Acad Sci* 1225:E94–E104.
- Zilles K, Schleicher A, Pehlemann F (1982): How many sections must be measured in order to reconstruct the volume of a structure using serial sections? *Microsc Acta* 86:339–346.
- Zilles K, Rehkaemper G (1988): The brain, with special reference to the telencephalon; in Schwartz JH (ed): *Orang-Utan Biology*. Oxford, Oxford University Press, pp 157–176.