UNIVERSITY OF CALIFORNIA, SAN DIEGO

Comparative Analysis of Broca's Area in Hominoids

A Dissertation submitted in partial satisfaction of the
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In

Anthropology

by

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The Dissertation of Natalie Marie Schenker is approved, and it is acceptable in quality and form for publication on microfilm:

Chair

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DEDICATION

To my mother, for continually reminding me that I can do anything to which I set my mind; to my father, for his unfailing support; and to my brother, for providing a stellar example of how to be a successful human being.

[W]e argue that language grows out of more primitive primate and mammalian neuro-behavioral complexes, including those that govern face-to-face interaction, categorization, and symbolization…[T]he key to the transition from primate vocal and visible gesture systems to language…is the introduction of iconic, visible gestures at some point in hominid evolution…. [S]yntax… emerges out of such visual-gestural systems.

David Armstrong, William Stokoe, and Sherman Wilcox
Gesture and the Nature of Language, 1995

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Emmorey, K., Allen, J., Bruss, J., Schenker, N., and H. Damasio. 2003. A morphometric analysis of auditory brain regions in congenitally deaf adults. *Proceedings of the National Academy of Sciences*. 100(17):10049-10054.

Schenker, N. M., Desgouttes, A., and K. Semendeferi. 2005. Neural connectivity and cortical substrates of cognition in hominoids. *Journal of Human Evolution.* 49(5):547-569.

Schenker, N. M., Sherwood, C. C., Hof, P. R., and K. Semendeferi. 2007. Microstructural asymmetries of the cerebral cortex in humans and other mammals. In Hopkins, W. D. (ed.) *The Evolution of Hemispheric Specialization in Primates*. Academic Press.

Schenker, N. M., Buxhoeveden, D. P., Amunts, K., Zilles, K., and K. Semendeferi. (in revision). A comparative quantitative analysis of cytoarchitectonics and minicolumnar organization in Broca's area in humans and great apes.

CONFERENCE ABSTRACTS

Schenker, N. M., Buxhoeveden, D. P., Amunts K., Zilles, K. and Semendeferi, K. 2007. A comparative analysis of cytoarchitecture and vertical organization of Broca's area in humans, great apes and macaques. American Association of Physical Anthropology Meetings.

Schenker, N. M., Buxhoeveden, D. P., and K. Semendeferi. 2006. Minicolumns in Broca’s area in apes and humans. American Association of Physical Anthropology Meetings.

Barger, N., Stefanacci, L., Schenker, N., and K. Semendeferi. 2004. Comparative morphometric analysis of the ape and human amygdala and its basolateral division. *Society for Neuroscience Abstracts.* 30.

Buxhoeveden, D., Semendeferi, K., Schenker, N., and E. Courchesne. 2004. Decreased cell column spacing in Autism. *Society for Neuroscience Abstracts*. 30.

Teffer, K., Schenker, N., and K. Semendeferi. 2004. A comparative volumetric analysis of the cornu ammonis, dentate gyrus, and subiculum of the hippocampus in the hominoids. *Society for Neuroscience Abstracts*. 30.

Barger, N., Stefanacci, L., Schenker, N., and K. Semendeferi. 2004. Comparative volumetric analysis of the hominoid amygdala. *American Journal of Physical Anthropology*. 120(S36):59.

Teffer, K., Schenker, N., and K. Semendeferi. 2004. A comparative volumetric analysis of the human and ape hippocampus. *American Journal of Physical Anthropology.* 120(S36):194.

Emmorey, K., Allen, J., Schenker, N., Bruss, J., and H. Damasio. 2003. A morphometric analysis of auditory brain regions in congenitally deaf adults. Cognitive Neuroscience Abstracts.

Semendeferi, K., Schenker, N., and H. Poje. 2002. Neural Substrates of Cognition in Monkeys and Apes: Preliminary Observations. *American Journal of Physical Anthropology*. 117(S34):139.

Semendeferi, K. and N. Schenker. 2001. Temporal and Frontal Lobes in Monkeys, Apes, and Humans. *Society for Neuroscience Abstracts*. 27.

ABSTRACT OF THE DISSERTATION

Comparative Analysis of Broca's Area in Hominoids

by

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Doctor of Philosophy in Anthropology

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At the heart of the study of the evolution of language ability is the question how to produce testable hypotheses regarding the antecessors of language in our hominid ancestors and the presence or absence of such traits in our closest living relatives, the apes. One region that is consistently implicated in modern functional studies of language, and was implicated even in early lesion studies, is the region known as Broca's area (Brodmann's areas 44 and 45). The region's involvement in language function in humans is the reason that Broca's area is

expected to express different anatomical characteristics from other prefrontal areas and to have unique characteristics in humans. In order to assess the degree to which the human brain has changed in relation to other hominoids, we need to have detailed knowledge, not only of the human brain, but also as many closely related species as possible.

Previous studies have reported data on the comparative gross morphology of the inferior frontal gyrus, in which Broca's area is located in chimpanzees, bonobos, and gorillas (Cantalupo and Hopkins, 2001), but studies of the cytoarchitecture of Broca's area are limited to humans (Brodmann, 1909; von Economo, 1929; Sarkissov et al., 1955; Amunts et al., 1999; 2003) and chimpanzees (Bailey et al., 1950; Sherwood et al., 2003).

The purpose of this dissertation was to assess, both qualitatively and quantitatively, the cytoarchitectonic structure of Brodmann's areas 44 and 45 in human, chimpanzee, bonobo, gorilla, orangutan, and gibbon specimens.

Quantitative measurements of minicolumnar organization, volume, neuron number, and neuron density revealed only limited differences between humans and apes. While volume of and neuronal number in Brodmann's areas 44 and 45 were larger in humans, neither were larger than expected. Furthermore, the relative size of the areas was not larger in humans than in apes and did not differentiate among ape species. Neuron density and grey level index (within minicolumns) were decreased in humans relative to apes, and only limited evidence for any asymmetry was observed.

The variation among species in Brodmann's areas 44 and 45 suggests that this region behaves much as expected in response to changes in brain size. Despite Broca's area's involvement in language related behavior in humans, the current data provides no evidence that

there are differences in information processing occurring in this region, and no pattern emerges that requires explanations other than those related to increases in brain size.

This suggests Broca's area involvement in non-linguistic activities that are just as crucial to apes as they are to humans. Alternatively, there may be differences among species that are not distinguishable using the parameters examined in this research. Future research should focus on expanding the sample to encompass individual variation within species and expand the parameters examined to subpopulations of neurons to gain a more detailed view of the anatomical structure of Broca's area within and among species.

INTRODUCTION

At the heart of the study of the evolution of language ability is how to produce testable hypotheses regarding the antecessors of language in our hominid ancestors and the presence or absence of such traits in our closest living relatives, the apes. Language is a complex behavior that is an amalgam of disparate cognitive abilities that combine to produce what we see and hear as linguistic communication. As a result, the neural substrates underlying linguistic interaction are also varied. One region that is consistently implicated in modern functional studies of language, and was implicated even in early lesion studies, is the region known as Broca's area. This term is most often used, and will be used here, to refer to the area comprising Brodmann's cytoarchitectonic areas 44 and 45.

Some have argued that language, as a unique trait of humans, should be regarded as a trait that is discontinuous from animal communication systems that are visible in other species (Chomsky, 1992; Bickerton, 2003), a hypothesis that is reliant on the idea that language is indivisible. If we accept that idea, then we are confronted with the insurmountable task of determining how such an indivisible element of cognition evolved through natural processes. However, if we take the stand that language is not indivisible but that it is instead composed of diverse elements, each of which can be acted upon separately, each of those elements can then be addressed individually. These individual components can then be regarded as the parts that come together to create the emergent behavior we see as language. Among these elements, there are those that clearly have aspects present in other primates, such as semantic knowledge (or knowledge of "what" objects are (Donaldson et al., 2007; Zuberbuhler et al., 1999; Gifford et al., 2003) and recognition and differential processing of conspecific vocalizations (Gil-da-Costa et al., 2006; Cohen et al., 2007; Beecher et al., 1979; May et al.,

1989), while other aspects are less clearly connected, such as those that may be related to behaviors that involved hierarchical processing.

The regions that have been implicated in linguistic processing have also been shown to be involved in additional cognitive tasks –further suggesting little segregation between language and other cognition. For instance, Broca's area in humans is not only activated in language-related activities, but is also involved in additional cognitive tasks that require hierarchical processing and sequencing. Multiple studies suggest that Broca's area is less selective regarding the type of stimuli than once believed. While the region is clearly involved in language-related behaviors, it is also activated during tasks that involve manipulation of non-linguistic auditory sequences (Gelfand and Bookheimer, 2003), tasks that require particular attention to the relationship between object location, speed, and timing (Schubotz and von Cramon, 2001), and goal-directed motion and recognizing deviations from expected sequences (Nishitani et al., 2005). It is clear that these processes are also utilized in linguistic settings, as in the manipulation of linguistic sequences, identifying the relationship between words in a grammatical sequence, and recognizing deviations from expected grammatical sequences. There is also evidence that Broca's area and its right hemisphere homolog are utilized in general executive functions for creating plans of action (Koechlin and Jubault, 2006). All of this evidence indicates the presence of non-linguistic cognitive abilities that have been co-opted by linguistic processing and exhibit some continuity with abilities observed in nonhuman primates.

Despite evidence that Broca's area is involved in non-linguistic cognitive tasks (Schubotz and von Cramon, 2001; Gelfand and Bookheimer, 2003; Koechlin and Jubault, 2006), it is because of the region's involvement in language function in humans that Broca's

area has typically been expected to express different anatomical characteristics from other prefrontal areas. Broca's area's position as transitional cortex between the unimodal premotor association cortex and higher order prefrontal association cortex also suggests its intermediate status both functionally and structurally. While it has long been assumed that regions involved in language function must have unique characteristics in humans, there are little published data from this region of the frontal cortex in apes. Furthermore, only a few recent studies have been accomplished, and most existing cytoarchitectonic information is derived from the classical maps of humans. In order to assess the degree to which the human brain has changed in relation to our closest relatives, we need to have detailed knowledge, not only of the human brain, but also as many closely related species as possible.

While previous studies have addressed the comparative gross morphology of the inferior frontal gyrus, in which Broca's area is located (Cantalupo and Hopkins, 2001) in chimpanzees, bonobos, and gorillas, gross morphological data give only a general idea of interspecies differences without providing a more detailed view of microstructural differences. Furthermore, qualitative descriptions of Broca's area in humans (Brodmann, 1909; von Economo, 1929; Sarkissov et al., 1955; Amunts et al., 1999; 2003) and chimpanzees (Bailey et al., 1950; Sherwood et al., 2003) have shown that the boundaries of Brodmann's areas 44 and 45 vary considerably between individuals and do not correspond directly to gross morphological features (Amunts et al., 1999; Sherwood et al., 2003). Qualitative descriptions can identify the presence or absence of cortical areas in the brain, but quantitative data on cytoarchitecture provides us with another level on which to base comparisons among species. However, because of the time-consuming nature of the research, and the limited supply of suitable

histological specimens, quantitative cytoarchitectural data exists only for humans (Amunts et al., 1999; Amunts et al., 2003; Uylings et al., 2005; Uylings et al., 2006) and not for any of the apes.

The purpose of this research was to assess, both qualitatively and quantitatively, the cytoarchitectonic structure of Brodmann's areas 44 and 45 (also known as Broca's area) in human, chimpanzee, bonobo, gorilla, orangutan, and gibbon specimens.

Because Broca's area is known to be functionally asymmetrical (Habib and Demonet, 1996; Price, 2000), and existing histological data (Amunts et al., 1999; Amunts et al., 2003; Uylings et al., 2006) suggest structural asymmetry as well, chapter one addresses the extent of microstructural asymmetries in human and nonhuman brains, not only in Broca's area, but in many regions of the cortex. The chapter reviews known structural asymmetries in the human cerebral cortex, with special attention to the relatively small literature on microstructural asymmetries in the brains of other mammals. Also discussed are the implications of current data to our understanding of the evolution of functional cortical asymmetries in humans.

Chapters two and three are primary data reports addressing the qualitative and quantitative description and analysis of the structure of Broca's area across species. Chapter two focuses on the basic cytoarchitectural description of Brodmann's areas 44 and 45 in humans and great apes, and also the quantification of the minicolumnar structure of both areas in humans and great apes. Minicolumnar organization in the adult brain is thought to be derived from ontogenetic columns and the migration of neuroblasts from the ventricular and subventricular zones into radial columns during development (Rakic, 1995). Furthermore, functional studies of minicolumns in motor cortex indicate preferential selection of direction in arm reach tasks, (Amirikian and Georgopoulos, 2003; Georgopoulos et al., 2007). It is not

unreasonable to expect that minicolumns in Broca's area maintain similar functional preferences. As the width of columns is not static throughout development, the spacing between cells in layer III (where minicolumns are assumed to be one-cell wide; Seldon, 1981) may reflect the degree of connectivity among minicolumns achieved in adult brains. If there are unique functional adaptations in Broca's area in humans related to connectivity, those should be reflected in the spacing of minicolumns.

A quantitative approach was used by measuring mean horizontal spacing distance (between cells) and the fraction of the area that is occupied by cell bodies (as measured by grey level index or GLI). Two primary hypotheses were tested. The first was that horizontal spacing distance and grey level index in these areas in both hemispheres indicate greater distance between cells in humans than in great apes, as has been reported in the temporal language cortex (Buxhoeveden et al., 2001b) and in the frontal pole (Buxhoeveden and Semendeferi, 2005). The second hypothesis was that spacing and the fraction of area occupied by cell bodies are lateralized in humans in areas 44 and 45, but not in great apes. This hypothesis is derived from the reported asymmetry of GLI in humans (Amunts et al., 1999; 2003) and the region's involvement in lateralized language-related activity, in addition to findings of lateralization of minicolumns in temporal lobe language areas (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001a).

Chapter three presents boundary criteria of Brodmann's areas 44 and 45 in human, chimpanzee, bonobo, gorilla, orangutan, and gibbon specimens. Volumes were estimated using the Cavalieri principle in all specimens and total neuron number was estimated with the

stereological optical fractionator method; neuronal density was calculated using volume and neuron number.

Volume is assumed to be correlated with the importance of a cortical region and how much the region is used. Therefore, if a region occupies a greater proportion of cortex in one species than another, then there are also concomitant differences in the behavioral usage of that region (e.g. size of primary somatosensory cortex: Krubitzer and Kaas, 2005). Thus, volumetric data were used to test the hypothesis that Broca's area occupies a greater proportion of the hemisphere in humans than in other apes, because of the region's involvement in language production that is absent in nonhumans.

Neuronal numbers are a measure of the number of computational units in a region, and indirectly may imply the complexity of the computational ability of the region. Neuronal densities reflect the ratio of neurons to volume, and unlike GLI do not reflect cell size. Previous studies have reported estimates of neuronal densities in the prefrontal cortex of humans (total frontal cortex: Pakkenberg, 1993; areas 9 & 46: Rajkowska and Goldman-Rakic, 1995; area 13: Semendeferi et al., 1998; area 10: Semendeferi et al., 2001; areas 9 & 44: Selemon et al., 2003) and two prefrontal areas in ape species (area 13: Semendeferi et al., 1998; area 10: Semendeferi et al., 2001).

Given the reported presence of asymmetry in both volume and neuron number in Brodmann's area 44 in humans (Uylings et al., 2006), and the reported gross asymmetry of the inferior frontal gyrus in chimpanzees, gorillas, and orangutans (Cantalupo and Hopkins, 2001), the hypothesis that both volume and neuron number area asymmetrical in the all species examined was also tested.

These basic quantitative measures provide a first good overview of the nature of Broca's area across hominoids. This research reports not only on the presence of a Broca-like cytoarchitectural area in the brains of hominoids, but also seeks to quantify variation within that area across species, allowing one to test for similarities and differences in an area that is activated during language tasks in humans. Are there differences that might be related to language tasks? Or, if there are no or only limited differences, what hypotheses can be made regarding other behavioral tasks utilize the region that might explain why differences are absent?

REFERENCES

Amirikian B, Georgopoulos AP. 2003. Modular organization of directionally tuned cells in the motor cortex: is there a short-range order? Proceedings of the National Academy of Sciences of the United States of America 100:12474-12479.

Amunts K, Schleicher A, Burgel U, Mohlberg H, Uylings HBM, Zilles K. 1999. Broca's region revisited: Cytoarchitecture and intersubject variability. Journal of Comparative Neurology 412:319-341.

Amunts K, Schleicher A, Ditterich A, Zilles K. 2003. Broca's region: Cytoarchitectonic asymmetry and developmental changes. Journal of Comparative Neurology 465:72-89.

Bailey P, von Bonin G, McCulloch WS. 1950. The Isocortex of the Chimpanzee. Urbana: University of Illinois Press.

Beecher MD, Petersen MR, Zoloth SR, Moody DB, Stebbins WC. 1979. Perception of Conspecific Vocalizations by Japanese Macaques-Evidence for Selective Attention and Neural Lateralization. Brain Behavior and Evolution 16:443-460.

Bickerton D. 2003. Language evolution without evolution. Behavioral and Brain Sciences 26:669-670.

Brodmann K. 1909. Vergleichende Lokalisationslehre der Grobhirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig: Johann Ambrosius Barth.

Buxhoeveden D, Casanova M. 2000. Comparative lateralisation patterns in the language area of human, chimpanzee, and rhesus monkey brains. Laterality 5:315-330.

Buxhoeveden DP, Semendeferi K. 2005. Cell Columns in Frontal Cortex of Apes and Humans. American Journal of Physical Anthropology S40:81.

Buxhoeveden DP, Switala AE, Litaker M, Roy E, Casanova MF. 2001a. Lateralization of Minicolumns in Human Planum temporale is Absent in Nonhuman Primate Cortex. Brain Behavior and Evolution 57:349-358.

Buxhoeveden DP, Switala AE, Roy E, Litaker M, Casanova MF. 2001b. Morphological differences between minicolumns in human and nonhuman primate cortex. American Journal of Physical Anthropology 115:361-371.

Cantalupo C, Hopkins WD. 2001. Asymmetric Broca's area in great apes. Nature 414:505.

Chomsky N. 1992. On the nature, use and acquisition of language. In: Pütz M, Editor. Thirty years of linguistic evolution: studies in honour of René Dirven on the occasion of his sixtieth birthday. Amsterdam: J Benjamins. p 3-30.

Cohen YE, Theunissen F, Russ BE, Gill P. 2007. Acoustic features of rhesus Vocalizations and their representation in the ventrolateral prefrontal cortex. Journal of Neurophysiology 97:1470-1484.

Donaldson MC, Lachmann M, Bergstrom CT. 2007. The evolution of functionally referential meaning in a structured world. Journal of Theoretical Biology 246:225-233.

Gelfand JR, Bookheimer SY. 2003. Dissociating neural mechanisms of temporal sequencing and processing phonemes. Neuron 38:831-842.

Georgopoulos AP, Merchant H, Naselaris T, Amirikian B. 2007. Mapping of the preferred direction in the motor cortex. Proceedings of the National Academy of Sciences of the United States of America 104:11068-11072.

Gifford GW, Hauser MD, Cohen YE. 2003. Discrimination of functionally referential calls by laboratory-housed rhesus macaques: Implications for neuroethological studies. Brain Behavior and Evolution 61:213-224.

Gil-da-Costa R, Martin A, Lopes M, Munoz M, Fritz J, Braun A. 2006. Species-specific calls activate homologs of Broca's and Wernicke's areas in the macaque. Nature Neuroscience 9:1064-1070.

Habib M, Demonet J-F. 1996. Cognitive neuroanatomy of language: The contribution of functional neuroimaging. Aphasiology 10:217-234.

Koechlin E, Jubault T. 2006. Broca's area and the hierarchical organization of human behavior. Neuron 50:963-974.

Krubitzer L, Kaas J. 2005. The evolution of the neocortex in mammals: how is phenotypic diversity generated? Current Opinion in Neurobiology 15:444-453.

May B, Moody DB, Stebbins WC. 1989. Categorical Perception of Conspecific Communication Sounds by Japanese Macaques, Macaca fuscata. Journal of the Acoustical Society of America 85:837-847.

Nishitani N, Schurmann M, Amunts K, Hari R. 2005. Broca's region: From action to language. Physiology 20:60-69.

Pakkenberg B. 1993. Total Nerve Cell Number in Neocortex in Chronic Schizophrenics and Controls Estimated Using Optical Disectors. Biological Psychiatry 34:768-772.

Price CJ. 2000. The anatomy of language: contributions from functional neuroimaging. Journal of Anatomy 197 Pt 3:335-359.

Rajkowska G, Goldman-Rakic PS. 1995. Cytoarchitectonic Definition of Prefrontal Areas in the Normal Human Cortex: I. Remapping of Areas 9 and 46 using Quantitative Criteria. Cerbral Cortex 5:307-322.

Rakic P. 1995. Radial Versus Tangential Migration of Neuronal Clones in the Developing Cerebral-Cortex. Proceedings of the National Academy of Sciences of the United States of America 92:11323-11327.

Sarkissov SA, Filimonoff IN, Kononowa EP, Preobraschenskja IS, Kukuew LA. 1955. Atlas of the Cytoarchitectonics of the Human Cerebral Cortex. Moscow: Medgiz.

Schubotz RI, von Cramon DY. 2001. Functional organization of the lateral premotor cortex: fMRI reveals different regions activated by anticipation of object properties, location and speed. Cognitive Brain Research 11:97-112.

Seldon HL. 1981. Structure of Human Auditory-Cortex. 1. Cytoarchitectonics and Dendritic Distributions. Brain Research 229:277-294.

Selemon LD, Mrzljak J, Kleinman JE, Herman MM, Goldman-Rakic PS. 2003. Regional specificity in the neuropathologic substrates of schizophrenia - A morphometric analysis of Broca's area 44 and area 9. Archives of General Psychiatry 60:69-77.

Semendeferi K, Armstrong E, Schleicher A, Zilles K, Van Hoesen GW. 1998. Limbic frontal cortex in hominoids: a comparative study of area 13. Am J Phys Anthropol 106:129-155.

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.

Sherwood CC, Broadfield DC, Holloway RL, Gannon PJ, Hof PR. 2003. Variability of Broca's Area Homologue in African Great Apes: Implications for Language Evolution. Anatomical Record Part A 271A:276-285.

Uylings HBM, Jacobsen AM, Zilles K, Amunts K. 2006. Left-right asymmetry in volume and number of neurons in adult Broca's area. Cortex 42:652-658.

Uylings HBM, Malofeeva LI, Bogolepova IN, Jacobsen AM, Amunts K, Zilles K. 2005. No postnatal doubling of number of neurons in human Broca's areas (Brodmann areas 44 and 45)? A stereological study. Neuroscience 136:715-728.

von Economo C. 1929. The Cytoarchitectonics of the Human Cerebral Cortex. London: Oxford University Press.

Zuberbuhler K, Cheney DL, Seyfarth RM. 1999. Conceptual semantics in a nonhuman primate. Journal of Comparative Psychology 113:33-42.

MICROSTRUCTURAL ASYMMETRIES OF THE CEREBRAL CORTEX IN HUMANS AND OTHER MAMMALS

ABSTRACT

The human brain shows marked gross anatomical and microstructural asymmetries that are presumably related to lateralized motor and cognitive functions. This chapter generally summarizes the extant data on gross morphological asymmetries in human and nonhuman brains. In addition, the evidence of microstructural asymmetries, such as gray-level index, mini-column width, and cellular organization, are presented. Although there are few studies of microstructrual asymmetries in nonhuman primates, it is argued that such studies are important for validating morphological asymmetries as well as for understanding the cellular basis for hemispheric specialization in primates, including humans.

INTRODUCTION

The cerebral cortex can be parcellated into areas that differ in their cytoarchitecture [Brodmann 1909], chemoarchitecture [Krubitzer and Huffman 2000; Krubitzer and Kahn 2003], connectivity [Barbas and Rempel-Clower 1997], and distribution of receptors for neurotransmitter molecules [Zilles et al. 2002, 2004]. It is well known that such regional variation in cortical microstructure contributes to the many distinct functional specializations of the cortex. For example, differential activation of cortical areas known to vary in microstructure has been demonstrated in numerous studies in humans, using functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and electroencephalography (EEG) [e.g. Azari et al. 2001; Beauregard et al. 2001; Bookheimer et al. 1997; Dagher et al. 1999; Grabowski et al. 2001; Pesenti et al. 2001; Rowe and Passingham 2001].

The cerebral hemispheres are also often differentially recruited in divergent functional activities. Thus, a region that is recognized as homotopic between hemispheres on the basis of topology and basic cytoarchitectural organization may participate in different information processing in each hemisphere. The best documented example of such cerebral lateralization is language, where 95% of humans are left hemisphere dominant for the production and comprehension of language [Branche et al. 1964; Ojemann 1991; Petersen et al. 1988]. As there are structural differences between cortical areas that exhibit functional variation, interhemispheric functional asymmetry in the cerebral cortex arises, in part, from differences in the connectivity and microstructure of homotopic cortical areas [Hutsler and Galuske 2003]. Therefore, important insights into the anatomical substrates of the lateralized functions of the cerebral cortex can be gained through the incorporation of microstructural data.

At present, however, the majority of studies concerning human brain asymmetries are at the level of macrostructure. For example, the human brain exhibits large-scale asymmetries in the protrusion of the frontal and occipital lobes, called petalias [Holloway and de Lacoste-Lareymondie 1982]. Furthermore, it is known that regions associated with language function are also asymmetric in their gross anatomy. Still, it is not entirely clear how such large-scale asymmetries are reflected in the microstructure of the cerebral hemispheres. It has been suggested that volumetric differences are primarily caused by changes in the number of cells, rather than by changes in cell density [Galaburda et al. 1986; Rosen 1996]. However, other types of microstructural asymmetries also exist. Asymmetries in cell size, columnar organization, the complexity of dendritic arbors, and chemoarchitectural organization have been reported [Buxhoeveden et al. 2001; Garcia et al. 2004; Hayes and Lewis 1995; Scheibel et al.

1985]. Here we review known structural asymmetries in the human cerebral cortex, with special attention to the relatively small literature on microstructural asymmetries in the brains of other mammals. We discuss the implications of current data to our understanding of the evolution of functional cortical asymmetries in humans.

GROSS ANATOMICAL ASYMMETRIES

Pioneering research on the human brain by Paul Broca drew attention to the functional asymmetries of the human inferior frontal cortex through evidence that lesions to the left cerebral hemisphere tend to result in language impairments [Broca 1861]. These initial observations inspired an explosion of studies concerning the distribution of gross anatomical asymmetries of the human brain as they relate to functional asymmetries. While early efforts were constrained to rely on small samples of postmortem brains, in recent years, the advent of magnetic resonance imaging (MRI) and voxel-based morphometry methods have allowed measurement of cerebral asymmetries in larger samples. With this proliferation of data, much has been learned regarding how gross anatomical asymmetries in the human cortex vary with age, sex, psychiatric, and neurological conditions. As a rule, these gross morphological data are interpreted to reflect corresponding changes in some aspect of underlying neural circuitry. Many asymmetries are measured using sulcal landmarks, which may [Brodmann 1909] or may not [Amunts et al. 1999; Sherwood et al. 2003] reflect the boundaries of cortical areas as defined by microstructural details. Nonetheless, if we assume that gross morphological asymmetries emerge from some underlying factor that causes the displacement of tissue volumes, sulci, and gyri, then consideration of cortical asymmetry at the macroanatomical level yields suggestive

evidence regarding regions where microstructural architecture may express asymmetry as well. Studies of this variety have primarily addressed the length of particular sulci as well as the volume of areas defined by sulcal boundaries [Tomaiuolo et al. 1999; White et al. 1997a].

Asymmetries in sulcal lengths and trajectories in humans have been described for the central sulcus and the Sylvian fissure. In one study, the length of the central sulcus at the cortical surface was found not to evince a consistent pattern of lateral asymmetry [White et al. 1997a]. The depth of the central sulcus, however, as measured on horizontal MR images, displays an interaction with sex and handedness [Amunts et al. 2000]. Asymmetry of the contralateral central sulcus was most pronounced in right-handed men, with a decreased degree of asymmetry in mixed- and left-handed men. In contrast, asymmetries in central sulcus depth did not differ across handedness in women. Likewise, the “hand knob” of primary cortex in chimpanzees does not display a significant population-level asymmetry [Hopkins and Cantalupo 2004]. However, a correlation between the volumetric asymmetry of the hand knob in motor cortex and hand preference in a specific tool task was reported, with a tendency for leftward volumetric asymmetry to be associated with preference for using the right hand. Similarly, in male capuchin monkeys, asymmetries in the depth of the central sulcus are also correlated with hand dominance on a coordinated bimanual task [Phillips and Sherwood 2005], suggesting that this neuroanatomical relationship to handedness may be more widespread among primates.

The length of the Sylvian fissure was found to be longer in the left hemisphere in humans [Blanton et al. 2001; Foundas et al. 1999], partly because of the difference in the shape

of the sulcus between the hemispheres. The right sulcus typically contains an upward bend at the posterior end, whereas the left sulcus remains relatively horizontal [Foundas et al. 1999].

A few regions have been found to express volumetric asymmetry on the basis of gross morphological criteria. In humans, the cytoarchitectural subdivisions composing Broca's area, a region involved with language production, frequently lie within the morphological boundaries of the inferior frontal gyrus (IFG). Within the IFG, the ascending (vertical) ramus of the Sylvian fissure separates pars opercularis (Brodmann's area 44) from pars triangularis (area 45), and the anterior (horizontal) ramus separates pars triangularis from pars orbitalis (area 47). Numerous studies have investigated macrostructural asymmetry in Broca's area using these sulcal landmarks to subdivide the region. However, the results from these studies differ markedly, depending on methodology and anatomical definitions. While measures of the convexity of the cortical surface area of the frontal operculum (including the pars opercularis and the posterior portion of the pars triangularis) have not revealed significant population-level leftward dominance [Wada et al. 1975], asymmetries are significant when intrasulcal cortex is included [Falzi et al. 1982; Tomaiuolo et al. 1999]. Furthermore, some volumetric MRI-based studies have found that both pars triangularis and pars opercularis [Foundas et al. 1998, 2001] are leftward dominant, but others have not found volumetric asymmetry in pars opercularis [Knaus et al. 2006; Tomaiuolo et al. 1999]. Thus, a consensus has yet to be reached regarding macrostructural asymmetries in the human inferior frontal gyrus. Nonetheless, population-level leftward asymmetry of fronto-orbital sulcal length, an external morphologic feature in this region of great apes, has been reported in chimpanzees [Cantalupo and Hopkins 2001].

A multi-species MRI analysis of living ape brains found volumetric asymmetries in two structurally-defined subdivisions of the frontal lobe: the dorsal sector (composed of most of the cortex on the lateral surface of the lobe) showed a rightward asymmetry, while the medial cortex (composed of the entire cortex on the medial surface of the lobe) showed a leftward asymmetry. The orbital sector showed no asymmetry [Schenker et al. 2005].

Gross morphological studies have also identified volumetric asymmetries of the planum temporale (PT, including the posterior part of Brodmann's area 22; also known as Tpt), a temporal lobe region involved in language processing, in both humans and chimpanzees using MRI [Emmorey et al. 2003; Hopkins and Cantalupo 2004; Hopkins et al. 1998; Penhune et al. 1996] and postmortem specimens [Anderson et al. 1999; Gannon et al. 2001]. A third region, the angular gyrus in the inferior parietal lobe, recently confirmed as a region in a language circuit [Catani et al. 2005], also exhibits the same left greater than right volumetric asymmetry [Watkins et al. 2001]. Furthermore, the region shows reversed (right greater than left) volumetric asymmetry in schizophrenic patients [Buchanan et al. 2004; Niznikiewicz et al. 2000]. A nearby region, occupying the posterior bank of the posterior ascending branch of the Sylvian fissure, shows a significant interaction between handedness and sex in the analysis of volumetric asymmetry. Right-handed men and left-handed women show a strong rightward asymmetry. Right-handed women also have a rightward asymmetry (but not as strong), while left-handed men show a weak leftward asymmetry [Jancke et al. 1994]. A study of the temporal lobes in MR images of living ape brains reported limited evidence of hemispheric asymmetry in gyrification and surface area [Rilling and Seligman 2002]. Another study found no evidence of asymmetry in images of postmortem chimpanzee brains [Zilles et al. 1996].

Gross anatomical studies of asymmetry have focused predominantly on areas that demonstrate functional asymmetry. Such studies have revealed asymmetries in motor cortex related to hand preference in both humans and chimpanzees. Studies of language related areas (IFG, PT, angular gyrus) report a tendency for these regions to exhibit leftward asymmetry, matching the prevalence of left hemisphere dominance for language, particularly among right-handed individuals. However, repeated studies of the IFG, with varying conclusions, reveal the continuing lack of consensus on the presence and magnitude of such macrostructural asymmetries.

MICROSTRUCTURAL ASYMMETRIES

While gross anatomical analyses can provide an overview of where structural asymmetries may exist, microstructural studies are necessary to elucidate particular hemispheric specializations of neural wiring that underlie functional lateralization. In addition, although comparative microarray analyses of gene expression in the brain can reveal interesting differences between the transcriptomes of humans and other primates [Caceres et al. 2003; Enard et al. 2002; Uddin et al. 2004], this approach is relatively insensitive to subtle variation in gene expression levels among small populations of cells [Geschwind 2000]. This is especially problematic in analyses of regional differences in the cerebral cortex because of the cellular heterogeneity of its composition and large degree of interindividual variation. In this regard, it is noteworthy that a recent study, looking for differentially expressed genes, was unable to distinguish among three regions known to differ in function: human Broca's area in the left hemisphere, its homotopic counterpart in the right, and left dorsolateral prefrontal cortex

[Khaitovich et al. 2004]. This same study also could not detect greater differences in transcript levels between human and chimpanzee Broca's area as compared to several other cortical areas. Therefore, to reveal the correlates of functional cortical lateralization, it is necessary to examine interhemispheric differences in microstructural organization. Several microstructural studies of histological specimens, including investigations of regional volume, cell density, dendritic structure, and cell size, have been performed to investigate asymmetries in human brain areas that have well-established patterns of population-wide gross morphological asymmetry. Figure 1.1 shows interhemispheric comparisons of human cortex in three of these regions demonstrating that variation between the hemispheres is expressed in quite subtle details of histological architecture. Before reviewing the current evidence concerning histological asymmetries in the cerebral cortex, it is important to note that very limited sample size is a problem that plagues all such studies. Indeed, it is not uncommon for samples to be smaller than n=10. Thus, interpretation of negative findings should be considered with caution and should not necessarily be taken as definitive evidence of lack of asymmetry in the larger population.

Volume

Volumetric studies of histological samples based on cytoarchitectural criteria have been conducted in several regions of cortex, including primary motor (Brodmann's area 4), primary visual (area 17), and language related cortices (areas 44, 45, and part of 22).

In a study of six postmortem human brains of unknown handedness, five had a leftward asymmetry in the total volume of primary motor cortex, while the sixth had a larger motor area

in the right hemisphere [Zilles et al. 1996]. However, another study of the same region, using twenty postmortem brains, did not find a significant population level asymmetry in the total volume of primary motor cortex, with a similar number of brains having a leftward asymmetry as had a rightward one [White et al. 1997b]. When only the hand representation area of primary motor cortex was measured, there was a population-level leftward asymmetry that approached significance, but six of twenty specimens exhibited a rightward asymmetry [White et al. 1997b].

The primary visual cortex of humans shows a rightward asymmetry at the population level, although some individual brains show a leftward asymmetry. In a study of 31 brains, the volume of this region of cortex exhibited a significant rightward asymmetry across the sample [Murphy 1985]. The average asymmetry, regardless of direction, was 8%; 24 of the 31 postmortem brains exhibited a rightward asymmetry, while the remaining 7 had a leftward asymmetry. In another cytoarchitectural study, a similar right-hemispheric bias in the volume of primary visual cortex was found in 11 of 14 brains, with a mean asymmetry of 13.7% [Andrews et al. 1997].

Volumetric asymmetries have also been reported in both anterior and posterior language areas, involved in production and comprehension respectively. The anterior language area consists of Brodmann's areas 44 and 45. Using computer-assisted observer-independent quantification of laminar cytoarchitectural variation, recent studies have been able to parcellate and measure the volume of areas 44 and 45 on the basis of the multivariate distances between their quantitative cytoarchitectural profiles [Amunts et al. 1999; Schleicher et al. 1999; Uylings et al. 2006]. When this method of volumetric measurement was applied to 10 adult human brains of both sexes, a robust leftward volumetric asymmetry was found in area 44 (all 10 brains had a

larger area 44 in the left than in the right hemisphere). In area 45, the degree of asymmetry (without regard to direction) was similar to that found in area 44. The five female brains all displayed leftward asymmetry, but there was no significant asymmetry was detected among the five male brains [Amunts et al. 1999; Uylings et al. 2006]. In these studies, the location of cytoarchitectural boundaries among area 44, 45, and adjacent areas did not correspond to external sulcal landmarks. Of note, another study concerning the correspondence between the boundaries of area 44 in common chimpanzees as defined by cytoarchitecture, myeloarchitecture, and the distribution of nonphosphorylated neurofilament protein-immunoreactive neurons, also failed to reveal a correlation between the borders of this cortical area and external morphologic features [Sherwood et al. 2003].

The posterior language area includes part of Brodmann's area 22. A study of area Tpt (which comprises the posterior part of area 22) in four human brains found left hemisphere volumetric dominance in all four specimens based on qualitative assessment of cytoarchitectural boundaries [Galaburda and Sanides 1980]. A correlation between the volume of Tpt and the surface area of the grossly-defined planum temporale has also been reported [Galaburda et al. 1978]. One of the four brains in this study had a slight rightward asymmetry in the planum temporale, but this was the brain with the least asymmetric area Tpt.

Grey-level index (GLI)

Asymmetries of cell density have been analyzed indirectly by measurement of gray level index (GLI), which estimates the fraction of tissue volume that is occupied by Nissl-stained cell bodies versus neuropil space. In the region of hand representation in primary motor cortex

(area 4), the right hemisphere was found to have, on average, greater GLI than the left hemisphere in 12 postmortem human brains [Amunts et al. 1996]. This means that in the right hemisphere a greater percentage of the total tissue volume was occupied by cell soma than the corresponding region in the left hemisphere. In contrast, the left hemisphere contained relatively more neuropil space, which is occupied primarily by dendrites, axons, and synapses. This asymmetry was not confined to single layer, but was observed across the entire cortical depth. In young children, asymmetry of GLI is present in this region as a whole, although development of adult-like asymmetry in supragranular cortical layers (layers II and III), which are involved in corticocortical association projections, is delayed as compared to infragranular layers (layers V and VI), which are involved in projections to subcortical structures [Amunts et al. 1997b].

Similar analyses of asymmetries in GLI have been conducted in the inferior frontal gyrus. Amunts et al. [1999] found GLI to be greater in left area 44 than in the corresponding area in the right hemisphere in all of the male (n = 5) and three of the female brains (n = 5) studied. Thus, there may be a sex difference in the presence of asymmetry in this region. No asymmetry or sex difference in GLI was observed in area 45 [Amunts et al. 1999]. However, in a subsequent study, using a larger sample that included the brains from the earlier study, Amunts and colleagues [Amunts et al. 2003] reported a significantly greater GLI in areas 44 and 45 in the *right* hemisphere when the two areas were analyzed together. Furthermore, they found that asymmetry increases with age, with infants showing little or no asymmetry. GLI decreased in both areas with age, primarily during early childhood. However, the decrease differed between hemispheres, meaning that the adult pattern of asymmetry did not appear until relatively late in development (age 5 for area 45 and age 11 for area 44).

Pyramidal cell somatodendritic geometry

The size of pyramidal cell dendritic arbors, the number and complexity of their branches, and their spine density are known to vary among cytoarchitectonic areas in humans [Jacobs et al. 2001] and macaques [Elston 2000; Elston and Rockland 2002] as well as among species [Elston et al. 2001]. These parameters may provide a picture of the complexity of the integrative capacity of individual pyramidal cells.

Several studies of human cortex have focused specifically on measuring asymmetry in the dendritic arbors of pyramidal cells based on Golgi impregnations. Scheibel et al. [1985] analyzed the dendritic profiles of pyramidal cells in the orofacial region of primary motor cortex and found that, overall, the number of dendritic segments was greater in the left hemisphere in a sample of six right-handed adult human males. However, total dendritic length was slightly greater in the right hemisphere. They also found an interaction between hemisphere and order of the segment (which is the distance of the segment from the cell body in terms of the number of branching points). The number and total length of segments more proximal to the soma were greater in the right hemisphere, while higher-order segments were greater in the left hemisphere.

Scheibel et al. (1985) also analyzed the inferior frontal gyrus and found longer total dendritic length in the left frontal opercular region. However, as in the motor cortex, this difference was mainly due to the length of higher-order segments (those further from the cell body) in the left hemisphere, as lower-order segments were longer in the right [Scheibel et al. 1985]. In contrast, another study examined asymmetries in only the largest pyramidal cells in

layer III of area 45 in humans [Hayes and Lewis 1996]. This population of pyramidal neurons, known as magnopyramidal cells, mainly furnishes long corticocortical association projections. Total dendritic length, dendritic complexity (numbers of branches and maximal branch order) and spine densities were found to be greater in the right. Contrary to the authors' expectations, dendritic length was positively correlated with soma volumes of magnopyramidal cells only in the left hemisphere and not in the right [Hayes and Lewis 1996]. This finding suggests that there are additional factors that significantly contribute to the size of cells and the size of their dendritic arbors. Furthermore, these two studies of pyramidal cell geometry in anterior language-related cortical areas indicate that there are differences in the somatodendritic geometry of different cell populations within a single region.

Studies of dendritic parameters in the planum temporale are somewhat more difficult to interpret. One study found a slight leftward asymmetry in total dendritic length averaged across 20 human individuals [Jacobs et al. 1993]. Individually, only twelve of the twenty brains showed a leftward asymmetry. A similar asymmetry in the number of dendritic spines was observed, however, no asymmetry in mean dendritic segment length was found. Furthermore, there was a significant negative correlation between age and total dendritic length, and accompanying the decrease in length was a decrease in asymmetry. That is, in younger individuals (<50 years), total dendritic length was significantly greater in the left than in the right hemisphere, but a clear asymmetry was not present in the older sample [Jacobs and Scheibel 1993]. In contrast, another study found a right greater than left asymmetry in three dendritic parameters: total basal dendritic length, number of dendrite branches, and number of dendritic spines, in seven of nine individuals [Anderson and Rutledge 1996], but, like Jacobs and Scheibel [1993], these

authors also found a negative correlation between these three variables and age, with no corresponding difference in cell soma size.

Cell columns and connectivity

The isocortex (neocortex) is populated by vertically-oriented aggregates of cells with strong vertical interconnections among layers, forming fundamental structural and functional units known as minicolumns [Douglas and Martin 1992; Mountcastle 1997]. The emergence of columnar organization is related to the migration of neuroblasts from the ventricular and subventricular zones into radial columns during development [Rakic 1995]. Cellular minicolumns differ from pyramidal cell modules, which have also been identified as minicolumns [Rockland and Ichinohe 2004]. Such modules are formed by a core of apical dendrites surrounded by neurons that do not necessarily align in vertical rows [Peters and Kara 1987; Peters and Sethares 1991; Rockland and Ichinohe 2004]. In contrast, cellular minicolumns comprise single rows of neurons [Buxhoeveden and Casanova 2002; Mountcastle 1997]. The width of a cellular minicolumn is a measure of the size of the core region of the minicolumn, which contains the majority of the neurons and apical dendrites, and both myelinated and unmyelinated fibers [Buxhoeveden and Casanova 2002; Mountcastle 1997; Seldon 1981]. A cell-poor region, containing dendritic arbors, unmyelinated axons, and synapses, surrounds each minicolumn. The size of the cell-poor area is quantified as the distance between minicolumns. The width of these columns has been investigated in multiple auditory areas in the temporal lobe, including von Economo and Koskina's areas TA, TB, and TC [Seldon 1981]. Both the width and the distance between minicolumns were found to be greater in the left

hemisphere than in the right throughout auditory cortex. In most auditory cortical areas, the length of basal dendrites was found to compensate for asymmetric differences in distance between columns, but *not* in the planum temporale. In the planum temporale, the tangential extent of dendrites was increased in the left hemisphere, but not to a degree that completely compensated for the differences in minicolumn spacing between hemispheres. Some of these findings have been replicated by recent studies of area Tpt, which found a greater width of minicolumns and a relatively larger volume of neuropil space in the left hemisphere of humans, but no such asymmetry in chimpanzees and rhesus macaques [Buxhoeveden et al. 2000, 2001].

Interconnectivity among cell columns in posterior area 22 has also been studied using carbocyanine dye to anterogradely label axons and retrogradely label cells in postmortem human specimens [Galuske et al. 2000]. Labeled terminal axon arbors and labeled cells were found to be superimposed, forming regularly spaced clusters surrounding the injection site. The average size of the clusters was the same in the two hemispheres, but the distance *among* clusters was significantly larger in the left hemisphere. Such an asymmetry did not exist in primary auditory cortex. Galuske et al. (2000) suggest that the labeled clusters represent different subsystems of interconnected columns and that the greater distance between clusters in the left hemisphere implies the presence of more subsystems within area 22 in the left hemisphere than in the right. However, it is also possible that larger cell columns may account for the distance between clusters in the left hemisphere. This would mean that axons in the right and left hemispheres reach across the same number of columns and that subsystems in the left hemisphere are simply more spread out, but not more numerous.

Using postmortem specimens and gross morphological boundaries, Anderson et al. [1999] found a volumetric asymmetry in white matter within the posterior superior temporal gyrus corresponding to a part of area Tpt in humans and then examined the contribution of various microstructural factors to this asymmetry. Although no interhemispheric differences were found in the number of nonphosphorylated neurofilament protein-immunoreactive neurons, the relative volume of glial cells, or the diameters of axons, the axons in the left hemisphere had significantly thicker myelin sheaths than their counterparts in the right hemisphere. It would be interesting to know whether comparable asymmetries in myelination might explain interhemispheric asymmetries in neuropil space reported in various other cortical areas.

Cell size and number

Another parameter in which asymmetries have been investigated is the size of individual cells. Neuronal soma volume is determined by the biosynthetic and metabolic requirements of the entire cell, including its dendritic arbors and axon [Kaas 2000], suggesting that differences in cell volume may represent changes in the thickness and ramifications of cells' neurites or differences in metabolic activity. Asymmetries in cell sizes have been found in several regions throughout human cortex. In primary visual cortex, the left hemisphere tends to have larger neurons than the right, an asymmetry that is markedly absent in dyslexic patients [Jenner et al. 1999]. However, this region has greater numbers of neurons in the right hemisphere in rhesus macaques [Suner and Rakic 1996].

In frontal cortex, the presence and direction of asymmetry differs by the region analyzed. In dorsal area 4, no interhemispheric differences were found in the mean size of layer III magnopyramidal cells. In area 45, layer III magnopyramidal cells were larger in the left hemisphere, while in area 46 these cell types were larger in the right hemisphere. Within the right hemisphere, no difference in cell size was observed among the three areas. However, cells in left area 45 were significantly larger than those in either left area 46 or left area 4 [Hayes and Lewis 1995].

Furthermore, asymmetry within area 45 is observable only in the largest pyramidal cells in layer IIIb [Hayes and Lewis 1995]. If all pyramidal cells are sampled equally, there is no difference in mean cell size between area 45 in the right and left hemispheres. This suggests that the distribution of cell sizes differs between the two hemispheres. If the mean size of all pyramidal cells is equal in the two hemispheres, than left area 45 must also have more small cells than right area 45 in order to counterbalance the larger magnopyramidal cells. Total neuron count in the inferior frontal gyrus of humans may also be asymmetrical [Uylings et al. 2006]. In a study of 10 human brains, Uylings et al. (2006) found neuron numbers in area 44 to be greater in the left than right hemisphere in all ten brains, but the difference only reached significance within the 5 male brains. In area 45, neuron numbers were leftwardly asymmetric in all 5 female brains, but only in 2 of 5 male brains. However, no asymmetry was found in neuron *density* in this study.

A study of pyramidal cells in the superior temporal lobe found greater numbers of magnopyramidal cells throughout auditory cortex in the left hemisphere than in the right, including primary and secondary auditory cortex, as well as regions within Wernicke's area,

such as the planum temporale and the supramarginal gyrus. The largest interhemispheric differences were seen in the anterior regions (Heschl's gyrus and anterior planum temporale) and the magnitude decreased posteriorly [Hutsler and Galuske 2003].

Cell size asymmetries have also been found in particular subpopulations of pyramidal cells. Hutsler and Gazzaniga [1996] found size asymmetries in acetylcholinesterase-enriched cells in lower layer III of several regions in the posterior superior temporal lobe, not restricted to putative language areas. Another study also reports greater size of acetylcholinesterase-rich layer III magnopyramidal cells that is restricted to left area 45 of humans [Garcia et al. 2004]. Acetylcholinesterase is an enzyme responsible for the deactivation of acetylcholine. Density of the labeled cells was symmetrical, but differed among cortical areas. Interestingly, however, a previous study of choline acetyltransferase, an enzyme that facilitates the formation of acetylcholine, found that the concentration of this enzyme was significantly greater in the left hemispheres than in the right hemispheres of four individuals [Amaducci et al. 1981]. Peak concentration seemed to be located within layers II and IV in both hemispheres.

DISCUSSION

Functional anatomy

Microstructural asymmetries have been primarily reported in four regions of the human brain, including primary motor cortex, primary visual cortex, and both anterior (Broca's) and posterior (Wernicke's) language areas.

Motor control is one of the most conspicuously lateralized functions. Most humans exhibit a preference for using one hand rather than the other for most tasks, with the majority showing right hand dominance. Functional studies of primary motor cortex show that the

strongest activation is generally within the primary motor cortex contralateral to the movement [Rao et al. 1995]. Greater usage is known to be related to the size of cortical areas within motor cortex in both humans [Amunts et al. 1997a; Karni et al. 1995; Pascual-Leone et al. 1995] and squirrel monkeys [Nudo et al. 1996]. Thus, if volumetric asymmetry of motor cortex reflects population-level right-hand dominance, we might find a leftward asymmetry in the total size of the motor cortex. The presence of such an asymmetry may [Zilles et al. 1996] or may not [White et al. 1997b] exist. However, it is likely that such an asymmetry exists within the hand representation region of motor cortex [White et al. 1997b]. Other parameters also display asymmetry in portions of primary motor cortex. Relative neuropil volume seems to be greater in the left hemisphere in the hand region of area 4 [Amunts et al. 1996], which is particularly interesting if the same region expresses a volumetric asymmetry. Thus, in humans greater macrostructural asymmetry of the hand representation in the left hemisphere may be due in part to elaboration of interconnections in the dominant hemisphere. The complexity of connectivity also differs between hemispheres in the orofacial region as represented by dendritic geometry [Scheibel et al. 1985], but no interhemispheric difference has been found in cell size [Hayes and Lewis 1995].

Vision is also functionally lateralized, with one eye being dominant. As with motor cortex, visual cortex is more strongly activated contralaterally [Miki et al., 2000, 2001]. However, despite the higher prevalence of right eye dominant individuals [Annett 2000], visual cortex more frequently exhibits a rightward volumetric asymmetry [Murphy 1985] suggesting that the anatomical asymmetry is unrelated to eye dominance. Murphy [1985] has suggested the rightward bias in volume might underlie right hemisphere/left visual field superiority for a

number of visual tasks [see Kimura and Durnford 1974]. An asymmetry in the reverse direction is observed in cell size; the left hemisphere tends to have larger neurons than the right, an asymmetry that is markedly absent in dyslexic patients [Jenner et al. 1999].

The finding of an asymmetry in visual cortex that relates to language is interesting because language is an aspect of cognition that has garnered significant attention regarding both functional and anatomical lateralization. Broca [1861] and Wernicke [1874] first identified regions in the left hemisphere of the brain that are crucial to proper language function. Since those classical studies, the functional lateralization of these regions (inferior frontal gyrus and posterior superior temporal) has been confirmed via countless studies using the lesion method and/or functional imaging. Furthermore, asymmetries have been reported in at least one language area for each microstructural parameter reviewed here.

Volumetric asymmetries have been reported in both anterior and posterior language areas. Population level asymmetries were found in areas 44 and Tpt [Amunts et al. 1999; Galaburda and Sanides 1980]. Asymmetry was also reported at the individual level in area 45, but not at the population level [Amunts et al. 1999]. Volumetric asymmetry in the white matter of the posterior superior temporal lobe has been found to be related to the thickness of the myelin sheaths encompassing axons [Anderson et al. 1999]. There is a greater percentage of neuropil space per unit volume in the dominant hemisphere in the inferior frontal gyrus [Amunts et al. 2003].

Reports regarding dendritic parameters differ among cell populations and among regions. In the inferior frontal gyrus, total dendrite length is greater in the left hemisphere [Scheibel et al. 1985], but if only magnopyramidal cells are examined, then dendritic parameters

are greater in the right hemisphere [Hayes and Lewis 1996]. In the temporal language area, there seems to be substantial individual variation in dendritic parameters. Asymmetries with left [Jacobs et al. 1993] or right [Anderson and Rutledge 1996] hemisphere dominance have both been reported.

Cell columns have been found to be larger in the left planum temporale than in the right [Buxhoeveden et al. 2000]. This may correspond to reports that patterns of interconnectivity differ between the two hemispheres in this region [Galuske et al. 2000]. To our knowledge, cell columns and related interconnectivity have yet to be analyzed in the inferior frontal gyrus.

The size of magnopyramidal cell soma in layer III displays asymmetry in area 45, such that this subpopulation of the largest pyramidal cells is larger in the left hemisphere. This is in contrast to a neighboring region with no known language involvement, area 46, in which the corresponding cells are larger in the right hemisphere [Hayes and Lewis 1995]. Similar asymmetries have been observed in temporal auditory and language areas, with greater numbers of layer III magnopyramidal cells present in the left hemisphere [Hutsler and Galuske 2003]. Also, acetylcholinesterase-enriched cells are larger in left auditory areas and area 45 than in the corresponding regions in the right hemisphere [Garcia et al. 2004; Hutsler and Gazzaniga 1996].

Individual variability and population-level asymmetry

In all of the above studies that report individual values, there is strong evidence that individual variability is present. Thus, even where substantial asymmetry exists in individuals, it does not necessarily follow that population-level asymmetry is present. For example, in a

sample of ten human brains, nine exhibited strong asymmetry in the volume of area 45 (> 6%), but four favored the right hemisphere and five favored the left, meaning that no significant asymmetry was seen at the population level [Amunts et al. 1999].

Similarly, the presence of a population-level asymmetry does not rule out the presence of individual variability. In fact, the presence of substantial interindividual variability means that the population-level findings of a study can change with the addition of more individuals. This is evident by a comparison of Amunts and colleagues' studies (1999, 2003) on asymmetries in Broca's area. In the earlier study, the sample size was 10, which in histological studies is quite large. Nonetheless, the subsequent study included additional adult individuals (sample size of 16) and resulted in different conclusions. Furthermore, the differences between hemispheres in these two studies are quite small when compared with interindividual variability.

Asymmetry in nonhumans

Very limited evidence is currently available to examine whether humanlike asymmetries are present in nonhuman species [Buxhoeveden et al. 2001; Gannon et al. 2000; Kheck et al. 1999; Rosen et al. 1993; Sherwood et al. 2005]. In a direct comparison of the microstructure of area Tpt in humans, chimpanzees, and rhesus macaques, Buxhoeveden and colleagues (2001) reported that only humans have left dominant asymmetry of neuropil volume and minicolumn widths, suggesting that the microstructure of the dominant hemisphere has been reorganized in humans for its involvement in language function. Small samples of nonhuman species in this study, however, give reason to be cautious in concluding that such histological asymmetries are entirely absent. If population-level asymmetries are present, but to a lesser magnitude than

found in humans, they would be more difficult to detect statistically in a small sample. In this regard, it is notable that another study that examined volumetric asymmetries of cytoarchitecturally-defined area Tpt in long-tailed macaques, revealed significantly greater volume of this cortical area on the left (Gannon et al., 2000). Interestingly, asymmetries have also been described in the distribution of calcium-binding protein-immunoreactive inhibitory interneuron subtypes within area Tpt of macaques (Kheck et al., 1999). Unfortunately, comparable data on interneuron distributions within area Tpt of humans does not exist. Taken together, these findings suggest that asymmetry of the size and some aspects of microcircuitry in area Tpt of humans may be an ancestral homology that is shared with other Old World primates. This interpretation is consistent with observations based on behavior, functional imaging, and lesion studies indicating that macaques are left hemisphere dominant for the processing of acoustic features in conspecific vocal calls [Hauser and Andersson 1994; Heffner and Heffner 1984; Petersen et al. 1984; Petersen et al. 1978; Poremba et al. 2004].

It has been suggested that, given the conduction delays associated with interhemispheric transfer, functional and structural asymmetries evolve as an adaptation to preserve temporal fidelity in the processing of complex streams of serial information, such as the vocal calls of conspecifics and the performance of fine motor sequences [Ringo et al. 1994]. Thus, it might be expected that due to these network constraints, lateral asymmetries will emerge among any species that relies heavily on acoustic communication in its social interactions or that displays high dexterity of movements. Indeed, house mouse mothers exhibit a right ear preference in their orientation response to the ultrasonic distress calls of their pups [Ehret 1987] and electrophysiological mapping reveals a greater extent of auditory cortex

surface area in the left hemisphere compared to the right [Stiebler et al. 1997]. Furthermore, data from multiunit recordings in starlings shows lateralization in the strength of neuronal activation in response to the presentation of species-specific songs, but not other artificial sounds, with interindividual variation in the dominant hemisphere [George et al. 2002]. Although the results of these studies would seem to suggest that humanlike asymmetry of auditory cortex is prevalent among vertebrates, a cytoarchitectural study of auditory cortical area DSCF in mustached bats did not find population-level asymmetries in neuronal densities, glial-neuron ratios, or the distribution of magnopyramidal cells [Sherwood et al. 2005]. This is in spite of the fact that mustached bats use a complex repertoire of vocalizations, with heteroharmonic calls that can last a second or more [Kanwal et al. 1994] and some auditory cortical neurons display specialized response properties for social vocalizations [Esser et al. 1997; Ohlemiller et al. 1996].

While functional asymmetry may be a common feature in processing social vocal communication among vertebrates, the microstructural correlates of this phenomenon have yet to be fully elucidated. Evidence from mice and macaques suggest that the volumetric extent of auditory cortical areas may express left hemisphere dominance. However, most studies of the intrinsic microstructure of the cortex in nonhumans have failed to reveal patterns of asymmetries that are homologous with humans. Some suggest that the particular pattern of asymmetries present in the human cortex is the defining characteristic of the human species [Crow 1998a, b]. The argument is that there are species-level asymmetries in humans, showing a consistency in the direction of asymmetry across individuals that may be absent in other species. Furthermore, certain psychological disorders, such as schizophrenia, may be

associated with abnormal or absent asymmetries [Buchanan et al. 2004; Crow 2004; Irle et al. 2005; Niznikiewicz et al. 2000] of the cortex, suggesting that typical asymmetries are important for normal human brain function. However, one must consider that the absence of evidence for asymmetries in nonhuman primates is not evidence of absence. Given the paucity of comparative data concerning microstructural cortical asymmetries, it is premature to assume that such asymmetries are absent among nonhumans.

Future directions

There is ample evidence for microstructural asymmetries in human neocortex. However, the evidence is neither consistent nor uniformly distributed. No parameter has been analyzed equivalently across many cortical areas, and repeated analyses of a single measure in one cortical area sometimes produce differing results. Furthermore, our knowledge of the presence or absence of asymmetries in nonhumans is even more limited than our knowledge of asymmetries in humans.

There are tremendous opportunities for additional research in this area. Much is not yet known regarding how asymmetry in a particular region reflects the function of that region, how much individual variability there is in asymmetry and how that variability is reflected in population-level asymmetry, nor how cortical asymmetries have evolved over time. Future studies are needed to fill this gap. Ongoing investigations include the analysis of cell columns in humans and their closest relatives (great apes and gibbons) in multiple regions and the mapping of individual cortical areas across species. Additional future studies should include increased investigations of both humans and nonhumans and examination of microstructural

parameters that can be compared with existing studies. Further studies of humans that can replicate and expand upon existing findings will help to elucidate the functional anatomy of asymmetry, while additional comparative analyses will serve to illuminate the evolution of asymmetry and further shed light on possible correlations with known functional asymmetry.

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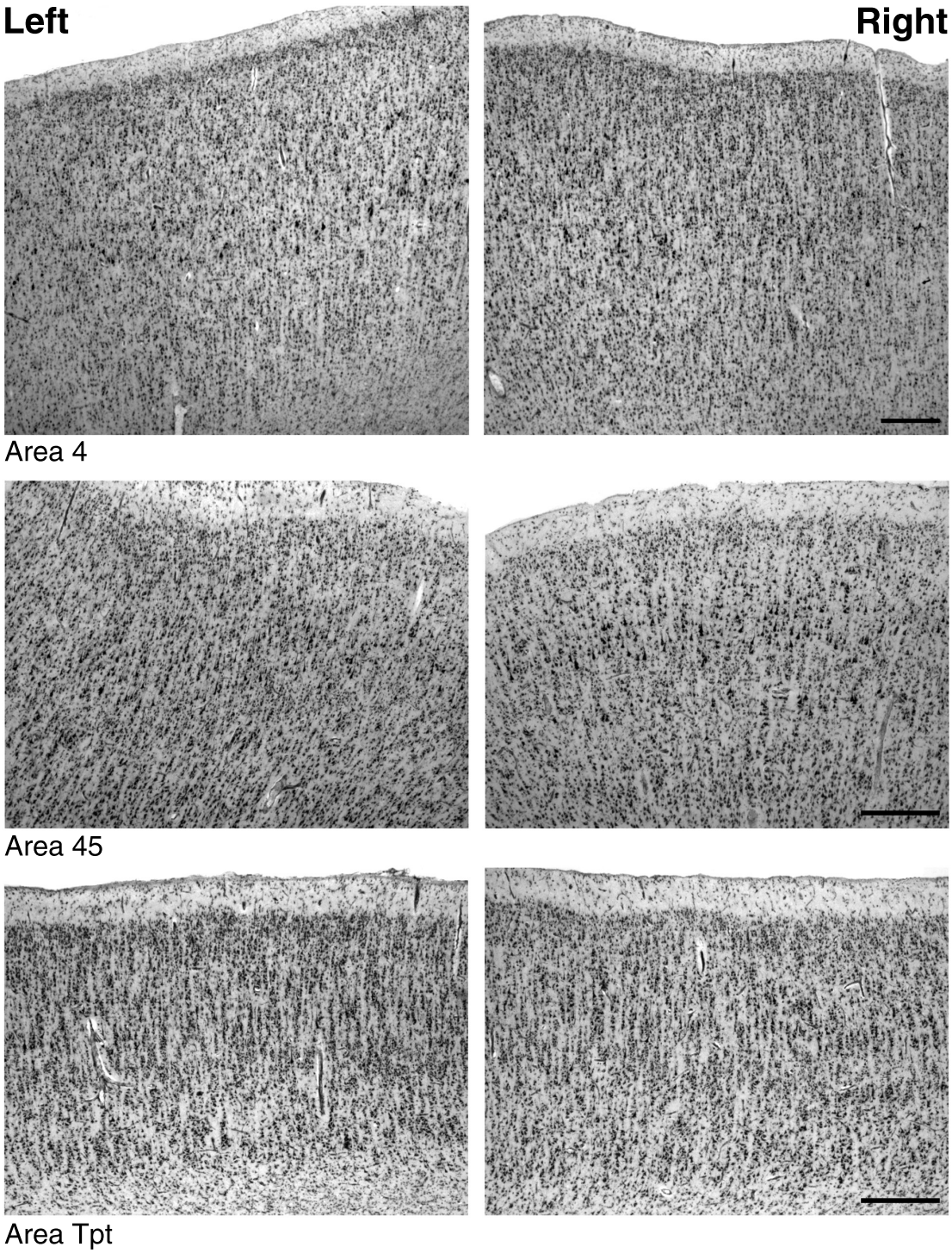

Figure 1.1 Representative micrographs of cortex from the left and right hemispheres of a human brain in areas 4, 45, and Tpt. Notice the overall similarity between cortical areas in each hemisphere. The asymmetries that have been detected have relied on quantitative methods that are capable of measuring subtle variations in histological features such as cell sizes, cell densities, and the space between minicolumns.

REFERENCES

Amaducci L, Sorbi S, Albanese A, Gainotti G. 1981. Choline-Acetyltransferase (ChAT) Activity Differs in Right and Left Human Temporal Lobes. Neurology 31(7):799-805.

Amunts K, Jancke L, Mohlberg H, Steinmetz H, Zilles K. 2000. Interhemispheric asymmetry of the human motor cortex related to handedness and gender. Neuropsychologia 38(3):304-312.

Amunts K, Schlaug G, Jancke L, Steinmetz H, Schleicher A, Dabringhaus A, Zilles K. 1997a. Motor cortex and hand motor skills: Structural compliance in the human brain. Human Brain Mapping 5(3):206-215.

Amunts K, Schlaug G, Schleicher A, Steinmetz H, Dabringhaus A, Roland PE, Zilles K. 1996. Asymmetry in the human motor cortex and handedness. Neuroimage 4(3):216-222.

Amunts K, Schleicher A, Burgel U, Mohlberg H, Uylings HBM, Zilles K. 1999. Broca's Regions Revisited: Cytoarchitecture and Intersubject Variability. Journal of Comparative Neurology 412:319-341.

Amunts K, Schleicher A, Ditterich A, Zilles K. 2003. Broca's region: Cytoarchitectonic asymmetry and developmental changes. Journal of Comparative Neurology 465(1):72-89.

Amunts K, SchmidtPassos F, Schleicher A, Zilles K. 1997b. Postnatal development of interhemispheric asymmetry in the cytoarchitecture of human area 4. Anatomy and Embryology 196(5):393-402.

Anderson B, Rutledge V. 1996. Age and hemisphere effects on dendritic structure. Brain 119:1983-1990.

Anderson B, Southern BD, Powers RE. 1999. Anatomic asymmetries of the posterior superior temporal lobes: A postmortem study. Neuropsychiatry, Neuropsychology, and Behavioral Neurology 12(4):247-254.

Andrews TJ, Halpern SD, Purves D. 1997. Correlated size variations in human visual cortex, lateral geniculate nucleus, and optic tract. Journal of Neuroscience 17(8):2859-2868.

Annett M. 2000. Predicting combinations of left and right asymmetries. Cortex 36(4):485-505.

Azari NP, Nickel J, Wunderlich G, Niedeggen M, Hefter H, Tellmann L, Herzog H, Stoerig P, Birnbacher D, Seitz RJ. 2001. Neural correlates of religious experience. European Journal of Neuroscience 13(8):1649-1652.

Barbas H, Rempel-Clower N. 1997. Cortical structure predicts the pattern of corticocortical connections. Cerebral Cortex 7(7):635-646.

Beauregard M, Levesque J, Bourgouin P. 2001. Neural correlates of conscious self-regulation of emotion. Journal of Neuroscience 21(18):U11-U16.

Blanton RE, Levitt JG, Thompson PM, Narr KL, Capetillo-Cunliffe L, Nobel A, Singerman JD, McCracken JT, Toga AW. 2001. Mapping cortical asymmetry and complexity patterns in normal children. Psychiatry Research-Neuroimaging 107(1):29-43.

Bookheimer SY, Zeffiro TA, Blaxton T, Malow BA, Gaillard WD, Sato S, Kufta C, Fedio P, Theodore WH. 1997. A direct comparison of PET activation and electrocortical stimulation mapping for language localization. Neurology 48(4):1056-1065.

Branche C, Milner B, Rasmussen T. 1964. Intracarotid sodium amytal for the lateralization of cerebral speech dominance. Journal of Neurosurgery 21:399-405.

Broca P. 1861. Remarques sur le siège de la faculté du langage articulé, suivies d'une observation d'aphémie. Bulletin de la Société Anatomique Paris 36:330-357.

Brodmann K. 1909. Vergleichende Lokalisationslehre der Grobhirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Garey LJ, translator. Leipzig: Johann Ambrosius Barth.

Buchanan RW, Francis A, Arango C, Miller K, Lefkowitz DM, McMahon RP, Barta PE, Pearlson GD. 2004. Morphometric assessment of the heteromodal association cortex in schizophrenia. American Journal of Psychiatry 161(2):322-331.

Buxhoeveden D, Armstrong E, Casanova M. 2000. Lateralization patterns of minicolumns in the language area of human and non-human anthropoids. Laterality 5(4):315-30.

Buxhoeveden D, Switala A, Litaker M, Roy E, Casanova M. 2001. Lateralization of minicolumns in human planum temporale is absent in nonhuman primate cortex. Brain, Behavior and Evolution 57:349-358.

Buxhoeveden DP, Casanova MF. 2002. The minicolumn hypothesis in neuroscience. Brain 125:935-951.

Caceres M, Lachuer J, Zapala M, Redmond J, Kudo L, Geschwind D, Lockhart D, Preuss T, Barlow C. 2003. Elevated gene expression levels distinguish human from non-human primate brains. Proceedings of the National Academy of Sciences of the United States of America 100:13030-13035.

Cantalupo C, Hopkins WD. 2001. Asymmetric Broca's area in great apes. Nature 414:505.

Catani M, Jones D, ffytche D. 2005. Perisylvian Language Networks of the Human Brain. Annals of Neurology 57:8-16.

Crow TJ. 1998a. Sexual selection, timing and the descent of man: A theory of the genetic origins of language. Cahiers De Psychologie Cognitive-Current Psychology of Cognition 17(6):1079-1114.

Crow TJ. 1998b. Why cerebral asymmetry is the key to the origin of Homo sapiens: How to find the gene or eliminate the theory. Cahiers De Psychologie Cognitive-Current Psychology of Cognition 17(6):1237-1277.

Crow TJ. 2004. Cerebral asymmetry and the lateralization of language: core deficits in schizophrenia as pointers to the gene. Current Opinion in Psychiatry 17(2):97-106.

Dagher A, Owen AM, Boecker H, Brooks DJ. 1999. Mapping the network for planning: a correlational PET activation study with the Tower of London task. Brain 122:1973-1987.

Douglas R, Martin K. 1992. Exploring cortical microcircuits: A combined anatomical, physiological, and computational approach. In: Zornetzer S, editor. Single Neuron Computation. San Diego and London: Academic Press. p 381-412.

Ehret G. 1987. Left hemisphere advantage in the mouse brain for recognizing ultrasonic communication calls. Nature 325:249-251.

Elston GN. 2000. Pyramidal cells of the frontal lobe: All the more spinous to think with. Journal of Neuroscience 20(18):RC95.

Elston GN, Benavides-Piccione R, DeFelipe J. 2001. The pyramidal cell in cognition: A comparative study in human and monkey. Journal of Neuroscience 21(17):RC163.

Elston GN, Rockland KS. 2002. The pyramidal cell of the sensorimotor cortex of the macaque monkey: Phenotypic variation. Cerebral Cortex 12(10):1071-1078.

Emmorey K, Allen JS, Bruss J, Schenker N, Damasio H. 2003. A morphometric analysis of auditory brain regions in congenitally deaf adults. Proceedings of the National Academy of Sciences of the United States of America 100(17):10049-10054.

Enard W, Khaitovich P, Klose J, Zollner S, Heissig F, Giavalisco P, Nieselt-Struwe K, Muchmore E, Varki A, Ravid R et al. 2002. Intra- and interspecific variation in primate gene expression patterns. Science 296:340-343.

Esser K, Condon C, Suga N, Kanwal J. 1997. Syntax processing by auditory cortical neurons in the FM-FM area of the mustached bat *Pteronotus parnellii*. Proceedings of the National Academy of Sciences of the United States of America 94:14019-14024.

Falzi G, Perrone P, Vignolo LA. 1982. Right-Left Asymmetry in Anterior Speech Region. Archives of Neurology 39(4):239-240.

Foundas AL, Eure KF, Luevano LF, Weinberger DR. 1998. MRI asymmetries of Broca's area: The pars triangularis and pars opercularis. Brain and Language 64(3):282-296.

Foundas AL, Faulhaber JR, Kulynych JJ, Browning CA, Weinberger DR. 1999. Hemispheric and sex-linked differences in Sylvian fissure morphology: A quantitative approach using volumetric magnetic resonance imaging. Neuropsychiatry, Neuropsychology, and Behavioral Neurology 12(1):1-10.

Foundas AL, Weisberg A, Browning CA, Weinberger IR. 2001. Morphology of the frontal operculum: A volumetric magnetic resonance imaging study of the pars triangularis. Journal of Neuroimaging 11(2):153-159.

Galaburda A, Sanides F. 1980. Cytoarchitectonic Organization of the Human Auditory-Cortex. Journal of Comparative Neurology 190(3):597-610.

Galaburda AM, Aboitiz F, Rosen GD, Sherman GF. 1986. Histological Asymmetry in the Primary Visual-Cortex of the Rat: Implications for Mechanisms of Cerebral Asymmetry. Cortex 22(1):151-160.

Galaburda AM, Sanides F, Geschwind N. 1978. Human-Brain: Cytoarchitectonic Left-Right Asymmetries in Temporal Speech Region. Archives of Neurology 35(12):812-817.

Galuske RAW, Schlote W, Bratzke H, Singer W. 2000. Interhemispheric asymmetries of the modular structure in human temporal cortex. Science 289(5486):1946-1949.

Gannon PJ, Hof PR, Kheck N. 2000. Brain language area evolution: Human-like pattern of cytoarchitectonic, but not gross anatomic, L>R hemispheric asymmetry of planum temporale homolog in *Macaca fascicularis*. American Journal of Physical Anthropology Supplement 30:155.

Gannon PJ, Kheck N, Hof P. 2001. Language areas of the hominoid brain: a dynamic communicative shift on the upper east side planum. In: Gibson K, editor. Evolutionary Anatomy of the Primate Cerebral Cortex. Cambridge: Cambridge University Press. p 216-240.

Garcia R, Montiel J, Villalon A, Gatica M, Aboitiz F. 2004. AChE-rich magnopyramidal neurons have a left-right size asymmetry in Broca's area. Brain Research 1026:313-316.

George I, Cousillas H, Richard J, Hausberger M. 2002. Song perception in the European starling: hemispheric specialisation and individual variations. Comptes Rendus Biologie 325:197-204.

Geschwind D. 2000. Mice, microarrays, and the genetic diversity of the brain. Proceedings of the National Academy of Sciences of the United States of America 97:10676-10678.

Grabowski TJ, Damasio H, Tranel D, Ponto LLB, Hichwa RD, Damasio AR. 2001. A role for left temporal pole in the retrieval of words for unique entities. Human Brain Mapping 13(4):199-212.

Hauser M, Andersson K. 1994. Left hemisphere dominance for processing vocalizations in adult, but not infant, rhesus monkeys: field experiments. Proceedings of the National Academy of Sciences of the United States of America 91:3946-3948.

Hayes TL, Lewis DA. 1995. Anatomical Specialization of the Anterior Motor Speech Area: Hemispheric-Differences in Magnopyramidal Neurons. Brain and Language 49(3):289-308.

Hayes TL, Lewis DA. 1996. Magnopyramidal neurons in the anterior motor speech region: Dendritic features and interhemispheric comparisons. Archives of Neurology 53(12):1277-1283.

Heffner H, Heffner R. 1984. Temporal lobe lesions and perception of species-specific vocalizations by macaques. Science 226:75-76.

Holloway RL, de Lacoste-Lareymondie MC. 1982. Brain Endocast Asymmetry in Pongids and Hominids: Some Preliminary Findings on the Paleontology of Cerebral-Dominance. American Journal of Physical Anthropology 58(1):101-110.

Hopkins WD, Cantalupo C. 2004. Handedness in chimpanzees (*Pan troglodytes*) is associated with asymmetries of the primary motor cortex but not with homologous language areas. Behavioral Neuroscience 118(6):1176-1183.

Hopkins WD, Marino L, Rilling J, MacGregor L. 1998. Planum temporale asymmetries in great apes as revealed by magnetic resonance imaging (MRI). Neuroreport 9:2913-2918.

Hutsler J, Galuske RAW. 2003. Hemispheric asymmetries in cerebral cortical networks. Trends in Neurosciences 26(8):429-435.

Hutsler JJ, Gazzaniga MS. 1996. Acetylcholinesterase staining in human auditory and language cortices: Regional variation of structural features. Cerebral Cortex 6(2):260-270.

Irle E, Lange C, Sachsse U. 2005. Reduced size and abnormal asymmetry of parietal cortex in women with borderline personality disorder. Biological Psychiatry 57(2):173-182.

Jacobs B, Schall M, Prather M, Kapler E, Driscoll L, Baca S, Jacobs J, Ford K, Wainwright M, Treml M. 2001. Regional dendritic and spine variation in human cerebral cortex: a quantitative Golgi study. Cerebral Cortex 11(6):558-571.

Jacobs B, Schall M, Scheibel AB. 1993. A Quantitative Dendritic Analysis of Wernicke Area in Humans. 2. Gender, Hemispheric, and Environmental-Factors. Journal of Comparative Neurology 327(1):97-111.

Jacobs B, Scheibel AB. 1993. A Quantitative Dendritic Analysis of Wernicke Area in Humans. 1. Life-Span Changes. Journal of Comparative Neurology 327(1):83-96.

Jancke L, Schlaug G, Huang YX, Steinmetz H. 1994. Asymmetry of the Planum Parietale. Neuroreport 5(9):1161-1163.

Jenner AR, Rosen GD, Galaburda AM. 1999. Neuronal asymmetries in primary visual cortex of dyslexic and nondyslexic brains. Annals of Neurology 46(2):189-196.

Kaas J. 2000. Why is brain size so important: design problems and solutions as neocortex gets bigger or smaller. Brain and Mind 1:7-23.

Kanwal J, Matsumura S, Ohlemiller K, Suga N. 1994. Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. Journal of the Acoustical Society of America 96:1229-1254.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG. 1995. Functional MRI Evidence for Adult Motor Cortex Plasticity During Motor Skill Learning. Nature 377(6545):155-158.

Khaitovich P, Muetzel B, She X, Lachmann M, Hellmann I, Dietzsch J, Steigele S, Do H, Weiss G, Enard W et al. 2004. Regional patterns of gene expression in human and chimpanzee brains. Genome Research 14:1462-1473.

Kheck N, Hof PR, Deftereos M, Lo T, Gannon PJ. 1999. Brain language area evolution IV: Chemoarchitectonic interhemispheric asymmetries of the planum temporale (PT) homolog in Old World monkeys. Society of Neuroscience Abstracts 25:105.

Kimura D, Durnford M. 1974. Normal studies in the function of the right hemisphere in vision. In: Beaumont J, editor. Hemisphere Function in the Brain. New York: Halstead. p 25-47.

Knaus TA, Bollich AM, Corey DM, Lemen LC, Foundas AL. 2006. Variability in perisylvian brain anatomy in healthy adults. Brain and Language 97(2):219-232.

Krubitzer L, Huffman KJ. 2000. Arealization of the neocortex in mammals: Genetic and epigenetic contributions to the phenotype. Brain, Behavior and Evolution 55(6):322-335.

Krubitzer L, Kahn DM. 2003. Nature versus nurture revisited: an old idea with a new twist. Progress in Neurobiology 70(1):33-52.

Miki A, Liu GT, Englander SA, van Erp TGM, Bonhomme GR, Aleman DO, Liu CSJ, Haselgrove JC. 2001. Functional magnetic resonance imaging of eye dominance at 4 tesla. Ophthalmic Research 33(5):276-282.

Miki A, Liu GT, Raz J, Van Erp TGM, Liu CSJ, Leuthardt EC, Haselgrove JC. 2000. Contralateral monocular dominance in anterior visual cortex confirmed by functional magnetic resonance imaging. American Journal of Ophthalmology 130(6):821-824.

Mountcastle VB. 1997. The columnar organization of the neocortex. Brain 120 (Pt 4):701-22.

Murphy GM. 1985. Volumetric Asymmetry in the Human Striate Cortex. Experimental Neurology 88(2):288-302.

Niznikiewicz M, Donnino R, McCarley RW, Nestor PG, Iosifescu DV, O'Donnell B, Levitt J, Shenton ME. 2000. Abnormal angular gyrus asymmetry in schizophrenia. American Journal of Psychiatry 157(3):428-437.

Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM. 1996. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. Journal of Neuroscience 16(2):785-807.

Ohlemiller K, Kanwal J, Suga N. 1996. Facilitative responses to species-specific calls in cortical FM-FM neurons of the mustached bat. Neuroreport 7:1749-1755.

Ojemann G. 1991. Cortical organization of language. Journal of Neuroscience 11:2281-2287.

Pascual-Leone A, Wassermann EM, Sadato N, Hallett M. 1995. The role of reading activity on the modulation of motor cortical outputs to the reading hand in Braille readers. Annals of Neurology 38(6):910-915.

Penhune VB, Zatorre RJ, MacDonald JD, Evans AC. 1996. Interhemispheric anatomical differences in human primary auditory cortex: Probabilistic mapping and volume measurement from magnetic resonance scans. Cerebral Cortex 6(5):661-672.

Pesenti M, Zago L, Crivello F, Mellet E, Samson D, Duroux B, Seron X, Mazoyer B, Tzourio-Mazoyer N. 2001. Mental calculation in a prodigy is sustained by right prefrontal and medial temporal areas. Nature Neuroscience 4(1):103-107.

Peters A, Kara DA. 1987. The Neuronal Composition of Area 17 of Rat Visual-Cortex. 4. The Organization of Pyramidal Cells. Journal of Comparative Neurology 260(4):573-590.

Peters A, Sethares C. 1991. Organization of Pyramidal Neurons in Area-17 of Monkey Visual-Cortex. Journal of Comparative Neurology 306(1):1-23.

Petersen M, Beecher M, Zoloth S, Green S, Marler P, Moody D, Stebbins W. 1984. Neural lateralization of vocalizations by Japanese macaques: communicative significance is more important than acoustic structure. Behavioral Neuroscience 98:779-790.

Petersen M, Beecher M, Zoloth S, Moody D, Stebbins W. 1978. Neural lateralization of species-specific vocalizations by Japanese macaques (*Macaca fuscata*). Science 202:324-327.

Petersen S, Fox P, Posner M, Minton M, Raichle M. 1988. Positron emission tomographic studies of the cortical anatomy of single-word processing. Nature 331:585-589.

Phillips KA, Sherwood CC. 2005. Primary motor cortex asymmetry is correlated with handedness in Capuchin monkeys (Cebus apella). Behavioral Neuroscience 119(6):1701-1704.

Poremba A, Malloy M, Saunders R, Carson R, Herscovitch P, Mishkin M. 2004. Species-specific calls evoke asymmetric activity in the monkey's temporal poles. Nature 427:448-451.

Rakic P. 1995. Radial Versus Tangential Migration of Neuronal Clones in the Developing Cerebral-Cortex. Proceedings of the National Academy of Sciences of the United States of America 92(25):11323-11327.

Rao SM, Binder JR, Hammeke TA, Bandettini PA, Bobholz JA, Frost JA, Myklebust BM, Jacobson RD, Hyde JS. 1995. Somatotopic Mapping of the Human Primary Motor Cortex with Functional Magnetic Resonance Imaging. Neurology 45(5):919-924.

Rilling JK, Seligman RA. 2002. A quantitative morphometric comparative analysis of the primate temporal lobe. Journal of Human Evolution 42(5):505-533.

Ringo J, Doty R, Demeter S, Simard P. 1994. Time is of the essence: a conjecture that hemispheric specialization arises from interhemispheric conduction delay. Cerebral Cortex 4:331-343.

Rockland KS, Ichinohe N. 2004. Some thoughts on cortical minicolumns. Experimental Brain Research 158(3):265-277.

Rosen GD. 1996. Cellular, morphometric, ontogenetic and connectional substrates of anatomical asymmetry. Neuroscience and Biobehavioral Reviews 20(4):607-615.

Rosen GD, Sherman GF, Galaburda A. 1993. Neuronal subtypes and anatomic asymmetry: changes in neuronal number and cell-packing density. Neuroscience 56:833-839.

Rowe JB, Passingham RE. 2001. Working memory for location and time: Activity in prefrontal area 46 relates to selection rather than maintenance in memory. Neuroimage 14(1):77-86.

Scheibel AB, Paul LA, Fried I, Forsythe AB, Tomiyasu U, Wechsler A, Kao A, Slotnick J. 1985. Dendritic Organization of the Anterior Speech Area. Experimental Neurology 87(1):109-117.

Schenker NM, Desgouttes A-M, Semendeferi K. 2005. Neural connectivity and cortical substrates of cognition in hominoids. Journal of Human Evolution 49(5):547-569.

Schleicher A, Amunts K, Geyer S, Morosan P, Zilles K. 1999. Observer-independent method for microstructural parcellation of cerebral cortex: A quantitative approach to cytoarchitectonics. Neuroimage 9(1):165-177.

Seldon HL. 1981. Structure of Human Auditory-Cortex. 1. Cytoarchitectonics and Dendritic Distributions. Brain Research 229(2):277-294.

Sherwood CC, Broadfield DC, Holloway RL, Gannon PJ, Hof PR. 2003. Variability of Broca's Area Homologue in African Great Apes: Implications for Language Evolution. Anatomical Record Part A 271A:276-285.

Sherwood CC, Raghanti MA, Wenstrup JJ. 2005. Is humanlike cytoarchitectural asymmetry present in another species with complex social vocalization? A stereologic analysis of mustached bat auditory cortex. Brain Research 1045(1-2):164-174.

Stiebler I, Neulist R, Fichtel I, Ehret G. 1997. The auditory cortex of the house mouse: left-right differences, tonotopic organization and quantitative analysis of frequency representation. Journal of Comparative Physiology A, Sensory, Neural, and Behavioral Physiology 181:559-571.

Suner I, Rakic P. 1996. Numerical relationship between neurons in the lateral geniculate nucleus and primary visual cortex in macaque monkeys. Visual Neuroscience 13(3):585-590.

Tomaiuolo F, MacDonald JD, Caramanos Z, Posner G, Chiavaras M, Evans AC, Petrides M. 1999. Morphology, morphometry and probability mapping of the pars opercularis of the inferior frontal gyrus: an in vivo MRI analysis. European Journal of Neuroscience 11(9):3033-3046.

Uddin M, Wildman D, Liu G, Xu W, Johnson R, Hof PR, Kapatos G, Grossman L, Goodman M. 2004. Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. Proceedings of the National Academy of Sciences of the United States of America 101:2957-2962.

Uylings HBM, Jacobsen AM, Zilles K, Amunts K. 2006. Left-right asymmetry in volume and number of neurons in adult Broca's area. Cortex 42(4):652-658.

Wada JA, Clarke R, Hamm A. 1975. Cerebral Hemispheric Asymmetry in Humans: Cortical Speech Zones in 100 Adult and 100 Infant Brains. Archives of Neurology 32(4):239-246.

Watkins KE, Paus T, Lerch JP, Zijdenbos A, Collins DL, Neelin P, Taylor J, Worsley KJ, Evans AC. 2001. Structural asymmetries in the human brain: a voxel-based statistical analysis of 142 MRI scans. Cerebral Cortex 11(9):868-877.

Wernicke C. 1874. Das Aphasiche Symptomenkomplex. Breslau, Poland: Cohn and Weigart.

White LE, Andrews TJ, Hulette C, Richards A, Groelle M, Paydarfar J, Purves D. 1997a. Structure of the human sensorimotor system. 1. Morphology and cytoarchitecture of the central sulcus. Cerebral Cortex 7(1):18-30.

White LE, Andrews TJ, Hulette C, Richards A, Groelle M, Paydarfar J, Purves D. 1997b. Structure of the human sensorimotor system. 2. Lateral symmetry. Cerebral Cortex 7(1):31-47.

Zilles K, Dabringhaus A, Geyer S, Amunts K, Qu M, Schleicher A, Gilissen E, Schlaug G, Steinmetz H. 1996. Structural asymmetries in the human forebrain and the forebrain of non-human primates and rats. Neuroscience and Biobehavioral Reviews 20(4):593-605.

Zilles K, Palomero-Gallagher N, Grefkes C, Scheperjans F, Boy C, Amunts K, Schleicher A. 2002. Architectonics of the human cerebral cortex and transmitter receptor fingerprints: reconciling functional neuroanatomy and neurochemistry. European Neuropsychopharmacology 12(6):587-599.

Zilles K, Palomero-Gallagher N, Schleicher A. 2004. Transmitter receptors and functional anatomy of the cerebral cortex. Journal of Anatomy 205(6):417-432.

A COMPARATIVE QUANTITATIVE ANALYSIS OF CYTOARCHITECTONICS AND MINICOLUMNAR ORGANIZATION IN BROCA'S AREA IN HUMANS AND GREAT APES

ABSTRACT

Broca's area was identified in the inferior frontal gyrus of chimpanzee, bonobo, gorilla, and orangutan brains through direct cytoarchitectonic comparison to human brains. Across species, Broca's area comprises Brodmann's areas 44 and 45, which are characterized by relatively thick cortex and the presence of magnopyramidal cells in layers IIIc and Va. Layer IV is consistently narrower in area 44 than in area 45. These areas are distinguished from neighboring regions by differences in pyramidal cell size in layers III and V and in the thickness of layer IV.

We analyzed the minicolumnar organization of cells in layer III of Broca's area in eleven human and nine ape specimens using a quantitative approach. A semi-automated method was used to analyze digitized images of histological sections stained for Nissl substance. Images were analyzed for horizontal spacing distance and grey level index (GLI).

We found greater horizontal spacing distance in humans than in great apes ($p<0.002$); GLI was lower in humans than in great apes ($p<0.001$). Neither parameter exhibited hemispheric asymmetry at the population-level, although human females exhibited a leftward asymmetry in GLI. Increased cell spacing in the cortex primarily reflects greater space for neuropil, where synapses, dendrites and axons are located. More space for connectivity in layer III may suggest that Broca's area in humans is better able to integrate information from a variety of cortical regions, although horizontal spacing is smaller relative to brain size in humans than in great apes.

INTRODUCTION

Language is a pervasive component of human behavior and cognition. While language processes have been shown to recruit the involvement of diverse regions composing extensive systems in the brain (Price, 2000; Damasio et al., 2004), three areas in particular remain the chief foci of investigations. Studies of the inferior frontal gyrus of the human brain indicate its participation in language motor control, sequencing, planning, syntax, phonological processing and other components of language (Ojemann, 1991; Burton, 2001; Foundas et al., 2001; Bookheimer, 2002; Amunts et al., 2004; Indefrey et al., 2004; Hagoort, 2005; Caplan, 2006; Friederici et al., 2006). The superior posterior temporal lobe, including the planum temporale, has been linked to the processing of perceptual linguistic signals (Ojemann, 1991; Foundas et al., 2001; Bookheimer, 2002; Devlin et al., 2003). Recently, a region of anterior parietal cortex has been confirmed as part of a network involving the inferior frontal gyrus and the superior posterior temporal gyrus (Catani et al., 2005). This region, previously identified for its association with connection dysphasia has now been shown to have distinct connections to the other language areas.

Early studies of language control and the brain in humans implicated part of the inferior frontal gyrus (Broca, 1861). This region in humans is considered by many researchers to be part of a network that has its roots in preexisting working memory (Aboitiz et al., 2006) and mirror neuron (Fadiga and Craighero, 2006) networks in monkeys. Functional studies of nonhuman primates also suggest the contribution of the inferior frontal gyrus to salient, species-relevant behavior, such as the identification and imitation of conspecific actions (Rizzolatti and Arbib, 1998), abilities that are thought to be important to language learning in humans; a recent

PET study found activation in the inferior frontal region in rhesus macaques in response to species-specific calls (Gil-da-Costa et al., 2006). Additionally, the inferior frontal region receives input from multiple modalities in rhesus macaques (Barbas and Pandya, 1989; Petrides and Pandya, 2001), suggesting it is a potential region for exaptation for both manual and vocal linguistic processing during hominid evolution.

The inferior frontal gyrus in humans is a region containing cortex that is transitional between unimodal association cortex and higher-order association prefrontal cortex (von Economo, 1929). Two cytoarchitectonic areas, Brodmann's areas 44 and 45, comprise what is known as Broca's area, and typically occupy part of the inferior frontal gyrus. These areas are defined using traditional histological criteria such as differences in laminar thickness, cell densities, and cell types (Brodmann, 1909; von Economo, 1929; Sarkissov et al., 1955, Fig. 2.1a-c). It has long been assumed that regions involved in language function must have unique characteristics in humans. However, there is little published data from this region of frontal cortex in apes to test this assumption. Although descriptions of the cytoarchitectonics of Brodmann's areas 44 and 45 exist for the brains of chimpanzees (Bailey et al., 1950, Fig. 2.1d; Sherwood et al., 2003), the only quantitative data are derived from studies of magnetic resonance images of gross anatomical features in chimpanzees, bonobos, and gorillas (Cantalupo and Hopkins, 2001); no cytoarchitectonic analyses exist for bonobos, gorillas, and orangutans.

While traditional cytoarchitectonic studies typically focus on the horizontal lamination of the cortex, another important component of organization, the spatial patterns of vertical arrays of cells, is promising for quantitative analyses. The isocortex (neocortex) is populated by

vertically-oriented aggregates of cells with strong vertical interconnections among layers, forming fundamental structural and functional units known as minicolumns (Douglas and Martin, 1992; Mountcastle, 1997). Minicolumnar organization in the adult brain is thought to be derived from ontogenetic columns and the migration of neuroblasts from the ventricular and subventricular zones into radial columns during development (Rakic, 1995a).

Minicolumns comprise single rows of neurons traversing layers II-VI, though some degree of sublaminar specialization may also occur (Mountcastle, 1997; Buxhoeveden and Casanova, 2002). They provide fine-tuning within cortical columns (larger units of cortex including multiple minicolumns), are capable of information processing (Kaas et al., 1981; Lee et al., 1992; Favorov and Kelly, 1994; Sugimoto et al., 1997; Rao et al., 1999), and are assumed to be one cell wide in layers III, V, and VI (Seldon, 1981). Minicolumns and their interactions within larger cortical columns have been a rich source for computer modeling of brain function (Fukai, 1994; Li et al., 2005; Neilson and Neilson, 2005; Johansson and Lansner, 2007). As part of computer models of neural networks, "minicolumns can act as monolithic functional units for purposes of critical, fast decisions and learning, [and are] able to organize their collective inputs without supervision by Hebbian plasticity into selective receptive field shapes, thereby becoming classifiers for input patterns" (Lucke and von der Malsburg, 2004:501)

Functional properties of minicolumns may vary according to region-specific circuits. While there are no physiological studies of the micro-vertical organization in Broca's motor area, microelectrode experiments in the primary motor cortex of monkeys have demonstrated that individual minicolumns are engaged in preferred directional selection in arm reaching tasks

(Amirikian and Georgopoulos, 2003; Georgopoulos et al., 2007). Interestingly, no two minicolumns within a cortical column exhibit the same directional preference, although the pattern of preferences across minicolumns is replicated in adjacent cortical columns. It is not unreasonable to expect that minicolumns in Broca's area maintain similar properties related to the production of language.

The width of minicolumns has been investigated comparatively in temporal lobe language areas (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001a). Those studies found minicolumns to be generally wider in humans when compared with those in chimpanzees and rhesus macaques. Minicolumns also were found to be wider in the left hemisphere than in the right throughout auditory cortex in humans (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001a); no asymmetry was observed in nonhuman primates.

In this study, we analyzed the cytoarchitectonic and minicolumnar organization in Brodmann's areas 44 and 45 in both hemispheres of the inferior frontal gyrus in the ventrolateral frontal cortex of human, chimpanzee, bonobo, gorilla, and orangutan brains. We used a quantitative approach by measuring mean horizontal spacing distance and the fraction of the area that is occupied cell bodies (as measured by grey level index or GLI). We test the hypothesis that horizontal spacing distance and grey level index in these areas in both hemispheres indicate greater distance between cells in humans than in great apes, as has been reported in temporal language cortex (Buxhoeveden et al., 2001b) and in the frontal pole (Buxhoeveden and Semendeferi, 2005). We also hypothesize that spacing and the fraction of area occupied by cell bodies are lateralized in humans in areas 44 and 45, but not in great

apes. This hypothesis is derived from the reported asymmetry of GLI in humans (Amunts et al., 1999; 2003) and the region's involvement in lateralized language-related activity, in addition to findings of lateralization of minicolumns in temporal lobe language areas (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001a).

MATERIALS

Our sample comprised 20 complete series of histologically processed brains of individuals from five hominoid species (Table 2.1). It included both hemispheres of eleven humans, three chimpanzees, two bonobos, two gorillas, and one orangutan, with an additional right hemisphere of another orangutan specimen.

All specimens were fixed in either 4% buffered formalin (pH 7.4) or a Bodian mixture (formalin, glacial acetic acid, and ethanol) within 24 hours after the natural death of the subject and were then processed and stained in a consistent manner (Semendeferi et al., 1998; Amunts et al., 1999). All brains were paraffin-embedded and serially sectioned in the coronal plane into 20μm thick sections (except for one chimpanzee, YN89, which was cut in the axial plane into 15μm thick sections) producing complete series through the entirety of each brain. Every tenth to thirtieth section was mounted on a glass slide and stained with a modification of the Gallyas silver stain for neuronal perikarya (Gallyas, 1971; Merker, 1983). One specimen (gorilla A375) was stained with cresyl violet. In no case was a neural pathology the cause of death.

The ape specimens in our collection (UCSD) were donated by the Busch Gardens Zoo, the Henry Doorly Zoo, the Milwaukee County Zoo, and Yerkes National Primate Research Center following the natural deaths of the animals. Eight ape specimens and two human

specimens (SN207 and H988) were examined in our collection (UCSD). The other nine human specimens and two ape specimens (1548 and A375) were examined at the Vogt Insitute in Duesseldorf, Germany. Only two ape specimens (gorilla A375 and orangutan Briggs) were wild born; no information is available on the origin of one specimen (chimpanzee 1548). One specimen had been exposed to some language training (bonobo YN86). Individuals ranged from 2-89 years of age and represented both sexes.

METHODS

Identification of areas 44 and 45

In humans, Brodmann's areas 44 and 45 lie mostly within the inferior frontal gyrus and comprise three major subdivisions: the pars opercularis, the pars triangularis, and the pars orbitalis. These three regions are divided by prominent sulci in most human brains (Ono et al., 1990; Duvernoy, 1991, Fig. 2.2a). The most posterior region, the opercular cortex, forms the pars opercularis, which is bounded by the inferior precentral sulcus, the inferior frontal sulcus, the ascending ramus of the Sylvian fissure and the Sylvian fissure. The pars triangularis is a triangular lobule, lying directly anterior to the pars opercularis, and is bordered by the ascending ramus of the Sylvian fissure, the inferior frontal sulcus, and the horizontal ramus of the Sylvian fissure. The pars orbitalis is located ventral to the triangularis, and is delimited by the horizontal ramus of the Sylvian fissure, the inferior frontal sulcus, and the lateral orbital sulcus.

In chimpanzees, areas 44 and 45 can also be found within the inferior frontal gyrus (Bailey et al., 1950; Sherwood et al., 2003, Fig. 2.2b). The areas lie almost exclusively anterior to the precentral sulcus and ventral to the inferior frontal sulcus, although, as with the human brain, individual variation exists in the extent of these areas on the lateral surface of the brain.

Brodmann's areas 44 and 45 are similar cytoarchitectonically (Fig. 2.3) in humans (von Economo, 1929; Amunts et al., 1999) and in chimpanzees (Bailey et al., 1950; Sherwood et al., 2003). Shared cytoarchitectonic characteristics include magnopyramidal cells in lower layer III, medium large pyramidal cells in upper layer V, and decreased cell density in lower layer V. There is a size gradient in the cells of layer III, with the smallest cells at its border with layer II and the largest near layer IV. The main cytoarchitectonic difference between areas 44 and 45 is the prominence of layer IV and the total thickness of cortex. In particular, layer IV in area 44 is dysgranular —visible but with few cells, and appears to be invaded by cells from III and V. In area 45, layer IV is more prominent, and creates a clear barrier between layers III and V. The cortex is notably thinner in area 45 than in area 44. The boundaries of areas 44 and 45 in those human brains used in previous studies (Amunts et al., 1999; Uylings et al., 2006, see Table 2.1) were defined using an observer-independent methodology based on GLI (Schleicher and Zilles, 1990).

Following a similar approach to that used to identify other frontal cortices (Brodmann's areas 10 and 13) across hominoid species (Semendeferi et al., 1998; 2001), the above topographical and cytoarchitectonic criteria were applied to identify Brodmann's areas 44 and 45 in all species available in the present study.

Quantification of Minicolumns

Multiple regions of interest from representative areas within each region were magnified, photographed, and digitized with a final resolution of 1.47μm/pixel (UCSD) or 1.64μm/pixel (Vogt Institute). Scale calibration was obtained by the use of a micrometer photographed at the same resolution and magnification as the images to be studied. Using

software modeled on the ImageJ program, each digitized image underwent the processes of thresholding (to exclude any cells smaller than 20 pixels) and watershedding (for edge detection), and was converted into a binary image. Each step was performed automatically by a series of computer algorithms, with operator input being limited to the determination of the threshold level and the boundary of the region of interest within each image. The use of a threshold eliminates small cells such as glia and interneurons, focusing instead on the pyramidal cells which constitute most of layer III.

Measurements are based on pixel density in the y-dimension collapsed onto the horizontal plane measured over multiple levels of the image. The parameters used were averages based on the entire width of the region of interest. Mean horizontal spacing distance (MSD) was calculated based on edge-to-edge measures of cells and represents the average spacing distance between them. Because minicolumns are one cell wide in layer III, MSD reflects both the width of the minicolumn and any space between minicolumns. The advantage of this approach is that it does not attempt to impose a minicolumn structure onto the cell bodies in a region of interest (Buxhoeveden et al., 2006). The grey level index (GLI, Schleicher and Zilles, 1990) is an independent measure of the area fraction of the image occupied by Nissl-stained cells. It provides information on both the horizontal and vertical axes, through an estimation of the percentage of the area of an image occupied by cell bodies. GLI may vary in relation to cell size, cell number, or cell spacing, or any combination of the three.

All parameters were measured in layer III of cortex, an approach that matches that taken in our previous work (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001a; 2001b). Layer III was chosen because it typically displays the clearest linearity of all cortical

lamina. Since minicolumns are vertical arrays traversing layers II-VI, the distance between cell arrays in layer III reflect, to some degree, the distance between cells in other layers as well. In addition, the long apical dendrites from layer V pyramidal cells pass through layer III, and the pyramidal cells in these layers contribute their apical dendrites to the same bundles before ascending into layers I and II. Pyramidal cells in layer III are also vertically interconnected with the infragranular layers by descending axons. Furthermore, cells in the supragranular layers are critical in transcolumnar and corticocortical processing (Colombo and Reisin, 2004). In particular, they are involved with corticocortical connections and internal communication among different regions of cortex, whereas other layers of cortex are predominantly connected with extracortical structures such as the thalamus, basal ganglia, cerebellum, or the peripheral nervous system. Thus, differences in minicolumn size in layer III may be related to differences in higher order associative functions.

Analysis

We analyzed our data for differences between taxonomic groups and for asymmetry between the hemispheres within taxonomic groups. The Kruskal-Wallis nonparametric test was used to test for differences among species.

To relate minicolumn size to brain size, we approximated an average cross-sectional area associated with each minicolumn, by calculating the area of a hexagon with the diameter equal to MSD, using the equation $[(3*\sqrt{3}/8)*MSD^2]$, because hexagons have the lowest circumference to area ratio of a regular polygon that can be tiled without gaps between shapes; due to surface pressure, objects that would otherwise be circular become hexagonal (e.g. the wax cells in a honeycomb; Hales, 2001). Ideally, we would compare measures of like units,

such as the area of a minicolumn with the surface area of the brain. If the number of minicolumns in a brain is stable, then one would expect the area of a minicolumn to scale with total surface area. However, surface area measures are not available for the specimens in this sample. Therefore, we calculated a ratio of area to volume using the cross-sectional area associated with a minicolumn and total brain volume.

Asymmetry coefficients for each area (Brodmann's areas 44 and 45) within each individual were calculated using the following equation: [100*(L-R)/(L/2+R/2)]. Asymmetry coefficients were analyzed in relation to sex, taxonomic group (great apes or humans), age and area using multi-way ANOVAs. We corrected for multiple comparisons by using a standard Bonferroni correction. All statistical tests were conducted using JMP IN 4.02 (SAS Institute, 2000).

RESULTS

Cytoarchitectonics

In the great ape (chimpanzee, bonobo, gorilla, and orangutan) specimens, Brodmann's areas 44 and 45 were found within the inferior frontal gyrus. In all species, both areas display the characteristic features as seen in humans (see description in Methods). Allowing for the existence of individual variation, the basic cytoarchitectonics of areas 44 and 45 were similar across species (Fig. 2.4). However, there were a few notable differences. In the bonobos, we noticed a wider layer I in both areas and a very dysgranular layer IV in area 44. In humans, chimpanzees, and bonobos, the cortex in area 45 was noticeably thinner than in area 44, whereas in gorillas and orangutans, the cortex was of similar thickness in both areas. Overall, the cortex appeared to be thinner in gorillas than it did in any of the other great apes.

Minicolumns

Across species all individuals had an average MSD greater than 30μm (Fig. 2.5a). Average MSD (including both hemispheres) was larger in humans in both cortical areas (area 44: 45.9-68.5μm; area 45: 46.4-62.6μm) than in the great apes (area 44: 34.6-57.6μm; area 45: 44.6-55.3μm). The difference in MSD between humans and great apes was significant (Kruskal-Wallis test, X^2=9.9408, df=1, p=0.0016).

Grey level index (GLI) was lower in humans (area 44: 0.15-0.23; area 45: 0.17-0.21μm) than in great apes (area 44: 0.18-0.28; area 45: 0.19-0.29; Fig. 2.5b). Again, the difference between humans and great apes was significant (Kruskal-Wallis test, X^2=11.4300, df=1, p=0.0007). For each of the parameters, only one individual (the male gorilla) had values that fell within the range of the human values. Comparisons were made between areas 44 and 45 using both parameters (MSD, GLI), and no notable differences between areas were observed in these parameters. Furthermore, we analyzed the contributions of age, sex, species (among the great apes), and brain size (within humans) and observed no significant differences in these parameters.

We also investigated the contribution of brain size across species, by examining a ratio between MSD and brain size (see Methods), which showed that MSD was smaller relative to brain size in humans than in great apes (Kruskal-Wallis test, X^2=13.1167, df=4, p=0.0107; Fig. 2.6).

Asymmetry.

No parameter in this sample exhibited hemispheric asymmetry at the population level (p>0.05). However, at the individual level, there were notable interhemispheric differences that

were indicated by the size of the asymmetry coefficients (Fig. 2.7). Furthermore, there was greater range in interhemispheric differences in MSD and GLI in humans than in great apes, as demonstrated by the greater range of asymmetry coefficients within the human sample.

Further investigation of the difference in variation between humans and great apes revealed an interaction between sex and taxonomic group (human or great apes) in the GLI asymmetry values ($F_{1,34}$=9.0875, p=0.0048). Human females exhibited greater leftward asymmetry (meaning a higher GLI in the left hemisphere) than any other group (human males, or great apes of either sex). However, human females also had the greatest range of values. No interaction between GLI and area (44 vs 45) was observed.

An interaction was also observed in the degree of asymmetry in MSD between group and cortical area ($F_{1,34}$=11.3548, p=0.0020). There was greater variation in MSD asymmetry in Brodmann's area 44 than in area 45 in humans, whereas the variation in asymmetry was greater in area 45 in the apes than in area 44.

DISCUSSION

Previous studies have described Brodmann's areas 44 and 45 in humans (von Economo, 1929; Amunts et al., 1999; Bogolepova et al., 1999) and the inferior frontal cortex in certain nonhuman primate species (Walker, 1940; Bailey et al., 1950; Watanabe-Sawaguchi et al., 1991; Sherwood et al., 2003); a few studies have investigated the region via comparisons between two species (e.g. galago and rhesus macaque, Preuss and Goldman-Rakic, 1991; human and rhesus macaque, Petrides and Pandya, 1994; Petrides, 2006). The studies of nonhuman primates have primarily focused on a few species of prosimians, in which Brodmann's areas 44 and 45 have not been identified, and some species of monkeys, in which

the presence of area 45 is established, although the presence of area 44 is debated. Therefore, there has existed a phylogenetic gap in our knowledge regarding the existence and structure of Broca's area in hominoids.

The current study analyzed the cytoarchitectonics of Broca's area in multiple hominoid species including human and great ape specimens. The areas exhibit the same diagnostic criteria in all species examined, as described in the methods. There were qualitative differences in cortical thickness and in laminar thicknesses observed among species. In particular, the total thickness of cortex varied among species, with gorillas and orangutans displaying less difference in thickness between the two areas than chimpanzees and bonobos did. Additionally, we quantified aspects of minicolumnar organization of the cortex, including mean horizontal spacing distance and the fraction of the area occupied by cell bodies (as measured by GLI).

Comparative analysis of minicolumns

Historically, emphasis has been placed on the differences in number of radial units, or minicolumns, rather than their size. This is a result of the relationship between cortical surface area and the number of radial ontogenetic columns produced in the embryonic brain (Rakic, 1995b; Rakic and Kornack, 2001), as well as the fact that minicolumn size only varies from 20μm to 80μm across species (Mountcastle, 1997), while surface area varies a thousand-fold. However, the three- to four-fold difference in minicolumn size among different species is not insignificant, and cortical surface area is a reflection of the width of the radial units in addition to their number.

Humans:

The current study is part of a growing database revealing that increased horizontal spacing between cells and lower grey level index may be hallmarks of human cortex. This study of Brodmann's areas 44 and 45 joins previous studies of the planum temporale (Buxhoeveden et al., 2001a; 2001b) and the frontal pole (Buxhoeveden and Semendeferi, 2005) that found increased spacing and lower grey level index in humans than in great apes.

Although MSD values in humans are larger than those found in great apes, they are actually smaller relative to brain volume (Fig. 2.6). As a result, despite the increased size of minicolumns, a human's cortex contains more minicolumns than the cortex of a great ape. When brains enlarge, there is always a trade-off necessary to balance the needs of connecting a larger number of neurons with the space needs of greater numbers of connections (Hofman, 1989; Ringo, 1991), and the decreased efficiency of longer connections (Kaas, 2000). For instance, maintaining less horizontal spacing between cells would allow for individual cells to be connected to *more* cells within a given radius, without forcing the cells to have longer or more complex dendritic structures. The resulting well-connectedness at the local level might allow for greater efficiency and integration of processing. However, the presence of smaller columns may also indicate that the processes are less likely to have thick myelination (which would increase spacing and/or width), meaning that the transmission time will be longer, thus reducing efficiency.

In our analysis of GLI, we have found evidence of an asymmetry in human females (with increased spacing in the right hemisphere) in the opposite direction to the asymmetry found in temporal language areas (Seldon, 1981; Buxhoeveden et al., 2001a). The rightward

asymmetry of spacing observed in this sample in Broca's area suggests the possibility of alternate underlying mechanisms acting on minicolumn size in the inferior frontal gyrus than those acting in temporal language areas. Elsewhere, we have indicated that the expansion of minicolumns provides greater space for dendritic branching, spines and other features generally found in the neuropil surrounding minicolumns, although the space may also be due to increased numbers of non-pyramidal cells (DeFelipe, 2005). In particular, we suggest that one contribution to increased spacing and width of minicolumns in the human brain is increased elaboration and complexity of dendritic branching (Buxhoeveden and Casanova, 2002). Although differences in dendritic branching and other features of local unmyelinated connections may contribute to minicolumn asymmetries in the human temporal cortex as well (Buxhoeveden and Casanova, 2002), it is more likely that this asymmetry reflects known hemispheric differences in the thickness of myelin sheaths (Anderson et al., 1999), which aid in the speed of signal transduction in a region associated with rapid temporal processing (Zatorre and Belin, 2001).

Furthermore, the asymmetry in Brodmann's areas 44 and 45 in humans may be related to a rightward asymmetry in parameters relating to connectivity in the largest pyramidal cells in layer III of Brodmann's area 45 (Hayes and Lewis, 1996). Total dendritic length, dendritic complexity (numbers of branches and maximal branch order) and spine densities were found to be greater in the right hemisphere. This population of magnopyramidal neurons mainly furnishes long corticocortical association projections and these large neurons may furnish a significant portion of the connections that fill the neuropil. However, the complexity of dendritic structure does not necessarily correspond to changes in soma size (Elston and Rockland,

2002). As the overall *size* of these large cells in layer III of area 45 is not larger in the right hemisphere (in fact, the magnopyramidal cells are smaller in the right hemisphere, Hayes and Lewis, 1996), the resulting impact on minicolumn parameters would manifest in an increase in MSD and an accompanying decrease in GLI (assuming that the numbers of cells do not vary significantly between hemispheres). This may be of particular import because of our focus on minicolumns in layer III. Cells in the supragranular layers are critical in transcolumnar and corticocortical processing (Colombo and Reisin, 2004). In particular, they are involved with corticocortical connections and internal communication between different regions of cortex, whereas other layers of cortex are predominantly connected with extracortical structures such as the thalamus, basal ganglia, cerebellum, or the peripheral nervous system. Thus, differences in minicolumn size in layer III may be related to differences in higher order associative functions. In the specific areas examined in this study, the differences in horizontal spacing distance in layer III are likely related to the extensive intra-areal connections within layers II and III that are found in Brodmann's areas 44 and 45 (Tardif et al., 2007) and also to the strong inter-areal connections (among Broca's area, the posterior superior temporal gyrus, and the angular gyrus, Catani et al., 2005; Anwander et al., 2007) that support language function.

While differences in connectivity and volume of neuronal connections affect the horizontal spacing distance, the grey level index may also be affected by changes in pyramidal cell size and cell number. In the current sample, MSD and grey level index in layer III are tightly, and inversely, correlated across all individuals (Spearman's Rho=-0.9556, $p<0.0001$). This indicates that either both cell number and cell size are constant or cell number and cell size vary in opposition to each other and maintain a constant area occupied by cells. Given that

average cell size is reported to be constant across the species examined here (Haug, 1987), the first option may be more plausible. Furthermore, although *average* cell size is constant, the *range* of cell sizes varies by species (Haug, 1987). We hypothesize that there is a greater range of cell size in humans than in the great apes in Broca's area, with average pyramidal cell size being larger in humans, particularly in deep layer III. However, this suspicion awaits further examinations of cell size and number in the regions of interest.

Apes:

It is clear from reports in the literature that all four great ape species have complex cognitive capabilities in multiple realms. The patterns of social organization observed range from the semi-solitary orangutans to the fission-fusion groups of chimpanzees and bonobos, with the gorillas forming groups typically comprising multiple females with offspring and one or two males (McGrew et al., 1996). Tool use has been reported repeatedly in studies in the wild of all of the great apes (Panger, 2007) along with social learning of skills and behaviors (Whiten et al., 2001; van Schaik et al., 2003).

Additionally, all four species of great apes have been part of relatively successful enculturation projects (chimpanzees, Gardner and Gardner, 1969; gorillas, Patterson and Cohn, 1990; orangutans, Miles, 1994; bonobos, Savage-Rumbaugh and Fields, 2000) in which individual apes acquired some functional usage of symbols (sign language, lexigrams, and/or vocal comprehension). It has been suggested that Broca's area in humans is involved in complex, sequential and hierarchical processing (Fiebach and Schubotz, 2006), and there is ample evidence for hierarchical processing in great apes in the wild (e.g. Byrne et al., 2001; Corp and Byrne, 2002; Nakamichi, 2004). Therefore, it is unsurprising that Broca's area is

present in all great ape species and that, across species, the patterns of horizontal cell spacing are remarkably similar.

The exception to this may be the gorillas. Both of the gorillas in this sample had MSD values greater than any of the other great apes, and one (A375) of the two had MSD values that fell within the range of human values. Although the fact that one of the gorillas was stained with a different protocol (see Methods) may account in part for the different values, no methodological concerns exist for the second individual. Whether these two individuals portray an accurate representation of the normal range of spacing distances in gorillas or whether the larger of the two individuals is an outlier or artifact awaits further investigation with additional samples. Developmental age may also be an important factor. The two-year old bonobo (YN86), despite having been exposed to human enculturation, had relatively decreased spacing. This is likely because of age, as horizontal cell spacing increases throughout development in humans (Buxhoeveden and Casanova, 2002). Another important life history factor is whether or no an individual was wild-born. However, there is not a clear effect of being born wild in this smaple. Although one of the wild-born individuals may stand out (A375) the other (Briggs) does not.

The presence of variation within and among species with similar brain sizes and with varying life histories emphasizes the importance of analyzing multiple individuals from multiple great ape species whenever possible, rather than focusing on a single species (e.g. chimpanzees) when addressing the evolution of the human brain.

CONCLUSION

Multiple studies of Brodmann's areas 44 and 45 in humans have been published (von Economo, 1929; Amunts et al., 1999; Bogolepova et al., 1999), and some studies have been made of the same regions in particular nonhuman primate species (Walker, 1940; Watanabe-Sawaguchi et al., 1991; Sherwood et al., 2003). However, similar data on multiple hominoids has not been available. In this study, we examined all extant species of great apes to compare with human specimens. Furthermore, our current research contributes additional data to the expanding literature on the characterization of these areas in humans. Since reorganization of underlying cortices (Holloway, 1968) is likely to have accompanied the changes in gross morphology that occurred in hominid evolution (Falk, 1983; Broadfield et al., 2001), observed similarities and differences between humans and other primates in areas 44 and 45 can shed light not only on how cognition is organized in the human brain, but also on what changes may have occurred during the evolution of cognition since the split with other hominoids.

We report the finding of asymmetry in the minicolumnar organization of Broca's area in humans. Although asymmetries are present in both parameters (MSD and GLI), significance is only reached for GLI in the human females. We also report the presence of larger minicolumns in humans than in great apes, with greater horizontal spacing between neurons in layer III of cortex in Brodmann's areas 44 and 45. While it is not yet clear what the advantages and disadvantages of selection for different minicolumn widths would be in the course of evolution, we presume that the increased horizontal spacing in humans is utilized for increased numbers of input and output pathways and for increased microcircuitry. We expect that differences in microcircuitry may be attributable to differences in the complexity of dendritic branching of the

large pyramidal cells that occupy lower layer III (Hayes and Lewis, 1996). However, the mechanisms responsible for differences in minicolumn width may vary according to the situation. It may be one thing to have different sized minicolumns among species, yet another to have differences within regions of the same brain, and still another when minicolumn size is asymmetric within one brain. Furthermore, the size of minicolumns relative to brain volume is actually smaller in humans than in the other hominoids, so that greater numbers of minicolumns are present in a human's cortex than in the cortex of a great ape. This combination of size and number of minicolumns in human cortex differentiates it from other primates, and may demonstrate greater computational power at the local level, as well as at regional levels due to a greater abundance of minicolumns.

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Table 2.1: Specimen data

Species	Specimen ID	Hemispheres	Sex	Age	Brain Weight (grams)
Homo sapiens (Human)	H988	Both	M	21	1633
	SN146[a]	Both	M	37	1437
	SN16[a]	Both	M	54	1757
	SN189[a]	Both	M	56	1270
	SN382[a]	Both	F	59	1142
	SN281[a]	Both	M	69	1360
	SN56[a]	Both	F	72	1216
	SN207[a]	Both	M	75	1349
	SN544[a]	Both	F	79	1360
	SN68[a]	Both	F	79	1110
	SN2[a]	Both	F	85	1046
Pan troglodytes (Chimpanzee)	Chimp1	Both	F	22	440
	YN89[b]	Both	M	22	420
	1548	Both	F	Adult	N/A
Pan paniscus (Bonobo)	YN86	Both	F	2	392
	Zahlia	Both	F	11	324
Gorilla gorilla (Gorilla)	A375	Both	M	Juv	450
	YN82	Both	F	20	376
Pongo pygmaeus (Orangutan)	YN85	Both	M	16	369
	Briggs	Right	M	34	345

[a] Specimen was used in Amunts et al. (1999; 2003) and Uylings (2006).

[b] YN89 was cut in the axial plane at 15µm. All others were cut in the coronal plane at 20µm.

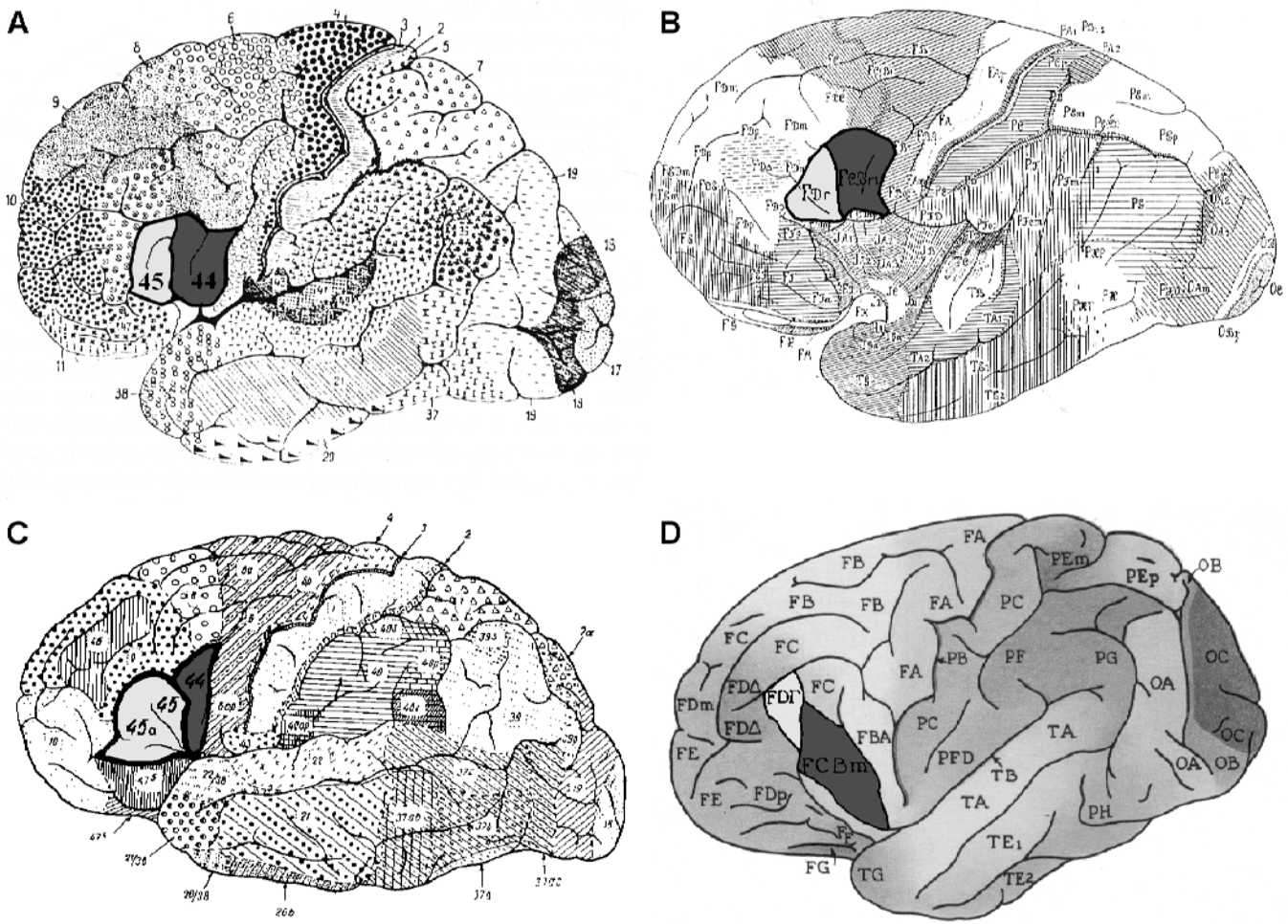

Figure 2.1: Cytoarchitectonic maps of the human brain modified from A) Brodmann, 1909, B) von Economo, 1929, and C) Sarkisov et al, 1949 and of the chimpanzee brain modified from D) Bailey et al., 1950 with areas 44 (dark) and 45 (light) highlighted.

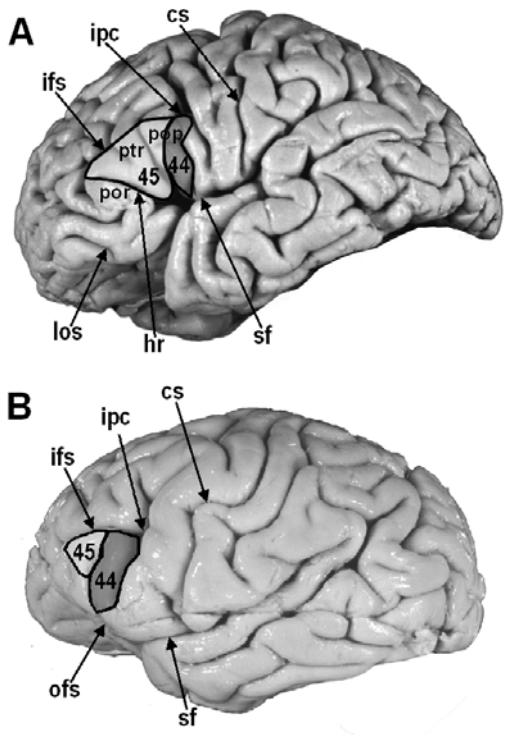

Figure 2.2: Photographs of A) a human brain and B) a chimpanzee brain with relevant structures marked. Images are not to scale.

Abbreviations:
ar – ascending ramus of the Sylvian fissure;
cs – central sulcus;
hr – horizontal ramus of the Sylvian fissure;
ifs – inferior frontal sulcus;
ipc – inferior precentral sulcus;
los – lateral orbital sulcus;
ofs – orbitofrontal sulcus;
pop – pars opercularis;
por – pars orbitalis;
ptr – pars triangularis;
sf – Sylvian fissure

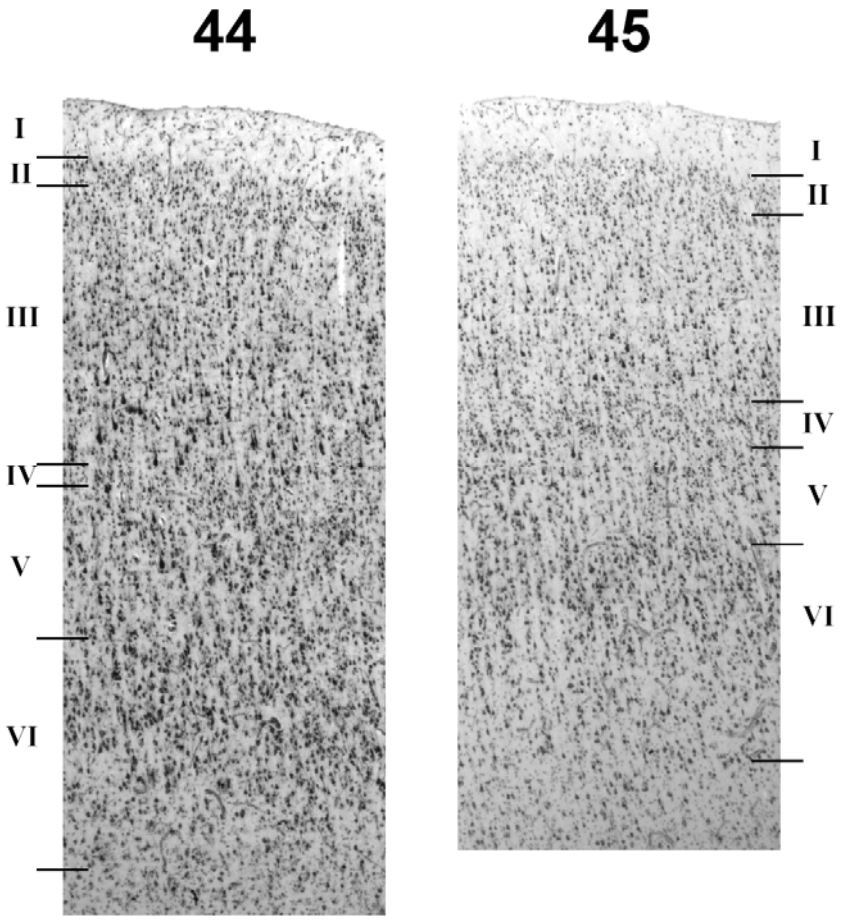

Figure 2.3: Representative images of the full depth of the cortex in Brodmann's areas 44 and 45 of a human brain.

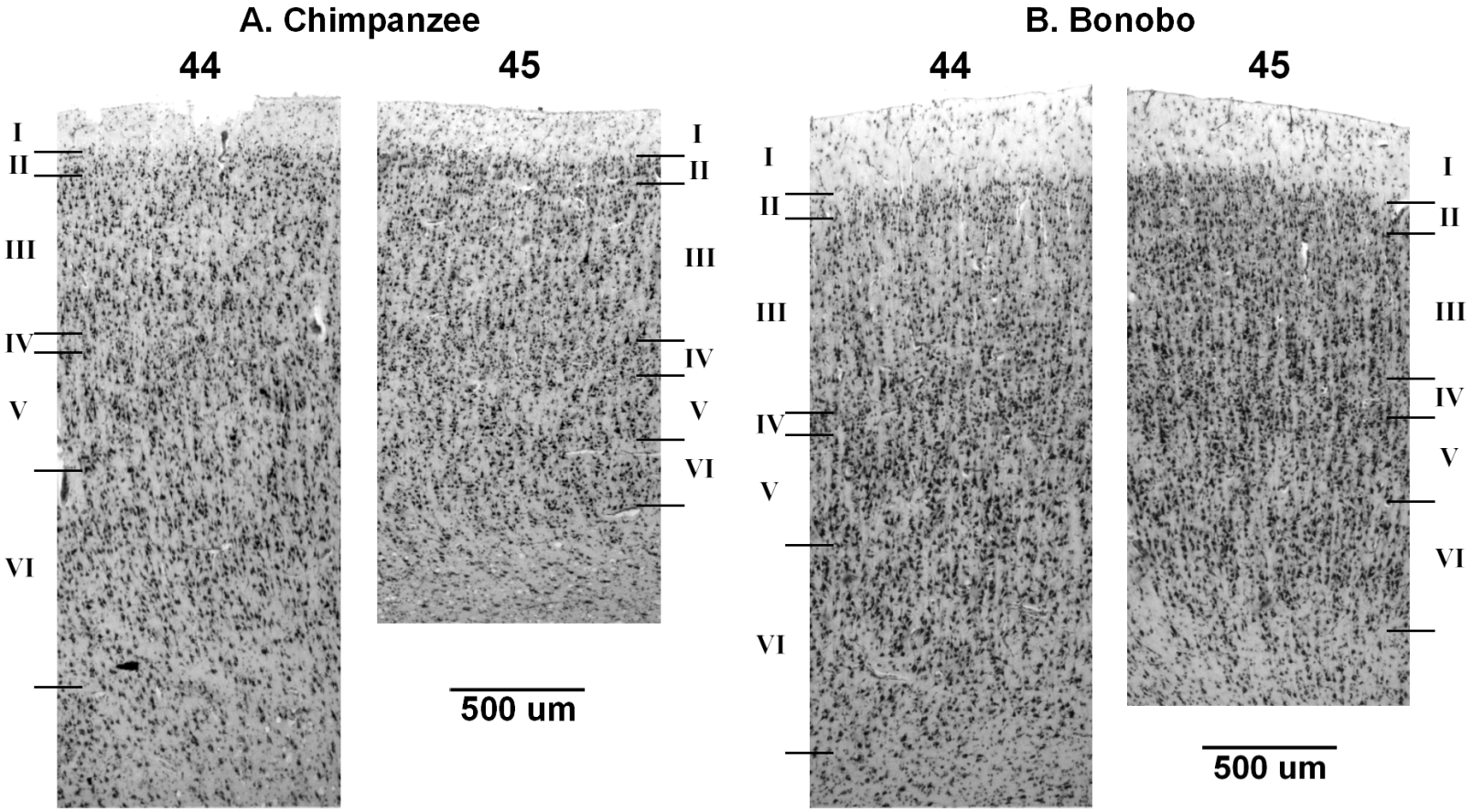

Figure 2.4: Representative images of the full depth of the cortex in Brodmann's areas 44 and 45 of A) chimpanzee, B) bonobo, C) gorilla, and D) orangutan brains.

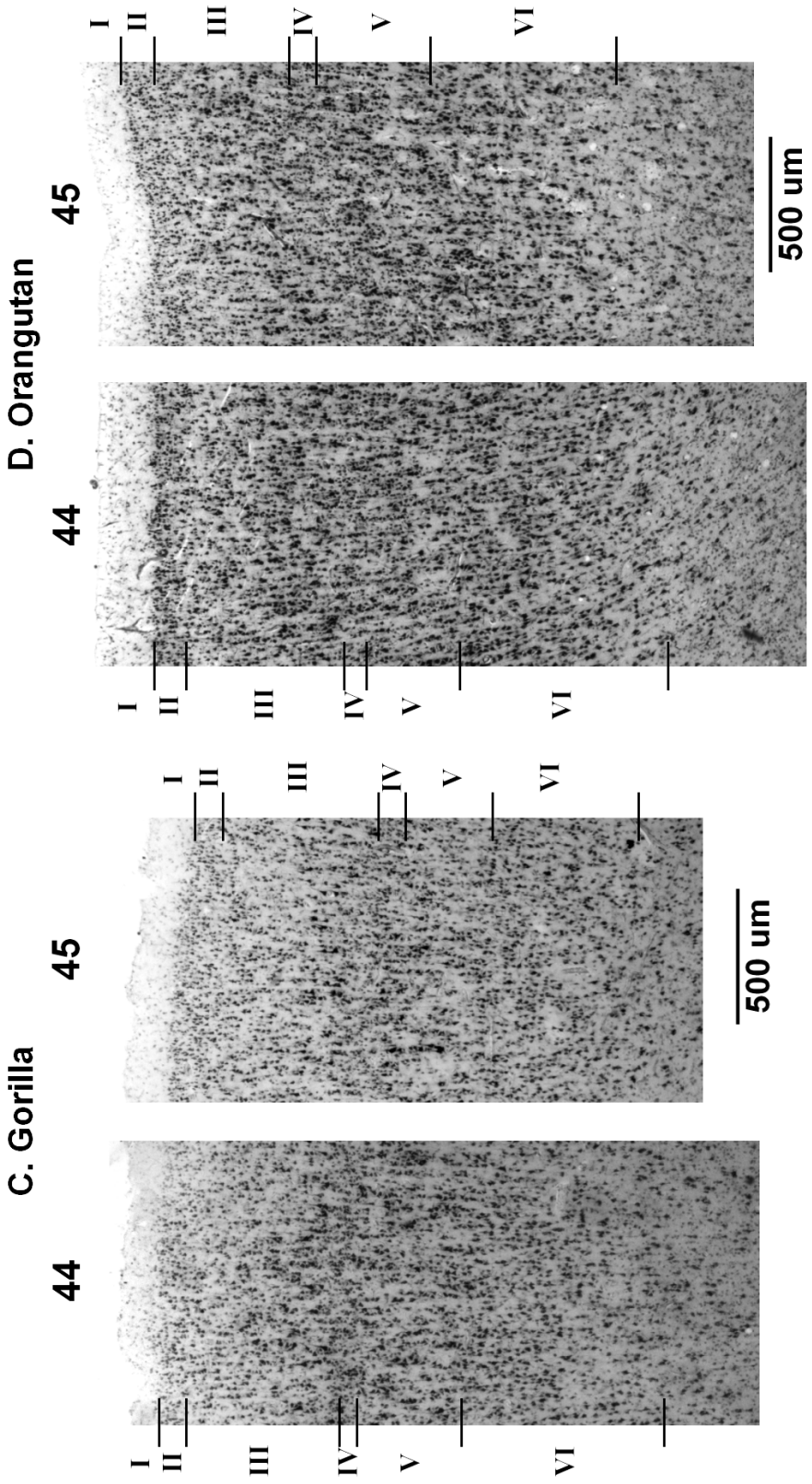

Figure 2.4 (cont.)

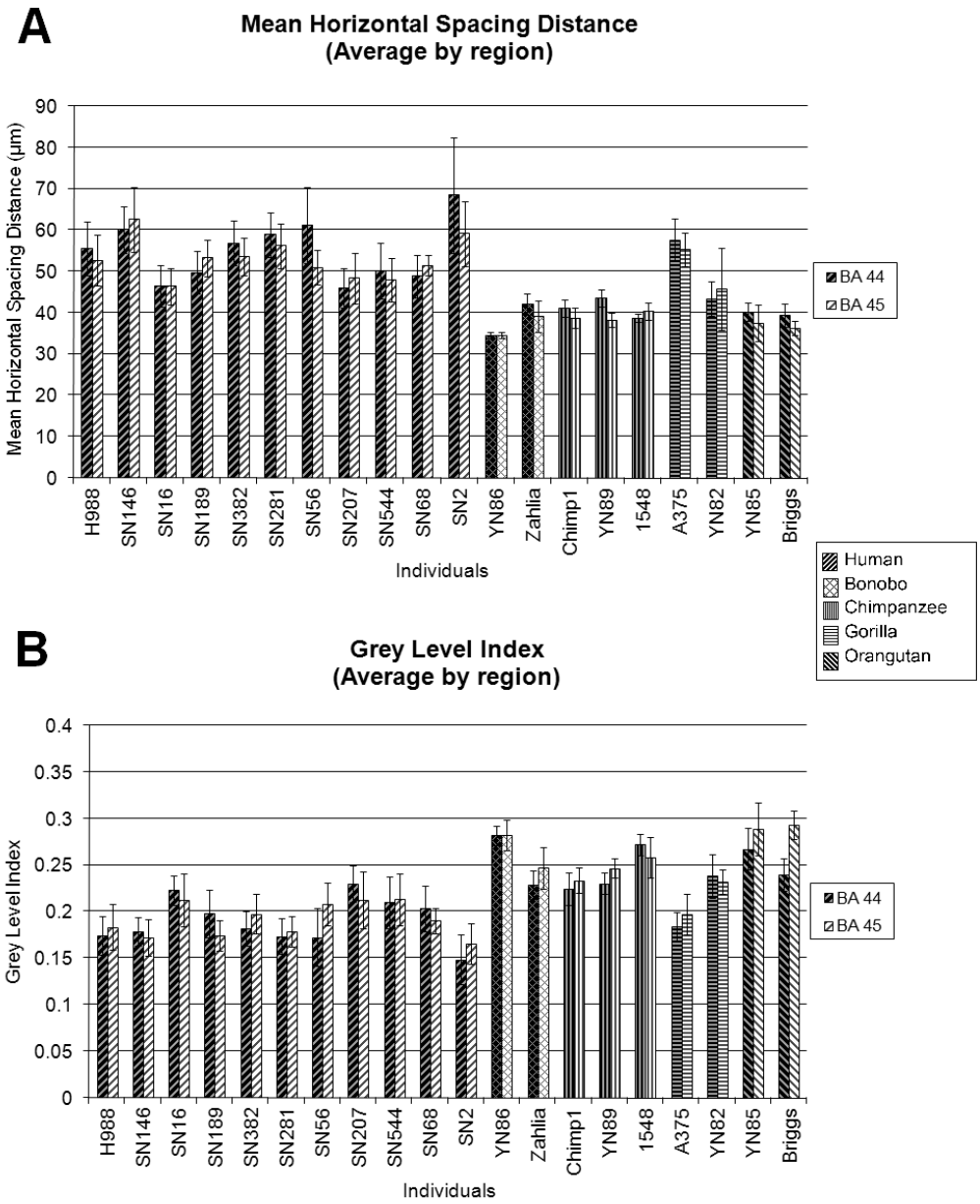

Figure 2.5: Charts of A) mean horizontal spacing distance and B) grey level index by individual and area

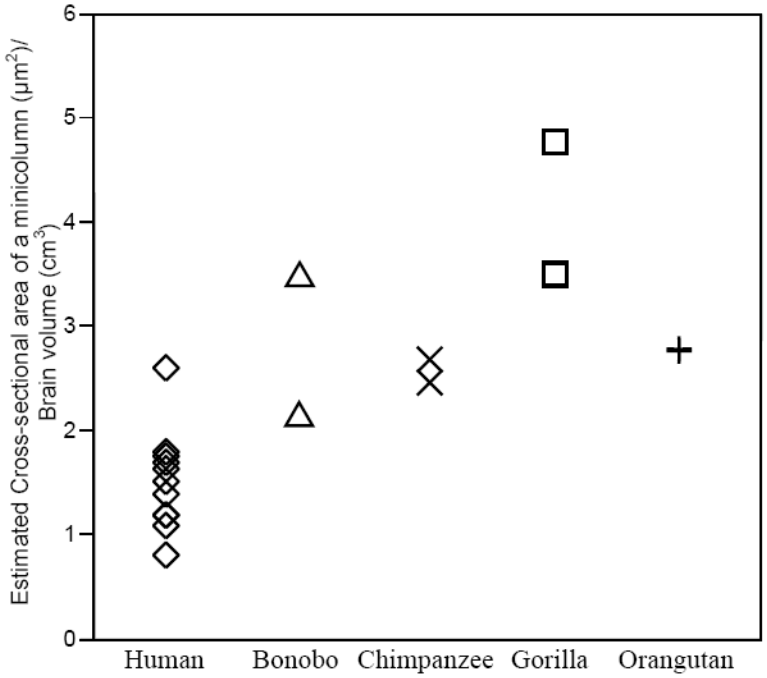

Figure 2.6: Estimated cross-sectional area of a minicolumn relative to brain size

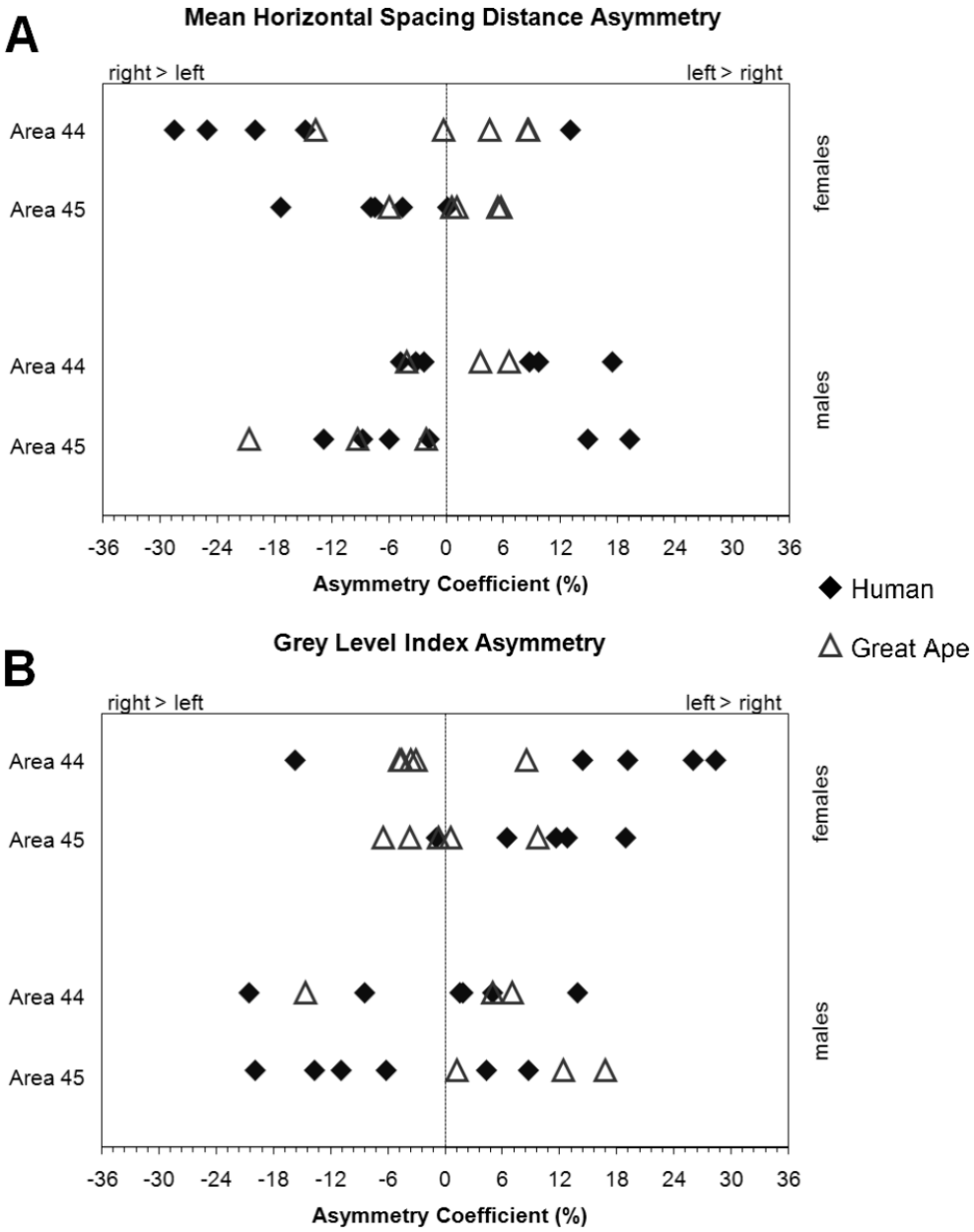

Figure 2.7: Plots of asymmetry coefficients for A) mean horizontal spacing distance, and B) grey level index, grouped by sex, area, and species group

REFERENCES

Aboitiz F, Garcia R, Brunetti E, Bosman C. 2006. The Origin of Broca's Area and Its Connections from an Ancestral Working Memory Network. In: Grodzinsky Y, Amunts K, Editors. Broca's Region. Oxford: Oxford University Press. p 3-16.

Amirikian B, Georgopoulos AP. 2003. Modular organization of directionally tuned cells in the motor cortex: is there a short-range order? Proceedings of the National Academy of Sciences of the United States of America 100:12474-12479.

Amunts K, Schleicher A, Burgel U, Mohlberg H, Uylings HBM, Zilles K. 1999. Broca's region revisited: Cytoarchitecture and intersubject variability. Journal of Comparative Neurology 412:319-341.

Amunts K, Schleicher A, Ditterich A, Zilles K. 2003. Broca's region: Cytoarchitectonic asymmetry and developmental changes. Journal of Comparative Neurology 465:72-89.

Amunts K, Weiss PH, Mohlberg H, Pieperhoff P, Eickhoff S, Gurd JM, Marshall JC, Shah NJ, Fink GR, Zilles K. 2004. Analysis of neural mechanisms underlying verbal fluency in cytoarchitectonically defined stereotaxic space - The roles of Brodmann areas 44 and 45. Neuroimage 22:42-56.

Anderson B, Southern BD, Powers RE. 1999. Anatomic asymmetries of the posterior superior temporal lobes: A postmortem study. Neuropsychiatry, Neuropsychology, and Behavioral Neurology 12:247-254.

Anwander A, Tittgemeyer M, von Cramon DY, Friederici AD, Knosche TR. 2007. Connectivity-based parcellation of Broca's area. Cerebral Cortex 17:816-825.

Bailey P, von Bonin G, McCulloch WS. 1950. The Isocortex of the Chimpanzee. Urbana: University of Illinois Press.

Barbas H, Pandya DN. 1989. Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. Journal of Comparative Neurology 286:353-375.

Bogolepova IN, Malofeeva LI, Uylings HBM. 1999. Structural Asymmetry of Motor Speech Areas 44 and 45 in Human Cerebral Cortex during Postnatal Ontogeny. Bulletin of Experimental Biology and Medicine 128:1066-1069.

Bookheimer S. 2002. Functional MRI of language: New approaches to understanding the cortical organization of semantic processing. Annual Review of Neuroscience 25:151-188.

Broadfield DC, Holloway RL, Mowbray K, Silvers A, Yuan MS, Marquez S. 2001. Endocast of Sambungmacan 3 (Sm 3): A new Homo erectus from Indonesia. Anatomical Record 262:369-379.

Broca P. 1861. Remarques sur le siège de la faculté du langage articulé, suivies d'une observation d'aphémie. Bulletin de la Société Anatomique Paris 36:330-357.

Brodmann K. 1909. Vergleichende Lokalisationslehre der Grobhirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig: Johann Ambrosius Barth.

Burton MW. 2001. The role of inferior frontal cortex in phonological processing. Cognitive Science 25:695-709.

Buxhoeveden D, Casanova M. 2000. Comparative lateralisation patterns in the language area of human, chimpanzee, and rhesus monkey brains. Laterality 5:315-330.

Buxhoeveden DP, Casanova MF. 2002. The minicolumn hypothesis in neuroscience. Brain 125:935-951.

Buxhoeveden DP, Semendeferi K. 2005. Cell Columns in Frontal Cortex of Apes and Humans. American Journal of Physical Anthropology S40:81.

Buxhoeveden DP, Switala AE, Litaker M, Roy E, Casanova MF. 2001a. Lateralization of Minicolumns in Human Planum temporale is Absent in Nonhuman Primate Cortex. Brain Behavior and Evolution 57:349-358.

Buxhoeveden DP, Switala AE, Roy E, Litaker M, Casanova MF. 2001b. Morphological differences between minicolumns in human and nonhuman primate cortex. American Journal of Physical Anthropology 115:361-371.

Buxhoeveden DR, Hasselrot U, Buxhoeveden NE, Booze RM, Mactutus CF. 2006. Microanatomy in 21 day rat brains exposed prenatally to cocaine. International Journal of Developmental Neuroscience 24:335-341.

Byrne RW, Corp N, Byrne JME. 2001. Estimating the complexity of animal behaviour: How mountain gorillas eat thistles. Behaviour 138:525-557.

Cantalupo C, Hopkins WD. 2001. Asymmetric Broca's area in great apes. Nature 414:505.

Caplan D. 2006. Why is Broca's area involved in syntax? Cortex 42:469-471.

Catani M, Jones D, ffytche D. 2005. Perisylvian Language Networks of the Human Brain. Annals of Neurology 57:8-16.

Colombo J, Reisin H. 2004. Interlaminar astroglia of the cerebral cortex: a marker of the primate brain. Brain Research 1006.

Corp N, Byrne RW. 2002. Leaf processing by wild chimpanzees: Physically defended leaves reveal complex manual skills. Ethology 108:673-696.

Damasio H, Tranel D, Grabowski T, Adolphs R, Damasio A. 2004. Neural systems behind word and concept retrieval. Cognition 92:179-229.

DeFelipe J. 2005. Reflections on the Structure of the Cortical Minicolumn. In: Casanova M, Editor. Neocortical Modularity and the Cell Minicolumn. New York: Nova Science Publishers, Inc. p 57-91.

Devlin JT, Raley J, Tunbridge E, Lanary K, Floyer-Lea A, Narain C, Cohen I, Behrens T, Jezzard P, Matthews PM, Moore DR. 2003. Functional asymmetry for auditory processing in human primary auditory cortex. Journal of Neuroscience 23:11516-11522.

Douglas R, Martin K. 1992. Exploring cortical microcircuits: A combined anatomical, physiological, and computational approach. In: McKenna T, Davies J, Zornetzer S, Editors. Single Neuron Computation. San Diego and London: Academic Press. p 381-412.

Duvernoy J. 1991. The Human Brain: Surface, Three-Dimensional Sectional Anatomy and MRI. Vienna: Springer-Verlag.

Elston GN, Rockland KS. 2002. The pyramidal cell of the sensorimotor cortex of the macaque monkey: Phenotypic variation. Cerebral Cortex 12:1071-1078.

Fadiga L, Craighero L. 2006. Hand actions and speech representation in Broca's area. Cortex 42:486-490.

Falk D. 1983. Cerebral Cortices of East-African Early Hominids. Science 221:1071-1074.

Favorov OV, Kelly DG. 1994. Minicolumnar Organization within Somatosensory Cortical Segregates .1. Development of Afferent Connections. Cerebral Cortex 4:408-427.

Fiebach CJ, Schubotz RI. 2006. Dynamic anticipatory processing of hierarchical sequential events: A common role for Broca's area and ventral premotor cortex across domains? Cortex 42:499-502.

Foundas AL, Weisberg A, Browning CA, Weinberger IR. 2001. Morphology of the frontal operculum: A volumetric magnetic resonance imaging study of the pars triangularis. Journal of Neuroimaging 11:153-159.

Friederici AD, Fiebach CJ, Schlesewsky M, Bornkessel ID, von Cramon DY. 2006. Processing linguistic complexity and grammaticality in the left frontal cortex. Cerebral Cortex 16:1709-1717.

Fukai T. 1994. A Model of Cortical Memory Processing Based on Columnar Organization. Biological Cybernetics 70:427-434.

Gallyas F. 1971. A principle for silver staining of tissue elements by physical development. Acta Morphologica Academiae Scientiarum Hungaricae 19:57-51.

Gardner RA, Gardner BT. 1969. Teaching sign language to a chimpanzee. Science 165:664-672.

Georgopoulos AP, Merchant H, Naselaris T, Amirikian B. 2007. Mapping of the preferred direction in the motor cortex. Proceedings of the National Academy of Sciences of the United States of America 104:11068-11072.

Gil-da-Costa R, Martin A, Lopes M, Munoz M, Fritz J, Braun A. 2006. Species-specific calls activate homologs of Broca's and Wernicke's areas in the macaque. Nature Neuroscience 9:1064-1070.

Hagoort P. 2005. On Broca, brain, and binding: a new framework. Trends in Cognitive Sciences 9:416-423.

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 180:126-142.

Hayes TL, Lewis DA. 1996. Magnopyramidal neurons in the anterior motor speech region: Dendritic features and interhemispheric comparisons. Archives of Neurology 53:1277-1283.

Hofman MA. 1989. On the Evolution and Geometry of the Brain in Mammals. Progress in Neurobiology 32:137-158.

Holloway RL. 1968. The evolution of the primate brain: some aspects of quantitative relations. Brain Research 7:121-172.

Indefrey P, Hellwig F, Herzog H, Seitz RJ, Hagoort P. 2004. Neural responses to the production and comprehension of syntax in identical utterances. Brain and Language 89:312-319.

Johansson C, Lansner A. 2007. Towards cortex sized artificial neural systems. Neural Networks 20:48-61.

Kaas J, Nelson R, Sur M, Merzenich M. 1981. Organization of somatosensory cortex in primates. In: Schmitt FO, Worden FG, Adelman G, Dennis SG, Editors. The organization of the cerebral cortex. Cambridge, MA: MIT Press. p 237-261.

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.

Lee CJ, Whitsel BL, Tommerdahl M. 1992. Mechanisms underlying somatosensory cortical dynamics: II. In vitro studies. Cerebral Cortex 2:107-133.

Li S, Qi XL, Hu H, Wang YJ. 2005. Synchronized oscillation in a modular neural network composed of columns. Science in China Series C-Life Sciences 48:6-17.

Lucke J, von der Malsburg C. 2004. Rapdi processing and unsupervised learning in a model of the cortical macrocolumn. Neural Computation 16:501-533.

McGrew WC, Marchant LF, Nishida T. 1996. Great Ape Societies. Cambridge: Cambridge University Press.

Merker B. 1983. Silver staining of cell bodies by means of physical development. Journal of Neuroscience Methods 9:235-241.

Miles HL. 1994. Chantek: The language ability of an enculturated orangutan (*Pongo pygmaeus*). In: Ogden J, Perkins L, Sheeran L, Editors. Proceedings of the international conference on "Orangutans: The neglected ape". San Diego: Zoological Society of San Diego. p 209-219.

Mountcastle VB. 1997. The columnar organization of the neocortex. Brain 120 (Pt 4):701-722.

Nakamichi M. 2004. Tool-use and tool-making by captive, group-living orangutans (Pongo pygmaeus abelii) at an artificial termite mound. Behavioural Processes 65:87-93.

Neilson PD, Neilson MD. 2005. Motor maps and synergies. Human Movement Science 24:774-797.

Ojemann GA. 1991. Cortical Organization of Language. Journal of Neuroscience 11:2281-2287.

Ono M, Kubik S, Abernathey CD. 1990. Atlas of the Cerebral Sulci. Stuttgart/New York: Thieme Verlag.

Panger M. 2007. Tool Use and Cognition in Primates. In: Campbell CJ, Fuentes A, MacKinnon KC, Panger M, Bearder SK, Editors. Primates in Perspective. New York: Oxford University Press. p 665-677.

Patterson FG, Cohn RN. 1990. Language acquisition by a lowland gorilla: Koko's first ten years of vacabulary development. Word 41:97-142.

Petrides M. 2006. Broca's Area in the Human and the Nonhuman Primate Brain. In: Grodzinsky Y, Amunts K, Editors. Broca's Region. Oxford: Oxford University Press. p 31-46.

Petrides M, Pandya DN. 1994. Comparative architectonic analysis of the human and the macaque frontal cortex. In: Boller F, Grafman J, Editors. Handbook of Neuropsychology. Amsterdam: Elsevier Science Ltd. p 17-58.

Petrides M, Pandya DN. 2001. Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. European Journal of Neuroscience 16:291-310.

Preuss TM, Goldman-Rakic PS. 1991. Myelo- and Cytoarchitecture of the Granular Frontal Cortex and Surrounding Regions in the Strepsirhine Primate *Galago* and the Anthropoid Primate *Macaca*. Journal of Comparative Neurology 310:429-474.

Price CJ. 2000. The anatomy of language: contributions from functional neuroimaging. Journal of Anatomy 197 Pt 3:335-359.

Rakic P. 1995a. Radial Versus Tangential Migration of Neuronal Clones in the Developing Cerebral-Cortex. Proceedings of the National Academy of Sciences of the United States of America 92:11323-11327.

Rakic P. 1995b. A Small Step for the Cell, a Giant Leap for Mankind - a Hypothesis of Neocortical Expansion During Evolution. Trends in Neurosciences 18:383-388.

Rakic P, Kornack DR. 2001. Neocortical expansion and elaboration during primate evolution: a view from neuroembryology. In: Falk D, Gibson K, Editors. Evolutionary Anatomy of the Primate Cerebral Cortex. Cambridge, UK: Cambridge University Press. p 30-56.

Rao SG, Williams GV, Goldman-Rakic PS. 1999. Isodirectional tuning of adjacent interneurons and pyramidal cells during working memory: Evidence for microcolumnar organization in PFC. Journal of Neurophysiology 81:1903-1916.

Ringo JL. 1991. Neuronal Interconnection as a Function of Brain Size. Brain Behavior and Evolution 38:1-6.

Rizzolatti G, Arbib MA. 1998. Language within our grasp. Trends in Neurosciences 21:188-194.

Sarkissov SA, Filimonoff IN, Kononowa EP, Preobraschenskja IS, Kukuew LA. 1955. Atlas of the Cytoarchitectonics of the Human Cerebral Cortex. Moscow: Medgiz.

Savage-Rumbaugh ES, Fields W. 2000. Linguistic, cultural and cognitive capacities of bonobos (*Pan paniscus*). Culture & Psychology 6:131-153.

Schleicher A, Zilles K. 1990. A Quantitative Approach to Cytoarchitectonics - Analysis of Structural Inhomogeneities in Nervous Tissue Using an Image Analyzer. Journal of Microscopy 157:367-381.

Seldon HL. 1981. Structure of Human Auditory-Cortex. 1. Cytoarchitectonics and Dendritic Distributions. Brain Research 229:277-294.

Semendeferi K, Armstrong E, Schleicher A, Zilles K, Van Hoesen GW. 1998. Limbic frontal cortex in hominoids: a comparative study of area 13. Am J Phys Anthropol 106:129-155.

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.

Sherwood CC, Broadfield DC, Holloway RL, Gannon PJ, Hof PR. 2003. Variability of Broca's Area Homologue in African Great Apes: Implications for Language Evolution. Anatomical Record Part A 271A:276-285.

Sugimoto S, Sakurada M, Horikawa J, Taniguchi I. 1997. The columnar and layer-specific response properties of neurons in the primary auditory cortex of Mongolian gerbils. Hearing Research:175-185.

Tardif E, Probst A, Clarke S. 2007. Laminar Specificity of Intrinsic Connections in Broca's Area. Cerebral Cortex 10.1093/cercor/bhm021.

Uylings HBM, Jacobsen AM, Zilles K, Amunts K. 2006. Left-right asymmetry in volume and number of neurons in adult Broca's area. Cortex 42:652-658.

van Schaik CP, Ancrenaz M, Borgen G, Galdikas B, Knott CD, Singleton I, Suzuki A, Utami SS, Merrill M. 2003. Orangutan Cultures and the Evolution of Material Culture. Science 299:102-105.

von Economo C. 1929. The Cytoarchitectonics of the Human Cerebral Cortex. London: Oxford University Press.

Walker AE. 1940. A Cytoarchitectural Study of the Prefrontal Area of the Macaque Monkey. Journal of Comparative Neurology 73:59-86.

Watanabe-Sawaguchi K, Kubota K, Arikuni T. 1991. Cytoarchitecture and Intrafrontal Connections of the Frontal Cortex of the Brain of the Hamadryas Baboon (*Papio hamadryas*). Journal of Comparative Neurology 311:108-133.

Whiten A, Goodall J, McGrew WC, Nishida T, Reynolds V, Sugiyama Y, Tutin CEG, Wrangham RW, Boesch C. 2001. Charting cultural variation in chimpanzees. Behaviour 138:1481-1516.

Zatorre RJ, Belin P. 2001. Spectral and temporal processing in human auditory cortex. Cerebral Cortex (Cary) 11:946-953.

STEREOLOGICAL QUANTIFICATION OF BROCA'S AREA IN HOMINOIDS

INTRODUCTION

At heart of the study of the evolution of language ability is how to produce testable hypotheses regarding the antecessors of language in our hominid ancestors and the presence or absence of such traits in our closest living relatives, the apes. Language is a complex behavior that is an amalgam of disparate cognitive abilities that combine to produce what we see and hear as linguistic communication. As a result, the neural substrates underlying linguistic interaction are also varied. One region that is consistently implicated in modern functional studies of language, and was implicated even in early lesion studies, is the region known as Broca's area. This term is most often used, and will be used here, to refer to the area comprising Brodmann's cytoarchitectonic areas 44 and 45.

In addition to the linguistic implications for Broca's area, upon which most studies of the region have focused, there is evidence that the region is involved in additional cognitive tasks that require hierarchical processing and sequencing. Multiple studies suggest that Broca's area is less selective regarding the type of stimuli than once believed. While the region is clearly involved in language-related behaviors, it is also activated during tasks that involve manipulation of non-linguistic auditory sequences (Gelfand and Bookheimer, 2003), tasks that require particular attention to the relationship between object location, speed, and timing (Schubotz and von Cramon, 2001), and goal-directed motion and recognizing deviations from expected sequences (Nishitani et al., 2005). This translates into the ability to imitate (Iacoboni et al., 1999) and convert what we see into action (Grezes et al., 2003). However, the function of attention to timing and detail may come more from planning of actions than from the online organization of actions. Koechlin and Jubault (2006:963) suggest that "Broca's area and its

right homolog process hierarchically structured behaviors regardless of their temporal organization, suggesting a fundamental segregation between prefrontal executive systems involved in the hierarchical and temporal organization of goal-directed behaviors." This general executive function for creating plans of action seems to be distinct from a function for organization of temporal sequences of actions.

The general executive function of Broca's area may have significant implications for theories on the evolution of linguistic communication. If one looks for behaviors in closely related species that can provide a glimpse at possible precursors of language (Hewes, 1973; Armstrong et al., 1995; Dunbar, 1996; Calvin and Bickerton, 2000; Rizzolatti et al., 2001; King, 2004), it may be necessary to consider the idea that language utilizes nonlinguistic cognitive resources. For instance, Calvin (2000) has suggested that language employs some of the same planning and execution abilities that can be seen in throwing actions that are thought to be important during the course of hominid evolution. Others suggest a continuity related to imitation (Rizzolatti et al., 2001), gesture (Hewes, 1973; Armstrong et al., 1995), or co-regulation of social interactions (Dunbar, 1996; King, 2004).

If there are continuities in the cognitive abilities that utilize Broca's area, then we would expect to see continuity in the neuroanatomical structure of the region as well; some evidence of continuity already exists. In old world monkeys, the presence of both areas 44 and 45 has been the subject of debate. Walker (1940), in a study that became the foundation of subsequent analyses of prefrontal cortex in anthropoid primates, identified Brodmann's area 45 in rhesus macaques. Area 44 was first delineated in macaques by Petrides and Pandya (1994). Prior to Petrides and Pandya (1994), numerous studies had described the region, but none had

specifically delineated area 44. Barbas and Pandya (1987; 1989) examined the cytoarchitecture of premotor and prefrontal cortex in rhesus macaques, providing a classification that is in general agreement with that reported by Walker (1940), although they suggest that Walker's area 45 should be identified as a ventral subdivision of area 8, because of its similarity with more dorsal cortices. A later study by Preuss and Goldman-Rakic (1991) of rhesus macaques parcellated granular frontal cortex using primarily myeloarchitectural criteria, resulting in a map that closely matches that of Barbas and Pandya (1989). However, Preuss and Goldman-Rakic (1991) also observed additional divisions of the premotor cortex. In particular, they indicate a division that was later delineated by Petrides and Pandya (1994) as area 44. In great apes and humans, Brodmann's areas 44 and 45 have been previously described in studies of human (Brodmann, 1909; von Economo, 1929; Sarkissov et al., 1955; Amunts et al., 1999) and chimpanzee brains (Bailey et al., 1950; Sherwood et al., 2003).

Here we identified the boundaries of Brodmann's areas 44 and 45 in human, chimpanzee, bonobo, gorilla, orangutan, and gibbon specimens. We estimated volume using the Cavalieri principle in all specimens and estimated total neuron number with the stereological optical fractionator method. We test the hypothesis that Broca's area occupies a greater proportion of the hemisphere in humans than in other apes, because of the region's involvement in language production that is absent in nonhumans. Furthermore, given the reported presence of asymmetry in both volume and neuron number in Brodmann's area 44 in humans (Uylings et al., 2006), and the reported gross asymmetry of the inferior frontal gyrus in chimpanzees, gorillas, and orangutans (Cantalupo and Hopkins, 2001), we test the hypothesis that both volume and neuron number area are asymmetrical in all the species examined.

MATERIALS

Our sample comprised 14 complete series of histologically processed brains of individuals from five hominoid species (Table 3.1). It included both hemispheres of two humans, four chimpanzees, two bonobos, two gorillas, two orangutans, and one gibbon with one additional hemisphere of a human.

All specimens were fixed in either 4% buffered formalin (pH 7.4) or a Bodian mixture (formalin, glacial acetic acid, and ethanol) within 24 hours after the natural death of the subject and were then processed and stained in a consistent manner (Semendeferi et al., 1998; Amunts et al., 1999). Most brains were paraffin-embedded and serially sectioned in the coronal plane into 20μm thick sections producing complete series through the entirety of each brain. Every tenth to sixteenth section was mounted on a glass slide and stained with a modification of the Gallyas silver stain for neuronal perikarya (Gallyas, 1971; Merker, 1983). One chimpanzee, YN89, was cut in the axial plane into 15μm thick sections with every 30th section mounted and stained. One gorilla (A375) was stained with cresyl violet. One human (CS025) was cut in the coronal plane at 80μm and the sections were stained with thionin. In no case was a neural pathology the cause of death.

The ape specimens in our collection (UCSD) were donated by Busch Gardens Zoo, Milwaukee County Zoo, Metro Washington Park Zoo, Gladys Porter Zoo, and Yerkes National Primate Research Center following the natural deaths of the animals. Three ape specimens (chimpanzee Bathsheba, gorilla A375, and orangutan Harry) were wild born; no information is available on the origin of one specimen (chimpanzee 1548). Two specimens had limited

exposure to language training (chimpanzee Bathsheba and bonobo YN86). Individuals (including humans) ranged from 2-75 years of age and represented both sexes.

METHODS

In both hemispheres, the boundaries of Brodmann's areas 44 and 45 were hand traced in serial sections with the Stereo Investigator software (Micro Bright Field, Williston, VT) using the 1x objective (N.A. 0.4) of the Nikon 80i microscope.

Anatomical regions of interest

Brodmann's areas 44 and 45 comprise what is known as Broca's area, and typically occupy part of the inferior frontal gyrus in humans. These areas are defined using traditional histological criteria such as differences in laminar thickness, cell densities, and cell types (Brodmann, 1909; von Economo, 1929; Sarkissov et al., 1955). Shared cytoarchitectonic characteristics that can be seen across species (Schenker et al., submitted) include magnopyramidal cells in lower layer III, medium large pyramidal cells in upper layer V, and decreased cell density in lower layer V. There is a size gradient in the cells of layer III, with the smallest cells at its border with layer II and the largest near layer IV. The main cytoarchitectonic difference between areas 44 and 45 is the prominence of layer IV and the total thickness of cortex. In particular, layer IV in area 44 is dysgranular —visible but with few cells, and appears to be invaded by cells from III and V. In area 45, layer IV is more prominent, and creates a clear barrier between layers III and V. The cortex is notably thinner in area 45 than in area 44. Definitions of boundaries of the ROIs for this study were made in consultation with the anatomical descriptions of macaques (Petrides, 2006), chimpanzees (Bailey et al., 1950;

Sherwood et al., 2003) and humans (Brodmann, 1909; von Economo, 1929; Amunts et al., 1999).

In humans, Brodmann's areas 44 and 45 lie mostly within the inferior frontal gyrus and comprise three major subdivisions: the pars opercularis, the pars triangularis, and the pars orbitalis divided by prominent sulci in most human brains (Ono et al., 1990; Duvernoy, 1991). Areas 44 and 45 can also be found within the inferior frontal gyrus in the great apes (chimpanzees, Bailey et al., 1950; Sherwood et al., 2003; bonobos, gorillas, orangutans, Schenker et al., submitted). The areas lie almost exclusively anterior to the precentral sulcus and ventral to the inferior frontal sulcus, although, as with the human brain, individual variation exists in the extent of these areas on the lateral surface of the brain, and there is some variation between species.

Adjacent areas.

Adjacent regions were differentiated from areas 44 and 45 on the basis of cytoarchitectonic criteria. The following are observations on the changes in architecture as one leaves Brodmann areas 44 and 45 and moves into surrounding areas.

Anterior: Anterior to BA 45, the cortex is quite narrow. Layer II is narrow and dense. Cells in lower layer III become smaller with little or no gradient from upper to lower III. Layer IV is visible. Cells in layer V are similar in size to those in lower layer III (Figure 3.1a).

Anterior superior: Layer II is not easily distinguishable from layer III. There is a slight gradient in the size of cells in layer III, with larger cells near the boundary with layer IV. Layer IV is also present, but not as noticeably prominent as in BA 45. Pyramidal cells in layers III and V

are more uniform in size than in BA 45. There is no noticeable boundary between layers V and VI (Figure 3.1b).

Posterior Dorsal: Cortex is similar in thickness to area 44. Layer III cells are not as large as those in found in area 44. Layer IV is barely present, and layer V cells are larger than those seen in layer III (Figure 3.1c).

Posterior: Posterior to area 44, the overall thickness of cortex is greater. Cells in layer III are large, but they are not markedly larger than those in layer V. Furthermore, the absence of a defined layer IV means that layer III is continuous with layer V (Figure 3.1d).

Posterior Ventral: Cortex is quite narrow. Layers II and IV are present and distinguishable. Medium cells are present in both lower layer III and upper layer V. A pale band separates layers V and VI (Figure 3.1e).

Anterior Ventral: The thickness of cortex here is somewhat narrower than 45. Scattered large cells are found in lower layer III, but generally cell size is smaller that in BA 45. Layer IV is very well-developed. Layers V and VI have small-to-medium cells. A cell-sparse band can be seen in lower layer V (Figure 3.1f).

Volumes

Volumetric data was collected for each area in each hemisphere with the Cavalieri method (Gundersen et al., 1988), using a 250 μm (apes) or 500 μm (humans) point counting grid in the Stereo Investigator program. In each hemisphere, 9 to 17 sections were analyzed at intervals of 400 to 1200 μm for each ROI between sections in the apes, depending on absolute brain size. In the humans, 9-19 sections were analyzed at intervals of 600 to 1920 for each ROI between sections. As required by the Cavalieri method, sampled sections from each specimen

were chosen at standard intervals for each ROI and the starting section was picked randomly from the first interval. The coefficient of error (Gundersen et al., 1999, m=1) was less than 0.015 for each ROI in each specimen; such low values indicate that the precision of the volume estimates was high and that the sampling parameters were sufficient.

We used correction factors for every specimen as calculated previously for each individual specimen to account for much of the shrinkage which occurs during tissue processing. Although correction factors cannot account entirely for the possibility that white and gray matter shrink differentially (Kretschmann et al., 1982), calculating them for individual specimens is the best means available to us for nullifying variation due to processing. For each specimen, the pre-processing brain volume was divided by the post-processing volume, and we then multiplied ROIs by this factor to obtain corrected volumes. Brain volumes before processing were determined by dividing brain weight by the specific gravity of brain tissue (1.036). Fixed brain volumes were estimated using the Cavalieri method on approximately 50 sections in each series. Two correction factors were obtained by NS (CS025 & H988). Three correction factors were provided by Carol MacLeod (Langara College, Vancouver; Chimp1, 1548, & A375). The remainder of the correction factors were provided by Nicole Barger (Barger et al., 2007). Both uncorrected and corrected values are reported.

Neuron counts

To estimate neuron numbers, we used the optical fractionator method (West et al., 1991) with a 50 µm counting frame and a 1100-1300 µm grid (in the apes) or a 1300-2600 µm grid (in the humans). Size of the grid depended on the size of the particular ROI and was designed to optimize the efficiency of counting. Section thickness was measured at every site

during counting. We used a standard dissector depth of 14 μm in the specimens cut at 20 μm, and a dissector depth of 9 μm in the specimen cut at 15 μm (YN89), so as not to count the top and bottom of the tissue where significant sectioning artifacts may occur. The same sections used for volumetric estimates were used for cell counts. Cells were counted if a clear nucleolus came into focus, as per the principles of the optical fractionator method (West et al., 1991). The calculated coefficients of error (Gundersen et al., 1999, m=1) were within the optimal range of <0.1 for each ROI in each specimen. Neuron densities were calculated using total neuron numbers and uncorrected volumes, as is common practice in the literature. Densities calculated using corrected volumes are also reported.

Data analysis

Reported volume measures for each ROI are provided for both the right and left hemispheres. The relative volume of the ROI is this volume divided by the volume of the relevant hemisphere, in other words, the percent of the hemisphere occupied by the ROI. The cerebral hemispheres were defined as the whole brain minus cerebellum, midbrain, and brainstem, and the left and right hemispheres were divided using a line bisecting the corpus callosum and other midline anatomical structures. To assess the possibility of lateralization, the relative degree of asymmetry of each ROI in each individual was measured as: |(Left–Right)/[(Left + Right)/2]|. Asymmetry coefficients were analyzed in relation to sex, taxonomic group (apes or humans), age and area using multi-way ANOVAs. We corrected for multiple comparisons by using a standard Bonferroni correction. All statistical tests were conducted using JMP IN 4.02 statistical software (SAS Institute, 2000).

Regression analyses were performed using log-transformed data. Equations and 95% confidence intervals from standard least squares regression (SLS) were obtained using JMP IN 4.02. In previous studies (Semendeferi and Damasio, 2000), we found only minor differences between SLS and reduced major axis regressions, so here we used only the more common SLS. We also performed independent contrasts (IC) analysis with the PDAP program (Garland et al., 1999; Garland and Ives, 2000), using Purvis's (1995) phylogeny to set branch lengths (which were squared to ensure that standardized contrasts did not correlate with their standard deviation). Regressions drawn from the PDTREE module of PDAP were mapped back into original data space with individual values for each specimen plotted over this regression line. For the independent variable, ROI volume was subtracted from hemisphere volume to avoid statistical artifacts related to regressing an ROI against a region of which it is a part (Deacon, 1990). We report results from both regressions focusing on the allometric relationship between ROI volume and hemisphere volume in two conditions: including or excluding the human datum. In all cases, SLS regressions yielded considerably narrower 95% confidence intervals (CI) than IC analysis. Differences between slopes were tested using a modified version of the t-test (Zar, 1996: section 18.1). Because this sample is unlikely to satisfy the requirements of parametric analyses, *e.g.*, normal distribution, the nonparametric Kruskal-Wallis test was used to test for differences between means in JMP IN 4.02.

RESULTS

Cytoarchitecture

There were slight differences in the location of the regions among species. The primary difference was between the African apes (chimpanzees, bonobos, and gorillas) and the

Asian great apes (orangutans). Although the differences were slight, the pattern observed in the African apes was more similar to that of the humans than was the pattern in the two orangutans. In the African apes, area 44 tended to lie directly anterior to the inferior precentral sulcus, with area 45 lying immediately anterior to area 44 (Figure 3.2). In contrast, while area 44 was found directly anterior to the inferior precentral sulcus in the orangutans, area 45 was located somewhat dorsally as well as anterior (Figure 3.2d). In the gibbon, the location of Brodmann's areas 44 and 45 corresponds approximately to the locations described by Petrides (2006) in rhesus macaques (Figure 3.2e), although the extent of area 45 is not as great in the gibbon as that described in the aforementioned study, but corresponds more closely to the more circumscribed extent described in other studies (Walker, 1940; Barbas and Pandya, 1987; 1989).

Uncorrected volumes

Humans had the largest volumes for areas 44 and 45, ranging from 468.30 (R44) to 1565.76 (L44) mm^3, with substantial intra-individual variation between hemispheres and area and also variation among individuals. Volumes in the great apes ranged from 50.48 (R44 in a chimpanzee) to 431.14 (R45 in a bonobo) mm^3. In the gibbon individual, volumes ranged from 53.05 (L45) to 108.73 (R44) mm^3 (Table 3.1; Figure 3.3a). Corrected volume data displayed the same pattern as uncorrected volume data (Figure 3.3b).

The size relationship between areas 44 and 45 was not consistent across individuals, or even between hemispheres within the same individual. While in the majority of hemispheres area 45 is larger than area 44, the difference is not significant, as there are six hemispheres (four individuals) in which area 44 is larger than area 45.

Regression

No significant difference was observed between regressions calculated using standard least squares and those using phylogenetically independent contrasts. Furthermore, no significant differences were seen between the regressions made with and without humans. No species stands out in relation to the regression lines. Furthermore, no regression displayed a significant deviation from isometry, given the size of the sample (Figure 3.4).

Relative volumes

The volume of individual areas relative to the volume of the hemisphere ranged from 0.12% for left area 44 in a gorilla to 0.49% for right area 45 in the gibbon. The range of relative volumes overlapped among all species (Figure 3.5).

Neuronal Numbers and Density

Humans have greater numbers of neurons in left area 44 than do the other species. However, total numbers of neurons are overlapping among all species for the other three areas (Table 3.2). Also, the number of neurons is greater in both left areas together in the humans than in the other species (Figure 3.6).

Humans have lower neuronal density in all regions than do the apes, with the ranges being non-overlapping. This is true both for densities calculated using uncorrected volumes (Figure 3.7a) and for those calculated using corrected volumes (Figure 3.7b). However, sample sizes are too small to be significant.

Asymmetry

Only limited evidence of asymmetry is present within the current sample. The only significant volumetric asymmetry observed was in the gorillas, where volumes in the right

hemisphere were greater than those in the left; the asymmetry was most prominent in Brodmann's area 44. No significant asymmetries were observed in the relative volume, neuron number, or neuron density of either area 44 or area 45 in any species.

Individual variation

All variables were examined for relationships with sex, age, life history (wild or captive born), and brain size. No patterns were observed between volume, neuron number, or neuron density and any of the above parameters.

DISCUSSION

Variation

In concordance with previous investigations of human (Brodmann, 1909; Amunts et al., 1999) and chimpanzee (Sherwood et al., 2003) brains, we found individual variation in the extent of areas 44 and 45 and their location in relation to sulci. Furthermore, the general sulcal morphology of the inferior frontal gyrus varies, not just among individuals, but among species as well. The sulcal pattern in both orangutan specimens was different than that in the African ape specimens. The literature reports extensive variability in the frontal lobe sulci of orangutan brains; one of the described patterns includes an X created by the inferior precentral sulcus, the inferior frontal sulcus, and the horizontal branch of the inferior precentral sulcus (Connolly, 1950). This pattern may be common in orangutans, but it is not reported to be common among chimpanzees and gorillas (Connolly, 1950; Sherwood et al., 2003), and may reflect differences in the associated cortical areas.

Individual variation is also present in the stereological data obtained in this study. While no relationship was observed between this variation and known life history traits,

differences were observed in the uncorrected volumes between specimens that were processed in different ways. One human (CS025) was sectioned and stained in a different manner, and this is apparent in the larger uncorrected volumes obtained. However, this difference disappears after correction is made for shrinkage, emphasizing the importance of attempting to correct for variation in shrinkage among specimens.

Comparison with previous studies

Previous studies have reported estimates of neuronal densities in the prefrontal cortex of humans (total frontal cortex: Pakkenberg, 1993; areas 9 & 46: Rajkowska and Goldman-Rakic, 1995; area 13: Semendeferi et al., 1998; area 10: Semendeferi et al., 2001; areas 9 & 44: Selemon et al., 2003) and two prefrontal areas in ape species (area 13: Semendeferi et al., 1998; area 10: Semendeferi et al., 2001). These data are presented in Table 3.3. The figures for neuron densities from the current study (Table 3.2) are lower than the values reported in areas 9 and 46, and somewhat lower than those reported for total frontal cortex, but they are comparable to the results for areas 10 and 13. Five of the six specimens measured by Semendeferi et al. (1998; 2001) were also included in this study (the sixth was not usable for neuron counting in Broca's area), and the numbers from those studies and the current one are the most comparable (Table 3.4), despite some differences in the methodological approach. This suggests that individual variation is an important factor in the measurement of neuronal density, in addition to variation between different regions of cortex within the same brain.

An additional study of areas 44 and 45 in humans (Uylings et al., 2006), provides estimates of neuron counts and corrected volumes from which densities were calculable (Table 3.3). The range of values in Uylings et al. (2006) for volumes of areas 44 and 45 and for

neuron numbers clearly demonstrates the presence of substantial individual variation. The current reported numbers (Table 3.2) fall in the lower range of those seen in the Uylings study. Furthermore, we suspect there may be differences between the current study and the Uylings study in the factors used to correct for shrinkage, which could account for some of the variation between results.

While one might expect that the variation in neuronal density is directly related to brain size and allometry, there is no relationship between brain size and density among the ape specimens. However, it is possible that age plays a role. An interesting difference between areas 44 and 45 and areas 10 and 13 is noticeable in the neuron density in one of the bonobos (YN86). This individual was only two years old. The density of neurons in areas 44 and 45 in this individual is greater than in the other bonobo and than in the same individual in areas 10 and 13. However, the difference between individuals disappears when the density is corrected for shrinkage, suggesting that there may be differences within the individual between regions (44/45 vs. 10/13) that are not as present in other individuals that are older.

CONCLUSION

The argument that Broca's area is involved with the regulation and planning of organized sequences of sounds has been used as evidence for why chimpanzees would not need a Broca's area, since their natural vocalizations are not made by varying the sequential order of individual units (Passingham, 1981). However, if the hierarchical processing achieved by Broca's area is found beyond the linguistic realm, as has been suggested (Fiebach and Schubotz, 2006), then the hierarchical processing of actions observed in the great apes (Byrne et al., 2001; Corp and Byrne, 2002; Nakamichi, 2004) may utilize these resources for non-

linguistic behaviors. To engage in these complex hierarchical behaviors, great apes need to be able to create and also to parse complicated sequences of motor and/or oral behaviors. This possibility provides a potential starting point from which one can argue that language may have evolved through piggy backing on neural systems involved in other types of processing, such as tool-making. We have demonstrated here that Broca's area cytoarchitectural morphology is present in all apes, suggesting that the functional behaviors associated with the region are present in some form in nonhuman hominoids. Furthermore, the region does not occupy any greater or lesser percentage of the hemisphere in humans than in other species. However, the lower neuronal densities in humans suggest the possibility that there are basic differences in information processing within the region. Whether those differences are Broca's area specific or whether they may be seen on a wider scale throughout the human brain awaits further investigation of other regions.

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Table 3.1a: Uncorrected volumetric estimates

	Specimen ID	Age	Sex	Brain Weight	Hemisphere (cm^3)		Area 44(mm^3)		Area 45(mm^3)	
					L	R	L	R	L	R
Human	SN207/84	75	M	1350	278.04	275.47	842.57	468.30	1060.50	1279.35
	H9/88	21	M	1633	354.34	362.41	953.25	870.25	561.00	1032.50
	CS025	44	M	1640	604.71		1565.76	–	1399.68	–
Bonobo	Zahlia	11	F	324	68.28	73.95	205.60	295.73	327.49	431.14
	YN86	2	F	392	55.78	55.13	154.50	93.45	182.93	199.91
Chimpanzee	Schimp	22	F	440	78.57	80.33	153.15	124.73	187.50	186.08
	YN89	22	M	420	70.98	69.33	95.40	242.16	123.47	250.76
	Bathsheba	24	F	359.5	74.89	72.69	130.58	172.53	80.18	76.80
	1548	Adult	?	NA	–	–	75.53	50.48	118.20	75.28
Gorilla	YN82	20	F	376	68.65	69.36	81.76	109.72	187.92	234.42
	A375	Juvenile	M	450	–	–	80.81	107.33	101.23	132.98
Orangutan	YN85	16	M	369	65.02	68.53	189.30	143.74	306.23	159.64
	Harry	37	M	440	69.99	68.45	168.03	154.20	200.48	116.40
Gibbon	Disco	22	F	120	21.42	22.16	68.90	108.73	53.05	57.00

Table 3.1b: Corrected volumetric estimates

	Specimen ID	CF	Hemisphere (cm^3)		Area 44(mm^3)		Area 45(mm^3)	
			L	R	L	R	L	R
Human	SN207/84	2.08	578.01	572.67	1751.57	973.53	2204.62	2659.58
	H9/88	1.88	666.66	681.85	1981.66	1809.12	1166.23	2146.41
	CS025	1.31	791.51	–	3254.98	–	2909.72	–
Bonobo	Zahlia	1.74	119.06	128.95	427.41	614.77	680.80	896.27
	YN86	2.74	152.72	150.93	321.18	194.27	380.27	415.59
Chimpanzee	Schimp	1.99[a]	156.06	159.55	318.38	259.28	389.78	386.82
	YN89	2.32	164.84	161.01	198.32	503.41	256.67	521.30
	Bathsheba	2.00	149.66	145.26	271.45	358.65	166.67	159.66
	1548	2.10[b]	–	–	157.01	104.93	245.72	156.50
Gorilla	YN82	2.17	149.26	150.81	169.97	228.09	390.66	487.32
	A375	1.87	–	–	168.00	223.11	210.44	276.44
Orangutan	YN85	2.31	150.34	158.44	393.53	298.81	636.60	331.86
	Harry	2.45	171.20	167.43	349.30	320.56	416.76	241.98
Gibbon	Disco	2.14	45.82	47.39	143.23	226.02	110.28	118.49

[a]Estimated from other specimens
[b]Provided by Carol MacLeod

Table 3.2: Neuronal number and density estimates

		Neuron Count Estimates (x10^6)				Uncorrected Neuronal Densities (1000s/mm^3)				Corrected Neuronal Densities (1000s/mm^3)			
	Specimen ID	Area 44		Area 45		Area 44		Area 45		Area 44		Area 45	
		L	R	L	R	L	R	L	R	L	R	L	R
Human	SN207/84	24.3	13.9	30.4	31.4	28.847	29.676	28.694	24.530	13.876	14.275	13.803	11.800
	H9/88	25.6	19.6	12.9	28.1	26.813	22.500	23.054	27.209	14.252	11.959	12.253	14.462
	CS025	–	–	–	–	–	–	–	–	–	–	–	–
Bonobo	Zahlia	7.9	17.0	11.1	18.3	38.300	57.558	33.904	42.404	21.964	33.008	19.443	24.317
	YN86	11.3	6.7	12.7	14.0	72.895	72.090	69.595	70.165	26.626	26.331	25.420	25.628
Chimpanzee	Schimp	6.4	4.5	7.9	7.1	41.654	36.088	41.933	38.281	20.972	18.169	21.112	19.274
	YN89	7.6	16.4	11.0	24.3	79.180	67.897	89.303	96.906	34.093	29.235	38.452	41.725
	Bathsheba	8.0	9.0	6.8	5.0	61.321	52.454	84.796	65.634	30.687	26.249	42.434	32.845
	1548	–	–	–	–	–	–	–	–	–	–	–	–
Gorilla	YN82	3.3	4.0	7.4	8.7	40.181	36.669	39.465	37.141	18.481	16.866	18.151	17.083
	A375	–	–	–	–	–	–	–	–	–	–	–	–
Orangutan	YN85	8.6	6.2	15.7	10.5	45.381	43.210	51.181	65.994	19.628	18.689	22.136	28.543
	Harry	7.5	7.4	10.4	5.5	44.776	48.215	51.770	47.602	18.305	19.711	21.164	19.461
Gibbon	Disco	3.4	5.4	2.8	2.7	49.676	49.691	53.130	47.929	23.225	23.232	24.840	22.408

Table 3.3: Neuronal density (1000s of neurons/mm^3) estimates from previous studies

Species	Area 9[1]	Area 46[1]	Area 13[2]	Area 10[3]	Area 44[4]		Area 45[4]		Other frontal[5]
					Left	*Right*	*Left*	*Right*	
Human	45.80	55.38	30.35	34.01	42.09	42.98	38.20	47.71	32.86-46.40
Chimpanzee			50.69	60.47					
Bonobo			44.11	55.69					
Gorilla			54.78	47.30					
Orangutan			42.40	78.18					
Gibbon			53.83	86.19					

[1]Rajkowska & Goldman-Rakic, 1995
[2]Semendeferi et al., 1998
[3]Semendeferi et al., 2001
[4]Uylings et al., 2006 (calculated from published volumes using a standard correction factor of 2.0 for shrinkage by NS)
[5]Anderson & Harvey, 1996; Pakkenberg, 1993; Selemon et al., 2003

Table 3.4: Neuronal densities in specimens examined in this study and previous Semendeferi and colleague studies

Individual	Area					
	Left 44	*Right 44*	*Left 45*	*Right 45*	*Area 13[1]*	*Area 10[2]*
SN207	28,847	29,676	28,694	24,530	30,351	34,014
YN86	72,895	72,090	69,595	70,165	44,111	55,690
YN82	40,181	36,669	39,465	37,141	54,783	47,300
YN85	45,381	43,210	51,181	65,994	42,400	78,182
YN81	49,676	49,691	53,130	47,929	53,830	86,190

[1]Semendeferi et al., 1998
[2]Semendeferi et al., 2001

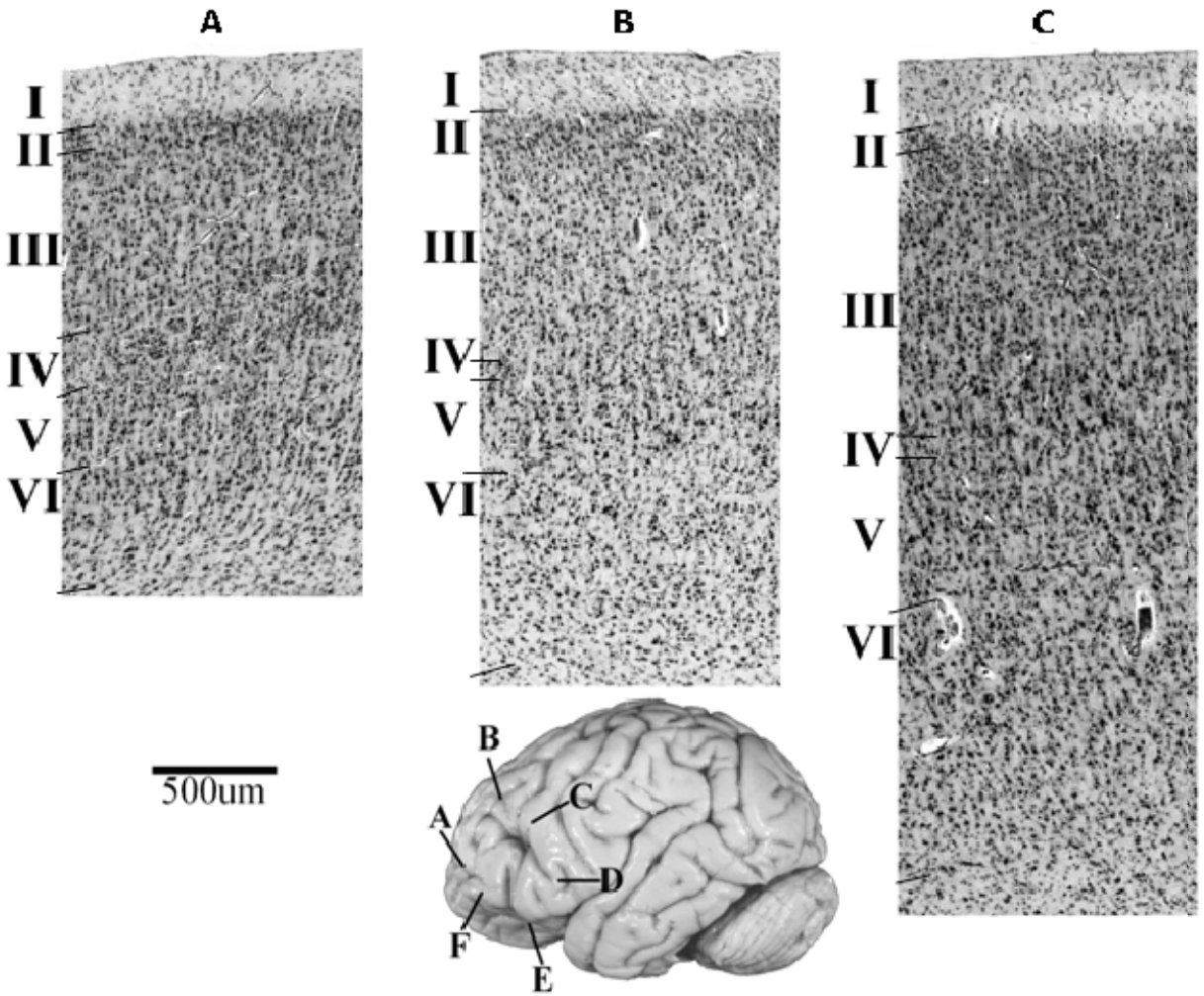

Figure 1: Images of the cytoarchitectonics of regions bordering Brodmann's areas 44 and 45. All images are taken from a bonobo and located where indicated on the inset photograph of the lateral side of the

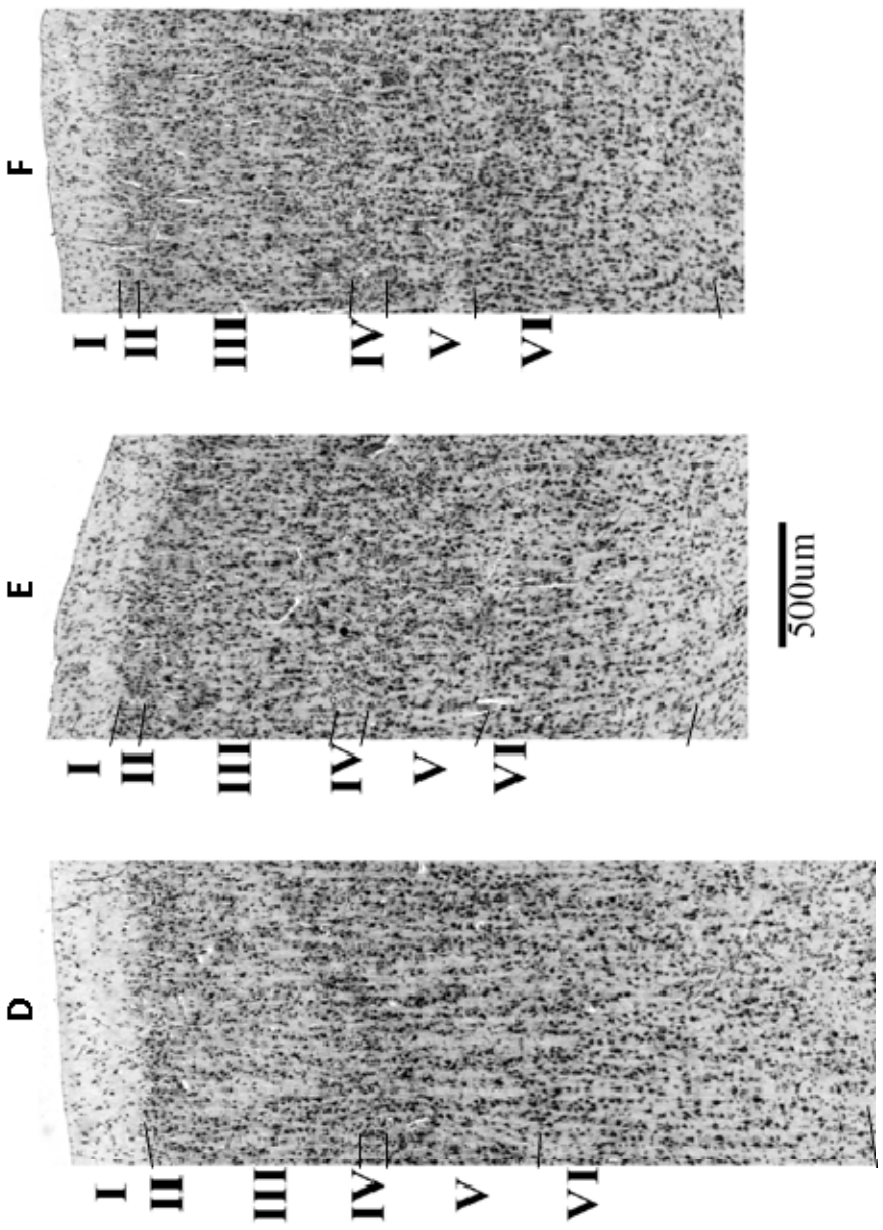

Figure 1 (cont.)

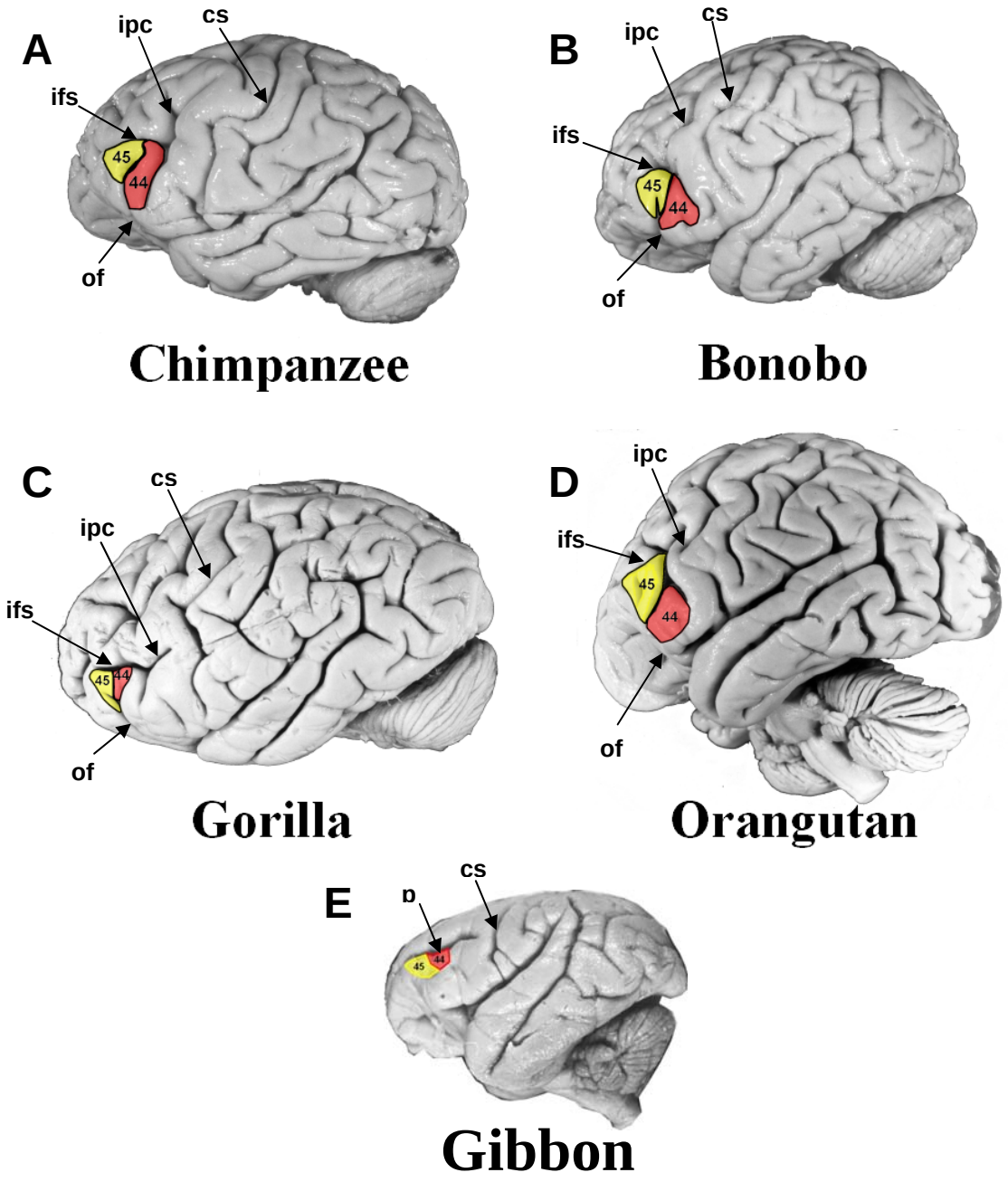

Figure 2: Photographs of the lateral side of a) chimpanzee, b) bonobo, c) gorilla, d) orangutan, and e) gibbon specimens with major sulci indicated. The extent of Brodmann's areas 44 and 45 are indicated in red and yellow respectively.

cs – central sulcus
ifs – inferior frontal sulcus
ipc – inferior precentral sulcus
ofs – orbitofrontal sulcus
ps – principal sulcus

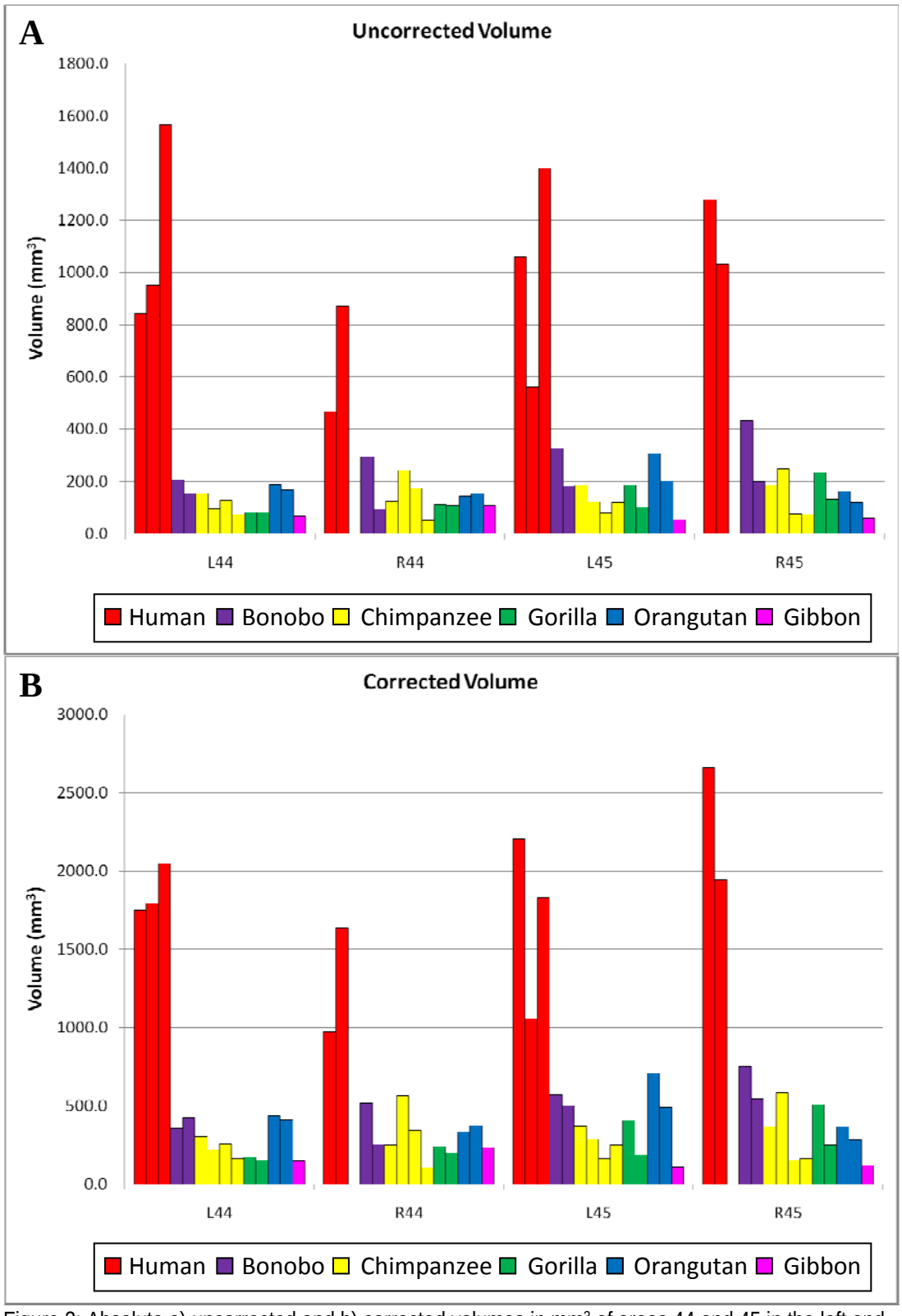

Figure 3: Absolute a) uncorrected and b) corrected volumes in mm^3 of areas 44 and 45 in the left and right hemispheres in all specimens.

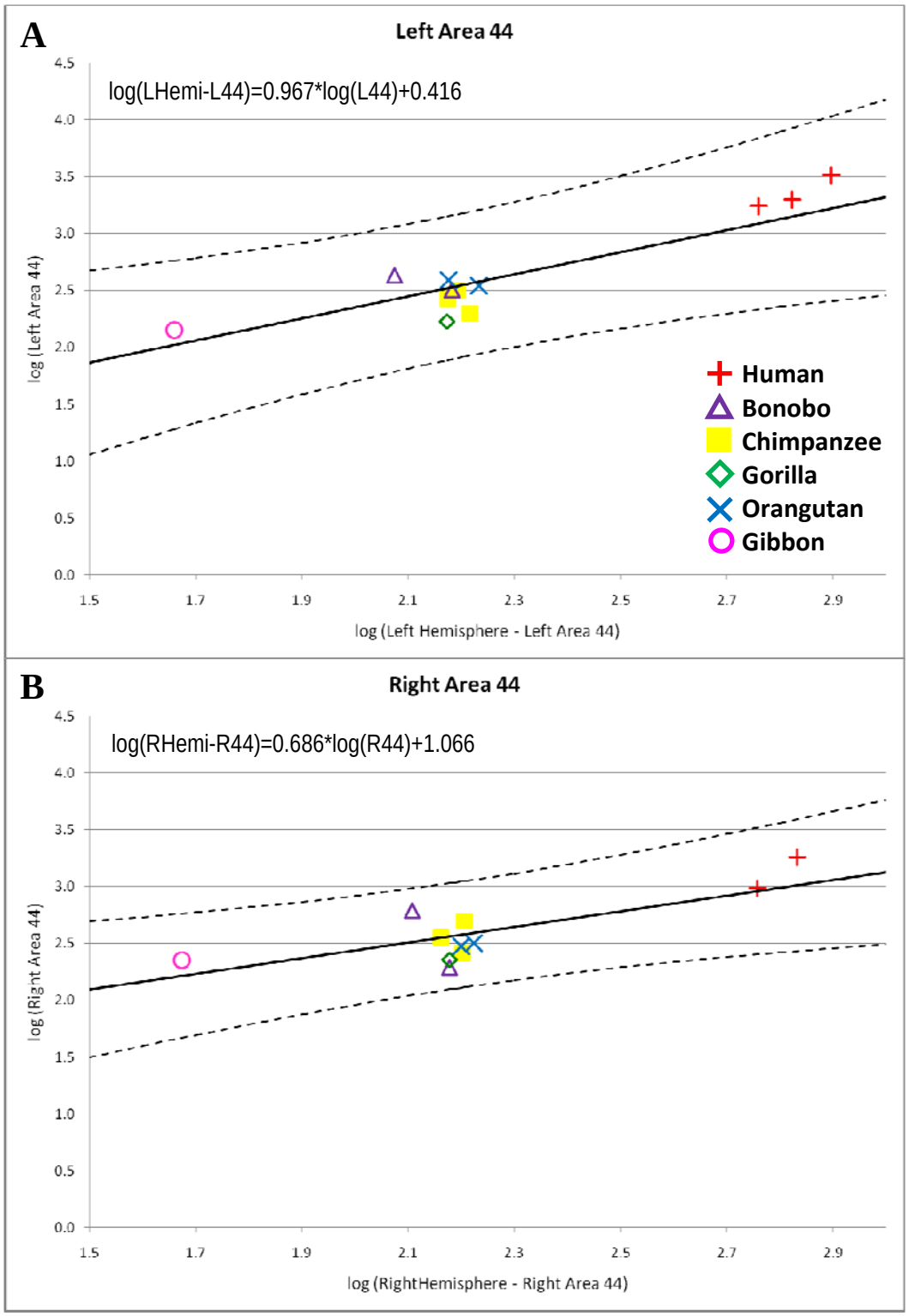

Figure 4: Log-log regressions drawn using standard least squares through species means of all hominoids. Plots include points for all individuals and individual confidence intervals for each regression line. a) Left 44 vs. (Left hemi-Left 44); b) Right 44 vs. (Right hemi-Right 44); c) Left 45 vs. (Left hemi-Left 45); d) Right 45 vs. (Right hemi-Right 45).

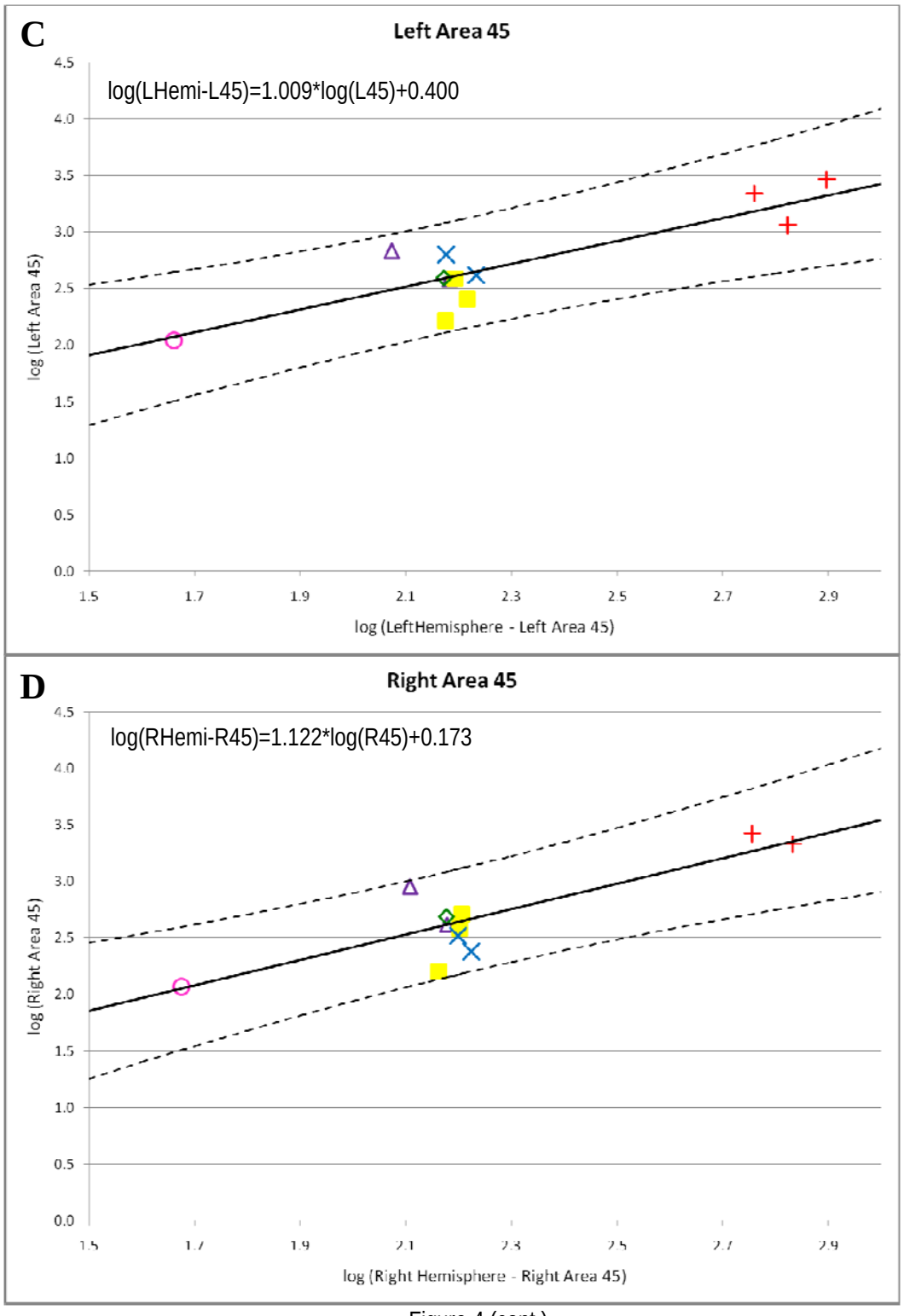

Figure 4 (cont.)

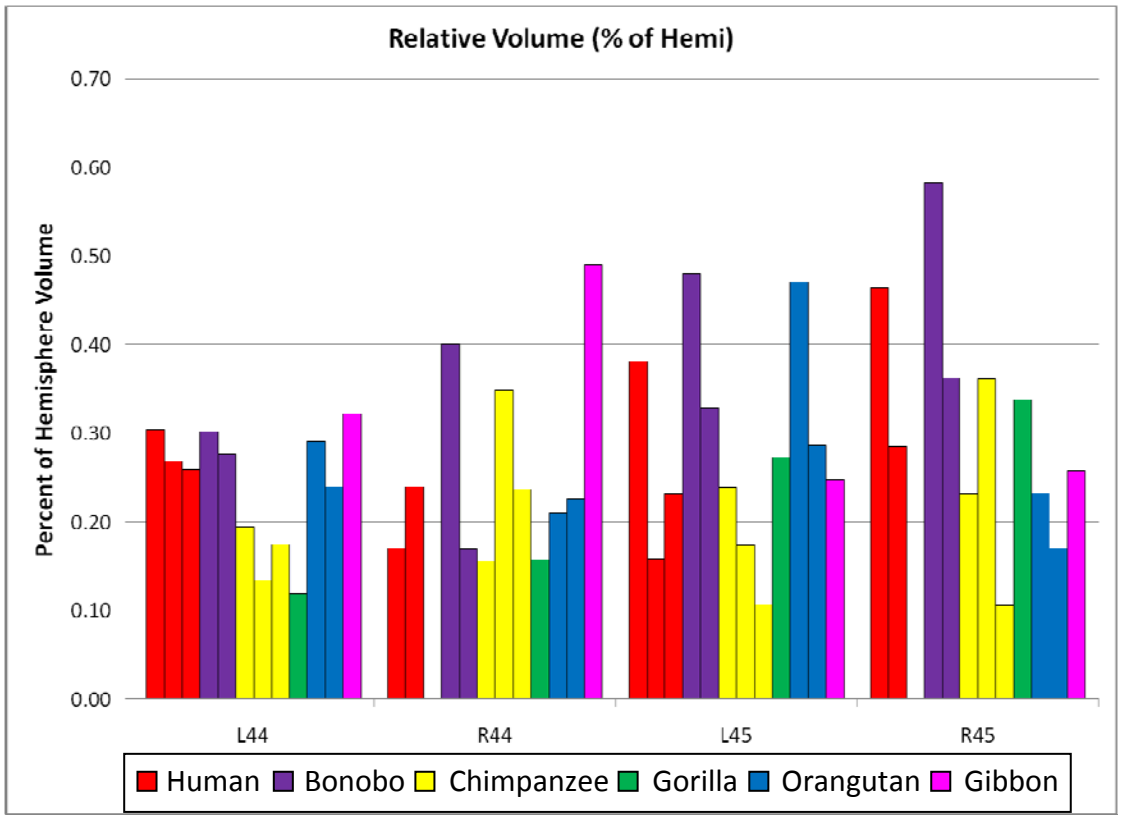

Figure 5: Relative volumes as a percentage of the hemisphere of areas 44 and45 in left and right hemispheres in all specimens.

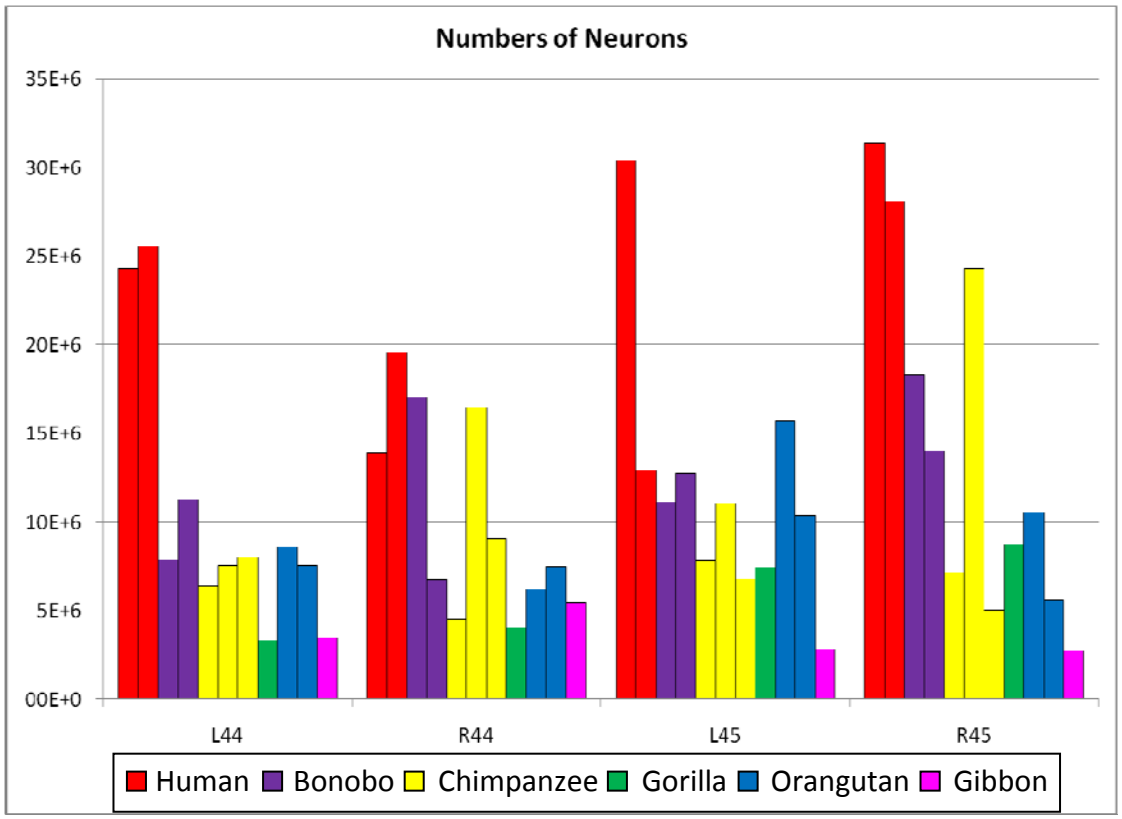

Figure 6: Numbers of neurons in areas 44 and 45 in left and right hemispheres in all specimens.

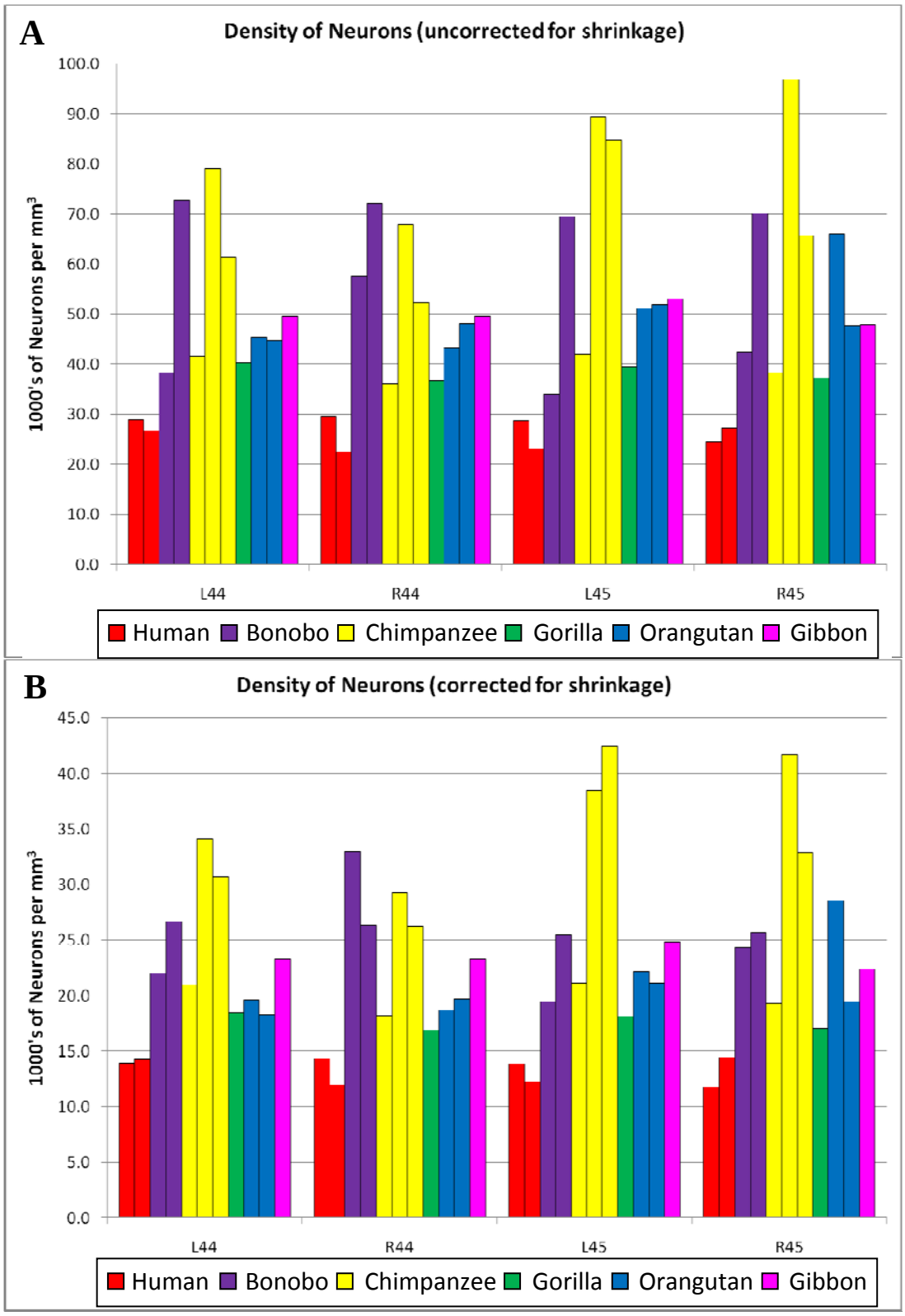

Figure 7: Density of neurons a) uncorrected and b) corrected for shrinkage in areas 44 and 45 in left and right hemispheres in all specimens.

REFERENCES

Amunts K, Schleicher A, Bürgel U, Mohlberg H, Uylings HBM, Zilles K. 1999. Broca's Region Revisited: Cytoarchitecture and Intersubject Variability. Journal of Comparative Neurology 412:319-341.

Armstrong DF, Stokoe WC, Wilcox SE. 1995. Gesture and the Nature of Language. Cambridge: Cambridge University Press.

Bailey P, von Bonin G, McCulloch WS. 1950. The Isocortex of the Chimpanzee. Urbana: University of Illinois Press.

Barbas H, Pandya DN. 1987. Architecture and Frontal Cortical Connections of the Premotor Cortex (area 6) in the Rhesus Monkey. Journal of Comparative Neurology 256:211-228.

Barbas H, Pandya DN. 1989. Architecture and Intrinsic Connections of the Prefrontal Cortex in the Rhesus Monkey. Journal of Comparative Neurology 286:353-375.

Barger N, Stefanacci L, Semendeferi K. 2007. A Comparative Volumetric Analysis of the Amygdaloid Complex and Basolateral Division in the Human and Ape Brain. American Journal of Physical Anthropology DOI 10.1002/ajpa.20684.

Brodmann K. 1909. Vergleichende Lokalisationslehre der Grobhirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig: Johann Ambrosius Barth.

Byrne RW, Corp N, Byrne JME. 2001. Estimating the complexity of animal behaviour: How mountain gorillas eat thistles. Behaviour 138:525-557.

Calvin WH, Bickerton D. 2000. Lingua ex machina : reconciling Darwin and Chomsky with the human brain. Cambridge, Mass.: MIT Press.

Cantalupo C, Hopkins WD. 2001. Asymmetric Broca's area in great apes. Nature 414:505.

Connolly CJ. 1950. External morphology of the primate brain. Springfield, Ill.: C. C. Thomas.

Corp N, Byrne RW. 2002. Leaf processing by wild chimpanzees: Physically defended leaves reveal complex manual skills. Ethology 108:673-696.

Deacon TW. 1990. Problems of Ontogeny and Phylogeny in Brain-Size Evolution. International Journal of Primatology 11:237-282.

Dunbar R. 1996. Grooming, Gossip and the Evolution of Language. Cambridge, MA: Harvard University Press.

Duvernoy J. 1991. The Human Brain: Surface, Three-Dimensional Sectional Anatomy and MRI. Vienna: Springer-Verlag.

Fiebach CJ, Schubotz RI. 2006. Dynamic anticipatory processing of hierarchical sequential events: A common role for Broca's area and ventral premotor cortex across domains? Cortex 42:499-502.

Gallyas F. 1971. A principle for silver staining of tissue elements by physical development. Acta Morphologica Academiae Scientiarum Hungaricae 19:57-51.

Garland T, Ives AR. 2000. Using the past to predict the present: Confidence intervals for regression equations in phylogenetic comparative methods. American Naturalist 155:346-364.

Garland T, Midford PE, Ives AR. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. American Zoologist 39:374-388.

Gelfand JR, Bookheimer SY. 2003. Dissociating neural mechanisms of temporal sequencing and processing phonemes. Neuron 38:831-842.

Grezes J, Armony JL, Rowe J, Passingham RE. 2003. Activations related to "mirror" and "canonical" neurones in the human brain: an fMRI study. Neuroimage 18:928-937.

Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, et al. 1988. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. Apmis 96:379-394.

Gundersen HJG, Jensen EBV, Kieu K, Nielsen J. 1999. The efficiency of systematic sampling in stereology-reconsidered. Journal of Microscopy-Oxford 193:199-211.

Hewes GW. 1973. Primate Communication and the Gestural Origin of Language. Current Anthropology 14:5-24.

Iacoboni M, Woods RP, Brass M, Bekkering H, Mazziotta JC, Rizzolatti G. 1999. Cortical mechanisms of human imitation. Science 286:2526-2528.

King BJ. 2004. The dynamic dance : nonvocal communication in African great apes. Cambridge, Mass.: Harvard University Press.

Koechlin E, Jubault T. 2006. Broca's area and the hierarchical organization of human behavior. Neuron 50:963-974.

Kretschmann HJ, Tafesse U, Herrmann A. 1982. Different Volume Changes of Cerebral-Cortex and White Matter During Histological Preparation. Microscopica Acta 86:13-24.

Merker B. 1983. Silver staining of cell bodies by means of physical development. Journal of Neuroscience Methods 9:235-241.

Nakamichi M. 2004. Tool-use and tool-making by captive, group-living orangutans (Pongo pygmaeus abelii) at an artificial termite mound. Behavioural Processes 65:87-93.

Nishitani N, Schurmann M, Amunts K, Hari R. 2005. Broca's region: From action to language. Physiology 20:60-69.

Ono M, Kubik S, Abernathey CD. 1990. Atlas of the Cerebral Sulci. Stuttgart/New York: Thieme Verlag.

Pakkenberg B. 1993. Total Nerve Cell Number in Neocortex in Chronic Schizophrenics and Controls Estimated Using Optical Disectors. Biological Psychiatry 34:768-772.

Passingham RE. 1981. Brocas Area and the Origins of Human Vocal Skill. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 292:167-175.

Petrides M. 2006. Broca's Area in the Human and the Nonhuman Primate Brain. In: Amunts K, Editor. Broca's Region. Oxford: Oxford University Press. p 31-46.

Petrides M, Pandya DN. 1994. Comparative architectonic analysis of the human and the macaque frontal cortex. In: Grafman J, Editor. Handbook of Neuropsychology. Amsterdam: Elsevier Science Ltd. p 17-58.

Preuss TM, Goldman-Rakic PS. 1991. Myelo- and Cytoarchitecture of the Granular Frontal Cortex and Surrounding Regions in the Strepsirhine Primate *Galago* and the Anthropoid Primate *Macaca*. Journal of Comparative Neurology 310:429-474.

Purvis A. 1995. A Composite Estimate of Primate Phylogeny. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 348:405-421.

Rajkowska G, Goldman-Rakic PS. 1995. Cytoarchitectonic Definition of Prefrontal Areas in the Normal Human Cortex: I. Remapping of Areas 9 and 46 using Quantitative Criteria. Cerbral Cortex 5:307-322.

Rizzolatti G, Fogassi L, Gallesse V. 2001. Neurophysiological mechanisms underlying the understanding and imitation of action. Nature Reviews Neuroscience 2:661-670.

Sarkissov SA, Filimonoff IN, Kononowa EP, Preobraschenskja IS, Kukuew LA. 1955. Atlas of the Cytoarchitectonics of the Human Cerebral Cortex. Moscow: Medgiz.

Schenker NM, Buxhoeveden DP, Amunts K, Zilles K, Semendeferi K. submitted. A comparative quantitative analysis of cytoarchitectonics and minicolumnar organization in Broca's area in humans and great apes. Journal of Comparative Neurology.

Schubotz RI, von Cramon DY. 2001. Functional organization of the lateral premotor cortex: fMRI reveals different regions activated by anticipation of object properties, location and speed. Cognitive Brain Research 11:97-112.

Selemon LD, Mrzljak J, Kleinman JE, Herman MM, Goldman-Rakic PS. 2003. Regional specificity in the neuropathologic substrates of schizophrenia - A morphometric analysis of Broca's area 44 and area 9. Archives of General Psychiatry 60:69-77.

Semendeferi K, Armstrong E, Schleicher A, Zilles K, Van Hoesen GW. 1998. Limbic frontal cortex in hominoids: a comparative study of area 13. Am J Phys Anthropol 106:129-155.

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.

Sherwood CC, Broadfield DC, Holloway RL, Gannon PJ, Hof PR. 2003. Variability of Broca's Area Homologue in African Great Apes: Implications for Language Evolution. Anatomical Record Part A 271A:276-285.

Uylings HBM, Jacobsen AM, Zilles K, Amunts K. 2006. Left-right asymmetry in volume and number of neurons in adult Broca's area. Cortex 42:652-658.

von Economo C. 1929. The Cytoarchitectonics of the Human Cerebral Cortex. London: Oxford University Press.

West MJ, Slomianka L, Gundersen HJG. 1991. Unbiased Stereological Estimation of the Total Number of Neurons in the Subdivisions of the Rat Hippocampus Using the Optical Fractionator. Anatomical Record 231:482-497.

Zar JH. 1996. Biostatistical analysis. Upper Saddle River, NJ: Simon and Schuster.

CONCLUSIONS

SUMMARY

The current research confirmed the presence of Brodmann's areas 44 and 45, as defined via cytoarchitectural characteristics, in all great ape species and one species of gibbon. Quantitative measurements of minicolumnar organization, volume, neuron number, and neuron density revealed limited differences between humans and apes. While the absolute size of Brodmann's areas 44 and 45 was substantially larger in humans, the areas were not larger than expected based on regressions, and the regressions did not deviate significantly from isometry. Furthermore, the relative size of the areas was not larger in humans than in apes and did not differentiate among ape species. Neuron number was also larger in humans, likely due to the increased absolute size of the areas, although neuron density was decreased in humans relative to apes. Matching the decreased neuron density was the finding of greater mean spacing distance in humans, accompanied by lower grey level index. The only parameter that exhibited any noticeable asymmetry in the current sample was grey level index, and then only in human females.

CONCLUSIONS

While asymmetries are present in many regions of human cortex, Broca's area is one region exhibiting some of the most conspicuous functional asymmetry. This functional asymmetry may be accompanied by a degree of structural asymmetry in various parameters, such as volume, dendritic branch lengths, and percentage of the area occupied by neuropil space (Scheibel et al., 1985; Hayes and Lewis, 1996; Amunts et al., 1999; Uylings et al., 2006). However, the absence of any population-level asymmetry in the current human sample seems to contradict the notion that functional asymmetry in language dominance corresponds to

profound structural asymmetry. Nevertheless, the human sample for volumes and neuron counts was substantially smaller than that for minicolumn analysis, and it is conceivable that a larger sample would reveal greater evidence for structural asymmetry in the region.

The variation among species in Brodmann's areas 44 and 45 suggests that this region behaves much as any other cortical region in response to changes in brain size. The documented evidence of decreased neuron density in larger brains is supported by the current data and the size of the areas relative to the cortical hemisphere increases isometrically with brain size. Furthermore, the increased size of minicolumns in humans accompanied by a decreased neuronal density is likely related to the presence of greater numbers of connections. Since no study to date has failed to find this difference in minicolumn spacing between humans and great apes in any area, the size difference is likely to be the result of a greater number of processing units (neurons) requiring connections, a factor that affects the entire human brain, and not just Broca's area. To test for differential increase in connections, a more comprehensive study of minicolumn spacing in multiple cortical areas across species would be necessary.

Volumetric data indicate that the relative size of Broca's area exhibits substantial interindividual variability, but no consistent differences between species. These data, taken with the data on density and minicolumn spacing, provide no evidence that there are different mechanisms for information processing occurring in this region despite Broca's area's involvement in language related behavior in humans that is absent in other species, and no pattern emerges that requires explanations other than those related to increases in brain size.

Therefore, these findings provide support for the idea that there is strong continuity in the evolution of the anatomy of Broca's area, and that the region is involved in non-linguistic activities, such as hierarchical processing, that are just as crucial to apes as they are to humans. The result is that the fundamental cytoarchitecture does not differ substantially between species. However, one must maintain an open mind regarding the possibility of differences among species that were not distinguishable on the parameters examined here. Future research will focus not only on the continuing need to expand sample size to encompass individual variation within species, but will also focus on additional parameters, such as subpopulations of neurons that express specific proteins, in order to gain a more detailed view of the anatomical structure of Broca's area within and among species. Additional comparisons will be made within specimens among areas involved in "higher" cognition (such as Broca's area) and those that are more "primary" (such as motor cortex). Investigation of all parameters in multiple cortical areas will further illuminate the position of Broca's area in relation to the evolution of the brain as a whole.

REFERENCES

Amunts K, Schleicher A, Burgel U, Mohlberg H, Uylings HBM, Zilles K. 1999. Broca's region revisited: Cytoarchitecture and intersubject variability. Journal of Comparative Neurology 412:319-341.

Hayes TL, Lewis DA. 1996. Magnopyramidal neurons in the anterior motor speech region: Dendritic features and interhemispheric comparisons. Archives of Neurology 53:1277-1283.

Scheibel AB, Paul LA, Fried I, Forsythe AB, Tomiyasu U, Wechsler A, Kao A, Slotnick J. 1985. Dendritic Organization of the Anterior Speech Area. Experimental Neurology 87:109-117.

Uylings HBM, Jacobsen AM, Zilles K, Amunts K. 2006. Left-right asymmetry in volume and number of neurons in adult Broca's area. Cortex 42:652-658.