

# Variability of Broca's Area Homologue in African Great Apes: Implications for Language Evolution

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## ABSTRACT

The cortical circuits subserving neural processing of human language are localized to the inferior frontal operculum and the posterior perisylvian region. Functional language dominance has been related to anatomical asymmetry of Broca's area and the planum temporale. The evolutionary history of these asymmetric patterns, however, remains obscure. Although testing of hypotheses about the evolution of language areas requires comparison to homologous regions in the brains of our closest living relatives, the great apes, to date little is known about normal interindividual variation of these regions in this group. Here we focus on Brodmann's area 44 in African great apes (*Pan troglodytes* and *Gorilla gorilla*). This area corresponds to the pars opercularis of the inferior frontal gyrus (IFG), and has been shown to exhibit both gross and cytoarchitectural asymmetries in humans. We calculated frequencies of sulcal variations and mapped the distribution of cytoarchitectural area 44 to determine whether its boundaries occurred at consistent macrostructural landmarks. A considerable amount of variation was found in the distribution of the inferior frontal sulci among great ape brains. The inferior precentral sulcus in particular was often bifurcated, which made it impossible to determine the posterior boundary of the pars opercularis. In addition, the distribution of Brodmann's area 44 showed very little correspondence to surface anatomy. We conclude that gross morphologic patterns do not offer substantive landmarks for the measurement of Brodmann's area 44 in great apes. Whether or not Broca's area homologue of great apes exhibits humanlike asymmetry can only be resolved through further analyses of microstructural components. *Anat Rec Part A 271A:276–285, 2003.* © 2003 Wiley-Liss, Inc.

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Broca's area, located in the inferior frontal gyrus (IFG) of humans, is a key component of the cortical circuitry that subserves language production. In approximately 95% of humans the left hemisphere is dominant for language (Branche et al., 1964), as demonstrated by functional imaging (Petersen et al., 1988) and cortical stimulation studies (Rasmussen and Milner, 1975; Ojemann, 1991). Whereas numerous studies of gross and microscopic structure have revealed anatomic asymmetries that may underlie this functional dominance in humans, an important unresolved question is whether this asymmetric pattern is evolutionarily novel to humans (autapomorphic) or is shared with our closest living relatives, the great apes (synapomorphic).

At the microstructural level, Broca's area is comprised of Brodmann's areas 44 and 45 (Aboitiz and Garcia, 1997). Studies of microstructural features in Broca's area of hu-

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man brains have revealed several significant asymmetries. Volumetric cytoarchitecture-based studies have shown that area 44, but not area 45, is leftward dominant (Galaburda, 1980; Amunts et al., 1999). Using Golgi impregnation of the posterior IFG, Scheibel and colleagues (Scheibel, 1984; Scheibel et al., 1985) found a greater number of higher-order branches on the basal dendrites of pyramidal neurons in the left hemisphere compared to the right, suggesting more integrative function in the dominant hemisphere. In another study, layer III magnopyramidal neurons of area 45 were found to be significantly larger, and to express nonphosphorylated neurofilament protein at higher frequencies in the left hemisphere compared to the right. This indicates a possible anatomical specialization of area 45 for language in the dominant hemisphere (Hayes and Lewis, 1995).

In humans, cytoarchitectural subdivisions of Broca's area generally fall within distinct morphological boundaries of the IFG. The ascending (vertical) ramus of the Sylvian fissure separates the pars opercularis (area 44) from the pars triangularis (area 45), and the anterior ramus divides the pars triangularis from the pars orbitalis (area 47). Numerous studies have investigated macrostructural asymmetry in Broca's area using these sulcal landmarks to subdivide the region. Results from these macrostructural studies, however, have differed markedly depending upon methodology and anatomical definitions. Although cortical surface area measures of the frontal operculum do not reveal significant population-level leftward dominance (Wada et al., 1975; Witelson and Kigar, 1992), asymmetries are significant when the intrasulcal portion of this cortex is included (Falzi et al., 1982; Tomaiuolo et al., 1999). Some volumetric MRI-based studies have concluded that both the pars triangularis (Foundas et al., 1998, 2001) and the pars opercularis (Foundas et al., 1998) are leftward dominant; however, others have not found volumetric asymmetry in the pars opercularis (Tomaiuolo et al., 1999). In sum, a consensus does not yet exist regarding macrostructural asymmetries of the human IFG.

In great apes, the fronto-orbital sulcus (not the ascending ramus) forms the anterior border of the pars opercularis (Connolly, 1950; Shantha and Manocha, 1969), and the pars triangularis is not consistently present (Connolly, 1950). Nevertheless, cytoarchitectural studies of the chimpanzee and orang-utan frontal cortex describe a dysgranular region lying just anterior to the inferior precentral sulcus designated Brodmann's area 44 (von Bonin, 1949), FCBm (Bailey et al., 1950), or areas 56 and 57 (Kreht, 1936). In chimpanzees, this region has been shown to receive projections from the dorsomedial nucleus of the thalamus (Walker, 1938). Additionally, physiological investigations of the chimpanzee brain describe evoked movements of the larynx and tongue when this region is stimulated (Bailey et al., 1950). Maps of the cortical surface of great apes show that the pars opercularis corresponds, at least in part, to this cytoarchitectural area (von Bonin, 1949; Bailey et al., 1950; Connolly, 1950). Thus, there is suggestive evidence that an anatomical and functional homologue of Brodmann's area 44, which is localized to the pars opercularis of the IFG, exists in great ape brains.

A recent structural magnetic resonance imaging (MRI) study by Cantalupo and Hopkins (2001) claimed that area 44 (defined by the sulcal boundaries of the pars opercu-

laris: the fronto-orbital sulcus and the inferior precentral sulcus) is asymmetric in great ape brains. Based on surface area measurements, the authors concluded that the majority of African great ape brains exhibit significant left hemisphere dominance in this region. Certainly, if this region of great ape brains is asymmetric, the evolution of human brain language areas will require reevaluation. Several problems, however, potentially mitigate against using MRI data to accurately measure asymmetries of pars opercularis or area 44 in these species. It is possible that, similar to humans, interindividual variability in the expression of sulcal landmarks (Tomaiuolo et al., 1999) and cytoarchitectural boundaries (Amunts et al., 1999) of the IFG may confound a direct relationship between external features and cytoarchitecture in this region. Although the sulci of the chimpanzee and gorilla IFG have been the subject of several studies (Cunningham, 1892; Le Gros Clark, 1927; Mingazzini, 1928; Tilney and Riley, 1928; Papez, 1929; Walker and Fulton, 1936; von Bonin, 1949; Bailey et al., 1950) there is little information available regarding variability in the frequencies of morphological patterns, and microstructural variability is not documented whatsoever. The purpose of the present study was to investigate the distribution of sulcal variation in the IFG of African great apes, and to map interindividual variation in the correspondence between cytoarchitecture and external morphology. The results of this study have implications for the use of surface morphology to assess asymmetries of anterior language areas of living great apes and fossil early hominins.

## MATERIALS AND METHODS

### Analysis of External Morphology

Postmortem cerebral hemispheres from a total of 90 African great apes, including 77 common chimpanzees (*Pan troglodytes*) and 13 Western lowland gorillas (*Gorilla gorilla gorilla*), were available for examination of surface morphology. Only adult brain specimens that were undamaged in the region of the IFG were analyzed. Thirty-seven hemispheres were obtained from the Smithsonian Institution's collection of wild-caught animal brains (from 29 chimpanzees and eight gorillas). Fifty-three brain hemispheres were obtained from animals (48 chimpanzees and five gorillas) that lived in research or zoological facilities.

Variability of IFG sulci was analyzed from photographic images. Each hemisphere was photographed with a digital camera in standard lateral anatomical orientation. Images were viewed on a computer monitor and IFG sulci were scored for morphological variations. We analyzed sulci that form the putative boundaries of the pars opercularis using descriptions and illustrations from the literature to identify relevant landmarks (Kreht, 1936; von Bonin, 1949; Bailey et al., 1950; Connolly, 1950). von Bonin's (1949) and Bailey et al.'s (1950) cortical surface maps of the chimpanzee precentral motor cortex illustrate area 44 (or FCBm) located on the free surface of the pars opercularis bounded anteriorly by the fronto-orbital sulcus, posteriorly by the inferior precentral sulcus (or the subcentral anterior sulcus), extending inferiorly onto the ventral surface, and terminating superiorly at the level of the uppermost tip of the fronto-orbital sulcus (Fig. 1). However, von Bonin's text does not clearly indicate how many individuals were studied, and interindividual differences in cytoarchitectural boundaries were not mapped.

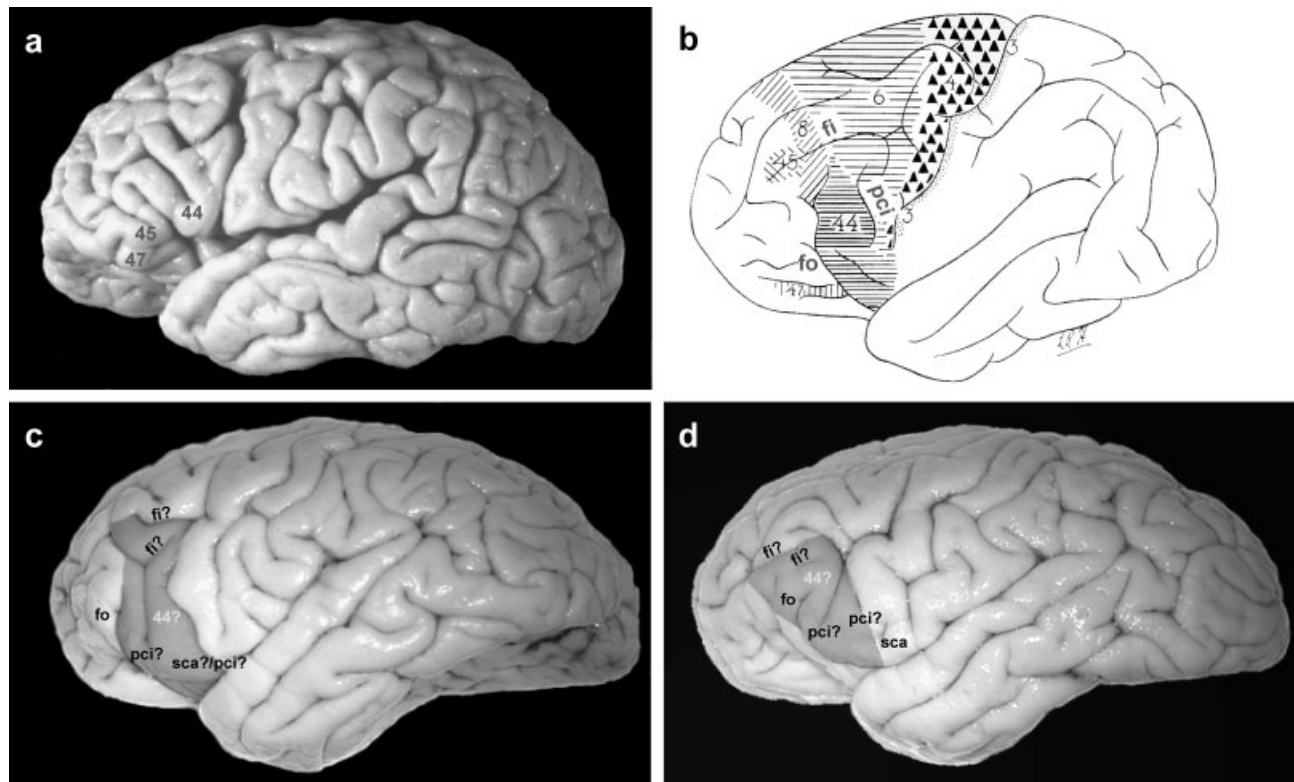


Fig. 1. The IFG of humans and sulcal variations in chimpanzees. **a:** Left lateral view of a human brain identifying the approximate location of Brodmann's areas 44, 45, and 47. **b:** Cytoarchitectural map for the chimpanzee identifying area 44 with the sulcal boundaries (after von Bonin, 1949). **c** and **d:** Left lateral views of chimpanzee brains identifying

the IFG and the possible boundaries of the pars opercularis (shaded). Abbreviations: fo, fronto-orbital sulcus; fi, inferior frontal sulcus; pci, inferior precentral sulcus; sca, subcentral anterior sulcus. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

Therefore, we also included the inferior frontal sulcus as a possible superior border for area 44 in our analysis because cytoarchitectural mapping of this area in humans, based on a large sample ( $n = 10$ ), shows that this sulcus forms the boundary between area 44 and areas 6 and 8 (Amunts et al., 1999). In the present study, the fronto-orbital, inferior frontal, and inferior precentral sulci were each scored as 1) complete, 2) interrupted/broken, or 3) bifurcated into separate terminal limbs. The intersection of the inferior frontal and inferior precentral sulci was scored as either 1) continuous or 2) discontinuous. Finally, the diagonal sulcus (or dimple) was scored as either 1) present or 2) absent.

### Microstructural Analysis

Five adult chimpanzee specimens (one male and four females) were processed for histological analysis. The brains were removed within 12 hr postmortem and immersed in 10% neutral buffered formalin. Only the left hemisphere was used from each brain. In some cases the hemisphere had already been blocked in the coronal plane. In these instances only blocks that contained the entire pars opercularis and adjacent sulci were used. Prior to sectioning, the brain surface was photographed with a digital camera, which made it possible to correlate histologically-defined area boundaries with external morphological landmarks. A 5–7-mm-thick block was dissected

from the IFG in either the horizontal or parasagittal plane. Each block traversed the precentral gyrus, inferior precentral sulcus, pars opercularis, and fronto-orbital sulcus (as well as the diagonal sulcus and the subcentral anterior sulcus when present). Blocks were cryoprotected by infiltration with graded sucrose solutions (up to 30%), and 40- $\mu$ m-thick sections were cut on a freezing sliding microtome. Horizontal sections were obtained from four brains, and a parasagittal series was obtained from one brain. All sections were immediately collected and stored in strict serial order. From each block, every 10th section was stained for Nissl substance with thionin, and every 20th section was stained for myelin with Black-Gold (Schmued and Slikker, 1999).

Immunohistochemistry was performed on every 50th section. Free-floating sections were stained for nonphosphorylated neurofilament protein with the monoclonal antibody SMI-32 (1:4,000 dilution; Sternberger Monoclonals, Baltimore, MD), which recognizes a nonphosphorylated epitope on the medium- (168 kDa) and heavy- (200 kDa) molecular-weight subunits of the neurofilament triplet protein. Briefly, prior to immunostaining, sections were pretreated for antigen retrieval by incubation in citrate buffer (pH 8.5) at 95°C in a water bath. Then endogenous peroxidase activity was quenched by incubation in a solution of absolute methanol and 3% hydrogen peroxide (75/25 vol/vol). Sections were incubated in the primary

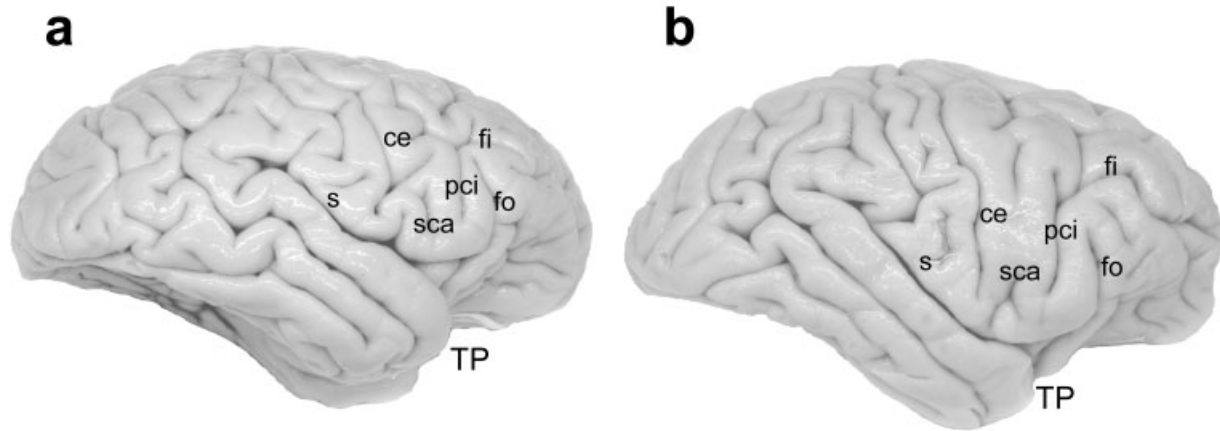


Fig. 2. Common sulcal patterns of the IFG in chimpanzees and gorillas. **a:** Right lateral view of a chimpanzee brain. **b:** Right lateral view of a gorilla brain. Abbreviations: s, Sylvian fissure; ce, central sulcus; fo, fronto-orbital sulcus; fi, inferior frontal sulcus; pci, inferior precentral sulcus; sca, subcentral anterior sulcus; TP, temporal pole.

antibody in phosphate-buffered saline (PBS) containing 2% normal horse serum and 0.3% Triton X-100 for 48 hr at 4°C. After several rinses in PBS, sections were incubated in the secondary antibody (biotinylated anti-mouse IgG, diluted 1:200; Vector Laboratories, Burlingame, CA) and processed with the avidin-biotin method using a Vectastain ABC kit (Vector Laboratories). Immunoreactivity was visualized using 3,3'-diaminobenzidine as a chromogen. The specificity of the immunoreaction was confirmed by processing control sections as described above, excluding the primary antibody. Control sections were completely absent of immunostaining. Used in conjunction with Nissl and myelin preparations, immunohistochemistry allows for greater confidence in cortical areal parcellation based on the highly specific laminar pattern of labeled neurons and neuropil.

Cytoarchitectural boundaries were identified according to previous studies of the chimpanzee and human IFG (von Economo, 1929; Kreht, 1936; von Bonin, 1949; Bailey et al., 1950; Bailey and von Bonin, 1951; Braak, 1980; Galaburda, 1980; Amunts et al., 1999). The section outline of one representative section from each block was drawn under a 2.5x Plan-Neofluar (0.075 NA) objective using NeuroLucida morphometry software (MicroBrightfield, Williston, VT). Cytoarchitectural boundaries were then mapped onto the section outline by reference to the pattern of Nissl, myelin, and neurofilament protein staining in sections through the entire block. Two observers (C.C.S. and P.R.H.) independently mapped the location of cytoarchitectural transitions, and the resulting consensus maps were transferred to Adobe Photoshop version 6.0 for color coding of cytoarchitectural boundaries and labeling of sulci. Because cytoarchitectural area transitions extended over several millimeters, these regions were depicted as color gradations.

## RESULTS

### Sulcal Patterns

Variability was prominent in the sulcal patterns of the IFG in common chimpanzee and gorilla brain hemispheres (Fig. 1). Nevertheless, the IFG of these species

presented a basic sulcal template (Fig. 2), based upon which there were several variations. The fronto-orbital sulcus frequently had a short anterior limb at its terminus. A posterior limb encroaching on the pars opercularis was found in only 10% of the hemispheres (Table 1). When the subcentral anterior sulcus was present, it appeared either as a short dimple or as a longer sulcus that was sometimes confluent ventrally with the Sylvian fissure. Although the subcentral anterior sulcus was most often observed between the central sulcus and the inferior precentral sulcus, in one chimpanzee hemisphere it appeared anterior to the inferior precentral sulcus within the pars opercularis (specimen PTT 21 in Fig. 6). In chimpanzees, the fronto-orbital and inferior frontal sulci showed moderate frequencies of discontinuity or bifurcation (Table 1). Bifurcation of the inferior precentral sulcus was especially frequent in chimpanzees (43% on the left; 29% on the right), which made it impossible to determine the posterior boundary of the pars opercularis (Fig. 3). In these cases, the anterior bifurcating ramus was usually oriented more vertically than the posterior ramus, and the posterior ramus was frequently longer and inclined more posteriorly. However, several other configurations were also observed. In some of these instances it is likely that the posterior bifurcating limb of the inferior precentral sulcus was, in fact, the subcentral anterior sulcus extending ventrally and becoming confluent with the inferior precentral sulcus. However, in many cases both a bifurcated inferior precentral sulcus and a subcentral anterior sulcus were observed in the same hemisphere. Variability was less pronounced in the gorilla sample. In particular, the inferior precentral sulcus most often ran vertically in parallel to the central sulcus and did not exhibit a high frequency of bifurcation.

The diagonal sulcus is a short sulcus or dimple that is frequently present in the pars opercularis of human brains (Turner, 1948; Bailey and von Bonin, 1951). It may appear within the pars opercularis unjoined to other sulci or it may adjoin the inferior frontal sulcus dorsally, the inferior precentral sulcus caudally, or the Sylvian fissure inferiorly. Although reports of the frequency of this sulcus

TABLE 1. Ratio distribution of sulcal variations of the inferior frontal gyrus

	<i>Pan troglodytes</i>		<i>Gorilla gorilla gorilla</i>		Total (n = 90)
	L (n = 37)	R (n = 40)	L (n = 6)	R (n = 7)	
Fronto-orbital (fo)					
Incomplete	1/37	2/40	0/6	0/7	3/90
Bifurcated	5/36	2/38	1/6	1/7	9/87
Inferior frontal (fi)					
Incomplete	5/37	5/40	1/6	0/7	11/90
Bifurcated	3/32	6/35	0/6	0/7	9/79
Inferior precentral (pci)					
Incomplete	2/37	2/40	0/6	0/7	4/90
Bifurcated	15/35	11/38	1/6	0/7	27/86
Inferior precentral-inferior frontal (pci-fi) discontinuous	7/37	5/40	1/6	0/7	13/90
Diagonal dimple or sulcus present	9/37	12/40	0/6	0/7	21/77

Sample size refers to the number of hemispheres studied. Incomplete sulci were broken in the portion that would form a border for pars opercularis. The fronto-orbital sulcus, for example, commonly had a short accessory limb running anteriorly away from pars opercularis. This variation was not scored as a bifurcation. Bifurcated sulci had a ramus that impinged on the territory of pars opercularis (individuals with an incomplete sulcus were removed from the sample examined for a bifurcated sulcus).

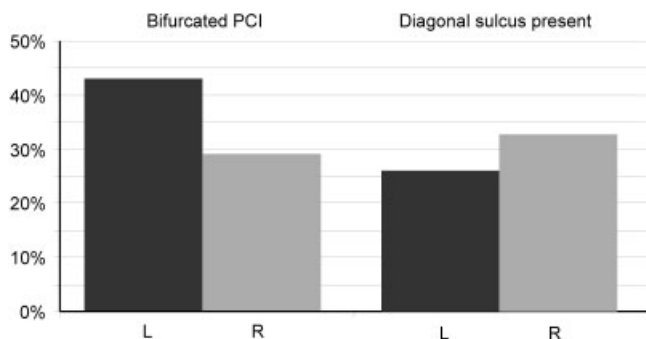


Fig. 3. Frequencies of sulcal variations in chimpanzees. A bifurcated precentral sulcus occurs more frequently in the left hemisphere. The diagonal sulcus or dimple appears more often in the right hemisphere.

in human brains vary (Galaburda, 1980; Ono et al., 1990; Tomaiuolo et al., 1999), it is consistently reported to occur more frequently in the left hemisphere, with differences in left–right frequencies ranging from 2% (Tomaiuolo et al., 1999) to 14% (Galaburda, 1980). The chimpanzee brain hemispheres we examined displayed a diagonal sulcus in 24% of the left hemispheres and 30% of the right hemispheres—a reversal of the pattern found in humans (Fig. 3).

### Cytoarchitecture, Myeloarchitecture, and Immunohistochemical Staining Patterns

In Nissl preparations, the cortex of the precentral gyrus (Brodmann's area 6) was easily identified by the absence of a distinct layer IV and the presence of large pyramidal cells in layers III and V. Brodmann's area 6 has a columnar appearance that is particularly conspicuous in infragranular layers. Area 44 also was characterized by a columnar pattern, but could be distinguished from the posteriorly adjacent premotor cortex (area 6) by the development of a thin layer IV and particularly large, clustered magnopyramidal neurons in the deep part of layer III (von Economo, 1929; Bailey and von Bonin, 1951; Braak, 1980), allowing for the recognition of two sublayers within layer

III. Layer IV in area 44 has an undulating appearance due to the invasion of pyramidal cells from layers III and V. Rostrally, area 45 is distinguished from area 44 by the presence of a more prominent layer IV, a more homogeneous distribution of pyramidal cells in the deep portion of layer III, and the absence of conspicuous cell columns (Fig. 4). Qualitatively, layer IV of area 45 appeared twice as thick as layer IV in area 44.

The myeloarchitecture of the IFG was significantly more homogeneous than the cytoarchitectural patterns (Fig. 5). Myelin staining in the IFG revealed a pattern of heavy myelination in deep cortical layers, and fewer myelinated fibers in superficial layers. Prominent vertically-oriented fiber bundles were observed in infragranular layers. Horizontally-running fibers were observed in layer IV, and more superficial layers exhibited a sparse plexus of fine myelinated fibers. Myelin staining patterns, however, were rather uniform throughout the IFG and did not allow for the recognition of distinct subareas.

As previously shown, neurofilament protein is found in a subpopulation of pyramidal neurons, mostly distributed in layers III, V, and VI, with specific regional patterns (Campbell and Morrison, 1989; Hof and Morrison, 1995). Because neurofilament protein immunoreactivity presents a "simplified" architectural pattern of pyramidal cell distributions, and more clearly exposes shifts in pyramidal cell size and spatial distributions among cortical areas, several studies have used the SMI-32 antibody as a tool to distinguish cortical area boundaries reliably (Campbell and Morrison, 1989; Del Río and DeFelipe, 1994; Hof and Morrison, 1995; Hof et al., 1995). Neurofilament protein staining of the chimpanzee IFG revealed several areas with distinct patterns of immunoreactivity (Fig. 5). All neurofilament protein-immunoreactive neurons expressed a typical pyramidal cell morphology, with an apical dendrite extending vertically toward layer I, as well as several basal dendritic arbors. In general, staining intensity was related to cell body size, with larger cells being labeled more intensely. The region that corresponds to area 6 showed a bilaminar staining pattern with an upper band of lightly stained, evenly distributed medium- and large-sized pyramidal neurons at the bottom of layer

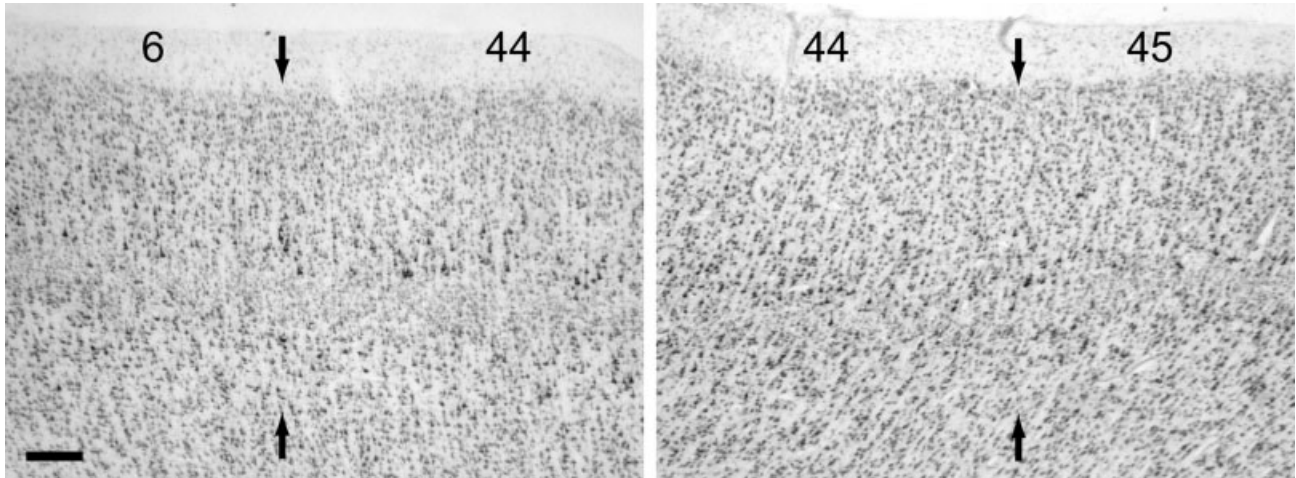


Fig. 4. Boundaries between cytoarchitectural areas. Note how the magnopyramidal cells in layer III form clusters in the transition to area 44. The transition from area 44 to area 45 is characterized by an increase in the thickness of layer IV. Scale bar = 200  $\mu\text{m}$ .

III, and a lower band of immunoreactive layer V cells and neuropil. Rostrally, area 44 was generally comparable to area 6, but could be distinguished from it by the presence of clusters of intensely stained magnopyramidal neurons in the deep part of layer III. In both area 6 and area 44 immunoreactive pyramidal cells were often observed to encroach upon the neurofilament protein-poor interlaminar zone. In contrast, neurofilament protein staining in area 45 was characterized by complete separation of immunoreactive neurons into upper (layer III) and lower (layer V) populations, and by the absence of the intensely stained magnopyramidal clusters characteristic in area 44.

#### Relationship Between Microstructure and Macrostructural Landmarks

Microstructural information from Nissl and neurofilament protein staining was used to determine the distribution of Brodmann's area 44 and its neighboring cortical areas. The resulting cytoarchitectural maps were then matched with surface anatomy to assess the extent of interindividual variability in the correlation between micro- and macrostructure (Fig. 6). In sum, there was a poor correspondence between cytoarchitectural borders and sulcal landmarks. In our sample of five chimpanzee hemispheres, the boundary between area 6 and area 44 was found on both banks of the inferior precentral sulcus, as well as within the subcentral anterior sulcus. The rostral boundary of area 44 with area 45 exhibited pronounced interindividual variability, being found within the inferior precentral sulcus, the subcentral anterior sulcus, the fronto-orbital sulcus, and the diagonal sulcus.

#### DISCUSSION

The primate brain exhibits a high degree of intraspecific variability in sulcal anatomy and cytoarchitectural boundaries (Geyer et al., 2001; Rademacher et al., 2001). We evaluated the macro- and microstructural variability of the pars opercularis of the IFG in African great ape brains and observed considerable interindividual variation in

sulcal patterns and cytoarchitectural boundaries, a finding that is consistent with previous studies of the IFG in great apes (Mingazzini, 1928; Walker and Fulton, 1936). Notably, the inferior precentral sulcus was bifurcated in several individuals. When a bifurcated inferior precentral sulcus was observed it was often present in only one hemisphere, and it was more frequent on the left. This situation can potentially bias surface anatomy-based estimates of asymmetry toward the left if the posterior ramus of the inferior precentral sulcus is presumed to represent the posterior border of the pars opercularis. Considering the significant variability of IFG sulci in these great apes, we conclude that it is not possible to reliably define the boundaries of the pars opercularis based on surface assessments from direct observation or MRI.

Furthermore, even if the pars opercularis could be reliably outlined, our cytoarchitectural analysis of area 44 in chimpanzees revealed pronounced interindividual variability in the relationship between cytoarchitectural boundaries and surface landmarks. Although previous studies of this region in great ape brains provided cortical surface maps of cytoarchitectural area distributions, these studies included samples of limited or unknown size and did not report variability in the precise correspondence of sulci to the distribution of area 44 (Brodmann, 1912; Kreht, 1936; von Bonin, 1949; Bailey et al., 1950). The amount of variability found in chimpanzees is consonant with a recent cytoarchitectural survey of this region in a large sample of human brains ( $n = 10$ ) that concluded that external sulci do not consistently demarcate the boundaries of cytoarchitectural areas 44, 45, and 47 (Amunts et al., 1999). In humans, for example, the border of areas 44 and 45 was found to fall anywhere between the fundus of the ascending ramus of the Sylvian fissure and the diagonal sulcus.

In this respect, the recent MRI study by Cantalupo and Hopkins (2001), which reported left dominant asymmetry of the pars opercularis in great apes, raises several methodological issues that deserve comment. First, the surface



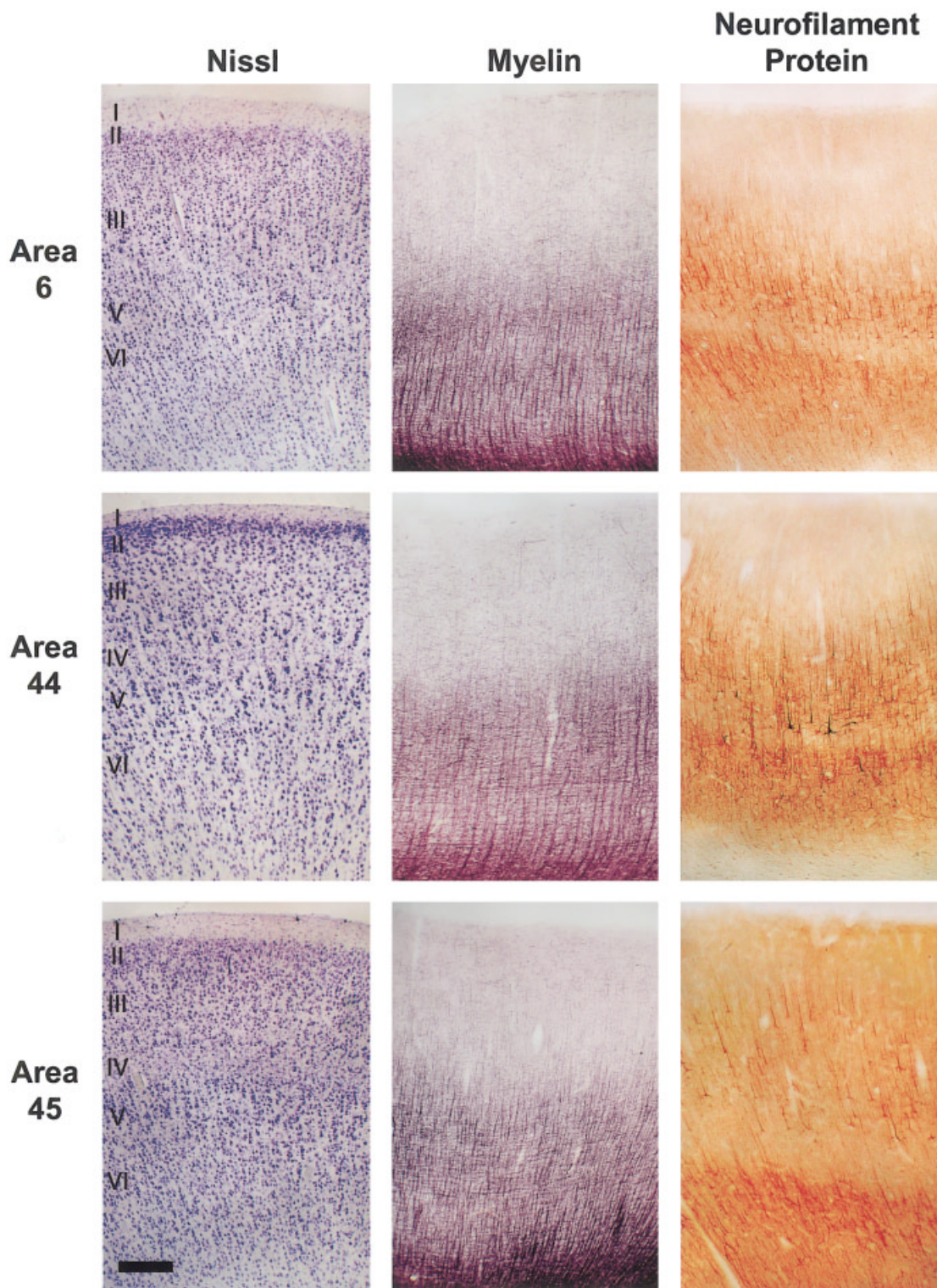


Fig. 5. Microstructure of the IFG in chimpanzees. Nissl: Area 6 does not have a visible layer IV. Area 44 has a thin layer IV and prominent magnopyramidal neurons in the deep part of layer III. Area 45 is distinguished by a clear layer IV and the absence of large pyramidal neurons in layer III. Myelin: Myeloarchitecture of the cortex of the IFG is fairly uniform across areas. Note the distinct vertically-oriented fiber bundles in infragranular layers. Neurofilament protein: Area 6 contains medium

neurofilament protein-immunoreactive neurons distributed evenly along layers III and V. Area 44 is distinguished by several clusters of large neurofilament protein-rich neurons in the deep portion of layer III. Area 45 has relatively fewer neurofilament protein-immunoreactive neurons in layers II and V. The neurofilament protein-poor interlaminar zone of area 45 is relatively thick compared to area 44. Scale bar = 200  $\mu$ m.

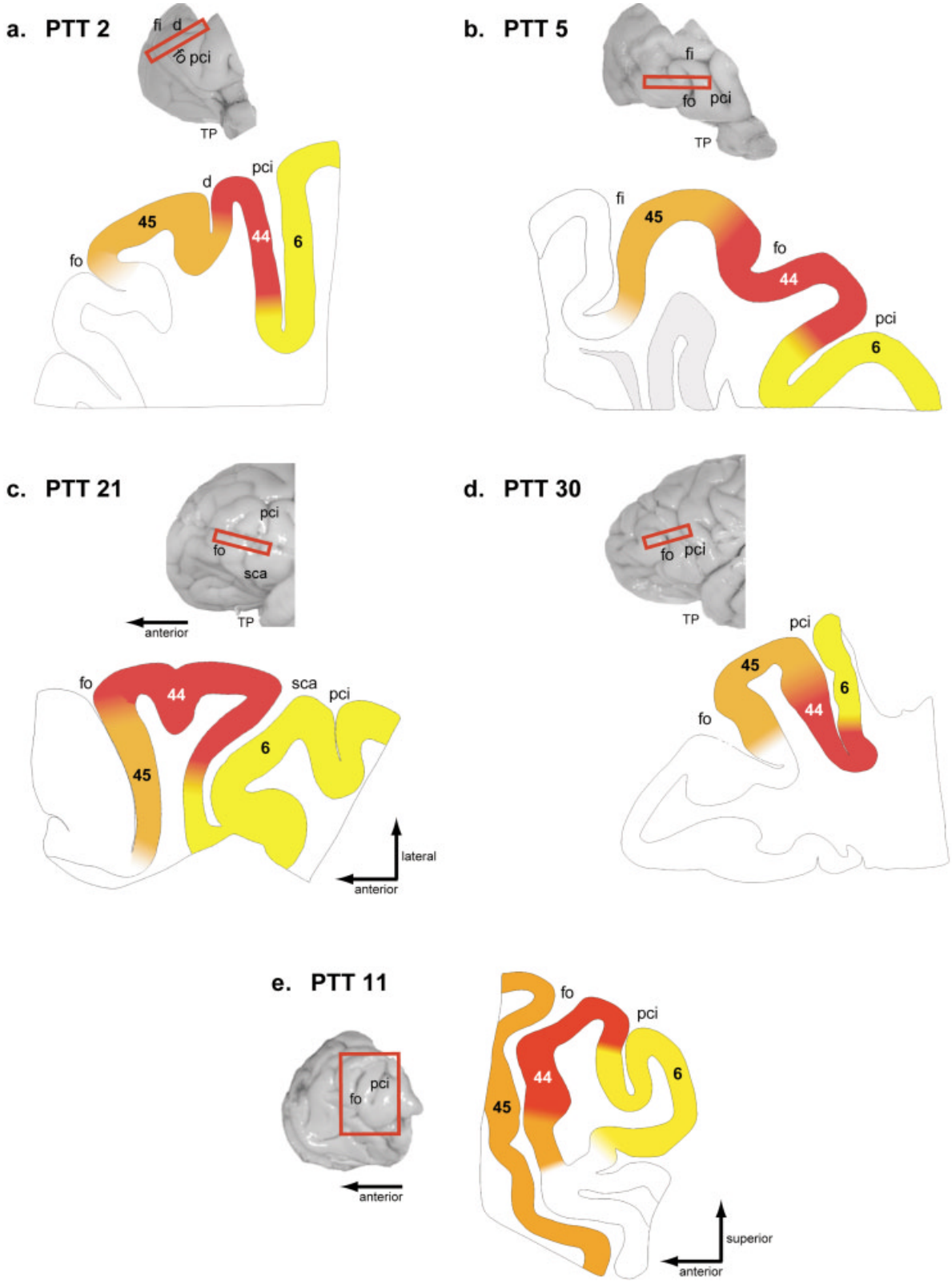


Fig. 6. Correlation of surface anatomy and cytoarchitectural area distributions in chimpanzees. **a–d**: Microstructural information was used to map the distribution of Brodmann's areas onto horizontal sections sampled from the IFG. The sampling location is depicted by the red box

on the brain's surface. **e**: Cytoarchitectural map from a parasagittal sample through the IFG. In sum, there was a great amount of interindividual variation in the relationship between cytoarchitectural area boundaries and sulcal landmarks.



area measurements reported in that study may not be adequate estimates of underlying tissue volume (Tomaiuolo et al., 1999). Second, in light of the variability of the IFG found in the present analysis, it is highly unlikely that the pars opercularis can be reliably outlined from the anatomic information available in MRI. This is especially important considering the discrepant results obtained from different MRI analysis methodologies applied to the quantification of pars opercularis asymmetry in humans (Foundas et al., 1998; Tomaiuolo et al., 1999). Third, considering the poor concordance between sulcal landmarks and cytoarchitectural boundaries in the IFG of human and chimpanzee brains, it is unlikely that the sulci used to define the pars opercularis coincided with the borders of cytoarchitectural area 44. Thus, cytoarchitecture-based volumetric analysis of a large sample of great ape brains is needed to adequately resolve this issue.

Nevertheless, even if more accurate quantification of asymmetries in great apes is obtained, several important questions will remain unanswered. The recent finding of humanlike asymmetry of the planum temporale in great apes (Gannon et al., 1998; Hopkins et al., 1998) suggests that grossly observable asymmetries of language-related brain areas may not offer much information to explain the unique neural wiring that supports human language. To this end, examination of the microstructural organization of great ape brains may yield contrasts between humans and nonhumans that are more meaningful for understanding how the human brain has been reorganized to process language. Indeed, several comparative studies of microstructure have revealed significant differences between humans and great apes in widespread regions of the cortex, including the primary visual cortex (Preuss et al., 1999; Preuss and Coleman, 2002), anterior cingulate cortex (Nimchinsky et al., 1999; Hof et al., 2001), Brodmann's area 10 (Semendeferi et al., 2001), and area Tpt (Buxhoeveden et al., 2001a, b). These results highlight the possibility that reorganization of circuits within a region, in the absence of dramatic volumetric change, may serve as an important substrate for the evolution of novel function. Therefore, it is likely that Brodmann's area 44 homologue in great apes, while similar in basic structure to that in humans, differs in subtle aspects of connectivity and lacks homologous function.

While both humans and great apes use a rich repertoire of gestural and vocal signals in communication, human language is unique in its combinatorial and symbolic properties. Recent data from functional imaging studies of the human brain reveal that in addition to its role in language, Broca's area is also activated during nonlinguistic tasks such as observation of finger movements (Binkofski et al., 2000) and recognition of manual gestures (Rizzolatti and Arbib, 1998). The close relationship between oral and manual motor representation in the brain is underscored by the finding that chimpanzees make sympathetic movements of the mouth while engaged in fine motor actions of the hand (Waters and Fouts, 2002). While the precise function of the frontal operculum, including the pars opercularis, of African great apes remains to be fully defined, we suggest that the language capacity of Broca's area evolved from a specialized premotor system designed to organize behavior based on the recognition of forelimb movements of social partners. We hypothesize that during human evolution, the learning of complex sequential motor patterns for tool manufacture and missile projection,

concomitant with increased dietary reliance on animal protein, served as a scaffold for the evolution of syntactically-rich symbolic communication (Holloway, 1969, 1976).

In conclusion, this study confirms the presence of Brodmann's area 44 and 45 in chimpanzees and demonstrates the general similarities of this region between chimpanzees and humans. Gross and microstructural variability of the IFG in great apes, however, prohibits reliable measurement of the pars opercularis or Brodmann's area 44 based on external landmarks, and thus makes it impossible to reliably determine whether humanlike asymmetry is found in this region of great ape brains. The results of this study point to the need for further investigation of the function and microstructure of this region in order to develop comparative analyses between apes and humans, that may illuminate scenarios concerning the evolution of language.

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