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Spatial Organization of Neurons in the Frontal Pole Sets Humans Apart from Great Apes

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Few morphological differences have been identified so far that distinguish the human brain from the brains of our closest relatives, the apes. Comparative analyses of the spatial organization of cortical neurons, including minicolumns, can aid our understanding of the functionally relevant aspects of microcircuitry. We measured horizontal spacing distance and gray-level ratio in layer III of 4 regions of human and ape cortex in all 6 living hominoid species: frontal pole (Brodmann area [BA] 10), and primary motor (BA 4), primary somatosensory (BA 3), and primary visual cortex (BA 17). Our results identified significant differences between humans and apes in the frontal pole (BA 10). Within the human brain, there were also significant differences between the frontal pole and 2 of the 3 regions studied (BA 3 and BA 17). Differences between BA 10 and BA 4 were present but did not reach significance. These findings in combination with earlier findings on BA 44 and BA 45 suggest that human brain evolution was likely characterized by an increase in the number and width of minicolumns and the space available for interconnectivity between neurons in the frontal lobe, especially the prefrontal cortex.

Keywords: chimpanzee, evolution, minicolumn, primate, pyramidal neuron

Introduction

There is an increased interest in human uniqueness and its genomic underpinnings (Varki et al. 2008); yet, only a few morphological differences have been identified so far that distinguish the human brain from the brains of our closest relatives, the apes, other than a more-than-3-fold difference in absolute size. The frontal lobe, a common site of comparative neuroanatomical inquiry given its participation in higher order cognitive processes such as language and executive function, is more than 3 times larger in absolute size in humans than it is in great apes but is not disproportionately enlarged in humans when scaled with brain size (Semendeferi and Damasio 2000). When white matter is considered separately from gray matter, the human frontal lobe also remains undistinguished from apes in terms of overall relative volume (Schenker et al. 2005). However, human brains have a greater distribution of white matter in gyral regions (Schenker et al. 2005) and it has been argued that the most rostral parts of the prefrontal cortex include more white matter in humans (Schoenemann et al. 2005, but also see Sherwood et al. 2005). Some cortical areas of the frontal lobe are enlarged in humans (Brodmann area [BA] 10; Semendeferi et al. 2001), while others are smaller than would be expected (BA 13; Semendeferi et al. 1998). Additionally, as a whole, the human prefrontal cortex is 25% more gyrified than it is in apes, as measured by the gyrification index (Armstrong et al. 1991, 1993; Rilling 2006).

Examination at the cellular level has revealed some variation among species. In general, apes and humans have modestly thicker cortex than smaller brained primates, and homologous cortical areas in humans tend to be thicker than in apes (Sherwood and Hof 2007). Differences in neurotransmitter innervation have been noted in the prefrontal cortex of humans and chimpanzees both when they are compared with the prefrontal cortex of macaques and with primary motor cortex in all 3 species. In humans and chimpanzees, BA 9 and BA 32, which are involved in working memory and theory of mind, respectively, possessed a higher number of dopaminergic afferents in layers III, V, and VI (Raghanti et al. 2008a) and greater density of serotonin transporter–immunoreactive axons in layers V and VI (Raghanti et al. 2008b). A specific class of neurons, Von Economo neurons, or spindle cells, was identified in the anterior cingulate cortex and frontal insula in humans (Allman et al. 2002; Fajardo et al. 2008) and great apes (Nimchinsky et al. 1999; Allman et al. 2010), as well as in several other species of social mammals, including elephants (Hakeem et al. 2009) and cetaceans (Butti et al. 2009). Primary motor cortex (BA 4) is similar across humans and other primates, but there are a few interspecific differences; great apes and humans possess a relatively thicker layer III and greater neuropil space compared with Old World monkeys (Sherwood et al. 2004b). Another clade-specific trait in BA 4 in apes and humans are the calretinin-immunoreactive pyramidal cells, involved in calcium signaling, in layer V (Hof et al. 1999; Sherwood et al. 2004a). The soma size of the large Betz cells found in layer V of BA 4 scales with brain size in hominoids, such that they are largest in humans and smallest in orangutans (Sherwood et al. 2003). In primary sensory cortex (BA 3), Meynert cells, the larger pyramidal cells in lower layer V, are relatively largest in chimpanzees and smallest in orangutans (Sherwood et al. 2003). The primary visual cortex (BA 17) is markedly reduced in size in humans (Holloway 1996) compared with other primates, and apes and humans exhibit several clade-specific adaptations to thalamic recipient layer IV (Preuss et al. 1999; Preuss and Coleman 2002).

Recent studies (Herculano-Houzel et al. 2008) also provided further support for the ideas that neuron number and density vary across species (Williams and Herrup 1988) as well as

within species (Pakkenberg and Gundersen 1997). There is also variation in column cell numbers between regions (Beaulieu 1993) and among species (Zilles et al. 1986; Haug 1987). Interspecific differences in dendritic structure have also been noted; pyramidal cells in the human prefrontal cortex are more branched and spinous than those in the temporal and occipital lobes, and are also more branched and spinous than those in the prefrontal cortex of macaques and marmosets (Elston et al. 2001). In the human prefrontal cortex, layer III projections possess more branched and spinous dendritic arbors than in temporal, occipital, or parietal cortex (Elston et al. 2001; Jacobs et al. 2001; Petanjek et al. 2008). The long-range corticocortical projections of layer IIIc neurons (Lewis et al. 2002) in particular are thought to be critical to working memory and other higher order cognitive processes in primates (Fuster 2003; Elston et al. 2006), suggesting that the reported differences in dendritic tree structure are related to cognitive differences (although see Zeba et al. 2008).

In the current study, we compared the spatial organization of neurons in the cortex of humans and great apes, an arena of research with considerable relevance to aspects of microcircuitry. Specifically, the horizontal spacing of neurons has been linked to the modular and vertical characteristics of the cortex, including morphological features of minicolumns (Mountcastle 1997; Galuske et al. 2001; DeFelipe 2005). Minicolumns are characterized as vertical aggregates of cells traversing layers II through VI of the cortex, typically consisting of 80–100 neurons (Buldyrev et al. 2000), and are assumed to be one neuron wide in layers III, V, and VI (Seldon 1981). Cell modules, consisting of groups of pyramidal cells in layers III and V whose apical dendrites form clusters, have been identified (Peters and Sethares 1991, 1996), suggesting a correlation between pyramidal cells and fiber bundles (Buxhoeveden and Casanova 2005b) throughout the extent of the column. This generally vertical arrangement of pyramidal cells represents the form of the basic minicolumn configuration, which is considered to be the remnant of fetal cell columns in the adult brain (Rakic 1995). Variation in spacing between minicolumns, as measured here by looking at layer III neurons, may be indicative of differences in dendritic tree structure; spatial differences in the horizontal plane necessarily impute changes in the anatomical configuration of the cortex. This is of strong importance for comparative neuroanatomy, as features of dendritic arborization may influence neuronal functioning, including size, branching pattern, and the number and distribution of inputs (Elston 2007).

Layer III, the focus of this study, is of particular interest in the study of horizontal spacing distance (HSD) as it is the origin of axons sent horizontally to other columns (Yabuta and Callaway 1998), although its pyramidal neurons also extend associational and long-range intrinsic projections (Lewis et al. 2002). In addition to pyramidal cells, layer III also contains axons from layer IV stellate cells, axons from layer II double bouquet cells, and apical dendrites from layer V (Buxhoeveden and Casanova 2005b). It is the origin and the target of callosal and commissural axons linking the 2 hemispheres and also the target of associational axons linking ipsilateral areas within each hemisphere (Herschkowitz et al. 1999; DeFelipe 2005).

One possible mechanism of evolutionary increase in brain size is the addition of minicolumns, as a result of either an increase in the neuroepithelial cell population or an increase in the duration of neurogenesis (Rakic 1995, 2008; but also see Allman 1990; Kaas 1995; Hansen et al. 2010 for other possible mechanisms of evolutionary increase in brain size). Indeed, only 3 additional symmetrical divisions of cells in the ventricular zone are required to achieve the 8- to 10-fold size increase between the human and monkey brain (Rakic 1995). Changes in the width of vertical organization between cortical areas therefore reflect changes in anatomical organization, including cell size, number and size of efferent and afferent pathways, intrinsic connections, or number of synapses, all of which may reflect or affect functionality in that part of the cortex (Hilgetag and Barbas 2005).

Although the precise significance of minicolumns as functional units in the adult human brain is debated (Horton and Adams 2005; Rakic 2008), their structural characteristics have been described for a variety of smaller mammals, including rats (Bruno et al. 2003; Lubke and Feldmeyer 2007), cats (Favorov and Diamond 1990; Tommerdahl et al. 1993), squirrel and macaque monkeys (Casanova et al. 2009; Georgopoulos et al. 2007), and humans (Buxhoeveden et al. 1996, 2006; Schlaug et al. 1995; Schenker et al. 2008). Human brains are shown to have the widest minicolumns as evidenced by, among other parameters, the increased HSD between neuronal cell bodies. Nevertheless, it remains unknown whether this is a human-specific characteristic or is related to the more-than-10-fold differences in absolute brain size with the smaller primates. Despite the close phylogenetic relationship that great apes share with humans, direct ape-to-human comparisons of the spatial organization of neurons are scarce. Human minicolumns are reported to be wider in the lateral superior temporal cortex (BA 22) than in chimpanzees (Buxhoeveden et al. 2001) and larger in BA 44/45 (Broca's area) than in all great ape species (Schenker et al. 2008). The question raised by these findings is whether the presence of wider minicolumns in humans is a characteristic only of the cortical regions specifically involved in aspects of language-related function, or whether wider minicolumns may be found throughout the human cortex, regardless of its functional properties.

We measured HSD of neuronal cell bodies and the fraction of the area occupied by the neuronal cell bodies (gray-level ratio [GLR]) in layer III of 4 regions of human and ape cortex: BA 10 in the prefrontal cortex, and primary motor (BA 4), primary somatosensory (BA 3), and primary visual (BA 17) cortex. All 4 regions selected for examination in the current study display noteworthy interspecific diversity, as described above, and are thus of interest to comparative neuroanatomical studies as possible sites for uniquely human specializations. This study tested the hypothesis that the spatial organization of neurons differs between humans and apes (bonobo, chimpanzee, gorilla, orangutan, gibbon) in the frontal pole (Fig. 1), but not in BA 4, BA 3, or BA 17. The parameters used in this study, HSD and GLR, are free of assumptions about cell columnarity but provide information about the spatial organization of neurons that is also informative in the context of the increasing number of studies on aspects of minicolumnar organization of the cortex.

Materials and Methods

Specimens and Tissue Preparation

Our sample comprised 19 complete series of histologically processed brains of human and ape male and female individuals from all 6 extant hominoid species: humans, bonobos, chimpanzees, gorillas, orangutans, and gibbons (Table 1). Measurements included the right hemisphere of

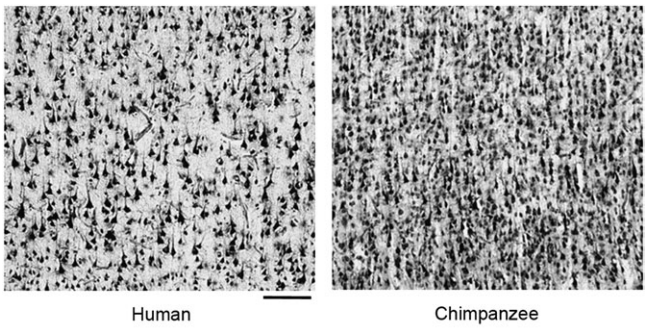

Figure 1. Photomicrograph of layer III in human and chimpanzee BA 10. Scale bar = 100 μm.

Table 1
Specimens

Species	Specimen	Sex	Age (years)	Brain weight (g)
Human	H988	M	21	1633
	SN146	M	37	1437
	SN16	M	54	1757
	SN189	M	56	1270
	SN382	F	59	1142
	SN281	M	69	1360
	SN56	F	72	1216
	SN207	M	75	1349
Bonobo	YN86	F	2	392
	Zahlie	F	11	324
Chimpanzee	Chimp 1	F	22	440
	YN89	M	22	420
	Bathsheba	F	24	359
Gorilla	YN82	F	20	376
Orangutan	YN85	M	17	369
	Briggs	M	34	345
	Harry	M	37	440
Gibbon	Disco	F	22	120
	YN81	F	Ad	92

one chimpanzee (YN89), both hemispheres of all other apes and 2 humans (H988 and SN207), and the left hemisphere of the remaining 6 humans. The ape specimens were donated following the natural death of the animals by the Busch Gardens Zoo, Henry Doorly Zoo, Milwaukee County Zoo, and Yerkes National Primate Research Center. None of the human or ape subjects died from a neural pathology. One orangutan (Briggs) was wild born and 1 bonobo (YN86) had been exposed to language training before its death at the age of 2. All human and most of the ape subjects were adult (ages 21–75 and 16.5–37 years, respectively) and 2 bonobos were 2 and 11 years old (Table 1). Although the 2-year-old bonobo is young, the extreme rarity of bonobo histological tissue makes its inclusion in this study valuable. We analyzed the results with and without this young individual to control for possible influences due to age. We also analyzed our results with and without the oldest specimens for the same reason (see Results).

All human and ape brain specimens included here were processed in the same manner at the Cecile and Oskar Vogt Institute for Brain Research in Düsseldorf (Amunts et al. 1999). They were fixed within 24 h after the natural death of the subject in either a 4% formalin or a Bodian fixative (mixture of formalin, glacial acetic acid, and ethanol), and were then processed in a consistent manner. All specimens were subsequently embedded in paraffin and serially sectioned into 20-μm sections in the coronal plane, except for one chimpanzee specimen, YN89, which was sectioned into 15-μm sections in the axial plane (the inclusion of this specimen did not significantly change our findings; see Results). In all specimens, every 10th to 20th section was mounted on a glass slide and stained with a modification of the Gallyas silver stain for neuronal perikarya (Merker 1983).

Quantification of Spatial Organization

A combination of strict topographical and cytoarchitectonic criteria were used to identify the regions of interest in all specimens across species according to previously published work (Geyer et al. 1996, 2000; Amunts et al. 2000; Amunts and Zilles 2001; Semendeferi et al. 2001). For BA 3 and BA 4, we employed the delineations formerly made by Geyer et al. (1996, 2000) and specifically analyzed regions located in BA 3b and BA 4a or 4p, depending on tissue quality, orientation (vertically cut cortex), and non-tangential locations away from the crown and fundus of the sulci. For BA 10 (Fig. 1), we were guided by previously published work that specifically targeted the cytoarchitecture of BA 10 in humans and apes (Semendeferi et al. 1994, 2001) and gave close consideration to the descriptions of the human prefrontal cortex made by Economo and Koskinas (1925), Sanides (1964, 1970), and Kononova (1938, 1949, 1955). Although these studies identified variability within the frontopolar cortex, they did not claim differences large enough to form separate cortical areas. Images of the frontal pole in the human and great ape brains were captured from throughout the extent of BA 10, including the dorsal, mesial, and orbital surface. The size of each captured ROI is 700–900 × 1100 μm, so there are many locations even in the most convoluted parts of the cortex where the minicolumnar formations can be seen. With respect to the gibbon BA 10, we obtained images only from the orbital and ventromesial parts of the frontal pole, as by definition the extent of BA 10 in that species does not include the dorsal surface of the frontal pole (Semendeferi et al. 2001).

A minimum of 5–10 images (with dimensions of 700–900 × 1100 μm) from 3 to 5 individual sections from each region of interest (ROI) were captured and digitized, with a final resolution of 1.02 μm/pixel. The sampling scheme was nonstereological, as the collection of images from large segments of layer III depended on the quality of the tissue in the large human and ape brains. Nevertheless, data collection was carried out in a strict and consistent manner. All specimens were processed in an identical manner by the same laboratory (Amunts and Zilles 2001). Images were captured in a consistent fashion; no tangential cuts or locations were selected given that cortical areas and cortical layers had to be clearly identifiable.

All parameters were measured in layer III of cortex, an approach that matches that taken in our previous work (Buxhoeveden et al. 2001; Schenker et al. 2008). Layer III has been extensively targeted in studies of minicolumns because it typically displays the clearest linearity, cell arrays within it are one cell wide, columns in layer III are generally descriptive, although not identical, of the size of a minicolumn throughout the depth of the cortex in the adult cortex (Buxhoeveden et al. 1996), and the supragranular layers play a critical role in transcolumnar and corticocortical processing. In the present study, layer III was targeted also because results can be compared with a large database obtained in other regions of the normal brain, all of which are based on studies in layer III (Buxhoeveden et al. 1996, 2002; Buxhoeveden and Casanova 2004; Schenker et al. 2008). The study of additional cortical layers is desirable but has not been accomplished yet.

Photomicrographs were obtained using a Nikon H600L microscope with a ×10 CFI Plan Apochromat lens (N.A. 0.20), attached to a Dell workstation via an Optronics MicroFire video camera. We obtained scale calibration from the use of a micrometer photographed at the same resolution and magnification as the images. All images were coded before analysis and raters were blinded to the specimen investigated. Using software modeled on the ImageJ program, each digitized image underwent the process of thresholding (to exclude cells smaller than 20 pixels), and watershedding (for edge detection), and was then converted into a binary image (Fig. 2). The use of a threshold eliminates small cells, such as glia and smaller interneurons, and focuses on the pyramidal cells that comprise most of layer III. The parameters used were averages based on the entire width of the ROI. Each step was carried out automatically by a series of computer algorithms, and operator input was limited to the determination of the threshold level and the boundary of ROI within each image. HSD is calculated based on the edge-to-edge measures of cells in the horizontal axis and provides a measure of the average spacing distance between cells. The GLR calculates the fraction of the converted binary image that is gray, which

reveals how much of the image is occupied by stained cell bodies (Buxhoeveden et al. 2001). A higher value is indicative of a larger number of neurons, larger size of cell bodies, or a combination thereof. GLR is derived from the same binary image used to measure horizontal cell spacing distances but is computed as an independent variable. The GLR method is based on the same general principles that guide the Gray Level Index (GLI) method (Schleicher et al. 1999) but is a slightly different procedure in that it does not rescale the 255 gray values to 100 (gray value × 100/255) to express it in percent (which is the standard protocol of the GLI method). GLI and GLR values are thus not directly comparable. In general, the 2 parameters, HSD and GLR, correlate inversely and when cells are located further away from each other, HSD tends to be higher and GLR lower. HSD is a central measure in that it provides the mean spacing of cells from their edges across the horizontal plane in absolute numbers (microns). Nevertheless, GLR is more sensitive than HSD to possible differences in cell size in addition to spacing of cells. A higher GLR value may be indicative of greater density of neurons due to their number or their size, or a combination thereof. GLR is complimentary to HSD in that it describes the distribution of the neurons in the ROI and does so via a percentage that is a relative measure compared with a specific distance in microns. The relative measurement of GLR helps to diminish the effects of shrinkage and other factors that affect direct measures, especially when tissue is processed differently, which is not a concern to the present study.

The aforementioned method is a recent modification of previous minicolumn quantification techniques (Buxhoeveden et al. 2001; Casanova et al. 2006). The technique used here was an assumption-free approach to the presence of minicolumns in the adult human cortex and did not test the presence or absence of minicolumns. Specifically, the program used here to analyze images was free of assumptions about vertical cell linearity and measured HSD and the fraction of the area occupied by neuronal bodies in layer III. Nevertheless, these 2 parameters together are hypothesized to describe aspects of minicolumnar morphology, and therefore, we used the term "minicolumn" throughout. We used the term "wider minicolumns" to refer to an increase in the horizontal spacing between neuronal cell bodies that together with a decrease in GLR is indicative of increased intracolumnar and intercolumnar neuropil space in layer III. Whether or not such differences in width are consistent throughout cortical layers remains to be tested, given that dispersal of ontogenetic columns during development may be subject to layer-specific alterations related to connectivity. In other words, the width of minicolumns may prove to differ between layers in the adult cortex after the dispersal of cell bodies following the earlier formation of ontogenetic columns. Furthermore, even though early reports on minicolumns are of interest, the absolute values provided here are directly comparable only with those published more recently by our laboratory (Buxhoeveden et al. 2006; Schenker et al. 2008).

Analysis

HSD and GLR were analyzed for differences between humans and all great apes as a group. Wilcoxon 2-sample tests were used to test for difference between humans and great apes and for differences within the human brain. Mean and standard deviation values were obtained from measurements of the multiple images representing ROIs in individual specimens. SPSS (SPSS, Inc.) was used for all statistical calculations. We examined the relationship of HSD to brain size by calculating a ratio between the estimated cross-sectional area of a minicolumn [$(3 \times \sqrt{3}/8) \times \mathrm{HSD}^2$] and brain volume. This method (Schenker et al. 2008) was used to estimate minicolumn size as a hexagon because hexagons have the lowest circumference to area ratio of a regular polygon that can be configured 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).

Results

The current study found that HSD was greatest in human BA 10 compared to the other regions examined (although this difference did not reach significance in comparison to BA 4). HSD was also greatest in human BA 10 compared with apes BA 10 (see Table 2 and Fig. 3 for a summary of HSD data). In all species, primary visual cortex (BA 17) had the smallest HSD and largest GLR values in comparison with the other cortical areas examined (see Table 2 and Fig. 3 for a summary of HSD data, and Table 3 and Fig. 4 for a summary of GLR data). Within the

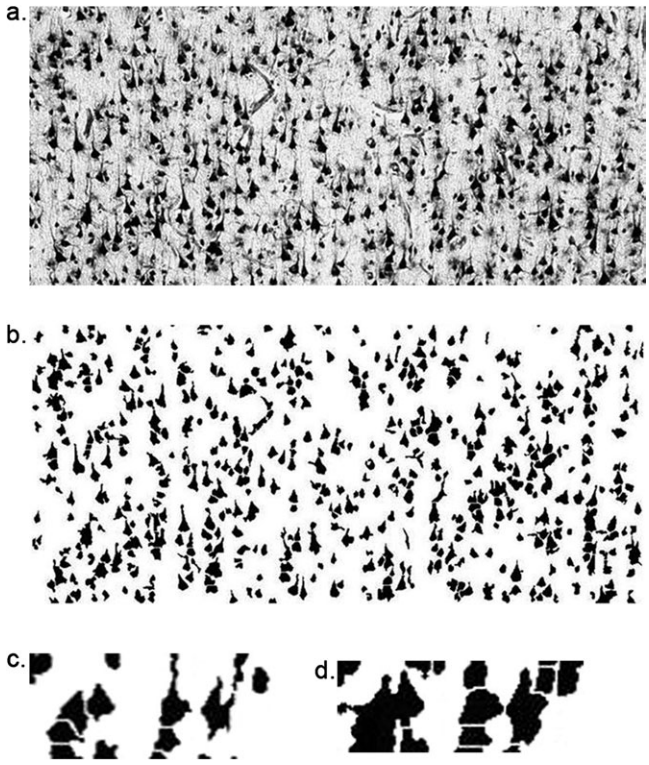

Figure 2. (*a*) Images were obtained from regions of interest. (*b*) Each image was digitized and thresholded to exclude cells smaller than 20 pixels, and was then converted into a binary image. The 2 parameters used (HSD and GLR) were averages based on the entire width of the ROI. Each step was carried out automatically by a series of computer algorithms. (*c*) HSD is calculated based on the edge-to-edge measures of cells in the horizontal axis and provides a measure of the average spacing distance between cells. GLR is the fraction of the converted binary image that is gray, which reveals how much of the image is occupied by stained cell bodies. Locations largely occupied by cells have high GLR values. HSD and GLR are generally correlated, and when cells are located further away from each other, HSD tends to be higher and GLR lower. Nevertheless, GLR is more sensitive than HSD to possible differences in cell size in addition to spacing of cells. (*d*) The binary image shown here comes from the exact same ROI shown in *c*, but the cells were artificially enlarged (fattened) to demonstrate the value of both measures. Despite the striking difference in appearance, the spacing of the cells as reflected in HSD remains identical between *c* and *d*, but GLR changes dramatically. A higher GLR value is indicative of greater density of neurons due to their number or their size, or combination thereof.

Table 2
HSD in all ROIs, averaged by species, including SD (in microns)

Species	BA 10 (SD)	BA 4 (SD)	BA 3 (SD)	BA 17 (SD)
Human	59.52 (12.77)	49.23 (7.72)	45.32 (8.48)	38.61 (8.94)
Bonobo	38.69 (3.23)	44.26 (3.83)	37.46 (2.06)	33.25 (3.93)
Chimpanzee	37.52 (3.44)	52.66 (6.83)	40.35 (3.53)	30.74 (2.90)
Gorilla	41.76 (3.58)	51.41 (4.60)	41.47 (3.17)	30.40 (1.35)
Orangutan	36.35 (3.39)	45.63 (5.37)	38.09 (3.22)	31.22 (2.52)
Gibbon	33.49 (3.39)	44.88 (7.68)	39.57 (4.17)	29.50 (2.42)

Note: Regions include BA 10 (frontal pole), BA 4 (primary motor cortex), BA 3 (primary somatosensory cortex), and BA 17 (primary visual cortex). SD, standard deviation.

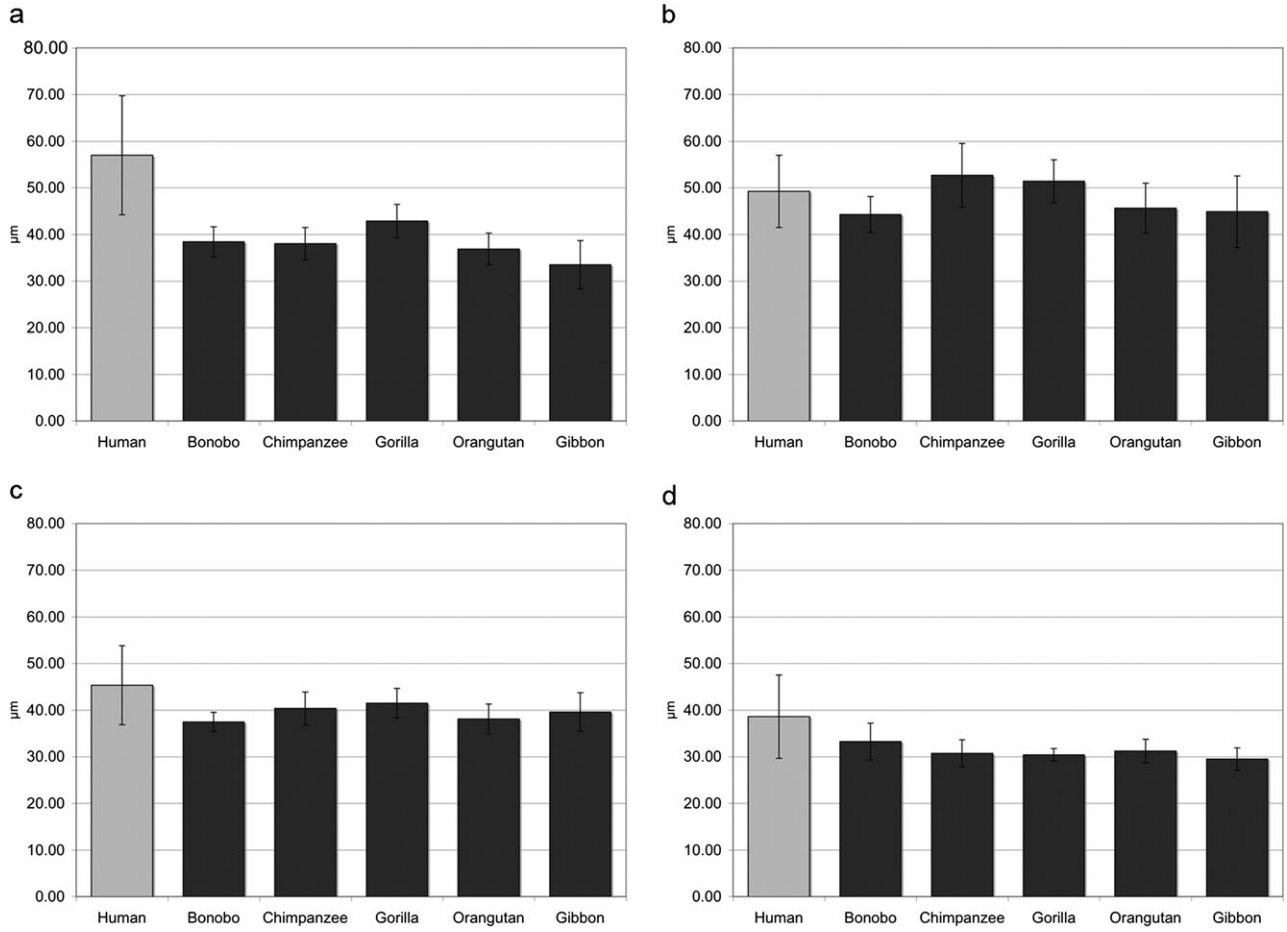

Figure 3. HSD in all species, by region. (*a*) BA 10 (frontal pole), (*b*) BA 4 (primary motor cortex), (*c*) BA 3 (primary somatosensory cortex), and (*d*) BA 17 (primary visual cortex) (in microns).

human brain, HSD is significantly smaller in BA 17 than it is in BA 10 (Wilcoxon 2-sample rank test, $Z = 3.361$, $P = 0.00016$), BA 4 ($Z = 3.361$, $P = 0.001$), and BA 3 ($Z = 2.415$, $P = 0.016$). In a near inverse of the pattern seen for human HSD, BA 17 has larger GLR values than BA 10 ($Z = 3.006$, $P = 0.001$) and BA 4 ($Z = 3.205$, $P = 0.001$). Within great apes as a group, HSD was also significantly smaller in BA 17 compared with BA 10 ($Z = 3.175$, $P = 0.001$), BA 4 ($Z = 3.578$, $P = 0.000346$), and BA 3 ($Z = 3.576$, $P = 0.000349$). Relatedly, GLR is significantly larger in BA 17 in great apes when it is compared with all 3 other regions examined; BA 17 GLR is larger than BA 10 ($Z = 3.382$, $P = 0.001$), BA 4 ($Z = 3.231$, $P = 0.001$), and BA 3 ($Z = 2.266$, $P = 0.023$).

HSD values in BA 17 for individual apes ranged from 27.90 μm in a gibbon specimen to 36.49 μm in a bonobo. Although this range overlapped with the range of human individual specimen values, 31.10–41.45 μm, the species mean value for human HSD (38.61 μm) was significantly higher than HSD in apes (Wilcoxon 2-sample rank test, $Z = 2.598$, $P = 0.008$). In apes, species means were lowest in gibbons (29.5 μm) and highest in bonobos (33.25 μm). The GLR values were similar between humans and apes, with human values being slightly smaller (see Table 3 and Fig. 4 for a summary of GLR data). The human mean (0.29) fell outside the range of means seen in the apes (from 0.31 in the chimpanzee specimens to 0.39 for the gibbons) but did not differ statistically from the ape means.

Table 3
GLR in all ROIs, averaged by species, including SD

Species	BA 10 (SD)	BA 4 (SD)	BA 3 (SD)	BA 17 (SD)
Human	0.18 (0.04)	0.22 (0.03)	0.23 (0.05)	0.29 (0.06)
Bonobo	0.24 (0.03)	0.29 (0.04)	0.33 (0.02)	0.31 (0.03)
Chimpanzee	0.24 (0.04)	0.23 (0.04)	0.30 (0.04)	0.35 (0.05)
Gorilla	0.21 (0.02)	0.23 (0.02)	0.28 (0.02)	0.38 (0.02)
Orangutan	0.27 (0.03)	0.27 (0.04)	0.32 (0.04)	0.37 (0.04)
Gibbon	0.30 (0.07)	0.28 (0.02)	0.31 (0.05)	0.39 (0.05)

Note: Regions include BA 10 (frontal pole), BA 4 (primary motor cortex), BA 3 (primary somatosensory cortex), and BA 17 (primary visual cortex).

In humans, primary somatosensory cortex (BA 3) had the next smallest HSD and highest GLR values after BA 17. Within the human brain, BA 3 exhibited significantly smaller HSD (Wilcoxon 2-sample rank test, $Z = 1.995$, $P = 0.046$) and larger GLR than BA 10 ($Z = 2.069$, $P = 0.039$). As described above, human BA 3 also had significantly greater HSD values than BA 17. In almost all ape specimens, BA 3 displayed slightly lower HSD than BA 4, and slightly greater HSD than BA 17 and BA 10. In great apes as a group, BA 3 exhibited significantly greater GLR than BA 10 ($Z = 3.059$, $P = 0.002$) and BA 4 ($Z = 2.799$, $P = 0.005$). The species mean value for humans was 45.32 μm, followed by gorillas (41.47 μm) and the rest of the apes, with the lowest value in bonobos (37.46 μm). GLR values were similar between humans and apes, with humans being

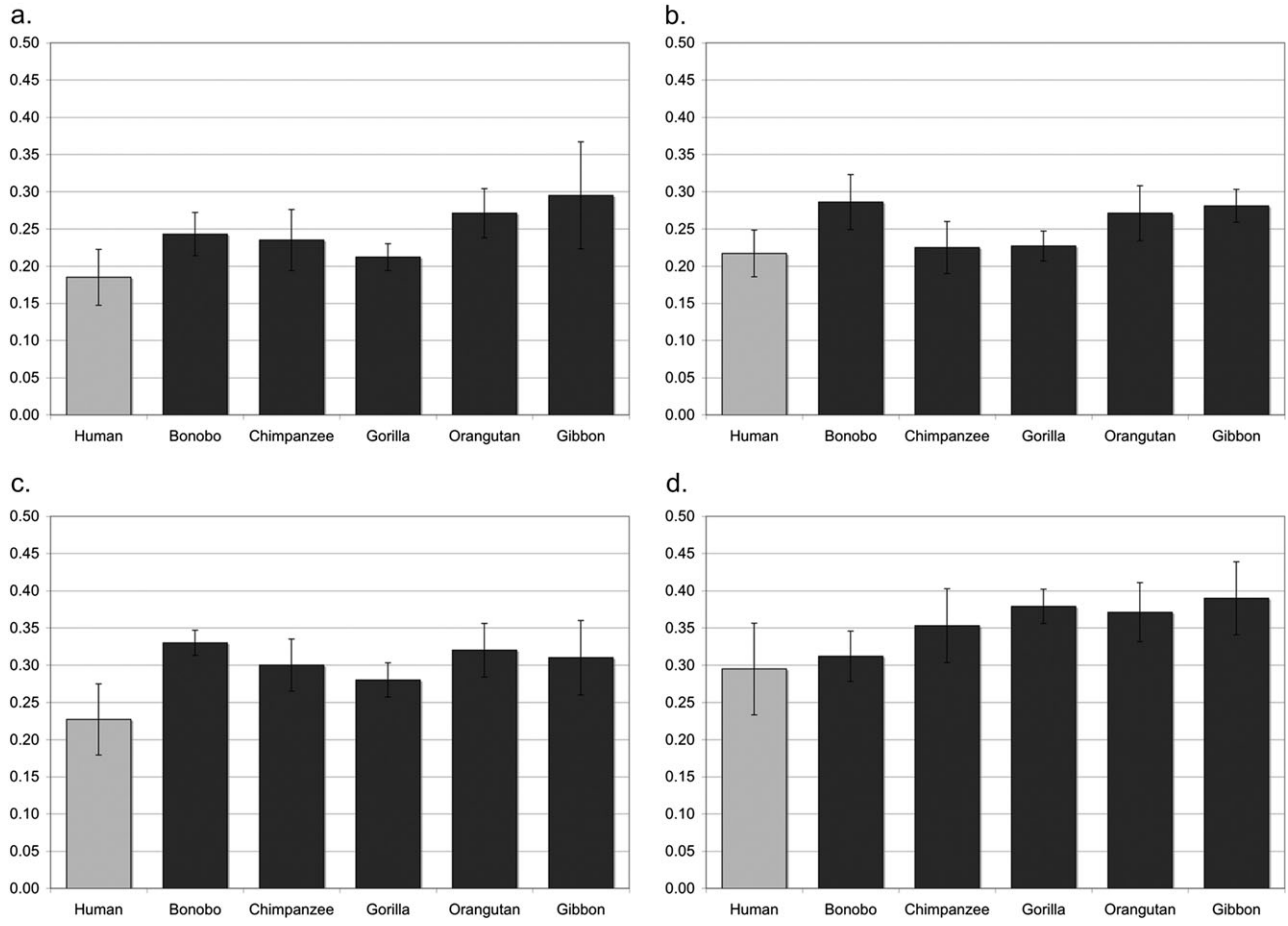

Figure 4. GLR in all species, by region. (*a*) BA 10 (frontal pole), (*b*) BA 4 (primary motor cortex), (*c*) BA 3 (primary somatosensory cortex), and (*d*) BA 17 (primary visual cortex).

somewhat, although insignificantly, smaller. The human mean (0.23) fell outside the range seen in the apes (from 0.28 in the gorilla to 0.33 in the bonobos) but did not differ statistically from the ape means.

The region with the greatest HSD in all individual ape specimens, as well as in 3 of 8 humans, was primary motor cortex (BA 4). Within the great apes as a group, HSD in BA 4 is significantly larger than in all 3 other ROIs; as described previously, BA 4 HSD is larger than BA 10 ($Z = 3.313$, $P = 0.001$), BA 3 ($Z = 3.225$, $P = 0.001$), and BA 17 ($Z = 3.578$, $P = 0.000346$). Also as noted previously, in humans BA 4 had significantly larger HSD than BA 17. Individual apes ranged between 40.23 and 56.91 μm and overlapped with the range of human specimens (42.98–62.43 μm). The mean human value (49.23 μm) fell well within the range of ape species mean values (from 44.26 μm in bonobos to 52.66 μm in chimpanzees). GLR values were also similar between humans and apes. The human mean (0.22) also fell within the range of means seen in the apes (from 0.23 in the gorilla specimen to 0.286 for the bonobos) and did not differ significantly from the ape means.

BA 10 values for HSD were significantly larger in humans (mean = 59.52 μm, range = 41.72–68.36 μm) than in great apes (Wilcoxon 2-sample rank test, $Z = 3.368$, $P = 0.0002$), where species means ranged from 36.35 μm in orangutans to 41.76 μm in gorillas. GLR was significantly lower in the human BA 10 (mean = 0.18) than in great apes, where the species means ranged from 0.21 in gorillas to 0.27 in orangutans ($Z = 2.791$, $P = 0.004$). In fact, GLR in the human BA 10 was lower than in any other region, in any species, examined in this study. As noted previously, the human trend of increased HSD in BA 10 reached significance in comparison with BA 3 ($Z = 1.995$, $P = 0.046$) and in comparison with BA 17 ($Z = 3.361$, $P = 0.00016$). Although the human species mean for HSD was greater in BA 10 than in BA 4, this difference was not statistically significant. The larger HSD seen in BA 10 of most of the human specimens is a result of apes having less widely spaced neurons in BA 10, rather than humans having smaller HSD values in BA 4, BA 3, or BA 17 compared with apes. In great apes, BA 10 HSD is also significantly larger than BA 17 HSD ($Z = 3.175$, $P = 0.001$), just as it is in humans.

Given the large differences in absolute brain size between humans and great apes, we examined the relationship of HSD to brain size by calculating a ratio between the estimated cross-sectional area of a minicolumn and brain volume (see Materials and Methods and Schenker et al. 2008). As can be easily seen in Figure 5 in all regions, humans possessed minicolumns that were significantly narrower than in great apes, relative to brain size (Wilcoxon 2-sample rank test, BA 10 $Z = 3.274$, $P = 0.001$; BA 4 $Z = 3.464$, $P = 0.001$; BA 3 $Z = 3.466$, $P = 0.001$, BA 17 $Z = 3.466$, $P = 0.001$).

Of particular note is the degree of interindividual variation in our study (Fig. 6). The human specimens in particular exhibited a great degree of interindividual variation in HSD, especially within BA 10, where the standard deviation was

larger than it was in any other species or region (±12.77, Table 2). No interaction with sex or age was found for any statistical measure, although in the BA 10, female apes had a slightly higher HSD and slightly lower GLR than male apes. However, because there was a wide range of ages in our human sample, we tested for the effects of age by removing the 3 oldest human specimens (69, 72, and 75), as their frontal lobes may have been subject to age-related changes in cell density that may skew parameters such as HSD. When these 3 oldest human specimens were removed from analysis, the species mean for BA 10 was, in fact, slightly larger (64.83 μm when only the 5 youngest brains are analyzed and 59.52 μm in the original analysis). BA 4 was similarly larger in the 5 youngest human specimens (53.73 μm compared with 49.23 μm). HSD in BA 3 was very slightly smaller in the 5 youngest brains, at 43.45 μm, compared with 45.32 μm in the original analysis. HSD in BA 17 was likewise very slightly smaller in the 5 youngest brains, at 36.59 μm, compared with 38.61 μm in our original analysis. One bonobo specimen was 2 years old but has been successfully incorporated in previous work (Schenker et al. 2008) and had HSD and GLR values for all 4 regions examined that fell well within the ranges in those regions reported for the great apes. We carried out tests after removing it from analysis and found that its exclusion did not substantially change the species mean or any of the comparisons carried out with the rest of the species. One chimpanzee specimen was sectioned at 15 μm rather than 20 μm, and in the axial rather than coronal plane. To ensure that this was not affecting the chimpanzee species results, we also removed it from analysis and found that its exclusion did not substantially change the species mean or any of the comparisons carried out with the rest of the species. For BA 10, removing YN89 gave chimpanzees a species mean HSD of 36.99 μm, compared with 37.52 μm in the original analysis. In BA 4, the species mean was 50.86 μm without YN89,

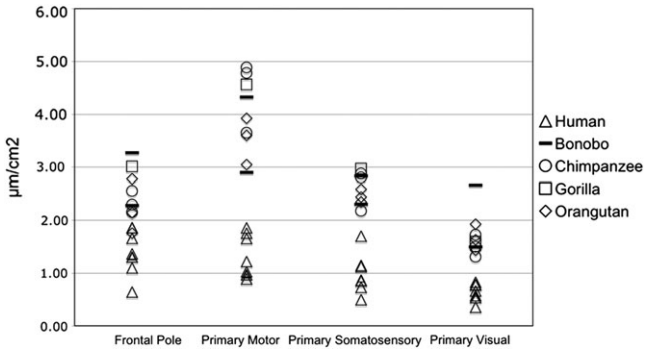

Figure 5. Estimated cross-sectional area of a minicolumn relative to brain size in all species and regions.

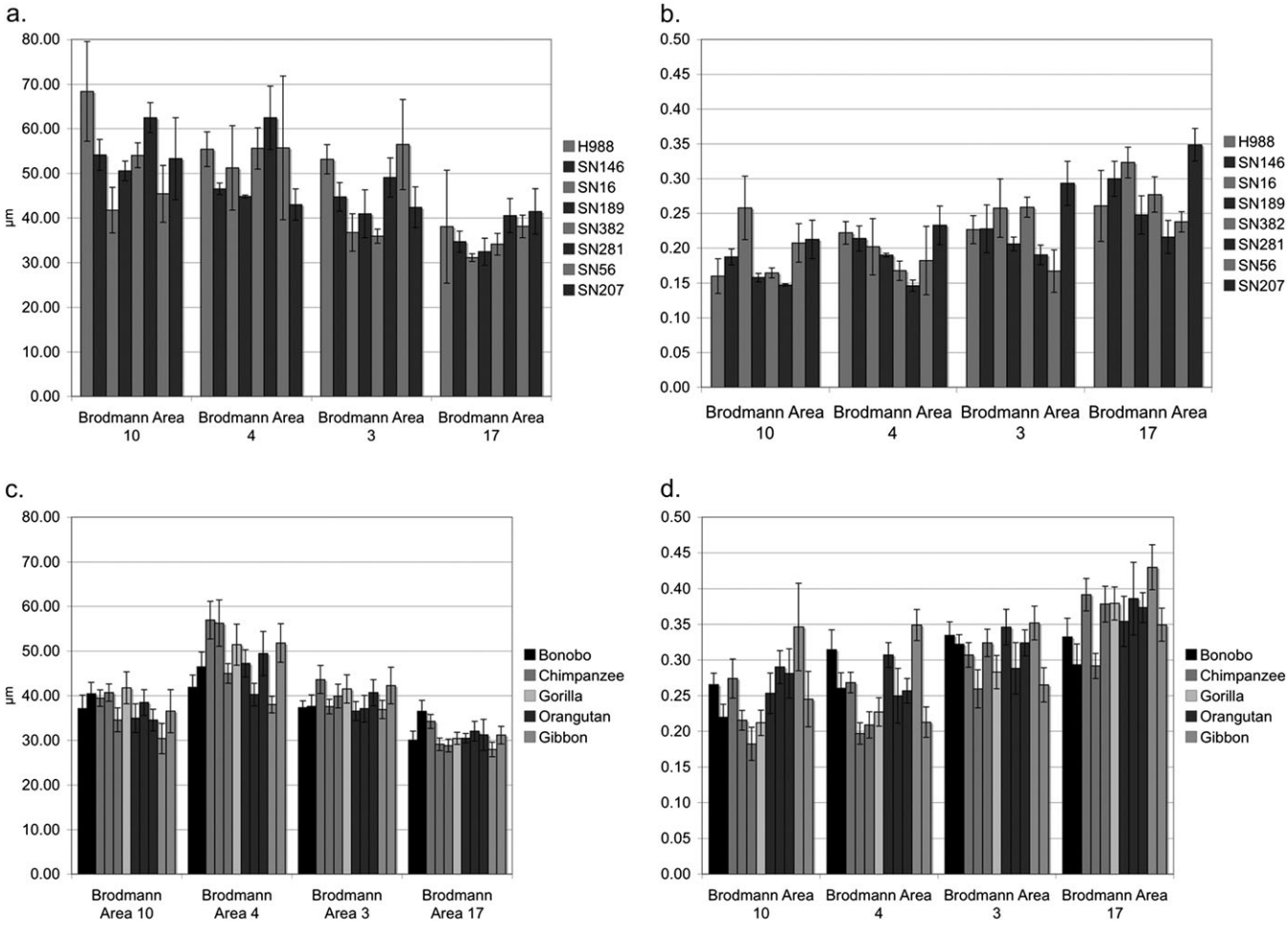

Figure 6. Human data by specimen for (*a*) HSD and (*b*) GLR, and ape data by specimen for (*c*) HSD and (*d*) GLR.

compared with 52.66 μm with it included. BA 3, the species mean was 41.76 μm without YN89, compared with 40.35 μm with. In BA 17, excluding YN89 gives a value of 31.75 μm, compared with 30.74 μm.

In summary, the current study identified significant differences in both parameters studied regarding the spatial organization of neurons between humans and apes in the frontal pole (BA 10). Additionally, in the human brain, significant differences were found between the frontal pole and 2 of the 3 regions studied (BA 3 and BA 17). Specifically, HSD was greatest in human BA 10, followed by BA 4, BA 3, and BA 17 (Fig. 3). In all of the apes, however, HSD was greatest in BA 4, followed by BA 3 or BA 10, with the smallest HSD again being found in BA 17. HSD in the human frontal pole in particular stood out by being 30% larger than in the frontal pole of the other species. As expected, the GLR, which indicates the fraction of area occupied by cell bodies, followed a nearly opposite pattern to that seen through HSD. GLR in the human BA 10 was 25–30% less than it was in the ape specimens (Fig. 4). With the exception of BA 10, humans shared overlapping HSD and GLR values with the apes for all areas, including BA 17, BA 3, and BA 4. HSD in BA 4, although relatively largest in apes, did not differ between humans and apes in absolute terms, suggesting that the absolute increase in the size of BA 10 human values was the cause of the observed disparity between humans and apes.

Discussion

The present findings demonstrated that, with the exception of BA 10, absolute HSD varies modestly across species, including humans. Despite large differences in overall brain size between species, HSD in BA 17 was close to 30 μm in lesser and great apes and 40 μm in humans. The smaller apes, gibbons, with a brain size of about 100–130 g, exhibited HSD values in BA 10 that were only slightly increased in comparison with BA 17, and similar to those seen in great apes, whose brains are 3-4 times larger. Across the apes, the region with the most widely spaced neurons was the primary motor cortex, BA 4, whereas BA 10 and BA 3 values were similar in the great apes. In all species, the region with the most closely spaced neurons was the primary visual cortex, BA 17.

The parameters used in our method (HSD and GLR) do not assume the existence of minicolumns and provide data that can be used either in connection to the minicolumn hypothesis (Buxhoeveden and Casanova 2002a, 2002b) or independent of that. Given that we find the columnar organization to be a very important feature of the cortex (Mountcastle 1997; DeFelipe 2005), we will continue to interpret our data in this light. The combination of increased HSD and decreased GLR values have been previously used to identify the presence of wider minicolumns in the cortex, reflecting increased intracolumnar and intercolumnar neuropil space in layer III (Buxhoeveden et al. 1996, 2001; also see Materials and Methods here). As in the current study, minicolumns were found to be wider in the prefrontal cortex (dorsal, mesial, and orbital regions) than in BA 17 in normal human adult brains, as compared with autistic brains (Buxhoeveden et al. 2006), while another comparison between human control and autistic brains (Casanova et al. 2006) reports interneuronal distance to be highest in prefrontal area BA 9, followed closely by BA 4, then BA 3 and BA 17. A study of BA 10 of humans and apes reports more neuropil space present in supragranular layers (II and III) in humans than in great apes or other primates (Semendeferi et al. 2001). Our previous study comparing minicolumnar organization in BA 44/45 in humans and apes (Schenker et al. 2008) also demonstrates that neurons in layer III are significantly more widely spaced in the human frontal lobe than in great apes. The HSD and GLR values in BA 44/45, which were obtained in an identical manner from the same individual specimens included here, are very close to the values obtained from BA 10. Although BA 10 and BA 45 form only part of the prefrontal cortex, the fact that both areas exhibit significantly wider minicolumns in humans than great apes may be indicative of some degree of reorganization characteristic of the human prefrontal cortex in general and possibly the frontal cortex as a whole. While the current study also found slightly wider minicolumns in human BA 17 (increased HSD) than in great ape BA 17, there is no evidence that there are significant differences in dendritic branching in human BA 17; studies examining both minicolumn spacing (Buxhoeveden et al. 2006; Casanova et al. 2006) and dendritic branching (Elston et al. 2006) found both wider minicolumns and more complex dendritic branching in the human prefrontal cortex compared with BA 17.

One interpretation of the functional significance of absolutely wider minicolumns, such as those noted in this and the previously mentioned studies, is that they are associated with being more generalized processors (Gustafsson 1997, 2004). Minicolumns are largely comprised of pyramidal neurons in layer III, along with their myelinated axons and apical dendrites, which have a vertical orientation (Peters and Sethares 1996; DeFelipe 2005). These neurons are present in large numbers in the cortex (Peters and Kara 1987) and are involved in corticocortical integration (Meyer 1987; Spruston 2008). Structural features of these pyramidal cells are associated with processing demands (Benavides-Piccione et al. 2002, 2006; Elston 2003; Elston and Zietsch 2005). Specifically, basilar dendrites are the target of connections from other parts of the cortex (Douglas et al. 1995), and thus, the extrasomatic parts of the neurons located in the space between the bodies of the cells may reflect the amount of information convergence on a neuron (Jacobs et al. 1997).

Thus, these observed differences may reflect a potential divergence in the integrative demands on these regions. The specificity and significance of laminar relationships of prefrontal connections to distinct classes of neurons in this and other parts of the cortex have been extensively documented (Barbas et al. 2005; Germuska et al. 2006). Cells in supragranular layers, such as layer III, are important for transcolumnar and corticocortical connectivity and processing (Fuster 2003), and there are important prefrontal microcircuitry differences in humans, due to the complexity of dendritic branching of their large pyramidal cells in layer III (Elston et al. 2006; Petanjek et al. 2008). Alterations of the genotype in mice suggest that increases in dendritic length in parts of the brain may be related to the human-specific Foxp2 (Enard et al. 2009). In a pattern similar to the differences in HSD among areas reported here, Jacobs et al. (2001) found greater dendritic/spine complexity in higher association cortex in humans, including BA 10, BA 11, BA 6b, and BA 39, than in primary somatosensory and motor cortex (BA 3 and BA 4). We likewise found an anterior to posterior gradation of human HSD values, as BA 10 had the greatest HSD values, followed closely by BA 4, then more distantly by BA 3, and then

BA 17. Also correspondingly, there is a considerably greater number of spines in the basal dendritic tree of pyramidal cells in the human prefrontal cortex in comparison with small primates (galago, marmoset monkey, vervet monkey, macaque monkey, and Chacma baboon), but there is no such increase present in BA 17 (Elston et al. 2006).

When considered in tandem with the aforementioned studies (Casanova et al. 2006; Schenker et al. 2008), our results suggested that humans have more space for connections between neuronal cell bodies in the frontal lobe compared with apes (Fig. 6), and also compared with BA 3 and BA 17 within the human brain. Given the arguments that modifications in human pyramidal cell structure are related to changes in cognition (Elston 2007), the more widely spaced neurons observed in the frontal lobe of the human specimens may be related to behavioral differences in associative functions, including the greater human capacity for language and executive functions. The greater HSD appears at this time to be a characteristic specific to some regions and not a cortex-wide feature of the human brain. Space for connections may be more conserved and similar across species in primary cortical areas, like primary somatosensory, visual, and auditory areas, because they are shared across mammals and were present in the common mammalian ancestor (Krubitzer 1995; Krubitzer and Kaas 2005). Future studies including all great ape species can explore whether the unique human feature identified in the frontal lobe (BA 10 here, BA 45 in our previous study, and BA 9 in Casanova et al. 2006) is shared by other higher order association areas in the temporal and parietal cortex as well.

We must consider the alternative hypothesis that horizontal spacing between neurons observed in this and other studies may be related in part to differences in neuron to glia ratios (Sherwood et al. 2006) rather than largely to increased dendritic complexity (Elston 2007). However, the human pattern of glia to neuron ratios does not vary significantly in humans from what is predicted based on brain size (Sherwood et al. 2006). The current study further demonstrates that the human frontal pole is not part of a general primate pattern but instead stands out from the great apes. The observed absolute increased spacing of neuronal cell bodies is more likely to be related to the increased dendritic branching already demonstrated in humans versus other primates but still unexamined in any ape species.

Additionally, the current study, in agreement with our previous work on BA 44 and BA 45, suggests that minicolumns are narrower than expected relative to brain size in humans. Wider minicolumns contain more elements, such as synapses, and may have greater processing potential. However, the increase in their width results in fewer of them per unit cortex and fewer interconnections, whether local or long distance. The large cortex found in humans permits both absolutely wider minicolumns and more of them (Buxhoeveden et al. 2001; Schenker et al. 2008); despite having an enlarged cortex, primates appear to have relatively narrower minicolumns compared with most other mammals with much smaller brains (Peters and Sethares 1991; Peters and Yilmaz 1993). When the relationship of HSD to brain size is taken into account, all 4 regions examined in this study display narrower minicolumns in humans than they do in any of the apes. Narrower minicolumns may provide more resolution by dividing the input coming into a region into more units (Seldon 1981; Gustafsson 1997). Rats and cats have much wider minicolumns in V1 than primates and this has been thought to be related to the complexity of primate vision (Peters and Yilmaz 1993; Peters and Sethares 1996).

As Buxhoeveden and Casanova (2005a) note, there are numerous advantages to the comparative study of minicolumns across closely related species. Evidence strongly suggests that minicolumns in the adult brain are largely the product of ontogenetic cell columns (Rakic 1995, 2007). Despite the presence of tangential migration during cortical development (Corbin et al. 2001), radial migration is the principal mode of migration within the developing cortex (Kornack and Rakic 1995; Parnavelas 2005; see review in Rakic and Kornack 2007), and pyramidal cells in particular almost entirely follow radial pathways (Marin and Lopez-Bendito 2007; Huang et al. 2009). Moreover, even if adult minicolumns are not entirely related to ontogenetic units, they are still an important level of analysis. In the adult brain, minicolumns have been proposed to function as input–output processing devices that maintain specific connections between aggregated minicolumns (macrocolumns), cortical regions, and subcortical structures (Mountcastle 1979). Even if minicolumns do not have a discrete function of their own, they represent a subunit of a larger functional unit, the macrocolumn (Mountcastle 1997). Thus, variations in minicolumn morphology, at minimum, suggest alterations in the composition of macrocolumns. Additionally, minicolumns are also of interest due to their potential to help address the question of how brains deal with increasing size and the ensuring connectivity issues. As brain size increases, they may become less efficient in terms of connectivity; there is a corresponding decrease in the percentage of neurons to which any individual neuron is linked (Hofman 2001; Striedter 2005).

In addition to the interest that minicolumns in and of themselves have, the 2 parameters of focus in this study, HSD and GLR, are also important as indices of possible differences in cell morphology that may translate into differences in cell communication and overall cortical input–output organization between areas and species. Development of the cortex entails many corresponding anatomical events; the modest enlargement of neurons, their expansion from their once tighter vertical array (as witnessed in fetal and young postnatal cortex), the development of myelination, intrinsic and extrinsic fibers, synaptogenesis, and other developmental events are all components of the reason why minicolumns will be the size that they are. As discussed previously, differences in spacing between cell bodies may be indicative of differences between arborization patterns in humans (Elston 2007), although no studies exist for the great apes yet. In sum, we suggest that a consistent increase in minicolumn width is a significant anatomical feature that can only be present as a result of differences in the set of features described previously. HSD and GLR are feasible measures on Nissl-stained sections, assuming the application of firm and consistent protocols as applied here, that can then point to locations of interest in the cortex where additional sampling with other techniques (e.g., Golgi stain) that target small numbers of selected cells can be promising. Thus, measures such as HSD and GLR are an important first step in identifying regions of potential species-specific specializations.

Developmental events may also be relevant to the human-specific pattern of neural organization. Buxhoeveden et al. (2006), examining spacing distance in minicolumns, reported

that a 2-year-old normal human possesses minicolumns that are 90% of the adult width in BA 17, but less than 75% of the adult width in the prefrontal cortex. In contrast, the 2-year-old bonobo in the present study had prefrontal cortex values within the range of the adult apes. Although a 2-year-old bonobo is closer to adulthood than a 2-year-old human and these specimens are therefore not directly comparable, both are young infants undergoing significant brain development and can be considered analogous. The parameters used here, HSD and GLR, are free of assumptions about vertical cell linearity in the adult cortex (see Materials and Methods). Nevertheless, they can be hypothesized to describe aspects of minicolumnar morphology that may prove to differ between layers in the adult cortex after the dispersal of cell bodies following the earlier formation of ontogenetic columns. A reasonable hypothesis is therefore that in humans both the remarkable increase in frontal pole HSD values and their departure from the ape pattern take place sometime after the age of 2 years. This increase is unique to the BA 10 and does not take place in BA 4 or BA 3, and only marginally in BA 17. In these 3 latter areas, HSD values are similar to those seen in apes. Until at least the age of 2, humans share similar absolute minicolumn values with great apes in the frontal pole and BA 4, BA 3, and BA 17 (Buxhoeveden et al. 2006). It is also of interest that Travis et al. (2005), who studied dendritic patterns of human pyramidal neurons in the developing human cortex, report that the developmental time course of basilar dendritic systems is heterochronous and more protracted for BA 10 than for areas BA 4, BA 3, and BA 17. Based on the radial unit hypothesis (Rakic 1995, 2007), one way that the surface area of cortex (or specific regions) can increase is due to changes in the number of ontogenetic units, the size of their adult form, or a combination of both. Thus, enlarging the width of adult columns will result in an increase in the size of a given cortical region without an increase in the number of ontogenetic units. The present finding that human BA 10 HSD is larger than that of the great apes in combination with a previous finding documenting that BA 10 is enlarged in the human brain when compared with great apes (Semendeferi et al. 2001) is possibly a reflection of both more ontogenetic units and an increase in their width and provides some support for this hypothesis.

In conclusion, the present findings support the idea (Semendeferi et al. 2002; Allen 2009) that human evolution, after the split from the common ancestor with chimpanzees, was accompanied by discrete modifications in local circuitry and interconnectivity of selected parts of the brain. In Figure 7, we depict an evolutionary reconstruction that includes proposed changes in the shared common ancestry with the apes. As can be seen, most of the evolutionary history of hominoids includes narrower minicolumns and increased cell packing in BA 17, followed closely by BA 10 and BA 3, with BA 4 having the largest neuronal spacing. It is only after the split from the last

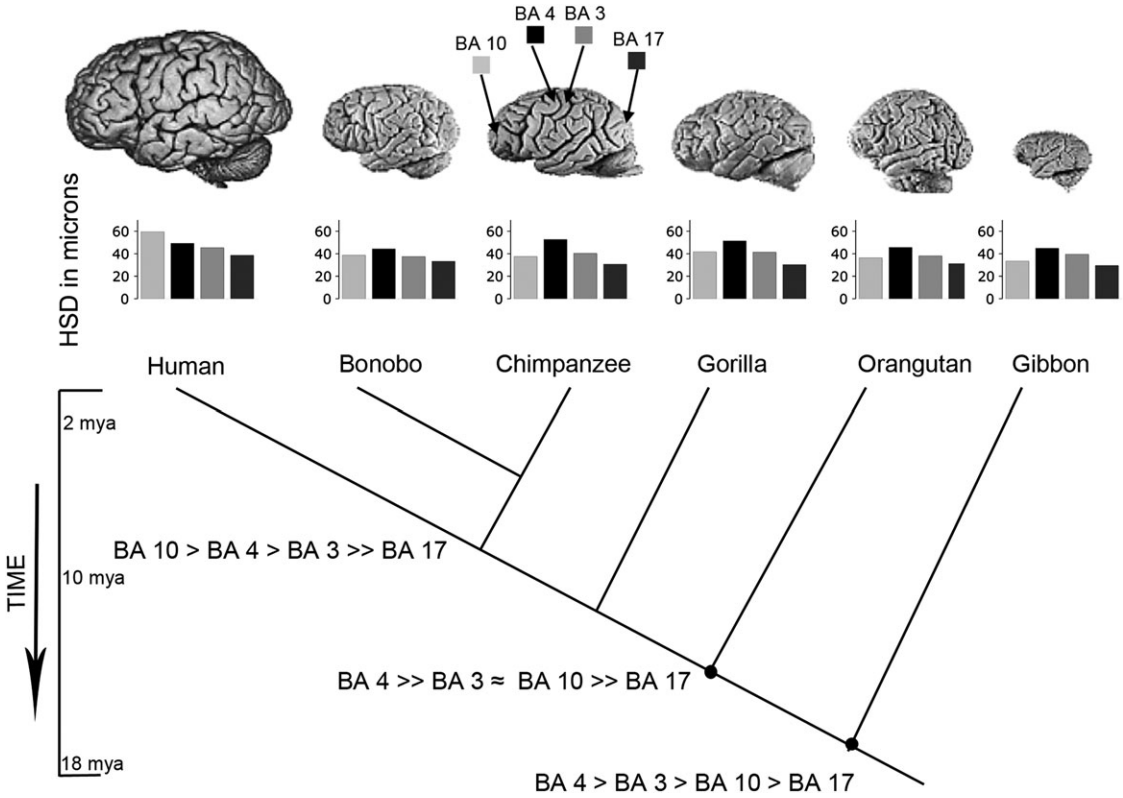

Figure 7. Cladogram showing lateral views of the human and ape brain and horizontal spacing of neurons in the cortex. Bars in the graphs represent the frontal pole (BA 10), primary motor cortex (BA 4), primary somatosensory cortex (BA 3), and primary visual cortex (BA 17) across human and ape brains. The evolutionary reconstruction includes proposed changes in the shared common ancestry with the apes. Most of the evolutionary history of apes and humans includes narrower minicolumns and increased cell packing in the primary visual cortex (BA 17), followed closely by the frontal pole (BA 10) and primary somatosensory cortex (BA 3), with primary motor cortex (BA 4) having the largest neuronal spacing. It is only after the split from the last common ancestor with the chimpanzees that BA 10 neuronal spacing became the largest compared with the other areas of the human brain and with the other apes. The symbol ">" indicates when HSD is larger in one area compared with another, ">>" indicates a statistically significant difference, and "≈" indicates that HSD in the 2 regions is roughly the same. Tests of significance for regional differences within the brain were carried out on all great ape specimens as a group and all human specimens as a group.

common ancestor with the chimpanzees that BA 10 HSD became the largest compared with the other areas of the human brain and with the other apes.

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